RESTING FOREARM BLOOD-TISSUE LACTATE AND GAS EXCHANGE KINETICS DURING SUPINE LEG EXERCISE

by

PETER CATCHESIDE

B.Sc. (Hons.) (University of Adelaide, 1989)

A thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

Department of Physiology,
University of Adelaide,
South Australia, 5005

December, 1999

For Britt,

Sam and Katherine

TABLE OF CONTENTS

ABSTRACT

DURING EXERCISE, active muscles exhibit large positive veno-arterial lactate concentration differences and are the primary site of lactate production. At the same time inactive muscles demonstrate substantial negative veno-arterial lactate differences. Without exception, this uptake has been interpreted to indicate a shift from metabolic production to disposal (principally oxidation) of lactate in resting muscles. Few authors have acknowledged that most of the lactate taken up under these conditions is unlikely to be metabolised.

The work described in this thesis was stimulated by the observation that during leg exercise resting forearm lactate kinetics appear to resemble closely the blood-tissue exchange kinetics of inert gases that have been reported in many organs during inert gas inhalation. Clearly, when the arterial concentration of any diffusible substance changes with time, substantial veno-arterial concentration differences need not imply metabolic disposal. Accordingly, passive lactate movements with little or no lactate disposal potentially dominate forearm lactate uptake during leg exercise.

Two mathematical models of forearm lactate uptake were developed, and three series of experiments were conducted to investigate the contribution and impact of passive (inert-like) lactate movements to veno-arterial concentration differences in the resting forearm during leg exercise.

In the first study, described in Chapter 3, resting forearm venous blood lactate changes associated with moderate intensity leg exercise were predicted from measurements of forearm volume, arterial concentration and blood flow using an integrated form of the Fick equation commonly applied to the study of inert gas uptake by tissues. Close agreement between the measured and predicted venous

blood lactate responses indicated that forearm lactate uptake could be predominantly accounted for in terms of passive lactate movements without disposal.

In the second study (Chapter 4), the influence of forearm lactate uptake on lactate threshold measurements was examined. The lactate threshold is an important measure of endurance exercise capacity and is operationally defined as the exercise intensity at which blood lactate accumulation begins, or an arbitrary fixed concentration of lactate is reached during an incremental exercise test. While arterial blood most accurately reflects the systemic blood lactate response to incremental exercise, the lactate threshold is more commonly derived using forearm venous blood and is therefore potentially affected by forearm lactate uptake. The log-log lactate threshold, 2 and 4 $mmol \cdot l^{-1}$ fixed concentration thresholds and the lactate slope index (LSI), a measure derived from an exponential model of lactate increase during incremental exercise, were determined from simultaneous arterial, forearm venous and finger prick blood lactate measurements and compared between the blood sampling sites. To investigate the distorting effects of forearm lactate uptake on the underlying arterial blood lactate response, the fitting ability of the conventional threshold (log-log) model, and the competing exponential model of blood lactate increase were compared between the blood sampling sites. Differences between lactate threshold determinations from forearm venous blood and those derived from arterial and finger prick blood were consistent with predictions based on passive lactate movements without forearm lactate disposal. Whilst the threshold and exponential models of lactate increase during incremental exercise fit equally well when applied to finger prick and forearm venous blood, the exponential model provided a significantly better fit when applied to arterial blood. These findings provide a theoretical basis for blood sampling site differences in lactate threshold determinations, support the hypothesis that blood lactate accumulates as an exponential function of exercise intensity, and highlight the importance of blood sampling site considerations in incremental exercise tests.

In the final study (Chapter 5), assumptions underlying the treatment of blood-tissue lactate exchange in a manner equivalent to an inert gas were more closely examined. While the general principles governing inert gas exchange apply to all membrane translocatable blood borne substances, blood-tissue exchanges of metabolic substrates and products are potentially more complex. Veno-arterial concentration differences in lactate, H^+, CO_2, O_2 and the metabolically inert gas nitrous oxide (N_2O) were examined during and after a 20 min period of moderate to high intensity leg exercise and N_2O inhalation. Veno-arterial N_2O concentration differences were relatively poorly predicted with the single compartment model conventionally applied to inert gas uptake. Consequently, the treatment of N_2O and other blood borne substances such as lactate with this model in the resting forearm may be inappropriate. However, a two compartment model incorporating a diffusion barrier to blood-tissue exchange simultaneously accounted for the majority of the observed veno-arterial concentration differences in N_2O, lactate and CO_2 and to a lesser degree H^+ and O_2, supporting the hypothesis that passive movements of blood borne substances dominate forearm veno-arterial concentration differences during leg exercise.

In summary, these studies demonstrate the difficulties associated with the interpretation of veno-arterial concentration differences measured during non-equilibrium conditions such as exercise. In the resting forearm, substantial lactate uptake, as well as increased CO_2 output are largely consistent with passive movements associated with exercise related changes in arterial blood chemistry and forearm blood flow. Consequently, the role that resting muscles play in disposing of lactate produced by active muscles may have been over-estimated.

PUBLICATIONS

Journal Article

Catcheside, P.G. and G.C. Scroop. Lactate kinetics in resting and exercising forearms during moderate-intensity supine leg exercise. Journal of Applied Physiology. 74(1): 435-443, 1993.

Paper Presented at a Conference

Catcheside, P.G. and G.C. Scroop. The effect of blood sampling site on the lactate threshold during incremental exercise. Proceedings of the Australian Physiological and Pharmacological Society. 24(2): 187P, 1993. Awarded the Clinical and Experimental Pharmacology and Physiology prize for best conference poster.

DECLARATION

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

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

Peter Catcheside
22 December, 1999

ACKNOWLEDGEMENTS

First and foremost, I am most grateful to Associate Professor Garry Scroop for providing me with the opportunity to work in his laboratory, for his expert guidance and for his patience and support.

I am very grateful to Dr Jon Buckley for his friendship, expert assistance in experiments and thoughtful discussions, and to Julia Pitcher, Ali Mark-Seymour, Jacob Ross, Stan Flavell, Maureen Crossman, Chris Amery, Janine Phillips and Craig Noel who at various times provided invaluable technical help.

I am also very grateful to Colin McClean for his advice and for making available the gas chromatographic facilities for measuring blood nitrous oxide levels.

I am also indebted to Michael Arnold, Ashley Rivett, Chris Wallace and Troy Porter who at various times provided invaluable advice regarding mathematical issues.

I am especially grateful to Associate Professor Doug McEvoy for granting me the opportunity to complete the final stages of this work.

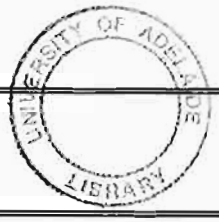
I am also very grateful to the subjects who participated in the experiments, to The University of Adelaide and the Department of Physiology for scholarship support, and to my colleagues and friends for their help and encouragement.

A final thankyou to my parents for their support and to Mum and Amy Jordan for proof reading.

GLOSSARY OF ABBREVIATIONS

[ ]	Concentration of a substance (given within braces)
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
Ca(t)	Arterial concentration at time t
Cb(t)	Blood (compartment) concentration at time t
C_{dpg}	Erythrocyte 2,3-diphosphoglycerate concentration
χ^2	Chi-squared
Ci(t)	Tissue concentration at time t
CO_2	Carbon dioxide
COHb	Carboxyhaemoglobin
Cv(t)	Venous concentration at time t
F(t)	Blood flow at time t
H^+	Hydrogen ions
Hbf	Fetal haemoglobin
HR	Heart rate
HR_{peak}	Peak heart rate
IAT	Individual anaerobic threshold
K_m	Michaelis-Menton equilibrium constant
λ	Blood-tissue partition coefficient
La	Lactate
LDH	Lactate dehydrogenase
LDH-H	Lactate dehydrogenase (heart specific isomer)
LDH-M	Lactate dehydrogenase (muscle specific isomer)
LSI	Lactate slope index
LT	Lactate threshold
m	Model diffusion term
Mb	Myoglobin
MbP_{50}	Tissue pO_2 at which myoglobin is 50% saturated
MetHb	Hemiglobin
MAP	Mean arterial blood pressure
MSC	Model selection criterion
N_2O	Nitrous oxide
NAD^+	Nicotinamide adenine dinucleotide (oxidised form)
NADH	Nicotinamide adenine dinucleotide (reduced form)
O_2	Oxygen
O_2Hb	Oxyhaemoglobin
ODC	Oxyhaemoglobin dissociation curve
P	Probability
pCO_2	Partial pressure of CO_2
pCO_2b	Partial pressure of CO_2 in the blood compartment
pCO_2i	Partial pressure of CO_2 in the tissue compartment
Pi	Inorganic phosphate
pO_2	Partial pressure of O_2
pO_2b	Partial pressure of O_2 in the blood compartment
pO_2i	Partial pressure of O_2 in the tissue compartment

PS	Permeability surface area product
Pyr	Pyruvate
Qb(t)	Substance quantity in the blood at time t
Qi(t)	Substance quantity in a tissue at time t
Qs(t)	Substance quantity in a system at time t
RdL	Rate of irreversible lactate disappearance
r^2	Coefficient of determination
RER	Respiratory exchange ratio
RSS	Residual sum of squares
sO_2	O_2 saturation (of Hb or Mb)
T	Temperature
t	Time
tCO_2b	Total blood compartment CO_2 content
tCO_2i	Total tissue CO_2 content
tCO_2p	Total plasma CO_2 content
tHb	Total haemoglobin concentration
tMb	Total myoglobin concentration
tO_2b	Total blood compartment O_2 content
tO_2i	Total tissue O_2 content
Vb	Blood compartment volume
$\dot{V}CO_2$	CO_2 output
$\dot{V}_E$	Minute ventilation
$\dot{V}_{E\ peak}$	Peak minute ventilation
Vi	Tissue compartment volume
V_{max}	Maximal reaction rate
$\dot{V}O_2$	O_2 uptake
$\dot{V}O_{2\ max}$	Maximal systemic O_2 uptake
$\dot{V}O_{2\ peak}$	Peak systemic O_2 uptake
WL_{peak}	Peak work load



CHAPTER 1 General Introduction

1.1 Background

LACTIC ACID is regarded as an important metabolic intermediate that undergoes substantial blood concentration changes during moderate to high intensity exercise. When the blood lactate concentration is elevated by exercise or by infusion, resting muscles have consistently been shown to take up lactate from the blood (4, 5, 40, 46, 95, 97, 104, 105, 161, 211, 247, 279, 282, 284). Without exception, this has been interpreted to indicate a shift from metabolic production to disposal of lactate in resting muscles. While resting muscles clearly possess the capacity to metabolise lactate, and there is some compelling evidence that resting muscle can become a net consumer of lactate when arterial lactate is elevated (13, 40, 46, 97), in all but one of these studies (97) non-equilibrium conditions predominated. Unless there is a steady-state in both arterial and venous lactate concentrations, an important component of resting muscle lactate uptake inevitably reflects blood-tissue equilibration and not metabolic disposal. Furthermore, it is very difficult to distinguish these two processes from veno-arterial concentration differences alone. Even with the benefit of tissue sampling and estimates of tissue lactate content, this alone may not account for the potential incorporation of lactate into glycolytic, glyconeogenic, citric acid cycle and other metabolic intermediates. Whilst many of these reactions could be regarded as metabolic disposal, particularly those involving irreversible reactions such as the oxidative decarboxylation of pyruvate to form acetyl-CoA, those involving glycolytic intermediates including pyruvate are reversible and potentially buffer lactate concentration changes without necessarily any change in net lactate production. The reliable interpretation of non-equilibrium veno-arterial lactate concentration differences clearly requires careful consideration of blood-tissue lactate equilibration processes.

This chapter examines the literature relating to the importance of lactate in exercise and the metabolic processes underlying blood lactate accumulation and disposal, particularly in resting muscles. In addition, the principles that govern the exchange of lactate and other substances between the blood and tissues and their potential significance with regard to resting muscle lactate uptake and gas exchange during exercise performed in other muscle groups are considered.

1.1.1 Terminology

For the work described in this thesis, it is important to clarify the meaning of several terms. Lactic acid has a dissociation constant of ~3.7 and is therefore almost completely dissociated into lactate and H^+ in the physiological range of pH (99). Consequently, lactate acid is generally referred to simply as lactate and this convention has been adopted throughout this thesis. The terms uptake (removal from the blood) and output (release into the blood) are used to indicate negative and positive veno-arterial concentration differences, without reference to the fate of the substance within muscle. Similarly, the terms wash-in and washout are used to describe passive substance movements (uptake and output respectively) associated with equilibration between the blood and tissues, while the terms disposal and production are used specifically to describe irreversible metabolic processes within muscle. Utilisation is used to indicate the irreversible metabolic disposal of a substance and not uptake associated with an increase in muscle content. The conversion of lactate to pyruvate catalysed by lactate dehydrogenase is not considered disposal because this reaction is near equilibrium and is readily reversible. These are important distinctions because some investigators imply that uptake is equivalent to disposal or that utilisation includes disposal as well as uptake within tissues.

1.1.2 Blood lactate accumulation and fatigue

The enormous interest in blood lactate concentration changes during exercise probably stems from the strong association between lactate accumulation, muscle fatigue and endurance exercise performance (119, 140, 141, 232, 257, 283). Many enzyme systems and reactions that utilise or produce hydrogen ions are strongly influenced by pH. For example, hydrogen ions could inhibit calcium binding to muscle regulatory proteins thereby interfering with force generation (143). Alternatively, hydrogen ions have been shown to inhibit the activity of enzymes such as phosphorylase and phosphofructokinase *in vitro* (6), although it appears that at least some of these effects are counteracted *in vivo* (222, 237). Whilst hydrogen ion accumulation associated with lactic acidosis is generally regarded the more important factor (14, 165, 166, 238), there is evidence to suggest that the lactate anion may also contribute to muscle fatigue (119). Hogan et al. (119) found that tension development in an electrically stimulated canine gastrocnemius muscle preparation was reduced during lactate infusion at a pH that produced no change in arterial or intramuscular pH, implicating the lactate anion itself as a cause of fatigue. Similarly, Mainwood et al. (166) showed that muscle fibers exposed to an elevated lactic acid concentration demonstrate some but not all of the responses seen in fatigue following contractions. In an earlier study by the same group (165), maximal tension was reduced in frog muscle exposed to a CO_2 induced acidosis, although the reduction in tension development was considerably less than in muscles fatigued by contraction to produce a similar intramuscular H^+ concentration.

Whilst the metabolic acidosis that accompanies lactate accumulation has been implicated as a cause of fatigue, the association between lactate and H^+ accumulation is much more complex than is widely appreciated. Contrary to common belief, when ATP formation and the likely electrical charges of magnesium with which ADP and ATP can be complexed are considered in the context of the intracellular pH, the breakdown of glucose or glycogen to lactate is unlikely to produce a net gain of protons (6, 69, 91). The accumulation of H^+ during moderate to

severe exercise is more likely a consequence of proton release during rapid ATP hydrolysis, in combination with a greater proportion of ATP resynthesis by glycolysis, rather than mitochondrial oxidative phosphorylation which is believed to favour net H^+ consumption (6). Nevertheless, even if perhaps largely associative, lactate formation coincides with metabolic acidosis and fatigue, and respiratory compensation for the metabolic acidosis adds further to the energy demands of exercise and could further contribute to fatigue. Furthermore, lactate accumulation is associated with accelerated glycolysis and therefore more rapid glycogen depletion, and glycogen is essential for sustaining high rates of ATP resynthesis. Whilst this would not limit short-term exercise, muscle glycogen depletion associated with prolonged moderate to high intensity exercise could also contribute to fatigue.

1.1.3 Patterns of blood lactate accumulation during exercise

1.1.3.1 Constant load exercise

The pattern of blood lactate increase during sustained submaximal exercise depends on exercise intensity. At exercise intensities below the lactate threshold, blood lactate changes very little from resting levels (62, 152). At moderate exercise intensities there is a progressive increase in blood lactate concentration reflecting a transient imbalance between the rates of lactate production and release, and uptake and disposal. Over time the rate of lactate removal approaches that of release giving rise to a more stable blood lactate concentration (112). During high intensity exercise the rate of lactate production may exceed the maximal rate of lactate removal such that lactate continues to accumulate to the point of exercise termination (112, 152).

1.1.3.2 Incremental exercise and the lactate threshold

During incremental exercise the blood lactate concentration changes little until the work rate reaches ~40-60% of maximal aerobic capacity ($\dot{V}O_{2\,max}$), beyond which

lactate rapidly accumulates in the blood. This pattern of increase, in combination with the traditional view that lactate production is a consequence of inadequate O_2 delivery to the tissues, led to the concept of an anaerobic or lactate threshold. Central to this concept is the existence of a threshold work load at which blood lactate accumulation begins. This concept has gained widespread acceptance perhaps most notably due to the work of Beaver et al. (22). These investigators proposed that the lactate threshold could be accurately identified from data obtained during an incremental exercise test by plotting the logarithm (log) of $\dot{V}O_2$ against the log of blood lactate concentration. This transformation appears to resolve the data into two intersecting linear segments, with the point of intersection determining the lactate threshold (22). However, this procedure, in common with the lactate threshold concept itself, assumes the existence of a threshold event such as O_2 requirement suddenly exceeding demand or perhaps a threshold pattern of motor unit recruitment (188). Although still a matter of debate, it appears that even during maximal exercise intracellular O_2 levels may not be limiting (53, 55), a finding that would be difficult to reconcile with the anaerobic threshold hypothesis. Nevertheless, this does not preclude the existence of a threshold in blood lactate accumulation.

The alternative hypothesis is that blood lactate accumulates in a progressive manner during incremental exercise to exhaustion and several investigators have advanced evidence to support this view. Hughson et al. (122) and Campbell et al. (43) reported that an exponential function provided a better fit to the blood lactate increase during incremental exercise when compared to the log-log model. Similarly, Dennis et al. (69) and Stanley et al. (246) have also suggested that lactate accumulates in the blood in an exponential manner. However, in all of these studies a somewhat empirical approach has been adopted and Morton (181) has cautioned that such an approach does not exclude a threshold process. Furthermore, systemic changes in blood lactate concentration represent a complex interaction between lactate production (and disposal) and net release in exercising muscles, and net uptake and disposal in other tissues. Whilst the intramuscular lactate concentration has been

shown by some to increase as a relatively linear function of stimulus frequency in an isolated muscle preparation (55), others have reported a threshold pattern of increase in exercising muscle lactate concentration during incremental exercise in humans (47). In combination, such findings suggest that a system approach is necessary to more thoroughly examine the pattern of blood lactate accumulation during incremental exercise and several models have been developed for this purpose (41, 182-184).

Whatever the true nature of the processes underlying the pattern of blood lactate increase during incremental exercise, the work load identified as the lactate threshold has repeatedly been shown to correlate well with endurance exercise performance (66, 110, 232, 250, 255, 283). Consequently, while not necessarily related to a threshold event, the lactate threshold does appear to provide a useful and objective measure of endurance exercise capacity.

1.2 Skeletal muscle lactate metabolism

As with any blood borne substance, the concentration of lactate in the blood at any given time reflects a balance between the rate of formation and release into, and the rate of removal from, the vascular compartment. Consequently, dynamic changes in blood lactate during exercise reflect the combined influences of lactate production, lactate disposal and blood-tissue lactate exchange processes occurring simultaneously in all of the tissue compartments.

1.2.1 Lactate dehydrogenase

Lactate dehydrogenase (LDH) is a tetrameric enzyme that catalyses the reaction:

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \overset{LDH}{\longleftrightarrow} \text{Lactate} + \text{NAD}^+$$

$$K_{LDH} = \frac{[\text{Pyruvate}][\text{NADH}][\text{H}^+]}{[\text{Lactate}][\text{NAD}^+]} \qquad \sim 1.11 \times 10^{-11}\ \text{M (260)}$$

This reaction is the final step in glycolytic lactate production and is effectively the only means by which endogenous or exogenous lactate can engage in skeletal muscle metabolism. LDH is therefore of fundamental importance to both lactate formation and disposal. There are five isozymes of the LDH tetramer, representing the possible combinations of the two subunit types, designated heart specific (H) and muscle specific (M) subunits (68). Predominantly H subunit isozymes have relatively low membrane binding affinities and favour the oxidation of lactate, while predominantly M subunit isozymes have a high membrane binding affinity and favour the reduction of pyruvate. In skeletal muscle, most LDH is of the M type and is located bound to the sarcoplasmic reticulum, the outer mitochondrial membrane and to a small extent solubilised within the cytosol. A small fraction of predominantly H-LDH is contained within the mitochondria (231).

Muscle LDH is critical for supporting high glycolytic flux. Several cases of muscle LDH deficiency have been described in which impaired glycolytic flux produces accumulation of glycolytic intermediates above the level of glyceraldehyde 3-phosphate dehydrogenase (138). As a result, ATP formation is impaired and rhabdomyolysis can occur during high intensity exercise.

LDH has high activity levels in skeletal muscle (12). Pyruvate reduction by LDH has a V_{max} 3-5 times higher than the highest rate of lactate formation observed *in vivo* (139), and the LDH reaction is generally believed to be in near equilibrium (189). Furthermore, the catalytic activity of LDH is many times greater than the combined activities of enzymes that catalyse the alternative reactions of pyruvate metabolism (31). Cytosolic NAD^+ produced in the forward LDH reaction serves to maintain glycolytic flux when the mitochondrial pyridine nucleotide shuttles do not replenish cytosolic NAD^+ at a rate compatible with ongoing glycolytic demands. At the same time a high muscle lactate concentration could also function as a substrate and/or redox buffer (52), providing readily accessible reducing equivalents and a carbon source to support mitochondrial oxidative phosphorylation. Furthermore, in human

muscle homogenates under optimal pH conditions, strong product inhibition of LDH activity has been demonstrated even at relatively low physiological lactate concentrations, leading Sjodin (231) to suggest that LDH may have a glycolytic regulatory function *in vivo*. It has also been proposed that the LDH reaction serves as an intracellular "lactate shuttle", facilitating the translocation of lactate within the cytosol from sites of glycolytic lactate production to sites of mitochondrial pyruvate consumption (36).

1.2.2 Lactate production

Lactate formation is intimately linked to the processes responsible for matching ATP replenishment to requirements. The exceptional capacity for skeletal muscle to hydrolyse ATP during muscular contraction places demands on these systems that promote lactate formation.

1.2.2.1 The role of hypoxia in lactate production

At a time when muscle preparations had been shown to produce lactate under hypoxic conditions (85), Hill and associates (115) observed blood lactate levels to rise slowly at first and then more rapidly at higher work loads during incremental exercise. This led to the belief that lactate production occurs as a consequence of inadequate oxygen delivery and tissue hypoxia. Under these conditions, where oxidative phosphorylation was believed to be limited by oxygen supply, accelerated glycolysis would serve to supplement ATP production. Although the precise role of hypoxia is still debated, several lines of evidence have emerged that refute a central role for hypoxia in lactate production. The most direct evidence has arisen from studies of mitochondrial redox state and intramuscular pO_2. Observations in isolated mitochondria indicate that the phosphorylation of ADP ceases when cytochrome *c* is more than 90% reduced, at a pO_2 of approximately 0.05 mmHg (45). The pO_2 above which the rate of phosphorylation is maximal is in the order of 0.1 – 0.2 mmHg in

isolated mitochondria (45) and 0.5 mmHg in intact cells (90, 146). Similarly, Connett et al. (51) demonstrated that the $\dot{V}O_{2\,peak}$ of dog gracilis muscle *in vivo* is independent of pO_2 above 0.5 mmHg.

Several studies using techniques such as surface NADH/NAD^+ fluorometry (126) and myoglobin cryomicroscopy (52-55) have shown lactate efflux from muscle despite an intramuscular pO_2 considerably in excess of 0.5 mmHg, even under maximal exercise conditions (53). Connett et al. (53) examined lactate efflux from muscles that were electrically stimulated at frequencies ranging from 1-8 Hz (10-100% $\dot{V}O_{2\,max}$) and later frozen *in situ* during steady-state contractions. Lactate efflux was found at twitch frequencies corresponding to approximately 50% $\dot{V}O_{2\,max}$ despite a median and lowest 5^{th} percentile intracellular pO_2 of 10 and 4 mmHg respectively, well above the level considered to limit oxidative phosphorylation. Anoxic intracellular loci were found in only 3 of 9 maximally stimulated muscles and, in each of these, accounted for only 15% of the cells examined. No anoxic loci whatsoever were found at lower twitch frequencies (4-6 Hz) despite considerable lactate efflux (53). Nevertheless, these observations do not exclude the existence of undetected intracellular hypoxic sites, which could still exist given the inhomogeneities in muscle capillarisation, blood flow, and intracellular mitochondrial distribution (129). Furthermore, surface NADH/NAD^+ fluorometry and cryomicrospectroscopy have been extensively criticised (77, 129, 142, 146). The signal penetrates only to a shallow depth and surface proximity could favour oxidation (77, 142). In addition, these techniques cannot distinguish between mitochondrial and cytosolic redox changes such that relative cytosolic oxidation could mask mitochondrial reduction.

Alternative evidence against a central role of hypoxia in lactate production derives from other properties of the exercise response. In both electrically stimulated muscle preparations and in human exercise, $\dot{V}O_2$ increases as a linear function of work rate, right to the point of maximal exercise. However, blood lactate accumulation begins at work rates corresponding to only 40 - 65% of maximal aerobic capacity ($\dot{V}O_{2\,max}$),

despite considerable aerobic reserve. Furthermore, blood lactate levels have been shown to vary with arterial pO_2 with no corresponding changes in $\dot{V}O_2$ (118, 163) and, following endurance training, $\dot{V}O_2$ at a given work load is the same as pre-training despite a lower blood lactate concentration and cardiovascular adaptations that should improve O_2 delivery (79, 120, 123). In fact, these adaptations are such that blood flow and therefore O_2 delivery to the exercising muscles is lower at a given absolute work rate after endurance training (79). Were mitochondrial phosphorylation to become limited by oxygen availability at submaximal work loads, the relationship between work rate and oxygen uptake might be expected to depart from linearity at the onset of oxygen limitation and to be altered with endurance training. Similarly, if lactate production serves to supplement mitochondrial ATP production to meet demand, then a non-linear relationship between work rate and $\dot{V}O_2$ would be predicted and oxygen availability should influence $\dot{V}O_2$. However, in humans and in electrically stimulated muscle preparations, there is no inflection in the linear increase in $\dot{V}O_2$ until near maximal work loads are reached, suggesting that mitochondrial oxidation is not limited in the normal physiological range of exercise intensity. On the other hand, glycolytic lactate formation is a poor means of supplementing ATP production, accounting for only an estimated 1-2% of the total ATP turnover even during high intensity exercise (53, 224). A corresponding decline in $\dot{V}O_2$ of this magnitude might be difficult to detect and need not occur if intracellular O_2 stores are not depleted as a linear function of work rate, if O_2 requirements concomitantly rise elsewhere or if mechanical efficiency simultaneously declines. Careful inspection of the data presented by Connett et al. (53) suggests that intracellular myoglobin stores of O_2 may actually increase with the onset of lactate efflux. This could indicate saturation behaviour in the ability of mitochondria to process O_2 at a time when O_2 delivery to the muscle is not limiting, although this seems unlikely given that $\dot{V}O_2$ continues to rise linearly with further increments in work rate. Such an observation, if reproducible, might be more consistent with a transitory phase of excessive ATP production via O_2 independent pathways.

There is little doubt that intracellular pO_2 declines as a function of exercise intensity, and while intracellular "hypoxia" *per se* may be unusual, a reduced pO_2 may well have an important influence on reactions indirectly linked to cytosolic redox state (210).

1.2.2.2 The contemporary view of lactate production

The contemporary view of lactic acid production is based on modern knowledge of the tight coupling between excitation-contraction processes and the cytosolic and mitochondrial pathways that regulate the cellular energy-state. An important component of this view is encompassed in the "near-equilibrium steady-state hypothesis" for the control of mitochondrial oxidative phosphorylation (277). In this hypothesis it is proposed that under physiological conditions, the reactions at the first two sites of oxidative phosphorylation (cytochromes *b* and *c*) are near equilibrium (81), and at the third site (cytochrome aa_3) are irreversible and therefore the rate-determining step in the overall process (93). At the first two sites the reaction rate depends on the intramitochondrial $[NAD^+]/[NADH]$, the mitochondrial phosphorylation potential, $[ATP]/[ADP]\cdot[Pi]$, and the relative concentrations of oxidised and reduced mitochondrial cytochrome *c*. At the third site the reaction rate is a function of the concentrations of O_2 and of reduced cytochrome *c*, the latter being a product of the near equilibrium reactions at the first two sites. Consequently, the rates of mitochondrial oxidative phosphorylation and the coupled O_2 consuming reactions of the electron transfer chain are believed to reflect the interaction of three main factors. These are, specifically, the intramitochondrial $[NAD^+]/[NADH]$ which depends on the activity of cytosolic and mitochondrial metabolic pathways and the glycerol phosphate and malate-aspartate shuttles, the mitochondrial phosphorylation potential which in turn is determined by the cytosolic phosphorylation potential and therefore ATP demand, and on the availability of molecular O_2.

With the onset of muscular contractions, ATP hydrolysis by the contractile elements consumes ATP and produces ADP and Pi. Changes in cytosolic [ATP]/[ADP] are largely buffered by a decrease in cytosolic [CrP]/[Cr], while Pi increases. Changes in mitochondrial [ATP]/[ADP] occur more slowly being dependent on the creatine, creatine kinase systems that facilitate the transfer of ADP and ATP between the myofibrils and the mitochondria and the dependence of this system on changes in creatine which take 1-2 min to reach steady-state (29, 240). At the same time, several intracellular events have a strong stimulatory influence on glycolytic rate. Calcium release from the sarcoplasmic reticulum produces a transient activation of phosphorylase kinase which covalently activates the less active *b* form of glycogen phosphorylase to the more active *a* form (64). This, in combination with additional phosphorylase activation via catecholamine induced changes in cyclic AMP and a Pi mediated increase in phosphofructokinase (PFK) activity, results in a more rapid increase in the rate of glycolysis relative to the rates of oxidative phosphorylation and transfer of reducing equivalents into the mitochondria. Subsequent NADH and pyruvate accumulation in the cytosol favours the reduction of pyruvate to lactate, thereby regenerating NAD^+ at a rate sufficient to maintain glycolysis. If the elevated rate of ATP hydrolysis is sustained creatine levels and subsequently the activity of the phosphorylcreatine shuttle approach a steady state, reducing the mitochondrial [ATP]/[ADP] and stimulating oxidative phosphorylation and electron transfer. The associated increase in mitochondrial NADH turnover facilitates the transfer of cytosolic reducing equivalents into the mitochondria thereby lowering the cytosolic [NADH]/[NAD^+] and the rate of lactate production. In addition, after approximately 5 minutes of exercise a reversal of the calcium mediated increase in phosphorylase activity (49) would tend to further reduce cytosolic [NADH]. These transient changes in cytosolic and mitochondrial metabolic drive, in combination with factors such as the rate of ATP hydrolysis and the concentrations of cytosolic and mitochondrial reactant and product pools, will determine the magnitude and time course of tissue lactate production. With low to moderate rates of ATP hydrolysis, lactate production early in exercise is frequently reversed or attenuated, and with continued exercise

gives rise to a balance between the rates of NADH and pyruvate production in the cytosol and their rates of mitochondrial consumption. This in turn gives rise to a stable rate of tissue lactate formation and, if balanced by the rate of tissue lactate efflux, a stable tissue lactate concentration. With higher intensity exercise the development of a steady state takes longer, and if the rate of NADH and pyruvate formation begins to exceed that of mitochondrial consumption or the rate of lactate formation exceeds the rate of tissue lactate efflux, then lactate will begin to accumulate in the muscle at a rate determined by the imbalance.

Accelerated glycolysis relative to oxidative phosphorylation appears important during early exercise, and essential for the performance of high intensity exercise. Patients with muscle phosphorylase deficiency (McArdle's disease) cannot effectively utilise glycogen, the principle substrate for glycolysis (160). The key features of this disorder are an inability to sustain exercise beyond a work load at which blood lactate accumulation normally begins, painful muscle cramps during early exercise and a lack of blood lactate accumulation during exercise (160), except under ischemic conditions in some patients (10). The relative absence of glycogenolysis is associated with extremely low levels of phosphorylated glycolytic intermediates and muscle pyruvate during exercise (262). The relative lack of pyruvate is likely to impair the rate of acetyl-CoA formation and limit the availability of citrate, both of which would impair citric acid cycle activity and subsequently oxidative phosphorylation during high ATP demand.

1.2.3 Fates of lactate within muscle

In the presence of LDH, pyruvate derived from lactate can engage in any of the reactions of pyruvate metabolism. For example, it can serve as a substrate for glycogen (35, 37) or alanine synthesis (195), be converted back to lactate, or be transported into the mitochondria and undergo complete oxidation in the citric acid cycle (37). This is clearly apparent from tracer studies that have shown incorporation

of labeled carbon from lactate into bicarbonate and/or CO_2 (24, 37, 169, 174, 247), glycogen (37, 174, 195, 228), alanine (37, 195, 228) and citric acid cycle products and intermediates such as glutamate, citrate, succinate and malate (27, 28).

1.2.3.1 Evidence from tracer studies

Several studies, using labeled lactate techniques, have shown skeletal muscles to simultaneously produce and release and extract and oxidise lactate, with the rates of extraction and release increasing with contractions in an exercise intensity dependent manner (24, 247). Using similar techniques, many studies have demonstrated incorporation of carbon label from lactate into a wide range of metabolic intermediates and end products, clearly demonstrating the diversity of reactions within which lactate can participate. However, some contend that tracer techniques have important limitations when applied to the study of lactate kinetics (144, 154, 220, 278). There can be apparent uptake of labeled lactate without net lactate utilisation, and labeled CO_2 production does not provide a measure of true lactate oxidation (144, 154). Furthermore, the position of the carbon label is well known to influence the outcome of lactate tracer studies (169). During a 2 h period of rest in humans, Mazzeo et al. (169) recovered 35.7% of a pre-injected dose of [1-^{13}C]lactate as $^{13}CO_2$ compared to only 13% when [2,3-^{13}C]lactate was used (168). Pyruvate derived from lactate can enter the citric acid cycle either via an anaplerotic carboxylation pathway to form oxaloacetate or via a cataplerotic decarboxylation to form acetyl-CoA and CO_2. $^{13}CO_2$ evolution from pyruvate derived from [1-^{13}C]lactate represents decarboxylation of the carboxylic acid carbon prior to entry into the citric acid cycle (169). Lower $^{13}CO_2$ recovery with [2,3-^{13}C]lactate presumably indicates that much of the lactate entering the citric acid cycle at rest is incorporated into other compounds with slower turnover times and is not fully oxidised.

A more important concern regarding lactate tracer techniques relates to studies that have shown rapid label equilibration between the lactate and pyruvate pools (212,

220, 278). These findings suggest that quantitative estimates of lactate kinetics based on tracer techniques more closely represent those of pyruvate. On the other hand, it has also been argued that tracer techniques are applicable to lactate metabolism (24, 157). In a recent study, Bergman et al. (24) pointed out that most of the tracer remains with lactate due to the action of LDH and large lactate-to-pyruvate ratios (263, 272). Nevertheless, a relatively large pool size would not appear to alleviate the concerns regarding rapid isotopic equilibration between pyruvate and lactate. Consequently, quantitative estimates of lactate disposal based on lactate tracer techniques should be treated with caution.

Given that carbohydrate oxidation accounts for ~40% of the systemic $\dot{V}O_2$ at rest (38) and perhaps as little as 17% of resting muscle $\dot{V}O_2$ (287), and given the high activity of LDH and the much greater size of the lactate compared to the pyruvate pool, a good deal of endogenous pyruvate could acquire label by passing through the lactate pool before engaging in the citric acid cycle and oxidative phosphorylation (278). Several studies based on lactate tracer techniques support this view. Stanley et al. (247), and more recently Bergman et al. (24), showed that both resting and exercising muscles extract significant quantities of lactate despite net lactate release. This finding clearly demonstrates that label appearance in citric acid cycle intermediates and in CO_2 does not necessarily indicate net lactate oxidation. Furthermore, when millimolar concentrations of carbon label are introduced via pyruvate and lactate together, the pattern of label accumulation in rat gastrocnemius muscle citric acid cycle intermediates is the same as that when labeled lactate alone is used (26-28). Consequently, although arteriovenous balance techniques reveal information regarding net and not absolute rates of extraction and release across specific tissues (57, 247), and this is sometimes considered a weakness (24), there are not the same concerns regarding the fate of the carbon label. Furthermore, unless combined with arteriovenous balance measurements, lactate tracer techniques are clearly limited in the study of the net effects of lactate production and release and uptake and disposal.

1.2.3.2 Lactate Oxidation

Oxidation is generally regarded as one of the primary fates of lactate within muscles (35, 37, 46, 70, 95, 161, 171), particularly in oxidative and mixed oxidative glycolytic muscle fibers (11, 195) and particularly during contractions (24, 34, 95, 97, 169, 171). Baldwin et al. (11) determined that glycolytic, oxidative-glycolytic and oxidative muscle homogenates oxidised lactate at 63%, 99% and 78% of their maximal capacity to oxidise pyruvate when exposed to 10 $mmol \cdot l^{-1}$ lactate, suggesting that different muscle types possess varying capacities to oxidise lactate.

Studies employing isotopically labeled lactate have shown lactate irreversible disposal and the isotopic enrichment of CO_2 to be linearly related to metabolic rate and curvilinearly related to the arterial lactate concentration (169, 247). Mazzeo et al. (169) found oxidation to account for 49.3%, 81.5% and 78.0% of the lactate irreversible disposal at rest and during easy (~50% $\dot{V}O_{2\ max}$) and hard (~75% $\dot{V}O_{2\ max}$) exercise respectively, following an intravenous bolus injection of [1-^{13}C]lactate. Similarly, Pagliassotti and Donovan (195), using lactate tracer techniques, demonstrated that resting fast-glycolytic, fast-oxidative-glycolytic and slow-oxidative rabbit skeletal muscle preparations oxidised ~30%, ~40% and ~55% respectively of the lactate removed during 2 h of perfusion with an elevated lactate concentration (~8 $mmol \cdot l^{-1}$).

More recently, Bertocci et al. (27, 28) examined lactate oxidation using an infusion of millimolar (as opposed to tracer) concentrations of ^{13}C lactate and acetate combined with nuclear magnetic resonance spectroscopy and isotopomer analysis in both intact anaesthetised rats (27) and an isolated perfused rat hindquarter preparation (28). These techniques are not subject to the difficulties associated with a non-steady-state and are therefore likely to provide more reliable estimates of lactate oxidation than traditional lactate tracer techniques. Bertocci et al. (27, 28) determined that ~20-25% of the rat soleus and gastrocnemius muscle acetyl-CoA pools derived from labeled lactate, both at rest and after ~30 min of fatiguing muscle

contractions. Over 50% of acetyl-CoA pool derived from acetate and less than 30% from unlabeled (endogenous) sources. In later experiments, the same authors demonstrated that when a mixture of labeled lactate and pyruvate was used in place of labeled lactate alone, the pattern of label incorporation was the same (26). These data strongly support the view that lactate and pyruvate oxidation are indistinguishable. Furthermore, although potentially confounded by the use of acetate and its effects on pyruvate dehdrogenase activity at rest (209), these data suggest that the fraction of resting muscle lactate disposal via oxidation may be less than several previous studies have suggested (5, 124, 169, 195).

Indirect evidence of lactate oxidation by active skeletal muscle derives from studies of active recovery from more strenuous exercise (23, 72, 108, 173, 213, 244, 245, 256). When light to moderate exercise is performed during recovery from strenuous exercise, the rate of blood lactate decline increases as a function of the recovery exercise intensity until a critical intensity (similar to the lactate threshold) is reached (23, 72, 173, 269). These findings are generally attributed to oxidation of lactate within the exercising muscles. However, a substantial component of accelerated blood lactate decline could reflect a greater blood flow and rate of lactate efflux in previously more active muscles, and therefore more rapid redistribution of lactate to alternative sites. McLoughlin et al. (175) examined the rate of blood lactate decline following maximal one-leg exercise during passive recovery and during active recovery with the previously active or inactive leg. Active recovery with either leg accelerated the decline in blood lactate, supporting the view that active muscle lactate disposal is primarily responsible for the increased rate of blood lactate decline during active recovery. However, although not significant, there was a suggestion that exercise in the previously active leg produced a somewhat faster decline in blood lactate compared to recovery exercise performed with the previously inactive leg. In addition, one-leg exercise has been shown to increase blood flow in the inactive leg (5) and this would promote lactate efflux from the previously active leg. Furthermore, while a significant fraction of lactate uptake by muscles during periods

of elevated blood lactate concentration is frequently considered disposal (46, 104, 161, 203, 247, 284), passive lactate movements into the muscle associated with equilibration are likely to account for the largest component of uptake (94), particularly over a short duration. This process could be accelerated by blood flow changes associated with light activity. Consequently, lactate oxidation by active muscles may constitute a smaller component of the accelerated blood lactate decline during active recovery than studies of recovery exercise suggest.

1.2.3.3 Glyconeogenesis

The substrate for glycogen synthesis is principally blood-borne glucose (15, 56). Nevertheless, several studies have indicated that lactate can serve as a substrate for skeletal muscle glyconeogenesis, particularly in fast-glycolytic and fast-oxidative-glycolytic muscle (9, 15, 35, 113, 174, 193, 195). Hermansen and Vaage (113), measured quadriceps muscle lactate and glycogen content, calf blood flow and arterial-femoral venous differences in lactate, glucose and alanine concentrations during 30 min of recovery from repetitive brief maximal leg exercise to exhaustion designed to deplete quadriceps muscle glycogen and elevate lactate. During recovery, muscle lactate disappearance coincided with glycogen synthesis both in magnitude and time course. On the basis that there was only a small efflux of lactate, no efflux of alanine and minimal uptake of glucose, it was concluded that the majority (75%) of the lactate present within quadriceps muscle at the end of exercise was converted to glycogen with less than 15% oxidised during recovery. However, Connett and Sahlin (56) have argued that different rates of glucose uptake and lactate release between muscle groups within the leg could also account for these findings, and further indicated that direct evidence for the conversion of lactate to glycogen in human skeletal muscle *in vivo* is lacking. On the other hand, several studies have shown incorporation of ^{14}C from lactate into glycogen (37, 174, 193, 195, 228), even in isolated skeletal muscle preparations perfused with a medium containing lactate as the only substrate (174). These findings are difficult to reconcile

in the absence of a skeletal muscle glyconeogenic pathway. Furthermore, the incorporation of ^{14}C from lactate into glycogen was shown to be faster and to account for a much greater fraction of ^{14}C removal in fast-glycolytic compared to slow-oxidative muscle (174, 193, 195). These findings, in combination with apparently greater lactate oxidation in oxidative compared to more glycolytic muscle (174, 193, 195), suggest that muscle fiber type differences are important in determining the metabolic fate of lactate taken up by skeletal muscle.

The rate of muscle glycogen synthesis appears to be inversely related to muscle glycogen concentration (30, 206), and glyconeogenesis from lactate in muscles depleted in glycogen by exercise appears to be a major pathway for lactate disposal during recovery (9). Early studies suggested that glycogen depletion was essential for net glycogen synthesis (25), and rapid incorporation of label from lactate into glycogen in resting glycogen depleted muscles is consistent with this view (174). However, more recent studies have shown ^{14}C from lactate to be incorporated into glycogen under conditions of low net glycogen synthesis (193, 228). In the study of Pagliassotti and Donovan (193), ~30%, ~10% and ~1% of ^{14}C from lactate removed by resting muscle was recovered in glycogen in fast-glycolytic, mixed fiber and slow-oxidative muscle preparations respectively following 2 h of perfusion with ~8 $mmol \cdot l^{-1}$ lactate. Whilst this finding could implicate glyconeogenesis as an important pathway for net lactate disposal within resting muscles exposed to a high lactate concentration (193, 203), others have failed to find net glycogen synthesis during lactate infusion (46, 97), and glycogen turnover would also explain these findings.

1.2.3.4 Alanine synthesis

Although quantitatively much less important than oxidation, the transamination of pyruvate to alanine and incorporation into proteins is another potentially important fate of lactate within skeletal muscle. Brooks and Gaesser (37) demonstrated that ~5% of ^{14}C from lactate was incorporated into protein constituents of resting rat

skeletal muscle. Although much more ^{14}C was recovered in CO_2 and bicarbonate, label incorporation into proteins appeared to account for more label than incorporation into glycogen. Similarly Pagliassotti and Donovan (195) demonstrated that between 20% and 30% of the labeled lactate removed by resting glycolytic, oxidative and mixed fiber muscle preparations perfused with ~8 $mmol \cdot l^{-1}$ lactate was recovered in proteins. At the same time, there was net alanine release from each resting muscle preparation. However, this is in contrast to a study by McLane and Holloszy (174) in which alanine release was too small to measure, and a study by Gladden et al. (97) in which alanine release was small and did not appear to be influenced by the perfusate lactate concentration. As with glycogen, substrate turnover could also account for label incorporation into alanine, and this may show little relationship to the net effects of lactate production and disposal.

1.2.3.5 Alternative end products and metabolic intermediates

Whilst alanine synthesis, glyconeogenesis and particularly oxidation are generally regarded as the most important fates of lactate within resting muscles, there are other alternatives. Due to the near equilibrium nature of the LDH reaction, an increase in muscle lactate concentration will lead to an increase in muscle pyruvate content. Whilst this will account for only a small quantity of lactate, an increase in muscle pyruvate is potentially associated with increases in other metabolic intermediates. Perchlorate extracts from muscles exposed to labeled carbon typically account for ~10-35% of labeled lactate taken up by resting muscles (37, 174, 195), not all of which can be accounted for by lactate, pyruvate and glucose. Furthermore, based on arteriovenous balance measurements and measurements obtained from biopsy samples of resting canine gastrocnemius-plantaris-soleus muscle perfused with ~10 $mmol \cdot l^{-1}$ lactate, Chin et al. (46) proposed that only 12-24% of the lactate taken up by the muscle was oxidised, with the majority involved in metabolic cycling along the glycolytic-glyconeogenic and/or triacylglycerol-free fatty acid pathways. In addition, these authors found a small but significant increase in glycerol release that

could account for ~4% of the lactate taken up by the muscle. Chin et al. (46) speculated that this reflected increased glycerol 3-phospate hydrolysis and lactate cycling upward in the glyconeogenic pathway and not an increase in triglyceride utilisation. Alternatively, the redox coupled conversion of lactate to pyruvate could, to some extent, inhibit glycolysis at the level of glyceraldehyde 3-phosphate, thereby leading to an increase in glycerol hydrolysis from dihydroxyacetone phosphate.

McLane and Holloszy (174) speculated that a portion of the carbon label contained within perchlorate extracts of muscle exposed to ~12 $mmol \cdot l^{-1}$ lactate consisted of citric acid cycle, glycolytic pathway and other metabolic intermediates derived from lactate. This possibility, at least with regard to citric acid cycle intermediates, has been confirmed in recent studies by Bertocci et al. (27, 28) in which carbon label from lactate was found incorporated into several citric acid cycle products and intermediates such as glutamate, citrate, malate and succinate.

Pyruvate derived from lactate could also contribute to the expansion of the citric acid cycle intermediate pool via one of several anaplerotic reactions, such as those catalysed by pyruvate carboxylase, malic enzyme and perhaps most importantly alanine aminotransferase (92). However, the importance of anaplerosis as a fate of lactate within resting muscles exposed to an elevated blood lactate concentration is unclear.

1.2.3.5a ***Carnitine buffering of acetyl-CoA***

Carnitine can act as an acceptor for acetyl groups from acetyl-CoA (58). In this manner, carnitine is potentially important in maintaining the catalytic activity of the pyruvate dehydrogenase complex, which is in part dependent on the mitochondrial acetyl-CoA/CoASH ratio (274). This is likely to be particularly important when the rate of acetyl-CoA condensation with oxaloacetate is less than the rate of acetyl-CoA formation from pyruvate. This most notably occurs in association with accelerated

glycolysis during exercise (221, 223), but could also occur within resting muscles when lactate infusion or exercise in remote muscle groups elevates intramuscular lactate and pyruvate. Sahlin and coworkers (221, 223) reported parallel increases in pyruvate, lactate and acetylcarnitine during moderate and high intensity exercise in humans, consistent with the view that acetylcarnitine provides a sink for pyruvate and acetyl-CoA. Similarly, Constantin-Teodosiu et al. (58) reported a strong relationship between acetyl-CoA and acetylcarnitine accumulation in exercising muscles during incremental exercise, with ~500 mmol acetyl groups binding to carnitine for each mmol of acetyl-CoA accumulated. From these studies it might be predicted that carnitine could provide a substantial sink for lactate taken up by resting muscles, although a recent study does not support this view (208). Putnam et al. (208) measured pyruvate dehydrogenase activity and acetyl-CoA, acetylcarnitine, lactate and pyruvate concentrations in the human resting deltoid muscle following repeated bouts of maximal leg exercise. Despite a two-fold increase in resting muscle lactate and pyruvate concentrations there was no accumulation of acetyl-CoA or acetylcarnitine. Furthermore, pyruvate dehydrogenase activity was augmented and therefore did not limit lactate and pyruvate oxidation within the inactive deltoid muscle.

1.2.3.6 Substrate competition and hormonal influences

Competition between oxidisable substrates and hormonal factors have been shown to exert a significant influence on lactate production and release as well as lactate uptake and disposal by skeletal muscle. For example, Essen et al. (82) demonstrated that on the day after one leg exercise to deplete muscle glycogen in one leg, two leg exercise was associated with lactate uptake in glycogen depleted leg and lactate output in the non-glycogen depleted leg. These findings are consistent with a reduced rate of endogenous pyruvate and lactate production from glycolysis in the glycogen depleted leg, which would favour lactate uptake from the circulation and oxidation within the active muscles.

The availability of circulating free fatty acids also appears to influence resting skeletal muscle lactate metabolism. Dunn and Critz (78), using a resting canine hindlimb preparation and lactate infusion to increase the arterial lactate concentration to ~2 $mmol \cdot l^{-1}$, found net lactate uptake when the arterial free fatty acid concentration was reduced by nicotinic acid, but no lactate uptake when the arterial free fatty acid concentration was elevated by infusion with a heparin-fat emulsion.

Circulating hormones, particularly catecholamines, can substantially influence the balance between lactate production and disposal in both exercising and resting skeletal muscles. For example, local β-blockade in one leg has been shown to abolish leg lactate release during rest and to decrease lactate release by ~50% during two leg exercise (137). Furthermore, sympathoadrenal activity and therefore the levels of circulating catecholamines increase as a function of exercise intensity (158, 170, 202). Epinephrine stimulates triacylglycerol breakdown in adipose tissue as well as glycogenolysis, glycolysis and endogenous lipid hydrolysis in skeletal muscle. In addition, epinephrine has been shown to preferentially stimulate glycogenolysis in glycolytic muscles and lipid hydrolysis in oxidative muscles at rest (196). The sympathetic nervous system response to exercise also plays a major role in diverting blood from inactive tissues to exercising skeletal muscle. In combination, these factors would promote glycolysis and lipid oxidation in resting glycolytic and oxidative muscles respectively and reduce lactate delivery to resting muscles in general, all of which would tend to shift the net balance between lactate production and disposal away from disposal.

Whilst the influence of exercise related changes in circulating hormone concentrations on contracting skeletal muscle lactate kinetics are relatively well known, their potential to modulate resting skeletal muscle lactate metabolism during exercise with other muscles has received little attention. Ross (215) found that non-selective β-blockade (via propranalol) restricted to one inactive forearm by arterial infusion was associated with greater lactate uptake compared to the non-blocked

resting forearm during moderate to high intensity supine leg exercise. This finding strongly supports the view that catecholamines limit resting muscle lactate metabolism during exercise with other muscle groups. This could be via stimulatory effects on glycogenolysis and glycolysis that promote endogenous lactate production, substrate competition associated with increased glycolysis and lipid hydrolysis, or perhaps both.

1.2.4 The lactate shuttle hypothesis

The traditional view of lactate as a metabolic waste product has been replaced by the widely accepted concept that lactate is an important and highly mobile metabolic intermediate. Based on emerging data concerning the readily oxidisable nature of lactate, particularly within exercising muscles, in combination with the established phenomena of the glucose paradox and the Cori and glucose-alanine cycles led Brooks (32-34, 36, 242) to develop the "lactate shuttle" hypothesis. In this hypothesis, skeletal muscle lactate production, and the subsequent distribution of lactate within muscle tissues and to other tissues via the circulation, serves as an important mechanism by which a readily oxidisable carbon source and gluconeogenic precursor can be rapidly and widely distributed to sites of increased requirements. In this manner, lactate produced within the cytosol of muscle fibers with high glycogenolytic and glycolytic rates could serve as an oxidisable substrate to support mitochondrial oxidation within the same cell (36, 242), in nearby oxidative fibers within the same muscle or in other tissues such as the heart or more remote skeletal muscles (34). At the same time, lactate released by skeletal muscle can also serve as a substrate for liver gluconeogenesis or glycogen synthesis.

1.3 Non-equilibrium blood-tissue lactate and gas exchange processes

Under equilibrium conditions, where both the arterial and venous concentrations of a given substance are stable, the presence of a veno-arterial concentration difference

across a tissue must indicate net production and release (positive veno-arterial differences) or net uptake and disposal (negative veno-arterial differences), with the magnitude of the difference being proportional to the rate of net production or disposal and inversely proportional to blood flow. This is the principle attributed to Fick (83), and is essentially the principle of mass balance. However, when the arterial concentration changes, such as occurs in many substances during moderate to high intensity exercise, the magnitude of the veno-arterial concentration difference is influenced by several additional factors. These include the rate of change in the arterial concentration, the physical arrangement and proportions of the component vascular and tissue structures, blood flow and the degree of blood flow heterogeneity and the ease with which the substance traverses blood-tissue barriers. These factors all have the potential to influence the rate at which a new equilibrium is reached and will inevitably interact to produce extracellular and intracellular concentration changes that may (or may not) affect the rate of production or metabolism.

The distinction between equilibrium and non-equilibrium conditions is very important. In an equilibrium state, factors such as blood flow and the membrane permeability of a given substance, while important determinants of venous, intra- and inter-cellular concentrations, may have little bearing on the rate of tissue production or disposal of the substance. For example, tissue oxygen uptake and carbon dioxide output are determined principally by metabolic rate, and changes in blood flow merely alter the concentrations of O_2 and CO_2 in tissue venous effluent over a wide range of flows. However, in a non-equilibrium state, such as exists following an abrupt change in tissue blood flow or the arterial concentration of the substance, blood-tissue concentration gradients are inevitably altered. Under these conditions, net blood-tissue substance movements, unrelated to although potentially influenced by metabolism, will continue until a new equilibrium is reached, with the rate of equilibration governed principally by blood flow and the ease with which the substance traverses blood-tissue barriers (178, 180). In highly permeable

substances, blood flow is the principal determinant of the rate of blood-tissue equilibration, and this relationship provides the basis of the Kety-Schmidt method for estimating tissue blood flow based on the wash-in or washout curve of an inert gas from the tissues of interest.

Whilst non-equilibrium blood-tissue exchange processes have been relatively well characterised for inert substances, they are difficult to study using substances capable of engaging in tissue metabolism, because tissue uptake (or release) related to metabolism is usually indistinguishable from that related to equilibration. This is perhaps particularly true for metabolic intermediates such as lactate that can readily engage in several reactions that may not constitute irreversible disposal. In a non-steady-state, even with the benefit of measurements of tissue lactate content, a significant quantity of lactate could reside within the tissues in the form of other metabolic intermediates that are returned to lactate during recovery to the baseline state. Consequently, the interpretation of tissue lactate uptake or release during the non-equilibrium conditions of exercise is problematic. Reliable inferences regarding changes in tissue metabolism based on non-equilibrium veno-arterial concentration differences, perhaps even with the benefit of tissue samples, depend on a thorough understanding of blood-tissue exchange processes in isolation. If the contribution of these processes to negative veno-arterial concentration differences measured during a non-equilibrium state is under-estimated, the role of metabolic disposal will be systematically over-estimated.

Blood-tissue exchanges of many substances such as respiratory and inert gases, pharmacological agents, as well as substrates for tissue metabolism have been studied extensively. Notable examples include Krogh's exploration of radial diffusion using the tissue cylinder concept (155), an extensive body of literature devoted to tissue wash-in and washout of inert gases and, more recently, more complex multiple tracer studies of the blood-tissue exchange of nutrients and metabolites (17, 19, 20,

61, 156). In all of these studies, mathematical models have been fundamental to the understanding of the blood-tissue exchange processes under investigation.

Some of the most important studies in the field of non-equilibrium blood-tissue exchange have examined inert gas kinetics in the absence of confounding metabolic influences. Morales and Smith (177-180, 235, 236) and perhaps most notably Kety and Schmidt (147-151) used simple conceptual models of tissues to develop mathematical expressions relating changes in the tissue venous concentration of an inert gas to arterial concentration changes, tissue volume, blood flow and, in more rigorous mathematical treatments, the product of gas permeability and the surface area for blood-tissue gas exchange (177-180). Using such an approach, Kety and Schmidt (150, 151) derived an integrated form of the Fick equation which they employed in the now classical method for estimating cerebral blood flow from the wash-in or washout of an inert gas in the cerebral circulation of humans. While such models are clearly simplistic and like all models have important limitations, there is generally close agreement between blood flow estimates based on the Kety-Schmidt model and those obtained using alternative methods (150) implying that even relatively simple blood-tissue exchange models have some validity.

1.3.1 Blood flow, tissue volume, and capillarisation

In virtually all models of blood-tissue exchange, blood flow relative to tissue volume, either at the whole tissue level (compartmental models) or in tissue units, are key determinants of non-equilibrium blood-tissue exchange, and only in relatively impermeable substances does blood flow theoretically become of relatively minor importance (178, 180).

Based on associations between blood flow and lactate release or uptake, several investigators have suggested that blood flow is an important determinant of the rate of blood-tissue lactate exchange (13, 103, 130). Bangsbo et al. (13) showed that

lactate uptake in the inactive leg doubled coincident with a two-fold increase in leg blood flow associated with arm exercise performed to elevate the arterial lactate concentration. Graham et al. (103) found that blood flow was negatively correlated with muscle lactate concentration ($r = -0.63$) and positively correlated with the rate of lactate efflux ($r = 0.50$) from contracting canine gastrocnemius muscle during the first 5 to 15 min of activity. Jorfeldt (130) found the rate of lactate uptake by the resting forearm to correlate more closely with the product of arterial lactate concentration and blood flow ($r = 0.94$) than with the arterial lactate concentration alone ($r = 0.88$), implicating blood flow as an important determinant of the rate of lactate uptake by inactive skeletal muscle. In contrast, on the basis that lactate efflux from supramaximally stimulated dog gastrocnemius muscle was ~3-fold higher and blood flow only 19% higher between 5 and 15 min of bicarbonate or trishydroxymethylaminomethane (THAM) induced alkalosis compared to HCl induced acidosis, Hirche et al. (116) concluded that blood flow was a relatively unimportant determinant of muscle lactate efflux. However, in addition to blood flow, several other factors differed between the acidotic and alkalotic preparations. For example, the arterial lactate concentration was higher and arterial pH lower during acidosis compared to alkalosis, both of which would tend to impede muscle lactate efflux and potentially confound the apparent importance of blood flow in determining the rate of muscle lactate efflux. Nevertheless, in a more recent study, Gladden et al. (96) demonstrated that canine gastrocnemius muscle contracting at 1 Hz and exposed to a stable arterial lactate concentration of ~10-13 $mmol \cdot l^{-1}$ by infusion took up lactate at similar rates at three different blood flow rates (~300 – 500 $ml \cdot kg^{-1} \cdot min^{-1}$). In these experiments blood flow was maintained constant for either 16 or 32 min and measurements obtained between 10-16 and 20-32 min respectively. With blood flows of ~300 and ~500 $ml \cdot kg^{-1} \cdot min^{-1}$, that were used in both the short and longer protocols, it appears that the rate of net lactate uptake was ~20-30% lower after 20-32 min compared to 10-16 min of infusion. Although this suggests that equilibration was incomplete, at least after 10-16 min, an important blood flow dependence of lactate uptake should be most apparent under non-equilibrium conditions and this

was not the case. Furthermore, higher blood flows should promote faster equilibration and therefore lower rates of lactate uptake at a given time during lactate infusion. In contrast, lactate uptake was significantly greater in the highest blood flow period (~650 $ml \cdot kg^{-1} \cdot min^{-1}$) compared to three lower blood flow rates (~300 – 500 $ml \cdot kg^{-1} \cdot min^{-1}$). Although these findings support the view that lactate uptake is relatively blood flow independent, at least at blood flow rates of ~300-500 $ml \cdot kg^{-1} \cdot min^{-1}$, the timing of measurements and the increased metabolic rate warrant further consideration. Lactate uptake associated with equilibration and that associated with metabolism are quite likely only loosely related. Assuming that blood-muscle equilibration was near complete in the study of Gladden et al. (96), blood flow independence of lactate uptake implies that lactate disposal processes in contracting muscles are relatively blood flow independent. However, this does not exclude an important blood flow dependence of the preceding blood-tissue equilibration process itself. Such a distinction, whilst not clear from the study of Gladden et al. (96), could explain much of the apparent conflict between studies that report significant associations between blood flow and skeletal muscle lactate uptake or release (13, 103, 130) and those that do not (96, 116, 117).

Based on experimental evidence and models of inert gas exchange in tissues, it is clear that blood flow is a major determinant of the rate at which a tissue equilibrates with a change in the arterial concentration of an inert gas. The Kety-Schmidt (integrated Fick) model in its simplest form models tissues as a single compartment in which the arterial blood enters, completely mixes with the contents of the tissue volume and then exits as venous blood. In this model, blood flow and therefore substance delivery to the tissue is a key determinant of the rate at which the venous concentration approaches that in the arterial blood. Despite the simplicity of this model there is generally close agreement between model predicted and experimental data, at least for highly diffusible inert gases such as N_2O. However, although this model appears to be a reasonable approximation in cerebral tissues, the relative importance of perfusion and diffusion processes will vary between substances with

different diffusive properties and between tissues such as the brain and skeletal muscle which have different vascular arrangements. Even in the relatively well perfused and homogenous cerebral tissues, the addition of a diffusion term has been shown to improve model fits to experimental data (74). Nevertheless, in simple compartmental models, as well as in more complex models that consider blood flow inhomogeneity by modeling numerous small tissue units (17, 19, 20), blood flow and tissue volume are potentially major determinants of the rate of blood-tissue exchange of many blood-borne substances.

The movements of molecules such as lactate and mannitol (a commonly used extravascular space marker) are generally considered not to be importantly restricted by the capillary membrane (63). However, the degree of capillarisation, the number of perfused capillaries and therefore the capillary surface available for exchange could be important determinants of the effective local muscle volume between which blood-tissue substance exchange will occur, the length of extracellular diffusion paths and the magnitude of blood-tissue lactate concentration gradients. Whilst the limiting surface area for blood-muscle lactate exchange is at the capillary (53), the relative importance of capillarisation and the open capillary surface for blood-tissue lactate exchange is difficult to evaluate and remains largely speculative (130, 258).

1.3.2 Diffusion and membrane transport

The translocation of gases across the capillary and sarcolemmal membranes is principally by diffusion and is therefore a relatively linear function of the transmembrane concentration (partial pressure) gradient. Whilst this is true also for CO_2, bicarbonate appears to facilitate CO_2 transport across biological membranes (109). Similarly, the chemical binding of O_2 to myoglobin in muscle, and in particular to haemoglobin in blood, effectively provides tissues and blood with the capacity to buffer changes in blood-tissue pO_2 gradients and has a major impact on blood-tissue

gas exchange. The passage of lactate between the tissues and blood is also considerably more complex than by straightforward diffusion.

1.3.2.1 Translocation of lactate across the sarcolemma

Considerable evidence exists to indicate that lactate moves across the sarcolemmal membrane principally via a transport system, as well as by diffusion (84, 98, 132, 135, 153, 216-218, 266, 267). Observations of muscle to extracellular lactate concentration gradients (95, 98, 116, 117, 214) and saturation kinetics for lactate uptake during periods of elevated blood lactate (97, 130), although suggestive, are not conclusive evidence for a saturable membrane hindrance to lactate translocation. These observations can also be explained on the basis of a Donnan equilibrium between lactate and other ions in combination with saturation of lactate utilisation (94). However, a number of studies have confirmed that lactate moves across the sarcolemma predominantly via a carrier-mediated transport system. Koch et al. (153) examined lactate influx into mouse diaphragm muscle *in vitro* and showed that a lactate carrier, inhibited by α-cyano-4-hydroxycinnamic acid (CIN), accounted for the majority of lactate uptake. Furthermore, uptake had an apparent K_m of ~7.6 $mmol \cdot l^{-1}$ and appeared to saturate when the external lactate concentration approached 10 $mmol \cdot l^{-1}$. In a later study, Juel et al. (133) demonstrated that lactate efflux from mouse soleus muscle during recovery from electrical stimulation was substantially inhibited by substances such as CIN, p-chloromercuribenzenesulphonic acid (pCMBS) and phloretin, all known blockers of lactate transport in other tissues (133). By following ^{14}C-labeled lactate movements into isolated soleus muscles in mice, Juel and Wibrand (136) showed lactate uptake to be inhibited by ~80% by CIN and pCMBS suggesting that the majority of lactate transport in muscle occurs via a lactate carrier. Similarly, Watt et al. (267) using the paired-tracer dilution technique in combination with lactate transport inhibitors concluded that lactate transport across the sarcolemma of the rat hindlimb occurs via both saturable and non-saturable pathways.

More recent studies have examined sarcolemmal lactate transport using sarcolemmal vesicle preparations (132, 134, 172, 216-219). In an extensive series of experiments Roth and Brooks (217-219) examined the influx of labeled lactate into sarcolemmal vesicles prepared from rat skeletal muscle and no lactate initially within the vesicles. The relationship between extravesicular lactate concentration and the initial rate of lactate influx displayed saturation kinetics with a K_m of 40.1 $mmol \cdot l^{-1}$ and V_{max} of 139.4 $nmol \cdot l^{-1} \cdot mg^{-1} \cdot min^{-1}$ and was markedly inhibited by CIN and pCMBS (218). Lactate influx was also inhibited by pyruvate, indicating the presence of a monocarboxylate rather than a lactate specific carrier. Furthermore, lactate influx was facilitated by an inwardly directed, and inhibited by an outwardly directed, hydrogen ion gradient, with the rate of influx depending on the magnitude of the pH gradient and not the absolute pH suggestive of a lactate and H^+ symport carrier (217). Roth (216) pointed out that this pH dependence would promote lactate and H^+ efflux from active muscles and uptake by other tissues such as the heart and inactive muscles. In a more recent study employing similar techniques, McDermott and Bonen (172) reported essentially very similar findings, with the exception of a much lower K_m (~12.5 $mmol \cdot l^{-1}$) and a V_{max} approximately three times higher than that reported by Roth and Brooks (218).

Juel and associates (132, 134, 135), using somewhat different techniques, examined lactate efflux from so-called giant sarcolemmal vesicles. Although the findings from these experiments were generally similar to those deduced from more conventional smaller vesicles (172, 217-219), lactate efflux displayed a lower K_m and much higher V_{max} compared to lactate transport in smaller vesicles.

Despite differences in apparent Michaelis-Menten constants, studies employing sarcolemmal vesicles (132, 134, 172, 216-219) strongly suggest that in the physiological range of lactate concentration gradients, lactate transport across muscle membranes occurs primarily via a bidirectional, pH gradient sensitive, saturable, sarcolemmal-bound monocarboxylate carrier that co-transports lactate and

hydrogen ions. However, it is not clear from these studies as to the relative importance of sarcolemmal lactate transport compared to other factors, such as capillary surface area and blood flow, that also potentially limit blood-tissue lactate exchange.

1.4 Resting skeletal muscle lactate metabolism under elevated arterial lactate conditions

Relatively inactive muscles constitute a large tissue mass even during exercise. Approximately 40% (28 kg in a typical non-obese 70 kg human) of the total body mass is muscle (7) of which approximately 30% (8 kg) is used during leg cycling exercise (161). This, in combination with the capacity for muscle to both produce and metabolise lactate, implicates inactive muscles as a potentially important site for modulating blood lactate levels during exercise.

Many studies have demonstrated that inactive muscles take up lactate when arterial blood lactate levels are elevated by lactate infusion (4, 13, 39, 46, 99, 279) or by exercise (5, 13, 16, 40, 88, 104, 111, 161, 162, 203, 247, 282, 284). At the same time, the lactate concentration within the resting muscles has been shown to increase (13, 46, 95, 140, 161, 195). This finding alone clearly demonstrates the capacity for resting muscles to act as a passive sink for lactate. Nevertheless, given the potential for muscle to consume lactate, it is likely that a proportion of the lactate taken up by resting muscles during exercise in other muscle groups is metabolised, and many investigators have advanced evidence to support this hypothesis.

1.4.1 Blood lactate elevated by infusion

Studies in which blood lactate levels have been elevated by infusion have provided convincing evidence that skeletal muscles dispose of lactate both at rest (46, 174, 195, 279) and in particular during contractions (95, 97). Nevertheless, as with any membrane translocatable substance, the initial period of uptake during infusion is dominated by blood-tissue equilibration and it is therefore difficult to determine the contribution of skeletal muscle metabolism to lactate uptake during the equilibration period. Chin et al. (46) and Gladden et al. (95) using perfused rat hindlimb and canine gastrocnemius muscle preparations respectively, found the peak rate of lactate uptake to occur within the first few minutes of lactate infusion. This was

followed by an exponential decline towards a steady-state level of uptake and an elevated muscle lactate content at the end of infusion. In combination, these findings clearly demonstrate that a significant component of uptake, at least during the early period of infusion, reflects blood-tissue equilibration. Furthermore, the asymptotic nature of the decline in lactate uptake makes it difficult to establish when blood-tissue equilibration is complete and uptake reflects the steady-state level of lactate disposal. Consequently, unless a steady-state can be convincingly demonstrated, quantitative estimates of lactate disposal based on the rate of lactate uptake may overestimate the true rate of muscle lactate disposal. In the study of Gladden et al. (95), it appears that a steady-state level of lactate uptake may not have been reached by the end of a 30 min period of lactate infusion to establish an arterial lactate concentration of ~8 $mmol \cdot l^{-1}$. In the study of Chin et al. (46), 30 min of exposure to an arterial lactate of ~10 $mmol \cdot l^{-1}$ was associated with resting muscle lactate uptake at a rate approximately 50% higher than that measured after ~40-50 min when a steady-state appeared to have been achieved. On the other hand, data presented by Pagliassotti and Donovan (195), obtained from rabbit skeletal muscle perfused with ~8 $mmol \cdot l^{-1}$ lactate, suggests that 30 min is sufficient to achieve a steady-state level of lactate uptake. Such between study comparisons must be treated cautiously given that differences between muscle preparations, such as lactate transport capacity, capillarisation, perfusate lactate concentration and perfusion flow rate, are potentially important determinants of the rate at which an equilibrium state is achieved. Nevertheless, equilibration processes clearly dominate resting muscle lactate uptake during the first ~20-30 min even in well perfused resting muscle preparations exposed to stable elevated arterial lactate concentrations. At the same time, given that steady-state levels of lactate uptake have been established in several studies (46, 195), there is no doubt that resting muscles do dispose of lactate when the intramuscular lactate concentration is elevated by infusion.

