OXYGEN UPTAKE AND LEG BLOOD FLOW AT THE ONSET OF KICKING EXERCISE IN HUMANS

by

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A thesis
presented to the University of Waterloo
in fulfilment of the
thesis requirement for the degree of
Doctor of Philosophy
in
Kinesiology

Waterloo, Ontario, Canada, 1998

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Abstract:

The main hypothesis of this thesis was that changes in the adaptation of leg blood flow (LBF) can impact on oxygen uptake ($\dot{V}O_2$) at the onset of moderate intensity kicking exercise. LBF and $\dot{V}O_2$ increase rapidly at the onset of exercise in humans, however, the specific regulatory mechanisms for these processes are unknown. Three studies were conducted in which the time course of change in LBF was determined at the onset of rhythmic leg kicking exercise using Doppler ultrasound. In addition, $\dot{V}O_2$ responses at the onset of exercise were measured. In the first study (Paper I) LBF kinetics were slowed when muscle perfusion pressure was less in the supine versus upright exercise position. This decrease in LBF kinetics in the supine position was associated with a slowing of $\dot{V}O_2$ at the onset of exercise. The LBF response was observed to precede the $\dot{V}O_2$ response, however, the dynamic responses of LBF and $\dot{V}O_2$ appeared to closely parallel each other at the onset of exercise, particularly in the supine position. In a second study (Paper II), the time course of LBF and $\dot{V}O_2$ at the onset of kicking exercise was unaffected by a single day of heavy exercise training. On the day of heavy exercise training, bouts of prior high intensity exercise resulted in an increase in the leg vascular conductance during both 0W and 50 W exercise, but did not alter the dynamic response of LBF during transitions between the two work rate levels. In the third study (Paper III), different gas breathing conditions altered the arterial oxygen content, however, adaptations in LBF and O_2 extraction resulted in no change in oxygen delivery or in $\dot{V}O_2$ at the onset of exercise. As part of the third study, the application of near infrared spectroscopy (NIRS) measures of Hb/ Mb O_2 saturation were compared to femoral venous O_2 saturation ($SfvO_2$) at the onset of exercise in different gas breathing conditions (Paper IV). There was a marked separation between the directly measured $SfvO_2$ and the indirect estimates of Hb/Mb O_2 saturation from NIRS. Alterations in the rate of adjustment of LBF to exercising muscle at the onset of moderate intensity large muscle mass, can induce adaptations in the dynamic response of $\dot{V}O_2$, however, regulatory mechanisms within the exercising muscles assist in maintaining LBF and $\dot{V}O_2$ at the onset of moderate intensity kicking exercise.

Acknowledgments

To my supervisor, Rich Hughson, for his patient support while I found my way from chemistry, to physiology, to medicine, and back. I know I took a winding road at times but your insight and guidance were reassuring signposts along the way. You have also shown me, by the way you enjoy your life and family, that the academic world is not one of just long hours at work and that the rewards are worth the effort.

A special thanks to Norm Ashton for helping me find my way into Rich's lab. Thank you to all of the members of my committee, especially Dr. H. Green, for including me in some very interesting experiments and Dr. M. Sharratt, for always being interested.

Thanks to everyone in the lab throughout my time in BMH. Although the faces have changed, the support, comradery and enthusiasm for sharing the scientific adventure, and some serious tobogganing parties, have been great. Special thanks to Kevin who always set such a tough example to follow and to Mike for asking such interesting, and long, questions. In you, I know, I have a friend. Thanks also to everyone who helped with the data collection and who participated in the studies. To "King"Dave, I learned so much from you and want you to know that I really appreciate all of the time and effort.

Thank you to Maria Hopman and everyone I worked with in Nijmegen. The experience was wonderful. To Mike and Cheri Houston, for making the transition to BC so smooth and being so understanding about the time it took me to complete this thesis.

To Mom and Dad, you always let me make my own decisions and I know that I changed my mind often! You were always interested in what I was doing even if it was a little hard to understand at times. I have been away from home for a long time now but I always know you are there for me if I need you. To Kirk, for reminding me that it is time to get out into the "real world". To Marlee, thanks for being a pilot subject may times and for being in prime physical condition. Most importantly, you showed me that any computer problem I might have was smaller than yours.

Especially to Stuart, for listening to me complaining, for reading first drafts, for encouraging me and for being my soul mate.