The fate of lactate taken up by muscles has been shown to be partly a function of the muscle fiber type composition (195). Furthermore, the rate of lactate disposal in

muscles exposed to elevated blood lactate levels has been shown to depend on the blood lactate concentration (195), muscle aerobic capacity (195) and muscle metabolic rate (95, 97). Pagliassotti and Donovan (195) examined lactate uptake in oxidative (soleus), glycolytic (gracilis) and mixed fiber type (gastrocnemius-plantaris-soleus) rabbit skeletal muscles infused with ^{14}C labeled lactate to achieve stable elevated arterial lactate concentrations of ~4 and ~8 mmol·l^{-1}. Steady-state levels of uptake were observed in all muscle preparations between 30 and 60 min of infusion, with uptake therefore reflecting the steady-state rate of lactate disposal. With an arterial lactate concentration of ~4 mmol·l^{-1} oxidative and mixed fiber muscles disposed of lactate (~0.05 mmol·kg^{-1}·min^{-1}) while glycolytic muscles demonstrated a near zero net lactate balance. When the arterial lactate concentration was further increased to ~8 mmol·l^{-1}, all preparations disposed of lactate with greater rates of disposal occurring in mixed (~0.2 mmol·kg^{-1}·min^{-1}) and oxidative (~0.15 mmol·kg^{-1}·min^{-1}) compared to glycolytic muscles (~0.05 mmol·kg^{-1}·min^{-1}). Most of the ^{14}C label was recovered in carbon dioxide (~55, 40 and 30% in oxidative, mixed and glycolytic muscles respectively), amino acids (~32, 27 and 20% respectively) and glycogen (~1, 11 and 30% respectively), with relatively minor quantities in glycerol, pyruvate and other perchlorate soluble products. However, the rates of tracer-estimated lactate disposal and total net carbon uptake were essentially similar in each muscle preparation, consistent with the view that [^{14}C]lactate turnover reflects essentially pyruvate turnover. Consequently, these estimates of the net fates of lactate inferred from ^{14}C data should be interpreted cautiously. Furthermore, although significant quantities of ^{14}C were recovered in glycogen in glycolytic and mixed muscles, there were only small increases in muscle glycogen content and it is not clear to what extent glycogen turnover contributes to ^{14}C incorporation into glycogen. Moreover, other studies have failed to demonstrate an increase in muscle glycogen content in resting muscles exposed to elevated blood lactate levels (46, 95, 97), suggesting that glycogen synthesis from lactate may be a limited pathway for lactate disposal in resting muscles. Similarly, although oxidation appears to be a primary pathway for lactate disposal in resting muscles, particularly in oxidative and

mixed muscles (195), resting muscle $\dot{V}O_2$ is unaffected by increased lactate delivery (97, 195). Consequently, the fractional contribution of oxidation to disposal declines with increasing arterial lactate concentrations, indicating that oxidation is also a limited pathway for lactate disposal in resting muscles.

From infusion studies it is clear that, when exposed to elevated blood lactate concentrations by infusion, resting muscles dispose of lactate at a rate proportional to the arterial lactate concentration. Furthermore, several studies have convincingly demonstrated that the lactate disposal capacity of muscle is a function of metabolic rate (95, 97). Gladden (95) using an *in situ* canine gastrocnemius-plantaris muscle preparation found that the steady-state level of lactate uptake increased as a function of contraction frequency in muscles exposed to stable elevated arterial lactate levels by sodium lactate infusion. More recently, in a particularly important study of skeletal muscle lactate uptake, Gladden et al. (97) examined the influence of both lactate concentration (~3-30 $mmol \cdot l^{-1}$) and metabolic rate on net lactate uptake in isolated perfused canine skeletal muscle. In this study (97), a strong relationship between plasma lactate concentration and net lactate uptake measured after 30 min of infusion approached a saturation limit with increasing plasma lactate concentration, consistent with saturation kinetics in membrane lactate transport, muscle lactate metabolism or both. Unfortunately, however, Gladden et al. (97) were unable to determine the relative contributions of membrane transport limitations versus muscle lactate disposal to the saturation behavior of lactate uptake measured after 30 min of infusion. Although steady-level conditions were reported to exist, this presumably referred only to blood flow and the arterial lactate concentration and not venous lactate levels because the authors concluded that membrane transport limitations were likely to account for a significant component of the observed saturation kinetics in lactate uptake (97). If venous levels were stable and blood-tissue equilibration complete after 30 min, continued uptake would reflect purely metabolic effects and saturation kinetics in lactate uptake would therefore indicate saturation in metabolic disposal. On the other hand, if venous lactate levels were still approaching an

asymptote after 30 min, a component of uptake would reflect continuing equilibration and this could account for much of the observed saturation kinetics in lactate uptake. As the time course of venous concentration changes was not reported it is not possible to confirm if equilibration was complete. However, this seems unlikely given the authors' conclusions (97) and that, in an earlier study (95) in which a similar preparation and perfusion rate (~200-400 $ml \cdot kg^{-1} \cdot min^{-1}$) were used, equilibration did not appear to be complete after 27 min of ~8 $mmol \cdot l^{-1}$ lactate infusion. Furthermore, sarcolemmal membranes are most likely the principal barrier to lactate uptake in whole skeletal muscle, and sarcolemmal lactate transport is well known to display saturation kinetics (132, 134, 172, 216-219). Consequently, high plasma lactate levels would be expected to impede and therefore prolong equilibration.

Although saturation kinetics in lactate uptake (97) are consistent with membrane transport limitations, a significant component of lactate uptake could reflect lactate disposal. However, there were no changes in muscle glycogen content or alanine output and only a minor increase in pyruvate output associated with increasing plasma lactate concentrations (97). Consequently, oxidation would seem the most likely metabolic fate of lactate within resting muscle under these conditions. However, resting muscle $\dot{V}O_2$ (~0.13 $mmol \cdot kg^{-1} \cdot min^{-1}$) did not change with increases in plasma lactate concentration. As 3 moles of O_2 are required to fully oxidise 1 mole of lactate, this level of $\dot{V}O_2$ could only support lactate oxidation at a rate of ~0.05 $mmol \cdot kg^{-1} \cdot min^{-1}$ even if lactate were the exclusive oxidisable substrate. While this could account for the majority of lactate uptake with plasma lactate concentrations of ~3-10 $mmol \cdot l^{-1}$, oxidation could account for only a fraction of lactate uptake (~0.6 $mmol \cdot kg^{-1} \cdot min^{-1}$) associated with the highest plasma lactate concentration (~30 $mmol \cdot l^{-1}$). Under these conditions, continued equilibration appears most likely to account for the majority of uptake, and under these conditions most of the lactate taken up by the muscle would merely increase the muscle lactate content. Unfortunately, this cannot be verified because muscle lactate concentration does not appear to have been measured (97).

Resting muscle lactate uptake has also been demonstrated in humans exposed to an increased blood lactate concentration by lactate infusion (4, 39, 130). Ahlborg et al. (4) concluded that skeletal muscles metabolised 35% of the lactate taken up during a 30 min lactate infusion associated with an arterial lactate concentration of ~3-5 $mmol \cdot l^{-1}$ in humans at rest. However, this estimate appears based on the assumption that all of the lactate taken up during infusion was metabolised by the muscles and ignores the quantity of lactate that merely increased the muscle lactate content during infusion and subsequently returned to the circulation during recovery. That this occurs, at least to some extent, has been demonstrated by Buckley (39). Buckley (39) examined lactate uptake in the resting untrained and endurance trained (dominant) forearms of racquet sportsmen during an incremental lactate infusion protocol to increase the arterial lactate concentration to ~5 $mmol \cdot l^{-1}$ over 18 min. Although both trained and untrained forearms took up lactate during infusion (trained, 41.5 ± 5.9 $\mu mol \cdot 100\ ml^{-1}$; untrained, 41.5 ± 7.8 $\mu mol \cdot 100\ ml^{-1}$), equivalent quantities were returned to the circulation during a subsequent 2 h period of recovery (trained, 26.3 ± 21.1 $\mu mol \cdot 100\ ml^{-1}$; untrained, 53.8 ± 31.3 $\mu mol \cdot 100\ ml^{-1}$).

Infusion studies such as those of Pagliassotti and Donovan (195) and Gladden et al. (97) strongly suggest that lactate disposal processes within muscles at rest are governed largely by the intramuscular lactate concentration. Consequently, the time course of change from net lactate production to net lactate disposal following the onset of an increase in arterial lactate concentration will principally be determined by the rate of muscle lactate influx. Although steady-state levels of resting muscle lactate uptake appear to be achieved within perhaps ~30 min of exposure to relatively stable arterial lactate concentrations with lactate infusion in isolated perfused resting muscle preparations (46, 95, 193, 195), equilibration in resting muscles exposed to increased arterial lactate levels during exercise with other muscle groups potentially takes longer.

In the resting forearm skeletal muscles of humans at rest, blood flow is generally measured to be ~2-5 ml·100 ml^{-1}·min^{-1} or ~20-50 ml·kg^{-1}·min^{-1} (127, 285) and decreases slightly during leg exercise (127). Similarly, resting leg blood flow is ~40 ml·kg^{-1}·min^{-1} (13). However, isolated muscle preparations are generally perfused at rates of ~20-40 ml·100 ml^{-1}·min^{-1} or ~200-400 ml·kg^{-1}·min^{-1} (46, 95-98, 194) such that muscle $\dot{V}O_2$ becomes flow independent. As a result, blood-muscle equilibration could be much faster compared to resting muscle uptake during exercise, particularly given the rapid onset of steady-state arterial concentrations generally employed with lactate infusion studies compared with the more gradual rise in the arterial blood lactate concentration that accompanies constant load exercise. Furthermore, whilst resting muscles are clearly a site of net lactate disposal when the blood lactate concentration is elevated by infusion, this is not necessarily the case during exercise with other muscle groups in which there are arterial concentration disturbances not only in lactate, but in many substances, such as catecholines, that potentially influence resting muscle lactate metabolism.

1.4.2 Blood lactate elevated by exercise

Many studies have suggested that resting muscles are an important site of net lactate disposal during exercise with other muscle groups (5, 13, 16, 40, 88, 104, 111, 161, 162, 203, 247, 282, 284). However, in all of these studies non-steady-state arterial and resting muscle venous lactate concentrations predominated and under these conditions arteriovenous lactate concentrations need not imply resting muscle lactate disposal. This is perhaps even more of a concern than in isolated perfused muscle preparations given the potentially lower rate of blood flow and the generally much longer period required for arterial lactate concentrations to stabilise during moderate to high intensity exercise.

One of the most widely cited studies supporting the view that resting muscles are an important site of lactate disposal during exercise with other muscle is that of

Poortmans et al. (203). These investigators examined arterial and forearm venous blood lactate concentrations during graded leg exercise and a subsequent 30 min recovery period. A small positive veno-arterial lactate concentration difference (output) at rest was replaced by a negative veno-arterial difference (uptake) throughout exercise. Uptake persisted until 5 min of recovery, after which the arterial and venous lactate concentrations were not different. Forearm blood flow was not measured, but, based on a value of 5 ml·100 g^{-1}·min^{-1}, an average veno-arterial lactate difference of 1 mmol·l^{-1} throughout 25 min of exercise and a total resting muscle mass of 20 kg (representing a 14 liter fluid compartment), Poortmans et al. (203) estimated that approximately 18% of the lactate taken up by resting muscles was metabolised. This figure appears to be based on the ratio of estimated total lactate uptake (25 mmol) and total resting muscle lactate content (140 mmol) predicted from the equilibration of a 14 liter fluid compartment with the venous lactate concentration at the end of exercise (10 mmol·l^{-1}). However, this would not determine the fraction of the lactate uptake that was metabolised by resting muscles, and in fact assumes that all of the lactate taken up (25 mmol) was metabolised. To satisfy the law of conservation of mass, any increase in resting muscle lactate content must balance the quantity of lactate removed from the arterial blood. Resting muscle lactate metabolism would be indicated only if the resting muscle lactate content at the end of exercise was less than the quantity taken up (ie < 25 mmol). To contain 140 mmol of lactate, having only taken up 25 mmol from the circulation, would require 115 mmol of lactate to have been produced by the resting muscles, or uptake to be underestimated by this amount.

Based on resting deltoid muscle biopsy samples and arterial and resting forearm venous blood samples obtained during and after leg cycling exercise in humans, Lindinger et al. (161) estimated that of the total quantity of lactate produced by the legs, < 1% was excreted by the kidneys, ~24% was distributed in ~20 kg of resting muscles, ~12% in the blood (~5 l) and ~25% in the active muscles (~8 kg) and speculated that the remaining ~38% was oxidised in tissues such as inactive muscle.

Whilst these calculations appear to be based on a lactate distribution space of ~50% of the body mass (227), which might be reasonable, such estimates are clearly speculative, appear to neglect disposal by the liver and could easily be over-estimated. Furthermore, assuming a constant forearm blood flow of 0.1 $l \cdot min^{-1}$ (based on 5 $ml \cdot 100\ ml^{-1} \cdot min^{-1}$, and a forearm volume of ~2 l), and using approximate values of arterio-venous lactate differences throughout the 23 min period of exercise and 5 min of recovery (obtained from Figure 4 of Lindinger et al. (161)), an estimated 9.5 mmol of lactate was taken up by the forearm. Distributed within a fluid volume of 1.5 l (75% tissue water) this would produce a forearm tissue lactate concentration of approximately 6.5 $mmol \cdot l^{-1}$, similar to that reported for the resting deltoid muscle (7.5 $mmol \cdot l^{-1}$) biopsied at the same time. In addition, the resting deltoid muscle lactate concentration continued to increase, attaining levels 2.5-fold higher than control after 25 min of recovery. Although no further biopsy samples were taken for the remainder of recovery, this observation strongly suggests that the post-exercise decline in resting muscle lactate content has a prolonged time course, and is consistent with the view that equilibration processes are an important and persistent component of resting muscle arteriovenous lactate concentration differences.

The relative absence of significant lactate release during recovery from a period of resting muscle lactate uptake is sometimes regarded as evidence for disposal of lactate within the resting muscles (111, 161, 203). However, in none of these studies had arterial or venous blood lactate concentrations returned to baseline levels by the end of recorded recovery. In the study conducted by Lindinger et al. (161), arterial and venous blood lactate concentrations remained approximately 3-fold higher than baseline levels even after 90 min of recovery following 4 brief bouts of intense leg exercise. Unless complete recovery can be demonstrated, inferences regarding the relative absence of significant lactate release, that may well occur relatively late in recovery when the arterial lactate concentration becomes lower than that within the muscle, do not appear entirely justified.

Whilst equilibration processes potentially account for much of the resting muscle lactate uptake attributed to disposal in several studies (104, 161, 203), this does not always appear to be the case (13, 40). In a recent study in which lactate uptake was measured across the resting leg during intermittent arm exercise to elevate the blood lactate concentration, Bangsbo et al. (13) concluded that most of the lactate taken up by the inactive leg was metabolised. After an initial small increase, resting muscle (vastus lateralis) lactate concentration remained relatively constant throughout the remaining period of arm exercise, supporting the view that lactate taken up by the inactive leg was metabolised and did not substantially contribute to an increase in muscle lactate content. Similarly, Buckley et al. (40) found that a significant quantity of lactate taken up by the resting forearm during high intensity (~85% $\dot{V}O_{2\,max}$) supine leg exercise was not returned to the circulation after 2 h of recovery, implicating lactate disposal within the forearm as the fate of much of the lactate taken up.

1.4.3 Prolonged exercise

Whilst relatively short-term moderate to high intensity exercise (~20-30 min) invariably results in resting muscle lactate uptake (5, 13, 16, 40, 88, 104, 111, 161, 162, 203, 247, 282, 284), prolonged exercise has been shown to increase resting muscle lactate release (2, 3). Ahlborg and Felig (3) demonstrated that the arteriovenous lactate concentration difference across the resting forearm became significantly more negative (lactate release) throughout 3.5 h of prolonged leg exercise (58% $\dot{V}O_{2\,max}$), reaching values ~3-fold greater than baseline levels of release. In a later study, Ahlborg (2) demonstrated that inactive forearm lactate release during prolonged leg exercise (50% $\dot{V}O_{2\,max}$) was attenuated by the arterial infusion of propranolol, implicating the ~9-10-fold increase in circulating catecholamine levels, particularly epinephrine, as the cause of increased resting muscle glycogenolysis and lactate release (2, 3). Whilst the low arterial lactate concentrations (~1-1.6 $mmol \cdot l^{-1}$) in these studies (2, 3) may have been insufficient to elicit net resting muscle lactate disposal, it is likely that circulating catecholamines

also promote resting muscle lactate production during higher intensity exercise associated with greater arterial lactate concentration disturbance. In support of this hypothesis, Ross (215) found greater lactate uptake in the β-blocked compared to the unblocked resting forearm during 20-min of supine leg exercise (~85% $\dot{V}O_{2\,peak}$, ~11 $mmol \cdot l^{-1}$ arterial lactate concentration). Wendling et al. (270) demonstrated that exercising muscle glycogenolysis was unaffected when 90 min of exercise at 65% $\dot{V}O_{2\,max}$ was performed with or without a ~2.5-fold increase in venous plasma epinephrine concentration produced by infusion. However, the venous lactate concentration was higher during the first 60 min of leg exercise combined with epinephrine infusion and, whilst speculative, it is possible that increased resting muscle glycogenolysis may have been partly responsible for this finding. More recently, Bergman et al. (24) concluded that inactive muscles and other tissues must release lactate during exercise to account for the maintenance of an elevated arterial lactate concentration despite net lactate release by active muscles falling close to zero after 45 min of exercise at 65% $\dot{V}O_{2\,max}$.

Such direct and indirect evidence for resting muscle lactate release during prolonged moderate intensity leg exercise clearly does not exclude resting muscles as an important site of net lactate disposal during shorter-term moderate to high intensity exercise. However, it is conceivable that the net exchange of lactate across resting muscles during prolonged exercise is more representative of forearm lactate metabolism during short-term exercise than lactate infusion studies on which the hypothesis of net resting muscle lactate disposal during exercise with other muscles is partly based. As exercise becomes prolonged, blood-tissue equilibration is unlikely to importantly influence the net exchange of lactate across resting muscles. Furthermore, as with shorter-term exercise, prolonged exercise is associated with many systemic disturbances, such as changes in circulating catecholamine levels, that are virtually absent in lactate infusion studies.

1.5 Summary and aims of the thesis

The traditional concept that lactate is a dead-end metabolite produced by exercising muscles due to limited O_2 availability has been replaced by the contemporary view of lactate as an important intermediary in exercise metabolism. Skeletal muscles clearly possess the capacity to simultaneously produce and metabolise lactate, the net effect depending on many factors, perhaps most importantly, the muscle metabolic rate.

Whilst there is good evidence that resting skeletal muscles dispose of lactate by complete oxidation and by alternative pathways when the intramuscular lactate concentration is elevated, this capacity may be quite small given the apparent metabolic rate dependence of oxidation and that increased lactate availability does not appear to increase resting muscle metabolic rate (97, 195). Furthermore, the time course of blood-tissue equilibration processes appears to be quite long, even in well perfused isolated muscle preparations exposed to a stable elevated arterial lactate concentration (46, 95). In studies of human exercise, moderate to high intensity exercise is typically sustained for ~30 min and arterial lactate concentration changes often take many minutes to stabilise. These factors, in combination with changes in resting muscle blood flow, circulating hormones and alternative substrate concentrations could interact to prolong resting muscle blood-tissue equilibration and inhibit the metabolic disposal of lactate within resting muscles.

Based on measurements of arteriovenous lactate concentration differences across resting muscle vascular beds and in some cases measurements of tissue lactate content, several investigators have suggested that resting muscles dispose of significant quantities of lactate under non-steady-state elevated arterial lactate conditions. However, although lactate is clearly a readily oxidisable substrate that can engage in many metabolic pathways, the net effect of lactate production and disposal within resting muscle is very difficult to establish unless equilibrium conditions exist. Such conditions are rare during exercise, perhaps existing only

during prolonged exercise of light to moderate intensity. Consequently, passive lactate movements are potentially the biggest component of resting muscle veno-arterial concentration differences during exercise. Even in studies that have inferred resting muscle lactate disposal from veno-arterial lactate concentration differences and in some cases muscle lactate content, wash-in was clearly the principal component of uptake at least within the first ~30 min of exposure to an elevated blood lactate concentration. Therefore, the precise role of resting muscles in metabolising blood lactate produced by other muscle groups remains unclear. While there is some compelling evidence to indicate that resting muscles do metabolise significant quantities of lactate under these conditions, there are some data to suggest that this may not always be the case. During prolonged moderate intensity exercise the arterial lactate concentration has been shown to remain elevated despite lactate release from exercising muscles falling close to zero after 45 min (24). Such a finding is not consistent with significant resting muscle lactate disposal and in fact Bergman et al. (24) concluded that inactive muscle and other tissues must release lactate to explain the maintenance of elevated arterial lactate concentrations.

Furthermore, while lactate has been shown to compete favourably with alternative oxidisable substrates within exercising muscles when lactate availability is increased by infusion, prior exercise or exercise in remote muscle groups, the rate of lactate oxidation appears to be determined principally by lactate concentration and muscle metabolic rate (97). Consequently, although an elevated lactate concentration would be expected to favour increased lactate oxidation, the low metabolic rate of muscle at rest may limit its capacity for lactate disposal. Furthermore, there are some data to suggest that resting muscle carbohydrate metabolism is not significantly augmented when arterial lactate levels are elevated (27, 28). Under these conditions, a change to net lactate disposal must rely principally on the inhibition of glycolytic pyruvate formation. Although increased lactate and H^+ availability would tend to inhibit glycolysis, a decline in resting muscle blood flow and catecholiminergic stimulation of

glycolysis may limit the capacity for glycolytic pyruvate formation to be reduced and therefore the capacity of resting muscle to become a site of net lactate disposal.

The primary aim of this doctoral study was to investigate the likely contribution of equilibration processes to the development of resting forearm muscle veno-arterial lactate and blood gas concentration differences during exercise with other muscle groups, in order to determine more reliably the role that changes in resting muscle metabolism play in the disposal of an arterial lactate load. A further aim was to explore the impact of these processes on the interpretation of blood lactate responses to incremental exercise that are frequently assessed using forearm venous blood.

CHAPTER 2 General methods

2.1 Subjects and experimental conditions

IN ALL, 18 male cyclists volunteered and provided written consent to participate in the three experimental series reported in this thesis. Subjects were informed regarding the nature of the study and the risks involved, and all studies were approved by the Committee on the Ethics of Human Experimentation at the University of Adelaide. Cyclists were recruited because of their familiarity with the cycle ergometer tasks to be undertaken and their ability to maintain a given cadence (± 1-2 RPM) and consequently work load. Eleven subjects were competitive cyclists training in excess of 200 km per week while the remaining subjects were from the general student population of the University of Adelaide and cycled at least twice a week. The physical characteristics of subjects participating in each separate study are reported in each relevant chapter.

Subjects attended the laboratory in the morning after an overnight fast and abstinence from caffeine and other stimulants, and a 36 h period without strenuous exercise. Throughout each experiment subjects lay supine on a comfortable catheterisation table in a temperature controlled laboratory (21 ± 1 °C, relative humidity 40-60%).

2.2 Choice of the forearm and supine leg exercise for the study of resting muscle metabolism during exercise with other muscles

The forearm is particularly well suited to the study of resting muscle metabolism during exercise with other muscle groups, and has been used for this purpose by many investigators (2-4, 104, 105, 131, 203, 284). Leg exercise, with which subjects are generally most familiar, and in particular supine leg exercise, provides a convenient and well controlled means to produce the neural and humoral

disturbances associated with exercise whilst leaving the forearm muscles protected from movement and at rest. Furthermore, the forearm is comprised mainly of muscle (1, 48, 60) and blood representative of that flowing into and out of the forearm muscles can easily be accessed for serial sampling by catheterisation of arterial and deep venous forearm vessels. Venous blood collected in this manner almost exclusively represents forearm muscle drainage at rest (48, 186). In addition, blood flow, a critical measurement for any investigation of tissue metabolism, can readily be measured in the forearm.

2.3 Experimental techniques

2.3.1 Measurement of forearm blood flow by venous occlusion plethysmography

2.3.1.1 Principles

In any study of tissue metabolism it is important that blood flow measurements are representative of the tissues from which venous blood is collected. In the studies reported in this thesis, venous occlusion plethysmography was considered best suited for this purpose and was used for all measurements of forearm blood flow. This method is based on the principle that blood flow through a limb segment is proportional to the rate of change in limb volume when the venous outflow of the limb segment is occluded (106, 185, 273). Venous occlusion plethysmography measures the combined blood flow through all of the tissues' vascular components, of which the forearm is comprised of approximately 60% muscle, 10% skin and subcutaneous tissue and 30% bone, fat and tendon (1, 60). Due to the comparatively avascular nature of these last three components, muscle accounts for approximately 82% of the vascular portion of the forearm with the remaining 18% reflecting cutaneous and subcutaneous tissues (1). Consequently, measurements of total forearm blood flow, which include both of these components, are not totally representative of blood flow through the skeletal muscles that are generally considered the principal source of

blood collected from deep forearm veins (48). Nevertheless, given the greater bulk of the forearm muscles and the forearm volume considerations detailed below, blood flow measurements in these studies were considered to represent principally blood flow in the forearm skeletal muscles from which deep forearm venous blood was likely to have been derived.

2.3.1.1a ***Forearm volume considerations***

Blood samples drawn from deep forearm veins drain only a portion of the total forearm volume. Consequently, it is important to consider the tissue components from which blood samples are obtained relative to those that contribute to the measurements of forearm blood flow. Total forearm blood flow, as measured by venous occlusion plethysmography, is conventionally expressed per unit (100 ml) volume of the whole forearm. However, this potentially leads to erroneous estimates of forearm muscle blood flow, expressed per unit volume of muscle, for two reasons. Firstly, the forearm volume includes the relatively avascular components of bone, tendon and fat within which there will be minimal expansion associated with interrupted venous outflow. Consequently, total blood flow through the distensible portion of the forearm (ie muscle and skin) will be systematically underestimated. Secondly, although skin blood flow is quantitatively less than muscle blood flow, the flow per unit volume of tissue is generally much greater in the skin (60). Consequently, total forearm blood flow measurements overestimate muscle flow and underestimate skin flow, expressed per unit volume of tissue. However, in combination, the underestimation of muscle flow per unit volume with the inclusion of the non-distensible volume of the forearm is counterbalanced by the overestimation associated with the inclusion of the skin component of flow. Consequently, in the resting forearm, reliable estimates of muscle blood flow are obtained from total forearm blood flow measurements expressed per unit volume of the whole forearm.

2.3.1.2 Procedures

On the forearm designated for blood flow measurements, the point of greatest forearm girth was identified (approximately 5-10 cm distal to the olecranon process) and the circumference measured using a 5 mm wide fiberglass measuring tape. This point, the middle wrist crease and a point 10 cm proximal to the olecranon process were marked with a water proof marker pen prior to measuring the hand and forearm volumes by water displacement. The hand was immersed, up to the middle wrist crease, into a large cylinder filled with water at room temperature. The volume of water displaced by the hand was directed via a spigot into a container resting on a digital scale (Mettler Toledo, 120 ± 0.02 kg) zeroed prior to immersion. The arm was then immersed up to the mark proximal to the olecranon process and the forearm volume determined by subtracting the mass of water displaced by the hand from that displaced by the whole arm, assuming a value of unity for the density of water.

After subjects adopted the supine position, both arms were outstretched and placed in a comfortable position, with the palms facing upwards and elbows slightly bent, on padded boards inclined 30° from horizontal (Figure 1.2 and Figure 1.3) to ensure adequate venous drainage between flow measurements (106). Forearm volume changes were detected using a strain gauge (Medasonics SG24), comprising silicone rubber tubing filled with an electro-conductive alloy, applied to the forearm at the point of greatest forearm circumference using a custom built and adjustable strain gauge support device. The venous occlusion cuff was placed around the upper arm 10 cm proximal to the olecranon process and inflated to between 50 and 60 mmHg three times each minute (13 seconds on, 7 seconds off) using an automated pneumatic system (Figure 1.3). With the exception of the experiments reported in Chapter 3, in which a radial artery was catheterised, a wrist cuff inflated to ≥ 200 mmHg was used to exclude the hand circulation from forearm blood flow measurements (159). The wrist cuff was periodically deflated between blood sampling periods to prevent ischemic injury to the hand. Changes in strain gauge length were amplified (Medasonics SPG16) and output to a pen chart recorder

(Tohshin). Calibrations were performed at the end of each experiment by recording pen deflections associated with known increments in strain gauge length, covering the full recorded range, using a micrometer based strain-gauge stretching device. Excluding vascular sampling periods and, in experiments employing a wrist cuff, periods when the wrist cuff was deflated, forearm blood flow (expressed as ml·100 ml of forearm^{-1}·min^{-1}) was determined from the angle formed by the inflow curve and the chart baseline (Figure 1.1), the chart speed (12 cm·min^{-1}) and the calibration strain gauge length change and pen deflection using the relationship (106):

$$\text{Flow} = \text{Tan}(\theta)\cdot\frac{2\cdot\text{Chart speed}\cdot\text{Calibration length change}}{\text{Forearm circumference}\cdot\text{Calibration pen deflection}}$$

Where all units of length or displacement are in cm. Forearm blood flow was subsequently calculated as the mean of all inflow measurements recorded each minute.

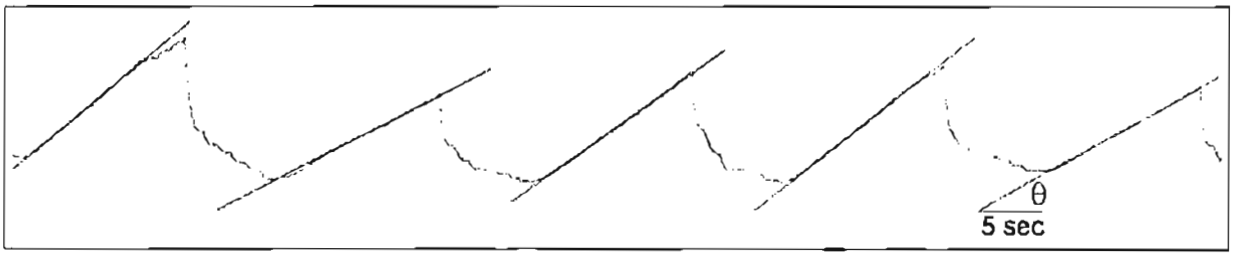


Figure 1.1. An example of a forearm plethysmography chart record showing five inflow curves. Forearm blood flow was determined from the slope of the inflow curve, the chart speed and the pen deflection associated with a known increment in strain-gauge length.

2.3.1.3 Potential limitations

All methods for the measurement of resting forearm blood flow have potentially important limitations. Arterial inflow measurements of blood flow, such as those obtained with venous occlusion plethysmography or thermodilution techniques, measure the combined flow of all the component tissues of the forearm and,

depending on the site of venous blood collection, may not be representative of blood flow in the tissues from which venous samples derive. Although the forearm is comprised principally of skeletal muscle (1, 48, 60), skin flow accounts for 30%-50% of the total forearm blood flow (60, 285). Furthermore, the contribution of skin flow can change independently from that of muscle flow, particularly in association with the release of skin vasoconstrictor tone and the enormous sweat-mediated skin vasodilatation associated with exercise (127, 285). Johnson and Rowell (127), using the injection and clearance of ^{125}I antipyrine to measure forearm muscle blood flow, showed forearm muscle flow to decline during 40 min of moderate intensity leg exercise. In contrast, total forearm blood flow increased approximately 5-fold, indicating a large and progressive exercise related increase in skin blood flow. Consequently, if forearm blood samples derive exclusively from muscle, blood flow determined from arterial inflow measurements such as venous occlusion plethysmography will over-estimate muscle flow. Under these conditions, methods based on the muscle clearance of a radiolabeled substance such as ^{125}I antipyrine (127) or ^{133}Xe (114, 190) would be more appropriate. However, while the forearm is comprised mainly of muscle, at least some of the blood collected from deep forearm veins is likely to derive from more superficial vascular beds (48). Furthermore, this contribution is likely to increase in proportion with thermoregulatory changes in skin flow associated with exercise. An exercise related rise in venous blood pO_2 and O_2 content was observed in all of the studies reported in this thesis, and is strong evidence for a contribution of skin blood to antecubital venous blood samples. If venous blood derived only from muscle, this observation would be indicative of an increase in muscle blood flow or a decline in forearm O_2 uptake, neither of which is consistent with previous reports (127, 285). Consequently, the venous occlusion plethysmographic measurements of forearm blood flow used in these studies were considered to be largely representative of the tissues from which antecubital blood was derived, and to be at least as reliable as alternative blood flow measurement techniques.

2.3.2 Vascular catheterisation

2.3.2.1 Procedures

To obtain samples of arterial and venous blood representative of the forearm tissues, Teflon catheters were inserted under local anaesthesia (lignocaine hydrochloride, 2%) using standard aseptic techniques and with the catheter tip directed upstream. Arterial catheters (Cathlon IV, 20-gauge, 1.05 mm OD x 44-mm length, Criticon) were inserted into a radial or brachial artery. Venous catheters (Insyte IV, 18-gauge, 1.2 mm OD x 44-mm length, Becton Dickinson Medical Pty Ltd) were inserted into the deep muscle branch (186) of an antecubital vein. The catheters were taped to the skin and 30 cm polyethylene extensions (Braun Melsungen) filled with sterile normal saline attached and sealed with three-way stopcocks (Braun Melsungen). At least 20 min elapsed between catheter insertion and the commencement of blood sampling.

2.3.2.2 Blood sampling

In all experiments, paired arterial and venous blood samples were withdrawn simultaneously at predetermined intervals detailed in each relevant chapter. Before each blood sample was collected, 1 ml of the blood-saline interface (corresponding to more than twice the dead space volume of the catheter and sample line) was withdrawn and discarded. Three ml blood samples for blood lactate and blood gas analysis were collected anaerobically into sterile glass syringes (Top Interchangeable) rinsed with heparin (5,000 international units·ml^{-1}) such that the dead space at the tip remained filled prior to blood sampling. Immediately prior to taking glass syringe samples in the experiments reported in Chapter 5, 1 ml blood samples were collected into 1 ml syringes (Becton Dickinson Medical Pty. Ltd.) for subsequent analysis of blood nitrous oxide (N_2O) concentration. To maintain catheter patency and blood volume, each blood sample was immediately replaced with an equivalent volume of sterile normal saline. In each experiment, the blood sampling procedures removed a total blood volume of between 200 and 250 ml.

2.3.3 Supine cycle ergometer exercise

All exercise tests were performed using a mechanically braked supine cycle ergometer (Jaquet, Switzerland) firmly bolted to a padded catheterisation table (Figure 1.2). Prior to each experiment, shoulder rests attached to the catheterisation table (Figure 1.3) were adjusted to suit the preferred cycling position of the subject and then firmly clamped in place. During rest and recovery periods, subjects lay relaxed with their feet out of the ergometer pedals and legs outstretched. In the few minutes preceding exercise, the subject's cycling shoes were firmly bound to the ergometer pedals using toe straps and adhesive tape or, more commonly, using the subjects own clip-less pedals and shoes. This was necessary to prevent subjects accidentally pulling their feet off the pedals during exercise. In all experiments subjects were instructed to target a pedalling cadence of 60 rpm using a digital tachometer suspended in prominent view of the subject and experimenters, and to rely only on the shoulder rests for positional support.

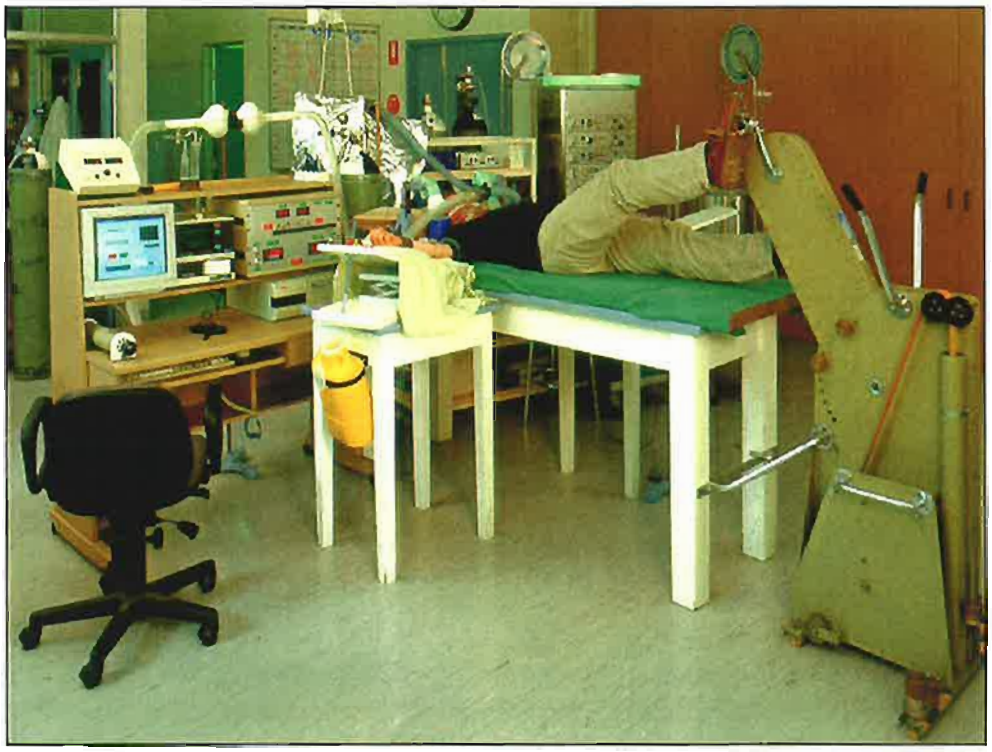


Figure 1.2. Experimental setup showing (clockwise from left); indirect calorimetry system consisting of ventilometer (top), mixing chamber, gas analysers and ECG monitor; the gas delivery system employed for the experiments described in Chapter 5; strain gauge plethysmography equipment; Grass polygraph; mechanically braked supine cycle ergometer; subject, catheterisation table with shoulder rests and arm supports.

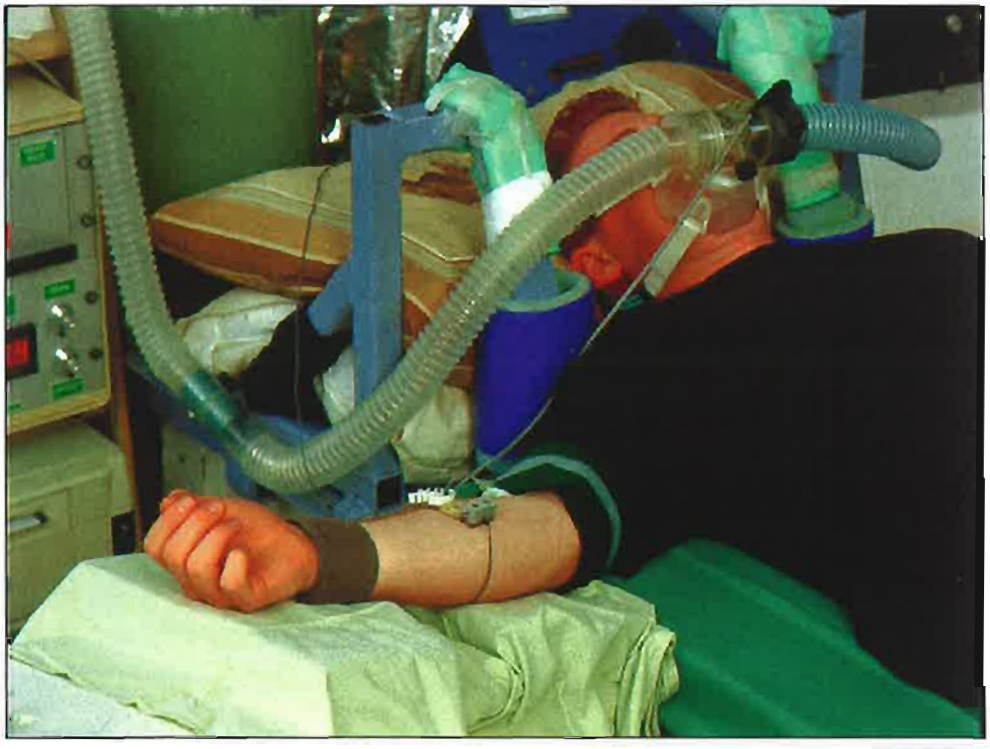


Figure 1.3. Closer view of the shoulder rests and forearm with arterial occlusion cuff at the wrist, upper arm venous occlusion cuff and strain gauge placed around the forearm.

2.3.4 Measurement of blood pressure and heart rate

Apart from the periods of arterial blood sampling, both pulsatile and mean arterial blood pressures (MAP) were recorded throughout each experiment by attaching the extension tubing of the arterial catheter to a pressure transducer (P23 ID, Gould-Statham) connected to a polygraph recorder (Model 7B, Grass). Heart rate was recorded continuously by accessing the electrical signal of arterial pressure to a tachograph preamplifier (7P4 EKG, Grass) and also as 15 sec averages using a heart rate monitor (Sport-Tester PE3000, Polar Electro, Finland). Both heart rate and the electrocardiogram were also displayed and monitored throughout each experiment using an intensive care unit monitor (Lifescope 6, Nihon Kohden, Figure 1.2).

2.3.5 Systemic gas exchange measurements

Minute ventilation ($\dot{V}_E$), systemic oxygen uptake ($\dot{V}O_2$) and carbon dioxide output ($\dot{V}CO_2$) were measured during rest and exercise with an on-line indirect calorimetry system (Figure 1.2). Subjects wore a nose clip and inspired room air through a unidirectional breathing valve (Hans Rudolph 2700) with a large turbine volume transducer (P.K. Morgan, England) attached to the inspiratory port. Expired gas was directed via 1 m of large bore tubing (Clean-Bor, Vacumed) to a 2.6 l mixing chamber (Sportech, Canberra, Australia) from which dried gas was sampled continuously and passed to oxygen (Rapid Zr, Morgan) and carbon dioxide (LB-2, Beckman) analysers calibrated with gases authenticated by micro-Scholander analysis (226) or with commercially prepared precision gas mixtures (BOC gases, Australia). Analog instrument outputs were processed using a personal computer-based data acquisition system employing standard algorithms to calculate 30 sec averages of $\dot{V}_E$ (BTPS), $\dot{V}O_2$ (STPD) and $\dot{V}CO_2$ (STPD) based on inspired minute volume $\dot{V}_I$ (ATPS) and the expired fractions of O_2 (FeO_2) and CO_2 ($FeCO_2$). The respiratory exchange ratio (RER) was determined as $\dot{V}CO_2$ divided by $\dot{V}O_2$.

2.3.6 Blood sample analyses

2.3.6.1 Lactate

Immediately after blood collection a 1 ml aliquot of each 3 ml blood sample was added to 2 ml of ice-cold 8% (w/v) perchloric acid in a 5 ml polystyrene tube, vortex mixed and then centrifuged for 10 min at 1500 g. The supernatant was then removed and stored frozen at -20 °C for subsequent determination of the whole blood lactate concentration.

In the studies reported in Chapter 4, finger prick blood samples were obtained at rest and at the end of each work load during incremental supine leg exercise to exhaustion. Prior to collecting each sample, a finger tip of the left hand was cleaned with alcohol, dried and then pricked with a lancet. The first drop of blood was removed with a dry swab. A 30 µl blood sample was then collected into a capillary tube and immediately deproteinated in 100 µl of ice-cold 8% perchloric acid. Samples were then centrifuged and the supernatant stored frozen for subsequent enzymatic blood lactate analysis. In the second preliminary experiment of the studies reported in Chapter 5, a drop of finger prick blood was obtained in a similar manner but the blood lactate concentration was determined using a portable blood lactate analyser (Accusport).

The lactate analysis of perchlorate extracts of whole blood was performed in duplicate using an enzymatic ultraviolet endpoint method (Sigma Diagnostics 826-UV) employing standard curves constructed from serially diluted stock standards and adjusting for the original sample dilution with perchloric acid. The coefficient of variation for blood lactate concentration measurements was 3.90%.

2.3.6.2 Blood gases

The 2 ml of blood remaining in each glass syringe following the lactate aliquotting procedure was stored capped on ice for a maximum of 60 min before being analysed for pO_2, pCO_2, pH, total haemoglobin concentration (tHb) and percentage oxygen saturation (sO_2). Glass syringes were used to avoid the changes in blood gas tensions that have been reported with the collection and short-term storage of blood in plastic syringes (187, 205, 281). Furthermore, preliminary tests indicated that, providing glass syringes were used and care was taken to eliminate gas bubbles following blood sampling and aliquotting procedures, samples could be stored capped on ice for at least 2 h with no significant changes in blood gas tensions.

In the experiments described in Chapter 3, blood gas analysis was performed using a Corning blood gas analyser (Corning 168) and a Radiometer (OSM2) haemoximeter. In the experiments detailed in Chapters 4 and 5 a Radiometer blood gas analyser (ABL510, Copenhagen) was used. Analysers were calibrated hourly and the performance with quality controls checked prior to each experiment using either tonometered whole blood (Corning 192 Precision Gas Mixer and Corning 184 Blood Gas Tonometer) or commercially available quality controls (Radiometer, Copenhagen). For each blood sample, total oxygen and carbon dioxide contents were determined using the equations of Siggaard-Andersen (229, 230).

2.3.6.3 Nitrous oxide

For the experiments described in Chapter 5, arterial and venous blood nitrous oxide (N_2O) concentrations were determined using a headspace gas chromatographic method similar to that described by Molloy (176). The principle of this method is that the concentration of N_2O dissolved within a blood sample can be determined from the gas (head-space) overlying and equilibrated with the blood sample within a gas-tight container.

The gas-tight containers were 5 ml screw cap (with a rubber septum) evacuated glass vials containing EDTA as an anticoagulant (Exetainer, EX5KE4.0, Johns) and would normally be used for blood collection via a multi-sample venepuncture needle. Prior to each experiment, each glass vial was numbered, weighed (Mettler Toledo, digital balance) and two needles (24-gauge) inserted through the rubber septum of the screw cap, one to serve as an injection needle and the other to vent gas displaced during the introduction of blood to the vial (Figure 1.4A).

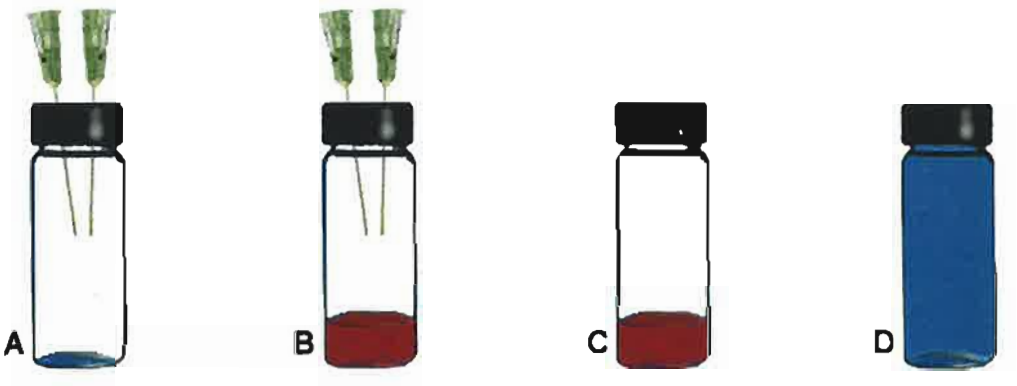


Figure 1.4. Head-space vial; prior to blood sample introduction (A, air filled), immediately after blood sample injection (B), after removal of the injection and vent needles (C) and after complete flushing and filling with distilled H_2O (via injection and vent needles) following gas chromatographic analysis of the head-space gas (D).

During each experiment, after collecting and discarding the sample line dead-space, approximately 1 ml of blood was withdrawn into a 1 ml syringe (Becton Dickinson Medical Pty. Ltd.) and immediately injected into the appropriately numbered glass vial (Figure 1.4B). Both needles were quickly removed (Figure 1.4C) and the vial contents mixed thoroughly using a vortex mixer. Vials were left at room temperature for a minimum of 3 h before gas chromatographic analysis of the head-space gas.

100 µl of head-space gas was withdrawn using a gas-tight glass syringe (Hamilton Co.) and introduced into the injection port of a gas chromatograph (Hewlett Packard, Model 5730A, Avondale, Pennsylvania). The separation of N_2O from other gases was achieved using a 2 m x 2 mm stainless steel column packed with Por-A-Pak QS

and using 10% methane in argon as a carrier gas (at a flow of 20 $ml \cdot min^{-1}$). N_2O detection was via an electron capture detector. The injection port, column and detector temperatures were maintained at 150 °C, 70 °C and 200 °C respectively. The retention time of N_2O was approximately 1.6 min. The area under the N_2O peak was determined using an integrator (Hewlett Packard, model 3380A) attached to the chromatograph. Calibration curves relating head-space N_2O concentration and area units were constructed by equilibrating 1, 5, 10, 25, 50, 75 and 100 µl volumes of 100% N_2O with 1 ml blood samples collected prior to N_2O inhalation.

To account for variability in empty vial volumes, as well as between sample variability in blood volume associated with the need for rapid sample collection and handling and volume inaccuracies of syringe sampling, each vial was completely flushed and filled with distilled water and re-weighed following chromatographic analysis (Figure 1.4D). The complete displacement of gas with water was achieved using an injection needle and a long vent needle (18-gauge venous catheter needle) extending to the base of the vial (inverted) during flushing. To avoid vial volume changes, care was taken to ensure that the screw cap was tight prior to each experiment and subsequently remained undisturbed. The head-space volume was determined by subtracting the mass of the vial plus blood from that of the water filled vial, with the density of water and blood considered unity. Similarly, the blood volume of each vial was calculated by subtracting the mass of the empty vial from that of the vial plus blood. Blood N_2O concentrations, corrected for blood and headspace volume differences between samples, were expressed in µl of N_2O per ml of blood at room temperature and atmospheric pressure ($\mu l \cdot ml^{-1}$). The coefficient of variation for duplicate N_2O analyses was 3.91%.

2.4 Calculations

2.4.1 Lactate threshold determinations

Incremental exercise tests were performed as part of each series of experiments in order to facilitate the selection of appropriate work loads to generate relatively stable and elevated arterial lactate concentrations. From these tests, the lactate threshold (LT) was determined as the exercise work load above which there was rapid blood lactate accumulation. This was achieved using the method described by Beaver et al. (22), modified in a manner similar to that described by Campbell et al. (43) to remove a subjective component from the analysis. The log-log method was chosen in preference to several alternatives due to its relative ease of measurement, its objective nature and its apparent widespread acceptance. The $\dot{V}O_2$ versus venous blood lactate data were log-log transformed and systematically divided into all possible combinations of two adjacent line segments on which linear regression analyses were performed. Regression lines both including and excluding a point common to both line segments were examined. The LT was determined as the point of intersection (expressed as a $\dot{V}O_2$ or $\%\dot{V}O_{2\,peak}$) between the two line segments that yielded the lowest overall residual sum of squares (RSS). When regression line pairs shared a common point, only the smallest squared difference between the point and each regression line was included in the calculation of overall RSS. Log-log LT determinations were performed using Excel (Excel Version 4, Microsoft Corporation) macros developed specifically for this purpose.

In the experiments reported in Chapter 4, additional lactate threshold measures were determined. These included the lactate slope index (LSI) described by Hughson et al. (122), the 2 $mmol \cdot l^{-1}$ (67, 110) and 4 $mmol \cdot l^{-1}$ (152, 250) fixed concentration thresholds, and the individual anaerobic threshold (IAT) described by Stegmann and Kindermann (251).

Hughson et al. (122), proposed that blood lactate increases as an exponential function during incremental exercise:

$$[\text{Lactate}] = A \cdot \exp(B \cdot \dot{V}O_2) + C \qquad (2.1)$$

Where [Lactate] is the blood lactate concentration in $mmol \cdot l^{-1}$, $\dot{V}O_2$ is in $l \cdot min^{-1}$, and A, B and C are constants that minimise the residual sum of squares (RSS) between measured lactate concentrations and those calculated from equation 2.1. In the data reported in Chapter 4, numerical optimisation (Excel 4 Solver, Microsoft Corporation, Seattle) was used to adjust A, B and C from initial values of unity in order to minimise the calculated RSS. The 2 $mmol \cdot l^{-1}$ and 4 $mmol \cdot l^{-1}$ lactate thresholds were subsequently determined as the $\dot{V}O_2$ corresponding to the appropriate lactate concentration using equation 2.2:

$$\dot{V}O_2 = \frac{\ln\left(\frac{[\text{Lactate}] - C}{A}\right)}{B} \qquad (2.2)$$

The LSI was determined as the point at which the slope of the lactate versus $\dot{V}O_2$ curve equalled unity (122) using equation 2.3:

$$\frac{d[\text{Lactate}]}{d \cdot \dot{V}O_2} = A \cdot B \exp(B \cdot \dot{V}O_2)$$

$$\Rightarrow \quad \text{LSI} = \frac{-\ln(A \cdot B)}{B} \qquad (2.3)$$

The IAT was determined by fitting third ($y = Ax^3+Bx^2+Cx+D$) and second ($y = Ax^2+Bx+D$) order polynomials to the exercise and recovery time versus blood lactate concentration curves respectively. For the exercise curve, recovery data were excluded. For the recovery curve, the first data point was considered the last point in exercise, followed by all sequential recovery data points until the first point at which

the blood lactate concentration was below that of the final exercise point. The IAT was subsequently determined as the point on the exercise curve at which the tangent line intersected the recovery curve where the lactate concentration became equal to that at the end of exercise (251).

2.4.2 Mathematical models of blood-tissue exchange in the resting forearm

In the studies described in this thesis two models of blood-tissue exchange were applied to the study of lactate and gas exchange in the resting forearm during moderate intensity leg exercise. The first and simplest (Kety-Scmidt or modified Fick model) was based on a model of inert gas exchange between the blood and tissues. In its simplest form this model considers a single well-mixed tissue compartment in which the arterial inflow completely mixes with the tissue contents before exiting in the venous outflow, and is therefore a single compartment perfusion limited model of blood-tissue exchange. However, this model includes a term allowing for the incorporation of a diffusion component to blood-tissue exchange, and it was this form of the model that was effectively used in the majority of the analysis (Chapters 3–5). The second model, applied only to the data reported in Chapter 5, was a 2-compartment model that considered the blood and extravascular compartments to be well-mixed and separated by a membrane barrier. This model is therefore a perfusion and diffusion limited model of blood-tissue exchange.

2.4.2.1 Kety-Scmidt (modified Fick) model

The principal method employed to predict the concentration of lactate and other blood borne substances in forearm venous blood during the non-equilibrium conditions associated with leg exercise was based on the classic technique developed by Kety and Schmidt (147-151) to measure blood flow in the cerebral circulation. This model was applied in all the studies reported in this thesis and was the only model used in the studies reported in Chapters 3 and 4.

2.4.2.1a ***Derivation of the integrated Fick equation***

Kety and Schmidt (147-151) reasoned that for a freely diffusible tracer substance such as nitrous oxide (N_2O), the time rate of change of the amount of tracer within the tissues is equal to the difference between the rate at which it is brought to the tissues in the arterial blood and the rate at which it is removed from the tissues in the venous blood. This is the Fick principle and is essentially a restatement of the law of conservation of mass:

$$\frac{dQi(t)}{dt} = F(t)[Ca(t) - Cv(t)] \qquad (2.4)$$

Where:
- $Qi(t)$ = The quantity of the tracer in the tissues at time, t.
- $F(t)$ = The rate of blood flow through the tissues at time, t.
- $Ca(t)$ = The tracer concentration in the arterial blood at time, t.
- $Cv(t)$ = The tracer concentration in the venous blood at time, t.
- t = A given time after the initial arterial tracer concentration change or appearance.

Dividing both sides by the volume of tissue, Vi, provides an expression for the concentration of tracer in the tissues:

$$\frac{dCi(t)}{dt} = \frac{F(t)[Ca(t) - Cv(t)]}{Vi} \qquad (2.5)$$

Where:
- $Ci(t)$ = The tissue concentration of the tracer at time, t.
- Vi = The tissue volume.

For a tissue which is homogenous with regard to blood perfusion rate and solubility of tracer within it, the following relationship between the arterio-venous and the arterial-tissue concentration differences has been postulated (149):

$$[Ca(t) - Cv(t)] = m[Ca(t) - \frac{Ci(t)}{\lambda}] \quad (2.6)$$

Where: λ = The blood-tissue partition coefficient for the tracer material (ie the ratio of the solubility of the tracer in the tissues and in the blood).

m = A constant representing the net effects of diffusion limitations, capillary impermeability and arterio-venous shunts which limit the equilibration of tissue with the blood.

$$Cv(t) = Ca(t) - mCa(t) + \frac{mCi(t)}{\lambda} \quad (2.7)$$

If it is assumed that there are no equilibration limitations then m = 1 (i.e. the concentration of tracer in the venous blood is in constant equilibrium with the concentration of the tissues which it drains) and the model effectively becomes a single compartment model.

Substituting equation 2.6 into 2.5 yields:

$$\frac{dCi(t)}{dt} = \frac{mF(t)[\lambda Ca(t) - Ci(t)]}{\lambda Vi} \quad (2.8)$$

and letting:

$$k = \frac{mF(t)}{\lambda Vi} \quad (2.9)$$

$$\frac{dCi(t)}{dt} = k[\lambda Ca(t) - Ci(t)]$$

$$\Rightarrow \frac{dCi(t)}{dt} + kCi(t) = \lambda kCa(t)$$

Which is a simple differential equation of the first order and first degree. Multiplying by an appropriate integrating factor $e^{\int k dt} = e^{kt}$:

$$\frac{dCi(t)}{dt}e^{kt} + kCi(t)e^{kt} = \lambda kCa(t)e^{kt}$$

Now:

$$\frac{d}{dt}Ci(t)e^{kt} = \frac{dCi(t)}{dt}e^{kt} + kCi(t)e^{kt} \qquad \text{(From the Product Rule*)}$$

$$\Rightarrow \frac{d}{dt}Ci(t)e^{kt} = \lambda kCa(t)e^{kt}$$

Integrating both sides over the limits t to 0 yields:

$$\int_0^t \frac{d}{dt}Ci(t)e^{kt}\,dt = \int_0^t \lambda kCa(t)e^{kt}\,dt$$

$$\Rightarrow Ci(t)e^{kt} = \lambda k\int_0^t Ca(t)e^{kt}\,dt$$

$$\Rightarrow \mathbf{Ci(t) = \lambda k e^{-kT}\int_0^t Ca(t)e^{kt}\,dt} \qquad \text{(2.10 Integrated form of the Fick equation)}$$

2.4.2.1b ***Assumptions related to the integrated Fick equation***

Several assumptions are required for the derivation and hence application of the integrated form of the Fick equation. Firstly, the blood at the point of sampling must be representative of the venous drainage from the tissues being studied. There can

---

* The Product Rule states that if: f(t) = g(t)p(t) then f'(t) = g(t)p'(t) + p(t)g'(t)

be no arterio-venous shunts or capillary bypasses. By sampling deep venous blood from the forearm it was assumed that these requirements were largely satisfied. Secondly, there must be homogeneity of blood flow throughout the tissues being studied. There can be no regional differences in perfusion rate, and any changes in blood flow must occur uniformly throughout the tissues. In considering the lower arm as a whole, perfusion rates are likely to vary between the different tissues such as skin, muscle and bone. Furthermore, changes in blood flow may occur independently in the different forearm tissues, as in leg exercise, where skin flow increases with rising body temperature while forearm muscle blood flow decreases (127). Therefore, it must be assumed that total forearm blood flow approximates mean blood flow through all of the tissues involved in the passive uptake and release of lactate. Furthermore, it is likely that a component of forearm deep venous blood samples is derived from the drainage of skin vascular beds, and that the proportion of this component increases along with skin blood flow during leg exercise. If such were the case then it is appropriate to include total forearm flow, rather than solely muscle flow, when considering wash-in and metabolism in the forearm. Thirdly, there must be homogeneity of tracer solubility within the tissues. It was assumed that this requirement was met when using gases and lactate as the tracer substance, and that these substances are equally soluble in the tissues and blood so that each blood-tissue partition coefficient, λ, equals unity. Finally, if m (in equation 2.6) is considered unity, it must be assumed that the equilibration of substance concentrations between the tissues and blood is limited predominantly by blood flow and not by the diffusion (or transport) of the substance across the capillary walls or throughout the extravascular tissues. Regarding this last assumption, there has been an accumulation of evidence for a carrier-mediated transport mechanism for the translocation of lactate across skeletal muscle membranes (9, 217). The high physiological K_m (40.1 $mmol \cdot l^{-1}$) and V_{max} (139.4 $nmol \cdot mg^{-1} \cdot min^{-1}$) for lactate in skeletal muscle sarcolemma, reported by Roth and Brooks (217), implies a mechanism for potentially rapid and voluminous bi-directional lactate flux in skeletal muscle. Therefore, principally blood flow and not the transport of lactate across the

capillary walls may well limit the equilibration of lactate between the tissues and the blood.

2.4.2.1c ***Application of the integrated Fick equation to predict venous concentrations during non-equilibrium conditions***

In a tissue bed of volume Vi, where the rate of blood flow through the tissues, F(t), and the concentrations of a diffusible substance in the arterial, Ca(t), and venous, Cv(t), blood are measured at different times, t, after an initial change in the arterial concentration of the substance (t = 0):

$$Ci(t) = \lambda k e^{-kt} \int_0^t Ca(t) e^{kt}\, dt \qquad \text{(from 2.10)}$$

From equation 2.7:

$$Cv(t) = Ca(t) - mCa(t) + \frac{mCi(t)}{\lambda}$$

$$\Rightarrow \quad Ci(t) = \frac{\lambda[Cv(t) + (m - 1)Ca(t)]}{m}$$

Substituting into equation 2.10:

$$\Rightarrow \quad \frac{\lambda[Cv(t) + (m - 1)Ca(t)]}{m} = \lambda k e^{-k_T} \int_0^t Ca(t) e^{kt}\, dt$$

$$\Rightarrow \quad Cv(t) + (m - 1)Ca(t) = mke^{-kt} \int_0^t Ca(t) e^{kt}\, dt$$

$$\Rightarrow \quad Cv(t) = mke^{-kt} \int_0^t Ca(t) e^{kt}\, dt + (1 - m)Ca(t) \qquad (2.11)$$

The non-steady state arterial concentrations (wash-in or washout phases) of many diffusible substances, including lactate (43, 122), are generally well described by a mono-exponential function:

$$Ca(t) = Ae^{-Bt} + C \qquad (2.12)$$

Where: $Ca(t)$ = The arterial concentration of the substance at time, t.

A, B, C = Constants that minimise the residual sum of squares.

Substituting equation 2.12 into 2.11 yields:

$$Cv(t) = mke^{-kt}\int_0^t (Ae^{-Bt} + C)e^{kt}\,dt + (1 - m)(Ae^{-Bt} + C)$$

$$\Rightarrow Cv(t) = mke^{-kt}\int_0^t [Ae^{(k-B)t} + Ce^{kt}]\,dt + (1 - m)(Ae^{-Bt} + C)$$

$$\Rightarrow Cv(t) = mke^{-kt}\left(\int_0^t Ae^{(k-B)t}\,dt + \int_0^t Ce^{kt}\,dt\right) + (1 - m)(Ae^{-Bt} + C)$$

$$\Rightarrow Cv(t) = Amke^{-kt}\int_0^t e^{(k-B)t}\,dt + Cmke^{-kt}\int_0^t e^{kt}\,dt + (1 - m)(Ae^{-Bt} + C)$$

$$\Rightarrow Cv(t) = Amke^{-kt}\left[\frac{e^{(k-B)t}}{k-B}\right]_0^t + Cmke^{-kt}\left[\frac{e^{kt}}{k}\right]_0^t + (1 - m)(Ae^{-Bt} + C)$$

$$\Rightarrow Cv(t) = Amke^{-kt}\left(\frac{e^{(k-B)t}}{k-B} - \frac{e^{(k-B)0}}{k-B}\right) + Cmke^{-kt}\left(\frac{e^{kt}}{k} - \frac{e^{k0}}{k}\right) + (1 - m)(Ae^{-Bt} + C)$$

Now the limit, as t tends towards zero, of e^t is 1

$$\Rightarrow Cv(t) = Amke^{-kt}\left(\frac{e^{(k-B)t} - 1}{k-B}\right) + Cmke^{-kt}\left(\frac{e^{kt} - 1}{k}\right) + (1 - m)(Ae^{-Bt} + C)$$

$$\Rightarrow \quad Cv(t) = \frac{Amke^{-kt}}{k-B}[e^{(k-B)t} - 1] + Cme^{-kt}(e^{kt} - 1) + (1 - m)(Ae^{-Bt} + C)$$

$$\Rightarrow \quad Cv(t) = \frac{Amke^{-kt}}{k-B}[e^{(k-B)t} - 1] - Cme^{-kt} + Cm + (1 - m)(Ae^{-Bt} + C)$$

$$Cv(t) = \frac{Amke^{-kt}}{k-B}[e^{(k-B)t} - 1] + (1 - m)(Ae^{-Bt} + C) - Cme^{-kt} + C \qquad (2.13)$$

If m = 1 then:

$$Cv(t) = \frac{Ake^{-kt}}{k-B}[e^{(k-B)t} - 1] - Ce^{-kt} + C$$

This expression (equation 2.13) was used in all experiments (Chapters 3-5). In the last study (Chapter 5), it became apparent that, in the earlier work reported in Chapters 3 and 4, an error had been made in the use of the expression for k (equation 2.9). In an attempt to convert Vi into units appropriate to the units of flow ($ml \cdot 100\ ml^{-1} \cdot min^{-1}$), Vi (ml) was erroneously divided by 100. The correct procedure would have been to retain the measured units of flow and use a Vi of 100, or to convert flow into the units of $ml \cdot min^{-1}$:

$$\text{Flow } (ml \cdot min^{-1}) = \frac{\text{Flow } (ml \cdot 100\ ml^{-1} \cdot min^{-1}) \cdot Vi\ (ml)}{100}$$

and retain the units of Vi (ml). Consequently, the effective value for m was not unity but:

$$m = \frac{100^2}{Vi\ (ml)}$$

While unfortunate, this error was serendipitous, being exactly equivalent to applying an apparently more appropriate two-compartment, perfusion and diffusion limited

model of blood-tissue exchange to the forearm, albeit with a somewhat arbitrary value for m.

To examine the impact of this on the ability of the Kety-Schmidt model (equation 2.13) to predict venous concentrations, two additional values for m were used in the analysis of the data reported in Chapter 5. These were m = 1, in which the model becomes a single compartment perfusion limited model (traditional Kety-Schmidt model), and m optimised to produce the best fit to measured venous data. This was achieved using numerical optimisation methods equivalent to those described for equation 2.14 below.

2.4.2.2 Two-compartment model

The two-compartment model described below was applied only to the data presented in Chapter 5. If a tissue is considered to contain a blood compartment separated from a tissue compartment by a diffusion barrier then the quantity of a given substance within the system at any given instant, Qs(t), must equal the sum of the quantities of the substance in the blood, Qb(t), and tissue, Qi(t), compartments to satisfy the law of conservation of mass.

$$Qs(t) = Qb(t) + Qi(t)$$

and

$$\frac{dQs(t)}{dt} = \frac{dQb(t)}{dt} + \frac{dQi(t)}{dt}$$

The rate of change in the quantity of the substance in the blood compartment equals the rate at which the substance is delivered to the tissue in the arterial blood minus the rates at which the substance is lost to the venous outflow and to the tissues by diffusion. In the tissue compartment, the rate of change in the quantity of the

substance equals the rate of diffusion loss to (or from) the blood plus the rate of production (or disposal) of the substance by the tissue. Consequently, the following differential equations can be written to describe the rates of change in the quantity of any given substance within each compartment:

$$\frac{dQb(t)}{dt} = F(t)[Ca(t) - Cb(t)] - PS\left(Cb(t) - \frac{Ci(t)}{\lambda}\right)$$

$$\frac{dQi(t)}{dt} = PS\left(Cb(t) - \frac{Ci(t)}{\lambda}\right) + \frac{dQ_T(t)}{dt}$$

Where:

$F(t)$ = The rate of blood flow at time, t.

$Ca(t)$ = The arterial concentration of the substance at time, t.

$Cb(t)$ = The concentration of the substance in the blood compartment at time, t.

$Ci(t)$ = The concentration of the substance in the tissue compartment at time, t.

PS = The product of the permeability coefficient of the substance in the diffusion barrier and the total diffusion barrier surface area.

λ = The blood-tissue partition coefficient of the substance.

$dQ_T \cdot dt^{-1}$ = The rate of tissue production or disposal of the substance (zero for an inert substance).

Dividing by the respective volumes of the blood (Vb) and tissue (Vi) compartments provides the following expressions for the rates of change in concentration of the substance:

$$\frac{dCb(t)}{dt} = \frac{F(t)}{Vb}[Ca(t) - Cb(t)] - \frac{PS}{Vb}\left(Cb(t) - \frac{Ci(t)}{\lambda}\right) \quad (2.14)$$

$$\frac{dCi(t)}{dt} = \frac{PS}{Vi}\left(Cb(t) - \frac{Ci(t)}{\lambda}\right) + \frac{1}{Vi}\frac{dQ_T(t)}{dt} \quad (2.15)$$

Unfortunately, it is not practical to obtain analytic solutions for these equations (2.14 and 2.15). Consequently numerical methods were used to simultaneously calculate Cb(t) and Ci(t) from these expressions.

2.4.2.2a ***Numerical methods for calculating venous and tissue concentrations in a two-compartment model of blood-tissue exchange***

Equations 2.14 and 2.15 represent a system of differential equations that can be considered as an initial value problem (167):

$$\frac{dCb}{dt} = f(t, Cb, Ci)$$

$$\frac{dCi}{dt} = g(t, Cb, Ci)$$

Where f(t, Cb, Ci) and g(t, Cb, Ci) are functions of time (equations 2.14 and 2.15) and at time zero:

$$Cb(t_0) = Cb(0)$$
$$Ci(t_0) = Ci(0)$$

For simplicity, these were solved numerically using fourth order Runge-Kutta equations (167):

$$Cb_{k+1} = Cb_k + \frac{h}{6}(f_1 + 2f_2 + 2f_3 + f_4)$$
$$Ci_{k+1} = Ci_k + \frac{h}{6}(g_1 + 2g_2 + 2g_3 + g_4)$$

Where:

h is an interval width in time

$$f_1 = f(t_k, Cb_k, Ci_k) \qquad g_1 = g(t_k, Cb_k, Ci_k)$$

$$f_2 = f\left(t_k + \frac{h}{2}, Cb_k + \frac{h}{2}f_1, Ci_k + \frac{h}{2}g_1\right) \qquad g_2 = g\left(t_k + \frac{h}{2}, Cb_k + \frac{h}{2}f_1, Ci_k + \frac{h}{2}g_1\right)$$

$$f_3 = f\left(t_k + \frac{h}{2}, Cb_k + \frac{h}{2}f_2, Ci_k + \frac{h}{2}g_2\right) \qquad g_3 = g\left(t_k + \frac{h}{2}, Cb_k + \frac{h}{2}f_2, Ci_k + \frac{h}{2}g_2\right)$$

$$f_4 = f(t_k + h, Cb_k + hf_3, Ci_k + hg_3) \qquad g_4 = g(t_k + h, Cb_k + hf_3, Ci_k + hg_3)$$

These equations were implemented using custom written software developed in the Visual Basic environment of Excel (Excel97, Microsoft Corporation). Briefly, linear interpolation was used to generate arrays of arterial concentration and blood flow versus time using a time interval width of 0.1 min. These, in combination with initial estimates of the remaining parameters (Vb, Vi, and *PS*) and 4th order Runge-Kutta formulas, were used to calculate the venous and tissue concentrations corresponding to the original measured time points. An additional parameter, V%, was employed to allow for the probability that only a fraction (assumed to be a constant ≤ 1) of the total forearm blood flow perfused the tissues from which venous blood was sampled. In all cases λ was considered to equal unity. The rate of tissue production (or disposal), $dQ_T \cdot dt^{-1}$, of each substance considered was calculated from the product of blood flow and the arterio-venous concentration difference recorded during an initial 10 min baseline period common to all experiments. This value was subsequently assumed to be a constant throughout the entire experiment. Similarly, values for Cb(0) were determined from baseline data. Based on published accounts where possible, initial values for tissue concentrations were considered:

$[N_2O]i(0) = 0\ \mu l \cdot ml^{-1}$

$[Lactate]i(0) = 0.389 \cdot [Lactate]v(0) + 1.9\ mmol \cdot l^{-1}$ (99)

$[CO_2]i(0) \doteq 33\ mmol \cdot l^{-1}$ corresponding to a pCO_2 of ~52 mmHg.

$[O_2]i(0) = 0.223\ mmol \cdot l^{-1}$ corresponding to a pO_2 of 2.5 mmHg and a muscle myoglobin concentration of 0.5 $mmol \cdot l^{-1}$ (89).

$[H^+]i(0) = 89\ nmol \cdot l^{-1}$ corresponding to a pH of 7.05 (50).

In one subject (Appendix, Subject C), there was a negative veno-arterial lactate difference at rest (Figure A.3C) indicating net lactate disposal. In this subject the initial tissue lactate concentration was assumed to be equal to that in venous blood.

Maximum likelihood estimates of the remaining model parameters (Vb, Vi, V% and *PS*) were obtained by chi-square (χ^2) fitting. Essentially, this involved summing the squared differences between the measured and calculated venous concentrations, each divided by the measurement standard deviation (uniformly set to 1 in these studies), over all of the measured time points. This is analogous to the residual sum of squares statistic, and is exactly equivalent when the standard deviation is assumed to equal unity. The unknown parameters were then adjusted in order to minimise χ^2. This was achieved using the numerical optimisation routines built into Excel (Excel97 Solver, Microsoft Corporation, USA), called from the routines programmed to calculate the venous and tissue concentrations. The initial blood and tissue volumes were set to 10% and 90% of the measured forearm volume and *PS* was initially set to unity. V% was fixed at 1 unless an initial fit produced a Vi exceeding the measured forearm volume, in which case V% was subsequently included as a fitting parameter.

N_2O data were fit to the two-compartment model first. The rationale for this was that, for an inert substance, the substance concentration in the venous drainage of a tissue is a product of blood-tissue exchange phenomena in the complete absence of tissue metabolic processes. With the assumption that a given model is appropriate, the various model parameters can subsequently be obtained by fitting the model to experimental data. Substance independent model parameters (Vb, Vi and V%) obtained in this manner were then considered constants, and χ^2 fitting employed to obtain estimates of the last parameter (*PS*), specific to each of the remaining substances (lactate, H^+, CO_2 and O_2). Theoretically, for CO_2 and O_2, this parameter could be estimated based on knowledge of the relative solubilities and therefore permeabilities in biological membranes of CO_2 and O_2 compared to N_2O. For lactate

this is not possible, and for N_2O, CO_2 and O_2 published values of solubility were found only for soap films (207) and water (Ostwald solubility: CO_2, 0.631; O_2, 0.0271; N_2O, 0.455 ml gas·ml water^{-1} at 37°C (268)), although these appear very similar to those reported for blood (268). In addition, bicarbonate appears to facilitate CO_2 diffusion such that knowledge of the permeability of CO_2 relative to other gases is probably not sufficient to adequately account for blood-tissue CO_2 exchange (109). Furthermore, the model's fitting ability is also dependent on the assumed value for the initial tissue concentration, and with the exception of N_2O this was clearly uncertain. Consequently the parameter *PS* was included as a fitted parameter. While lactate translocation across membranes is clearly more complex than by simple diffusion (84, 98, 132, 135, 153, 216-218, 266, 267), as with diffusion, it is still likely to be a near linear function of the transmembrane concentration gradient over the range of blood-tissue gradients predicted from these studies (≤ 4 mmol·l^{-1}). Consequently, while clearly simplistic, the fitting approach adopted in this analysis was considered reasonable.

2.4.2.3 Model Selection Criterion

The Model Selection Criterion (MSC), a modified Akaike Information Criterion (261) was used as a measure of goodness-of-fit for comparisons between model fits:

$$MSC = \ln\left(\frac{\sum_{i=1}^{n} w_i(Y_{obs_i} - \bar{Y}_{obs})^2}{\sum_{i=1}^{n} w_i(Y_{obs_i} - Y_{cal_i})^2}\right) - \frac{2p}{n}$$

Where n is the number of data points, w_i are weights applied to each point (assumed unity in the studies described in this thesis), p is the number of fitted parameters and Y_{obs_i}, $\bar{Y}_{obs}$, and Y_{cal_i} are the observed values, the weighted mean of the observed values, and the fitted values respectively. The MSC is suited to the comparison of competing models as it adjusts for the number of parameters, and is independent of the ordinate scale. A similar analysis was used recently by Doolette et al. (74) to evaluate tissue models of cerebral N_2O kinetics.

2.4.2.4 Respiratory blood gas considerations

For the respiratory gases (O_2 and CO_2), predictions of venous concentrations are considerably more difficult due to the nature of respiratory blood gas chemistry. As with any gas, blood-tissue partial pressure gradients determine the rates of gas exchange between the blood and extravascular compartments. However, haemoglobin and the bicarbonate reaction provide blood with the capacity to buffer changes in the plasma partial pressures of O_2 and CO_2. In a similar manner, muscle myoglobin provides tissues with the capacity to buffer changes in tissue pO_2. Consequently, total blood contents and blood and tissue partial pressures of O_2 and CO_2 must be considered simultaneously. For this purpose, blood contents of O_2 and CO_2 were determined from the measured values of pO_2, pCO_2, pH, tHb and sO_2 using the equations of Siggaard-Andersen et al. (229, 230). Subsequently, the rate of diffusional exchange of O_2 and CO_2 between the blood and tissue compartments was calculated using expressions relating partial pressures to blood and tissue contents. These expressions were derived principally from the equations of Siggaard-Andersen et al. (229, 230).

For all calculations, partial pressures were in kPa (1 kPa = 7.500638 mmHg) and tHb in $mmol \cdot l^{-1}$ (1 $mmol \cdot l^{-1}$ = 1.61140 $g \cdot dl^{-1}$) as required by the equations of Siggaard-Andersen et al. (229, 230). Otherwise all partial pressures are expressed in mmHg.

2.4.2.4a Calculation of CO_2 content, and pCO_2 from CO_2 content

Total blood CO_2 (tCO_2b) is the sum of dissolved CO_2, bicarbonate (HCO_3^-), and CO_2 contained within erythrocytes (229):

$$HCO_3^- = 0.23 \cdot pCO_2 \cdot 10^{(pH - pKp)}$$

Where:

$$pKp = 6.125 - \log(1 + 10^{(pH-8.7)})$$

$$tCO_2p = 0.23 \cdot pCO_2 + HCO_3^-$$

$$tCO_2b = 0.23 \cdot pCO_2 + tHb(1 + 10^{(pH_{Ery} - pK_{Ery})}) + tCO_2p\left(1 - \frac{tHb}{21}\right)$$

Where:

$$pH_{Ery} = 7.19 + 0.77(pH - 7.4) + 0.035(1 - sO_2)$$

$$pK_{Ery} = 6.125 - \log(1 + 10^{(pH_{Ery} - 7.84 - 0.06 \cdot sO_2)})$$

$$tHb = \text{Total haemoglobin concentration}$$

Combining equations:

$$tCO_2b = 0.23 \cdot pCO_2 \cdot tHb(1 + 10^{(pH_{Ery} - pK_{Ery})}) + 0.23 \cdot pCO_2(1 + 10^{(pH - pKp)})\left(1 - \frac{tHb}{21}\right)$$

$$tCO_2b = 0.23 \cdot pCO_2\left(tHb(1 + 10^{(pH_{Ery} - pK_{Ery})}) + (1 + 10^{(pH - pKp)})\left(1 - \frac{tHb}{21}\right)\right) \quad (2.16)$$

By rearrangement, an expression to calculate the blood compartment pCO_2 (pCO_2b) from blood CO_2 content was derived:

$$pCO_2b = \frac{tCO_2b}{0.23\left(tHb(1 + 10^{(pH_{Ery} - pK_{Ery})}) + (1 + 10^{(pH - pKp)})\left(1 - \frac{tHb}{21}\right)\right)} \quad (2.17)$$

Total tissue CO_2 (tCO_2i) was considered to equal the sum of dissolved CO_2 and bicarbonate:

$$tCO_2i = 0.23 \cdot pCO_2 + 0.23 \cdot pCO_2 \cdot 10^{(pH - pKp)} \quad (2.18)$$

By rearrangement, an expression to calculate tissue pCO_2 (pCO_2i) from tissue CO_2 content was derived:

$$pCO_2i = \frac{tCO_2i}{0.23(1 + 10^{(pH - pKp)})} \quad (2.19)$$

2.4.2.4b ***Calculation of O_2 content, and pO_2 from O_2 content***

The total oxygen content of the blood (tO_2b) is equal to the sum of dissolved O_2 and O_2 bound to haemoglobin within the blood (229):

$$tO_2b = 0.00983 \cdot pO_2 + tHb \cdot (1 - MetHb - COHb) \cdot sO_2 \quad (2.20)$$

sO_2 is a function of pO_2, such that equation 2.20 cannot simply be re-arranged to solve for pO_2. Consequently, numerical methods are necessary to calculate pO_2 from tO_2 and for this purpose the mathematical model of the haemoglobin-oxygen dissociation curve (ODC) described by Siggaard-Andersen et al. (229, 230) was used. In this model a function, incorporating a series of equations, is used to represent the ODC curve:

$$sO_2 = ODC(pO_2, pH, pCO_2, C_{dpg}, COHb, MetHb, Hbf, T)$$

Where: C_{dpg} = The concentration of 2,3-diphosphoglycerate in the erythrocytes ($mmol \cdot l^{-1}$).

COHb = The carboxyhaemoglobin fraction (this was assumed to equal 0.005 (229)).

MetHb = The hemiglobin fraction (this was assumed to equal 0.005 (229)).

Hbf = The fetal haemoglobin fraction (this was assumed to be zero (229)).

T = Blood temperature (this was assumed to equal 37 °C).

$$sO_2 = \frac{e^y}{1 + e^y} \tag{2.21}$$

$$y = 1.875 + x - x_0 + h \cdot T_h \tag{2.22}$$

$$T_h = \tanh[0.5343 \cdot (x - x_0)]$$

$$h = 3.5 + a$$

$$x_0 = 1.946 + a + b$$

$$a = -0.72 \cdot (pH - 7.4) + 0.09 \cdot \ln\left(\frac{pCO_2}{5.33}\right) + (0.07 - 0.03 \cdot Hbf) \cdot (C_{dpg} - 5) - 0.368 \cdot COHb - 0.174 \cdot MetHb - 0.28 \cdot Hbf$$

$$b = 0.055 \cdot (T - 37)$$

$$x = \ln(pO_2) \tag{2.23}$$

$$n = 1 + 0.5343 \cdot h \cdot (1 - T_h^2)$$

C_{dpg} was estimated from measured arterial values of sO_2 and pO_2 using a Newton-Raphson iterative procedure:

$$C_{dpg\,k+1} = C_{dpg\,k} + \left[\ln\left(\frac{sO_2}{1-sO_2}\right) - y\right] \cdot \left(\frac{1}{0.07 - 0.03 \cdot Hbf}\right) \cdot \left(\frac{1}{T_h - n}\right) \tag{2.24}$$

Where:

k = The iteration number. Two iterations were used, starting with an initial value for C_{dpg} of 5 $mmol \cdot l^{-1}$ (229).

sO_2 = The measured value for arterial sO_2.

y, T_h and n = Values calculated from the ODC function using the measured value of arterial pO_2.

The pO_2 of blood within the blood compartment (pO_2b) was subsequently calculated from tO_2b using the procedures described by Siggaard-Andersen et al. (230):

$$pO_2b_{k+1} = pO_2b_k + \frac{tO_2b - tO_2b_k}{0.00983 + tHb \cdot n_k\left(\frac{1 - sO_{2k}}{pO_{2k}}\right)} \qquad (2.25)$$

Where: k = The iteration number.

sO_{2k} was calculated from the ODC function, using an initial value (k = 0) for pO_2b_k of 3 kPa. First the parameters of the ODC were calculated from pH, pCO_2 and the value of C_{dpg} calculated from the measured arterial values of pO_2 and sO_2 using equation 2.24. x was then calculated from pO_{2k} using equation 2.23, y from x using equation 2.22, and sO_{2k} from y using equation 2.21. n_k was calculated from the ODC function and tO_{2k} using equation 2.20. Iteration was continued until the absolute difference between tO_2b and tO_2b_k was less than 0.001 $mmol \cdot l^{-1}$, at which point the estimate of pO_2b was obtained.

For expressions yielding partial pressures from content (essentially equations 2.17, 2.19, 2.25 and 2.27), in which other parameters such as blood or tissue pH or pCO_2 are necessary, the relevant arterial values were used. While this is clearly a simplification, predictions of venous contents based even partially on measured venous parameters were not considered justified. An alternative would be to simultaneously model the blood-tissue exchanges of O_2, CO_2 and H^+, and to use predicted values of venous pH and pCO_2 where necessary. However, this would be considerably more complex and was not attempted in this analysis.

The total oxygen content of the tissues (tO_2i) is equal to the sum of dissolved O_2 and O_2 bound to myoglobin (Mb) within the tissues. Unlike haemoglobin, myoglobin is a monomer and therefore exhibits no cooperativity for O_2 binding. In addition, the Mb

dissociation curve is unaffected by pH or pCO_2 (53, 89). Consequently, myoglobin O_2 saturation is well characterised by the classic Hill equation (225):

$$tO_2i = 0.00983 \cdot pO_2 + tMb \cdot \left(\frac{pO_2i}{pO_2i + MbP_{50}} \right) \quad (2.26)$$

Where: pO_2i = Tissue partial pressure of O_2.

tMb = Total myoglobin concentration. This was assumed to equal 0.5 $mmol \cdot l^{-1}$ (89).

MbP_{50} = The tissue pO_2 at which myoglobin is 50% saturated with O_2. This was assumed to equal 0.333 kPa (2.5 mmHg at 37 °C), based on reported values for human myoglobin determined at 35 °C (2.09 mmHg) and 40 °C (3.02 mmHg) (8).

As with blood O_2 content, equation 2.26 cannot easily be rearranged to solve pO_2i from tO_2i. Consequently, an iterative procedure, analogous to that described by Siggaard-Andersen et al. (230) for blood, was used for this purpose:

$$pO_2i_{k+1} = pO_2i_k + \frac{tO_2i - tO_2i_k}{0.00983 + tMb \cdot \left(\frac{1 - sO_{2k}}{pO_2i_k} \right)} \quad (2.27)$$

Where: k = The iteration number

$$sO_{2k} = \frac{pO_2i_k}{pO_2i_k + MbP_{50}}$$

Iteration was continued until the absolute difference between tO_2i and tO_2i_k was less than 0.001 $mmol \cdot l^{-1}$, at which point the estimate of pO_2i was obtained.

2.4.3 Substance fluxes across the resting forearm

Fluxes of lactate, H^+, CO_2, O_2 and N_2O across the resting forearm were calculated from the product of forearm blood flow and the respective veno-arterial concentration differences. Consequently, negative and positive values of flux indicate forearm uptake and output respectively. The terms flux, uptake and output are specifically used to indicate blood-tissue movements without reference to the fate of the substance within the tissues.

Measured and predicted venous concentrations were used to calculate measured and predicted values for flux, and the difference, adjusted for baseline flux:

$$\text{"Corrected" Flux} = \text{Measured Flux} - \text{Predicted Flux} + \text{Baseline Flux} \qquad (2.28)$$

used as an index of net production (positive values) or net disposal (negative values) of the substance in the forearm. It should be noted that estimates of disposal or production derived in this manner are subject to the limitations of the modelling analysis. However, it should also be borne in mind that failure to adequately account for the passive movements of a substance inevitably produce inflated estimates of tissue metabolism.

2.4.4 Total net uptake or output during exercise and recovery

The total flux of a given substance across the forearm during exercise and during the subsequent recovery was calculated from the total net area under the flux versus time curve. In this manner the total quantity of the substance taken up (or released) during exercise was compared to the total quantity returned to, or taken up from, the circulation during recovery. If a steady-state with respect to the arterial, venous and tissue concentrations of the substance is reached prior to the end of the recovery period, the difference between total net uptake and total net release can be considered to reflect tissue metabolism alone.

2.5 Statistical analyses

Time series measurements were analysed using analysis of variance (ANOVA) for repeated measures. The Greenhouse-Geisser estimate of Box's epsilon (ε) was used to adjust ANOVA degrees of freedom for the inflated risk of type I errors associated with the common failure of time series measures to meet the multisample sphericity assumptions of ANOVA (164). Basically, multisample sphericity, also known as circularity or compound symmetry, is the property of outcome measures to exhibit the same variance at each repetition and the same degree of correlation between all repetitions. This assumption is rarely fulfilled with time series measurements in which it is more common for the variance to increase along with the outcome measure and for neighbouring repetitions to be more highly correlated than repetitions spaced further apart. ANOVA main effects and interactions were considered the principle tests of a given hypothesis. In some cases in which significant ANOVA effects were identified, selected pairwise comparisons were made using Tukey's post-hoc tests, although in general these were avoided because they are insensitive, often counter-intuitive and do not test the principle hypothesis (164).

Two sample contrasts in normally distributed data were performed using Student's paired *t*-tests. Equivalent contrasts in non-normally distributed data were performed using Wilcoxon tests. In some cases, multiple hypotheses were tested using paired tests. When this was the case the Dunn-Sidak procedure was used to adjust for the inflated Type I error rate (164):

$$p' = 1 - (1 - p)^k$$

In which p' and p are the corrected and uncorrected p-values and k is the number of pairwise contrasts performed.

Most of the statistical analyses were performed using SPSS for Windows (Release 6.1.3, SPSS Inc. Chicago, IL), except for pairwise contrasts, linear regression

analyses and goodness-of-fit calculations which were performed using Excel (Excel4 - Excel97, Microsoft Corporation, USA).

In all statistical tests, the level of significance was $P < 0.05$, unless otherwise indicated. All group data are reported as means ± SEM.

CHAPTER 3 Resting forearm lactate kinetics during moderate-intensity supine leg exercise

3.1 Introduction

SEVERAL STUDIES employing lactate tracer techniques and measurements of veno-arterial lactate differences across specific tissue beds have suggested that resting skeletal muscle plays an important role in the removal of lactate from the blood during exercise (4, 5, 46, 87, 88, 95, 97, 161, 203, 247). While a negative veno-arterial difference is generally equated with net metabolic disposal of lactate by the muscles, this is only true when both arterial and venous lactate levels are stable. When lactate levels are changing, such as during exercise, concentration differences between blood and tissues can induce passive lactate movements and create both negative and positive veno-arterial differences, without implying metabolic disposal or production. With regard to lactate removal by a tissue, the veno-arterial difference due only to the passive uptake of lactate can be predicted if the physical characteristics of the tissue can be appropriately modelled. The simplest approach to achieve this is by using an integrated form of the Fick equation, more conventionally applied to the study of inert gas kinetics and the measurement of regional blood flow (149). In this approach it must be assumed that tissue lactate metabolism (net production or disposal) is constant, such that lactate is effectively treated as an inert diffusible tracer. In this manner, veno-arterial lactate differences reflecting passive lactate movements alone without forearm lactate disposal are predicted. It follows that the difference between the measured and predicted veno-arterial lactate difference provides an index of the net metabolic disposal (or production) of lactate by the muscle during non-steady-state arterial lactate conditions.

The purpose of the experiments reported in this Chapter was to re-examine the role of resting forearm skeletal muscle in the removal of an arterial lactate load generated by moderate-intensity supine leg exercise, taking into consideration the passive exchange of lactate between tissues and blood when the arterial lactate levels are changing with time. The terms uptake (removal from the blood) and output (release into the blood) are used to indicate negative and positive veno-arterial differences in blood lactate, without reference to its fate within the tissues. Lactate flux refers to the product of veno-arterial difference and forearm blood flow. The terms wash-in and washout are used to describe passive lactate movements associated with equilibration between the tissues and the blood while the terms net disposal and net production are used specifically to describe the net effects of muscle lactate production and metabolic disposal.

3.2 Methods

3.2.1 General

The subjects were 5 right-handed male volunteers. Each subject attended the laboratory on 2 separate occasions one week apart. On the first visit, the subject's peak oxygen uptake ($\dot{V}O_{2\,peak}$) and lactate threshold (LT) were determined. Following insertion of a catheter into the antecubital vein of the left arm, subjects completed an incremental exercise test to exhaustion on a supine ergometer (Jacquet, Switzerland). The initial work load was 100 watts followed by 25 watt increments every 2 min until exhaustion, which usually occurred within 10-14 min. In the last 15 sec of each 2 min interval blood samples (1 ml) were taken and subsequently analysed to determine blood lactate concentration. The exercise intensity (watts and $\%\dot{V}O_{2\,peak}$) necessary to elevate blood lactate (LT) was determined from log-log plots of blood lactate concentration, work load, and $\dot{V}O_2$ (22). In all subjects there was a clear inflection point in blood lactate at a given work load, and a work load 25 watts above this was subsequently chosen for the main experimental exercise protocol.

On the second laboratory visit subjects performed the main experiment. Following measurements of forearm volume and circumference, catheters were inserted into the left radial artery and the antecubital veins of both forearms. Each subject then completed 20 min of supine ergometer exercise at the work load established from the first laboratory visit (67.0 ± 3.5% $\dot{V}O_{2\,peak}$). Simultaneous samples of arterial and forearm venous blood were taken at rest (protocol min 2.5 and 10), during exercise (protocol min 12.5, 15, 17.5, 20, 22.5, 25, 27.5 and 30) and during recovery (protocol min 32.5, 35, 37.5, 40, 45, 50, 55 and 60) for analysis of blood gases and lactate concentration. Throughout the experiment the left forearm was maintained at rest, while the right forearm was used to perform 20 min of light (15% maximal voluntary contraction) static handgrip exercise coincident with leg exercise. Data related to the exercising forearm will not be considered in this study specific to resting muscles, but have been reported previously (44). Forearm blood flow, arterial blood pressure,

heart rate, systemic oxygen uptake ($\dot{V}O_2$) and carbon dioxide output ($\dot{V}CO_2$) were measured throughout the experiment.

In these experiments, the hand circulation was not excluded during forearm flow measurements because of the radial artery catheter at the left wrist, and the inclusion of light forearm exercise in the right arm. Consequently, forearm blood flow is expressed as ml blood per 100 ml arm volume (forearm and hand) below the venous occlusion cuff per minute. Given the cool and unchanging room temperature (21 ± 1 °C) the contribution of hand flow responses (principally a skin vascular bed) to total flow at rest is likely to be small (59, 275).

3.2.2 Calculations

The method employed to calculate the disposal (metabolism) or production of lactate by the forearm is based on the principles that govern the exchange of a diffusible tracer substance between the blood and the tissues, additional details of which are described in Chapter 2 (pp. 69). Briefly, Kety (149), using the Fick principle, derived the following relationship between the concentration of a tracer in the arterial blood and that in the venous drainage representative of the tissues:

$$Cv(t) = ke^{-kt}\int_0^t e^{kt}\, Ca(t)\, dt \qquad (2.10)$$

$$\text{in which: } k = \frac{m\, F(t)}{\lambda Vi}$$

where t is a given time after the onset of circulation of the tracer substance, Cv(t) is the concentration of tracer in the venous blood draining the tissues at time t, Ca(t) is the concentration of tracer in the arterial blood at time t, λ is the partition coefficient (ratio of the solubility of the tracer in tissue and in the blood, assumed to equal unity), Vi is the tissue volume, F(t) is the rate of blood flow through the tissues at time t, and m is essentially a diffusion term effectively equal to $100^2 \cdot Vi^{-1}$ in this study. Lactate

levels in the exercise and recovery phases were considered separately and the baseline adjusted such that the arterial lactate concentration at the start of each phase was zero. The individual baseline adjusted exercise and recovery arterial lactate curves were fitted by least squares to an exponential decay function of the form:

$$Ca(t) = Ae^{-Bt} + C \qquad (2.12)$$

in which A, B and C are constants that optimise the fit. Substituting equation 2.12 into equation 2.10 and integrating over the limits of t to 0 provided the following relationship to predict the change in venous lactate from baseline, Cv(t), at a given time, t, for both the exercise and recovery phases:

$$Cv(t) = \frac{Ake^{-kt}}{k-B}[e^{(k-B)t} - 1] - Ce^{-kt} + C + D \qquad (2.13)$$

D is a baseline correction that adjusts for output (or uptake) at time zero, and is the difference between the venous and arterial concentrations at time zero. The product of the measured veno-arterial lactate concentration difference and blood flow was used to determine forearm lactate uptake (negative values) or release (positive values). Similarly, the predicted uptake or release of lactate due to passive movements was calculated from the product of the predicted veno-arterial lactate concentration difference and blood flow. This analysis was also applied to determine forearm fluxes of H^+, CO_2 and O_2. To ascertain the likely contribution of forearm metabolism alone to veno-arterial differences, "corrected" fluxes were determined from equation 2.28. Several assumptions inherent to this analysis are detailed in Chapter 2 (pp. 67).

In addition to the modelling approach, the total net quantities of lactate, H^+, CO_2 and O_2 taken up (or released) throughout exercise and recovery were determined from

the net area under the time versus the respective flux curves. These values were compared to the total net flux that would have been measured had the resting levels of flux continued throughout exercise, recovery and exercise and recovery:

$$\text{Expected Total Net Flux} = \text{Baseline Flux (units·minute}^{-1}) \cdot \text{Duration (min)}$$

3.2.3 Statistical Analysis

Comparisons between arterial and measured venous concentrations, between measured and predicted venous concentrations, and between measured and predicted veno-arterial concentration differences and flux were made using two-way analysis of variance (ANOVA) for repeated measures. Time effects for a given parameter were examined using one-way ANOVA for repeated measures. Differences between resting forearm net uptake during leg exercise and net output during recovery were examined using Student's paired *t*-tests. The level of significance used throughout was $P < 0.05$. All data are reported as means ± SEM.

3.3 Results

3.3.1 General

The physical characteristics of the subjects are reported in Table 3.1. During the main experiment, subjects exercised at 180.0 ± 9.4 (range 150-200) watts.

Table 3.1. Subject characteristics.

Age (years)	Mass (kg)	Height (cm)	$\dot{V}O_{2\ peak}$ ($ml \cdot kg \cdot min^{-1}$)	LT ($\%\dot{V}O_{2\ peak}$)	Left forearm (+hand) volume (ml)
20.2 ± 0.7	72.9 ± 2.8	184.1 ± 2.1	59.3 ± 3.7	50.7 ± 3.7	1724.0 ± 78.9

Values are means ± SEM, n = 5.

3.3.2 Cardiorespiratory variables

From a resting value of 82.1 ± 3.3 mmHg, MAP increased immediately with the onset of exercise and achieved a stable value within 2.5 min. This was maintained for the duration of exercise (mean value, 113.3 ± 4.0 mmHg), and returned to resting values within 2.5 min of exercise termination. HR increased progressively throughout the 20 min exercise period from a resting value of 61.5 ± 2.9 $beats \cdot min^{-1}$ to reach a peak value of 174.6 ± 6.6 $beats \cdot min^{-1}$ at the end of exercise before returning to resting values within 10 min. The mean HR during the last 10 min of exercise was 160.3 ± 5.2 $beats \cdot min^{-1}$ (84.6 ± 1.5 $\%HR_{peak}$). Both $\dot{V}O_2$ and $\dot{V}CO_2$ increased immediately with the onset of exercise and showed a non-significant upward trend before returning to a stable value within 2.5 min after exercise. The mean $\dot{V}O_2$ during the exercise period was 39.3 ± 1.3 $ml \cdot kg^{-1} \cdot min^{-1}$ (67.0 ± 3.5 $\%\dot{V}O_{2\ peak}$).

Resting forearm blood flow initially decreased with the onset of cycle ergometer exercise, but within 10 min began to increase and continued to do so progressively

for the remainder of the exercise period. There was a gradual decline in forearm blood flow post-exercise, with pre-exercise levels being reached only by the end of the 30 min recovery period (Figure 3.1).

3.3.3 Model fits

The Model Selection Criterion and degree of correlation between measured and predicted venous concentrations of lactate, H^+, CO_2 and O_2 are presented in Table 3.2. The modified Fick equation was of reasonable predictive value for lactate and CO_2, but was of little value for H^+ and O_2.

Table 3.2. Model Selection Criterion (MSC) and coefficient of determination (r^2) for measured compared to model predicted values of venous lactate, H^+, CO_2 and O_2.

Substance	MSC	r^2
Lactate	1.51 ± 0.31	0.828 ± 0.038
H^+	0.50 ± 0.44	0.752 ± 0.067
CO_2	1.43 ± 0.42	0.813 ± 0.084
O_2	-0.34 ± 0.17	0.509 ± 0.150

Values are means ± SEM, n = 5.

3.3.4 Blood lactate

Arterial blood lactate increased with the onset of cycle ergometer exercise reaching stable values by 10 min of exercise which were ~11-fold higher compared with rest (Figure 3.2A). Forearm venous blood lactate increased more slowly during leg exercise such that there was a clear veno-arterial difference at the early sampling time points (arterial versus venous concentration by time interaction, P = 0.048, Figure 3.2A). For the final 12.5 min of exercise and throughout the recovery period the arterial and venous levels were not significantly different, although both measured and predicted venous levels tended to be higher than arterial throughout recovery. There was no significant difference (P = 0.998) and no interaction with time (P = 0.739) between the measured venous lactate concentration and that

predicted using the modified Kety (149) formula (equation 2.13). In addition, the measured and predicted values were highly correlated throughout the entire experimental period ($P < 0.001$, Table 3.2), indicating that changes in resting forearm veno-arterial lactate concentration differences throughout the protocol could be explained largely on the basis of passive equilibration with the extravascular compartment of the forearm. There was also no significant difference between measured and predicted veno-arterial differences in lactate concentration ($P = 0.998$, Figure 3.2B) or lactate flux ($P = 0.624$, Figure 3.2C). Although measured lactate flux did not change significantly with time ($P = 0.375$), when "corrected" for passive lactate movements lactate flux tended towards baseline levels of flux at most time points, and also did not change with time ($P = 0.484$, Figure 3.2C).

3.3.5 Blood hydrogen ions

The arterial H^+ concentration increased rapidly during exercise to reach values approximately 40% higher than at rest by the end of exercise (Figure 3.3A). A large positive veno-arterial H^+ concentration difference at rest was substantially reduced throughout exercise and recovery such that overall there was no significant difference between arterial and measured venous values ($P = 0.130$, Figure 3.3A). Although the modified Fick equation produced relatively poor fits of venous H^+ concentrations (Table 3.2), there were no overall differences between measured and predicted venous concentrations or veno-arterial concentration differences ($P = 0.267$, Figure 3.3A and B) or between measured and predicted forearm H^+ flux ($P = 0.165$, Figure 3.3C). However, all of these parameters were quite variable. Nonetheless, the time course of H^+ concentration changes was such that during the first 10 min of exercise there was a transient reduction in both measured and predicted forearm H^+ flux, followed by a rise in the second half of exercise and a gradual return towards baseline during recovery (Figure 3.3C).

3.3.6 Resting forearm respiratory gas exchange

3.3.6.1 CO_2 exchange

With the onset of leg exercise arterial carbon dioxide content decreased rapidly, reaching values ~60% of those at rest by the end of exercise, before returning towards baseline during recovery (Figure 3.4A). A significant difference between arterial and venous CO_2 content was maintained throughout the experiment ($P = 0.013$), although there was no interaction between this difference and changes in CO_2 content associated with time ($P = 0.194$). There was no difference between measured CO_2 content and that predicted from the modified Fick equation (Figure 3.4A), and no difference between the measured and predicted veno-arterial CO_2 difference (main effect $P = 0.320$, interaction with time $P = 0.129$, Figure 3.4B). Similarly, there was no difference between measured and predicted forearm $\dot{V}CO_2$ (main effect $P = 0.385$, interaction with time $P = 0.139$, Figure 3.4C), despite significant changes with time in all of these measures ($P < 0.05$). Both measured and predicted forearm CO_2 flux increased significantly during exercise ($P = 0.044$ and $P = 0.015$ respectively). However, when "corrected" for passive CO_2 movements, forearm $\dot{V}CO_2$ approached baseline levels and did not significantly increase during exercise ($P = 0.139$, Figure 3.4C).

3.3.6.2 O_2 exchange

Arterial O_2 content increased significantly during leg exercise ($P = 0.009$) but these changes were relatively small (at most ~15% higher) compared to baseline (Figure 3.5A). A substantial veno-arterial O_2 content difference was maintained throughout the experiment ($P = 0.004$, Figure 3.5B). During the first 10 min of exercise there was a small drop in venous blood O_2 content (Figure 3.5A) and an increase in forearm O_2 extraction (Figure 3.5B) coincident with a transient decline in forearm blood flow. However, with continued exercise, and during early recovery, this was replaced by a rise in venous O_2 content (Figure 3.5A) and consequently a reduction

in veno-arterial O_2 content difference (Figure 3.5B), although none of these effects reached statistical significance ($P > 0.063$). There were no significant differences between measured and predicted venous O_2 contents or veno-arterial content differences ($P = 0.729$) or between measured and predicted forearm O_2 flux ($P = 0.282$, Figure 3.5C). Although forearm $\dot{V}O_2$ appeared to increase during exercise, this effect was small and not significant and was reversed when O_2 flux was "corrected" for passive O_2 movements (Figure 3.5C). However, venous O_2 content, and therefore the veno-arterial O_2 difference and O_2 flux, were relatively poorly predicted on the basis of passive O_2 movements (Table 3.2, Figure 3.5).

3.3.7 Total net flux of lactate, H^+, CO_2 and O_2 in the resting forearm.

Prior to leg exercise the resting forearm exhibited a small non-significant net production of lactate, a significant net production of H^+ and CO_2, and net consumption of O_2 (Table 3.3). During exercise, recovery and combined exercise and recovery the measured total fluxes of all substances were similar to those predicted on the basis of passive movements, although all of these measures were highly variable. With the exception of O_2, there were no significant differences between measured values of total net flux and those expected on the basis of the measured baseline flux and time. Consequently, the quantities of lactate, H^+ and CO_2 unaccounted for (measured minus expected total net flux) were not significantly different from zero after 30 min of recovery. Although a significant quantity of O_2 was unaccounted for, the total quantity of O_2 taken up was less than expected. This would be consistent with a reduction in $\dot{V}O_2$ or an inflated estimate of baseline $\dot{V}O_2$, the latter perhaps more likely on the basis of a calculated steady-state resting forearm gas exchange ratio of 0.59 ± 0.06. Nonetheless, these data suggest it unlikely that there was an overall increase in resting forearm $\dot{V}O_2$ associated with leg exercise.

There was a significant correlation between total net flux measured during exercise and that measured during recovery for CO_2 and O_2 only, although the degree of association in each parameter was similar in measured compared to predicted values (Table 3.3).

Table 3.3. Total net flux of lactate, H^+, CO_2 and O_2 across the resting forearm during rest, supine leg exercise and recovery.

	Lactate (μmol·100 ml^{-1})	H^+ (pmol·100 ml^{-1})	CO_2 (μmol·100 ml^{-1})	O_2 (μmol·100 ml^{-1})
Rest	1.4 ± 0.9	129.8 ± 45.9 †	40.9 ± 6.5 †	-71.9 ± 12.5 †
Rest *(/min)*	0.3 ± 0.2	26.0 ± 9.2 †	8.2 ± 1.3 †	-14.4 ± 2.5 †
Exercise (20 min)				
Measured	-81.7 ± 66.1	417.7 ± 91.9 †	402.4 ± 66.8 †§	-374.4 ± 72.0 †
Predicted	-79.5 ± 18.9 †§	697.0 ± 356.6	438.1 ± 60.8 †§	-518.9 ± 118.4 †§
"Corrected"	-2.5 ± 63.6 ‡	24.7 ± 188.2	46.0 ± 50.4 ‡	-13.0 ± 94.8 ‡
Expected	5.6 ± 3.7	519.2 ± 183.5	163.8 ± 26.1 ‡	-287.5 ± 50.0
Recovery (30 min)				
Measured	43.3 ± 66.7	699.2 ± 233.4 †	517.9 ± 147.7 †§	-492.3 ± 110.0 †§
r^2 (vs Exercise)	0.022	0.500	0.800 *	0.849 *
Predicted	69.9 ± 14.3 †§	1296.3 ± 430.2 †‡	277.4 ± 68.9 †§	-615.7 ± 117.3 †§
r^2 (vs Exercise)	0.029	0.823 *	0.993 *	0.926 *
"Corrected"	-27.1 ± 66.6 ‡	-141.2 ± 60.4 ‡§	362.9 ± 128.1 ‡§	-112.9 ± 122.6 ‡§
Expected	33.8 ± 22.4	3115.2 ± 1101.2	982.8 ± 156.5 ‡	-1724.8 ± 300.2 ‡
Exercise & Recovery (140 min)				
Measured	-38.3 ± 100.6	1116.9 ± 305.4 †	920.3 ± 209.6 †	-866.7 ± 178.5 †§
Predicted	-9.6 ± 21.7 §	1993.3 ± 768.4 §	715.5 ± 129.5 †§	-1134.6 ± 233.5 †§
"Corrected"	-29.6 ± 91.3	-116.5 ± 207.8	408.9 ± 170.2 ‡§	-126.0 ± 216.2 ‡§
Expected	39.4 ± 26.1	3634.4 ± 1284.7 †	1146.6 ± 182.6 †	-2012.3 ± 350.2 †‡
Unaccounted	-77.8 ± 90.3	-2517.5 ± 989.7	-226.3 ± 166.6	1145.5 ± 305.4 †

Positive values indicate net output, and negative values net uptake. Measured, predicted and "corrected" values were calculated as the net area under the time versus respective flux curves. Expected values were calculated by multiplying the resting flux (/min) by the corresponding duration of exercise, recovery or both. Unaccounted values were calculated as the difference between measured and expected values. * indicates a significant degree of association, † a difference from a value of zero, ‡ from measured and § from expected ($P < 0.05$). All values are means ± SEM, n = 5.

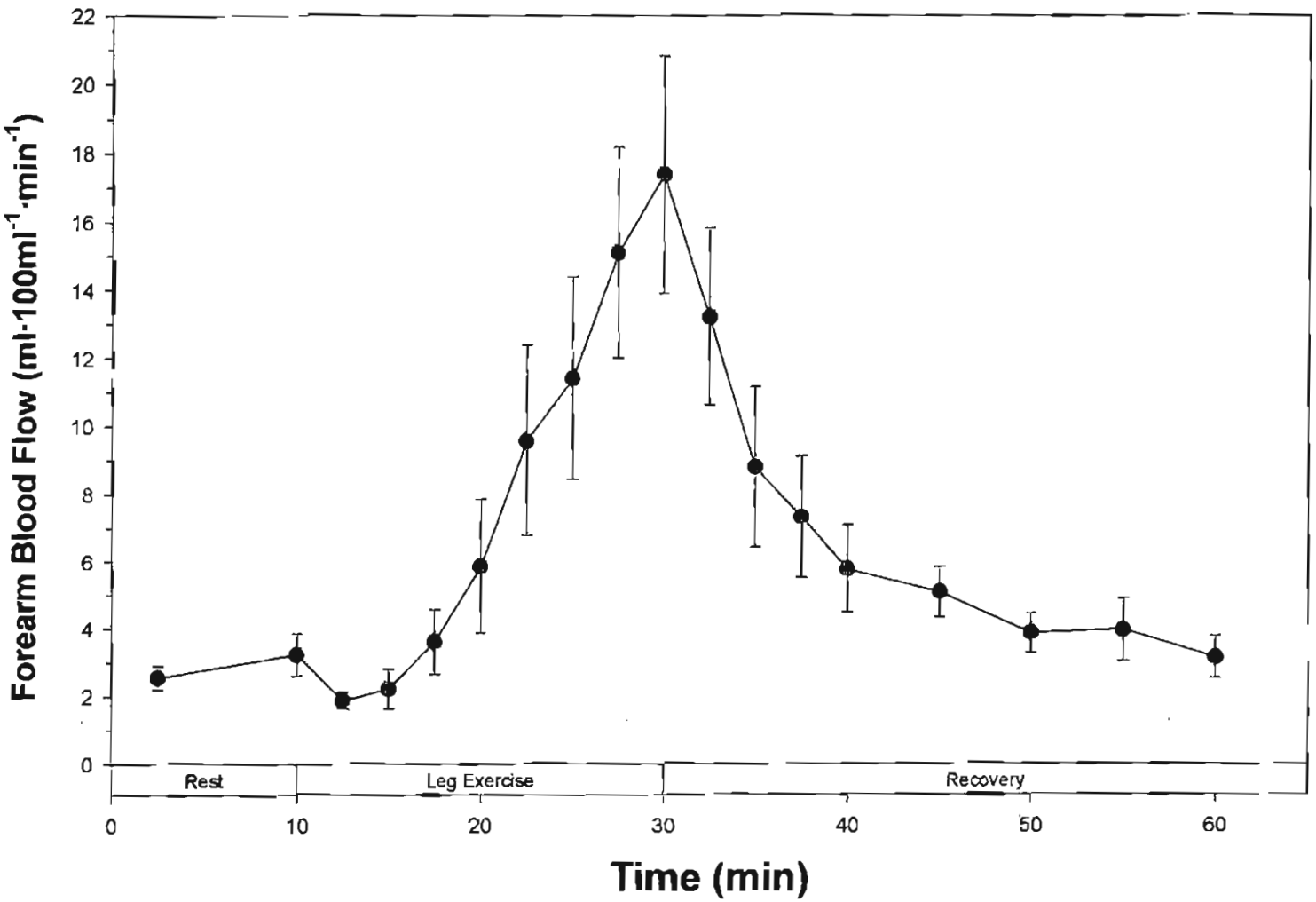


Figure 3.1. Resting forearm blood flow before during and after supine leg exercise (20 min at ~65% $\dot{V}O_{2\,peak}$). Values are means ± SEM, n = 5.

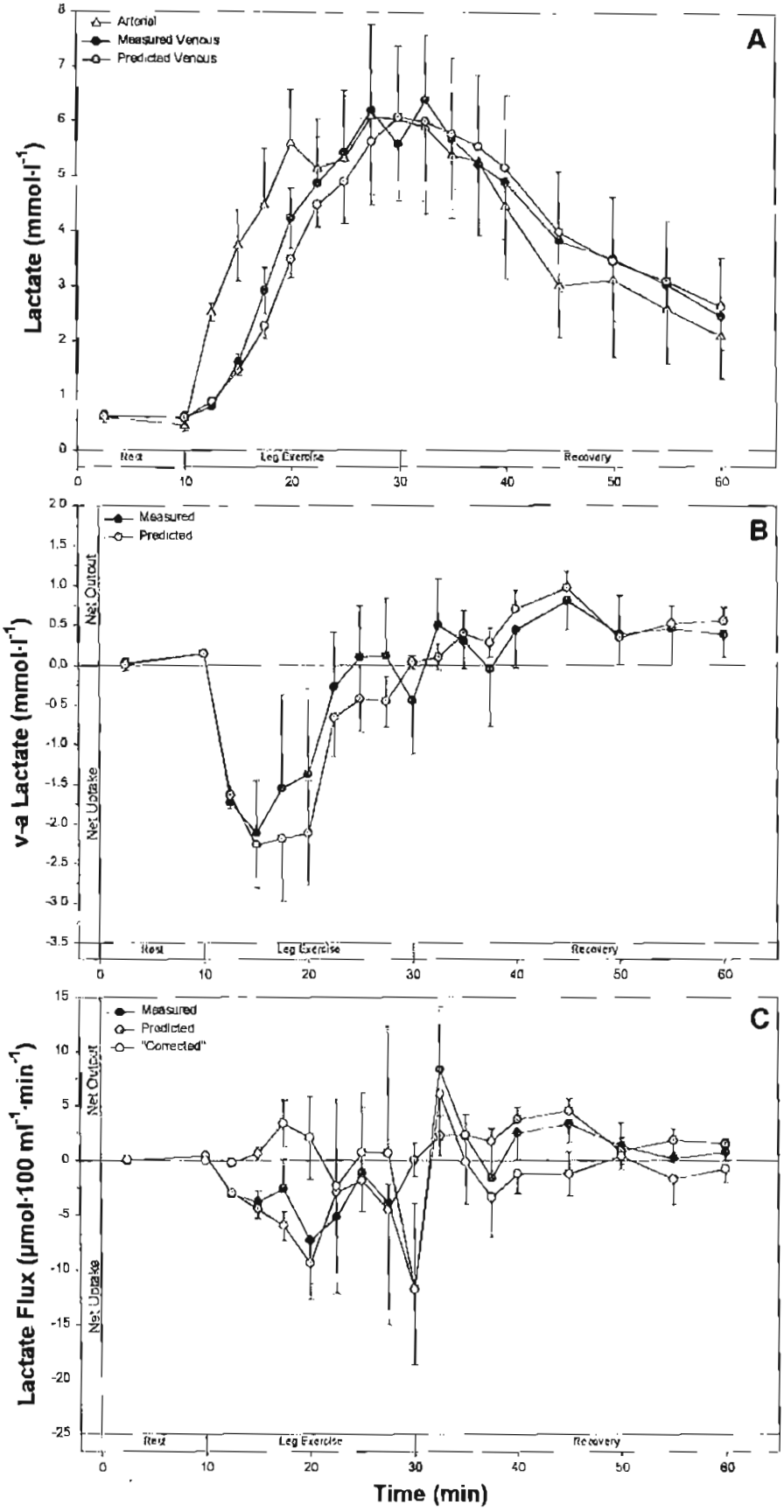


Figure 3.2. Blood lactate concentrations (A), veno-arterial concentration differences (B) and flux (C) across the resting forearm before, during and after supine leg exercise. "Corrected" = Measured – Predicted + Baseline. Values are means ± SEM, n = 5.

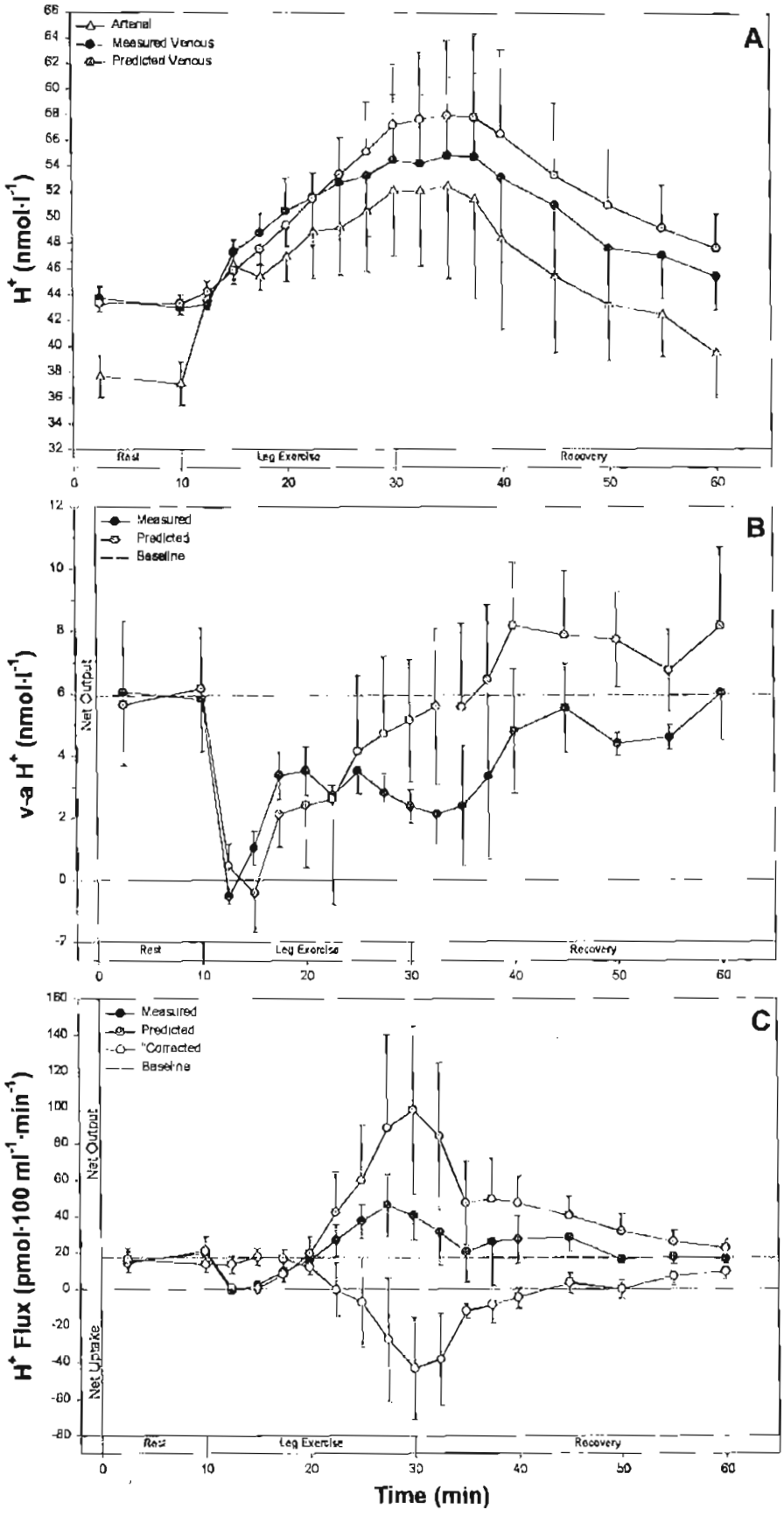


Figure 3.3. Blood H^+ concentrations (A), veno-arterial H^+ concentration difference (B) and H^+ flux (C) across the resting forearm before, during and after supine leg exercise. "Corrected" = Measured – Predicted + Baseline. Values are means ± SEM, n = 5.

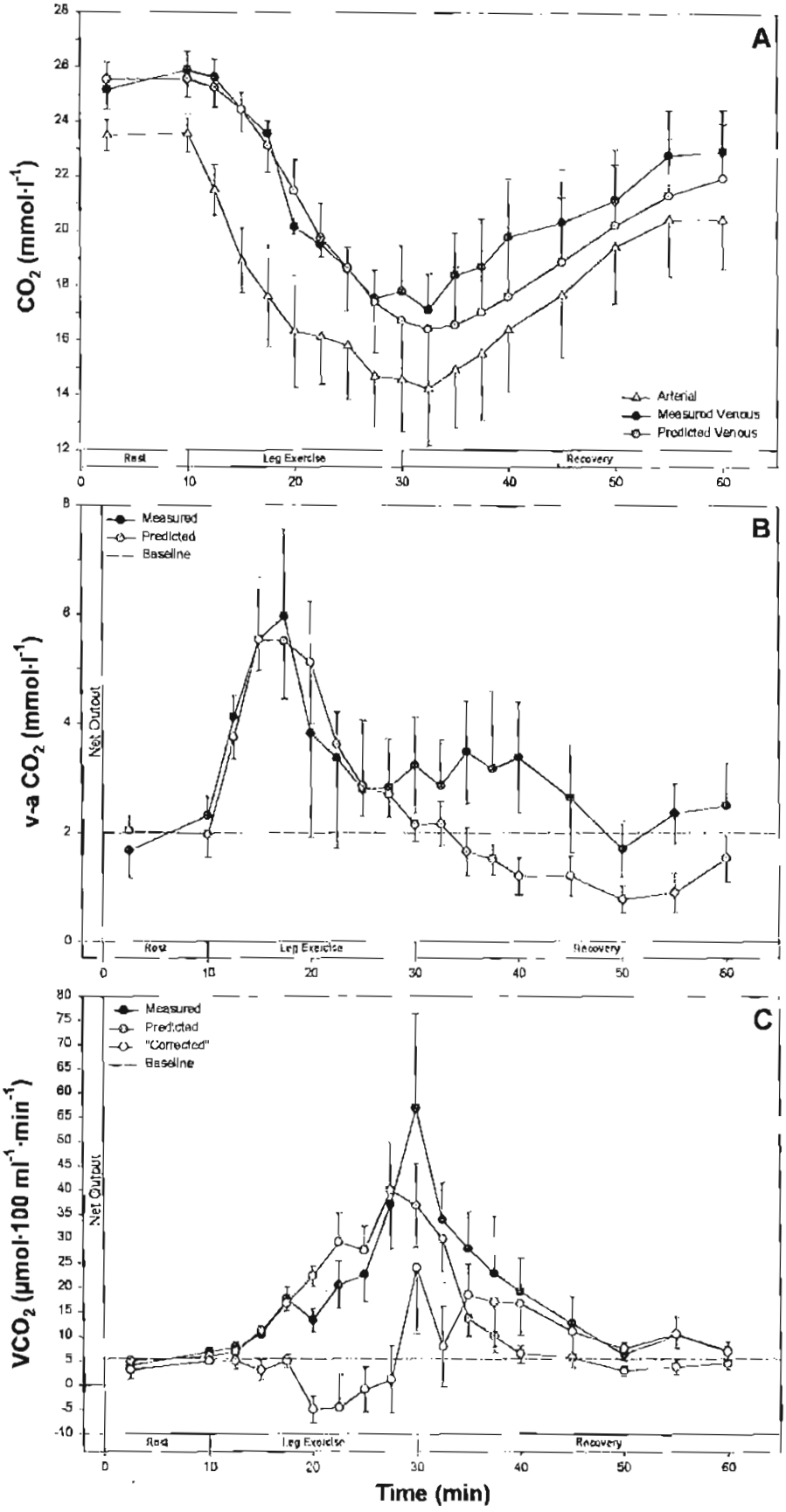


Figure 3.4. Blood CO_2 contents (A), veno-arterial CO_2 content difference (B) and $\dot{V}CO_2$ (CO_2 flux, C) across the resting forearm before, during and after supine leg exercise. "Corrected" = Measured – Predicted + Baseline. Values are means ± SEM, n = 5.

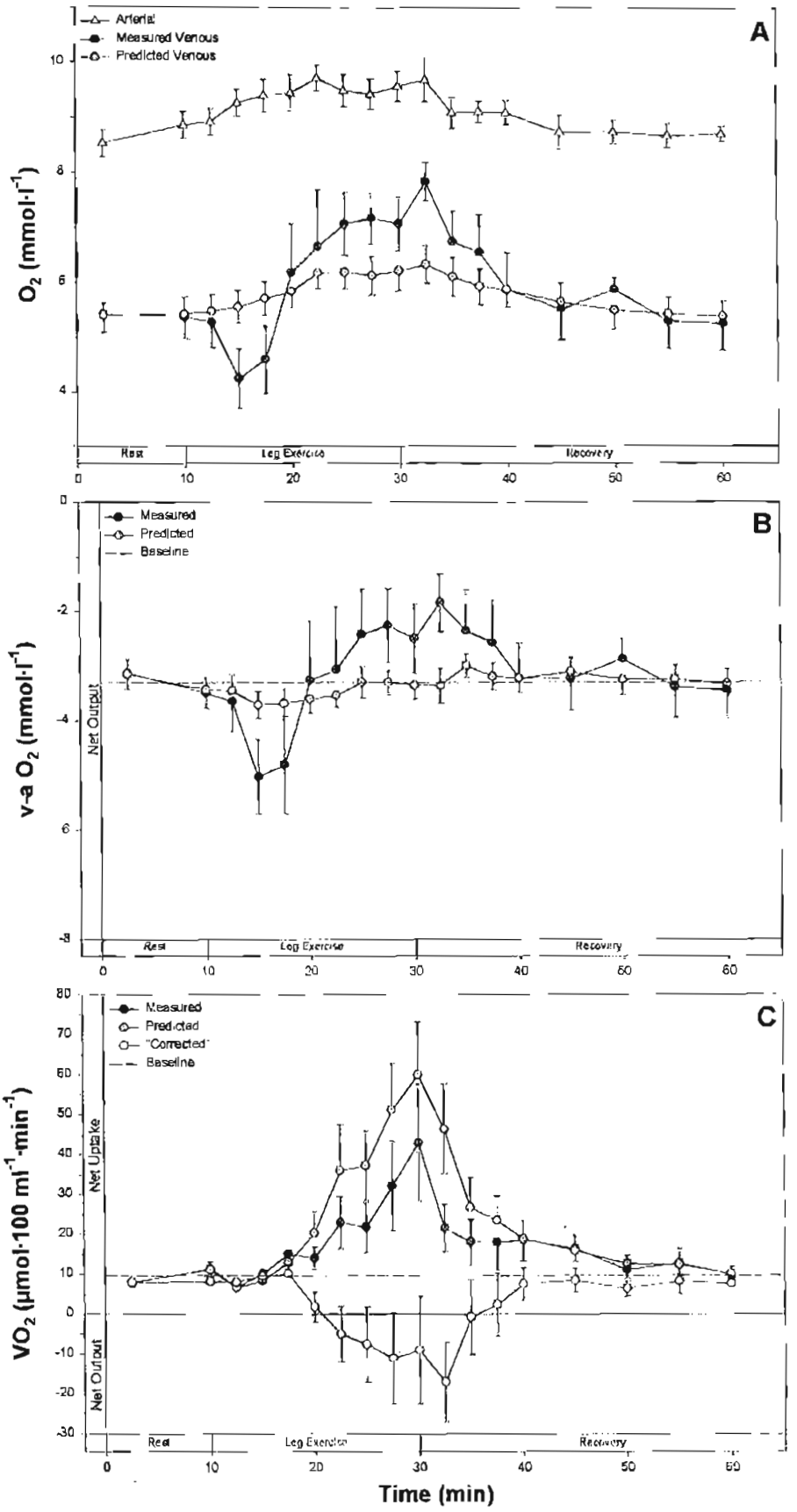


Figure 3.5. Blood O_2 contents (A), veno-arterial O_2 content difference (B) and $\dot{V}O_2$ (-ve O_2 flux, C) across the resting forearm before, during and after supine leg exercise. "Corrected" = Measured – Predicted + Baseline. Values are means ± SEM, n = 5.

3.4 Discussion

The principal finding of this study was that resting forearm lactate uptake during leg exercise could be explained entirely on the basis of passive lactate movements associated with the prevailing non-equilibrium conditions. Previous studies in humans have generally ignored the possibility of passive lactate uptake altogether (284) or speculated, based on simple calculations, that passive movements of lactate do not account for all of the measured lactate uptake (161, 203).

Investigations into the role that skeletal muscle plays in the disposal of an arterial lactate load rely on measurements of arterial and venous lactate levels and flow, and inevitably involve prolonged periods in the non-steady-state. However, to reliably quantify net lactate disposal by a muscle requires steady-state lactate conditions, and only then does net uptake imply metabolism by the muscle. In the non-steady-state, where the arterial lactate concentration increases with time, uptake can no longer be considered simply as metabolism (286, 287). Blood flow dependent equilibration, either with or without an additional diffusion or membrane transport component limiting equilibration, will inevitably result in passive lactate uptake under these conditions. Furthermore, even with direct measurements of intramuscular lactate levels, it is difficult to quantify how much of the lactate washed into the muscle is subsequently metabolised. However, using the analysis and assumptions detailed in Chapter 2, the venous levels of a substance resulting from passive movements alone, during non-steady-state arterial concentration conditions, can be predicted from arterial concentration and blood flow measurements. Differences between the predicted and observed venous levels subsequently reflect metabolic changes within the muscle, provided the assumptions are reasonable.

Employing this analysis in the present study produced predicted values for venous lactate, CO_2, and to a lesser extent H^+ and O_2 that displayed similar values, and a similar time course, to measured venous levels. These are important findings

because leg exercise is associated with substantial changes in arterial blood chemistry and forearm blood flow that result in apparent increases in forearm lactate and O_2 uptake, and CO_2 output with the application of the conventional Fick principle. While there are clear warnings against the interpretation of such uptake or output to indicate disposal or production under non-steady-state conditions (241, 286, 287), frequently too little consideration is given to this phenomenon. The potential for very large errors in estimates of disposal (or production) of a substance under non-equilibrium conditions is clearly apparent when inert substances are considered (149). In this setting, the same principles are routinely employed to determine tissue blood flow based on the substantial wash-in or washout that is observed with such substances.

Previous studies into lactate disposal by resting skeletal muscle have, in general, largely neglected the effects of wash-in during the non-steady-state, thereby attributing a positive arterio-venous lactate difference principally to increased lactate metabolism (3-5, 87, 88). Other investigators have alluded to wash-in under non-steady-state conditions but have only been able to estimate the total amount of lactate washed into inactive muscle based on broad assumptions, and could say nothing regarding the relative rates of lactate wash-in and metabolism in the muscle (94, 161, 192, 203). In all of these studies the greatest arterio-venous lactate gradients occurred within the first few minutes of exercise (or infusion) and diminished with time. This observation alone strongly suggests that inactive muscles are an important passive sink for lactate (94). Poortmans et al. (203) found a significant positive arterio-venous lactate difference across the inactive forearm during 25 min of incremental leg exercise and attributed 82% of the total lactate uptake during exercise to passive wash-in, the remaining 18% representing metabolism by the forearm. However, this figure appears to be based on the ratio of estimated total lactate uptake (25 mmol) and total resting muscle lactate content (140 mmol), predicted from the equilibration of a 14 liter fluid compartment with the

venous lactate concentration at the end of exercise (10 $mmol \cdot l^{-1}$). This calculation does not determine the fraction of lactate uptake subsequently metabolised. Any increase in resting muscle lactate content must balance the quantity of lactate removed from the arterial blood in order to conserve mass. Resting muscle lactate metabolism would be indicated only if the muscle lactate content at the end of exercise was less than the quantity taken up (ie < 25 mmol). To contain 140 mmol of lactate having only taken up 25 mmol from the circulation would require 115 mmol of lactate to have been produced by the resting muscles, or uptake to be underestimated by this amount. More likely is that the resting muscle lactate content was considerably less than predicted. In addition, Poortmans and co-workers (203) found no arterio-venous lactate difference across the forearm after 5 min of recovery and suggested that this indicated a striking reduction in the rate of lactate uptake after exercise. However, this finding does not support such a conclusion. During recovery, where there is a rapid decline in arterial lactate concentration, venous lactate levels would be expected to exceed arterial levels in association with muscle lactate washout. The absence of a negative arterio-venous lactate difference must therefore indicate either significant forearm lactate uptake (either in association with metabolism, or a lower than arterial muscle lactate concentration) during recovery, or sufficient blood flow (and/or a redistribution of flow) to favour rapid blood-tissue equilibration. In view of the high work loads (up to 270 watts) employed in the study of Poortmans et al. (203), it is possible that such changes in forearm blood flow occurred following exercise, resulting in no arterio-venous lactate difference being detected. Perhaps more likely is that the resting muscle lactate concentration was considerably lower than arterial during the majority of the post exercise period. Such a finding would be consistent with data reported by Lindinger et al. (161). The presence of a positive veno-arterial lactate difference in the present study may relate to the lower work load (180 ± 9 watts) which was accompanied by a decrease in total forearm blood flow during the recovery period. Alternatively, the much greater arterial lactate load reported by Poortmans and associates (203) compared with the

present study (12 $mmol \cdot l^{-1}$ compared with 6 $mmol \cdot l^{-1}$ at the end of leg exercise), might, via the inhibitory effects of associated hydrogen ions on glycolytic regulatory enzymes (64, 259), attenuate muscle glycolysis as an energy source. This would promote the oxidation of pyruvate derived from lactate by mass action, thereby accelerating the rate of lactate disposal.

Gladden (95), using canine skeletal muscle preparations *in situ*, demonstrated lactate uptake in both resting and exercising muscles exposed to stable elevated arterial lactate levels attained by sodium lactate infusion. At rest, lactate infusion was associated with substantial muscle lactate uptake, peaking within the first few minutes and then declining, apparently monotonically, over the course of the remaining 30 minutes of infusion. A steady-state with respect to the venous lactate concentration was not reached before the end of infusion, and it is therefore not possible to determine if there was any metabolic lactate disposal, although passive uptake clearly dominated. After 30 min the muscle preparation was electrically stimulated, during which stable elevated values of lactate uptake were observed that increased further when the stimulus intensity was increased. In view of the steady-state, lactate uptake by the active muscles cannot be attributed to passive lactate movements and must therefore indicate lactate disposal. However, increases in blood lactate during strenuous exercise are associated with systemic changes that are not present during exogenous lactate infusion studies and could well influence lactate disposal. For example, an exercise related rise in catecholamine levels would promote glycolysis in resting as well as exercising skeletal muscles (2). This would tend to counteract mass action pyruvate formation from lactate, tending to inhibit the disposal of lactate taken up from the circulation. Differences in arterial pH between infusion and exercise studies are also potentially important determinants of the fate of arterial lactate. Hydrogen ion gradients promote sarcolemmal lactate transport (217), such that exercise or low pH infusates would be expected to promote resting muscle lactate uptake, and therefore lactate disposal. Furthermore, changes in

resting muscle pH associated with systemic exercise, the lactate infusate pH, or the alkalinising effect of lactate disposal itself are also likely to influence many of the enzyme systems directly or indirectly involved in lactate disposal. While it is difficult to predict the net effects of these processes, it is possible that sodium lactate infusions favour muscle lactate disposal, particularly at rest in the absence of the catecholaminergic glycolytic stimulus, while systemic exercise does not.

Several investigators, employing constant infusion tracer techniques, have demonstrated an increase in the rate of irreversible lactate disappearance (RdL) with low to moderate intensity skeletal muscle activity and have attributed this to enhanced muscle lactate oxidation (124, 169, 247). However, in addition to the problems associated with passive lactate movements under non-steady-state arterial lactate conditions, tracer methods for measuring lactate kinetics are complicated by the reversible conversion of lactate to pyruvate (249, 278). As a result, oxidation of non-lactate derived pyruvate may contribute considerably to estimates of lactate oxidation rates based on labeled CO_2 measurements. In addition, Stanley and Lehman (248, 249), who analysed a four-compartment model of lactate metabolism, suggested that only under the conditions where the rate of pyruvate formation de novo and the tracer and tracee concentrations are in a steady-state can RdL be estimated using these techniques. Since it is questionable that such conditions existed in any of these studies, their findings must be viewed with some caution. On the other hand there is little doubt that with activity skeletal muscles can and do metabolise considerable quantities of lactate when arterial lactate levels are elevated by infusion (46, 95) or by exercise (95). However, the contribution of resting muscles to lactate disposal is more difficult to demonstrate and on the basis of the present study would appear to be minor.

Passive movements between a given tissue bed and the blood during transient arterial phases apply not only to lactate but to all diffusible blood constituents. For

this reason, any investigation into the rate of uptake or release of a diffusible substance must consider the effects of passive wash-in to avoid potentially large errors in the estimated rate of metabolism of the substance by the tissues. For example, in association with exercise hyperpnea (197), the arterial carbon dioxide content decreased significantly during exercise and subsequently increased in recovery. Consequently, forearm $\dot{V}CO_2$, calculated without consideration to passive wash-in, was overestimated in the resting forearm during leg exercise (at most, by an estimated 220% between 5 and 10 min of exercise) and underestimated in recovery (by approximately 50% after 20 min of recovery). While this analysis could be criticised on the basis of several assumptions and associated limitations, there is additional and independent evidence highlighting the dominance of passive CO_2 movements. Firstly, the measured total quantity of CO_2 released from the resting forearm over the whole period of study was not different from that expected based on the resting rate of CO_2 output. Secondly, the forearm respiratory exchange ratio calculated from measured values of CO_2 output and O_2 uptake, exceeded the metabolic maximum of unity for the entire period of exercise (mean 1.15 ± 0.05, peak value 1.66 ± 0.30). Whilst a compensatory increase in O_2 uptake might be predicted on the basis of passive O_2 movements, the changes in arterial O_2 content, and in particular pO_2, were minor. Consequently, passive O_2 movements are less likely to have substantially influenced forearm O_2 uptake. Similar changes in resting muscle $\dot{V}CO_2$ and respiratory exchange ratio have been reported previously in association with exercise (161), and a perfusate designed to simulate the metabolic and ionic characteristics of arterial blood following 30 min of intense exercise (46). In both of these reports increased CO_2 output was attributed, at least in part, to increased lactate oxidation. Based on predictions from the present study it would appear that such a conclusion might not be entirely justified.