List of Papers

This thesis is based on the following papers. They will be referred to throughout by their chapter designation.

Chapter II: Paper I Alveolar oxygen uptake and femoral artery blood flow dynamics in upright and supine leg exercise in humans

Chapter III: Paper II Effect of 16 Hours of intermittent heavy exercise on oxygen uptake and blood flow responses to submaximal leg exercise

Chapter IV: Paper III Effect of hyperoxia and hypoxia on oxygen uptake and leg blood flow responses to submaximal exercise

Chapter V: Paper IV Comparison of femoral blood gases and muscle near infrared spectroscopy at the onset of exercise in humans

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List of Abbreviations

(with units where appropriate)

$\dot{V}O_2$ - rate of oxygen uptake (mL/min)

$\dot{V}O_2$alv - rate of alveolar oxygen uptake (mL/min)

$\dot{V}O_2$mus - rate of muscle oxygen uptake (mL/min)

$\dot{V}CO_2$ - rate of carbon dioxide output (mL/min)

$\dot{V}_E$ - expired ventilation (L/min)

$\dot{Q}$ - flow rate (mL/min)

T_{VENT} - ventilatory threshold

MBV - mean blood velocity (cm/sec)

LBF - leg blood flow (mL/min)

VC_{fa} - femoral artery vascular conductance (mL/min/mmHg)

CO - cardiac output (L/min)

PO_2 - partial pressure of oxygen (mmHg)

FIO_2 - fractional concentration of oxygen in inspired air

CaO_2 - arterial oxygen content (mL/100mL)

CvO_2 - venous oxygen content (mL/100mL)

O_2 del - oxygen delivery (L/min)

a-vDO_2 - arteriovenous oxygen content difference (mL O_2/l)

SO_2 - oxygen saturation (%)

NIRS - near infrared spectroscopy

IR-SO_2 - oxygen saturation estimated by near infrared spectroscopy

$SfvO_2$ - femoral venous oxygen saturation (%)

Hb - haemoglobin

Mb - myoglobin

ABS - able bodied subjects

SCI - spinal cord injured

CHAPTER I

Introduction

The energy demands of the muscle increase immediately at the start of exercise and oxidative phosphorylation is the most sustainable method of supplying the energy needed to continue exercise. Blood flow to the exercising muscle must increase rapidly to perfuse the contracting muscle with oxygen rich arterial blood. At the onset of exercise in humans blood flow in exercising muscle can increase from about 2-3 mL/100g/min to over 300 mL/100g/min and oxygen uptake ($\dot{V}O_2$) can increase from about 350 mL/min to over 5000 mL/min, when exercise is performed by a small muscle mass (Rowell, 1988). In maximal whole body exercise performed by an adult of average fitness, typical peak muscle blood flow is estimated at about 70 mL/100g/min (Rowell, 1988), therefore the control systems for blood flow and $\dot{V}O_2$ must adapt to the demands and requirements of various tissues, both non-working and working, at the onset of exercise. Examination of non steady-state situations, such as the onset of exercise, provide an opportunity to increase our understanding of regulation of these dynamic control mechanisms.

While energy demand at the working muscle increases immediately following step changes in work rate, $\dot{V}O_2$ does not respond as rapidly (Krogh and Lindhard, 1913). Two opposing viewpoints exist for characterizing limitations to, and regulation of, oxidative metabolism at the onset of exercise. The first proposes that the rate at which oxygen is delivered to the working muscle is the primary process limiting the rate of increase in muscle $\dot{V}O_2$ at the onset of exercise (Linnarsson, 1974; Wagner, 1991). According to this theory, the limitation to $\dot{V}O_2$ at the onset of exercise can be attributed to several factors, ranging from the uptake of oxygen at the lung to the

diffusion of oxygen into the mitochondria of the working muscle (Hughson and Morrissey, 1983; Wagner, 1991). The second viewpoint states that the limitation to oxidative metabolism is intrinsic to skeletal muscle metabolism, and is due to the inertia of the enzymatic regulation of oxygen utilization (Barstow *et al.*, 1994; Casaburi *et al.*, 1977; Hill *et al.*, 1924; Mahler, 1985; Pendergast *et al.*, 1980).