On the basis of radioactive lactate tracer studies and the enzyme profiles of the different fibre types it has been suggested that muscles can take up, oxidise and

release lactate all at the same time, with the contribution of each depending on the proportion of slow-twitch and fast-twitch fibres in the muscle (195). The forearm muscles contain approximately 50% slow-twitch fibres (128) and might therefore be expected to have a considerable capacity to take up and oxidise lactate. However, an increase in resting muscle lactate oxidation must either be associated with an increase in $\dot{V}O_2$, all other things being equal, or a decrease in non-carbohydrate metabolism with little or no change in $\dot{V}O_2$. In the present study the small non-significant increase in resting forearm $\dot{V}O_2$ during leg exercise could only account for a small amount of additional (above resting) carbohydrate metabolism, and may well be overestimated due to passive O_2 movements. Energy in the resting forearm is derived from predominantly non-carbohydrate metabolism (7). If, during exercise, pyruvate derived from lactate became the principal substrate for oxidative phosphorylation there may be little or no change in $\dot{V}O_2$. Assuming a constant resting forearm $\dot{V}O_2$ of 10 $\mu mol \cdot 100\ ml^{-1} \cdot min^{-1}$ and a respiratory quotient of unity, approximately 3 $\mu mol \cdot 100\ ml^{-1} \cdot min^{-1}$ of lactate could be metabolised in this manner, equivalent to only 26% of the highest rate of lactate uptake by the resting forearm in the present study. Even if the increase in forearm $\dot{V}O_2$ during leg exercise could confidently be associated with enhanced metabolism, the increases were only sufficient to account for complete oxidation of lactate uptake after 10 min of exercise. Combined with the finding that all of the lactate uptake by the forearm during leg exercise could be accounted for in terms of wash-in alone, it is unlikely that the small increases in forearm $\dot{V}O_2$ could be associated with elevated lactate metabolism in the forearm.

The apparent time course of forearm H^+ flux was also not entirely consistent with increased forearm lactate oxidation, which should have an alkalinising effect on forearm tissues (99) and therefore promote forearm H^+ uptake. Although there was a reduction in forearm H^+ output during the initial stages of exercise, consistent with lactate disposal, this was reversed to increased H^+ output in the second half of

exercise at a time when lactate uptake was still recovering towards baseline levels. Furthermore, although the modified Fick equation predicted venous H^+ concentrations relatively poorly, the time course of changes in measured and predicted H^+ concentrations, and therefore veno-arterial H^+ concentration differences and H^+ flux were quite similar. This may indicate that the Kety-Schmidt model is basically sound but somewhat inaccurate in predicting passive movements of H^+ in the resting forearm during and after moderate intensity leg exercise.

The analysis employed in this study is clearly limited by the need to adopt several simplifying assumptions. The simplest alternative is to determine the net area under the measured flux curve for the whole period of study. If measurements are continued until steady-state baseline levels are attained post exercise, this procedure provides a measure of the total net quantity of the substance produced or metabolised by the forearm. However, such an analysis requires a protracted recovery period that was inadequate in this study and many like it (46, 161, 203). Furthermore, such measures incorporate cumulative experimental and measurement errors, are highly variable, and therefore of limited value in the study of metabolism (164). Nevertheless, in this study, the total quantities of lactate, CO_2 and H^+ exchanged with the forearm over the course of exercise and recovery were consistent with baseline levels of metabolism. While forearm O_2 uptake was less than expected, this could indicate an overestimation of baseline $\dot{V}O_2$ or be symptomatic of a short recovery period.

The predictive approach to the assessment of the likely contribution of passive movements to measured veno-arterial differences adopted in this study, potentially provides a much more sensitive means of assessing non-steady-state resting muscle metabolism than has previously been possible with measures of total net flux. In addition, changes in metabolism with time are potentially detectable. However, the reliability of the entire analysis depends on the accuracy of several underlying

assumptions. One potentially important limitation relates to the use of forearm blood flow measurements. Thermoregulatory increases in the skin component of flow during leg exercise may not reflect flow in the bulk of the forearm tissues (*ie* muscle), which reportedly decreases in leg exercise (127). However, based on an increase in forearm venous pO_2 and O_2 content coincident with forearm blood flow changes, it appears that a significant component of venous blood samples derived from skin vascular beds. If this were the case then measurements of total forearm rather than muscle blood flow are likely to be most appropriate. Conversely, if venous samples were solely derived from muscle then the use of blood flow measurements incorporating a skin component would produce inflated estimates of measured flux, and produce predicted venous values closer to arterial than would otherwise be derived based on lower estimates of flow.

This work (44) has been criticised on the basis that whole blood lactate, and not plasma lactate concentrations were measured (162). To the extent that the plasma phase of blood is in contact with the capillary surface, and that red blood cell and plasma lactate concentrations are not equivalent, this is a valid criticism. However, throughout the physiological range of blood lactate levels, there appears to be a strong correlation (87) and a relatively constant ratio between whole blood and plasma lactate levels (234). This is suggestive of rapid equilibration between the erythrocyte and plasma compartments and is consistent with relatively rapid lactate transport across red blood cell membranes under physiological conditions (76) and the extensive red blood cell exchange surface. Consequently, although whole blood lactate concentration measurements appear to systematically under-estimate plasma lactate levels (87, 161, 276), this would merely alter the apparent baseline of arterial, tissue and venous concentrations and not the magnitude or time course of changes associated with exercise and subsequent recovery. More importantly, arteriovenous concentration differences would be similar when calculated from whole blood or plasma concentrations, as long as the distribution of ions contributing to the Donnan

equilibrium distribution of lactate between plasma and blood was similar in arterial compared to venous blood. This is a more complex issue that could be addressed with more complex models incorporating two blood compartments. However, Lindinger et al.'s (161) own data indicate that resting forearm arteriovenous lactate concentration differences during and after leg exercise are similar when determined from whole blood and plasma, suggesting that passive lactate uptake is quantitatively an important component of veno-arterial concentration differences however measured. This is apparent when total net lactate uptake is determined from the area under the lactate flux curve over the whole exercise and recovery period. Although variable and therefore relatively insensitive, this measurement does not depend on complex models, nor does it matter if whole blood or plasma lactate measurements are employed. In the present study essentially all of the lactate taken up during exercise (81.7 ± 66.1 μmol·100 ml^{-1}) was returned to the circulation by 30 min of recovery.

The findings of this study suggest that resting muscles may not actively buffer changes in arterial blood chemistry as has previously been suggested (161), or that this role is minor compared to the effects of equilibration with arterial blood. From a teleological perspective, lactate disposal by resting muscles could be considered an inefficient use of available monocarboxylates, particularly during moderate intensity exercise, and particularly if associated with an increase in resting muscle $\dot{V}O_2$ that would add to systemic gas exchange demands. On the other hand, during high intensity exercise, the alkalinising effect of lactate disposal would appear beneficial.

In summary, in the experiments reported in this Chapter, where the rate of passive lactate wash-in during non-steady-state arterial lactate conditions was estimated, all of the lactate taken up by the inactive forearm during leg exercise could be accounted for in terms of passive uptake alone. At the moderate intensity of leg exercise adopted in the present study and given the limitations discussed with regard

to the mathematical analysis adopted, it is suggested that the role of resting skeletal muscle in the disposal of arterial lactate is minor, with most of the lactate taken up merely increasing the lactate content of the muscle rather than being metabolised.

CHAPTER 4 Forearm tissues modify the blood lactate response to incremental exercise and therefore influence lactate threshold determinations

4.1 Introduction

ENDURANCE EXERCISE CAPACITY is frequently assessed using indices obtained from the blood lactate response to incremental exercise (283). These indices include the log-log lactate threshold described by Beaver et al. (22) and the work load at which certain reference blood lactate levels are reached [eg 2 $mmol \cdot l^{-1}$ (67, 110) and 4 $mmol \cdot l^{-1}$ lactate thresholds (67, 152, 250)] and are commonly measured using arterial (71, 265, 284), forearm venous (42, 211, 254) or finger prick blood (232, 233). Despite these thresholds being largely governed by the absolute lactate concentration at any given time or the time course of lactate concentration change, only rarely is any consideration given to possible differences between blood collection sites (80, 211). Arterial blood will most accurately reflect systemic lactate kinetics. Blood collected from forearm veins, by finger prick or from alternative sites is potentially modified by lactate metabolism during its passage through the tissues proximal to the site of collection. Furthermore, during moderate to high intensity exercise, in which arterial lactate levels become substantially elevated over time, blood-tissue lactate concentration gradients can induce passive lactate movements between the blood and forearm tissues. These processes could substantially influence any measure derived from the blood lactate response to incremental exercise.

From the work reported in Chapter 3 of this thesis it appears that passive lactate movements alone can account for all of the resting forearm veno-arterial lactate difference associated with 20 minutes of constant load leg exercise of moderate

intensity. As with moderate intensity exercise, incremental exercise to exhaustion is dominated by non-equilibrium arterial blood and therefore presumably tissue lactate concentrations. Consequently, substantial arteriovenous lactate concentration differences in the inactive forearm during incremental exercise (211, 282, 284) are likely to reflect equilibration processes and not forearm lactate metabolism as has previously been suggested (282, 284). The principal aim of the experiments reported in this Chapter was to investigate the contribution of passive blood-tissue lactate movements to forearm veno-arterial lactate concentration differences during incremental exercise, and the likely impact of this phenomenon on several of the most commonly used measures of the lactate threshold.

A further concern regarding lactate kinetics is the controversy that exists regarding the nature of the blood lactate response to exercise. Beaver et al. (22) formalised the concept that arterial lactate increases as a threshold phenomenon, while others have suggested that blood lactate increases in an exponential manner during incremental exercise (43, 69, 122, 246, 282). These models have only been assessed using arterial or arterialised blood samples (22, 43, 122). However, both are frequently applied to characterise the venous blood lactate response to incremental exercise (69, 211, 254) without consideration for the possible effects that forearm tissue lactate uptake, and potentially changes in metabolism, may have on the fitting ability of the model employed. Stanley et al. (246) suggested that lactate appearance and disappearance in arterial blood displayed first order exponential kinetics during incremental exercise. In combination, these two processes would produce a simple exponential pattern of blood lactate increase. However, simultaneous lactate appearance and disappearance from multiple body compartments arranged in series and in parallel could produce a pattern of arterial lactate increase more closely resembling a threshold. Conversely, the combined effects of multiple compartments could be to mask an underlying threshold process of production, disposal or a combination of both. Therefore, a further aim of the study reported in this Chapter was to investigate the relative abilities of the two

leading models of blood lactate increase during incremental exercise to fit the patterns of lactate increase in arterial blood and in blood that has traversed the resting forearm tissues. Differences in model fits between blood collection sites in the forearm could be indicative of similar processes influencing the systemic blood lactate response to incremental exercise.

4.2 Methods

4.2.1 General

Six right-handed male volunteers participated in the study. Each subject attended the laboratory in the morning after a 12 h fast and a 36 h period without strenuous exercise. Measurements of right forearm dimensions were made before catheters were inserted into the left brachial artery and the deep muscle branch of the right antecubital vein. After a 10 min rest period, each subject performed an incremental exercise test to exhaustion on a supine cycle ergometer (Jaquet, Switzerland). Exercise was begun at 100 watts and increased by 25 watt increments every 3 min until the subject was unable to continue. Simultaneous arterial and venous blood samples were obtained at rest (protocol min 5 and 10), in the last 15 sec of each work load during exercise and at regular intervals during 120 min of recovery (recovery min 2.5, 5, 7.5, 10, 15, 20, 25, 30 then every 10 min). These blood samples were subsequently used for blood lactate and blood gas analysis as described in Chapter 2 (pp. 58, 59). Finger prick blood samples (30 µl) were obtained from the left hand during the rest and exercise periods coincident with the arterial and venous blood sampling procedures. These samples were immediately deproteinated in 100 µl of ice-cold 8% perchloric acid, centrifuged and the supernatant frozen for subsequent lactate analysis.

Right forearm blood flow, pulsatile and mean arterial blood pressures, heart rate and respiratory parameters ($\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$, RER) were measured throughout the experiment as detailed in Chapter 2 (pp. 51, 57). In this series of experiments, right hand blood flow was excluded from forearm blood flow measurements with the use of an arterial occlusion cuff at the wrist. Peak $\dot{V}O_2$ ($\dot{V}O_{2\,peak}$) was determined as the highest $\dot{V}O_2$ recorded during the final exercise work load.

4.2.2 Analytical methods

Venous and finger prick blood lactate concentrations were predicted using the integrated form of the Fick equation (equation 2.13, detailed in Chapter 2, pp. 69). This analysis assumes that forearm tissue lactate metabolism is constant and predicts tissue effluent (venous and capillary) levels based on tissue volume, blood flow and changes in arterial lactate concentration. For venous predictions, the measured forearm volume was used, while for finger prick predictions the skin volume was considered to equal 10% of the measured forearm volume (1, 60).

The log-log lactate threshold (LT), originally described by Beaver et al. (22), was determined as detailed in Chapter 2 (pp. 62). Relationships between arterial, measured and predicted venous and finger prick blood lactate concentrations and $\dot{V}O_2$ during exercise were also examined using the exponential model described by Hughson et al. (122), as detailed in Chapter 2 (pp. 63). The 2 $mmol \cdot l^{-1}$ and 4 $mmol \cdot l^{-1}$ lactate thresholds were determined as the $\dot{V}O_2$ (expressed in absolute terms and as a percentage of $\dot{V}O_{2\,peak}$) corresponding to the appropriate lactate concentration using equation 2.1 (pp. 63). The lactate slope index (LSI) was determined as the point at which the slope of the lactate versus $\dot{V}O_2$ curve equalled unity using equation 2.3 (122) (pp. 63).

In order to compare the fitting ability of the log-log and exponential models of blood lactate increase during incremental exercise, the residual sum of squares (RSS) associated with the best log-log fit was subsequently recalculated in rectilinear coordinates. This was to allow comparisons with the RSS associated with the exponential model of blood lactate increase (122). The RSS was used as the goodness of fit criterion rather than the mean square error (MSE) term as used by Hughson et al. (122), because the use of MSE has been considered misleading (181). However, the RSS does not take into consideration the additional fitting parameter afforded to the log-log model. Consequently, both models were also compared using the Model Selection Criterion (261) as detailed in Chapter 2 (pp. 77).

Wherever possible, the individual anaerobic threshold (IAT), originally described by Stegmann and Kindermann (251), was determined for arterial, measured and predicted venous blood using the method detailed in Chapter 2 (pp. 63).

The modified Fick equation was also employed to examine the likely contribution of passive movements to veno-arterial differences in H^+, CO_2 and O_2 associated with incremental exercise. Forearm fluxes were calculated as the product of forearm blood flow and the veno-arterial concentration differences in lactate, H^+, CO_2 and O_2 at each time point (conventional Fick equation). Consequently, positive and negative fluxes indicate output and uptake respectively. Predicted forearm fluxes were calculated as the product of forearm blood flow and predicted veno-arterial concentration differences. Measured minus predicted forearm fluxes, adjusted for baseline flux (equation 2.28, pp. 84), were subsequently used as an index of net production (positive values) or net metabolism (negative values) of substances in the forearm, largely independent of the effects of passive movements associated with the non-equilibrium conditions of incremental exercise.

The total net quantities of lactate, H^+, CO_2 and O_2 taken up (or released) by the forearm throughout exercise and recovery were determined from the net area under the time versus the respective flux curves. These values were compared to the total net flux that would have been measured had the resting levels of flux continued throughout exercise, recovery and exercise and recovery:

$$\text{Expected Total Net Flux} = \text{Baseline Flux (units} \cdot \text{minute}^{-1}) \cdot \text{Duration (min)}$$

4.2.3 Statistical analysis

Differences in lactate concentrations between blood sampling sites and with time were evaluated using 2-way ANOVA for repeated measures. As exercise times differed between subjects separate ANOVAs were used for exercise and recovery.

Differences in the log-log, 2 $mmol \cdot l^{-1}$ and 4 $mmol \cdot l^{-1}$ lactate thresholds and the LSI between blood sampling sites were also examined using 2-way ANOVA. Selected post-hoc comparisons were made using Tukey's post-hoc tests. Due to missing values, statistical comparisons between IAT measurements in arterial and venous blood and between other measures of the LT were not possible. The RSS and MSC of the log-log and exponential models of blood lactate increase during incremental exercise were compared using Student's paired *t*-tests. These were also used in comparisons involving calculated total net flux. Comparisons between arterial and measured venous concentrations, between measured and predicted venous concentrations and between measured and predicted veno-arterial concentration differences and flux were made using two-way analysis of variance (ANOVA) for repeated measures. Time effects for a given parameter were examined using one-way ANOVA for repeated measures. In all statistical tests the null hypothesis was rejected if $P < 0.05$, unless otherwise indicated. All data are reported as means ± SEM.

4.3 Results

4.3.1 General

Descriptive characteristics of the subjects and peak values of $\dot{V}O_2$, heart rate and work load are presented in Table 4.1.

Table 4.1. Subject characteristics.

						Right forearm	
Age	**Mass**	**Height**	$\dot{V}O_{2\ peak}$	HR_{peak}	WL_{peak}	**volume**	**circumference**
(years)	(kg)	(cm)	($ml \cdot kg \cdot min^{-1}$)	($b \cdot min^{-1}$)	(watts)	(ml)	(cm)
19.5	73.1	181.8	59.6	191.4	287.5	1676.7	27.60
± 0.6	± 1.9	± 3.7	± 2.9	± 3.0	± 10.7	± 90.5	± 0.65

Values are means ± SEM, n = 6.

4.3.2 Cardiorespiratory variables

$\dot{V}O_2$, $\dot{V}CO_2$, RER and $\dot{V}_E$ increased progressively during incremental exercise from resting values of 4.8 ± 0.3 $ml \cdot kg^{-1} \cdot min^{-1}$, 3.9 ± 0.3 $ml \cdot kg^{-1} \cdot min^{-1}$, 0.81 ± 0.04 and 8.4 ± 0.5 $l \cdot min^{-1}$ to reach peak values of 59.6 ± 2.9 $ml \cdot kg^{-1} \cdot min^{-1}$, 64.5 ± 2.2 $ml \cdot kg^{-1} \cdot min^{-1}$, 1.09 ± 0.04 and 137.9 ± 4.7 $l \cdot min^{-1}$ respectively at the end of the last work load completed by each subject (range 250-325 watts, Figure 4.1). Similarly, HR and MAP increased progressively from resting values of 65.5 ± 9.4 $beats \cdot min^{-1}$ and 102.3 ± 3.6 mmHg to reach peak values of 191.4 ± 3.0 $beats \cdot min^{-1}$ and 134.7 ± 3.7 mmHg at the end of exercise. HR recovered to pre-exercise levels within approximately 40 min of recovery. MAP decreased very rapidly post-exercise, falling to values lower than at rest within 10 min of recovery. MAP remained significantly lower ($P < 0.001$) than resting values throughout the remainder of recovery, although there was a slow upward trend towards resting values.

Forearm blood flow remained at pre-exercise levels (4.4 ± 0.6 ml·100 ml^{-1}·min^{-1}) until approximately 15 min of exercise after which there was a trend for an increase above pre-exercise levels (P = 0.069, Figure 4.2). Forearm blood flow reached a peak of 8.6 ± 2.7 ml·100 ml^{-1}·min^{-1} in the third highest work load completed by each subject (204.5 ± 41.2 watts) before declining slightly to 6.0 ± 1.9 ml·100 ml^{-1}·min^{-1} at the highest completed work load. Forearm blood flow had returned to pre-exercise levels by 10 min of recovery, after which levels slightly lower than resting levels were maintained (Figure 4.2). There was a strong positive relationship between forearm blood flow and venous blood O_2 content ($r = 0.77 \pm 0.05$, $r^2 = 0.61 \pm 0.07$, $P < 0.001$) supporting the view that forearm blood flow measurements were representative of tissues from which venous blood was collected.

4.3.3 Model fits

The Model Selection Criterion and degree of correlation between measured and predicted venous concentrations of lactate, H^+, CO_2 and O_2 are presented in Table 4.2. The modified Fick equation provided reasonable fits to venous lactate, H^+ and CO_2 data, but was of little predictive value when applied to O_2.

Table 4.2. Model Selection Criterion (MSC) and r^2 for measured compared to model predicted values of venous lactate, H^+, CO_2 and O_2.

Substance	MSC	r^2
Lactate (Venous)	2.13 ± 0.14	0.944 ± 0.015
Lactate (Finger Prick)	2.89 ± 0.47	0.966 ± 0.012
H^+	2.17 ± 0.31	0.929 ± 0.019
CO_2	1.88 ± 0.32	0.924 ± 0.014
O_2	-0.19 ± 0.25	0.673 ± 0.096

Values represent combined exercise and recovery data with the exception of finger prick lactate in which recovery data were not obtained. Values are means ± SEM, n = 6.

4.3.4 Blood lactate

4.3.4.1 Exercise response and lactate threshold determinations

Arterial, venous and finger prick blood lactate concentrations at rest and throughout incremental exercise are shown in Figure 4.3. Finger prick blood lactate levels were significantly higher than arterial and venous levels at rest (finger prick, 1.59 ± 0.18; arterial, 0.62 ± 0.03; venous, 0.66 ± 0.07 $mmol \cdot l^{-1}$, $P < 0.01$) and throughout incremental exercise ($P < 0.012$, Figure 4.3). Arterial and forearm venous lactate levels were similar at rest, but there was a more rapid rise in the arterial lactate concentration during exercise, such that, overall, forearm venous levels were significantly lower than arterial ($P = 0.024$). The patterns of lactate increase in both venous and finger prick blood were very similar to those predicted on the basis of passive equilibration between the forearm tissues and the rising lactate concentrations in the arterial blood (Figure 4.3). Consequently, there were no significant differences between measured and predicted venous or finger prick blood lactate concentrations throughout the period of rest and exercise ($P > 0.895$). There were strong relationships ($r^2 > 0.88$, $P < 0.001$) between lactate concentrations determined from blood obtained from each of the sampling sites (Figure 4.4A-C). However, venous blood lactate levels were systematically ~30% and ~35% lower compared with arterial and finger prick blood respectively (Figure 4.4A,B). Unlike venous blood lactate levels, which were proportionally lower, those in finger prick blood were systematically ~1 $mmol \cdot l^{-1}$ higher compared to arterial concentrations (Figure 4.4C). Measured venous and finger prick blood lactate levels were highly correlated and similar to those predicted on the basis of passive lactate movements, with linear regression lines demonstrating near zero ordinate and abscissa intercepts and slopes not different from unity (Figure 4.4D).

The time, absolute and relative work loads ($\dot{V}O_2$, $\%\dot{V}O_{2\ peak}$) and lactate concentrations coincident with the log-log, 2 and 4 $mmol \cdot l^{-1}$ LTs, the LSI and the IAT determined from blood sampled from the different forearm sites are presented in

Table 4.3. The log-log lactate threshold occurred later, at higher absolute and relative work loads, and was associated with a higher lactate concentration in finger prick compared to venous blood. The lactate concentration coincident with the log-log LT was also higher in finger prick compared to arterial blood, although the time and work load associated with the log-log LT were not different when determined from arterial and finger prick blood. In association with the systematically higher finger prick compared to arterial and venous blood lactate levels, fixed concentration thresholds tended to occur earlier and therefore at lower work loads in finger prick compared to arterial and venous blood. There were no significant differences in the LSI determined from arterial, venous or finger prick blood.

In order to determine the IAT it is essential that blood lactate levels peak post-exercise such that the point in recovery at which blood lactate levels equal that at exercise termination can be identified. Determinations of the IAT from finger prick blood were therefore not possible, as recovery samples were not obtained. It was also not possible to obtain measurements of the IAT from arterial blood in 4 subjects, and from venous blood in 1 subject because blood lactate levels had already begun to decline when the first recovery blood sample was obtained. In the remaining subjects, blood lactate levels reached peak values between 2.5 and 20 min of recovery (arterial blood, 2.5 – 7.5 min; venous blood 2.5 – 20 min). In two subjects in whom there were post-exercise peaks in both arterial and venous blood lactate levels, the peak was later in venous compared to arterial blood (by 5 and 12.5 min). As a result of the reduced number of data points, it was not possible to make meaningful statistical comparisons between the IAT measured from arterial and venous blood, or between the IAT and other measures of the LT. However, IAT determinations obtained from the measured and predicted venous blood lactate responses to incremental exercise were very similar ($P > 0.3$, $n = 5$, Table 4.3). Although exercise-termination levels of blood lactate concentration appeared to occur later during recovery in predicted compared to measured venous blood, this was not

significant (measured, 18.2 ± 6.4 min; predicted, 22.1 ± 6.4 min, P = 0.130, Figure 4.5A).

In venous and finger prick blood, the values of time, exercise intensity and lactate concentration associated with each of the LTs were similar to values obtained from the predicted venous and finger prick blood lactate responses to incremental exercise (P > 0.05, Table 4.3). In general, fixed concentration thresholds occurred later and therefore at higher exercise intensities compared to the log-log LT and the LSI.

Table 4.3. Time, $\dot{V}O_2$, $\%\dot{V}O_{2\,peak}$ and blood lactate concentrations at the log-log, 2 and 4 $mmol \cdot l^{-1}$ fixed concentration lactate thresholds, lactate slope index (LSI) and individual anaerobic threshold (IAT) determined from finger prick and/or arterial and venous blood.

	Log-log LT	2 $mmol \cdot l^{-1}$ LT	4 $mmol \cdot l^{-1}$ LT	Lactate Slope Index	IAT
Time (min)					
Arterial	20.68 ± 1.04	22.08 ± 1.02 *	27.79 ± 0.86 *†	18.47 ± 1.63 *§	21.35 ± 6.70
Venous	19.66 ± 0.95 *	23.68 ± 0.47 *†	29.80 ± 0.46 *†‡	19.74 ± 0.84 *§	21.33 ± 2.19
Finger Prick	22.74 ± 1.42	17.11 ± 0.52 †	25.47 ± 0.67 †‡	18.44 ± 0.76 †§	-
Predicted					
Venous	18.07 ± 0.93	24.20 ± 0.76	30.19 ± 0.51	20.11 ± 0.96	22.71 ± 2.23
Finger Prick	21.22 ± 0.90	16.87 ± 2.50	25.57 ± 1.40	18.33 ± 1.50	-
$\dot{V}O_2$ ($l \cdot min^{-1}$)					
Arterial	2.54 ± 0.21	2.73 ± 0.22 *	3.46 ± 0.23 *†	2.28 ± 0.30 *§	2.32 ± 0.75
Venous	2.41 ± 0.14 *	2.96 ± 0.20 *†	3.72 ± 0.21 *†‡	2.47 ± 0.20 *§	2.49 ± 0.31
Finger Prick	2.82 ± 0.18	2.22 ± 0.16 †	3.16 ± 0.19 ‡	2.29 ± 0.14 †§	-
Predicted					
Venous	2.19 ± 0.14	3.01 ± 0.20	3.78 ± 0.23	2.50 ± 0.22	2.68 ± 0.36
Finger Prick	2.61 ± 0.19	2.17 ± 0.38	3.17 ± 0.28	2.26 ± 0.28	-
$\%\dot{V}O_{2\,peak}$					
Arterial	58.33 ± 3.31	62.81 ± 3.93 *	79.45 ± 2.98 *†	52.12 ± 5.71 *§	62.14 ± 26.07
Venous	55.72 ± 3.38 *	67.94 ± 3.08 *†	85.59 ± 2.23 *†‡	56.70 ± 3.35 *§	58.90 ± 7.06
Finger Prick	65.09 ± 3.42	48.42 ± 1.92 †	72.75 ± 2.81 ‡	52.72 ± 2.45 †§	-
Predicted					
Venous	50.82 ± 3.66	69.32 ± 3.79	86.89 ± 3.43	57.42 ± 4.04	62.62 ± 6.60
Finger Prick	59.96 ± 2.95	46.83 ± 6.51	72.65 ± 4.33	51.78 ± 5.18	-
Lactate concentration ($mmol \cdot l^{-1}$)					
Arterial	1.44 ± 0.07 *	2.00	4.00 †‡	1.39 ± 0.12 *§	2.61 ± 1.53
Venous	1.07 ± 0.16 *	2.00 †	4.00 †‡	1.35 ± 0.06 *§	1.68 ± 0.31
Finger Prick	2.84 ± 0.43	2.00 †	4.00 †‡	2.39 ± 0.21 §	-
Predicted					
Venous	0.83 ± 0.08	2.00	4.00	1.31 ± 0.06	1.82 ± 0.34
Finger Prick	2.36 ± 0.17	2.00	4.00	2.34 ± 0.13	-

Values are means ± SEM. n = 6, except for the IAT in which n = 2 and n = 5 for arterial and venous blood respectively. Significant differences are indicated; * compared to finger prick blood, † compared to the log-log LT, ‡ compared to the 2 $mmol \cdot l^{-1}$ LT and, § compared to the 4 $mmol \cdot l^{-1}$ LT ($P < 0.05$).

4.3.4.2 Models of blood lactate increase during incremental exercise

Curve fit parameters and goodness-of-fit criteria for the log-log and exponential models of blood lactate increase determined from arterial, venous and finger prick blood in each subject, are presented in Table 4.4 and Table 4.5. In the lower line segment of the log-log model, the log-log slope (parameter a) was significantly lower in venous compared to arterial blood (Table 4.4). In addition, the log lactate concentration intercept (parameter b) was significantly higher in finger prick compared to arterial and venous blood in the lower line segment. These differences were consistent with the early development of an arteriovenous lactate concentration difference during exercise and the higher blood lactate concentrations in finger prick compared to arterial and venous blood (Figure 4.3). There were no other differences in log-log or exponential model curve fit parameters between the blood sampling sites, with the exception of parameter C in the exponential model which was lower in venous compared to finger prick blood, again consistent with the higher finger prick blood lactate concentrations. In both venous and finger prick blood, there were no differences in any model parameter determined from measured and predicted blood lactate concentrations.

In arterial blood, the exponential model of blood lactate increase during incremental exercise provided a better fit, being associated with a significantly lower RSS ($P = 0.041$) and a trend for a higher MSC ($P = 0.055$), when compared to the log-log model (Table 4.5). In contrast, the log-log and exponential models fit equally well when applied to venous (RSS, $P = 0.749$; MSC, $P = 0.768$) and finger prick blood (RSS, $P = 0.162$; MSC, $P = 0.310$). When applied to the predicted venous blood lactate response to exercise, both models also fit equally well, although the fits were significantly better when compared to those obtained from measured venous data. When applied to predicted finger prick data, the exponential model provided a superior fit, although the RSS and MSC were similar when compared to values obtained from measured finger prick data.

Table 4.4. Log-log and exponential model parameter estimates of the $\dot{V}O_2$ (x) vs lactate (y) increase during incremental exercise in arterial, venous and finger prick blood.

		Log-Log Model $y = 10^{a \log(x) + b}$						Exponential Model $y = Ae^{Bx} + C$		
		Lower Segment			Upper Segment					
Blood	**Subject**	**a**	**b**	**r^2**	**a**	**b**	**r^2**	**A**	**B**	**C**
Arterial	1	0.364	0.037	0.971	3.928	-1.262	0.994	0.060	1.331	0.627
	2	0.442	-0.034	0.999	3.559	-1.526	0.967	0.011	1.380	0.912
	3	0.373	-0.064	0.936	4.021	-1.302	0.946	0.008	1.973	0.788
	4	0.750	-0.063	0.984	2.206	-0.454	0.978	0.992	0.535	-1.006
	5	0.405	0.039	0.923	4.383	-1.667	0.980	0.030	1.406	0.812
	6	0.503	-0.076	0.906	3.965	-1.830	0.949	0.010	1.354	0.928
	Mean	**0.473**	**-0.027** †		**3.677**	**-1.340**		**0.185**	**1.330**	**0.510**
	± SEM	**± 0.059**	**± 0.021**		**± 0.313**	**± 0.198**		**± 0.162**	**± 0.187**	**± 0.306**
Venous	1	0.182	-0.015	0.530	3.977	-1.456	0.989	0.107	1.081	0.248
	2	0.115	-0.077	0.576	3.922	-1.858	0.930	0.002	1.735	0.861
	3	0.201	-0.040	0.425	4.015	-1.393	0.980	0.009	1.850	0.756
	4	0.448	-0.141	0.898	2.075	-0.603	0.969	0.412	0.603	-0.143
	5	0.470	0.055	0.917	3.357	-1.129	0.984	0.075	1.126	0.572
	6	0.143	-0.312	0.999	3.325	-1.489	0.970	0.232	0.675	-0.260
	Mean	**0.260** *	**-0.088** †		**3.445**	**-1.321**		**0.139**	**1.178**	**0.339** †
	± SEM	**± 0.064**	**± 0.052**		**± 0.302**	**± 0.173**		**± 0.064**	**± 0.213**	**± 0.192**
Finger Prick	1	0.123	0.279	0.808	4.017	-1.296	0.994	0.068	1.293	1.149
	2	0.362	0.189	0.998	3.168	-1.270	0.994	0.061	0.977	1.229
	3	0.132	0.320	0.738	3.134	-0.732	0.951	0.008	1.993	1.998
	4	0.403	0.435	0.934	3.410	-1.080	0.994	0.062	1.172	2.512
	5	1.141	0.037	0.784	3.482	-1.124	0.989	0.038	1.328	1.471
	6	0.281	0.150	0.456	2.621	-0.792	0.970	0.138	0.883	0.912
	Mean	**0.407**	**0.236**		**3.305**	**-1.049**		**0.062**	**1.274**	**1.545**
	± SEM	**± 0.154**	**± 0.057**		**± 0.188**	**± 0.097**		**± 0.018**	**± 0.160**	**± 0.245**
Predicted Venous	1	0.093	-0.046	0.472	3.491	-1.150	0.995	0.055	1.272	0.500
	2	0.162	-0.205	0.693	2.645	-1.166	0.999	0.092	0.796	0.292
	3	0.186	-0.164	0.694	3.679	-1.364	0.987	0.009	1.749	0.558
	4	0.291	-0.247	0.398	2.250	-0.622	0.996	0.459	0.631	-0.369
	5	0.245	-0.032	0.824	3.560	-1.282	0.988	0.071	1.137	0.453
	6	0.155	-0.218	0.746	3.184	-1.404	0.987	0.042	1.027	0.389
	Mean	**0.189**	**-0.152**		**3.135**	**-1.164**		**0.121**	**1.102**	**0.304**
	± SEM	**± 0.029**	**± 0.037**		**± 0.233**	**± 0.116**		**± 0.069**	**± 0.160**	**± 0.140**
Predicted Finger Prick	1	0.198	0.303	0.946	3.319	-0.881	0.993	0.080	1.256	1.398
	2	0.182	0.292	0.933	2.738	-0.940	0.963	0.027	1.173	1.768
	3	0.136	0.337	0.965	2.908	-0.658	0.934	0.010	1.888	2.042
	4	0.232	0.396	0.932	1.597	-0.017	0.977	0.847	0.562	0.781
	5	0.237	0.271	0.890	3.767	-1.269	0.980	0.039	1.338	1.491
	6	0.379	0.024	0.849	3.787	-1.700	0.950	0.011	1.330	1.085
	Mean	**0.227**	**0.271**		**3.019**	**-0.911**		**0.169**	**1.258**	**1.427**
	± SEM	**± 0.034**	**± 0.052**		**± 0.334**	**± 0.232**		**± 0.136**	**± 0.173**	**± 0.186**

a and *b* are in log-log coordinates. A, B and C are in linear coordinates. Differences are indicated; * compared to arterial blood, † compared to finger prick ($P < 0.05$).

Table 4.5. Goodness-of-fit criteria for log-log and exponential models of blood lactate increase during incremental exercise.

		Log-Log Model				Exponential Model			
Blood	**Subject**	**r^2**	**RSS**	**df**	**MSC**	**r^2**	**RSS**	**df**	**MSC**
Arterial	1	0.992	1.347	5	3.758	0.995	0.882	6	3.963
	2	0.977	1.238	3	2.634	0.993	0.314	4	3.420
	3	0.933	4.726	5	1.771	0.946	3.661	6	2.249
	4	0.994	0.432	5	4.228	0.990	0.702	6	3.965
	5	0.987	2.959	7	3.557	0.990	2.254	8	4.011
	6	0.956	3.713	6	2.231	0.976	1.827	7	3.141
	Mean		**2.402**		**3.030**		**1.606** *		**3.458** †
	± SEM		**± 0.678**		**± 0.392**		**± 0.507**		**± 0.282**
Venous	1	0.981	1.474	6	3.078	0.988	2.310	7	2.829
	2	0.948	2.273	4	1.768	0.987	0.473	5	3.586
	3	0.977	0.877	5	2.851	0.985	0.568	6	3.507
	4	0.993	0.189	5	3.953	0.992	0.200	6	4.123
	5	0.996	0.446	7	4.855	0.978	2.643	8	3.258
	6	0.979	1.013	6	2.886	0.971	1.169	7	2.942
	Mean		**1.045**		**3.232**		**1.227** ‡		**3.374** ‡
	± SEM		**± 0.306**		**± 0.432**		**± 0.418**		**± 0.193**
Finger Prick	1	0.997	0.583	6	4.825	0.985	2.492	7	3.572
	2	0.997	0.084	4	4.974	0.978	0.717	5	3.081
	3	0.954	3.736	5	2.147	0.972	2.163	6	2.916
	4	0.995	0.499	5	4.338	0.990	0.899	6	3.972
	5	0.991	1.534	5	3.877	0.990	1.237	6	3.397
	6	0.971	4.024	6	2.605	0.990	1.174	7	4.037
	Mean		**1.743**		**3.794**		**1.447**		**3.496**
	± SEM		**± 0.704**		**± 0.479**		**± 0.292**		**± 0.186**
Predicted Venous	1	0.995	0.573	5	5.366	0.998	0.165	6	5.029
	2	0.999	0.018	3	5.034	0.995	0.066	4	4.146
	3	0.991	0.181	5	3.694	0.998	0.031	6	5.674
	4	0.994	0.254	6	4.149	0.993	0.268	6	4.317
	5	0.996	0.674	7	4.414	0.995	0.591	8	4.727
	6	0.981	1.138	6	2.935	0.997	0.155	7	5.129
	Mean		**0.473**		**4.265**		**0.213**		**4.837**
	± SEM		**± 0.167**		**± 0.362**		**± 0.083**		**± 0.230**
Predicted Finger Prick	1	0.991	1.568	6	3.862	0.994	1.034	7	4.479
	2	0.976	1.119	4	2.630	0.994	0.261	5	4.336
	3	0.941	3.549	5	1.895	0.959	2.381	6	2.516
	4	0.994	0.469	5	4.090	0.993	0.470	6	4.310
	5	0.990	2.126	7	3.808	0.992	1.572	8	4.292
	6	0.956	3.551	6	2.254	0.978	1.632	7	3.232
	Mean		**2.064**		**3.090**		**1.225** *		**3.861** †
	± SEM		**± 0.520**		**± 0.385**		**± 0.324**		**± 0.326**

RSS, residual sum of squares in rectilinear co-ordinates; df, degrees of freedom; MSC, Model Selection Criterion; r^2, coefficient of determination calculated from data in linear coordinates. Differences between the exponential and log-log models are indicated by * (RSS, $P \leq 0.041$), and † (MSC, $P \leq 0.055$). ‡ indicates a difference compared to values derived from the predicted blood lactate response to exercise ($P \leq 0.040$).

4.3.4.3 Blood-tissue lactate exchange in the forearm during exercise and recovery

Arterial and venous blood lactate concentrations, veno-arterial lactate concentration differences and lactate flux throughout the experiment, including 120 min of recovery, are presented in Figure 4.5. Throughout incremental exercise, blood lactate concentrations were significantly lower in venous compared with arterial blood ($P = 0.003$), with the concentration differences increasing with exercise intensity (arterial vs venous by time interaction, $P = 0.001$, Figure 4.5A; veno-arterial lactate concentration difference time effect, $P < 0.001$, Figure 4.5B). However, these differences were consistent with predictions based on passive lactate movements into the forearm tissues. There were no significant differences between measured and predicted venous lactate concentrations ($P = 0.956$, Figure 4.5A), veno-arterial lactate concentration differences ($P = 0.956$, Figure 4.5B) or lactate flux ($P = 0.379$, Figure 4.5C), and no time dependent differences in any of these parameters during incremental exercise ($P > 0.281$). Furthermore, when "corrected" for passive lactate movements, forearm lactate flux did not change with time during incremental exercise ($P = 0.281$, Figure 4.5C).

Although there were no systematic lactate concentration differences ($P = 0.108$), the time course of concentration changes was different in arterial compared to venous blood during recovery ($P = 0.001$, Figure 4.5A). Peak lactate concentrations occurred earlier during recovery in arterial (1.7 ± 1.2, range 0-7.5 min) compared to venous blood (6.7 ± 2.9, range 0-20 min, $P = 0.032$). In addition, blood lactate concentrations were lower in venous compared to arterial blood during the first half of recovery, and higher than arterial in the second half of recovery (Figure 4.5A). Consequently, during the first ~30 min of recovery there was significant net uptake of lactate by the resting forearm, which reversed to net output after ~50 min of recovery and did not return to baseline even after 120 min of recovery (Figure 4.5B,C). There were substantial differences between measured and predicted venous blood lactate concentrations during recovery (main effect $P = 0.006$, interaction with time

interaction, P = 0.007, Figure 4.5A), giving rise to significant and time dependent differences in veno-arterial lactate concentration differences and lactate flux ($P < 0.016$, Figure 4.5B,C). As a result, "corrected" forearm lactate flux indicated net uptake during the first ~50 min of recovery, although the changes with time were not statistically significant (P = 0.102, Figure 4.5C).

4.3.5 Blood hydrogen ions

The relationships between changes in blood H^+ concentrations in arterial and venous blood, veno-arterial H^+ concentration differences and forearm H^+ flux throughout exercise and recovery were similar to those observed for lactate, apart from a higher baseline H^+ concentration in venous compared to arterial blood (Figure 4.6). As with lactate, significant time dependent changes in veno-arterial H^+ concentration differences and H^+ flux during exercise ($P \leq 0.005$, Figure 4.6A,B) were consistent with predictions based on passive forearm H^+ uptake (measured vs predicted H^+ concentration and flux by time interactions, $P \geq 0.304$). During recovery, differences in measured and predicted H^+ concentrations (main effect P = 0.035, interaction with time $P < 0.001$, Figure 4.6A) gave rise to differences between measured and predicted veno-arterial H^+ concentration differences (main and interaction effects, $P \leq 0.035$, Figure 4.6B) and forearm H^+ flux ($P \leq 0.036$, Figure 4.6C), particularly during the first ~50 min of recovery. As with lactate, forearm flux "corrected" for passive H^+ movements indicated forearm H^+ uptake within the first ~50 min of recovery (P = 0.008, Figure 4.6C).

4.3.6 Resting forearm respiratory gas exchange

4.3.6.1 CO_2 exchange

Arterial CO_2 content declined progressively during incremental exercise ($P < 0.001$), reached minimum values ~45% of baseline at ~5 min of recovery and then gradually returned to baseline levels over a period of ~90 min (Figure 4.7A). A positive veno-

arterial CO_2 content difference was maintained throughout exercise and recovery (arterial vs venous, $P < 0.001$; interaction with time, $P \geq 0.227$, Figure 4.7A). There were small increases during exercise, and decreases during recovery in the veno-arterial CO_2 content difference and forearm CO_2 flux, although these effects were only significant for CO_2 flux ($P \leq 0.033$, Figure 4.7B,C). Measured and predicted CO_2 contents (main effect $P = 0.951$, interaction with time $P = 0.311$), and therefore veno-arterial content differences and flux, were similar during exercise. During recovery, the predicted CO_2 content, and consequently the veno-arterial CO_2 content difference, was lower compared to measured values (main effect $P = 0.045$, interaction with time $P = 0.088$, Figure 4.7B). However, this was not associated with differences between measured and predicted CO_2 flux (main effect $P = 0.154$, interaction with time $P = 0.093$, Figure 4.7C). When "corrected" for passive CO_2 movements, resting forearm CO_2 output did not change with time during exercise ($P = 0.211$). Similarly, although there was an initial trend for an increase, "corrected" CO_2 output did not change with time during recovery ($P = 0.093$, Figure 4.7C).

4.3.6.2 O_2 exchange

There was a small but significant increase in arterial O_2 content during incremental exercise ($P < 0.001$) and subsequent decline during recovery ($P < 0.001$, Figure 4.8A). A significant veno-arterial O_2 content difference at rest was maintained throughout exercise and recovery (main effects $P < 0.001$, interactions with time $P \geq 0.140$). During the first half of exercise there was a small decline in venous O_2 content, and consequently a wider veno-arterial O_2 difference (Figure 4.8B) and increased O_2 flux (Figure 4.8C). However, as exercise continued this was replaced by a rise in venous O_2 content and reduced forearm O_2 uptake, although none of these effects reached statistical significance ($P \geq 0.129$). During recovery, there was a significant drop in venous O_2 content ($P = 0.037$), but this was not associated with a significantly widened veno-arterial O_2 content difference ($P = 0.140$). In addition, these effects were offset by a reduction in forearm blood flow such that the measured

forearm O_2 uptake was very similar to baseline levels throughout recovery (Figure 4.8C). Although there were no significant differences in measured and predicted venous O_2 contents, veno-arterial O_2 content differences or O_2 flux during exercise or recovery (main effects $P \geq 0.167$, interactions with time $P \geq 0.086$), venous O_2 content was poorly predicted on the basis of passive O_2 movements (Table 3.2, Figure 4.8). On the other hand, there were no significant changes with time in either measured or "corrected" forearm O_2 flux during exercise or recovery indicating only minor disturbances in resting forearm $\dot{V}O_2$ associated with incremental exercise however forearm $\dot{V}O_2$ was quantified ($P \geq 0.086$, Figure 4.8C).

The forearm RER determined from measured fluxes of CO_2 and O_2 increased throughout incremental exercise ($P = 0.013$), reaching a peak value of 7.8 ± 3.9 before declining over the first ~30 min of recovery to reach values below baseline that were then maintained for the remainder of recovery (Figure 4.9). There were no significant differences between measured and predicted RER during exercise (main effect $P = 0.392$, interaction with time $P = 0.210$) or recovery (main effect $P = 0.093$, interaction with time $P = 0.299$), although measured RER appeared to be higher than predicted throughout. When "corrected" for passive movements of CO_2 and O_2, RER did not change with time during exercise ($P = 0.524$) or recovery ($P = 0.104$, Figure 4.9), and was not different from unity overall ($P \geq 0.404$).

4.3.7 Total net flux of lactate, H^+, CO_2 and O_2 in the resting forearm

At rest there was a small non-significant net production of lactate, a significant net production of H^+ and CO_2, and net consumption of O_2 in the resting forearm (Table 4.6). During exercise, recovery and combined exercise and recovery, measured and predicted net fluxes were similar in all substances with the exception of lactate. In contrast to predicted net lactate output there was net lactate uptake during recovery, and combined exercise and recovery. The total net fluxes of H^+, CO_2 and O_2 were similar to values expected had pre-exercise levels of flux continued throughout the

entire period of study such that there were no significant quantities of these substances that could not be accounted for. In contrast, measured net lactate flux was significantly less than expected such that a significant quantity of lactate could not be accounted for. This could indicate either significant forearm lactate disposal or that 120 min of recovery is insufficient to allow lactate taken up by the forearm during exercise to be returned to the circulation during recovery.

The calculated forearm RER was 0.91 ± 0.10 at rest, and 0.93 ± 0.03 over the combined exercise and recovery period (P = 0.854).

The degree of association between total net flux measured during exercise and that measured during recovery for each of the substances examined was relatively weak, but reached statistical significance for CO_2 (Table 4.6).

Table 4.6. Total net flux of lactate, H^+, CO_2 and O_2 across the resting forearm during rest, supine leg exercise and recovery.

	Lactate (μmol·100 ml^{-1})	H^+ (pmol·100 ml^{-1})	CO_2 (μmol·100 ml^{-1})	O_2 (μmol·100 ml^{-1})
Rest	1.2 ± 1.9	60.4 ± 15.6 †	39.1 ± 6.8 †	-44.3 ± 7.5 †
Rest */min)*	0.2 ± 0.4	12.1 ± 3.1 †	7.8 ± 1.4 †	-8.9 ± 1.5 †
Exercise (25.2 ± 1.1, range 21 – 28 min)				
Measured	-106.9 ± 22.2 †§	134.8 ± 42.5 †	390.2 ± 63.4 †§	-274.3 ± 40.3 †
Predicted	-105.1 ± 19.6 †§	137.8 ± 93.0	440.0 ± 86.3 †§	-356.3 ± 97.6 †
"Corrected"	-5.6 ± 7.6	131.9 ± 71.6	38.4 ± 57.1	-3.4 ± 2.3 ‡§
Expected	6.2 ± 10.2	301.2 ± 75.2	199.1 ± 37.3	-227.6 ± 44.4
Recovery (120 min)				
Measured	-65.6 ± 34.8	677.0 ± 198.5 †	804.6 ± 117.5 †	-996.0 ± 135.7 †
r^2 (vs Exercise)	0.252	0.658 *	0.553	0.278
Predicted	120.0 ± 31.8 †‡§	1170.1 ± 197.1 †	447.1 ± 136.6 †	-735.2 ± 183.5 †
r^2 (vs Exercise)	0.107	0.331	0.507	0.295
"Corrected"	-156.2 ± 56.5 ‡§	956.0 ± 398.5	1296.5 ± 208.3	-1323.8 ± 246.8
Expected	29.4 ± 46.3	1449.1 ± 373.9	938.9 ± 163.5	-1063.0 ± 179.9
Exercise & Recovery (140 min)				
Measured	-172.5 ± 49.8 †§	811.8 ± 223.9 †	1194.8 ± 172.9 †	-1270.3 ± 167.9 †
Predicted	14.8 ± 42.5 ‡	1308.0 ± 259.6 †	887.0 ± 199.2 †	-1091.5 ± 262.1 †
"Corrected"	-161.8 ± 60.0 §	1087.9 ± 415.5 §	1334.9 ± 236.0	-1327.3 ± 248.7
Expected	35.5 ± 56.5 ‡	1750.3 ± 448.6 †	1138.0 ± 200.2 †	-1290.6 ± 223.9 †
Unaccounted	-208.1 ± 63.0 †	-938.5 ± 527.7	56.8 ± 258.0	20.3 ± 201.7

Positive values indicate net output, and negative values net uptake. Measured, predicted and "corrected" values were calculated as the net area under the time versus respective flux curves. Expected values were calculated by multiplying the resting flux (/min) by the corresponding duration of exercise, recovery or both. Unaccounted values were calculated as the difference between measured and expected values. * indicates a significant degree of association, + a difference from a value of zero, ‡ from measured and § from expected ($P < 0.05$). All values are means ± SEM, n = 6.

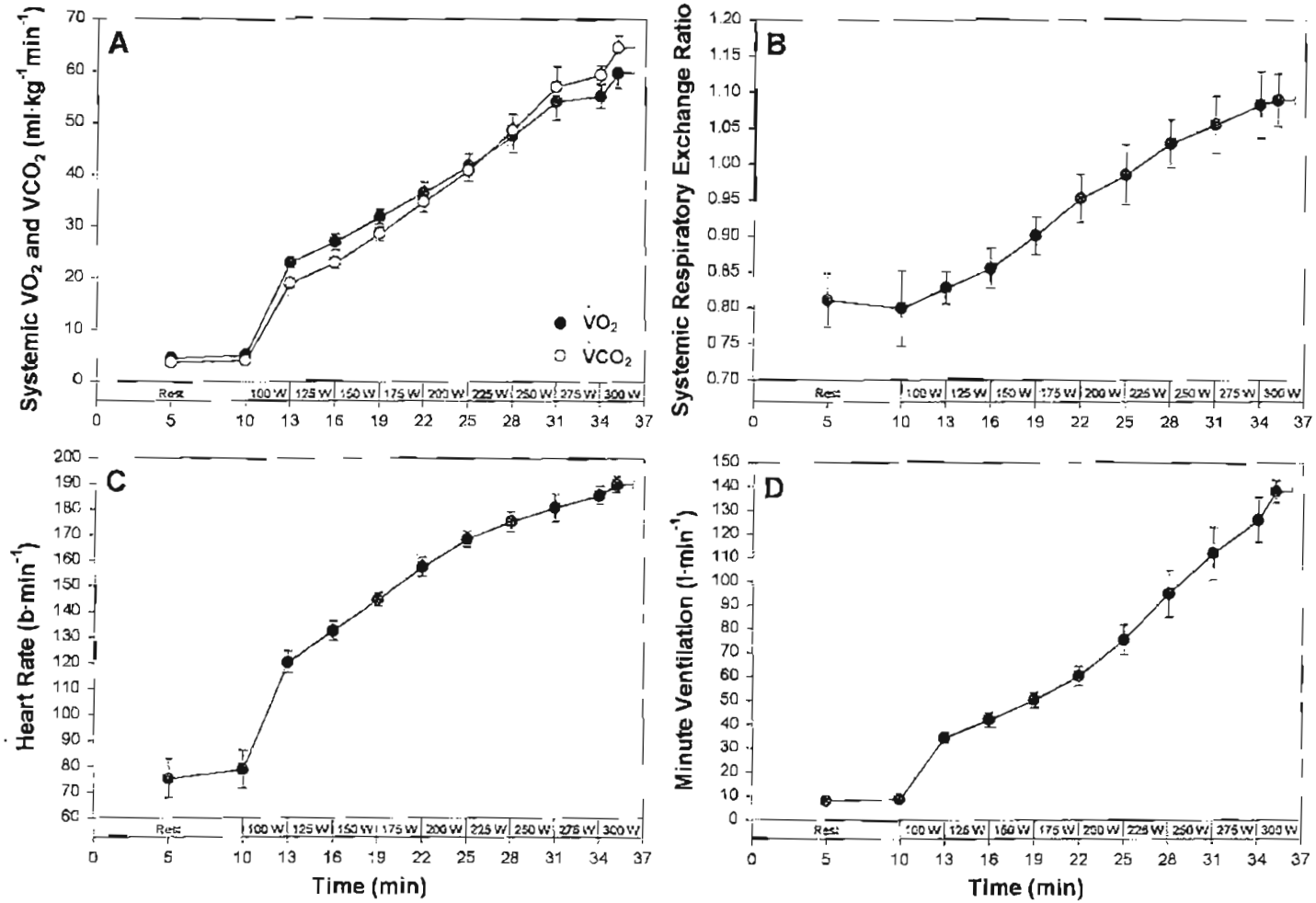


Figure 4.1. Systemic $\dot{V}O_2$ and $\dot{V}CO_2$ (A), RER (B), heart rate (C), and $\dot{V}_E$ (D) during incremental exercise to exhaustion. Values are means ± SEM, n = 6, except at the 275 watt work load in which n = 5. The final data point represents the mean ± SEM time and ordinate value associated with the highest work load completed by each subject.

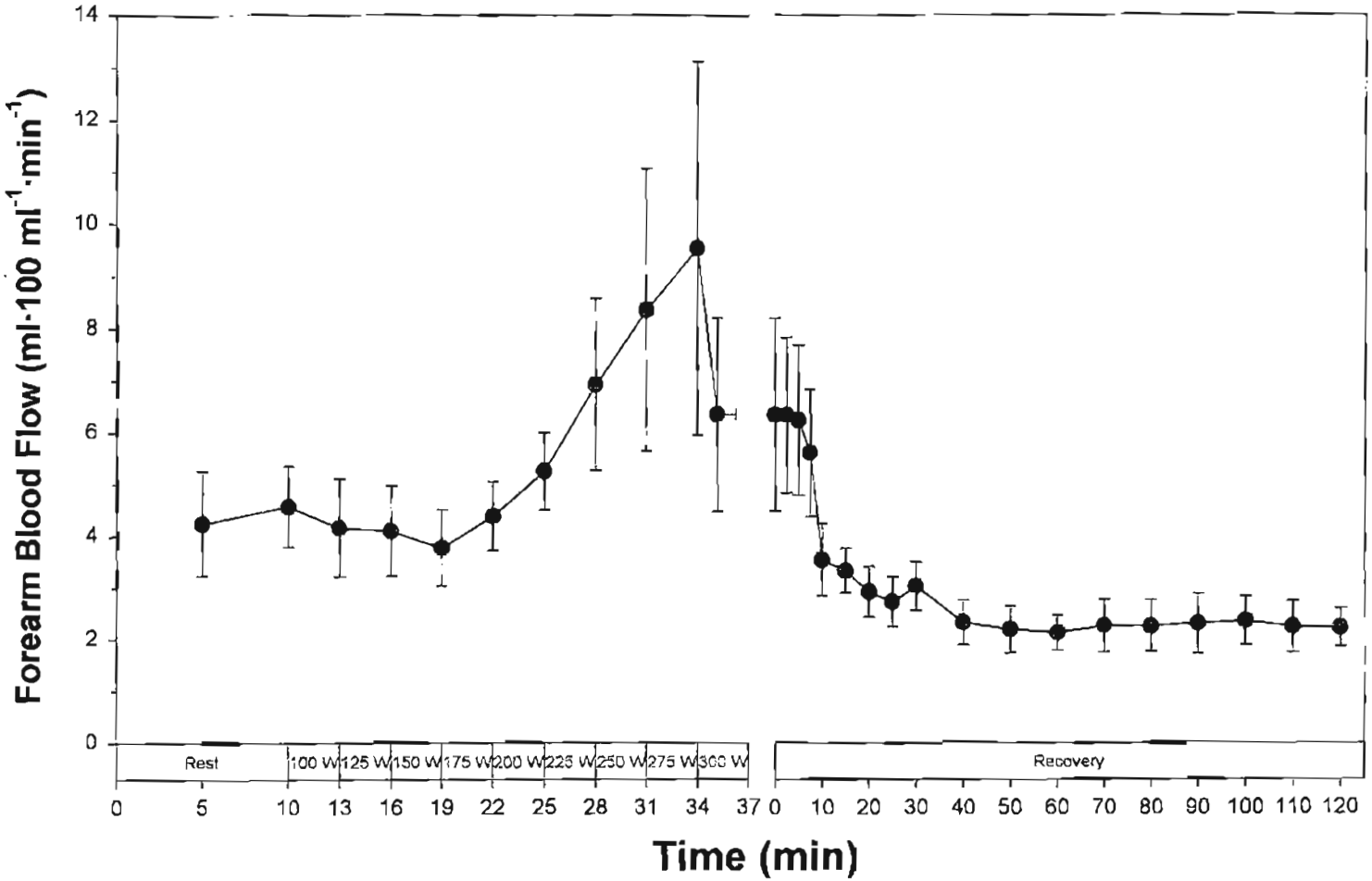


Figure 4.2. Resting forearm blood flow during incremental supine leg exercise and 120 min of recovery. Values are means ± SEM, n = 6, except at the 275 watt work load in which n = 5. The final data point in exercise represents the mean ± SEM time and blood flow associated with the highest work load completed by each subject.

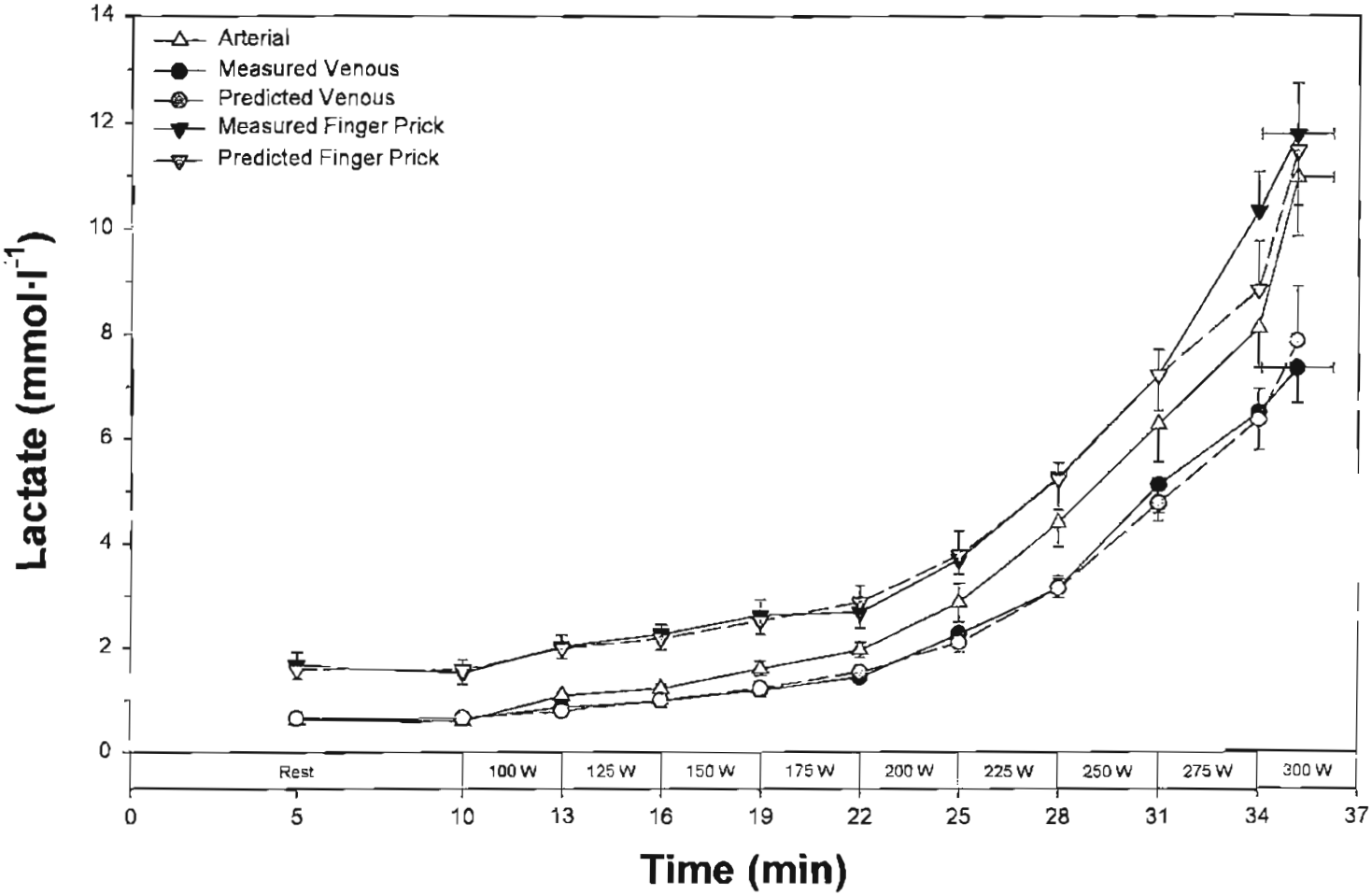


Figure 4.3. Arterial, venous and finger prick blood lactate concentrations during incremental supine leg exercise to exhaustion. Values are means ± SEM, n = 6 except at the 275 watt work load in which n = 5. The final data point represents the mean ± SEM time and lactate concentration associated with the highest work load completed by each subject.

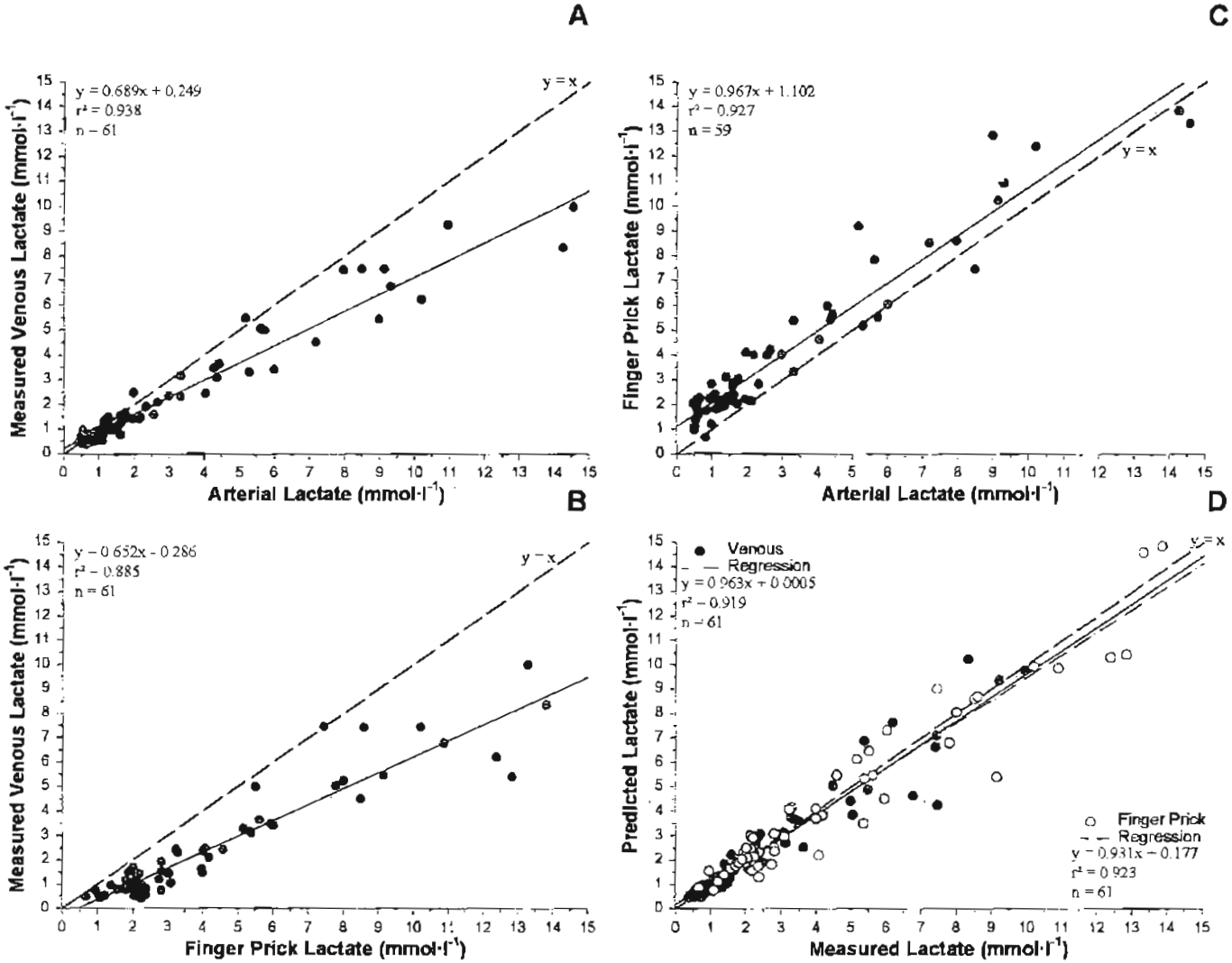


Figure 4.4. Relationships between blood lactate concentrations determined from arterial, forearm venous and finger prick blood samples (A-C), and between measured venous and finger prick concentrations and those predicted on the basis of passive lactate movements between the blood and the forearm tissues during incremental exercise to exhaustion (D). n = 6 subjects.

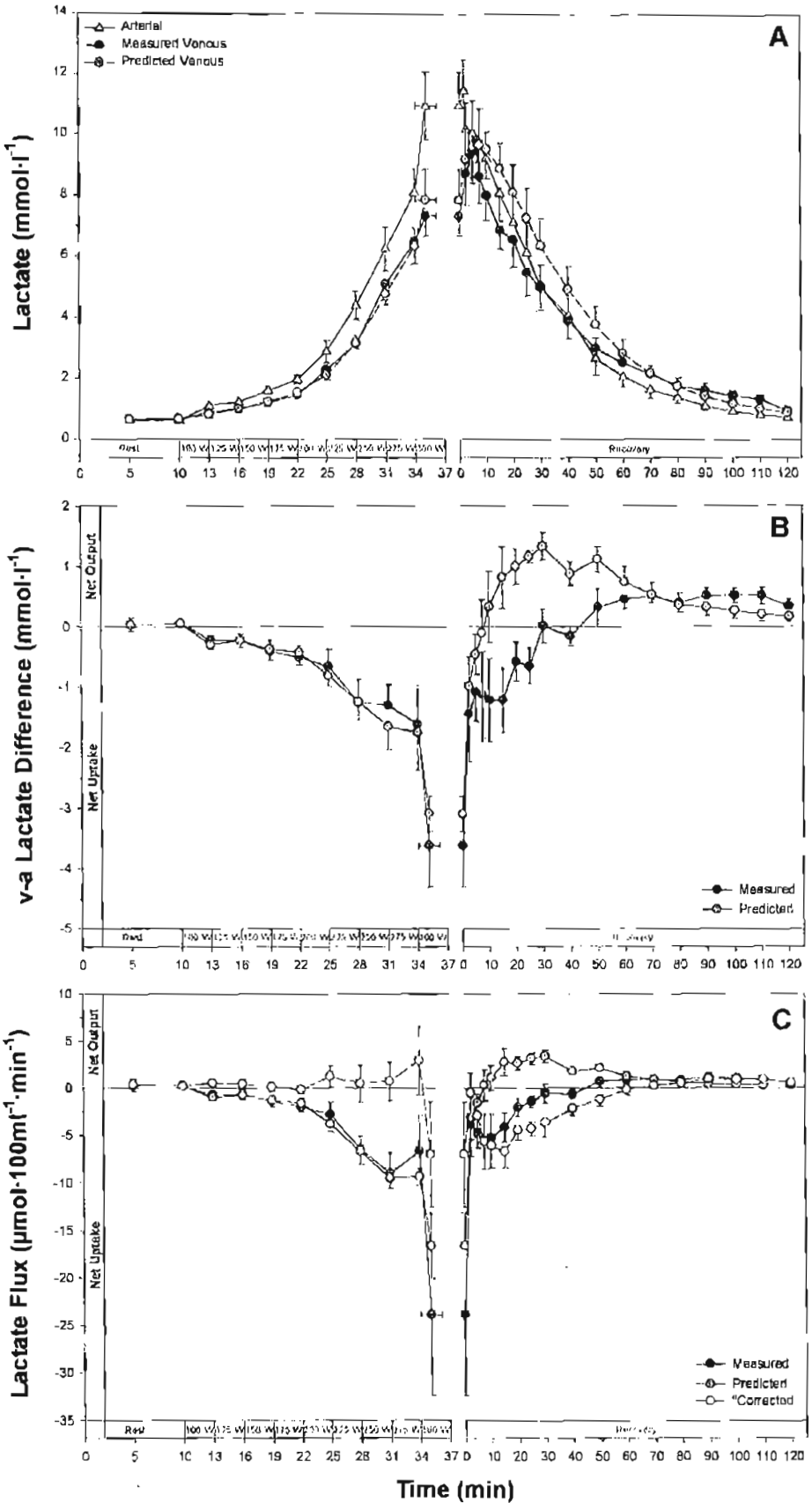


Figure 4.5. Blood lactate levels (A), veno-arterial lactate difference (B) and lactate flux (C) across the resting forearm before, during and after supine leg exercise. Values are means ± SEM, n = 6. "Corrected" = Measured – Predicted + Baseline.

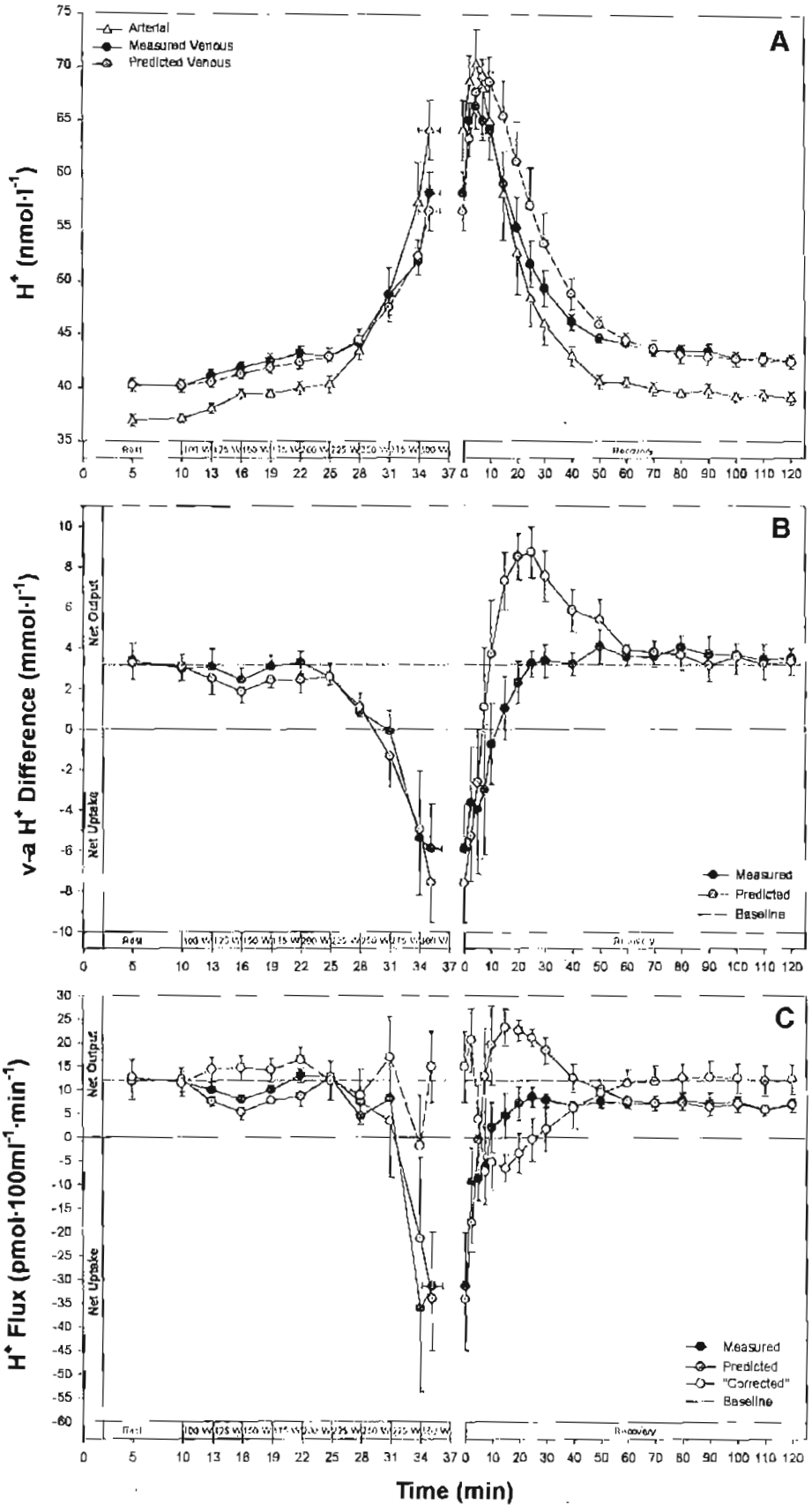


Figure 4.6. Blood H^+ concentrations (A), veno-arterial H^+ concentration difference (B) and H^+ flux (C) across the resting forearm before, during and after supine leg exercise. Values are means ± SEM, n = 6. "Corrected" = Measured – Predicted + Baseline.

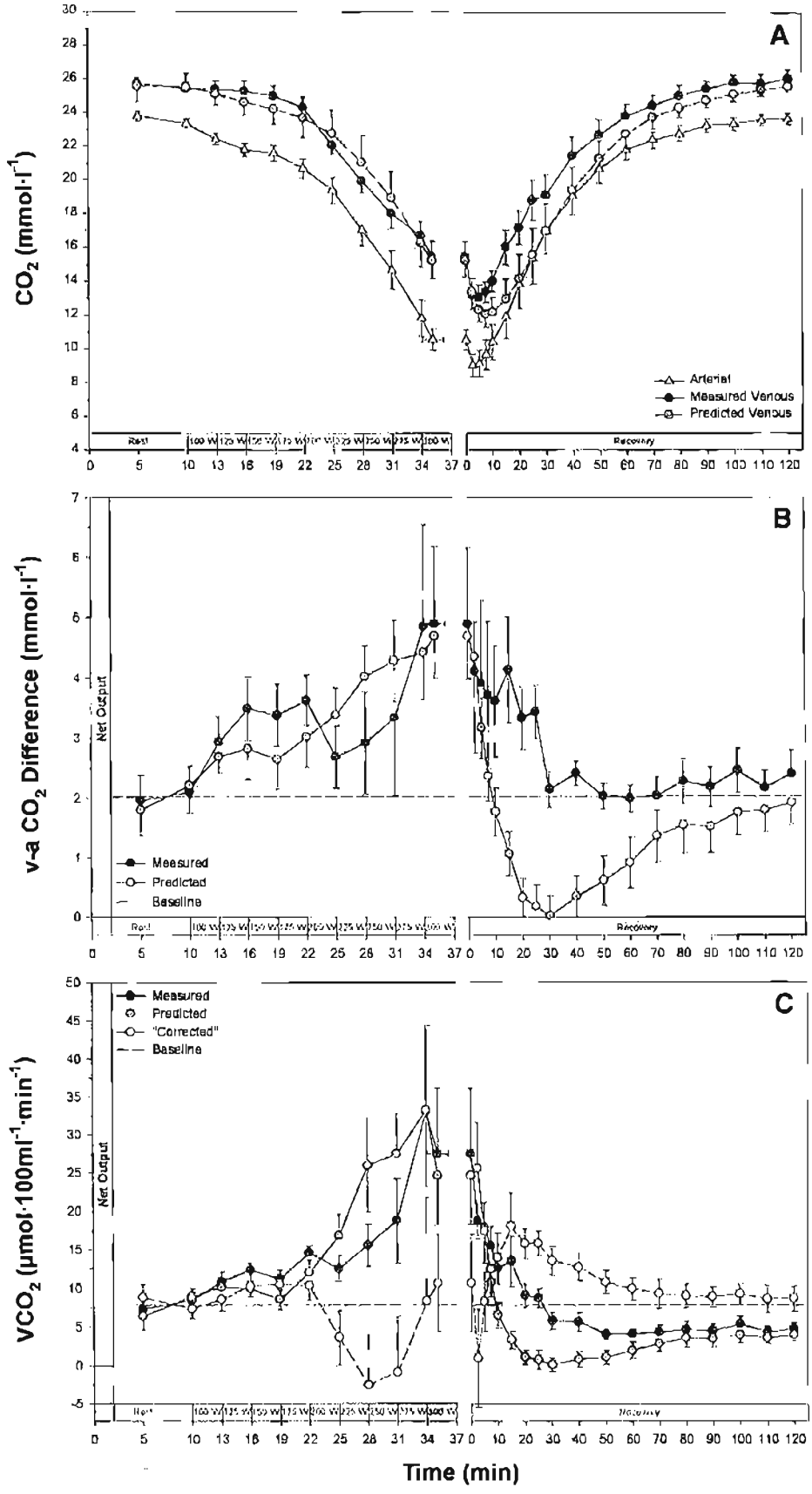


Figure 4.7. Blood CO_2 contents (A), veno-arterial CO_2 content difference (B) and $\dot{V}CO_2$ (CO_2 flux, C) across the resting forearm before, during and after supine leg exercise. Values are means ± SEM, n = 6. "Corrected" = Measured – Predicted + Baseline.

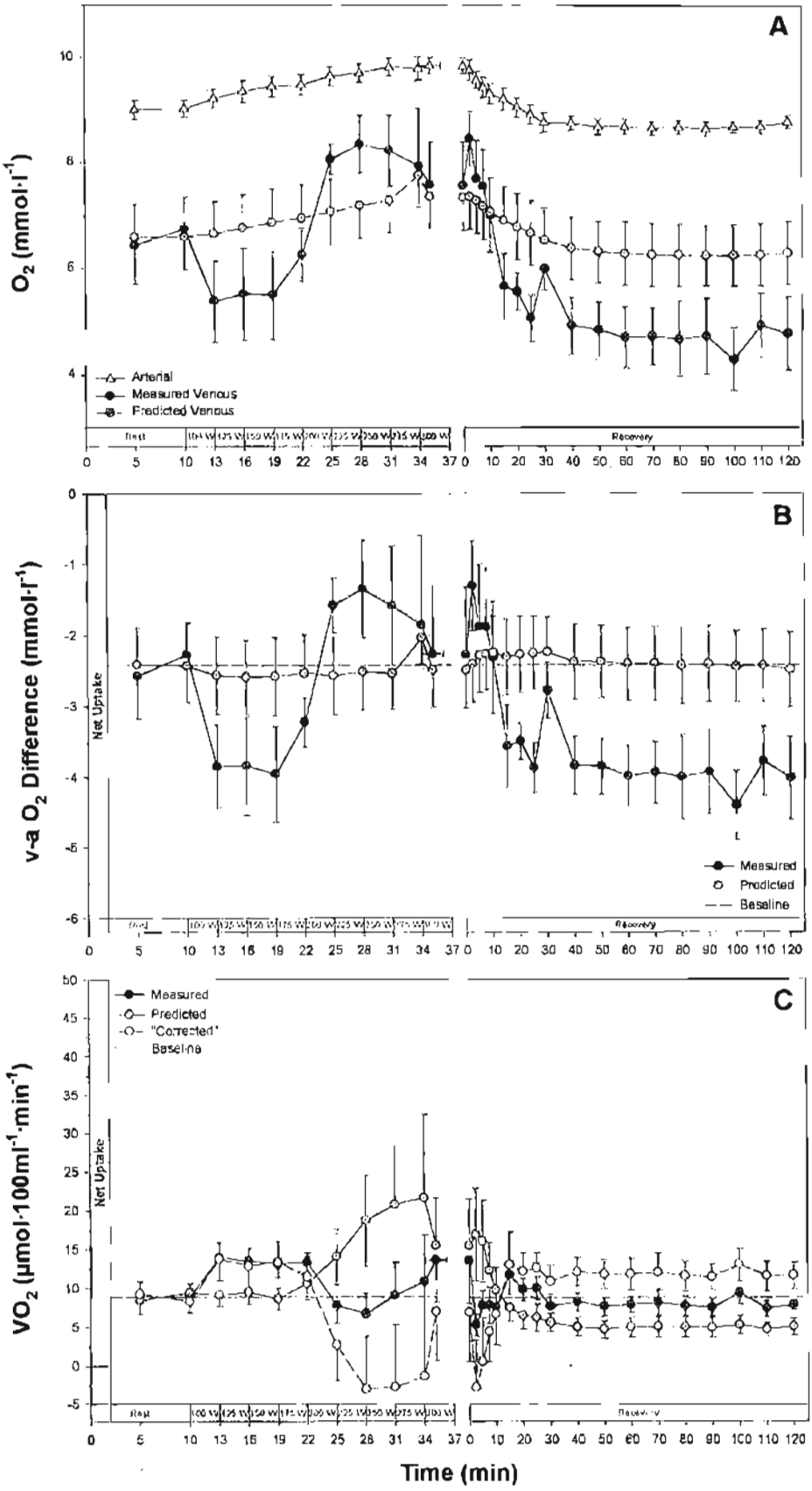


Figure 4.8. Blood O_2 contents (A), veno-arterial O_2 content difference (B) and $\dot{V}O_2$ (-ve O_2 flux, C) across the resting forearm before, during and after supine leg exercise. Values are means ± SEM, n = 6. "Corrected" = Measured – Predicted + Baseline.

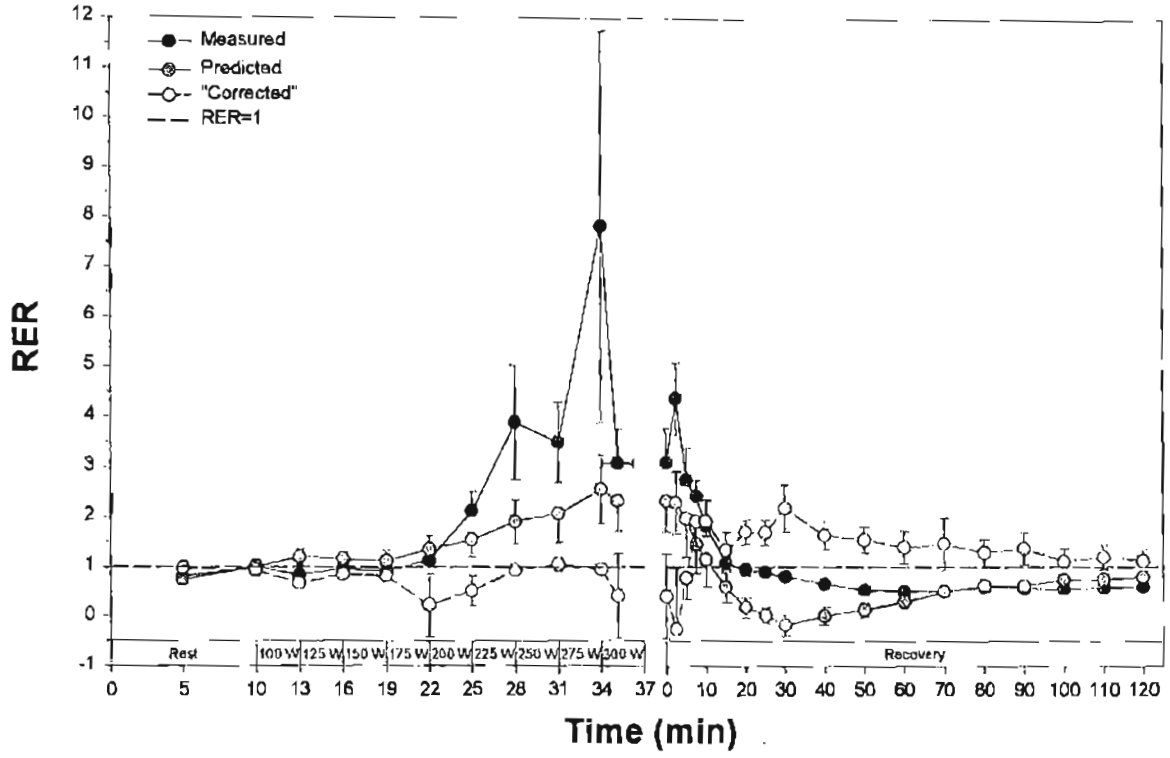


Figure 4.9. Forearm respiratory exchange ratio (RER) before, during and after supine leg exercise. Values are means ± SEM, n = 6. "Corrected" = Corrected $\dot{V}CO_2$ divided by corrected $\dot{V}O_2$.

4.4 Discussion

The main finding of the present study was that the forearm tissues significantly distort the blood lactate response to incremental exercise, and therefore confound interpretation of systemic lactate kinetics, including measures of the lactate threshold. Although this is in general agreement with similar studies (80, 86, 87, 211, 284), data from the present study indicate that forearm lactate metabolism is not responsible for these differences, as has previously been suggested (282, 284). Close agreement between measured forearm venous blood lactate levels and those predicted on the basis of passive lactate movements due to transient changes in arterial lactate concentrations suggests that equilibrium kinetics dominate arteriovenous blood lactate concentration differences under these conditions. In contrast, there were no significant time dependent differences in lactate concentration between arterial and finger prick blood, with a relatively stable rate of skin lactate production appearing to be superimposed on to exercise related changes in arterial lactate levels. However, this was also consistent with passive lactate movements because blood-tissue equilibration was predicted to be faster in the smaller tissue volume of the skin, compared to the presumably larger tissue volume from which antecubital venous blood was derived. In addition, a thermoregulatory increase in skin blood flow during exercise would further promote lactate concentration changes in arterial blood to be more faithfully transmitted to finger prick compared to forearm venous blood.

Although arteriovenous blood lactate concentration differences during exercise could be attributed to passive lactate movements, significant net uptake over the combined exercise and recovery period could indicate resting forearm lactate disposal overall. Based on blood lactate responses "corrected" to remove the predicted effects of passive forearm lactate movements, it would appear that forearm lactate disposal occurred during the first ~50 min of recovery, and not during leg exercise. While this appears consistent with the "corrected' time course of changes in H^+ and O_2 uptake, CO_2 output and RER, which might be expected to increase in parallel with lactate

disposal via oxidation, there was no evidence that total net forearm flux of H^+, CO_2 or O_2 changed from baseline levels overall. These apparently disparate results most likely indicate limitations in the modified Fick equation to predict accurately forearm venous concentration changes during the non-equilibrium conditions associated with incremental exercise and recovery. If one accepts that passive movements were responsible for the observed changes in forearm flux, as is strongly supported by total net flux data, at least for H^+, CO_2 and O_2, it follows that venous levels were sometimes under-predicted and at other times over-predicted. Furthermore, the modified Fick equation produced poor predictions of venous O_2 levels suggesting that its predictive ability is somewhat substance dependent. This could reflect differences in the magnitude of arterial concentration disturbances between substances, or indicate a limitation in the Fick model to predict the membrane hindrance component of blood-tissue exchange. Arterial changes in O_2 content (and pO_2) were relatively minor (at most ~1.09 x baseline) compared to changes in lactate (~14 x baseline), H^+ (~1.7 x baseline) and CO_2 content (~0.45 x baseline). In addition, while the modified Fick equation used in the analysis was effectively a 2 compartment model incorporating a membrane hindrance component (m) to blood-tissue equilibration, the value for this term was effectively an arbitrary constant for all substances ($100^2 \cdot Vi^{-1}$). This would undoubtedly limit the ability of the model to predict venous concentrations of different substances. Although such limitations would distort the apparent time course of changes in passive and consequently metabolically related flux, these distortions are likely to be less than would be apparent if passive movements were ignored altogether. Furthermore, measured total net flux is independent of predictions and would not be affected by these limitations. Predicted total net flux would also be unaffected because, by definition, the net area under the passive flux curve equals the net area under the baseline, provided areas are calculated up to the point of complete recovery.

Despite the apparent limitations in the integrated form of the Fick equation, passive substance movements in the resting forearm during and after leg exercise clearly

dominate changes in the forearm flux of CO_2, H^+ and almost certainly lactate. Consequently, passive movements cannot be ignored in the interpretation of the time course of the fluxes of these substances in the resting forearm under these conditions. If passive substance movements were under-estimated, with some uptake equated with disposal and output with production, it would appear that the resting forearm disposed of lactate and H^+ and produced CO_2 during leg exercise and early recovery. These are essentially the conclusions of several similar studies (46, 161, 203). However, it would also appear that after ~50 min, and for the remainder of recovery, lactate output predominated. At the same time, H^+ and CO_2 production appeared to be maintained at below resting levels. These observations are more consistent with passive substance movements than changes in forearm metabolism, and suggest that exercise related forearm tissue and venous lactate concentration disturbances might persist even beyond 2 h post-exercise. Consequently, it is likely that at least some of the total quantity of lactate that could not be accounted for in the present study remained sequestered within the forearm tissues and had not yet been returned to the circulation by the end of recovery. This possibility is difficult to confirm because very few studies have obtained arterial and resting forearm venous or tissue lactate concentration measurements during a sufficiently long recovery period. Perhaps the only comparable study is that of Lindinger et al. (161) who obtained arterial, forearm venous and resting muscle (deltoid) lactate concentration measurements during 90 min of recovery following repeated bouts of supramaximal leg exercise. There was no evidence of forearm lactate output during recovery, although venous lactate levels appeared to exceed arterial levels slightly at the last two sampling points (60 and 90 min). However, arterial lactate levels were higher (peak, ~18 $mmol \cdot l^{-1}$; 90 min recovery, ~3 $mmol \cdot l^{-1}$), and therefore the disturbances in forearm tissue and venous blood lactate concentrations probably greater, than in the present study (peak, ~11 $mmol \cdot l^{-1}$; 90 min recovery, ~1 $mmol \cdot l^{-1}$). This would tend to prolong recovery and delay prominent forearm lactate output. Furthermore, while peak lactate levels occurred around 5 min of recovery in venous and tissue samples, lactate concentrations appeared to have

declined in venous blood, but not in resting muscle, by the time the last biopsy sample was obtained at 25 min of recovery. The persistent elevation in tissue lactate concentration post exercise clearly demonstrates the capacity for forearm tissues to act as a passive sink for lactate, and suggests that subsequent lactate release continues over a protracted period, possibly well beyond 90-120 min of recovery.

Without the benefit of complete recovery measurements it is not possible to discount significant resting forearm lactate disposal as the fate of at least some of the lactate that could not be accounted for in the present study. However, this is not entirely consistent with other data. Unless buffered, forearm lactate oxidation should have an alkalinising effect on forearm tissues (99), and therefore be associated with an overall net H^+ uptake, or at least a reduction in overall net H^+ output. Although there was a small quantity of H^+ unaccounted for, this quantity was not significantly different from zero. In addition, over the ~2.5 h period of study there was no evidence that forearm total net O_2 or CO_2 flux, or RER changed from resting levels. A substantial increase in CO_2 output and therefore RER during exercise and early recovery, which has previously been attributed to increased carbohydrate (lactate) oxidation (46, 161), is more consistent with passive CO_2 movements alone. Furthermore, the presence of RER values far in excess of unity indicates that processes other than metabolism must dominate this measurement. Attempts to equate such values with increased carbohydrate metabolism are therefore difficult to reconcile.

If lactate oxidation had been the fate of the quantity of lactate that could not be accounted for by the end of recovery (~200 µmol·100 ml^{-1}), a total of ~600 µmol·100 ml^{-1} of O_2 and CO_2 would have been required and produced respectively:

$$200 \cdot (C_3H_5O_3^- + H^+ + 3O_2) = 200 \cdot (3CO_2 + 3H_2O) \cdot$$

As there was no evidence that forearm $\dot{V}O_2$ or $\dot{V}CO_2$ changed overall, this would be equivalent to ~50% of the total $\dot{V}O_2$ and $\dot{V}CO_2$ (~1200 μmol·100 ml^{-1}) measured over the combined exercise and recovery period. Whilst plausible, this appears unlikely. Carbohydrates are believed to be quantitatively less important than non-carbohydrates in supplying resting energy requirements (38), including those of resting muscles (7). Brooks et al. (38) suggested that ~40% of resting $\dot{V}O_2$ reflects carbohydrate oxidation. Consequently, even if lactate replaced all other carbohydrates as an energy source, the complete oxidation of the quantity of lactate that could not be accounted for in the present study could not have been achieved without an increase in $\dot{V}CO_2$ and RER and/or an increase in $\dot{V}O_2$. Others have speculated that lactate oxidation could account for between 12%-24% (46), ~18% (203), ~38% (161) and 70% (95) of resting muscle $\dot{V}O_2$. However, of necessity, such estimates are usually based on the assumption that oxidation is the primary fate of lactate that cannot be accounted for, and generally reflect upper limits of plausibility. Under-estimates of lactate disposal via alternative pathways such as gluconeogenesis or fatty acid synthesis, or an inflated estimate of total lactate disposal associated with failure to adequately account for equilibration processes would lead to over-estimates of lactate oxidation. Without the benefit of blood flow and arterial and venous lactate concentration measurements to the point of complete recovery, it is difficult to verify how much of the lactate taken up by resting muscles is subsequently oxidised. In the present study, lactate oxidation could theoretically account for as little as zero, or as much as ~50% of the total forearm $\dot{V}O_2$.

Despite evidence for a dominant role of passive lactate movements in forearm lactate uptake during leg exercise, an elevation in muscle lactate concentration in itself would be expected to favour lactate disposal via mass action pyruvate formation. However, the quantity of pyruvate so formed might be small given that the muscle LDH isomer favours lactate formation and normally sustains a high lactate to pyruvate ratio even at rest (231). Nonetheless, pyruvate and acetyl-CoA formed in this manner might be expected to compete favourably with glycolytically derived

pyruvate and acetyl-CoA from β-oxidation as substrates for processes such as oxidation, gluconeogenesis and alanine formation. However, the energy requirements of resting muscle are small, representing perhaps ~5% of muscle aerobic capacity (95). Furthermore, in resting glycogen replete muscle lactate incorporation into glycogen (193, 195, 228) may principally reflect glycogen turnover with a relatively minor contribution from net glycogen synthesis. In addition, for pyruvate derived from accumulated lactate to replace alternative substrates, the reactions from which pyruvate and acetyl-CoA would normally be derived must be inhibited. Despite the potential for pyruvate formation from lactate to influence cytosolic redox state and therefore potentially glycolysis, lactate inhibition of lactate dehydrogenase activity (231), combined with an already low rate of glycolysis, may limit the capacity for glycolytic flux to be further reduced. Similarly, acetyl-CoA accumulation in the absence of an increase in metabolic rate could, via an increase in citrate and the ratio of acetyl-CoA to free CoA, inhibit pyruvate dehydrogenase activity (209), thereby limiting the entry of pyruvate into the citric acid cycle. Consequently, the overall capacity for resting muscle to dispose of lactate could be quite small, particularly if exercise related blood flow redistribution away from resting muscles combined with catecholiminergic stimulation of glycolysis (239, 243) simultaneously promote glycolytic pyruvate and therefore lactate formation, as some data might suggest (3). In contrast, when metabolic rate and consequently pyruvate oxidation are augmented by exercise, there is clear evidence that muscles do dispose of significant quantities of lactate when arterial lactate concentrations are elevated (73, 95, 124, 247).

Irrespective of the fate of lactate within the forearm, arterial blood was modified on its passage through the forearm tissues such that there were differences in several measures of the LT determined from arterial, forearm venous and finger prick blood. Since the usual purpose of exercise testing is to characterise the systemic response to exercise, the choice of blood sampling site is potentially an important consideration in LT testing, particularly if training prescriptions are based on LT measurements.

Clearly, arterial samples most accurately reflect systemic lactate accumulation, but arterial sampling procedures are invasive. Venous samples, while less invasive, are clearly influenced by forearm lactate uptake, although in the present study this was not associated with LT determinations that were significantly different from those measured from arterial blood. Finger prick blood samples, whilst most convenient, were associated with the greatest LT differences compared with those determined from arterial, and in particular venous blood. On the other hand, LT differences were mainly confined to fixed concentration thresholds, which tended to occur earlier in finger prick compared to arterial and venous blood. LT determinations such as the log-log LT, LSI and perhaps the IAT, which are based on the time course of lactate concentration change, were little affected by blood sampling site. Furthermore, in many cases serial determinations of the LT are of greatest interest, in which case the choice of blood sampling site may be less important as long as it is consistent between serial measurements. In contrast, if training regimens are based on LT determinations, failure to consider the influence of the forearm tissues, particularly on fixed concentration LT measurements, could lead to the selection of sub-optimal training work loads. Although absolute blood lactate concentrations, and therefore fixed concentration lactate thresholds, are modified by training status, they can also be influenced by other factors. For example, it has long been recognised that arterial oxygenation has a profound effect on blood lactate levels and blood lactate responses to incremental exercise (115, 118). Similarly, antecubital venous, and presumably arterial blood lactate levels, are higher following glucose ingestion and lower following fat ingestion compared to fasting levels, and these differences are maintained throughout a subsequent incremental exercise test to exhaustion (125, 145, 264). As a result, fixed concentration thresholds may be more subject to pre-exercise dietary influences, and therefore of more limited value compared to LT measurements based on the time course of lactate concentration change.

Similar lactate concentration (65, 80, 87, 211, 284) and LT differences between blood sampling sites (80, 211, 284) have been reported in other studies in which cycling

exercise was performed. In contrast, Williams et al. (276) found no significant difference between arterial and venous blood lactate levels during incremental treadmill exercise. A methodological difference that could account for this discrepancy is that the forearm is more active during treadmill running compared to leg cycling exercise. Under these conditions forearm lactate production and blood flow might be augmented, tending to elevate venous lactate levels and hasten the equilibration between arterial and venous blood.

Many, and perhaps all LT measurements in popular use, including those examined in this study, reflect somewhat arbitrary parameters derived from empirical models of the blood lactate increase during incremental exercise. The log-log model of Beaver et al. (22) is based on the premise that blood lactate accumulates in a threshold manner, whilst the IAT and LSI are founded on the assumption that lactate accumulates exponentially. Fixed concentration thresholds have no theoretical basis whatsoever, but, as with many measures of the LT, strong associations between these and endurance exercise capacity have consistently been demonstrated (66, 110, 232, 250, 255, 283). It would therefore appear that such measures provide valuable tools for assessing endurance exercise capacity and optimising training protocols, although the parameters so derived are largely descriptive with no real biological meaning.

Despite the empirical approach, the arterial blood lactate response to incremental exercise was more consistent with progressive lactate accumulation than a threshold phenomenon, at least when assessed using a log-log model. While this observation cannot exclude the presence of an underlying threshold process (181), it does add support to other studies in which the arterial or arterialised blood lactate response to incremental exercise was better fit with an exponential model (43, 69, 122). In addition, the finding that both models fit equally well in venous and finger prick blood suggests that lactate uptake processes, at least in the forearm, significantly influence the apparent nature of the blood lactate response to incremental exercise. This is

potentially important, because similar processes operating throughout the systemic circulation will almost certainly modify the arterial blood lactate response to incremental exercise. From this viewpoint, the whole concept of a lactate threshold becomes unclear, since a threshold of production, disposal or both could theoretically exist. It could be argued that such processes might also produce an apparently progressive increase in arterial blood lactate concentrations. However, more sophisticated models based on a systems approach (41, 183, 184) also predict that arterial lactate concentrations increase in a progressive manner during exercise, and that small changes during early exercise may be difficult to detect experimentally due to equilibrium processes operating systemically (41). The simplest and most consistent explanation of the available data would appear to be that arterial lactate increases progressively, and not as a threshold, during incremental exercise.

Despite perhaps important limitations in the lactate threshold concept itself, LT measurements based on this concept are no more arbitrary than other parameters derived from the blood lactate response to incremental exercise. Consequently, whether a threshold exists or not, the LT appears to have merit as a measure of endurance exercise capacity (283), particularly given that venous and finger prick log-log LT measurements were similar to arterial in the present study. Furthermore, the approach of systematically dividing log $\dot{V}O_2$ and log lactate concentration data into all possible combinations of 2 line segments appears to be a useful approach in the identification of the LT (43). This approach obviates the need for an observer to identify the division point between two line segments, is therefore more objective than the original method (22) and potentially avoids inter-observer differences that have been reported in LT determinations (100, 101). This approach also ensured that both the log-log and exponential models were fit optimally. Without this consideration, the exponential model would tend to be favoured due simply to the optimisation approach of least squares.

A potentially important methodological consideration revealed by this approach relates to the inclusion of the data point between the two selected line segments. In the original description of the log-log procedure, Beaver et al. (22) state that:

> "The data points are divided into two segments, and the data point between them (the division point) is assumed to belong to both segments, since there is no statistical reason for assigning it to one or the other segment."

However, in only one of the 30 log-log fits obtained from arterial, measured and predicted venous and finger prick blood in this study was the optimal fit obtained when there was a data point common to both line segments. This suggests that the optimal fit generally occurs when there is no point common to both segments, at least with 3 min work load increments.

In summary, the present study has demonstrated that forearm tissues significantly distort the blood lactate response to incremental exercise and can therefore influence lactate threshold determinations, particularly those based on a fixed blood lactate concentration. Furthermore, passive lactate movements between arterial blood and the forearm tissues associated with non-equilibrium lactate concentration conditions could entirely account for the observed changes in finger prick and forearm venous blood lactate concentrations during incremental exercise. Similarly, substantial CO_2 output and a shift towards H^+ uptake in the resting forearm during leg exercise were also well predicted on the basis of passive CO_2 and H^+ movements associated with arterial concentration changes during exercise. Although there were differences between measured and predicted forearm lactate, H^+ and CO_2 flux during recovery that could be interpreted to indicate changes in forearm metabolism, these most likely reflected limitations in the modelling analysis. Supporting this view were the findings that total net forearm $\dot{V}O_2$, $\dot{V}CO_2$ and H^+ flux over the entire period of exercise and recovery were consistent with baseline levels of flux. Whilst a significant quantity of lactate taken up by the forearm during leg exercise could not be accounted for by

the end of recovery, there was evidence that the forearm muscle lactate content remained elevated even after 120 min of recovery. Consequently, although increased forearm lactate disposal could not be excluded, these data strongly suggest that passive substance movements between the blood and forearm tissues dominate veno-arterial concentration differences during and for a long time after supine leg exercise.

CHAPTER 5 Nitrous oxide, lactate, and respiratory gas exchange in the resting forearm during and after moderate intensity supine leg exercise and N_2O inhalation

5.1 Introduction

SKELETAL MUSCLE can both produce and metabolise lactate, the net effect within a given muscle depending on many factors including muscle metabolic rate (73, 95, 97, 124, 247), aerobic capacity and fiber type composition (11, 193, 195), degree of capillarisation (258), lactate concentration (97, 258) and to some extent blood flow (96, 266). In exercising muscles, the balance is usually in favour of production, with the rate of production being governed largely by exercise intensity and duration. When arterial lactate levels are elevated by infusion, prior exercise or exercise in remote muscle groups, exercising muscle has been shown to take up lactate in an intensity dependent manner. In muscles not directly involved in exercise (ie "resting" muscles), in which metabolic rate is low, the net effects of lactate production and metabolism are less clear. Based on measurements of resting muscle arterio-venous lactate concentration differences, several authors have suggested that resting muscles are an important site for net lactate metabolism when arterial lactate levels are elevated by infusion (46, 94) or by exercise (161, 195, 203, 247). However, in all of these studies non-steady-state arterial and venous lactate concentrations prevailed. Under these conditions, a negative resting muscle veno-arterial lactate concentration difference (lactate uptake) does not necessarily indicate lactate disposal. Transient changes in arterial concentrations make the interpretation of any veno-arterial concentration difference extremely difficult. For example, for any blood borne substance capable of blood-tissue exchange, and in the absence of any other changes, a rapid increase in arterial concentration will produce a more gradual rise in resting tissue and venous concentrations simply due to equilibration processes.

Tissue uptake under these conditions reflects an increase in the arterial-tissue concentration gradient that persists until the tissues fully equilibrate with the new arterial concentration. The rate at which the venous concentration approaches that in the arterial blood will depend on several factors such as blood flow, the magnitude of the initial concentration change, the ease with which the substance exchanges between the blood and tissue compartments, and the volume and arrangement of the tissues components (ie the vascular network, interstitial space, fat depots, muscle, bone and connective tissues). If blood-tissue exchange is very rapid then principally blood flow and tissue volume govern the rate at which a new equilibrium is attained (178, 180). When there are significant diffusion or membrane transport limitations, these potentially dominate, and in many cases both membrane transport processes and perfusion will determine the time course of equilibration (178, 180). When the substance of interest is a metabolic substrate or metabolite such as lactate, arterial concentration or tissue blood flow changes that affect delivery have the potential to alter tissue metabolism itself, and therefore influence uptake. However, veno-arterial concentration changes associated with altered metabolism are difficult to distinguish from changes related to blood-tissue equilibration, and must therefore be interpreted with considerable caution.

Lactate uptake associated with equilibration between the arterial blood and resting forearm tissues is likely to dominate antecubital veno-arterial lactate concentration differences, because antecubital venous blood derives from a relatively large and poorly perfused vascular bed (48). Consequently, unless lactate metabolism can be otherwise measured, or arteriovenous concentration measurements be continued until venous and tissue concentrations stabilise, it is not possible to measure resting muscle net lactate disposal or production reliably without quantifying the effects of passive lactate movements when non-steady-state conditions prevail. Conventional lactate tracer techniques are also of no benefit under these conditions. Passive movements influence labeled and unlabeled lactate alike. Furthermore, the LDH reaction is near equilibrium, and label has been shown to rapidly equilibrate between

the lactate and pyruvate pools (278). Consequently, increased labeled CO_2 elimination could reflect steady-state pyruvate rather than net lactate oxidation, particularly given that reduced arterial CO_2 levels during leg exercise favour net CO_2 efflux from the forearm (Chapters 3 and 4).

There are essentially only three methods available to distinguish lactate uptake associated with equilibration from that associated with an increase in resting muscle lactate disposal based on non-steady-state arterial and venous concentration measurements. The first and simplest is to determine the quantity of lactate returned to the circulation over the time course of complete recovery of arterial, venous and resting muscle lactate concentrations. From previous reports (161) and the data presented in Chapters 3 and 4, it appears that more than 2 hours of recovery are necessary for this approach to be completely reliable following strenuous leg exercise. In addition, such measures are variable and therefore relatively insensitive for detecting overall changes in net metabolism, and provide no information regarding the time course of changes in net lactate production or disposal in a resting muscle. This is potentially the most important limitation of this approach because increased lactate production could follow a period of significant lactate disposal so that little or no overall change in net metabolism would be detected. The second approach is to measure resting tissue lactate content in addition to arterial and venous concentration and blood flow measurements. In the presence of lactate disposal, the increase in tissue lactate content associated with arterial-tissue equilibration would be less than the total quantity of lactate taken up. The third approach, and the subject of investigation in this thesis, is to estimate the magnitude and time course of lactate uptake associated with equilibration, in order to determine the underlying effects of changes in tissue metabolism.

In the studies reported in Chapters 3 and 4 of this thesis, a simple blood-tissue exchange model (150, 151) was modified to estimate venous concentration changes associated with blood-tissue equilibration during the non-equilibrium conditions of

supine leg exercise. An underlying assumption of this analysis was that lactate behaves like an inert diffusible substance, with the venous concentration changing as a function of forearm tissue volume, blood flow and the arterial lactate concentration, with no change in tissue metabolism. Despite close agreement between experimental data and model predictions, and general support from measurements of total net flux over the course of each study, it was not possible to verify independently the accuracy of the model. There was also some evidence that model predicted venous concentrations of several of the substances examined were at times over-predicted, and at other times under-predicted, potentially distorting the apparent time course of changes in forearm metabolism. However, the utility of the model to predict the time course of concentration changes related to blood-tissue equilibration alone cannot be verified unless the contribution from tissue metabolism is known. With substances potentially metabolised or produced by resting muscles, this is difficult to establish. Consequently, the purpose of the present study was to examine more closely the reliability of the modeling analysis adopted in Chapters 3 and 4, using an inert substance (N_2O) for which there is no impact of resting muscle metabolism on veno-arterial concentration differences.

5.2 Methods

5.2.1 General

Seven male cyclists participated in the study. The first subject completed a preliminary experiment in which the protocol was slightly different from that subsequently adopted for the remaining subjects. This experiment incorporated two periods of N_2O inhalation and was designed to investigate the practicality of the N_2O methodology, as well as the potential for inert gas inhalation to interfere with other measurements (75). Consequently, data from this subject were excluded from the main analysis.

Each subject attended the laboratory on three separate occasions, with each visit separated by approximately 1 week. During the initial visit, subjects completed an incremental supine cycle ergometer exercise test to exhaustion to establish their $\dot{V}O_{2\,peak}$ and lactate threshold (LT) from forearm venous blood. Exercise began with a work load of 100 watts, followed by 25 watt increments every 3 min until the subject was unable to maintain a pedal cadence of 60 rpm. Forearm venous blood samples (1 ml) were collected at rest and in the last 15 sec of each work load. These were subsequently analysed to determine whole blood lactate concentrations and the LT calculated using the method of Beaver et al. (22), modified as detailed in Chapter 2 (pp. 62).

On the second laboratory visit each subject performed 20 min of supine cycling exercise at a cadence of 60 rpm and a work load estimated to generate a relatively stable arterial blood lactate concentration of approximately 4-6 $mmol \cdot l^{-1}$ by the end of exercise. The initial work load was 50 watts above the subject's calculated LT. This was increased or decreased during the test based on the finger prick blood lactate concentration determined at 10 min of exercise (Accusport, portable blood lactate analyser), and the subject's ability to maintain the work load. $\dot{V}_E$ and systemic $\dot{V}O_2$

and $\dot{V}CO_2$ were measured throughout using the methods described in Chapter 2 (pp. 57).

On the final visit each subject completed the main experiment. Following measurements of the right forearm dimensions the subject lay on the table attached to the supine ergometer and left brachial artery and right forearm antecubital venous catheters were inserted.

A face mask was then attached with the inspiratory circuit modified so that either room air or gas contained within a 300 l foil bag (Scholle Industries, Adelaide, South Australia) could be delivered to the inspiratory port of the mask via a low resistance 3-way tap (Hans Rudolph) and tubing (Figure 1.2). Before recording commenced the bag was filled from a compressed gas cylinder containing 10% N_2O and 21% O_2 (balance N_2) via an adjustable (0-150 $l \cdot min^{-1}$) flow regulator.

Experiments commenced with 10 min of rest during which subjects breathed room air. Supine ergometer leg exercise was then commenced at the work load established during the second laboratory visit. At the same time, the inspirate was switched to the N_2O gas so that arterial blood lactate and N_2O concentrations were elevated simultaneously. Throughout this period, gas flow into the reservoir bag was regulated to maintain an adequate reserve of inspirate. After 20 min (± 1-2 sec) of exercise and N_2O inhalation, exercise was stopped and the inspirate immediately returned to room air. Recovery was then monitored for 120 min. Simultaneous arterial and venous blood samples were collected at rest (protocol min 5 and 10), during N_2O inhalation (preliminary experiment) and N_2O inhalation combined with exercise (inhalation min 0.5, 1, 2, 3, 4, 5, 7.5, 10, 12.5, 15, 17.5 and 20), and throughout recovery (recovery min 0.5, 1, 2, 3, 4, 5, 7.5, 10, 15, 20, 30, 40, 50, 60, 75, 90, and 120). Blood samples were analysed to determine blood lactate and N_2O concentrations, respiratory gases, total haemoglobin, percentage oxygen saturation and pH as described in Chapter 2 (pp. 58, 59).

Right forearm blood flow, pulsatile and mean arterial blood pressure (MAP), heart rate (HR) and respiratory parameters ($\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$ and RER) were recorded throughout the main experiment using the procedures detailed in Chapter 2 (pp. 57). Blood flow from the hand was excluded from forearm blood flow measurements using an arterial occlusion cuff at the wrist. To account for N_2O interference of FeO_2 measurements and consequently calculated $\dot{V}O_2$ and $\dot{V}CO_2$, FeO_2 values recorded during the period of N_2O inhalation were adjusted by subtracting a correction factor (1.34 ± 0.10%) that produced a steady-state $\dot{V}O_2$ equivalent to that recorded for the same steady-state work load in the second preliminary visit.

5.2.2 Modeling analysis

Two models of forearm blood-tissue exchange were used to predict the forearm venous concentrations of N_2O, lactate, H^+, CO_2 and O_2 during the non-equilibrium conditions associated with moderate intensity leg exercise. The first model (equation 2.13, pp. 71) was the same as that used in the studies reported in Chapters 3 (44) and 4, and is a modification of the Kety-Schmidt model (150, 151) described in detail in Chapter 2 (pp. 64). Briefly, this model is a general (integrated) form of the Fick equation, more conventionally applied to estimate tissue blood flow from arteriovenous concentration differences of an inert diffusible substance such as N_2O (147, 150, 151) or ^{133}Xe (191). This model assumes that during the phases of tissue uptake (wash-in) or release (washout), the rate of blood-tissue equilibration is a function of blood flow and tissue volume and not diffusion, although the model can incorporate a diffusion term (m). During the analysis of the data reported in this study, it became apparent that in the studies reported in Chapters 3 and 4 an error, detailed in Chapter 2 (pp. 71), was made in converting the units of tissue volume (ml) into units compatible with flow measurements ($ml \cdot 100\ ml^{-1} \cdot min^{-1}$). Consequently, the diffusion term (m) was effectively $100^2 \cdot Vi^{-1}$ and not unity as was the original intent. To examine the impact of this on the ability of the model to predict venous concentrations, two additional Kety-Schmidt models were used in the analysis. In

one, m was set to unity, and is therefore a single compartment perfusion limited model (traditional Kety-Schmidt model). In the other, m was optimised to produce the best fit to measured venous data.

The second model was derived from the principles of mass balance, and was solved numerically as detailed in Chapter 2 (pp. 72). This model is a two-compartment diffusion and perfusion limited model of blood-tissue exchange and is essentially equivalent to previous derivations (74, 235), except for the inclusion of a term (assumed constant in this analysis) to account for the baseline rate of substance metabolism (net disposal or production).

The ability of each model to fit the measured venous data of each substance examined was assessed using the Model Selection Criterion described by Wagner (261), detailed in Chapter 2 (pp. 77).

Forearm fluxes were calculated as the product of forearm blood flow and the veno-arterial concentration differences in N_2O, lactate, H^+, CO_2 and O_2 at each time point (conventional Fick equation). Consequently, positive and negative fluxes indicate output and uptake respectively. Predicted forearm fluxes were calculated as the product of forearm blood flow and veno-arterial concentration differences predicted from the $m = 100^2 \cdot Vi^{-1}$ Fick and two-compartment models. The Fick model ($m = 100^2 \cdot Vi^{-1}$) was selected to enable comparisons with previous studies (Chapters 3 and 4). The two-compartment model was chosen as it was the best model of forearm N_2O kinetics, and of similar predictive value to the Fick model for the remaining substances examined. Measured minus predicted forearm fluxes, adjusted for baseline flux (equation 2.28, pp. 84), were subsequently used as an index of net production (positive values) or net metabolism (negative values) of substances in the forearm, largely independent of the effects of passive movements associated with the non-equilibrium conditions of incremental exercise.

The total net quantities of N_2O, lactate, H^+, CO_2 and O_2 taken up (or released) by the forearm throughout exercise and recovery were determined from the net area under the time versus the respective flux curves. These values were compared to the total net flux that would have been measured had the resting levels of flux continued throughout exercise, recovery and exercise and recovery:

$$\text{Expected Total Net Flux} = \text{Baseline Flux (units} \cdot \text{minute}^{-1}) \cdot \text{Duration (min)}$$

5.2.3 Statistical analysis

For each substance examined (N_2O, lactate, H^+, CO_2 and O_2) the MSC was compared between models (Kety-Schmidt m = 1, m = fit and $m = 100^2 \cdot Vi^{-1}$, and two-compartment model) using Student's paired *t*-tests and the Dunn-Sidak procedure to correct for the inflated Type I error risk associated with multiple pairwise contrasts (164). Student's *t*-tests were also used in comparisons involving calculated total net flux. Comparisons between arterial and measured venous concentrations, between measured and predicted venous concentrations and between measured and predicted veno-arterial concentration differences and flux were made using two-way analysis of variance (ANOVA) for repeated measures. In all statistical tests the null hypothesis was rejected if $P < 0.05$. All data are reported as means ± SEM.

5.3 Results

5.3.1 General

Forearm blood flow and blood concentration data obtained from each experiment and examined using the Fick ($m = 100^2 \cdot Vi^{-1}$) and two-compartment models are presented in the appendix. In the preliminary experiment there was no evidence that N_2O inhalation alone influenced any measurement other than blood N_2O concentrations. Consequently, the resting period of N_2O inhalation was considered unnecessary and was excluded from the remainder of the experiments (n = 6).

Descriptive characteristics, and peak values of $\dot{V}O_2$, heart rate and work load measured during the initial laboratory visit for the 6 subjects who completed the full study are presented in Table 5.1. During the main experiment, subjects exercised at 241.7 ± 26.0 (range 170–330) watts for 20 min.

Table 5.1. Subject characteristics.

					Right forearm	
Age	**Mass**	**Height**	$\dot{V}O_{2\ peak}$	**LT**	**Volume**	**Circumference**
(years)	(kg)	(cm)	($ml \cdot kg \cdot min^{-1}$)	(%$\dot{V}O_{2\ peak}$)	(ml)	(cm)
23.2	75.5	182.6	53.2	53.3	1643.3	26.48
± 1.9	± 3.3	± 2.4	± 5.0	± 3.7	± 90.0	± 0.60

Values are means ± SEM, n = 6.

5.3.2 Cardiorespiratory variables

5.3.2.1 Mean arterial pressure (MAP)

From a resting value of 90.2 ± 1.0 mmHg, MAP increased immediately with the onset of exercise, reaching a peak value of 127.0 ± 2.1 mmHg in 3 min, before declining gradually to 119.2 ± 3.2 in the last min of exercise (vs peak P = 0.009). The mean

MAP during exercise was 122.2 ± 2.4 mmHg. MAP declined very rapidly following exercise termination, reaching pre-exercise levels or below within the first 2 min and for the remainder of recovery.

5.3.2.2 Heart rate (HR)

HR increased progressively throughout exercise from a resting value of 59.1 ± 2.8 beats·min^{-1} to reach a relatively stable value of 161.8 ± 5.7 beats·min^{-1} in the last min of exercise. The mean heart rate during the final 10 min of exercise was 160.6 ± 5.2 beats·min^{-1} (88.7 ± 1.5 %HR_{peak}).

5.3.2.3 $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$ and the respiratory exchange ratio (RER)

$\dot{V}_E$ increased rapidly from a resting value of 7.4 ± 0.8 l·min^{-1} to reach 77.5 ± 5.9 l·min^{-1} during the final 15 min of exercise. Similarly, $\dot{V}O_2$ and $\dot{V}CO_2$ increased immediately with the onset of exercise, reached stable values within 5 min and then returned to baseline levels within 7.5 min after exercise. The mean $\dot{V}O_2$ during the last 15 min of exercise was 43.9 ± 4.5 ml·kg^{-1}·min^{-1} (82.5 ± 2.3 %$\dot{V}O_{2\,peak}$). RER was 0.88 ± 0.02 at rest, reached a peak value of 1.17 ± 0.04 at 3 min of exercise, before declining slightly to reach a relatively stable value of 1.04 ± 0.03 in the last 15 min of exercise.

5.3.2.4 Forearm blood flow

At rest, forearm blood flow was 2.28 ± 0.20 ml·100 ml^{-1}·min^{-1}. Within the first 2 minutes of exercise, forearm blood flow had declined to 1.51 ± 0.22 ml·100 ml^{-1}·min^{-1} (vs rest P = 0.016), after which flow progressively increased throughout the remainder of exercise to reach a peak value of 12.2 ± 2.3 ml·100 ml^{-1}·min^{-1} (Figure 5.1). Forearm blood flow declined progressively during early recovery, reaching stable near baseline levels within 15-20 min.

5.3.3 Model fits

Model parameter estimates for the modified Fick and two compartment models of forearm blood-tissue exchange are presented in Table 5.2. The Model Selection Criterion and degree of correlation between measured and predicted venous concentrations of N_2O, lactate, H^+, CO_2 and O_2 are presented in Table 5.3. When the Fick model diffusion term (m) was obtained by fitting model predicted to measured venous data, m was not significantly different from $100^2 \cdot Vi^{-1}$ for all substances apart from N_2O and H^+. Consequently, values for the MSC were very similar between the optimised (m = fit) and $m = 100^2 \cdot Vi^{-1}$ Fick models, except for N_2O in which fitting was associated with a higher MSC ($P < 0.05$, Table 5.3, Figure 5.2). Forearm venous N_2O concentrations were best fit by the two-compartment model of blood-tissue exchange (Table 5.3, Figure 5.2). However, for all other substances the two-compartment model was no better than the optimised (m = fit) or $100^2 \cdot Vi^{-1}$ Fick models. In general, the single compartment Fick model (m = 1) was associated with the poorest fits. With the exception of O_2 and H^+, in which all models provided relatively poor fits, both the Fick ($m = 100^2 \cdot Vi^{-1}$ and m = fit) and two-compartment models provided quite good fits to measured venous concentration data, often accounting for ~90% of the overall variability in venous substance concentrations. However, all models appeared to systematically under- and over-estimate venous concentrations at various times throughout the protocol. Even with the best (two-compartment) model, venous N_2O concentrations appeared to be over- and then under-estimated during early and late N_2O inhalation respectively (Figure 5.2). Similar systematic differences between measured and predicted venous concentrations were also apparent with the remaining substances, particularly during recovery.

Table 5.2. Parameter estimates for models of venous N_2O, lactate, H^+, CO_2 and O_2 concentrations.

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Mean ± SEM
All models							
Forearm Volume (ml)	1860.0	1460.0	1960.0	1440.0	1500.0	1640.0	1643.3 ± 90.9
Fick model m (diffusion term)							
m = fit							
N_2O	2.66	2.20	3.06	4.32	3.09	3.89	3.20 ± 0.32 *
Lactate	4.92	4.55	6.28	9.12	6.76	1.61	5.54 ± 1.03
H^+	11.37	12.24	17.95	22.36	14.80	6.64	14.23 ± 2.24 *
CO_2	5.41	4.87	17.88	9.30	6.06	10.62	9.02 ± 2.00
O_2	7.16	3.86	23.14	7.07	3.92	4.92	8.35 ± 3.02
$m = 100^2 \cdot Vi^{-1}$							
All substances	5.38	6.85	5.10	6.94	6.67	6.10	6.17 ± 0.32
Two-Compartment model							
V%	1.00	1.00	0.14	1.00	0.67	1.00	0.80 ± 0.14
Vb (ml)	270.95	143.47	68.60	284.22	248.85	444.42	243.42 ± 52.73
Vi (ml)	991.14	947.65	1149.24	469.52	1336.79	1664.32	1093.11 ± 164.24
PS (permeability surface product)							
N_2O	37.59	124.08	11.92	28.38	37.73	18.11	42.97 ± 16.76
Lactate	14.95	14.82	4.63	3.40	3.71	22.46	10.66 ± 3.23
H^+	1.63	3.73	0.47	2.76	3.93	3.76	2.71 ± 0.57
CO_2	11.01	17.43	3.05	6.13	8.49	0.02	7.69 ± 2.51
O_2	3.36	4.32	1.06	5.07	6.74	4.31	4.14 ± 0.77

V%, fraction of total forearm flow received by the tissues from which venous blood was sampled; Vb, blood compartment volume; Vi, tissue compartment volume. In the Fick model, m parameter values above unity represent a diffusion limitation. * indicates a significant difference from $m = 100^2 \cdot Vi^{-1}$ ($P < 0.05$).

Table 5.3. Model Selection Criterion and coefficient of determination (r^2) for the Fick (m = 1, m included as a fitted parameter and m = $100^2 \cdot Vi^{-1}$) and two-compartment models.

	Model Selection Criterion							r^2
	Subject							
	1	2	3	4	5	6	Mean ± SEM	Mean ± SEM
Fick Model (m = 1)								
N_2O	0.41	0.86	0.37	0.83	0.78	0.67	0.65 ± 0.09	0.536 ± 0.026
Lactate	-0.06	0.44	0.19	0.47	0.19	0.00	0.21 ± 0.09	0.301 ± 0.047
H^+	-0.40	-0.20	-1.62	-1.05	-0.26	-1.26	-0.80 ± 0.24	0.023 ± 0.009
CO_2	0.12	0.40	-0.09	0.37	0.40	0.20	0.23 ± 0.08	0.308 ± 0.047
pCO_2	-1.05	-0.69	-0.52	0.01	-0.34	-0.50	-0.51 ± 0.14	0.106 ± 0.032
O_2	0.09	-0.04	-0.46	-0.31	-0.08	-0.11	-0.15 ± 0.08	0.409 ± 0.080
pO_2	-0.18	-0.31	-0.59	-0.65	-0.26	-0.39	-0.40 ± 0.08	0.132 ± 0.049
Fick Model (m = fit)								
N_2O	1.20	1.22	0.78	3.14	1.76	2.31	1.74 ± 0.35 *	0.898 ± 0.029
Lactate	1.42	1.80	2.17	3.09	2.11	0.12	1.79 ± 0.40 †	0.903 ± 0.044
H^+	1.52	0.37	-1.66	-0.44	1.04	-1.22	-0.07 ± 0.51	0.691 ± 0.085
CO_2	1.58	2.57	1.58	2.06	2.32	3.00	2.19 ± 0.23 †	0.927 ± 0.020
pCO_2	0.51	1.73	1.21	1.71	1.58	2.39	1.52 ± 0.26 †	0.844 ± 0.062
O_2	0.21	-0.02	-0.29	-0.30	-0.04	-0.04	-0.08 ± 0.08	0.638 ± 0.080
pO_2	-0.04	-0.29	-0.53	-0.67	-0.28	-0.44	-0.38 ± 0.09	0.166 ± 0.058
Fick Model (m = $100^2 \cdot Vi^{-1}$)								
N_2O	0.91	0.61	0.60	2.52	1.16	2.06	1.31 ± 0.33	0.909 ± 0.014
Lactate	1.54	1.58	2.20	3.07	2.20	0.24	1.81 ± 0.39 †	0.951 ± 0.016
H^+	1.25	0.44	-1.63	-0.48	0.86	-1.14	-0.12 ± 0.47	0.536 ± 0.105
CO_2	1.67	2.39	1.23	2.09	2.41	2.38	2.03 ± 0.20 †	0.900 ± 0.020
pCO_2	0.59	1.58	0.83	1.72	1.67	1.69	1.35 ± 0.20 †	0.785 ± 0.057
O_2	0.28	0.05	-0.24	-0.21	0.04	0.04	0.00 ± 0.08 †‡	0.595 ± 0.085
pO_2	0.02	-0.21	-0.46	-0.58	-0.20	-0.36	-0.30 ± 0.09 ‡	0.101 ± 0.036
Two-Compartment Model								
N_2O	3.41	2.73	2.66	3.96	3.13	2.36	3.04 ± 0.24 *†	0.960 ± 0.009
Lactate	1.99	2.23	2.68	2.33	2.29	0.94	2.08 ± 0.24 †	0.938 ± 0.005
H^+	1.74	2.84	0.50	0.07	0.88	0.59	1.10 ± 0.42 †	0.628 ± 0.101
CO_2	1.61	1.99	1.65	2.49	2.43	1.93	2.01 ± 0.15 †	0.891 ± 0.018
pCO_2	0.51	1.13	1.30	2.08	1.67	1.25	1.32 ± 0.22 †	0.748 ± 0.055
O_2	0.23	0.29	0.60	1.27	0.95	0.79	0.69 ± 0.16	0.612 ± 0.071
pO_2	-0.12	0.07	0.14	-0.24	0.60	-0.02	0.07 ± 0.12	0.396 ± 0.094

Better fits are associated with higher values of the MSC. Significantly better fits ($P < 0.05$) are indicated by; * compared to the Fick model (m = $100^2 \cdot Vi^{-1}$), † compared to the Fick model (m = 1), and ‡ compared to the Fick model (m = fit).

5.3.4 Blood N_2O

Arterial N_2O concentrations increased very rapidly with the onset N_2O inhalation and exercise, with peak levels being reached within 2 minutes (Figure 5.2). This was followed by a small decline, possibly reflecting lung equilibration processes, and then a stabilisation of arterial N_2O concentrations at ~45 $\mu l \cdot ml^{-1}$ consistent with a 10% N_2O gas and a blood-gas partition coefficient of 0.45 (268). Forearm venous N_2O concentrations increased more slowly and had not fully equilibrated with arterial levels by the end of N_2O inhalation (Figure 5.2). There was a significant inverse relationship between forearm blood flow and the magnitude of the arteriovenous N_2O concentration difference at the end of exercise and N_2O inhalation ($r = -0.925$, $r^2 = 0.856$, $P = 0.008$). With resumption of room air breathing, arterial and venous N_2O concentrations declined very rapidly at first and then more slowly over the remaining 120 min of recovery. At the end of the recovery period, N_2O was still detectable in arterial blood (0.29 ± 0.08 $\mu l \cdot ml^{-1}$), and a significant veno-arterial N_2O concentration difference persisted (2.07 ± 0.47 $\mu l \cdot ml^{-1}$, $P = 0.007$, Figure 5.2).

Even when optimised (m = fit), the Fick model was associated with significant overall and time dependent differences between measured and predicted venous N_2O concentrations, and therefore veno-arterial N_2O concentration differences (main effect $P = 0.021$, interaction with time $P = 0.035$, Figure 5.2B). These were more apparent with the $m = 100_2 \cdot Vi^{-1}$ Fick model (Figure 5.2C), such that there were significant time dependent differences between measured and predicted veno-arterial N_2O concentration differences ($P = 0.004$, Figure 5.3A) and flux ($P = 0.004$, Figure 5.3D). Consequently, N_2O flux "corrected" for the effects of forearm N_2O equilibration kinetics using the Fick model ($m = 100^2 \cdot Vi^{-1}$) also changed significantly with time ($P = 0.004$, Figure 5.3C). Similarly, even with the best (two-compartment) model of forearm N_2O kinetics there were significant time dependent differences between measured and predicted venous N_2O concentrations, and therefore veno-arterial concentration differences ($P = 0.040$, Figure 5.2D and Figure 5.3B) indicative of

some limitations to the model. However, there was no significant difference between measured and predicted N_2O flux, and therefore N_2O flux "corrected" for passive N_2O movements was not different from zero and did not change with time ($P = 0.130$, Figure 5.3D).

Forearm tissue N_2O concentrations predicted using the two-compartment model were a relatively small fraction of venous levels even after 20 min of N_2O inhalation (~35% of venous). Furthermore, a much longer period of recovery appeared necessary to cover the complete washout of N_2O from the forearm tissues (Figure 5.2D).

5.3.5 Blood lactate

From a resting value of 0.61 ± 0.03 $mmol \cdot l^{-1}$, arterial blood lactate concentrations increased rapidly over the first 5 min of exercise to reach a relatively stable mean value of 5.55 $mmol \cdot l^{-1}$ in the last 15 min of exercise (Figure 5.4). Forearm venous blood lactate increased more slowly such that there was a clear veno-arterial difference particularly during the first 10 min, followed by a plateau in venous lactate concentration (4.56 ± 0.39 $mmol \cdot l^{-1}$, arterial versus venous concentration by time interaction, $P < 0.001$). Throughout recovery, arterial and venous levels were very similar, both declining exponentially to reach baseline levels by ~90 min of recovery (Figure 5.4).

Although there were no significant time dependent differences in the optimised (m = fit) Fick model, the predicted levels were higher than measured overall (main effect $P = 0.011$, interaction with time $P = 0.098$). In the $m = 100^2 \cdot Vi^{-1}$ Fick model there were both overall and time dependent differences between measured and predicted concentrations, and therefore veno-arterial concentration differences (main effect $P = 0.020$, interaction with time $P = 0.033$, Figure 5.4C and Figure 5.5A) and flux (main effect $P = 0.030$, interaction with time $P = 0.017$, Figure 5.5C). Consequently, "corrected" flux showed a significant time dependent uptake ($P = 0.017$, Figure 5.5C).

The biggest differences between measured and predicted concentrations and flux, and therefore the greatest changes in "corrected" flux, occurred during late exercise and the first 30-40 min of recovery. In contrast, when predicted from the two-compartment model, there were no significant differences between measured and predicted veno-arterial concentration differences (main effect P = 0.128, interaction with time P = 0.433, Figure 5.5B) or flux (main effect P = 0.296, interaction with time P = 0.279, Figure 5.5D). Consequently, "corrected" flux did not change significantly from baseline levels with time (P = 0.279, Figure 5.5D).

After 20 min of leg exercise, the tissue lactate concentration predicted from the two-compartment model (2.32 ± 0.39 $mmol \cdot l^{-1}$) was lower compared to the venous lactate concentration at the same time point (4.63 ± 0.25 $mmol \cdot l^{-1}$, P = 0.002). Consequently, arterial lactate concentrations remained higher than predicted tissue levels for the first ~10 min of recovery, and this was associated with continued predicted forearm lactate uptake. Although it appeared that the predicted forearm tissue lactate concentration had not fully recovered by the end of recovery, the values at this point were not significantly different from predicted baseline levels (P = 0.083).

There was a significant linear association between measured venous blood lactate concentration and the venous-to-muscle lactate concentration gradient predicted from the two-compartment model ($r^2 = 0.906$, $P < 0.05$, Figure 5.6).

5.3.6 Blood hydrogen ions

From a resting value of 38.01 ± 0.48 $nmol \cdot l^{-1}$ (pH = 7.42 ± 0.01), the arterial H^+ concentration increased rapidly with the onset of leg exercise to reach a peak value of 48.57 ± 1.63 $nmol \cdot l^{-1}$ (pH = 7.31 ± 0.01) after 5 min, followed by a gradual decline with continued exercise. During the first 10 min of recovery, there was an initial rapid drop in arterial H^+ concentration, followed by a more gradual decline, with resting

levels being reached after 90 min of recovery (Figure 5.7). A large positive veno-arterial H^+ concentration difference at rest was substantially reduced throughout exercise and the majority of recovery (arterial vs venous concentration main effect P = 0.002, interaction with time P = 0.010, Figure 5.7).

There were significant time dependent differences between measured venous H^+ concentrations and those predicted using each of the models ($P \leq 0.025$, Figure 5.7), in particular during recovery. Consequently, there were also differences between measured veno-arterial H^+ concentration differences and those predicted from the $m = 100^2 \cdot Vi^{-1}$ Fick (main effect P = 0.036, interaction with time P = 0.001, Figure 5.12A) and two-compartment (main effect P = 0.540, interaction with time P = 0.025, Figure 5.12B) models. However, these were only associated with a significant difference between measured and predicted flux in the $m = 100^2 \cdot Vi^{-1}$ Fick model (main effect P = 0.012, interaction with time P = 0.001, Figure 5.12C; two-compartment model main effect P = 0.341, interaction with time P = 0.142, Figure 5.12D). Consequently, while there was a significant time dependent increase in "corrected" H^+ output predicted using the $m = 100^2 \cdot Vi^{-1}$ Fick model (P = 0.001, Figure 5.12C), this was not apparent when the two-compartment model was used (P = 0.142, Figure 5.12D).

Changes in tissue H^+ concentrations predicted using the two-compartment model were very small, and at no time point were they significantly different from the assumed baseline H^+ concentration of 89 $nmol \cdot l^{-1}$ (pH = 7.05, $P \geq 0.679$, data not shown).

5.3.7 Resting forearm respiratory gas exchange

5.3.7.1 CO_2 exchange

After 5 min of leg exercise, arterial CO_2 content and pCO_2 had declined by ~6.5 $mmol \cdot l^{-1}$ and ~2.3 mmHg respectively (Figure 5.9 and Figure 5.10). These levels

were maintained for the remainder of exercise and did not fully recovery until ~60 min after the termination of exercise. Although a positive veno-arterial CO_2 content difference was maintained throughout exercise and recovery (arterial vs venous, P = 0.004), the magnitude of the difference was dependent on time (P = 0.002, Figure 5.9). All but the single compartment Fick model (m = 1) appeared to produce good fits to measured venous data. However, only in the $m = 100^2 \cdot Vi^{-1}$ model was there no difference between measured and predicted venous CO_2 contents, and therefore veno-arterial CO_2 content differences (main effect P = 0.173, interaction with time P = 0.114, Figure 5.9 and Figure 5.11A). Although values predicted using the optimised Fick model (m = fit) appeared comparable to the $m = 100^2 \cdot Vi^{-1}$ Fick model, these differed from measured values in a time dependent manner (main effect P = 0.184, interaction with time P = 0.047, Figure 5.9B). Similarly, there was a significant interaction with time in differences between measured and two-compartment model predicted venous CO_2 contents, and therefore veno-arterial CO_2 content differences (main effect P = 0.088, interaction with time P = 0.038, Figure 5.9D and Figure 5.11B). In contrast, differences between measured and predicted forearm CO_2 flux were apparent in the $m = 100^2 \cdot Vi^{-1}$ Fick model (main effect P = 0.059, interaction with time P = 0.031, Figure 5.11C) but not the two-compartment model (main effect P = 0.794, interaction with time P = 0.083, Figure 5.11D). Consequently, time dependent changes in "corrected" forearm CO_2 flux were apparent in the Fick model (P = 0.031, Figure 5.11C) but not the two-compartment model (P = 0.083, Figure 5.11D).

There were significant time related changes in tissue CO_2 content (P = 0.027, Figure 5.9D) and in particular pCO_2 (P = 0.001, Figure 5.10D) predicted on the basis of the two-compartment model of blood-tissue exchange. Associated with the early decline in forearm blood flow, there was initially a marked increase in predicted tissue pCO_2 and, to a much smaller degree, CO_2 content. After ~5 min of exercise, pCO_2 and therefore CO_2 content began to decline, and this continued until ~10 min of recovery, at which point CO_2 content was slightly below baseline levels (31.36 ± 0.62 vs 33

$mmol \cdot l^{-1}$, P = 0.045, Figure 5.9D). Throughout the remainder of recovery, there was a very slight upward trend towards baseline in CO_2 content and pCO_2.

5.3.7.2 O_2 exchange

There were small but significant changes in arterial O_2 content and pO_2 associated with exercise and recovery ($P \leq 0.041$, Figure 5.12 and Figure 5.13). A significant veno-arterial O_2 content difference at rest was maintained throughout exercise and recovery, but the magnitude of the difference changed dramatically with time (main effect $P < 0.001$, interaction with time P = 0.001). During the first 5 min of exercise there was a small but significant decline in venous O_2 content (P = 0.044) and pCO_2 (P = 0.038) and consequently a wider veno-arterial O_2 difference (Figure 5.14A,B) and greater inward (uptake) O_2 flux (Figure 5.14C,D). However, as exercise continued, venous O_2 content and pO_2 rose, markedly reducing the forearm veno-arterial O_2 content difference. However, this was mostly offset by the rise in forearm blood flow, such that measured forearm O_2 flux did not significantly change with time (P = 0.208, Figure 5.14C,D).

None of the models examined accurately predicted the time course of change in venous O_2 content (Figure 5.12) or pO_2 (Figure 5.13), with even the best (two-compartment) model predictions demonstrating significant time dependent differences compared to measured data (main effect P = 0.556, interaction with time P = 0.027, Figure 5.12D). Consequently, there were significant interactions with time between measured and predicted veno-arterial O_2 content differences determined using the $m = 100^2 \cdot Vi^{-1}$ Fick model (P = 0.001, Figure 5.14A) and the two-compartment model (P = 0.027, Figure 5.14B). While this resulted in time dependent differences in measured and predicted O_2 flux with the Fick model (main effect P = 0.018, interaction with time P = 0.004, Figure 5.14C), this was not apparent with the the two-compartment model (main effect P = 0.569, interaction with time P = 0.134, Figure 5.14C). Consequently, while forearm flux "corrected" for passive O_2

movements using the Fick model ($m = 100^2 \cdot Vi^{-1}$) showed a dramatic increase in uptake during leg exercise ($P = 0.004$, Figure 5.14C), that predicted using the two-compartment model did not ($P = 0.134$, Figure 5.14D).

There was only a very small increase in tissue O_2 content (~0.2 $mmol \cdot l^{-1}$) predicted on the basis of changes in forearm blood flow and arterial O_2 content using the two-compartment model. Nonetheless, this was associated with substantial and long lasting changes in tissue pO_2 ($P = 0.009$, Figure 5.13D).

5.3.7.3 Resiratory exchange ratio

Forearm RER determined from measured fluxes of CO_2 and O_2 showed significant time related changes over the course of exercise and recovery ($P = 0.012$), reaching a peak value of 1.47 ± 0.17 at 7.5 min of exercise, before declining to below baseline values for the majority of recovery (Figure 5.15). There were no significant differences between measured and predicted forearm RER with either the Fick ($m = 100^2 \cdot Vi^{-1}$) or two-compartment models (main effects $P \geq 0.408$, interactions with time $P \geq 0.225$, Figure 5.15). Consequently forearm RER "corrected" for model predicted passive movements of CO_2 and O_2 did not significantly change from baseline with either model throughout exercise and recovery ($P \geq 0.186$).

5.3.8 Total net flux of N_2O, lactate, H^+, CO_2 and O_2 in the resting forearm

At rest there was a small non-significant net production of lactate, a significant net production of H^+ and CO_2, and net consumption of O_2 in the resting forearm (Table 5.4). During exercise, recovery and combined exercise and recovery, measured and predicted net fluxes were similar in all substances except H^+ and CO_2. The total net fluxes of N_2O, lactate, H^+, CO_2 and O_2 were similar to values expected had pre-exercise levels of flux continued throughout the period of exercise and recovery. Consequently, there was no significant quantity of any substance that could not be

accounted for, although that for lactate approached statistical significance (P = 0.069).

The calculated forearm RER was 0.65 ± 0.06 at rest, and 0.68 ± 0.04 over the combined exercise and recovery period (P = 0.503).

The degree of association between total net flux measured during exercise and that measured during recovery for each of the substances examined was relatively weak, but reached statistical significance for H^+. With each substance the measured degree of association was of similar magnitude to that predicted from the two-compartment model (Table 5.4).

Table 5.4. Total net flux of N_2O, lactate, H^+, CO_2 and O_2 across the resting forearm during rest, supine leg exercise and recovery.

	N_2O (*nl·100 ml^{-1}*)	Lactate (*µmol·100 ml^{-1}*)	H^+ (*pmol·100 ml^{-1}*)	CO_2 (*µmol·100 ml^{-1}*)	O_2 (*µmol·100 ml^{-1}*)
Rest	0.0 ± 0.0	2.6 ± 1.4	54.4 ± 9.6 †	22.0 ± 4.7 †	-40.0 ± 4.5 †
Rest *(/min)*	0.0 ± 0.0	0.5 ± 0.3	10.9 ± 1.9 †	4.4 ± 0.9 †	-8.0 ± 0.9 †
Exercise (20 min)					
Measured	-1618.5 ± 129.2 †§	-127.4 ± 30.1 †§	197.5 ± 71.2 †	238.0 ± 50.7 †§	-208.0 ± 37.3 †
Predicted	-1692.8 ± 154.4 †§	-111.0 ± 22.2 †§	114.6 ± 29.8 †	308.9 ± 51.0 †§	-246.3 ± 36.7 †§
"Corrected"	74.3 ± 55.2 ‡	-5.1 ± 15.5 ‡	295.3 ± 64.4 †‡	8.4 ± 36.4 ‡	-126.8 ± 35.5 †‡
Expected	0.0 ± 0.0 ‡	10.5 ± 5.6 ‡	217.6 ± 38.3 †	88.1 ± 18.8 †‡	-160.0 ± 18.2 †
Recovery (120 min)					
Measured	1303.5 ± 161.5 †§	33.7 ± 45.6	998.1 ± 193.5 †	478.9 ± 115.2 †	-957.0 ± 101.3 †
r^2 (vs Exercise)	0.006	0.320	0.748	0.786 *	0.510
Predicted	1294.9 ± 150.1 †§	133.5 ± 36.2 †§	1192.5 ± 196.2 †‡	288.5 ± 101.7 †‡§	-862.9 ± 94.0 †§
r^2 (vs Exercise)	0.115	0.113	0.734	0.616	0.756
"Corrected"	8.5 ± 46.8 ‡	-32.2 ± 48.6 ‡	1079.9 ± 172.8 †	666.5 ± 114.5 †‡§	-1085.0 ± 111.9 †‡
Expected	0.0 ± 0.0 ‡	63.2 ± 33.8	1305.7 ± 229.6 †	528.3 ± 112.9 †	-959.8 ± 109.1 †
Exercise & Recovery (140 min)					
Measured	-315.0 ± 198.6	-93.7 ± 67.3	1195.6 ± 246.1 †	716.9 ± 161.9 †	-1165.0 ± 135.6 †
Predicted	-397.9 ± 175.1	22.5 ± 48.4 §	1307.0 ± 216.3 †	597.4 ± 145.3 †	-1109.1 ± 127.1 †
"Corrected"	82.9 ± 54.5	-37.4 ± 59.2 ‡	1375.2 ± 221.5 †	674.9 ± 128.2 †	-1211.8 ± 138.4 †
Expected	0.0 ± 0.0	73.7 ± 39.4	1523.3 ± 267.8 †	616.4 ± 131.7 †	-1119.7 ± 127.3 †
Unaccounted	-315.0 ± 198.6	-167.4 ± 72.3	-327.7 ± 288.9	100.5 ± 73.9	-45.2 ± 94.9

Positive values indicate net output, and negative values net uptake. Measured, predicted and "corrected" values were calculated as the net area under the time versus measured, predicted (two-compartment model) and "corrected" flux curves respectively. Expected values were calculated by multiplying the resting flux (/min) by the corresponding duration of exercise, recovery or both. Unaccounted values were calculated as the difference between measured and expected values. * indicates a significant degree of association, † a difference from a value of zero, ‡ from measured and § from expected ($P < 0.05$). All values are means ± SEM, n = 6.

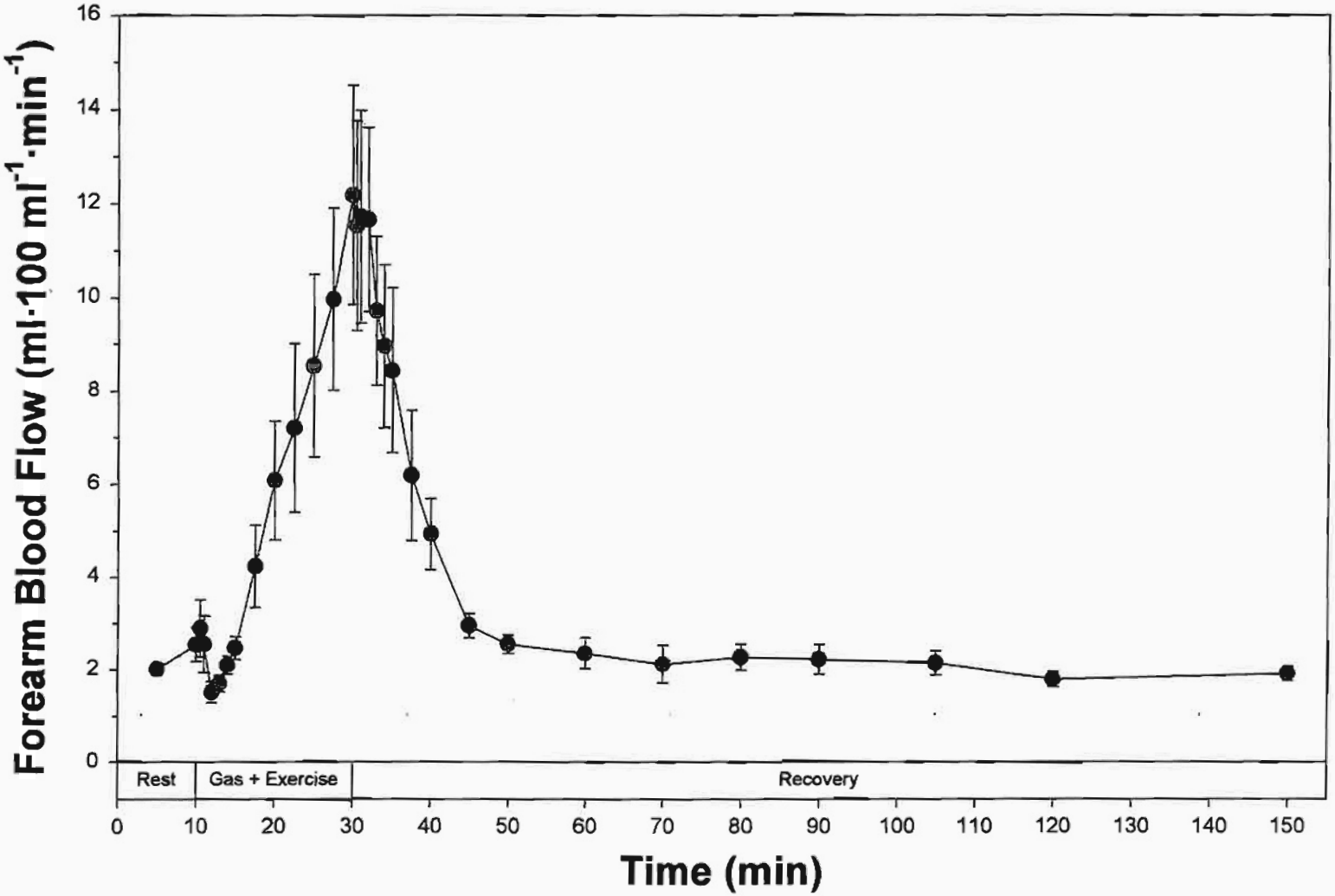


Figure 5.1. Resting forearm blood flow before, during and after 20 min of 10% N_2O inhalation and leg exercise (~80% $\dot{V}O_{2\,peak}$). Values are means ± SEM, n = 6.

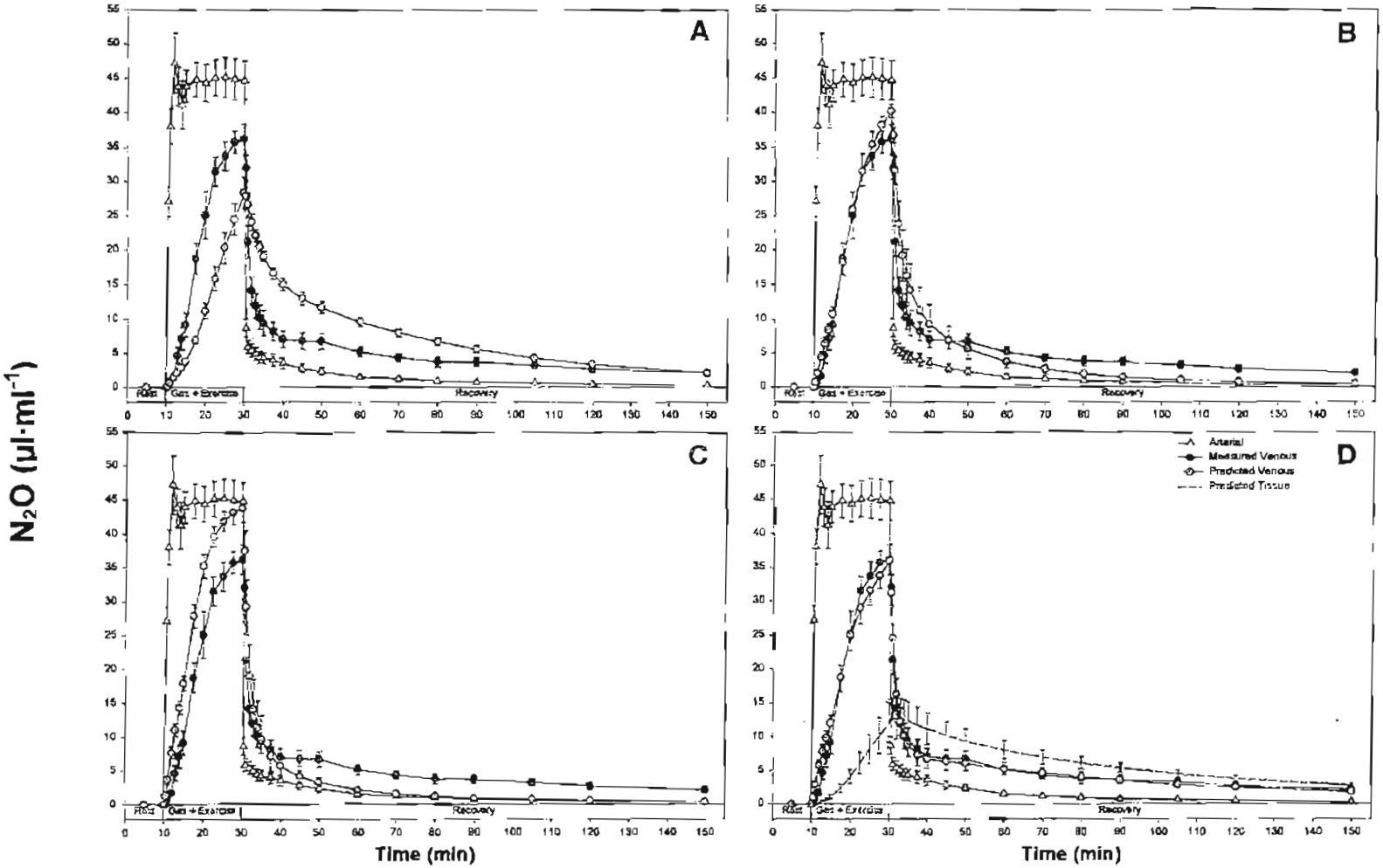


Figure 5.2. N_2O levels in the resting forearm before, during and after 20 min of N_2O inhalation and leg exercise. Predicted values were determined using a modified Fick model (A-C) in which the diffusion parameter (m) was 1 (A), optimised to best fit predicted to measured venous data (B), and $100^2 \cdot Vi^{-1}$ (C), and using a two-compartment model (D). Values are means ± SEM, n = 6.

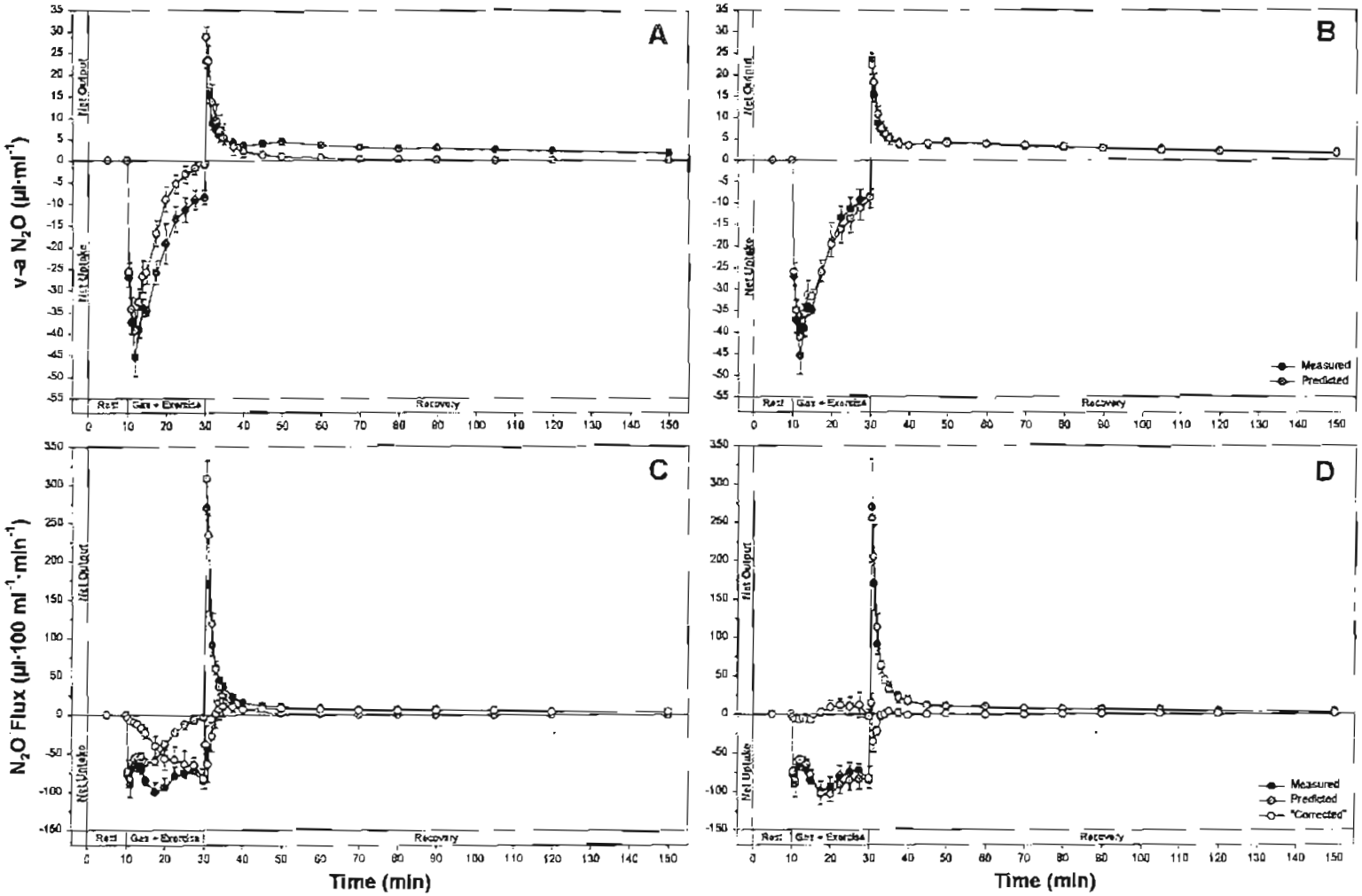


Figure 5.3. Veno-arterial N_2O concentration difference (A,B), and N_2O flux (C,D) in the resting forearm before, during and after 20 min of N_2O inhalation and leg exercise. Predicted values were determined using a modified Fick model (A,C) in which the diffusion parameter (m) was $100^2 \cdot Vi^{-1}$, and a two-compartment model (B,D). "Corrected" = Measured – Predicted + Baseline. Values are means ± SEM, n = 6.

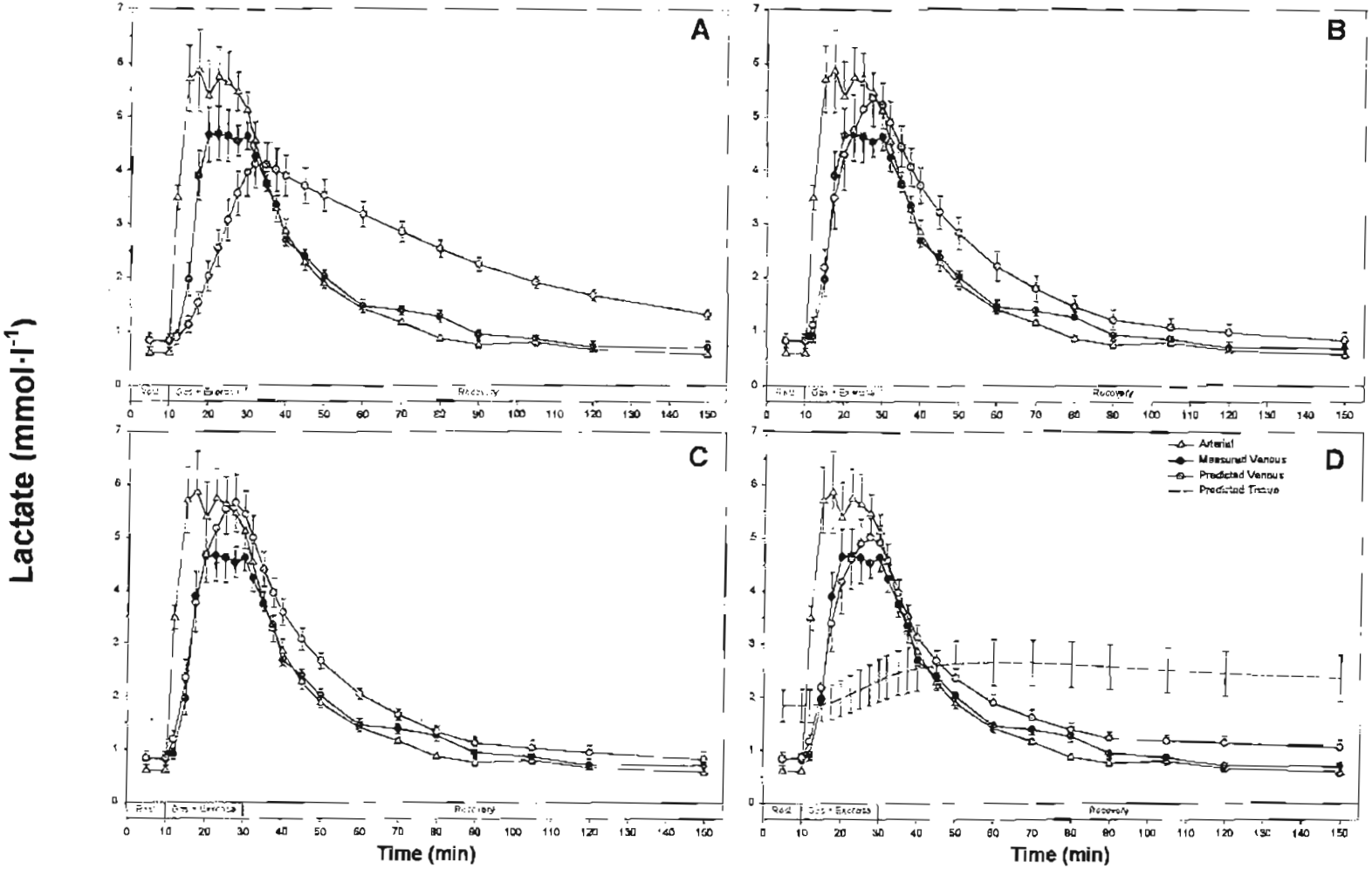


Figure 5.4. Resting forearm blood lactate levels before, during and after 20 min of N_2O inhalation and leg exercise. Predicted values were determined using a modified Fick model (A-C) in which the diffusion parameter (m) was 1 (A), optimised to best fit predicted to measured venous data (B), and $100^2 \cdot Vi^{-1}$ (C), and using a two-compartment model (D). Values are means ± SEM, n = 6.

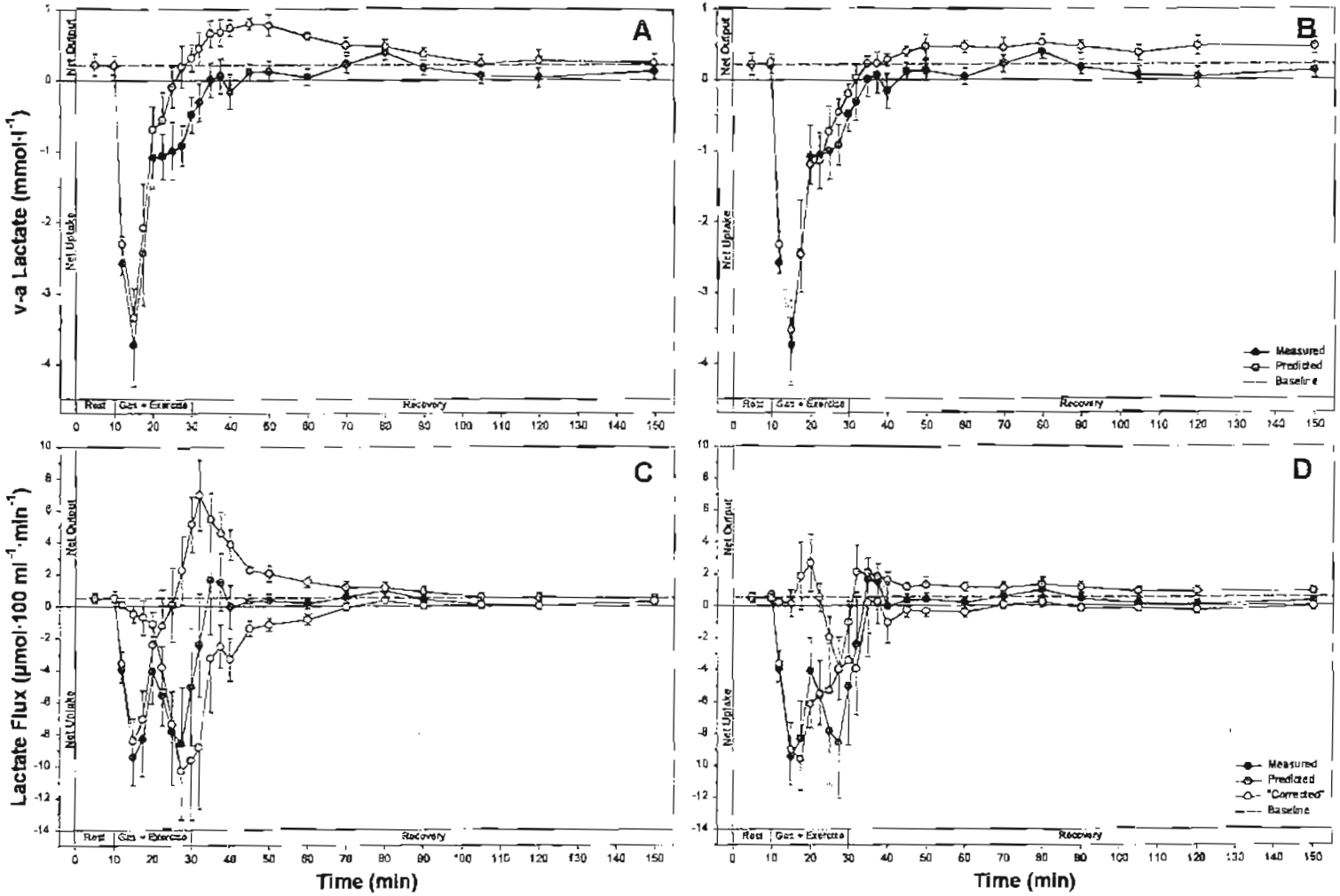


Figure 5.5. Veno-arterial lactate concentration difference (A,B), and flux (C,D) in the resting forearm before, during and after 20 min of N_2O inhalation and leg exercise. Predicted values were determined using a modified Fick model (A,C) in which the diffusion parameter (m) was $100^2 \cdot Vi^{-1}$, and a two-compartment model (B,D). "Corrected" = Measured – Predicted + Baseline. Values are means ± SEM, n = 6.

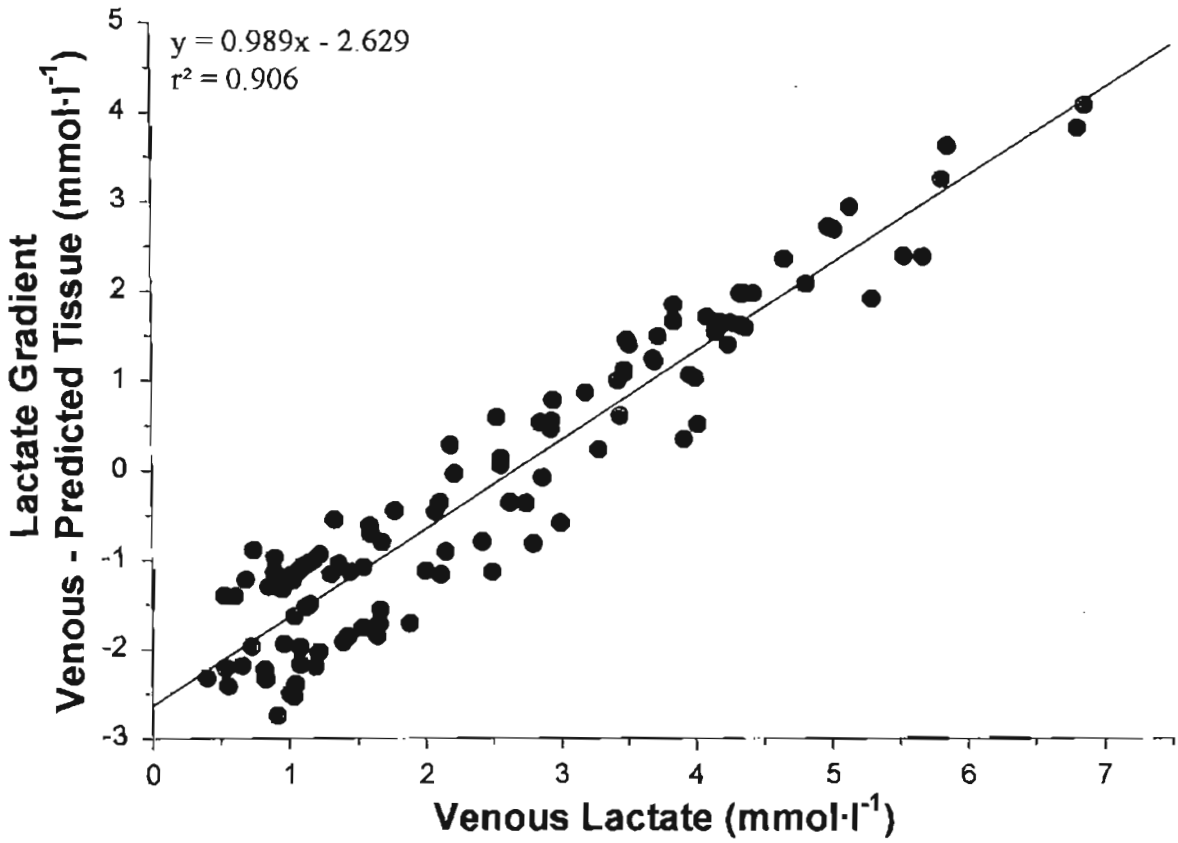


Figure 5.6. Measured forearm venous blood lactate concentration versus predicted lactate concentration gradient between forearm venous blood and forearm tissues. Predicted tissue values were determined using a two-compartment model of blood-tissue lactate exchange in the resting forearm, assuming an initial tissue concentration = 0.389·(resting venous lactate concentration) + 1.9 mmol·l^{-1} (99). In one subject there was resting forearm lactate uptake at rest and this expression was not used. Data from this subject were excluded. Rest, exercise and recovery values are included. n = 5 subjects.

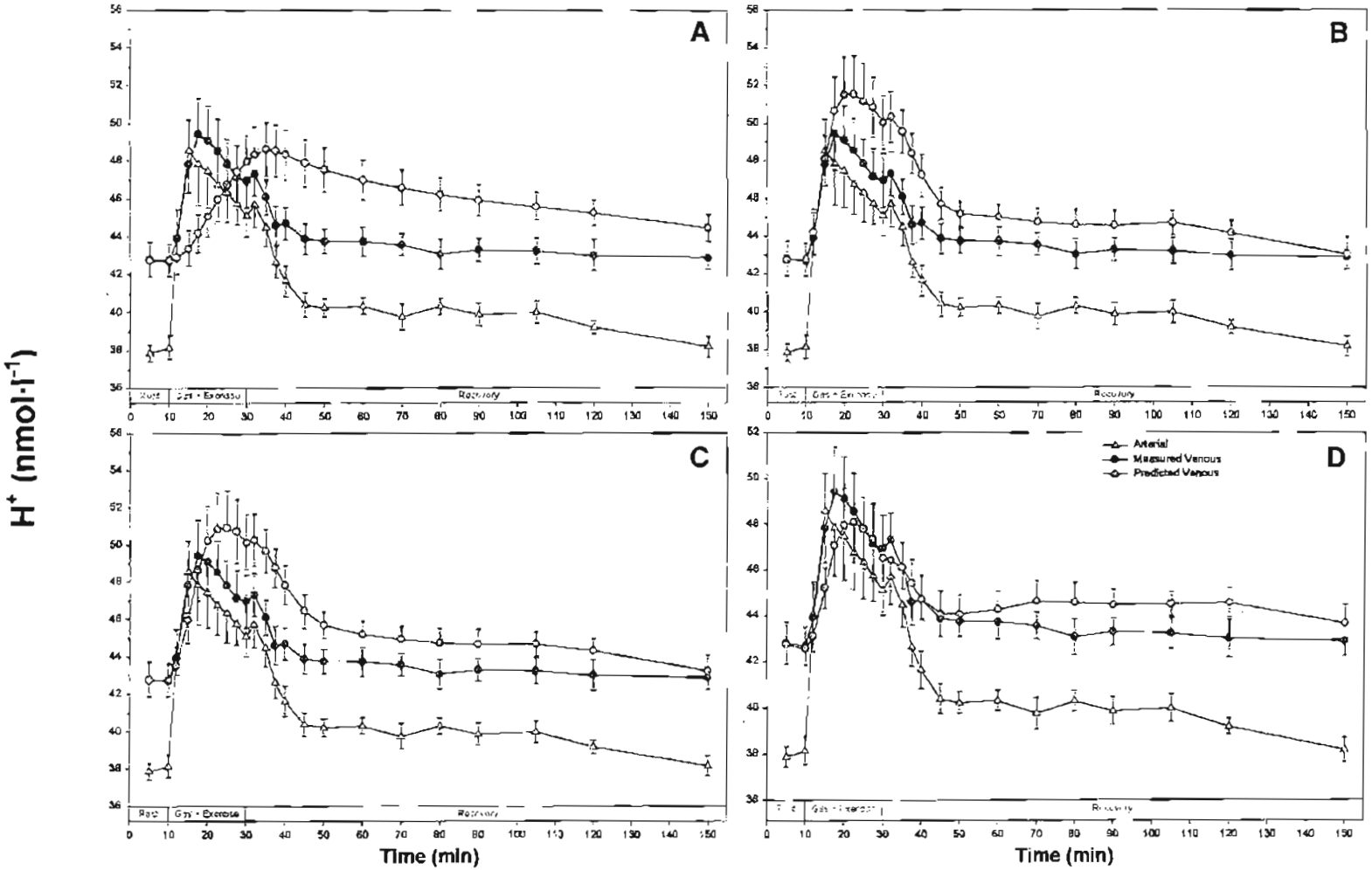


Figure 5.7. Resting forearm blood H^+ concentrations before, during and after 20 min of N_2O inhalation and leg exercise. Predicted values were determined using a modified Fick model (A-C) in which the diffusion parameter (m) was 1 (A), optimised to best fit predicted to measured venous data (B), and $100^2 \cdot Vi^{-1}$ (C), and using a two-compartment model (D). Values are means ± SEM, n = 6.

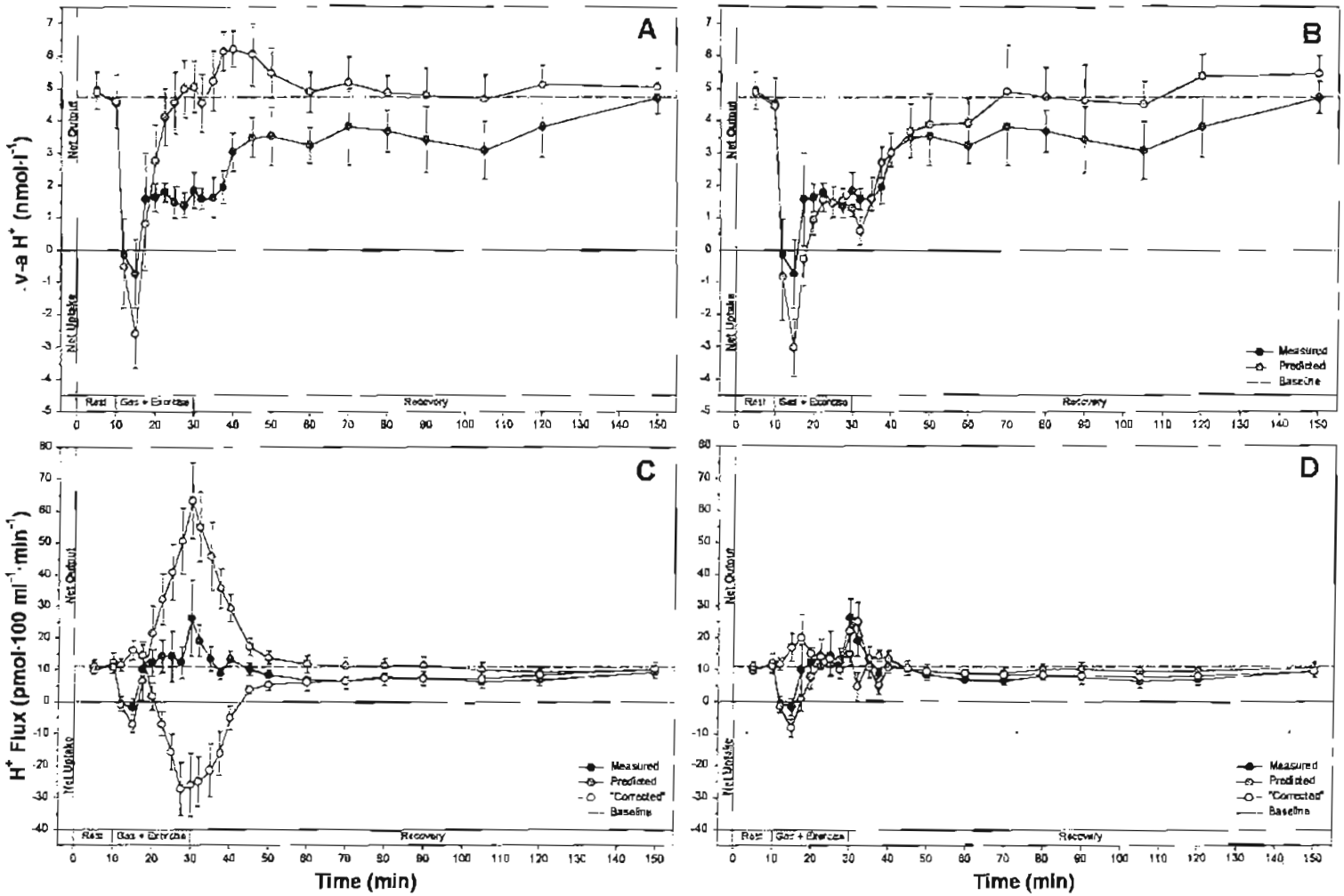


Figure 5.8. Veno-arterial H^+ concentration difference (A,B), and H^+ flux (C,D) in the resting forearm before, during and after 20 min of N_2O inhalation and leg exercise. Predicted values were determined using a modified Fick model (A,C) in which the diffusion parameter (m) was $100^2 \cdot Vi^{-1}$, and a two-compartment model (B,D). "Corrected" = Measured – Predicted + Baseline. Values are means ± SEM, n = 6.

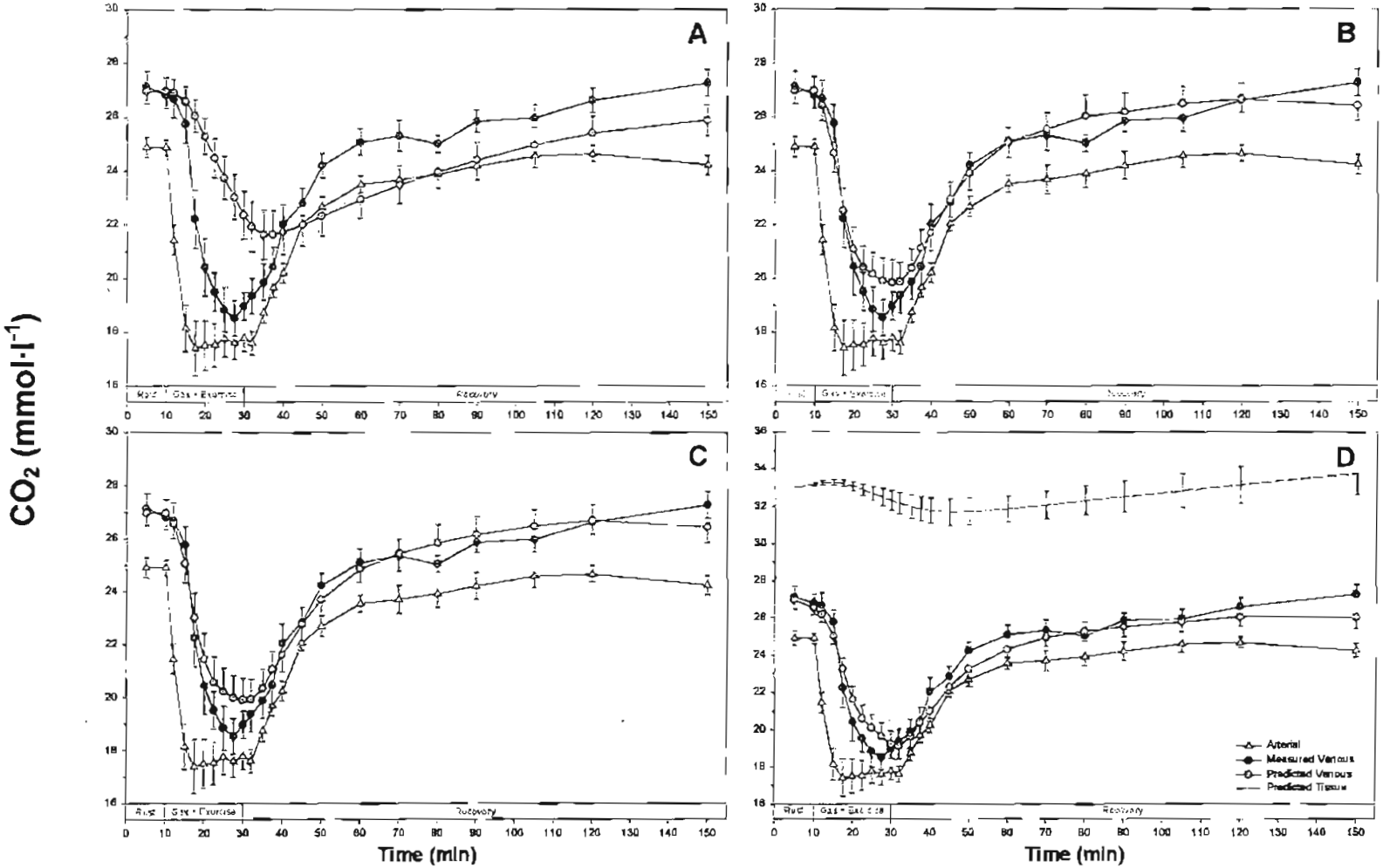


Figure 5.9. CO_2 content in the resting forearm before, during and after 20 min of N_2O inhalation and leg exercise. Predicted values were determined using a modified Fick model (A-C) in which the diffusion parameter (m) was 1 (A), optimised to best fit predicted to measured venous data (B), and $100^2 \cdot Vi^{-1}$ (C), and using a two-compartment model (D). Values are means ± SEM, n = 6.

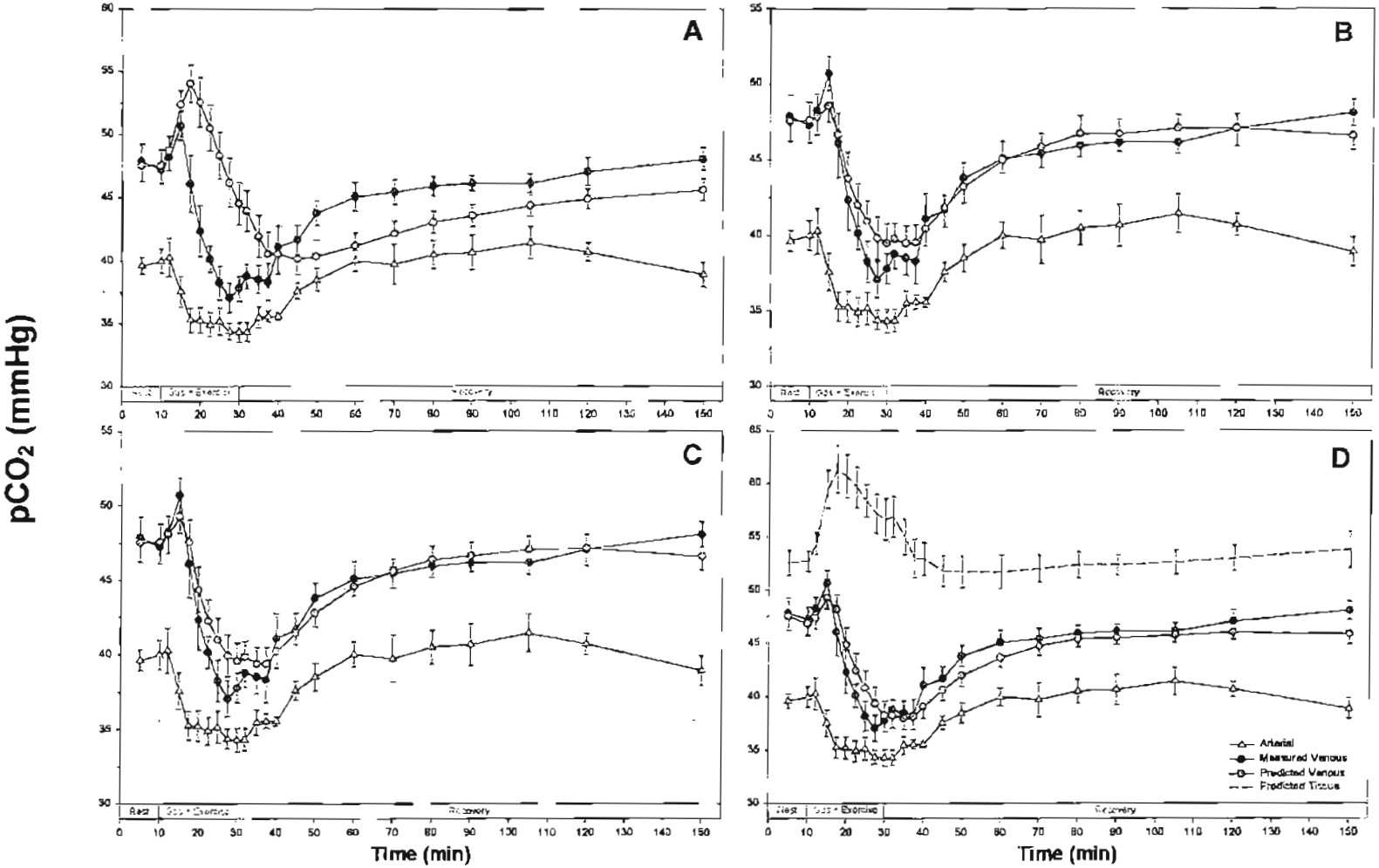


Figure 5.10. pCO_2 in the resting forearm before, during and after 20 min of N_2O inhalation and leg exercise. Predicted values were determined using a modified Fick model (A-C) in which the diffusion parameter (m) was 1 (A), optimised to best fit predicted to measured venous data (B), and $100^2 \cdot Vi^{-1}$ (C), and using a two-compartment model (D). Values are means ± SEM, n = 6.

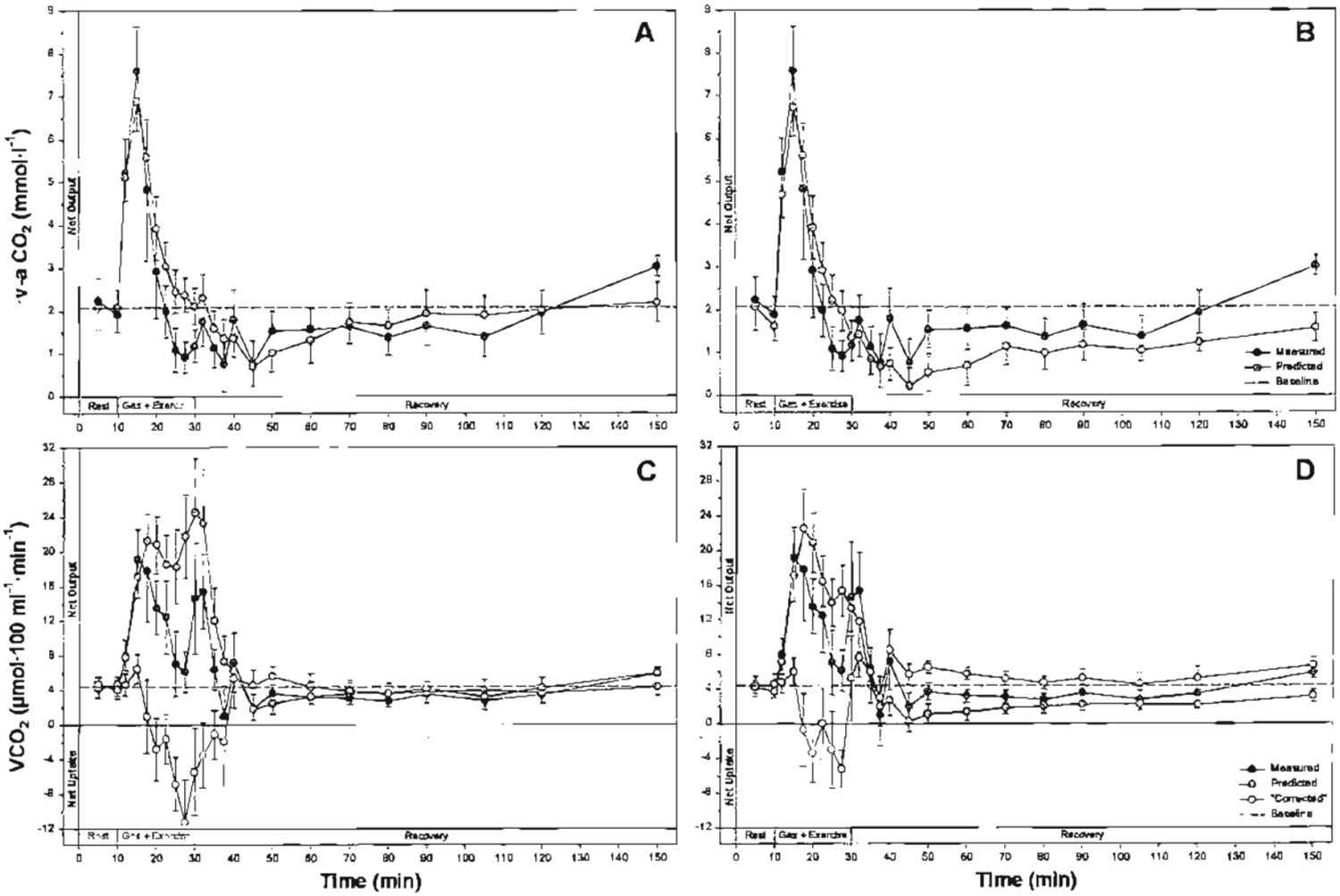


Figure 5.11. Veno-arterial CO_2 content difference (A,B), and $\dot{V}CO_2$ (CO_2 flux C,D) in the resting forearm before, during and after 20 min of N_2O inhalation and leg exercise. Predicted values were determined using a modified Fick model (A,C) in which the diffusion parameter (m) was $100^2 \cdot Vi^{-1}$, and a two-compartment model (B,D). "Corrected" = Measured – Predicted + Baseline. Values are means ± SEM, n = 6.

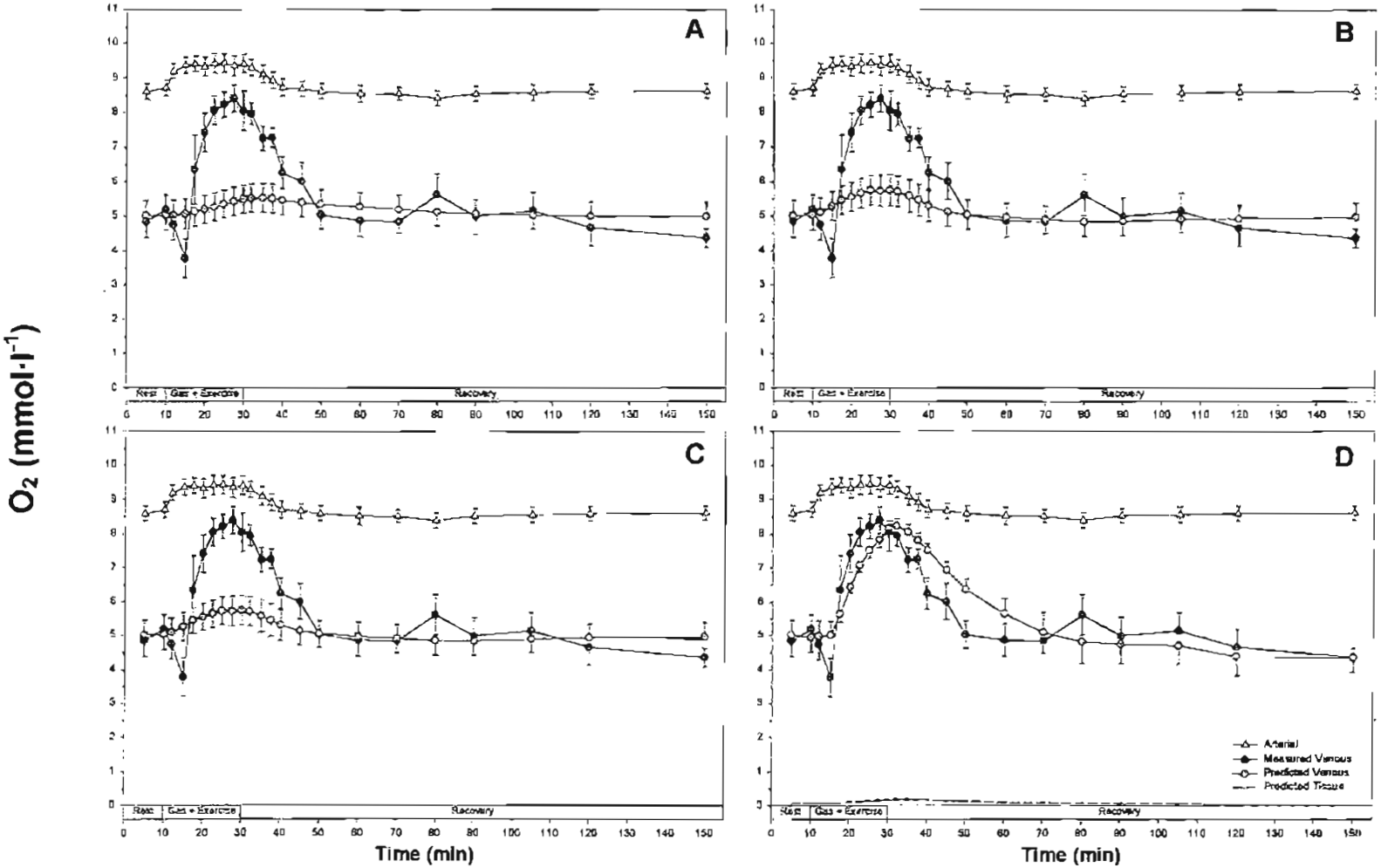


Figure 5.12. O_2 content in the resting forearm before, during and after 20 min of N_2O inhalation and leg exercise. Predicted values were determined using a modified Fick model (A-C) in which the diffusion parameter (m) was 1 (A), optimised to best fit predicted to measured venous data (B), and $100^2 \cdot Vi^{-1}$ (C), and using a two-compartment model (D). Values are means ± SEM, n = 6.

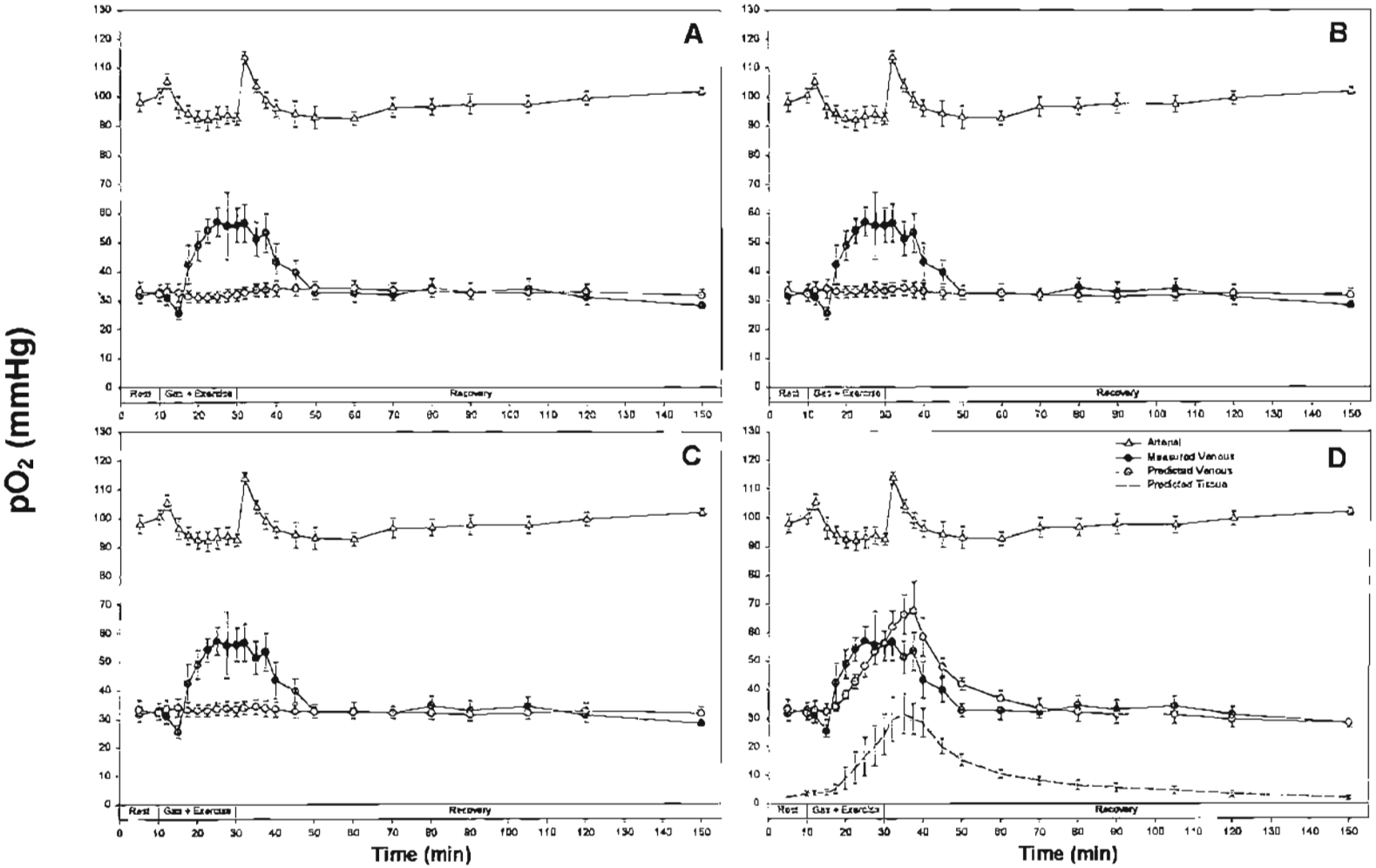


Figure 5.13. pO_2 in the resting forearm before, during and after 20 min of N_2O inhalation and leg exercise. Predicted values were determined using a modified Fick model (A-C) in which the diffusion parameter (m) was 1 (A), optimised to best fit predicted to measured venous data (B), and $100^2 \cdot Vi^{-1}$ (C), and using a two-compartment model (D). Values are means ± SEM, n = 6.

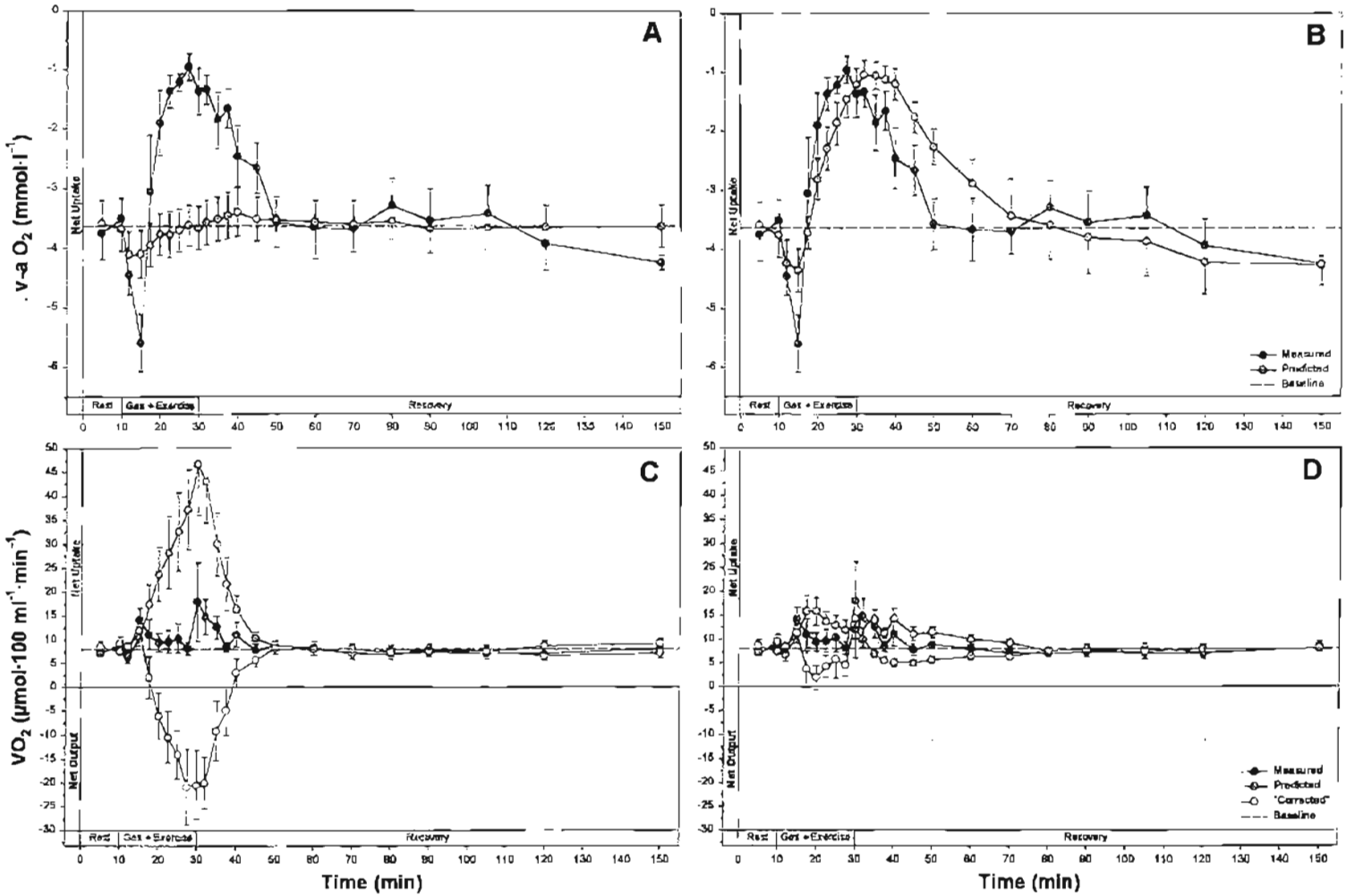


Figure 5.14. Veno-arterial O_2 content difference (A,B), and $\dot{V}O_2$ (-ve O_2 flux C,D) in the resting forearm before, during and after 20 min of N_2O inhalation and leg exercise. Predicted values were determined using a modified Fick model (A,C) in which the diffusion parameter (m) was $100^2 \cdot Vi^{-1}$, and a two-compartment model (B,D). "Corrected" = Measured – Predicted + Baseline. Values are means ± SEM, n = 6.

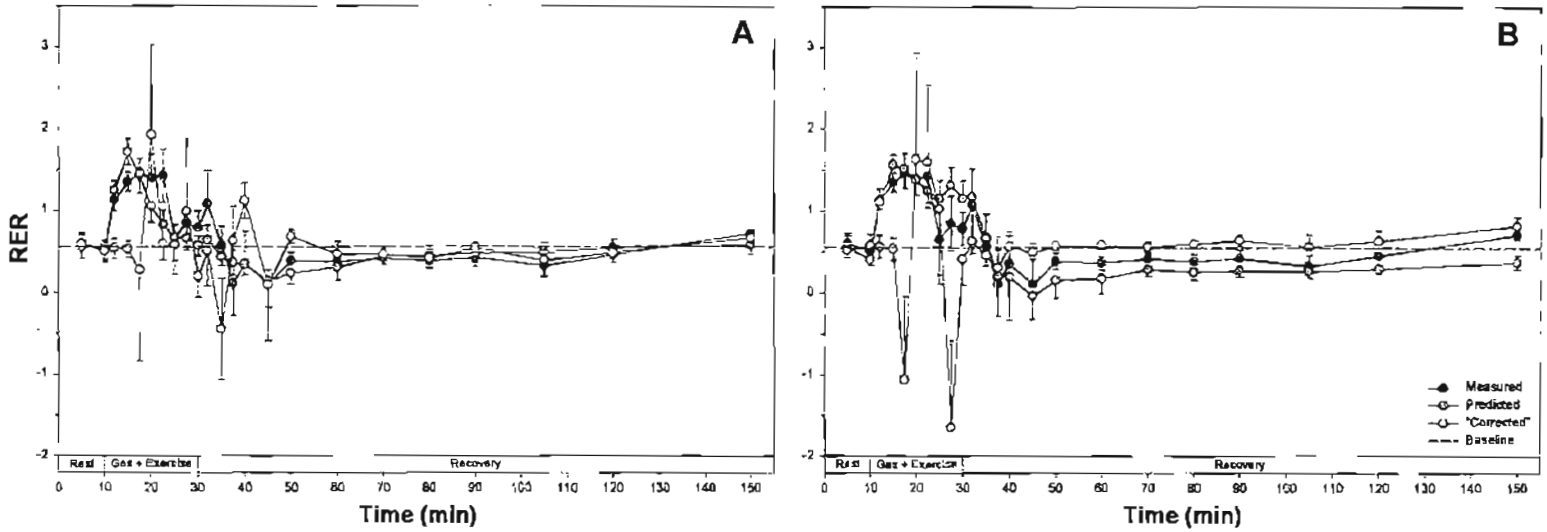


Figure 5.15. Forearm respiratory exchange ratio (RER) before, during and after supine leg exercise. Predicted and "Corrected" values were determined using a modified Fick model (A) in which the diffusion parameter (m) was $100^{2} \cdot \dot{V}i^{-1}$, and a two-compartment model (B). Values are means ± SEM, n = 6. "Corrected" = Corrected $\dot{V}CO_2$ divided by corrected $\dot{V}O_2$.

5.4 Discussion

The main finding of the present study was that during and after N_2O inhalation combined with high intensity leg exercise (~80% $\dot{V}O_{2peak}$) there was substantial uptake and then persistent release of N_2O in the resting forearm. As N_2O is metabolically inert and is relatively more soluble and therefore diffusible across biological membranes than virtually all other blood borne substances, this clearly demonstrates the importance of equilibration processes alone in producing resting forearm veno-arterial concentration differences associated with arterial concentration changes. Furthermore, N_2O was delivered to the forearm in exactly the same manner as other blood borne substances that simultaneously underwent substantial arterial concentration changes, suggesting that forearm equilibration may dominate veno-arterial concentration differences of many substances when the arterial concentration changes over time. Supporting this view was the finding that the same two-compartment model of blood-tissue exchange that could largely account for forearm N_2O kinetics, could also predominantly account for venous concentration changes in lactate, CO_2 and to a lesser extent H^+ and O_2. Changes in resting forearm metabolism were therefore unnecessary to account for forearm veno-arterial concentration differences and consequently flux. This was further supported by the finding that total net fluxes of lactate, H^+, CO_2 and O_2, calculated over the whole period of exercise and recovery, were all consistent with baseline levels of forearm metabolism.

As with any modelling approach, the modelling analysis adopted in this study is subject to many limitations, some of which have been detailed in Chapter 2. The models employed were simple compartmental models, and therefore ignore tissue and blood flow heterogeneity that certainly influence tissue uptake to some degree (17-19, 21, 107). Furthermore, although each of the models has a theoretical basis, the approach of fitting model predicted to measured venous concentration data is somewhat empirical, in that it presumes that a given model has validity. Consequently, although a reasonable model fit could indicate that a model has a

sound theoretical basis, it does not confirm that the model is "correct" or that the model parameters have any real biological meaning. On the other hand, a poor fit, despite optimisation of one or more model parameters, is clear evidence that a model is flawed and is grounds for rejection (204).

In the present study, treating the whole forearm volume as a single compartment produced poor fits with all of the substances examined. Whilst a smaller tissue volume would almost certainly have produced a better fit, this would still have been worse compared to the optimised (m = fit) Fick model in which there was an additional model parameter (m). This is consistent with the report of Novotny et al. (190) in which resting muscle xenon kinetics were poorly predicted by a model of multiple parallel perfusion-limited compartments. These investigators (190) did not explore alternative models, but speculated that diffusion processes, such as countercurrent diffusion, form an important component of inert gas kinetics in resting muscle.

Countercurrent diffusion processes have been suggested by several other investigators to account for more delayed inert gas tissue output kinetics than predicted from theoretical models (121, 198-201, 280). Although countercurrent diffusion was not formally investigated in this study, there was some evidence of its presence in the preliminary experiment (Figure A.2). At the termination of N_2O inhalation completed without the confounding influences of leg exercise and the associated thermoregulatory increase in forearm blood flow, there was a very brief dip in venous blood N_2O levels consistent with a diffusion shunt. However, this phenomenon was not observed when N_2O and exercise were terminated simultaneously, possibly reflecting the higher forearm (skin) blood flow immediately post exercise in combination with less equilibrated forearm tissues and therefore larger transient blood-tissue N_2O concentration gradients. Furthermore, diffusion shunts are likely to contribute significantly to blood-tissue exchange kinetics only with highly diffusible substances such as N_2O. Consequently, models incorporating

diffusion shunts were not explored and were not essential to account for the majority of forearm N_2O kinetics in this study.

A potential problem with using a diffusible gas such as N_2O as a tracer substance is that some N_2O can be lost percutaneously. Stoelting and Eger (252) showed the percutaneous loss of N_2O from the forearm to be 3.6 $ml \cdot min^{-1} \cdot m^{-2}$, representing ~6% of the total N_2O taken up during inhalation of 70% N_2O. Percutaneous losses in the present study would have tended to be reduced with the use of 10% N_2O, but increased in association with the thermoregulatory increase in skin blood flow. Whilst it is not possible to reliably predict the quantity of N_2O lost via the skin, ~80% of the N_2O taken up by the forearm during inhalation was recovered in forearm venous blood after 120 min of recovery. Furthermore, a significant veno-arterial N_2O concentration difference at the end recovery indicated that a significant quantity of N_2O remained within the forearm. In combination these findings suggest that the percutaneous losses of N_2O were minor.

In all of the substances examined, the inclusion of a diffusion component to forearm blood tissue-exchange significantly improved model fits over those obtained with the single compartment perfusion limited model, supporting the view that membrane transport process are an important determinant of resting muscle uptake and output kinetics. Although clearly the best model of resting forearm N_2O exchange, the two-compartment model was little better than the optimised (m = fit) or the $m = 100_2 \cdot Vi^{-1}$ Fick model when applied to lactate or CO_2. Furthermore, only with N_2O and H^+ was the optimised (m = fit) Fick model diffusion term different from $100^2 \cdot Vi^{-1}$. This suggests that the two-compartment Fick model adopted in the studies reported in Chapters 3 and 4, and in which a somewhat arbitrary diffusion term was used, may be a reasonable model of passive forearm lactate and CO_2 movements during and after supine leg exercise. Similarly, although there was an inverse relationship between the diffusion terms derived from the Fick and two-compartment models, the relative permeabilities of each substance were generally similar between models.

N_2O is relatively more soluble in water, and presumably biological membranes, than many other substances. The Ostwald solubility of N_2O is similar to that of CO_2 and considerably higher than that of O_2 (N_2O, 0.455; CO_2, 0.631; O_2, 0.0271; ml gas·ml water^{-1} at 37°C (268)). As diffusion is largely governed by membrane solubility, N_2O membrane translocation is likely to be considerably faster than in many other substances, including H^+, for which the sarcolemma is relatively impermeable, and molecules such as lactate that rely on both diffusion and transport systems for membrane translocation (98, 132-136, 156, 172, 216-218). However, diffusion is not the only process involved in the membrane translocation of lactate, H^+ or even CO_2 given that bicarbonate is likely to facilitate blood-tissue CO_2 exchange (109). Ion exchanges as well as membrane transport processes influence the membrane translocation of these substances. Consequently, blood-tissue equilibration associated with arterial blood concentration changes in these substances is far more complex than the models adopted in this study suggest. Nevertheless, although there is abundant evidence that sarcolemmal lactate transport occurs via both passive diffusion and a saturable carrier mediated H^+ symport process (98, 132-135, 153, 172, 217, 218), the importance of such processes in resting muscle blood-tissue equilibration is still unclear. In the present study, capillary and sarcolemmal lactate transport limitations were essentially combined into a single term, with the rate of entry or exit of lactate into the forearm tissues assumed to be a linear function of the blood-tissue lactate concentration gradient. While this assumption could limit the applicability of these models to lactate, there is considerable evidence that the K_m and V_{max} for sarcolemmal lactate transport is relatively high (134, 172, 217, 218). Consequently, membrane translocation processes may well be relatively linear in the range of the transmembrane concentration gradients encountered during this study, and in association with moderate intensity exercise in general. If sufficiently rapid, membrane transport processes could be much less important than blood flow in determining the time course of equilibration between arterial blood and the forearm tissues under non-equilibrium arterial concentration conditions (178), although it would appear that both are important in regard to resting muscle lactate uptake.

However, the relative contribution of blood flow and membrane transport processes, as well as tissue volume, to the rate of blood-tissue lactate exchange is difficult to predict. In the present study, the single compartment perfusion limited model of blood-tissue exchange could account for only ~30% of the variance in forearm venous blood lactate concentration, although this would have been somewhat greater had the tissue volume been assumed smaller. With the inclusion of a second compartment, more than 90% of the variance could be explained, but the relative contribution of blood flow, tissue volume and membrane transport processes to forearm lactate uptake is not explicit from this model. While there was only a weak relationship between forearm blood flow and forearm lactate flux ($r^2 = 0.21 \pm 0.09$, $P \geq 0.640$), and similar correlations have been proposed to reflect the contribution of blood flow to blood-tissue lactate exchange in the past (103, 130), such correlational analyses do not appear entirely sound because blood flow appears in both dependent and independent variables.

In this study, a strong linear relationship between venous blood lactate concentrations and the venous-to-tissue blood lactate concentration gradient was predicted on the basis of a two-compartment model of blood-tissue lactate exchange and is consistent with previous reports of blood-muscle lactate gradients (99, 103). While largely consistent with a study by Gladden (99), in which resting isolated dog gastrocnemious-plantaris muscle lactate concentrations were determined during lactate infusion, the venous-to-muscle lactate gradient was ~30% lower compared to the present study. Aside from obvious preparation and other methodological differences, this would be consistent with greater tissue lactate disposal in the muscle preparations studied by Gladden (95) or, perhaps more likely, too large a tissue compartment volume, and correspondingly different values for other parameters in the models used in the present study. Nevertheless, the finding that predicted tissue lactate levels were substantially lower than arterial in this study is consistent with data reported by Chin et al. (46) in the resting gastrocnemius-plantaris-soleus muscle group during perfusion with lactacid perfusate in rats, and by

Lindinger et al. (161) in the resting deltoid muscle following repetitive brief high intensity leg exercise in humans. Lindinger et al. (161) also found that deltoid muscle lactate levels were similar to end-exercise levels even after 25 min of recovery. This is also consistent with predictions from this study in which the tissue concentration disturbances of several substances including lactate and N_2O were predicted to have a prolonged time course of recovery. Although, at least intuitively, a relatively low tissue concentration appears to indicate tissue disposal, clearly this may not be the case.

In an intact tissue such as the forearm, there is considerable uncertainty regarding the likely tissue sources from which venous blood is derived (48), and this has potentially important implications for the modelling analysis adopted in this study and measurements of forearm substance fluxes in general. To be of any real use for determining measured or predicted forearm flux, blood flow measurements must be representative of the forearm tissues from which venous blood is sampled. During moderate to high intensity leg exercise thermoregulatory increases in skin blood flow dominate blood flow measurements derived from the whole forearm (127, 285). At the same time, forearm muscle blood flow has been shown to decrease (127, 285). Consequently, forearm flux calculated from whole forearm blood flow measurements would be grossly over-estimated if venous blood derived only from muscle. Similarly, if a component of venous blood derived from skin vascular beds, then muscle blood flow measurements, without a proportional skin component, would also be inappropriate. While blood from deep forearm veins is generally regarded to derive principally from muscle, a significant component could derive from more superficial tissues such as skin (48). In the present study, as well as those reported in Chapters 3 and 4, there was good evidence that a substantial component of venous blood was derived from skin sources, and that whole forearm blood flow measurements were therefore most appropriate. In each of these studies, there was no evidence that forearm $\dot{V}O_2$ increased overall. Had blood flow measurements been over-estimated with the inappropriate inclusion of a skin component of flow, $\dot{V}O_2$ determined from

more appropriate flow measurements would have decreased. In addition, unlike other substances, forearm $\dot{V}O_2$ appeared to be little affected by equilibration process due to the relatively minor changes in arterial pO_2 and O_2 content associated with leg exercise. Nevertheless, there were strong positive relationships between forearm blood flow and venous blood pO_2 and O_2 content (pO_2; $r = 0.69 \pm 0.07$, $P \leq 0.032$, O_2 content; $r = 0.75 \pm 0.06$, $P \leq 0.023$ in the present study), consistent with a significant skin contribution to venous blood samples (271). Furthermore, at the end of exercise and N_2O inhalation there was a strong inverse relationship between forearm blood flow and the magnitude of the arteriovenous N_2O concentration difference ($r = -0.925$, $r^2 = 0.856$, $P = 0.008$, $n = 6$). This suggests that tissue N_2O equilibration was relatively more complete in those subjects who demonstrated the greatest thermoregulatory increase in forearm blood flow, and is good evidence that forearm blood flow measurements were largely representative of the tissues from which antecubital venous blood was derived.

As in the study reported in Chapter 4, the Fick model appeared to overpredict venous lactate concentrations during recovery. While this could indicate metabolic lactate disposal during this period, it would also be consistent with a model limitation. Although the latter appears the more likely explanation given that the two-compartment model tended to predict venous levels more closely, even with this model predicted venous lactate concentrations were consistently higher than measured values during recovery. In the presence of a period of real change in forearm metabolism, the least squares approach of fitting predicted to measured venous concentration data would tend to produce model parameters that best fit both metabolic and equilibration phenomena. This could distort the apparent time course of changes in forearm metabolism, under-estimating the impact of metabolism during the period of metabolic change, and over-estimating its influence before and after the period of real change. Consequently, lower than predicted venous lactate concentrations during recovery could indicate an earlier period of forearm lactate disposal, such as during leg exercise, or more likely, during the period in which

resting muscle lactate concentrations were most elevated. Therefore, these data do not exclude the possibility of forearm lactate metabolism under conditions of elevated arterial lactate concentrations. Nevertheless, at least 90% of the variance in venous lactate concentrations could be explained on the basis of passive lactate movements. Furthermore, total net forearm flux of lactate over the combined period of exercise and recovery was consistent with baseline levels of lactate metabolism. In combination, these findings strongly suggest that lactate disposal is a minor component of resting muscle veno-arterial lactate concentration differences under conditions in which arterial lactate is elevated by leg exercise.

Very few investigators have attempted to distinguish passive lactate uptake from that associated with increased resting muscle lactate disposal when arterial lactate is elevated by infusion or by exercise in remote muscle groups. Consequently, in several reports (46, 161, 203), it is likely that some fraction of the lactate uptake that was attributed to disposal, instead reflected passive lactate movements. Similarly, substantial resting muscle CO_2 output that has previously been reported during exercise in remote muscle groups (161), and with a perfusate designed to replicate the arterial blood disturbances associated with heavy exercise (46), may not indicate a change in resting muscle CO_2 production as has been suggested (46, 161). In the present investigation, as in the studies reported in Chapters 3 and 4, changes in venous CO_2 content were remarkably well predicted on the basis of equilibration processes alone, without any change in resting forearm CO_2 production. Furthermore, in all of these studies there were times when the resting muscle respiratory exchange ratio greatly exceeded the metabolic limit of unity, clearly demonstrating the importance of equilibration processes under these conditions. The normalisation of RER values with the modelling analysis as well as the absence of change in RER, determined across the combined period of exercise and recovery, strongly suggest that resting forearm CO_2 production does not change during leg exercise. That equilibration within resting muscles has a prolonged time course was recently alluded to by Bergman et al. (24). These investigators (24) reported that

during a primed continuous infusion of [3-^{13}C]lactate, there was apparent $^{13}CO_2$ consumption across resting limbs even after 90 min, due to incomplete isotopic equilibration in CO_2 pools.

In the present study, as well as those reported in Chapters 3 and 4, venous disturbances in O_2 content were relatively poorly predicted on the basis of equilibration processes. However, the impact on venous O_2 levels of forearm equilibration with the relatively minor changes in arterial pO_2 and O_2 content associated with leg exercise are likely to be minimal. Furthermore, blood O_2 chemistry is far more complex and therefore difficult to model than most, and perhaps all, other substances. In this study, in which an attempt was made to account for haemoglobin and myoglobin O_2 binding and to some degree the influence of CO_2 and H^+ changes on haemoglobin O_2 affinity, the model predictions were closer to measured venous values compared to a more simple model of blood-tissue exchange.

In summary, substantial uptake and subsequent release of N_2O in the resting forearm during and after N_2O inhalation and leg exercise has a prolonged time course that can be relatively well accounted for by relatively simple two-compartment models of forearm blood-tissue exchange. Even without the modelling approach, these data clearly demonstrate that under non-equilibrium conditions, and in the absence of forearm metabolic influences, the resting forearm can produce substantial and persistent veno-arterial concentration differences even with a relatively diffusible substance such as N_2O. Equilibration processes are therefore likely to dominate forearm veno-arterial concentration differences in other substances under conditions in which the arterial concentration changes significantly with time. Consequently, when arterial lactate is elevated by infusion or by exercise, an important component of resting muscle lactate uptake is likely to represent equilibration, particularly given the size of resting muscle vascular compartments and their relatively low blood flow. Based on the modelling approach adopted in this study, substantial arteriovenous

concentration differences in lactate, CO_2, H^+ and O_2 in the resting forearm during and after supine leg exercise are largely consistent with blood-tissue equilibration processes with little or no contribution from changes in forearm metabolism.

CHAPTER 6 SUMMARY AND CONCLUSIONS

THE STUDIES REPORTED IN THIS THESIS clearly demonstrate the difficulties associated with the interpretation of non-equilibrium veno-arterial concentration differences such as those measured in the resting forearm during and after moderate to high intensity leg exercise.

Based on measurements of veno-arterial lactate concentration differences across resting muscles and in some cases resting muscle lactate content measurements, several investigators have suggested that resting muscle is an important site of net lactate disposal when the arterial lactate concentration is elevated by infusion (46, 95, 97, 279) or by exercise in remote muscle groups (4, 5, 40, 87, 88, 104, 161, 203, 211, 247, 282, 284). However, in all of these studies non-steady-state conditions prevailed, and under these conditions wash-in inevitably accounts for much of the uptake. While some investigators have considered the effects of wash-in at least to some degree (40, 46, 95, 161, 203, 279), several apparently have not.

Lactate uptake associated with blood-tissue equilibration is difficult to distinguish from that associated with an underlying shift towards resting muscle lactate disposal. One approach is to compare the total quantity of lactate taken up to tissue lactate content estimated from lactate concentrations in biopsy samples and the tissue volume or mass. However, only in a few studies does this approach appear to have been attempted (46, 95). Furthermore, the quantity of lactate taken up and resident within the muscle in the form of pyruvate, as well as other metabolic intermediates that potentially buffer lactate concentration changes, does not appear to have been considered. Nevertheless, these studies, as well as infusion studies in general, support the view that resting muscles are a site of significant net lactate disposal, at least when arterial lactate is elevated by infusion. A second approach to discerning resting muscle lactate uptake associated with wash-in from that related to disposal is

to determine the quantity of lactate returned to the circulation during the subsequent post-uptake recovery period. Very few authors (39, 40) appear to have used this approach, perhaps due to the requirement for a protracted recovery period and the relatively insensitive nature of this approach for detecting overall changes in metabolism (164). An alternative approach, and the basis of the studies reported in this thesis, is to predict the contribution to uptake of the process of blood-tissue equilibration. Given a suitable model and associated assumptions, differences between measured and model predicted uptake reflect underlying changes in tissue metabolism.

In the study reported in Chapter 3, a relatively simple two-compartment form of the Fick (Kety-Schmidt) model of blood-tissue exchange, more commonly applied to the study of blood-tissue inert gas kinetics, was employed to predict the contribution of wash-in to resting forearm lactate uptake when the arterial lactate concentration was elevated by leg exercise. Relatively close agreement between measured and model predicted venous lactate concentrations suggested that resting forearm lactate uptake could predominantly be accounted for in terms of passive lactate movements without disposal.

In Chapter 4, the same modelling analysis was employed to investigate the contribution of passive blood-tissue lactate movements to lactate concentration differences frequently reported in blood sampled from different sites during the non-equilibrium conditions associated with incremental leg exercise to exhaustion (65, 80, 87, 211, 247, 282, 284). Passive lactate movements between the arterial blood and the forearm tissues associated with a substantial increase in arterial lactate concentration could entirely account for changes in finger prick and forearm venous blood lactate concentrations during incremental leg exercise. As a result, differences in lactate threshold determinations, particularly those based on a fixed blood lactate concentration, were also largely consistent with passive blood-tissue lactate movements within the forearm. While similar blood sampling site differences in blood

lactate concentrations and thresholds have been reported previously (80, 211, 284), this is the first study to suggest that these differences are related primarily to passive lactate movements and not changes in forearm lactate metabolism.

Whilst the pattern of increasing lactate concentration during exercise in both finger prick and venous blood samples was compatible with a threshold model, the pattern in arterial blood, which presumably most closely reflects the rate of addition of lactate from the exercising muscles to the blood compartment, was better described by an exponential model. While these findings do not exclude a threshold phenomenon in the lactic acidosis of exercise, the contribution of passive blood-tissue equilibration processes to the structure of the lactate profiles must be considered.

In the study reported in Chapter 4, a significant quantity of lactate had not been returned to the circulation following 120 min or recovery from incremental leg exercise. This finding, in combination with significant time dependent differences between measured forearm lactate flux and that predicted on the basis of passive lactate washout during recovery, is consistent with significant resting forearm lactate disposal, an elevated forearm lactate content even after 120 min of recovery, or perhaps both. Lactate disposal may well account for these findings given that lactate disposal processes are almost certainly lactate concentration dependent, and that higher arterial lactate concentrations were achieved in this study (~11 $mmol \cdot l^{-1}$) compared to the studies reported in Chapters 3 and 5 (~6 $mmol \cdot l^{-1}$). This would be consistent with previous reports in which lactate uptake associated with high arterial lactate levels (>6 $mmol \cdot l^{-1}$) has been attributed, at least in part, to lactate disposal (40, 104, 203, 247). Furthermore, lactate uptake has been shown to increase as a function of the plasma lactate concentration (97). However, based on the studies reported in this thesis, it is not entirely clear if the 30 min period allowed for equilibration in the study reported by Gladden et al. (97) would have been sufficient to achieve a steady-state, particularly in muscle exposed to a very high plasma lactate concentration. Consequently, a prolonged period of blood-tissue equilibration

could be responsible for a significant component of lactate uptake under these conditions. Similarly, a limitation to the modelling analysis in combination with prolonged resting forearm lactate washout would also account for the findings reported in Chapter 4.

A further finding of this study was that a substantial increase in resting forearm CO_2 output during incremental leg exercise was consistent with passive CO_2 efflux from the forearm in association with a hyperpneic decrease in arterial CO_2 content. This was further supported by the finding that total net forearm CO_2 flux over the combined period of exercise and recovery was also consistent with baseline levels of CO_2 production having been maintained throughout exercise and recovery. This finding contrasts with previous reports in which a similar increase in resting muscle CO_2 output in association with a low CO_2 perfusate (46) and brief periods of high intensity leg exercise (161) were considered to reflect an increase in resting muscle carbohydrate metabolism. However, passive CO_2 movements were not considered in these studies (46, 161).

While forearm O_2 flux was relatively poorly predicted on the basis of passive O_2 movements, these are unlikely to significantly influence forearm O_2 flux given that there were only minor changes in arterial pO_2 and O_2 content during and after incremental leg exercise. Furthermore, the total net forearm O_2 flux throughout exercise and recovery was consistent with pre-exercise levels of forearm $\dot{V}O_2$. Consequently, there was no evidence for a net change in forearm RER over the period of exercise and recovery, supporting the view that resting forearm metabolism was not altered in favour of carbohydrate metabolism.

In Chapter 5, assumptions underlying the treatment of blood-tissue lactate exchange in a manner equivalent to an inert gas were examined more closely. Veno-arterial concentration differences in lactate, H^+, CO_2, O_2 and the metabolically inert gas N_2O were examined during and after a 20 min period of moderate intensity leg exercise

and N_2O inhalation. A two compartment model incorporating a diffusion barrier to blood-tissue exchange simultaneously accounted for the majority of the observed veno-arterial concentration differences in N_2O, lactate, CO_2 and H^+, supporting the hypothesis that passive movements of blood borne substances dominate forearm veno-arterial concentration differences during leg exercise.

Although veno-arterial N_2O concentration differences were less well predicted with the Fick model employed in the studies reported in Chapters 3 and 4 when compared to the more rigorous two-compartment model derivation, both models produced similar fits when applied to lactate and CO_2. Consequently, despite the accidental use of an arbitrary value for the diffusion term in the Fick model employed in Chapters 3 and 4, this did not appear to substantially alter model predictions, particularly with respect to CO_2. With regard to lactate, there was some evidence that the Fick model predictions were somewhat poorer, particularly during recovery. This, most likely, was largely responsible for the more marked differences between measured and predicted venous lactate concentrations observed during recovery in the study reported in Chapter 4. However, this possibility was not confirmed by applying the two-compartment model to the data obtained in Chapter 4 due principally to the absence of inert gas data with which to obtain the majority of the model parameter estimates.

In combination, these studies strongly support the view that passive movements dominate the blood-tissue exchange of membrane transportable blood-borne substances that undergo significant arterial concentration changes during leg exercise and recovery. Furthermore, it would appear that forearm equilibration with arterial concentration changes, even in a relatively freely diffusible inert gas, has a protracted time course, presumably because of a relatively low blood flow and metabolic rate. As uptake associated with wash-in is essentially indistinguishable from that associated with metabolism careful consideration for the potential effects of

wash-in is essential to avoid the over-estimation of resting muscle disposal of substances such as lactate during infusion and exercise studies.

The contemporary view is that lactate is an important metabolic intermediate, representing a readily oxidisable energy source and a gluconeogenic precursor capable of rapid transport between tissue sites. This "lactate shuttle" hypothesis represents a significant departure from earlier concepts of lactate as a metabolic waste product, and has important implications for the role of resting muscles in modulating blood lactate levels during exercise. The studies reported in this thesis are not incompatible with the lactate shuttle hypothesis in that resting muscle lactate disposal by complete oxidation, an important fate generally attributed to resting muscle lactate disposal (24, 27, 28, 34, 169, 171), could be considered an inefficient use of a limited carbohydrate resource, particularly during sustainable exercise of moderate intensity. Furthermore, increases in resting muscle $\dot{V}O_2$ and particularly $\dot{V}CO_2$, potentially associated with a shift towards resting muscle lactate oxidation, could significantly add to the ventilatory cost of exercise. Epinephrine has been shown to preferentially stimulate glycolysis in glycolytic muscle and endogenous lipid hydrolysis in oxidative muscle (196), both of which could offset the tendency for pyruvate derived from accumulated lactate by mass action to undergo oxidation in the citric acid cycle. Catecholamine release during exercise, in combination with a decrease in resting muscle blood flow could therefore serve to limit the loss of lactate to resting muscles. Furthermore, pyruvate dehydrogenase (PDH) activity is likely to be relatively low in resting muscles given the high ratios of ATP/ADP and $NADH/NAD^+$ (253). Although lactate and therefore pyruvate accumulation would tend to stimulate PDH activity, this could be more than offset by the inhibitory effects of acetyl-CoA and NADH accumulation and the simultaneous depletion of free CoA (209, 253). This would further limit the capacity of resting muscle to oxidise lactate.

A further possibility that could explain previous reports of significant net lactate disposal by resting muscles (40, 46, 104, 161, 203, 247) and its relative absence in

the studies reported in this thesis, relates to the potential for several reactions to effectively buffer changes in muscle lactate concentration. For example, carnitine has been shown to buffer changes in acetyl-CoA during muscle contractions (58, 221, 223) and could serve as a sink for acetyl CoA derived from pyruvate in association with an elevated intramuscular lactate concentration. Similarly, expansion of glyconeogenic and therefore potentially glycolytic intermediates, as well as those of the citric acid cycle, in combination, could account for a significant quantity of lactate taken up by muscles exposed to elevated intramuscular lactate with little or no change in energy requirements. Carbon accumulated in this manner could serve to support the energy requirements of the muscle during the subsequent period of blood lactate decline with reaction equilibria tending towards the baseline state. At the same time, a relative abundance of mitochondrial NADH could limit the capacity of the mitochondrial pyridine nucleotide shuttles to maintain the redox coupled exchange of pyruvate to lactate and a reduced glycolytic rate, potentially favouring net lactate production and release.

Clearly, the impact of lactate and consequently pyruvate accumulation on the time course and net effects of glycolysis and pyruvate consuming reactions within resting muscles during exercise with other muscle groups are very difficult to predict. Although the studies reported in this thesis suggest that passive movements dominate the blood-tissue exchange of lactate in the resting forearm, underlying changes in net lactate metabolism cannot be excluded.

The studies reported in this thesis, as with many in the field, have important limitations. In addition to uncertainty regarding the source and volume of the tissues from which venous blood was collected and the reliability of forearm blood flow measurements to be representative of the tissues under study, the modelling approach in itself has significant limitations. The models employed in these studies, while quite complex, are clearly simplistic both in terms of models that can be applied to blood-tissue exchange (17-19, 21, 102, 156) and the processes that underlie

blood-tissue exchange phenomena. Perhaps most importantly, the approach of assuming a constant rate of tissue metabolism and then fitting a model to measured venous data in order to examine passive substance movements favours an optimal fit to the data and is therefore somewhat self-fulfilling. However, given a good model fit, this approach, at the very least, demonstrates that equilibration processes can account for observed venous concentration changes. Furthermore, no fitting was employed in the Fick model employed in the studies reported in Chapters 3 and 4 and, except for N_2O, only a single model parameter was fit to each venous concentration curve in the alternative two-compartment model employed in Chapter 5. The ability of these models to fit a given venous concentration curve was therefore considerably restricted. More importantly, the total net forearm flux of each substance, determined over the combined period of exercise and recovery, was generally consistent with maintained baseline levels of flux. A notable exception was the total net forearm flux of lactate reported in Chapter 4 in association with the particularly high arterial lactate load encountered during and after incremental leg exercise. This could well reflect significant resting muscle lactate disposal.

In summary, these studies demonstrate the difficulties associated with the interpretation of veno-arterial concentration differences measured during non-equilibrium conditions such as exercise. In the resting forearm, substantial lactate uptake as well as increased CO_2 output and a shift towards H^+ and perhaps O_2 uptake are largely consistent with passive movements associated with exercise related changes in arterial blood chemistry and forearm blood flow. Consequently, the existing evidence that resting muscle plays an important role in disposing of lactate produced by active muscles should be treated cautiously.

APPENDIX Individual subject forearm blood flow and substance concentration responses during experiments reported in Chapter 5

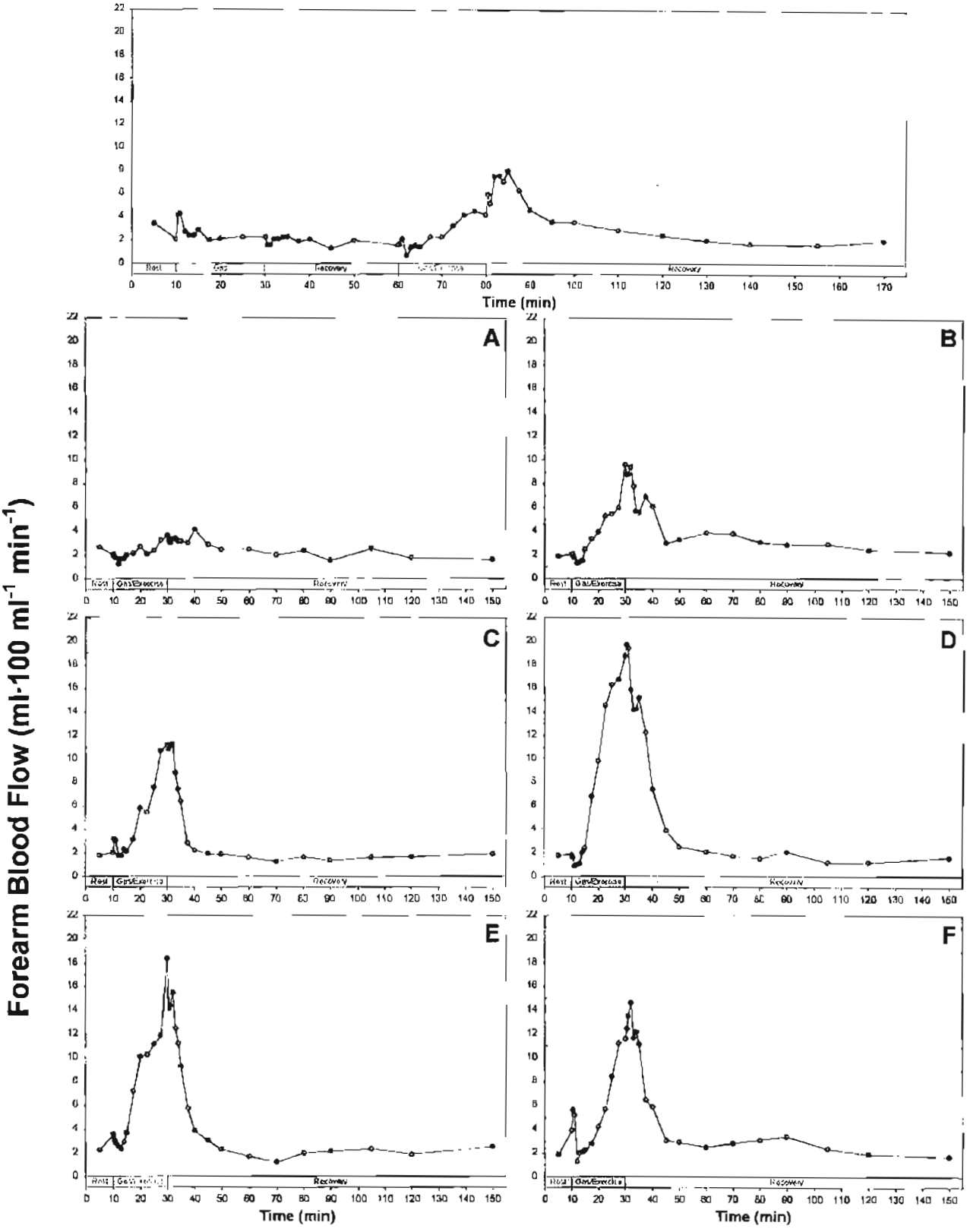


Figure A.1. Forearm blood flow before, during and after 20 min of 10% N_2O inhalation and supine leg exercise (~80% $\dot{V}O_{2\,peak}$) in a preliminary experiment (top), and 6 subsequent experiments (A-F).

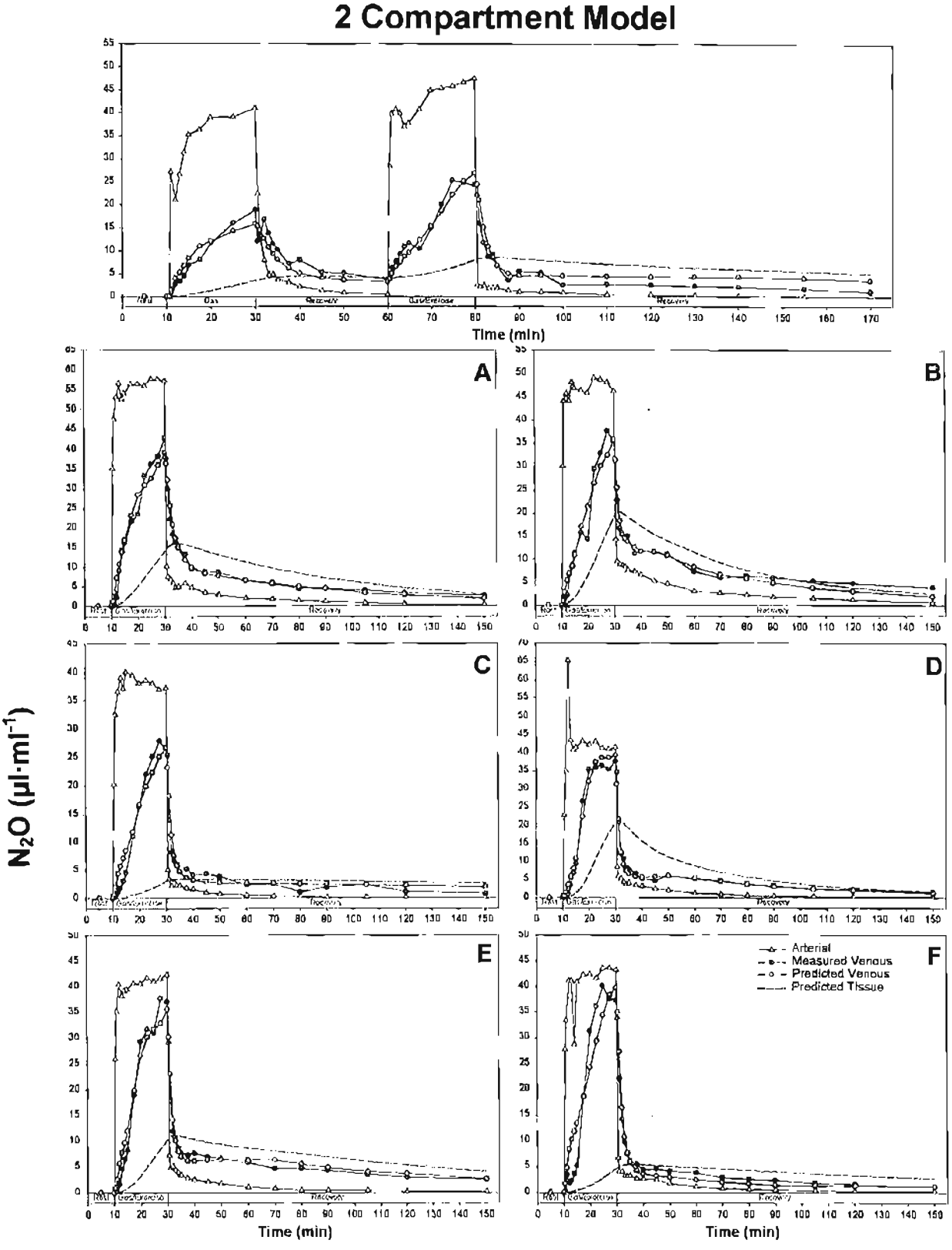


Figure A.2. Forearm N_2O concentrations before, during and after 20 min of 10% N_2O inhalation and supine leg exercise (~80% $\dot{V}O_{2\,peak}$) in a preliminary experiment (top), and 6 subsequent experiments (A-F). In subject F the inspiratory line to the mask became detached for ~10 sec during the first few min of exercise.

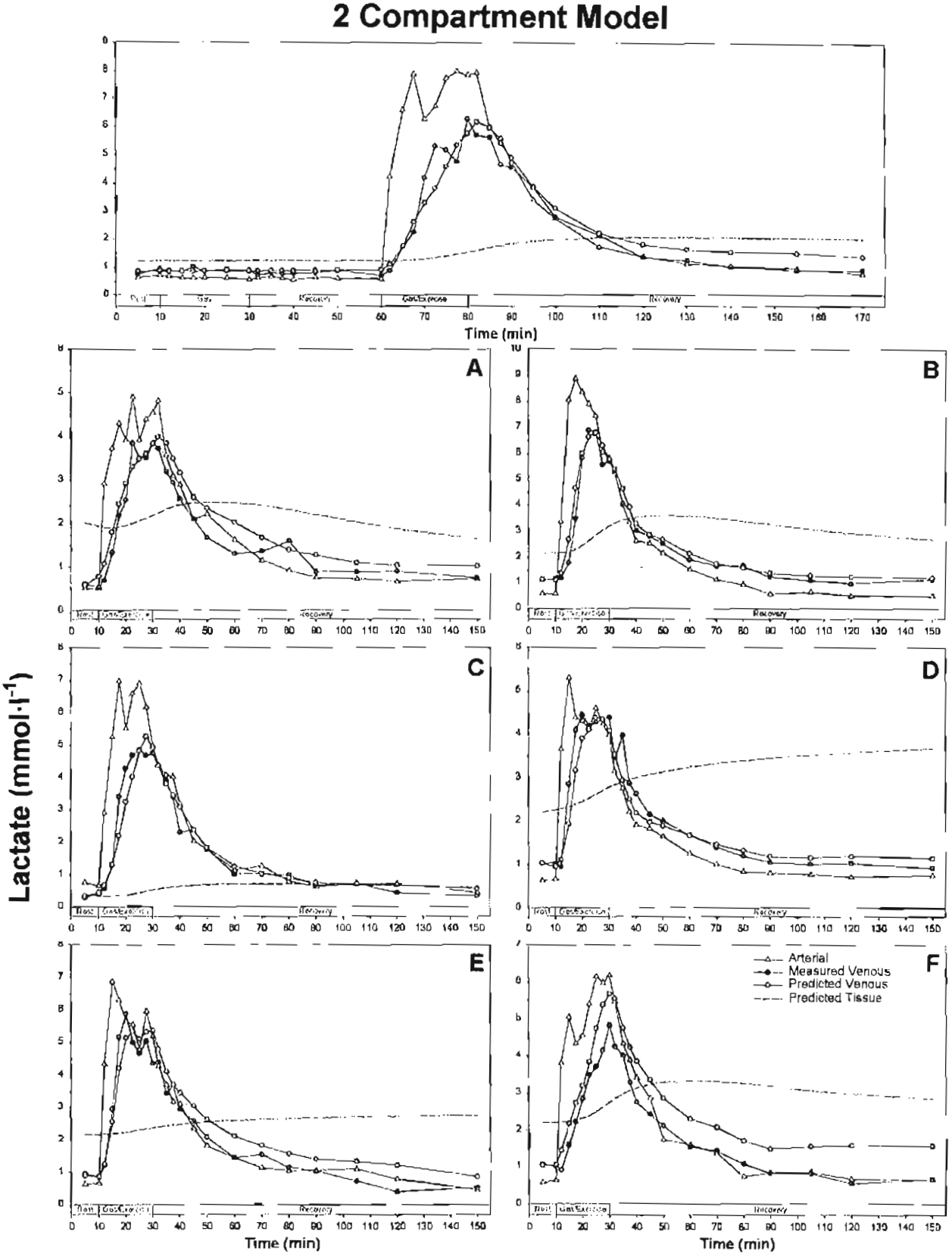


Figure A.3. Forearm lactate concentrations before, during and after 20 min of 10% N_2O inhalation and supine leg exercise (~80% $\dot{V}O_{2\,peak}$) in a preliminary experiment (top), and 6 subsequent experiments (A-F).

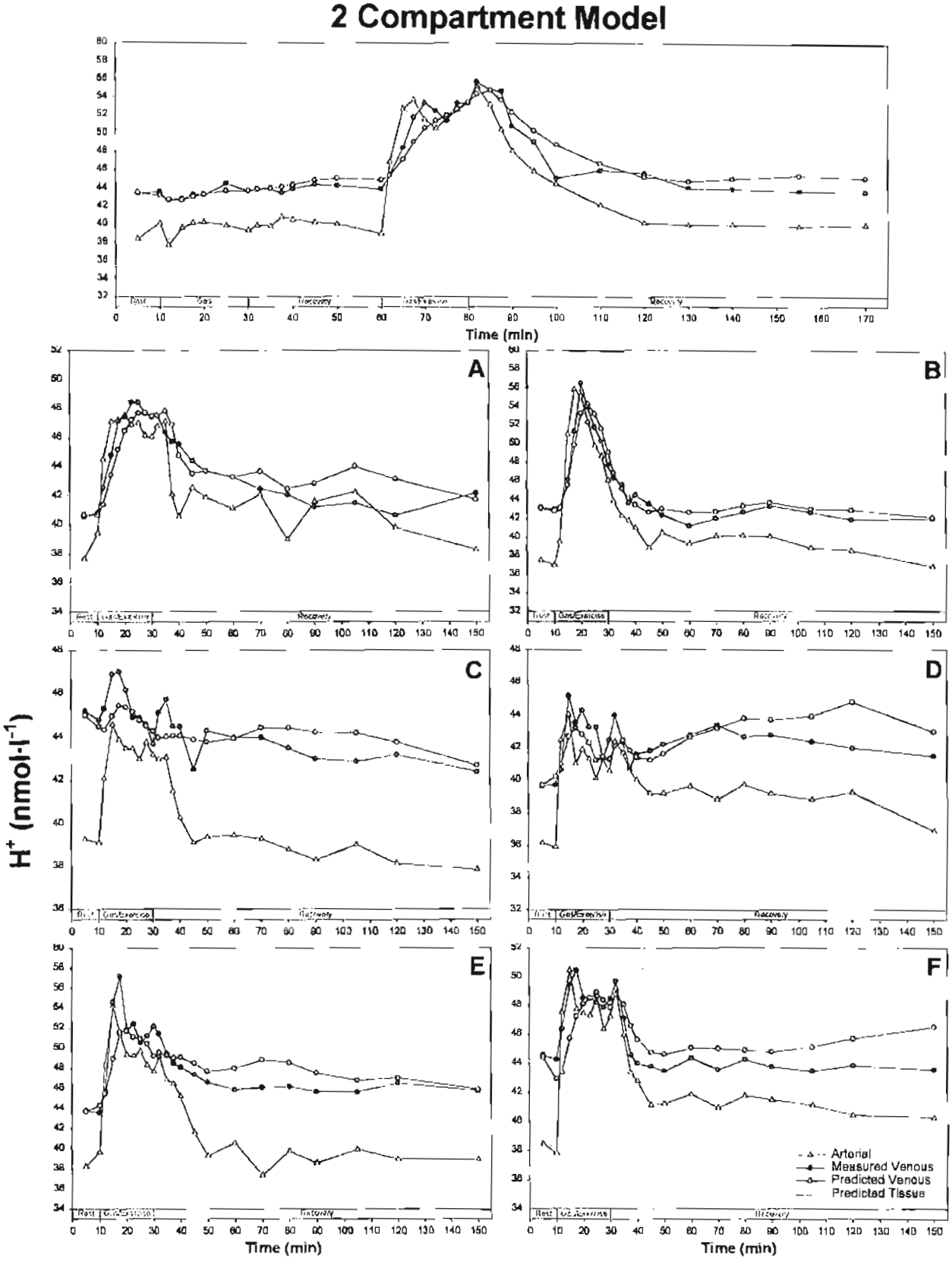


Figure A.4. Forearm H^+ concentrations before, during and after 20 min of 10% N_2O inhalation and supine leg exercise (~80% $\dot{V}O_{2\,peak}$) in a preliminary experiment (top), and 6 subsequent experiments (A-F).

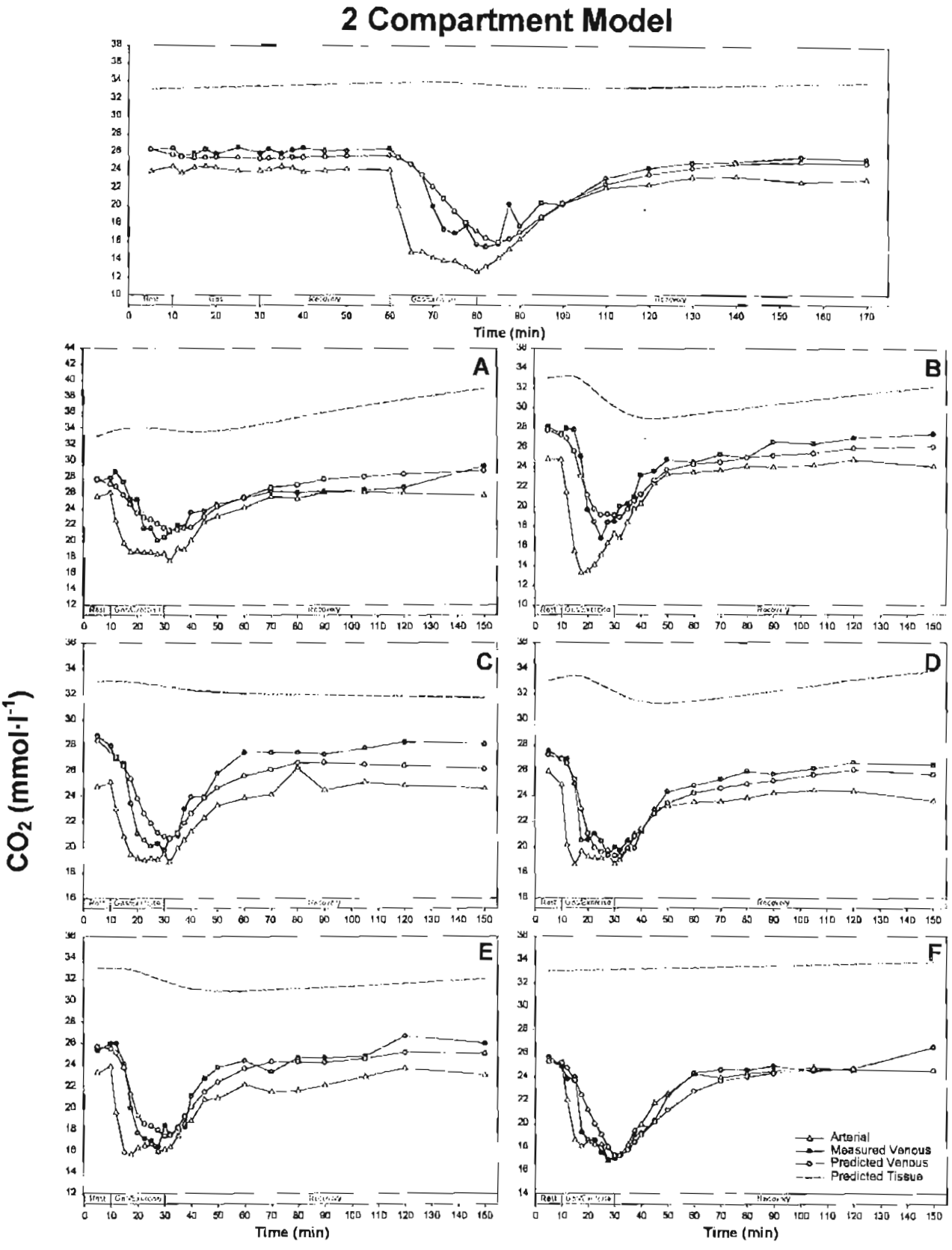


Figure A.5. Forearm CO_2 contents before, during and after 20 min of 10% N_2O inhalation and supine leg exercise (~80% $\dot{V}O_{2\,peak}$) in a preliminary experiment (top), and 6 subsequent experiments (A-F).

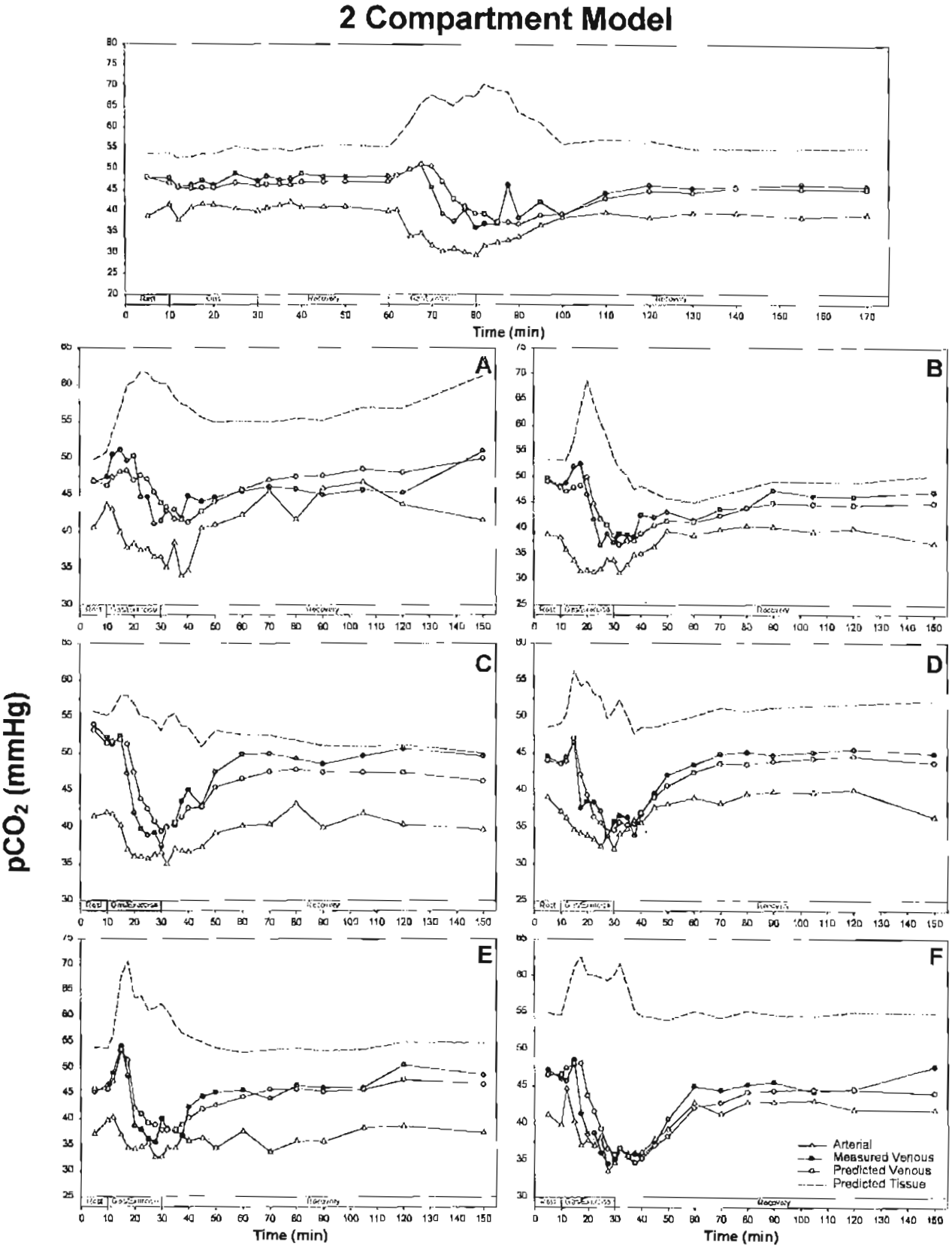


Figure A.6. Forearm pCO_2 before, during and after 20 min of 10% N_2O inhalation and supine leg exercise (~80% $\dot{V}O_{2\,peak}$) in a preliminary experiment (top), and 6 subsequent experiments (A-F).

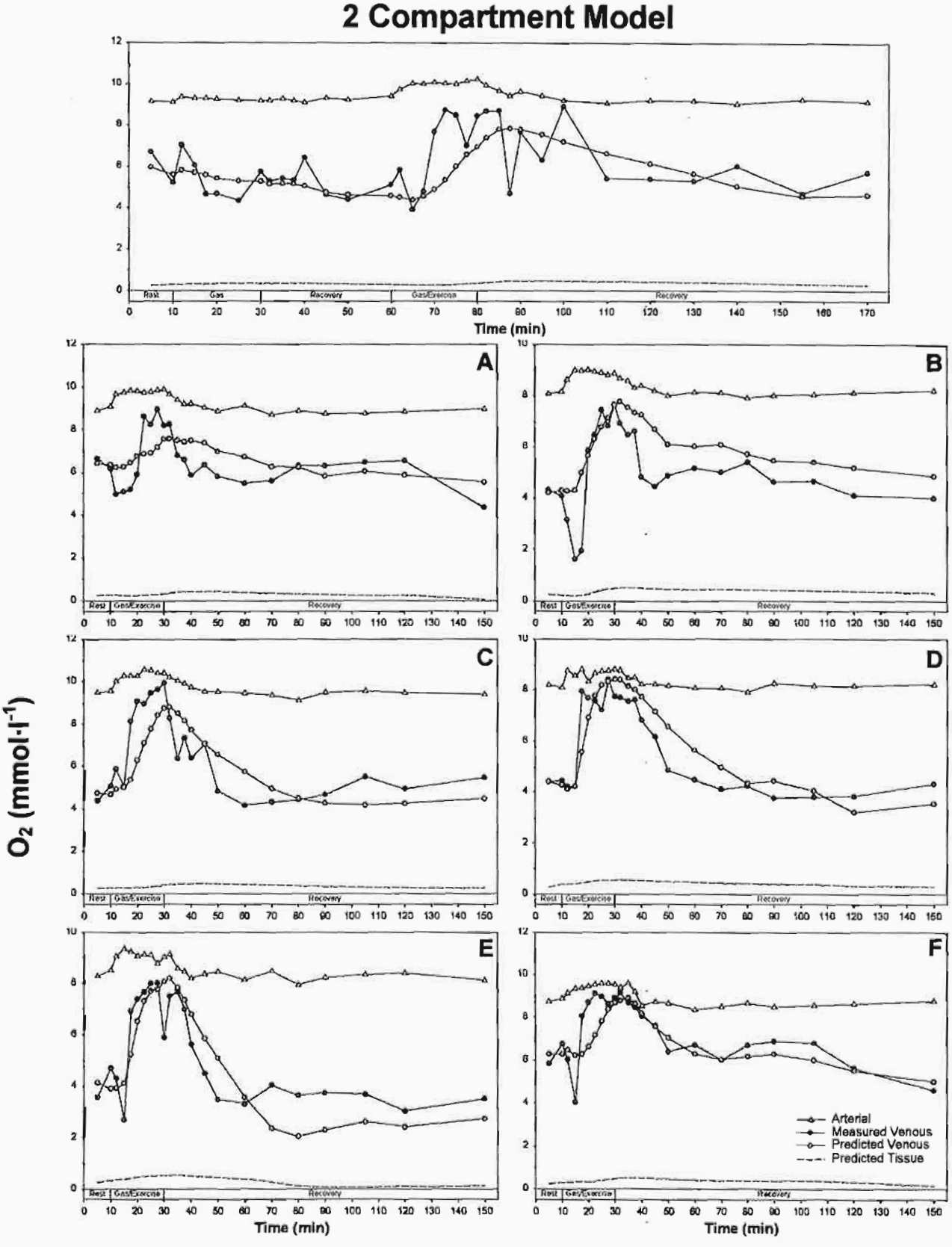


Figure A.7. Forearm $\dot{O}_2$ contents before, during and after 20 min of 10% N_2O inhalation and supine leg exercise (~80% $\dot{V}O_{2\,peak}$) in a preliminary experiment (top), and 6 subsequent experiments (A-F).

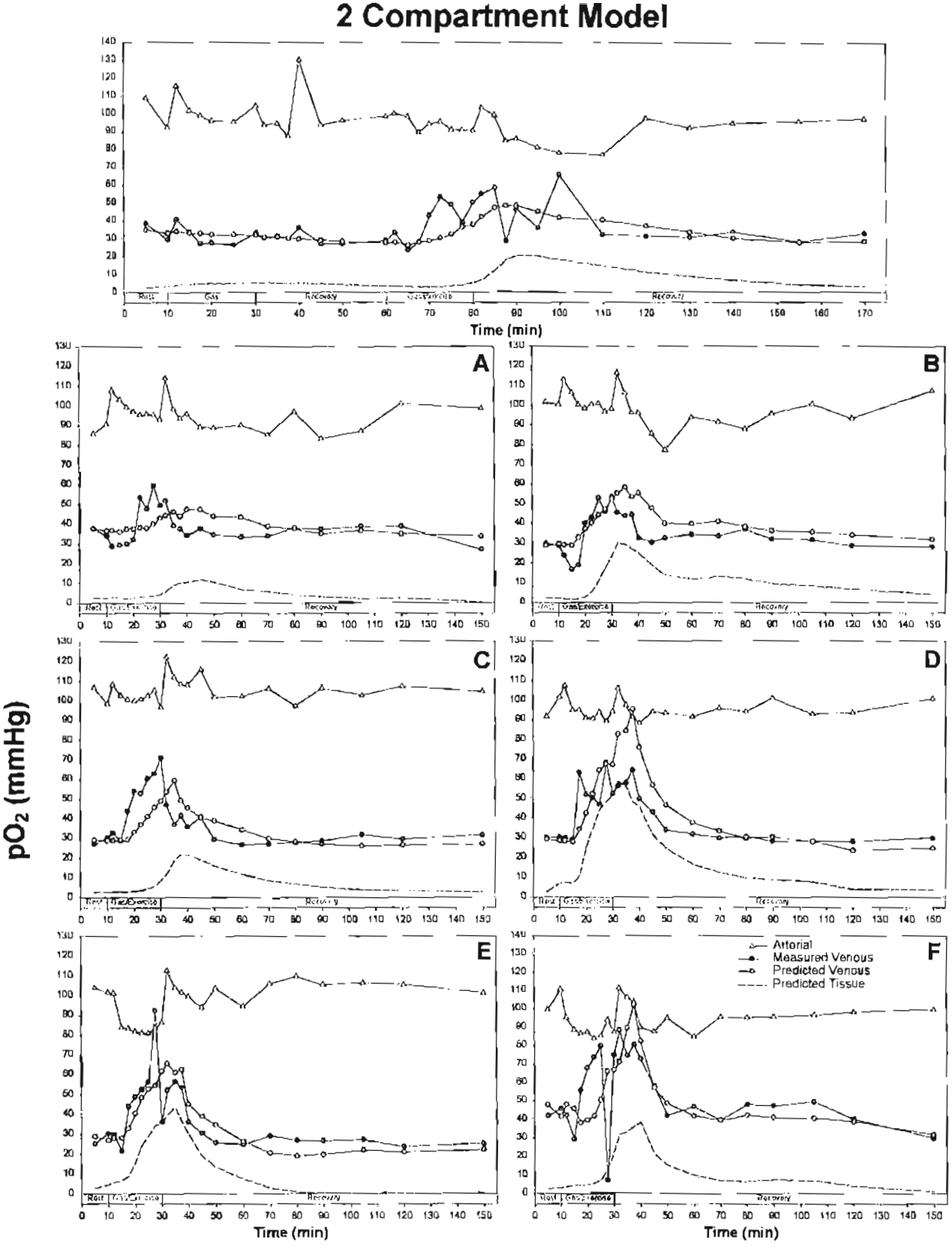


Figure A.8. Forearm pO_2 before, during and after 20 min of 10% N_2O inhalation and supine leg exercise (~80% $\dot{V}O_{2\,peak}$) in a preliminary experiment (top), and 6 subsequent experiments (A-F).

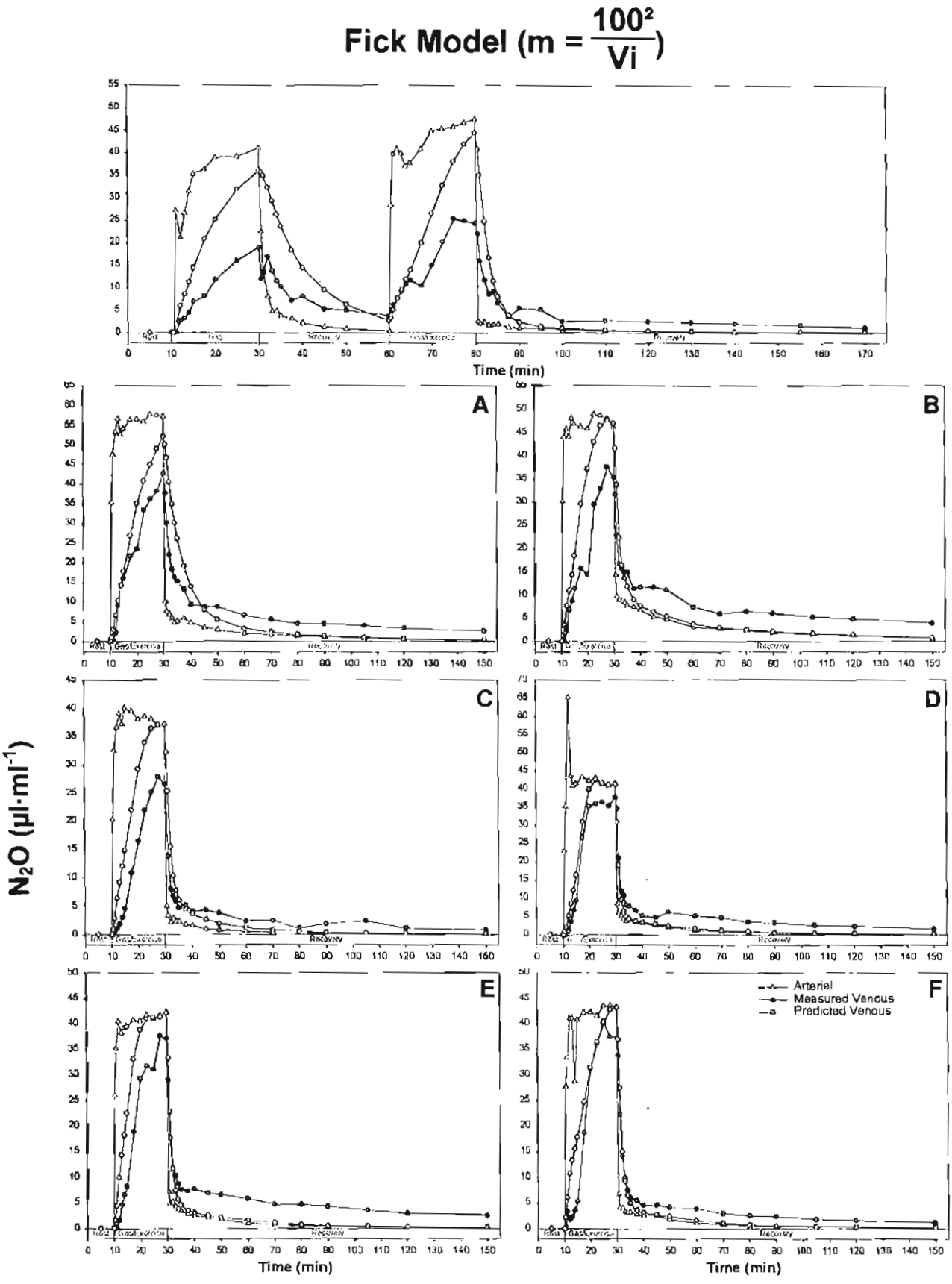


Figure A.9. Forearm N_2O concentrations before, during and after 20 min of 10% N_2O inhalation and supine leg exercise (~80% $\dot{V}O_{2\,peak}$) in a preliminary experiment (top), and 6 subsequent experiments (A-F).

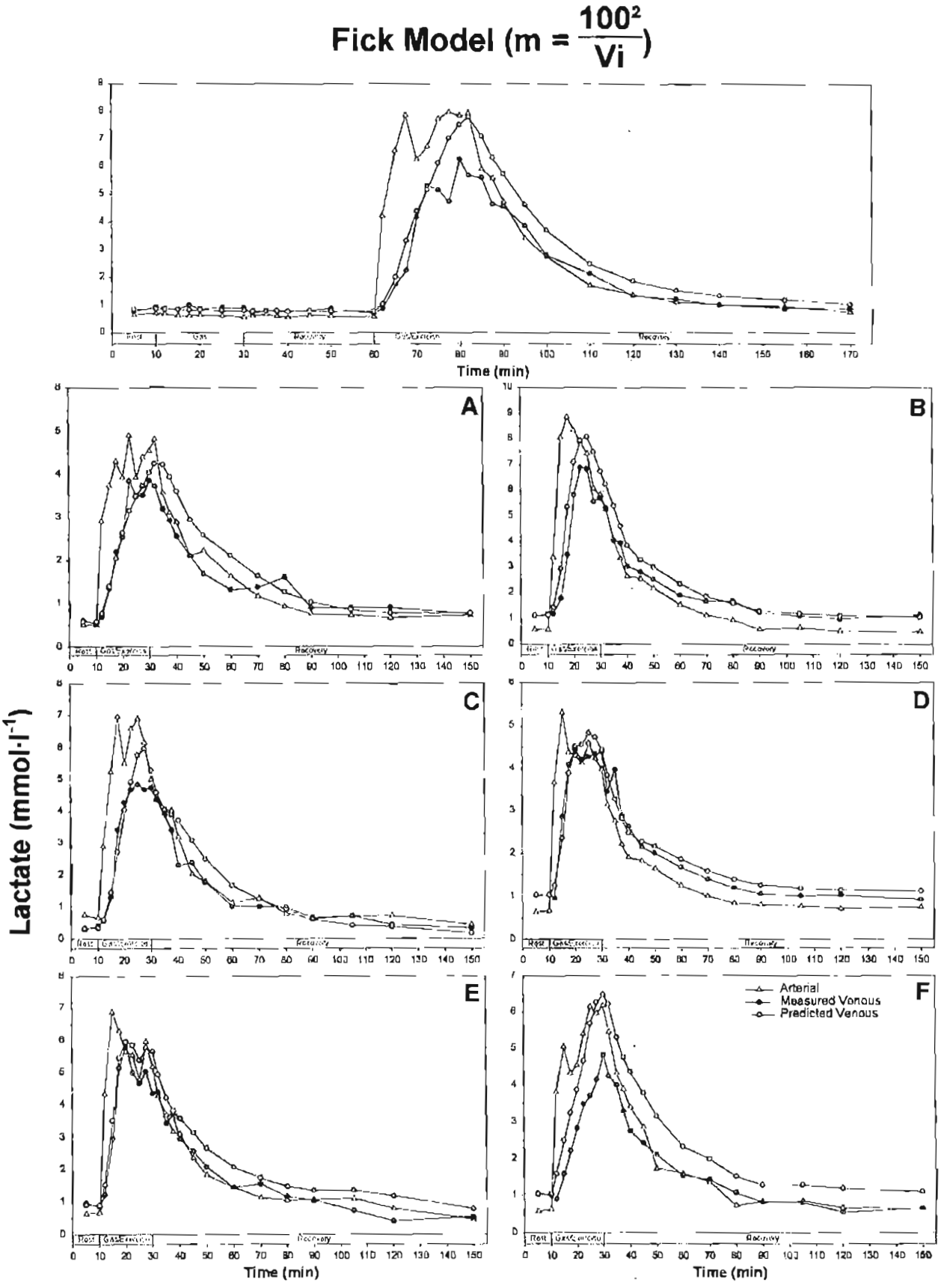


Figure A.10. Forearm lactate concentrations before, during and after 20 min of 10% N_2O inhalation and supine leg exercise (~80% $\dot{V}O_{2\,peak}$) in a preliminary experiment (top), and 6 subsequent experiments (A-F).

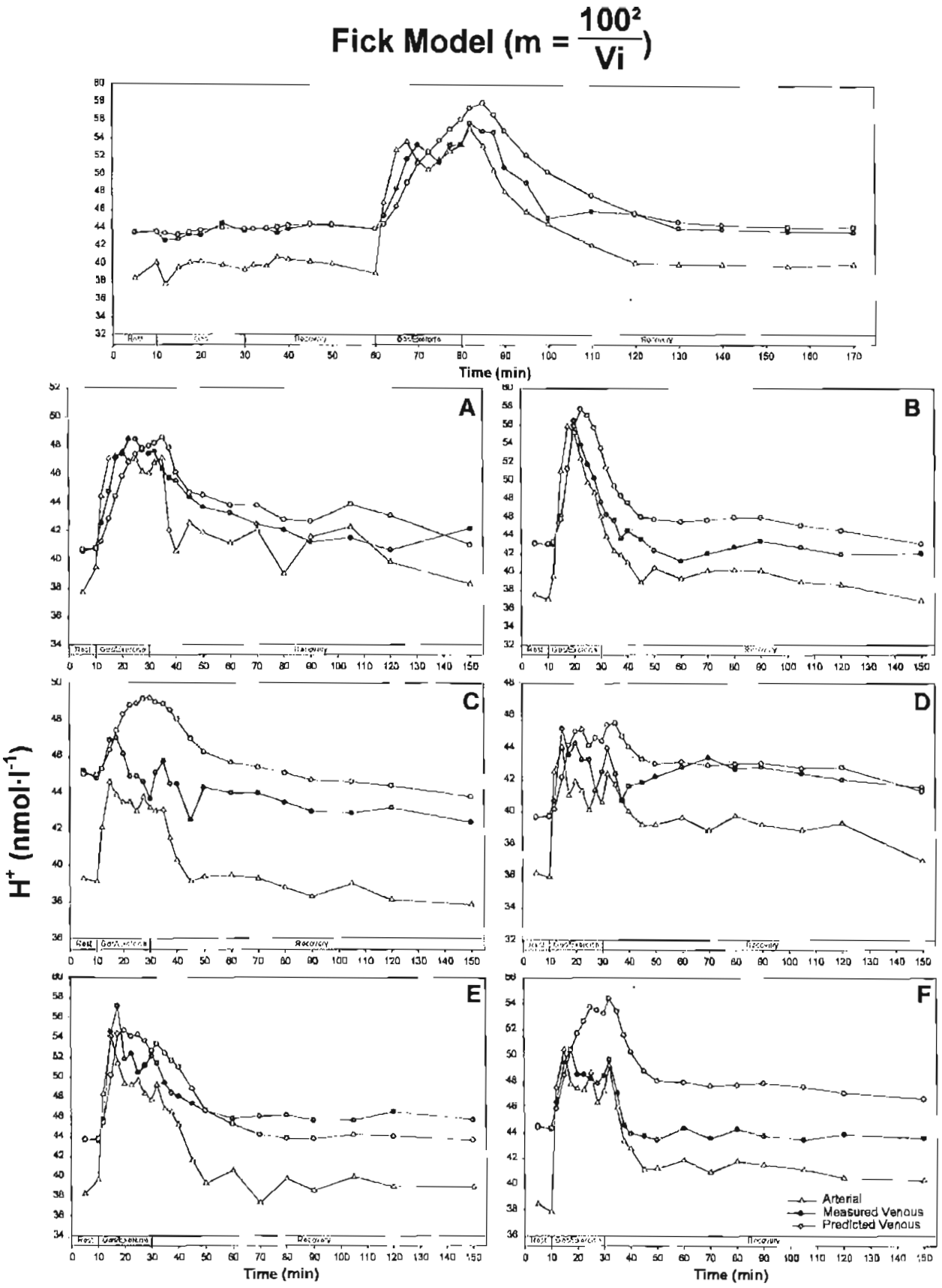


Figure A.11. Forearm H^+ concentrations before, during and after 20 min of 10% N_2O inhalation and supine leg exercise (~80% $\dot{V}O_{2\,peak}$) in a preliminary experiment (top), and 6 subsequent experiments (A-F).

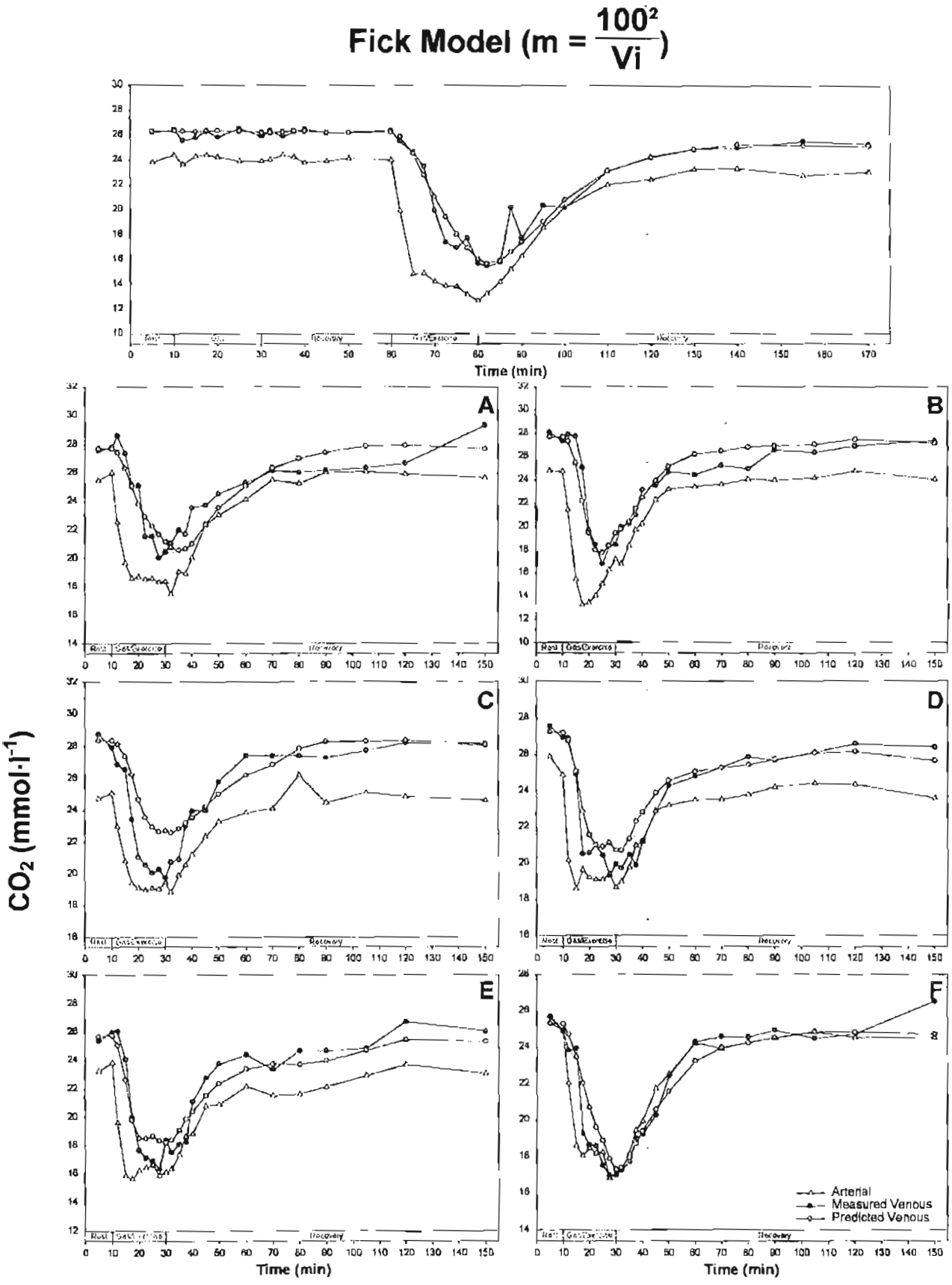


Figure A.12. Forearm CO_2 contents before, during and after 20 min of 10% N_2O inhalation and supine leg exercise (~80% $\dot{V}O_{2\,peak}$) in a preliminary experiment (top), and 6 subsequent experiments (A-F).

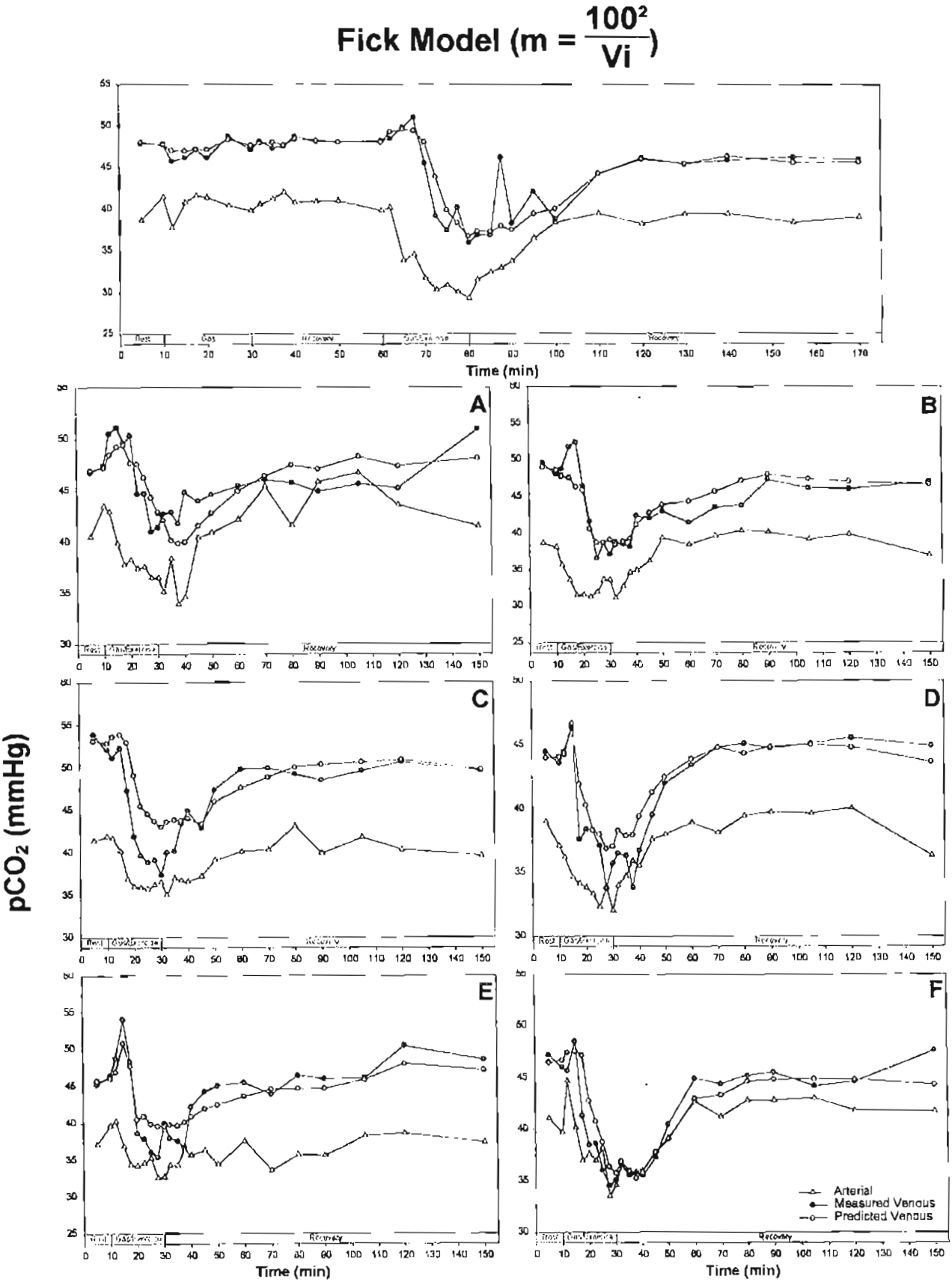


Figure A.13. Forearm pCO_2 before, during and after 20 min of 10% N_2O inhalation and supine leg exercise (~80% $\dot{V}O_{2\,peak}$) in a preliminary experiment (top), and 6 subsequent experiments (A-F).

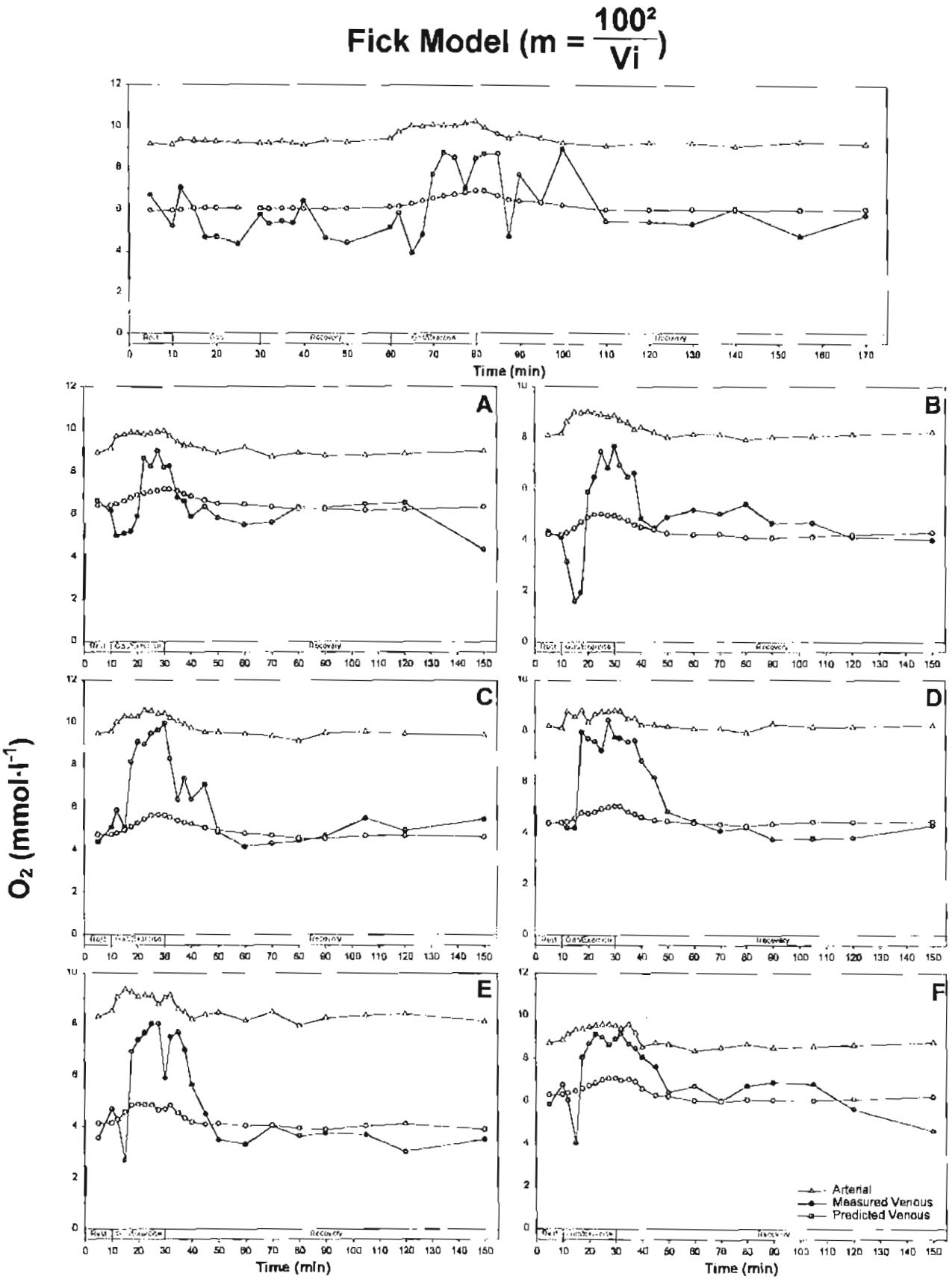


Figure A.14. Forearm O_2 contents before, during and after 20 min of 10% N_2O inhalation and supine leg exercise (~80% $\dot{V}O_{2\,peak}$) in a preliminary experiment (top), and 6 subsequent experiments (A-F).

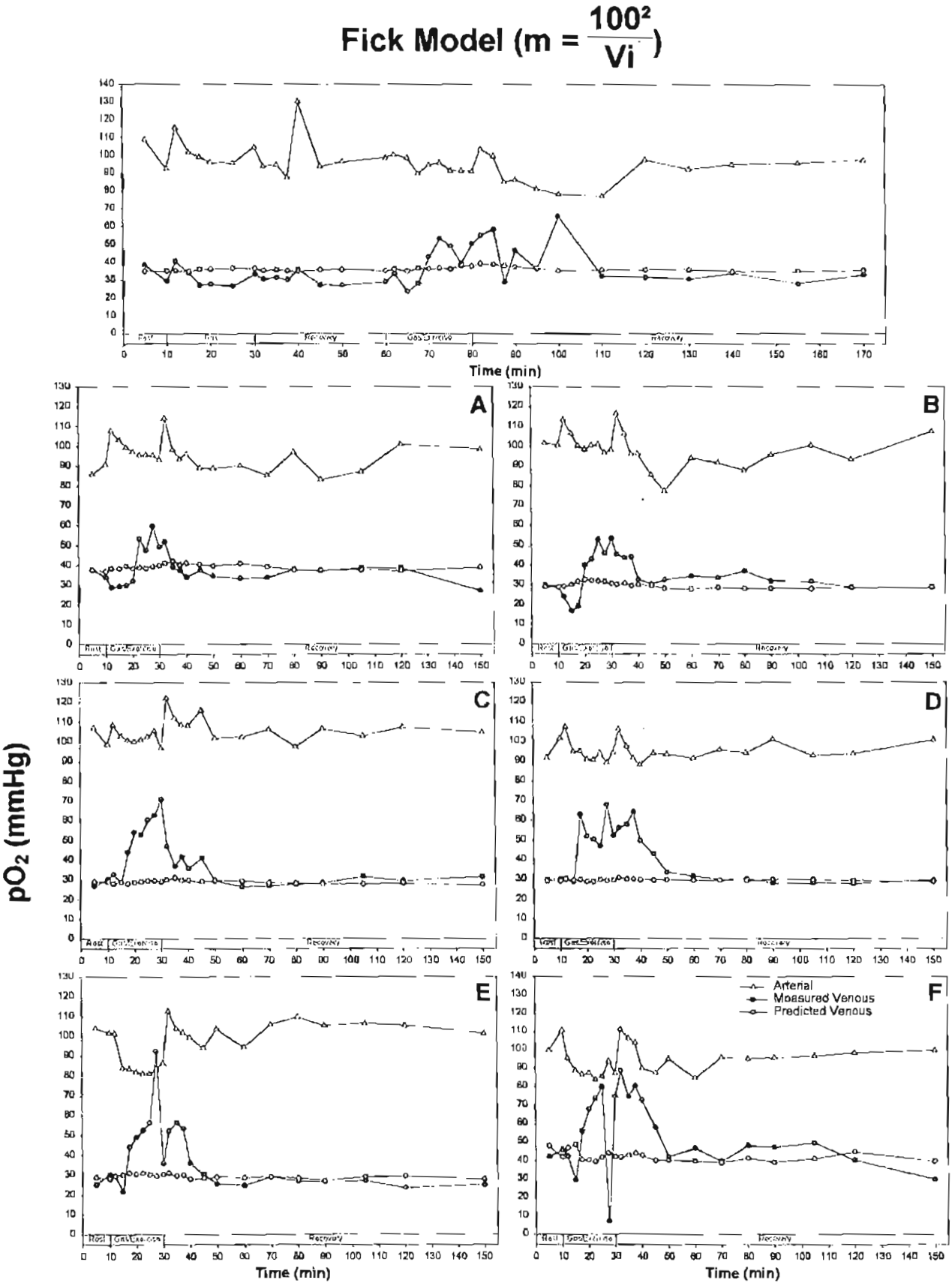


Figure A.15. Forearm pO_2 before, during and after 20 min of 10% N_2O inhalation and supine leg exercise (~80% $\dot{V}O_{2\,peak}$) in a preliminary experiment (top), and 6 subsequent experiments (A-F).

REFERENCES

1. Abramson, D. I., and E. B. J. Ferris. Responses of blood vessels in the resting hand and forearm to various stimuli. *Am. Heart J.* 19: 541-553, 1940.
2. Ahlborg, G. Mechanism for glycogenolysis in nonexercising human muscle during and after exercise. *Am. J. Physiol.* 248: E540-5, 1985.
3. Ahlborg, G., and P. Felig. Lactate and glucose exchange across the forearm, legs, and splanchnic bed during and after prolonged leg exercise. *J. Clin. Invest.* 69: 45-54, 1982.
4. Ahlborg, G., L. Hagenfeldt, and J. Wahren. Influence of lactate infusion on glucose and FFA metabolism in man. *Scand. J. Clin. Lab. Invest.* 36: 193-201, 1976.
5. Ahlborg, G., L. Hagenfeldt, and J. Wahren. Substrate utilization by the inactive leg during one-leg or arm exercise. *J. Appl. Physiol.* 39: 718-23, 1975.
6. Alberti, K. G. M. M., and C. Cuthbert. The hydrogen ion in normal metabolism: a review. In: *Metabolic acidosis*. London: Pitman Books Ltd, 1982, p. 1-19.
7. Andres, R., G. Cader, and K. L. Zierler. The quantitatively minor role of carbohydrate in oxidative metabolism by skeletal muscle in intact man in the basal state. Measurements of oxygen and glucose uptake and carbon dioxide and lactate production in the forearm. *J. Clin. Invest.* 35: 671-82, 1956.
8. Antonini, E., and M. Brunori. Specific aspects of the reactions of myoglobin with ligands. In: *Hemoglobin and myoglobin in their reactions with ligands*, edited by A. Neuberger and E. L. Tatum. Amsterdam: North-Holland, 1971, p. 219-234.
9. Åstrand, P. O., E. Hultman, A. Juhlin-Dannfelt, and G. Reynolds. Disposal of lactate during and after strenuous exercise in humans. *J. Appl. Physiol.* 61: 338-43, 1986.
10. Baksi, A. K., P. H. Buxton, P. Cochrane, and R. R. Hughes. Lactate production in McArdle's disease. *Postgrad. Med. J.* 53: 161-4, 1977.

11. Baldwin, K. M., A. M. Hooker, and R. E. Herrick. Lactate oxidative capacity in different types of muscle. *Biochem. Biophys. Res. Commun.* 83: 151-7, 1978.
12. Baldwin, K. M., W. W. Winder, R. L. Terjung, and J. O. Holloszy. Glycolytic enzymes in different types of skeletal muscle: adaptation to exercise. *Am. J. Physiol.* 225: 962-6, 1973.
13. Bangsbo, J., T. Aagaard, M. Olsen, B. Kiens, L. P. Turcotte, and E. A. Richter. Lactate and H+ uptake in inactive muscles during intense exercise in man. *J. Physiol. (Lond.)* 488: 219-29, 1995.
14. Bangsbo, J., K. Madsen, B. Kiens, and E. A. Richter. Effect of muscle acidity on muscle metabolism and fatigue during intense exercise in man. *J. Physiol. (Lond.)* 495: 587-96, 1996.
15. Bangsbo, J., K. Madsen, B. Kiens, and E. A. Richter. Muscle glycogen synthesis in recovery from intense exercise in humans. *Am. J. Physiol.* 273: E416-24, 1997.
16. Barr, D. P., and H. E. Himwich. Studies in the physiology of muscular exercise. II. Comparison of arterial and venous blood following vigorous exercise. *J. Biol. Chem.* 55: 525-537, 1923.
17. Bassingthwaighte, J. B. A concurrent flow model for extraction during transcapillary passage. *Circ. Res.* 35: 483-503, 1974.
18. Bassingthwaighte, J. B., I. S. J. Chan, and C. Y. Wang. Computationally efficient algorithms for convection-permeation diffusion models for blood-tissue exchange. *Ann. Biomed. Eng.* 20: 687-725, 1992.
19. Bassingthwaighte, J. B., R. B. King, J. E. Sambrook, and B. van Steenwyk. Fractal analysis of blood-tissue exchange kinetics. *Adv. Exp. Med. Biol.* 222: 15-23, 1988.
20. Bassingthwaighte, J. B., and T. Yipintsoi. Organ blood flow, wash-in, washout, and clearance of nutrients and metabolites. *Mayo Clin. Proc.* 49: 248-55, 1974.
21. Beard, D. A., and J. B. Bassingthwaighte. Power-law kinetics of tracer washout from physiological systems. *Ann. Biomed. Eng.* 26: 775-779, 1998.

22. Beaver, W. L., K. Wasserman, and B. J. Whipp. Improved detection of the lactate threshold during exercise using a log-log transformation. *J. Appl. Physiol.* 59: 1936-40, 1985.

23. Belcastro, A. N., and A. Bonen. Lactic acid removal rates during controlled and uncontrolled recovery exercise. *J. Appl. Physiol.* 39: 932-6, 1975.

24. Bergman, B. C., E. E. Wolfel, G. E. Butterfield, G. D. Lopaschuk, G. A. Casazza, M. A. Horning, and G. A. Brooks. Active muscle and whole body lactate kinetics after endurance training in men. *J. Appl. Physiol.* 87: 1684-1696, 1999.

25. Bergstrom, J., and E. Hultman. Muscle glycogen synthesis after exercise: an enhancing factor localized to the muscle cells in man. *Nature* 210: 309-10, 1966.

26. Bertocci, L. A. Manuscript in review. Personnal communication, 1999.

27. Bertocci, L. A., J. G. Jones, C. R. Malloy, R. G. Victor, and G. D. Thomas. Oxidation of lactate and acetate in rat skeletal muscle: analysis by ^{13}C-nuclear magnetic resonance spectroscopy. *J. Appl. Physiol.* 83: 32-9, 1997.

28. Bertocci, L. A., and B. F. Lujan. Incorporation and utilization of [3-^{13}C]lactate and [1,2-^{13}C]acetate by rat skeletal muscle. *J. Appl. Physiol.* 86: 2077-89, 1999.

29. Bessman, S. P., and P. J. Geiger. Transport of energy in muscle: the phosphorylcreatine shuttle. *Science* 211: 448-52, 1981.

30. Bloch, G., J. R. Chase, D. B. Meyer, M. J. Avison, G. I. Shulman, and R. G. Shulman. In vivo regulation of rat muscle glycogen resynthesis after intense exercise. *Am. J. Physiol.* 266: E85-91, 1994.

31. Brooks, G. A. Anaerobic threshold: review of the concept and directions for future research. *Med. Sci. Sports Exerc.* 17: 22-31, 1985.

32. Brooks, G. A. Current concepts in lactate exchange. *Med. Sci. Sports. Exerc.* 23: 895-906, 1991.

33. Brooks, G. A. Lactate production under fully aerobic conditions: the lactate shuttle during rest and exercise. *Fed. Proc.* 45: 2924-9, 1986.

34. Brooks, G. A. The lactate shuttle during exercise and recovery. *Med. Sci. Sports Exerc.* 18: 360-8, 1986.

35. Brooks, G. A., K. E. Brauner, and R. G. Cassens. Glycogen synthesis and metabolism of lactic acid after exercise. *Am. J. Physiol.* 224: 1162-6, 1973.

36. Brooks, G. A., H. Dubouchaud, M. Brown, J. P. Sicurello, and C. E. Butz. Role of mitochondrial lactate dehydrogenase and lactate oxidation in the intracellular lactate shuttle. *Proc. Nat. Acad. Sci. U.S.A.* 96: 1129-34, 1999.

37. Brooks, G. A., and G. A. Gaesser. End points of lactate and glucose metabolism after exhausting exercise. *J. Appl. Physiol.* 49: 1057-69, 1980.

38. Brooks, G. A., and J. Mercier. Balance of carbohydrate and lipid utilization during exercise: the "crossover" concept. *J. Appl. Physiol.* 76: 2253-61, 1994.

39. Buckley, J. D. Human lactate kinetics: training effects (PhD Thesis). In: *Department of Physiology*. Adelaide: University of Adelaide, 1997, p. 209.

40. Buckley, J. D., G. C. Scroop, and P. G. Catcheside. Lactate disposal in resting trained and untrained forearm skeletal muscle during high intensity leg exercise. *Eur. J. Appl. Physiol.* 67: 360-6, 1993.

41. Cabrera, M. E., G. M. Saidel, and S. C. Kalhan. Role of O_2 in regulation of lactate dynamic during hypoxia: mathematical model and analysis. *Ann. Biomed. Eng.* 26: 1-27, 1998.

42. Caiozzo, V. J., J. A. Davis, J. F. Ellis, J. L. Azus, R. Vandagriff, C. A. Prietto, and W. C. McMaster. A comparison of gas exchange indices used to detect the anaerobic threshold. *J. Appl. Physiol.* 53: 1184-9, 1982.

43. Campbell, M. E., R. L. Hughson, and H. J. Green. Continuous increase in blood lactate concentration during different ramp exercise protocols. *J. Appl. Physiol.* 66: 1104-7, 1989.

44. Catcheside, P. G., and G. C. Scroop. Lactate kinetics in resting and exercising forearms during moderate- intensity supine leg exercise. *J. Appl. Physiol.* 74: 435-43, 1993.

45. Chance, B., N. Oshino, T. Sugano, and A. Mayevsky. Basic principles of tissue oxygen determination from mitochondrial signals. *Adv. Exp. Med. Biol.* 37A: 277-92, 1973.

46. Chin, E. R., M. I. Lindinger, and G. J. F. Heigenhauser. Lactate metabolism in inactive skeletal muscle during lactacidosis. *Am. J. Physiol.* 261: R98-R105, 1991.

47. Chwalbinska-Moneta, J., R. A. Robergs, D. L. Costill, and W. J. Fink. Threshold for muscle lactate accumulation during progressive exercise. *J. Appl. Physiol.* 66: 2710-6, 1989.

48. Coles, D. R., K. E. Cooper, R. F. Mottram, and J. V. Occleshaw. The source of blood samples withdrawn from deep forearm veins via catheters passed upstream from the median cubital vein. *J. Physiol.* 142: 323-8, 1958.

49. Conlee, R. K., J. A. McLane, M. J. Rennie, and W. W. Winder. Reversal of phosphorylase activation in muscle despite continued contractile activity. *Am. J. Physiol.* 6: R291-6, 1979.

50. Connett, R. J. Cytosolic pH during a rest-to-work transition in red muscle: application of enzyme equilibria. *J. Appl. Physiol.* 63: 2360-5, 1987.

51. Connett, R. J., T. E. Gayeski, and C. R. Honig. An upper bound on the minimum PO_2 for O_2 consumption in red muscle. *Adv. Exp. Med. Biol.* 191: 291-300, 1985.

52. Connett, R. J., T. E. J. Gayeski, and C. Honig. Energy sources in fully aerobic rest-work transitions: a new role for glycolysis. *Am. J. Physiol.* 248: H922-9, 1985.

53. Connett, R. J., T. E. J. Gayeski, and C. R. Honig. Lactate efflux is unrelated to intracellular PO_2 in a working red muscle in situ. *J. Appl. Physiol.* 61: 402-8, 1986.

54. Connett, R. J., T. E. J. Gayeski, and C. R. Honig. Lactate production in a pure red muscle in absence of anoxia: mechanisms and significance. *Adv. Exp. Med. Biol.* 159: 327-35, 1983.

55. Connett, R. J., T. E. J. Gayeski, and C. R. Honig. Lactic acid accumulation in fully aerobic, working, dog gracilis muscle. *Am. J. Physiol.* 246: H120-8, 1984.

56. Connett, R. J., and K. Sahlin. Control of glycolysis and glycogen metabolism. In: *Handbook of Physiology*. Bethesda, MD: Am. Physiol. Soc., 1996, p. 870-911.

57. Consoli, A., N. Nurjhan, J. J. Reilly Jr, D. M. Bier, and J. E. Gerich. Contribution of liver and skeletal muscle to alanine and lactate metabolism in humans. *Am. J. Physiol.* 259: E677-84, 1990.

58. Constantin-Teodosiu, D., J. I. Carlin, G. Cederblad, R. C. Harris, and E. Hultman. Acetyl group accumulation and pyruvate dehydrogenase activity in human muscle during incremental exercise. *Acta Physiol. Scand.* 143: 367-72, 1991.

59. Cooper, C. J., J. D. Fewings, R. L. Hodge, G. C. Scroop, and R. F. Whelan. The role of skin and muscle vessels in the response of forearm blood flow to noradrenaline. *J. Physiol.* 173: 65-73, 1964.

60. Cooper, K. E., O. G. Edholm, and R. F. Mottram. The blood flow in skin and muscle of the human forearm. *J. Physiol.* 128: 258-67, 1955.

61. Corbelli, C., M. P. Saccomani, E. Ferrannini, R. A. Defronzo, R. Gelfand, and R. Bonadonna. A compartmental model to quantitate in vivo glucose transport in the human forearm. *Am. J. Physiol.* 257: E943-E958, 1989.

62. Costill, D. L. Metabolic responses during distance running. *J. Appl. Physiol.* 28: 251-255, 1970.

63. Crone, C., and D. G. Levitt. Capillary permeability to small solutes. In: *Handbook of Physiology. The Cardiovascular System, Microcirculation*. Bethesda, MD: Am. Physiol. Soc., 1984, p. 411-466.

64. Danforth, W. H. Activation of glycolytic pathway in muscle. In: *Control of energy metabolism*, edited by B. Chance, R. W. Estabrock and J. R. Williamson. New York: Academic Press, 1965, p. 287-297.

65. Dassonville, J., J. Beillot, Y. Lessard, J. Jan, A. M. Andre, C. Le Pourcelet, P. Rochcongar, and F. Carre. Blood lactate concentrations during exercise: effect

of sampling site and exercise mode. *J. Sports Med. Phys. Fitness* 38: 39-46, 1998.

66. Davis, J. Anaerobic threshold: review of the concept and directions for future research. *Med. Sci. Sports Exerc.* 17: 6-18, 1985.

67. Davis, J. A., V. J. Caiozzo, N. Lamarra, J. F. Ellis, R. Vandagriff, C. A. Prietto, and W. C. McMaster. Does the gas exchange anaerobic threshold occur at a fixed blood lactate concentration of 2 or 4 mM? *Int. J. Sports Med.* 4: 89-93, 1983.

68. Dawson, D. M., T. L. Goodfriend, and N. O. Kaplan. Lactate dehydrogenases: Functions of the two types. *Science* 143: 929-933, 1964.

69. Dennis, S. C., T. D. Noakes, and A. N. Bosch. Ventilation and blood lactate increase exponentially during incremental exercise. *J. Sports Sci.* 10: 437-49, 1992.

70. Depocas, F., Y. Minaire, and J. Chatonnet. Rates of formation and oxidation of lactic acid in dogs at rest and during moderate exercise. *Can. J. Physiol. Pharmacol.* 47: 603-10, 1969.

71. Dickstein, K., S. Barvik, T. Aarsland, S. Snapinn, and J. Karlsson. A comparison of methodologies in detection of the anaerobic threshold. *Circulation* 81: II38-46, 1990.

72. Dodd, S., S. K. Powers, T. Callender, and E. Brooks. Blood lactate disappearance at various intensities of recovery exercise. *J. Appl. Physiol.* 57: 1462-5, 1984.

73. Donovan, C. M., and G. A. Brooks. Endurance training affects lactate clearance, not lactate production. *Am. J. Physiol.* 244: E83-92, 1983.

74. Doolette, D. J., R. N. Upton, and C. Grant. Diffusion-limited, but not perfusion-limited, compartmental models describe cerebral nitrous oxide kinetics at high and low blood flows. *J. Pharm. Biopharm.* 26: 649-672, 1999.

75. Douguet, D., J. Raynaud, A. Capderou, C. Pannier, G. Reiss, and J. Durand. Muscular venous blood metabolites during rhythmic forearm exercise while

breathing air or normoxic helium and argon gas mixtures. *Clin. Physiol.* 8: 367-78, 1988.

76. Dubinsky, W. P., and E. Racker. The mechanism of lactate transport in human erythrocytes. *J. Membr. Biol.* 44: 25-36, 1978.

77. Duhaylongsod, F. G., J. A. Griebel, D. S. Bacon, W. G. Wolfe, and C. A. Piantadosi. Effects of muscle contraction on cytochrome a,a_3 redox state. *J. Appl. Physiol.* 75: 790-797, 1993.

78. Dunn, R. B., and J. B. Critz. Uptake of lactate by dog skeletal muscle in vivo and the effect of free fatty acids. *Am. J. Physiol.* 229: 255-9, 1975.

79. Ekblom, B., P. O. Astrand, B. Saltin, J. Stenberg, and B. Wallstrom. Effect of training on circulatory response to exercise. *J. Appl. Physiol.* 24: 518-28, 1968.

80. el-Sayed, M. S., K. P. George, D. Wilkinson, N. Mullan, R. Fenoglio, and J. Flannigan. Fingertip and venous blood lactate concentration in response to graded treadmill exercise. *J. Sports Sci.* 11: 139-43, 1993.

81. Erecinska, M., and D. F. Wilson. On the mechanism of regulation of cellular respiration. The dependence of respiration on the cytosolic [ATP], [ADP] and [Pi]. *Adv. Exp. Med. Biol.* 94: 271-8, 1978.

82. Essen, B., B. Pernow, P. D. Gollnick, and B. Saltin. Muscle glycogen content and lactate uptake in exercising muscles. In: *Metabolic Adaptations to Prolonged Physical Exercise*, edited by H. Howald and J. R. Poortmans. Basel: Birkhäuser Verlag, 1975, p. 130-134.

83. Fick, A. Über die Messung des Blutquantums in den Herzventrikeln. *Verhandl. d. phys.-med. Ges. zu Würzburg.* 2: xvi, 1870. Quoted in its entirety by Hoff, H. E. and Scott, H. J.; *New Eng. J. Med.*, 239: 122, 1948.

84. Fishbein, W. N. Lactate transporter defect: a new disease of muscle. *Science* 234: 1254-6, 1986.

85. Fletcher, W. M., and F. G. Hopkins. Lactic acid in amphibian muscle. *J. Physiol. (Lond.)* 35: 247-309, 1907.

86. Foxdal, P., A. Sjodin, B. Ostman, and B. Sjodin. The effect of different blood sampling sites and analyses on the relationship between exercise intensity and 4.0 mmol.l^{-1} blood lactate concentration. *Eur. J. Appl. Physiol.* 63: 52-4, 1991.

87. Foxdal, P., B. Sjodin, H. Rudstam, C. Ostman, B. Ostman, and G. C. Hedenstierna. Lactate concentration differences in plasma, whole blood, capillary finger blood and erythrocytes during submaximal graded exercise in humans. *Eur. J. Appl. Physiol.* 61: 218-22, 1990.

88. Freyschuss, U., and T. Strandell. Limb circulation during arm and leg exercise in supine position. *J. Appl. Physiol.* 23: 163-70, 1967.

89. Gayeski, T. E. J. Oxygen transport in rest-work transition illustrates new functions for myoglobin. *Am. J. Physiol.* 248: H914-H921, 1985.

90. Gayeski, T. E. J., R. J. Connett, and C. R. Honig. Minimum intracellular pO_2 for maximum cytochrome turnover in red muscle in situ. *Am. J. Physiol.* 252: H906-H915, 1987.

91. Gevers, W. Generation of protons by metabolic processes in heart cells. *J. Mol. Cell. Cardiol.* 9: 867-74, 1977.

92. Gibala, M. J., D. A. MacLean, T. E. Graham, and B. Saltin. Anaplerotic processes in human skeletal muscle during brief dynamic exercise. *J. Physiol. (Lond.)* 502: 703-13, 1997.

93. Gladden, L. B. Lactate transport and exchange during exercise. In: *Handbook of Physiology*. Bethesda, MD: Am. Physiol. Soc., 1996, p. 614-648.

94. Gladden, L. B. Lactate uptake by skeletal muscle. *Ex. Sport Sci. Rev.* 17: 115-155, 1989.

95. Gladden, L. B. Net lactate uptake during progressive steady-level contractions in canine skeletal muscle. *J. Appl. Physiol.* 71: 514-20, 1991.

96. Gladden, L. B., R. E. Crawford, and M. J. Webster. Effect of blood flow on net lactate uptake during steady-level contractions in canine skeletal muscle. *J. Appl. Physiol.* 72: 1826-30, 1992.

97. Gladden, L. B., R. E. Crawford, and M. J. Webster. Effect of lactate concentration and metabolic rate on net lactate uptake by canine skeletal muscle. *Am. J. Physiol.* 266: R1095-101, 1994.

98. Gladden, L. B., R. E. Crawford, M. J. Webster, and P. W. Watt. Rapid tracer lactate influx into canine skeletal muscle. *J. Appl. Physiol.* 78: 205-211, 1995.

99. Gladden, L. B., and J. W. Yates. Lactic acid infusion in dogs: effects of varying infusate pH. *J. Appl. Physiol.* 54: 1254-60, 1983.

100. Gladden, L. B., J. W. Yates, R. W. Stremel, and B. A. Stamford. Agreement between gas exchange and lactate anaerobic threshold determination among independent investigators. *Med. Sci. Sports Exerc.* 15: 121, 1983.

101. Gladden, L. B., J. W. Yates, R. W. Stremel, and B. A. Stamford. Gas exchange and lactate anaerobic thresholds: inter- and intraevaluator agreement. *J. Appl. Physiol.* 58: 2082-9, 1985.

102. Glenny, R. W., T. Robertson, S. Yamashiro, and J. B. Bassingthwaighte. Applications of fractal analysis to physiology. *J. Appl. Physiol.* 70: 2351-67, 1991.

103. Graham, T. E., D. G. Sinclair, and C. K. Chapler. Metabolic intermediates and lactate diffusion in active dog skeletal muscle. *Am. J. Physiol.* 231: 766-71, 1976.

104. Granier, P., H. Dubouchaud, B. Mercier, J. Mercier, S. Ahmaidi, and C. Prefaut. Lactate uptake by forearm skeletal muscles during repeated periods of short-term intense leg exercise in humans. *Eur. J. Appl. Physiol.* 72: 209-14, 1996.

105. Granier, P. L., H. Dubouchaud, B. M. Mercier, J. G. Mercier, S. Ahmaidi, and C. G. Prefaut. Effect of NaHCO3 on lactate kinetics in forearm muscles during leg exercise in man. *Med. Sci. Sports Exerc.* 28: 692-7, 1996.

106. Greenfield, A. D. M., R. J. Whitney, and J. F. Mowbray. Methods for the investigation of peripheral blood flow. *Br. Med. Bull.* 19: 101-9, 1963.

107. Grønlund, J., G. M. Malvin, and M. P. Hlastala. Estimation of blood flow distribution in skeletal muscle from inert gas washout. *J. Appl. Physiol.* 66: 1942-1955, 1989.

108. Gupta, S., A. Goswami, A. K. Sadhukhan, and D. N. Mathur. Comparative study of lactate removal in short term massage of extremities, active recovery and a passive recovery period after supramaximal exercise sessions. *Int. J. Sports Med.* 17: 106-10, 1996.

109. Gutknecht, J., M. A. Bisson, and F. C. Tosteson. Diffusion of Carbon dioxide through lipid bilayer membranes. Effects of carbonic anydrase, bicarbonate, and unstirred layers. *J. Gen. Physiol.* 69: 779-94, 1977.

110. Hagberg, J. M. Physiological implications of the lactate threshold. *Int. J. Sports Med.* 5: 106-9, 1984.

111. Harris, P., M. Bateman, and J. Gloster. The regional metabolism of lactate and pyruvate during exercise in patients with rheumatic heart disease. *Clin. Sci.* 23: 545-560, 1962.

112. Haverty, M., W. L. Kenney, and J. L. Hodgson. Lactate and gas exchange responses to incremental and steady state running. *Brit. J. Sports Med.* 22: 51-4, 1988.

113. Hermansen, L., and O. Vaage. Lactate disappearance and glycogen synthesis in human muscle after maximal exercise. *Am. J. Physiol.* 233: E422-9, 1977.

114. Hickner, R. C., D. Bone, U. Ungerstedt, L. Jorfeldt, and J. Henriksson. Muscle blood flow during intermittent exercise: comparison of the microdialysis ethanol technique and ^{133}Xe clearance. *Clin. Sci.* 86: 15-25, 1994.

115. Hill, A. V., C. N. H. Long, and H. Lupton. Muscular exercise, lactic acid and the supply and utilisation of oxygen. IV-VIII. *Proc. R. Soc. Lond. (Biol.)* 97: 84-176, 1924.

116. Hirche, H., V. Hombach, H. D. Langohr, U. Wacker, and J. Busse. Lactic acid permeation rate in working gastrocnemii of dogs during metabolic alkalosis and acidosis. *Pflugers Arch.* 356: 209-222, 1975.

117. Hirche, H., U. Wacker, and H. D. Langohr. Lactic acid formation in the working gastrocnemius of the dog. *Int. Z. angew. Physiol. einschl. Arbeitphysiol.* 30: 52-64, 1971.

118. Hogan, M. C., R. H. Cox, and H. G. Welch. Lactate accumulation during incremental exercise with varied inspired oxygen fractions. *J. Appl. Physiol.* 55: 1134-40, 1983.

119. Hogan, M. C., L. B. Gladden, S. S. Kurdak, and D. C. Poole. Increased [lactate] in working dog muscle reduces tension development independent of pH. *Med. Sci. Sports Exerc.* 27: 371-7, 1995.

120. Holloszy, J. O. Biochemical adaptations to exercise: aerobic metabolism. *Ex. Sport Sci. Rev.* 1: 45-71, 1973.

121. Homer, L. D., P. K. Weathersby, and S. Survanshi. How countercurrent blood flow and uneven perfusion affect the motion of inert gas. *J. Appl. Physiol.* 69: 162-70, 1990.

122. Hughson, R. L., K. H. Weisiger, and G. D. Swanson. Blood lactate concentration increases as a continuous function in progressive exercise. *J. Appl. Physiol.* 62: 1975-81, 1987.

123. Hurley, B. F., J. M. Hagberg, W. K. Allen, D. R. Seals, J. C. Young, R. W. Cuddihee, and J. O. Holloszy. Effect of training on blood lactate levels during submaximal exercise. *J. Appl. Physiol.* 56: 1260-4, 1984.

124. Issekutz, B. J., W. A. Shaw, and A. C. Issekutz. Lactate metabolism in resting and exercising dogs. *J. Appl. Physiol.* 40: 312-9, 1976.

125. Ivy, J. L., D. L. Costill, P. J. Van Handel, D. A. Essig, and R. W. Lower. Alteration in the lactate threshold with changes in substrate availability. *Int. J. Sports Med.* 2: 139-142, 1981.

126. Jöbsis, F. F., and W. N. Stainsby. Oxidation of NADH during contractions of circulated mammalian skeletal muscle. *Respir. Physiol.* 4: 292-300, 1968.

127. Johnson, J. M., and L. B. Rowell. Forearm skin and muscle vascular responses to prolonged leg exercise in man. *J. Appl. Physiol.* 39: 920-4, 1975.

128. Johnson, M. A., J. Polgar, D. Weightman, and D. Appleton. Data on the distribution of fibre types in thirty-six human muscles. An autopsy study. *J. Neurol. Sci.* 18: 111-29, 1973.

129. Jones, D. P. Intracellular diffusion gradients of O_2 and ATP. *Am. J. Physiol.* 250: C663-C675, 1986.

130. Jorfeldt, L. Metabolism of L(+)-lactate in human skeletal muscle during exercise. *Acta Physiol. Scand.* : 1-67, 1970.

131. Jorfeldt, L., and J. Wahren. Human forearm muscle metabolism during exercise. V. Quantitative aspects of glucose uptake and lactate production during prolonged exercise. *Scand. J. Clin. Lab. Invest.* 26: 73-81, 1970.

132. Juel, C. Human muscle lactate transport can be studied in sarcolemmal giant vesicles made from needle-biopsies. *Acta Physiol. Scand.* 142: 133-144, 1991.

133. Juel, C. Intracellular pH recovery and lactate efflux in mouse soleus muscles stimulated *in vitro*: the involvement of sodium/proton exchange and a lactate carrier. *Acta Physiol. Scand.* 132: 363-71, 1988.

134. Juel, C. Muscle lactate transport studied in sarcolemmal giant vesicles. *Biochim. Biophys. Acta* 1065: 15-20, 1991.

135. Juel, C., S. Kristiansen, H. Pilegaard, J. Wojtaszewski, and E. A. Richter. Kinetics of lactate transport in sarcolemmal giant vesicles obtained from human skeletal muscle. *J. Appl. Physiol.* 76: 1031-1036, 1994.

136. Juel, C., and F. Wibrand. Lactate transport in isolated mouse muscles studied with a tracer technique--kinetics, stereospecificity, pH dependency and maximal capacity. *Acta Physiol. Scand.* 137: 33-9, 1989.

137. Juhlin-Dannfelt, A., and H. Astrom. Influence of beta-adrenoceptor blockade on leg blood flow and lactate release in man. *Scand. J. Clin. Lab. Invest.* 39: 179-83, 1979.

138. Kanno, T., K. Sudo, M. Kitamura, S. Miwa, A. Ichiyama, and Y. Nishimura. Lactate dehydrogenase A-subunit and B-subunit deficiencies: comparison of the physiological roles of LDH isozymes. *Isozymes Curr. Top. Biol. Med. Res.* 7: 131-50, 1983.

139. Karlsson, J. Lactate and phosphagen concentrations in working muscle of man. *Acta Physiol. Scand. Suppl.* : 358, 1971.

140. Karlsson, J., F. Bonde-Petersen, J. Henriksson, and H. G. Knuttgen. Effects of previous exercise with arms or legs on metabolism and performance in exhaustive exercise. *J. Appl. Physiol.* 38: 763-7, 1975.

141. Karlsson, J., C. F. Funderburk, B. Essen, and A. R. Lind. Constituents of human muscle in isometric fatigue. *J. Appl. Physiol.* 38: 208-11, 1975.

142. Katz, A., and K. Sahlin. Regulation of lactic acid production during exercise. *J. Appl. Physiol.* 65: 509-18, 1988.

143. Katz, A. M. Contractile proteins of the heart. *Physiol. Rev.* 50: 63-158, 1970.

144. Katz, J. The application of isotopes to the study of lactate metabolism. *Med. Sci. Sports Exerc.* 18: 353-9, 1986.

145. Kelman, G. R., R. J. Maughan, and C. Williams. The effect of dietary modification on blood lactate during exercise. *J. Physiol.* 251: 34-35, 1975.

146. Kennedy, F. G., and D. P. Jones. Oxygen dependence of mitochondrial function in isolated rat cardiac monocytes. *Am. J. Physiol.* 250: C374-C383, 1986.

147. Kety, S. Cerebral circulation and its measurement by inert diffusible tracers. *Isr. J. Med. Sci.* 23: 3-7, 1987.

148. Kety, S. S. Study of the cerebral circulation by means of inert diffusible tracers. *Prog. Brain. Res.* 35: 375-85, 1919.

149. Kety, S. S. The theory and applications of the exchange of inert gas at the lungs and tissues. *Pharmacol. Rev.* 3: 1-41, 1951.

150. Kety, S. S., and C. F. Schmidt. The determination of cerebral blood flow in man by the use of nitrous oxide in low concentrations. *Am. J. Physiol.* 143: 53-66, 1945.

151. Kety, S. S., and C. F. Schmidt. The nitrous oxide method for the quantitative determination of cerebral blood flow in man: theory, procedure and normal values. *J. Clin. Invest.* 27: 476-83, 1948.

152. Kindermann, W., G. Simon, and J. Keul. The significance of the aerobic - anaerobic transition for the determination of work load intensities during endurance training. *Eur. J. Appl. Physiol.* 42: 25-34, 1979.

153. Koch, A., B. Webster, and S. Lowell. Cellular uptake of L-lactate in mouse diaphragm. *Biophys. J.* 36: 775-96, 1981.

154. Krebs, H. A., R. Hems, M. J. Weidemann, and R. N. Speake. The fate of isotopic carbon in kidney cortex synthesizing glucose from lactate. *Biochem. J.* 101: 242-249, 1966.

155. Krogh, A. The number and distribution of capillaries in muscles with calculations of the oxygen partial pressure head necessary for supplying the tissue. *J. Physiol. (Lond.)* 52: 405-415, 1919.

156. Kuikka, J., M. Levin, and J. B. Bassingthwaighte. Multiple tracer dilution estimates of D- and 2-deoxy-D-glucose uptake by the heart. *Am. J. Physiol.* 250: H29-H42, 1986.

157. Lehman, S. L. Measurement of lactate production by tracer techniques. *Med. Sci. Sports Exerc.* 23: 935-8, 1991.

158. Lehmann, M., J. Keul, G. Huber, and M. Da Prada. Plasma catecholamines in trained and untrained volunteers during graduated exercise. *Int. J. Sports Med.* 2: 143-7, 1981.

159. Lenders, J., G. J. Janssen, P. Smits, and T. Thien. Role of the wrist cuff in forearm plethysmography. *Clin. Sci.* 80: 413-7, 1991.

160. Lewis, S. F., and R. G. Haller. The pathophysiology of McArdle's disease: clues to regulation in exercise and fatigue. *J. Appl. Physiol.* 61: 391-401, 1986.

161. Lindinger, M. I., G. J. F. Heigenhauser, R. S. McKelvie, and N. L. Jones. Role of nonworking muscle on blood metabolites and ions with intense intermittent exercise. *Am. J. Physiol.* 258: R1486-94, 1990.

162. Lindinger, M. I., R. S. McKelvie, and G. J. F. Heigenhauser. K^+ and Lac^- distribution in humans during and after high-intensity exercise: role in muscle fatigue attenuation? *J. Appl. Physiol.* 78: 765-777, 1995.

163. Linnarsson, D., J. Karlsson, L. Fagraeus, and B. Saltin. Muscle metabolites and oxygen deficit with exercise in hypoxia and hyperoxia. *J. Appl. Physiol.* 36: 399-402, 1974.

164. Ludbrook, J. Repeated measurements and multiple comparisons in cardiovascular research. *Cardiovasc. Res.* 28: 303-11, 1994.

165. Mainwood, G. W., and J. M. Renaud. The effect of acid-base balance on fatigue of skeletal muscle. *Can. J. Physiol. Pharmacol.* 63: 403-16, 1985.

166. Mainwood, G. W., J. M. Renaud, and M. J. Mason. The pH dependence of the contractile response of fatigued skeletal muscle. *Can. J. Physiol. Pharmacol.* 65: 648-58, 1987.

167. Mathews, J. H. *Numerical methods for computer science, engineering, and mathematics*. London: Prentice-Hall International, 1987.

168. Mazzeo, R. S., G. A. Brooks, T. F. Budinger, and D. A. Scoeller. Pulse injection, ^{13}C tracer studies of lactate metabolism in humans during rest and two levels of exercise. *Biomed. Mass Spectrom.* 9: 310-4, 1982.

169. Mazzeo, R. S., G. A. Brooks, D. A. Schoeller, and T. F. Budinger. Disposal of blood [1-^{13}C]lactate in humans during rest and exercise. *J. Appl. Physiol.* 60: 232-41, 1986.

170. Mazzeo, R. S., and P. Marshall. Influence of plasma catecholamines on the lactate threshold during graded exercise. *J. Appl. Physiol.* 67: 1319-22, 1989.

171. McDermott, J. C., and A. Bonen. Glyconeogenic and oxidative lactate utilization in skeletal muscle. *Can. J. Physiol. Pharmacol.* 70: 142-9, 1992.

172. McDermott, J. C., and A. Bonen. Lactate transport by skeletal muscle sarcolemmal vesicles. *Mol. Cell. Biochem.* 122: 113-21, 1993.

173. McGrail, J. C., A. Bonen, and A. N. Belcastro. Dependence of lactate removal on muscle metabolism in man. *Eur. J. Appl. Physiol.* 39: 89-97, 1978.

174. McLane, J. A., and J. O. Holloszy. Glycogen synthesis from lactate in the three types of skeletal muscle. *J. Biol. Chem.* 254: 6548-6553, 1979.

175. McLoughlin, P., N. McCaffrey, and J. B. Moynihan. Gentle exercise with a previously inactive muscle group hastens the decline of blood lactate concentration after strenuous exercise. *Eur. J. Appl. Physiol.* 62: 274-8, 1991.

176. Molloy, M. J., I. P. Latto, and M. Rosen. Analysis of nitrous oxide concentrations in whole blood. *Br. J. Anaesth.* 45: 556-62, 1973.

177. Morales, M. F., and R. E. Smith. A note on the physiological arrangement of tissues. *Bull. Math. Biophysics* 7: 47-51, 1945.

178. Morales, M. F., and R. E. Smith. On the theory of blood-tissue exchange of inert gases: VI. Validity of approximate uptake expressions. *Bull. Math. Biophysics* 10: 191-200, 1948.

179. Morales, M. F., and R. E. Smith. On the theory of blood-tissue exchanges: III. Circulation and inert-gas exchanges at the lung with special reference to saturation. *Bull. Math. Biophysics* 6: 141-52, 1944.

180. Morales, M. F., and R. E. Smith. The physiological factors which govern inert gas exchange. *Bull. Math. Biophysics* 7: 99-106, 1945.

181. Morton, R. H. Detection of a lactate threshold during incremental exercise? *J. Appl. Physiol.* 67: 885-8, 1989.

182. Morton, R. H. Modelling human power and endurance. *J. Math. Biol.* 28: 49-64, 1990.

183. Morton, R. H. A three compartment model of human bioenergetics. *J. Math. Biol.* 24: 451-66, 1986.

184. Morton, R. H., and G. C. Gass. A systems model approach to the ventilatory anaerobic threshold. *Eur. J. Appl. Physiol.* 56: 367-73, 1987.

185. Mottram, R. F. Blood flow and muscle contraction in the forearm. *J. Physiol.* 182: 27P-8P, 1965.

186. Mottram, R. F. The oxygen consumption of human skeletal muscle *in vivo*. *J. Physiol.* 128: 268-76, 1955.

187. Muller-Plathe, O., and S. Heyduck. Stability of blood gases, electrolytes and haemoglobin in heparinized whole blood samples: influence of the type of syringe. *Eur. J. Clin. Chem. Clin. Biochem.* 30: 349-55, 1992.

188. Nagata, A., M. Muro, T. Moritani, and T. Yoshida. Anaerobic threshold determination by blood lactate and myoelectric signals. *Jpn. J. Physiol.* 31: 585-97, 1981.

189. Newsholme, E. A., and A. R. Leech. *Biochemistry for the medical sciences*. Chichester: John Wiley & Sons, 1988.

190. Novotny, J. A., D. L. Mayers, Y. J. Parsons, S. S. Survanshi, P. K. Weathersby, and L. D. Homer. Xenon kinetics in muscle are not explained by a model of parallel perfusion-limited compartments. *J. Appl. Physiol.* 68: 876-90, 1990.

191. Obrist, W. D., H. K. Thompson, C. H. King, and H. S. Wang. Determination of cerebral blood flow by inhalation of 133-xenon. *Circ. Res.* 20: 124-35, 1967.

192. Orok, C. J., R. L. Hughson, H. J. Green, and J. A. Thomson. Blood lactate responses to incremental exercise as predictors of constant load performance. *Eur. J. Appl. Physiol.* 59: 262-7, 1989.

193. Pagliassotti, M. J., and C. M. Donovan. Glycogenesis from lactate in rabbit skeletal muscle fiber types. *Am. J. Physiol.* 258: R903-11, 1990.

194. Pagliassotti, M. J., and C. M. Donovan. Influence of cell heterogeneity on skeletal muscle lactate kinetics. *Am. J. Physiol.* 258: E625-E634, 1990.

195. Pagliassotti, M. J., and C. M. Donovan. Role of cell type in net lactate removal by skeletal muscle. *Am. J. Physiol.* 258: E635-42, 1990.

196. Peters, S. J., D. J. Dyck, A. Bonen, and L. L. Spriet. Effects of epinephrine on lipid metabolism in resting skeletal muscle. *Am. J. Physiol.* 275: E300-9, 1998.

197. Piiper, J. Carbon dioxide-oxygen relationships in gas exchange of animals. *Boll. Soc. It. Biol. Sper.* 67: 635-58, 1991.

198. Piiper, J. Role of diffusion shunt in transfer of inert gases and O_2 in muscle. *Adv. Exp. Med. Biol.* 222: 55-61, 1988.

199. Piiper, J., and P. Haab. Oxygen supply and uptake in tissue models with unequal distribution of blood flow and shunt. *Respir. Physiol.* 84: 261-271, 1991.

200. Piiper, J., and M. Meyer. Diffusion-perfusion relationships in skeletal muscle: models and experimental evidence from inert gas washout. *Adv. Exp. Med. Biol.* 169: 457-66, 1984.

201. Piiper, J., M. Meyer, and P. Scheid. Dual role of diffusion in tissue gas exchange: Blood-tissue equilibration and diffusion shunt. *Respir. Physiol.* 56: 131-44, 1984.

202. Podolin, D. A., P. A. Munger, and R. S. Mazzeo. Plasma catecholamine and lactate response during graded exercise with varied glycogen conditions. *J. Appl. Physiol.* 71: 1427-33, 1991.

203. Poortmans, J. R., J. V. Bossche, and R. Leclercq. Lactate uptake by inactive forearm during progressive leg exercise. *J. Appl. Physiol.* 45: 835-9, 1978.

204. Press, W. H., S. A. Teukolsky, W. T. Vetterling, and B. P. Flannery. *Numerical recipes in fortran 77: The art of scientific computing.* Cambridge: Cambridge University Press, 1992. http://www.nr.com

205. Pretto, J. J., and P. D. Rochford. Effects of sample storage time, temperature and syringe type on blood gas tensions in samples with high oxygen partial pressures. *Thorax* 49: 610-2, 1994.

206. Price, T. B., D. L. Rothman, M. J. Avison, P. Buonamico, and R. G. Shulman. ^{13}C-NMR measurements of muscle glycogen during low-intensity exercise. *J. Appl. Physiol.* 70: 1836-44, 1991.

207. Princen, H. M., J. Overbeek, and S. G. Mason. The permeability of soap films to gases. II. A simple mechanism of monolayer permeability. *J. Colloid Inter. Sci.* 24: 125-30, 1967.

208. Putman, C. T., M. P. Matsos, E. Hultman, N. L. Jones, and G. J. Heigenhauser. Pyruvate dehydrogenase activation in inactive muscle during and after maximal exercise in men. *Am. J. Physiol.* 276: E483-8, 1999.

209. Putman, C. T., L. L. Spriet, E. Hultman, D. J. Dyck, and G. J. F. Heigenhauser. Skeletal muslce pyruvate dehydrogenase activity during acetate infusion in humans. *Am. J. Physiol.* 268: E1007-E1017, 1995.

210. Richardson, R. S. Oxygen transport: air to muscle cell. *Med. Sci. Sports Exerc.* 30: 53-9, 1998.

211. Robergs, R. A., J. Chwalbinska-Moneta, J. B. Mitchell, D. D. Pascoe, J. Houmard, and D. L. Costill. Blood lactate threshold differences between arterialized and venous blood. *Int. J. Sports Med.* 11: 446-51, 1990.

212. Romijn, J. A., D. L. Chinkes, J. M. Schwarz, and R. R. Wolfe. Lactate-pyruvate interconversion in blood: implications for in vivo tracer studies. *Am. J. Physiol.* 266: E334-40, 1994.

213. Rontoyannis, G. Lactate elimination from the blood during active recovery. *J. Sports Med. Phys. Fitness* 28: 115-23, 1988.

214. Roos, A. Intracellular pH and distribution of weak acids across cell membranes. A study of D- and L-lactate and of DMO in rat diaphragm. *J. Physiol. (Lond.)* 249: 1-25, 1975.

215. Ross, J. T. The effect of β-adrenoceptor blockade on resting forearm skeletal muscle lactate kinetics during leg exercise (Honours Thesis). In: *Department of Physiology*. Adelaide: University of Adelaide, 1993.

216. Roth, D. A. The sarcolemmal lactate transporter: transmembrane determinants of lactate flux. *Med. Sci. Sports Exerc.* 23: 925-34, 1991.

217. Roth, D. A., and G. A. Brooks. Lactate and pyruvate transport is dominated by a pH gradient-sensitive carrier in rat skeletal muscle sarcolemmal vesicles. *Arch. Biochem. Biophys.* 279: 386-94, 1990.

218. Roth, D. A., and G. A. Brooks. Lactate transport is mediated by a membrane-bound carrier in rat skeletal muscle sarcolemmal vesicles. *Arch. Biochem. Biophys.* 279: 377-85, 1990.

219. Roth, D. A., and G. A. Brooks. Training does not affect zero-trans lactate transport across mixed rat skeletal muscle sarcolemmal vesicles. *J. Appl. Physiol.* 75: 1559-65, 1993.

220. Sahlin, K. Lactate production cannot be measured with tracer techniques. *Am. J. Physiol.* 252, 1987.

221. Sahlin, K. Muscle carnitine metabolism during incremental dynamic exercise in humans. *Acta Physiol. Scand.* 138: 259-62, 1990.

222. Sahlin, K. Muscle fatigue and lactic acid accumulation. *Acta Physiol. Scand. Suppl.* 556: 83-91, 1986.

223. Sahlin, K., A. Katz, and S. Broberg. Tricarboxylic acid cycle intermediates in human muscle during prolonged exercise. *Am. J. Physiol.* 259: C834-41, 1990.

224. Sahlin, K., A. Katz, and J. Henriksson. Redox state and lactate accumulation in human skeletal muscle during dynamic exercise. *Biochem. J.* 245: 551-6, 1987.

225. Schenkman, K. A., D. R. Marble, D. H. Burns, and E. O. Feigl. Myoglobin oxygen dissociation by multiwavelength spectroscopy. *J. Appl. Physiol.* 82: 86-92, 1997.

226. Scholander, P. F. Analyser for accurate estimation of respiratory gases in one-half cubic centimeter samples. *J. Biol. Chem.* 167: 235-239, 1947.

227. Searle, G. L., and R. R. Cavalieri. Determination of lactate kinetics in the human analysis of data from single injection vs. continuous infusion methods. *Proc. Soc. Exp. Biol. Med.* 139: 1002-6, 1972.

228. Shiota, M., S. Golden, and J. Katz. Lactate metabolism in the perfused rat hindlimb. *Biochem. J.* 222: 281-92, 1984.

229. Siggaard-Andersen, O., P. D. Wimberley, N. Fogh-Andersen, and I. H. Gothgen. Measured and derived quantities with modern pH and blood gas equipment: calculation algorithms with 54 equations. *Scand. J. Clin. Lab. Invest.* 48: 7-15, 1988.

230. Siggaard-Andersen, O., P. D. Wimberley, I. Gothgen, and M. Siggaard-Andersen. A mathematical model of the hemoglobin-oxygen dissociation curve of human blood and of the oxygen partial pressure as a function of temperature. *Clin. Chem.* 30: 1646-51, 1984.

231. Sjodin, B. Lactate dehydrogenase in humans skeletal muscle. *Acta Physiol. Scand. Suppl.* 436: 1-32, 1976.

232. Sjodin, B., and I. Jacobs. Onset of blood lactate accumulation and marathon running performance. *Int. J. Sports Med.* 2: 23-6, 1981.

233. Sjodin, B., I. Jacobs, and J. Svedenhag. Changes in onset of blood lactate accumulation (OBLA) and muscle enzymes after training at OBLA. *Eur. J. Appl. Physiol.* 49: 45-57, 1982.

234. Smith, E. W., M. S. Skelton, D. E. Kremer, D. D. Pascoe, and L. B. Gladden. Lactate distribution in the blood during steady-state exercise. *Med. Sci. Sports Exerc.* 30: 1424-9, 1998.

235. Smith, R. E., and M. F. Morales. On the theory of blood-tissue exchanges: I. fundamental equations. *Bull. Math. Biophysics* 6: 125-31, 1944.

236. Smith, R. E., and M. F. Morales. On the theory of blood-tissue exchanges: II. Applications. *Bull. Math. Biophysics* 6: 133-9, 1944.

237. Spriet, L. L. Phosphofructokinase activity and acidosis during short-term tetanic contractions. *Can. J. Physiol. Pharmacol.* 69: 298-304, 1991.

238. Spriet, L. L., M. I. Lindinger, R. S. McKelvie, G. J. Heigenhauser, and N. L. Jones. Muscle glycogenolysis and H+ concentration during maximal intermittent cycling. *J. Appl. Physiol.* 66: 8-13, 1989.

239. Spriet, L. L., J. M. Ren, and E. Hultman. Epinephrine infusion enhances muscle glycogenolysis during prolonged electrical stimulation. *J. Appl. Physiol.* 64: 1439-44, 1988.

240. Stainsby, W. N. Biochemical and physiological bases for lactate production. *Med. Sci. Sports Exerc.* 18: 341-3, 1986.

241. Stainsby, W. N., W. F. Brechue, and D. M. O'Drobinak. Regulation of muscle lactate production. *Med. Sci. Sports Exerc.* 23: 907-11, 1991.

242. Stainsby, W. N., and G. A. Brooks. Control of lactic acid metabolism in contracting muscles and during exercise. *Ex. Sport Sci. Rev.* 18: 29-63, 1990.

243. Stainsby, W. N., C. Sumners, and P. D. Eitzman. Effects of catecholamines on lactic acid output during progressive working contractions. *J. Appl. Physiol.* 59: 1809-14, 1985.

244. Stamford, B. A., R. J. Moffatt, A. Weltman, C. Maldonado, and M. Curtis. Blood lactate disappearance after supramaximal one-legged exercise. *J. Appl. Physiol.* 45: 244-8, 1978.

245. Stamford, B. A., A. Weltman, R. Moffatt, and S. Sady. Exercise recovery above and below anaerobic threshold following maximal work. *J. Appl. Physiol.* 51: 840-4, 1981.

246. Stanley, W. C., E. W. Gertz, J. A. Wisneski, D. L. Morris, R. A. Neese, and G. A. Brooks. Systemic lactate kinetics during graded exercise in man. *Am. J. Physiol.* 249: E595-602, 1985.

247. Stanley, W. C., E. W. Gertz, J. A. Wisneski, R. A. Neese, D. L. Morris, and G. A. Brooks. Lactate extraction during net lactate release in legs of humans during exercise. *J. Appl. Physiol.* 60: 1116-20, 1986.

248. Stanley, W. C., and L. Lehman. Calculation of lactate disappearance with isotopic tracers using tissue lactate specific radioactivity. *Biochem. J.* 259: 935, 1989.

249. Stanley, W. C., and L. Lehman. A model for measurement of lactate disappearance with isotopic tracers in the steady state. *Biochem. J.* 256: 1035-8, 1988.

250. Stegmann, H., and W. Kindermann. Comparison of prolonged exercise tests at the individual anaerobic threshold and the fixed anaerobic threshold of 4 mmol.l^{-1} lactate. *Int. J. Sports Med.* 3: 105-10, 1982.

251. Stegmann, H., W. Kindermann, and A. Schnabel. Lactate kinetics and individual anaerobic threshold. *Int. J. Sports Med.* 2: 160-5, 1981.

252. Stoelting, R. K., and E. I. Eger. Percutaneous loss of nitrous oxide, cyclopropane, ether and halothane in man. *Anaesthesiology* 30: 278-283, 1969.

253. Stryer, L. *Biochemistry*. New York: W. H. Freeman and Company, 1988.

254. Systrom, D. M., D. J. Kanarek, S. J. Kohler, and H. Kazemi. ^{31}P nuclear magnetic resonance spectroscopy study of the anaerobic threshold in humans. *J. Appl. Physiol.* 68: 2060-6, 1990.

255. Tanaka, K., and Y. Matsuura. Marathon performance, anaerobic threshold, and onset of blood lactate accumulation. *J. Appl. Physiol.* 57: 640-3, 1984.

256. Taoutaou, Z., P. Granier, B. Mercier, J. Mercier, S. Ahmaidi, and C. Prefaut. Lactate kinetics during passive and partially active recovery in endurance and sprint athletes. *Eur. J. Appl. Physiol.* 73: 465-70, 1996.

257. Tesch, P. Muscle fatigue in man with special reference to lactate accumulation during short term intense exercise. *Acta Physiol. Scand. Suppl.* 480: 1-40, 1980.

258. Tesch, P. A., and J. E. Wright. Recovery from short term intense exercise: its relation to capillary supply and blood lactate concentration. *Eur. J. Appl. Physiol.* 52: 98-103, 1983.

259. Ui, M. A role of phosphofructokinase in pH-dependent regulation of glycolysis. *Biochim. Biophys. Acta* 124: 310-22, 1966.

260. Veech, R. L., J. W. R. Lawson, N. W. Cornell, and H. A. Krebs. Cytosolic phosphorylation potential. *J. Biol. Chem.* 254: 6538-6547, 1979.

261. Wagner, J. G. *Pharmacokinetics for the pharmaceutical scientist*. Lancaster: Technomic Publishing Co., 1993.

262. Wahren, J., P. Felig, R. J. Havel, L. Jorfeldt, B. Pernow, and B. Saltin. Amino acid metabolism in McArdle's syndrome. *N. Engl. J. Med.* 288: 774-777, 1973.

263. Wasserman, K., W. L. Beaver, J. A. Davis, J. Z. Pu, D. Heber, and B. J. Whipp. Lactate, pyruvate, and lactate-to-pyruvate ratio during exercise and recovery. *J. Appl. Physiol.* 59: 935-40, 1985.

264. Wasserman, K., A. L. Van Kessel, and G. G. Burton. Interaction of physiological mechanisms during exercise. *J. Appl. Physiol.* 22: 71-85, 1967.

265. Wasserman, K., B. J. Whipp, S. N. Koyal, and W. L. Beaver. Anaerobic threshold and respiratory gas exchange during exercise. *J. Appl. Physiol.* 35: 236-43, 1973.

266. Watt, P. W., L. B. Gladden, H. S. Hundal, and R. E. Crawford. Effects of flow and contraction on lactate transport in the perfused rat hindlimb. *Am. J. Physiol.* 267: E7-13, 1994.

267. Watt, P. W., P. A. MacLennan, H. S. Hundal, C. M. Kuret, and M. J. Rennie. L(+)-lactate transport in perfused rat skeletal muscle: kinetic characteristics and sensitivity to pH and transport inhibitors. *Biochim. Biophys. Acta* 944: 213-22, 1988.

268. Weathersby, P. K., and L. D. Homer. Solubility of inert gases in biological fluids and tissues: a review. *Undersea Biomed. Res.* 7: 277-96, 1980.

269. Weltman, A., B. A. Stamford, and C. Fulco. Recovery from maximal effort exercise: lactate disappearance and subsequent performance. *J. Appl. Physiol.* 47: 677-82, 1979.

270. Wendling, P. S., S. J. Peters, G. J. Heigenhauser, and L. L. Spriet. Epinephrine infusion does not enhance net muscle glycogenolysis during prolonged aerobic exercise. *Can. J. Appl. Physiol.* 21: 271-84, 1996.

271. Whelan, R. F. *Control of the peripheral circulation in man.* Springfield: Charles C Thomas, 1967.

272. Whipp, B. J., and K. Wasserman. Oxygen uptake kinetics for various intensities of constant-load work. *J. Appl. Physiol.* 33: 351-6, 1972.

273. Whitney, R. J. The measurement of volume changes in human limbs. *J. Physiol. (Lond.)* 121: 1-27, 1953.

274. Wieland, O. H. The mammalian pyruvate dehydrogenase complex: structure and regulation. *Rev. Physiol. Biochem. Pharmacol.* 96: 123-70, 1983.

275. Williams, C. A., and A. R. Lind. Measurement of forearm blood flow by venous occlusion plethysmography: Influence of hand blood flow during sustained and intermittent isometric exercise. *Eur. J. Appl. Physiol.* 42: 141-9, 1979.

276. Williams, J. R., N. Armstrong, and B. J. Kirby. The influence of the site of sampling and assay medium upon the measurement and interpretation of blood lactate responses to exercise. *J. Sports Sci.* 10: 95-107, 1992.

277. Wilson, D. F. Factors affecting the rate and energetics of mitochondrial oxidative phosphorylation. *Med. Sci. Sports Exerc.* 26: 37-43, 1994.

278. Wolfe, R. R., F. Jahoor, and H. Miyoshi. Evaluation of the isotopic equilibration between lactate and pyruvate. *Am. J. Physiol.* 254: E532-5, 1988.

279. Woll, P. J., and C. O. Record. Lactate elimination in man: effects of lactate concentration and hepatic dysfunction. *Eur. J. Clin. Invest.* 9: 397-404, 1979.

280. Wolpers, H. G., A. Hoeft, H. Korb, P. R. Lichtlen, and G. Hellige. Transport of inert gases in mammalian myocardium: comparison with a convection-diffusion model. *Am. J. Physiol.* 259: H167-73, 1990.

281. Wu, E. Y., K. W. Barazanji, and R. L. Johnson, Jr. Source of error on A-aDO_2 calculated from blood stored in plastic and glass syringes. *J. Appl. Physiol.* 82: 196-202, 1997.

282. Yeh, M. P., R. M. Gardner, T. D. Adams, F. G. Yanowitz, and R. O. Crapo. "Anaerobic threshold": problems of determination and validation. *J. Appl. Physiol.* 55: 1178-86, 1983.

283. Yoshida, T., M. Chida, M. Ichioka, and Y. Suda. Blood lactate parameters related to aerobic capacity and endurance performance. *Eur. J. Appl. Physiol.* 56: 7-11, 1987.

284. Yoshida, T., N. Takeuchi, and Y. Suda. Arterial versus venous blood lactate increase in the forearm during incremental bicycle exercise. *Eur. J. Appl. Physiol.* 50: 87-93, 1982.

285. Zelis, R., D. T. Mason, and E. Braunwald. Partition of blood flow to the cutaneous and muscular beds of the forearm at rest and during leg exercise in normal subjects and in patients with heart failure. *Circ. Res.* 24: 799-806, 1969.

286. Zierler, K. L. Theory of the use of arteriovenous concentration differences for measuring metabolism in steady and non-steady states. *J. Clin. Invest.* 40: 2111-25, 1961.

287. Zierler, K. L., A. Maseri, G. Klassen, D. Rabinowitz, and J. Burgess. Muscle metabolism during exercise in man. *Trans. Assoc. Am. Physicians* 81: 266-73, 1968.