Some controversy regarding this topic arises from comparisons of rate limiting factors at different work rates and with different types of exercise tests. Throughout this thesis, the emphasis will be on studies of exercising humans, with responses of animal models added as appropriate. The primary focus will be on exercise of moderate to heavy intensities. It is over this range of exercise intensities that rate limiting factors might change as different demands are placed on the cardiovascular and metabolic systems. Considerable effort has gone into the examination of the proposed regulatory mechanisms.

Findings which support an oxygen transport limitation at the onset of exercise include those which show faster $\dot{V}O_2$ kinetics at the onset of exercise with hyperoxic gas breathing (Linnarsson, 1974; MacDonald *et al.*, 1997; Pedersen, 1987), in rest to work transitions (Hughson and Morrissey, 1983), in supine exercise with lower body negative pressure (Hughson *et al.*, 1993a) and with prior high intensity exercise (Gausche *et al.*, 1989; Gerbino *et al.*, 1996; Pedersen, 1987). The response of $\dot{V}O_2$ in these situations, in which a wide range of work rates was examined, suggests that alterations in blood perfusion of the exercising muscle and oxygen delivery at the onset of exercise have significant metabolic consequences.

Studies which support the concept of an O_2 utilization limitation have shown faster $\dot{V}O_2$

kinetics with long term training (Boning *et al.*, 1991; Cerretelli *et al.*, 1979; Hagberg *et al.*, 1980; Hickson *et al.*, 1978; Zhang *et al.*, 1991) and studies which have estimated the rate of acceleration of biochemical processes to be similar to the observed rate of increase in $\dot{V}O_2$ at the onset of exercise (Barstow *et al.*, 1994; Mahler, 1985; McCully *et al.*, 1994; Whipp and Ward, 1990; Yoshida and Watari, 1993). These findings suggest that $\dot{V}O_2$ at the onset of exercise is limited by the inertia of the proposed biochemical regulators of oxidative phosphorylation.

Very little information is available on the time course of increases in blood flow at the onset of exercise. Most previous studies of regulation of oxidative metabolism have examined situations in which O_2 delivery was in excess (Erecinska and Wilson, 1982; Mahler, 1985) or have inferred the response of oxygen delivery from measurements of heart rate and cardiac output (Pendergast *et al.*, 1983; Pendergast *et al.*, 1980). Few methods have been available to accurately determine blood flow at the onset of exercise. Without measures of blood flow to the working muscle it is difficult to quantitatively determine the actual changes in oxygen delivery at the level of the working muscle.

The studies described in this thesis focus on the measurement of oxygen delivery and utilization at the onset of exercise. They were designed to explore regulation of $\dot{V}O_2$ at the onset of exercise. The measurement of blood flow and $\dot{V}O_2$ and the response of these variables at the onset of exercise will be discussed.

Oxygen Uptake at exercise onset

Measurement of Oxygen Uptake Dynamics

Krogh and Lindhard (1913) first described two phases of $\dot{V}O_2$ at the onset of exercise in

1913. They observed an initial rapid increase followed by more gradual increases to a steady-state.

Research following the work of Krogh and Lindhard focussed on the recovery from exercise (Henry, 1951; Hill *et al.*, 1924) . However, DeMoor (1954), Henry and DeMoor (1955) and Margaria (1963) determined that the increase in O_2 uptake at the onset of exercise followed an exponential pattern and the rate of increase was directly proportional to the intensity of exercise. All early research on whole body $\dot{V}O_2$ was conducted using Douglas bag and mixing box techniques. More detailed examinations of $\dot{V}O_2$ kinetic responses at the onset of exercise were made possible with the development of breath-by-breath technology (Auchincloss *et al.*, 1966). Refinements in this technology made the calculation of alveolar $\dot{V}O_2$ possible (Beaver *et al.*, 1981) and permitted measures of the precise time course of gas exchange at the onset of exercise. Whipp and Wasserman (1972) determined that the kinetics of $\dot{V}O_2$ at the onset of exercise were independent of work rate within an intensity domain. The upper limit of this intensity domain was defined in relation to the ventilatory threshold (T_{VENT}). T_{VENT}[1] is the point in a ramp exercise test where there is an increase in the slope of the relationship between $\dot{V}CO_2$ and $\dot{V}O_2$ (Beaver *et al.*, 1986). Linarsson (1974) performed extensive investigations of $\dot{V}O_2$ kinetics at relative and absolute workloads and during different gas breathing conditions and was the first to characterize the results using an exponential model which contained a time delay. The exact nature

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[1] Some investigators continue to call this point the anaerobic threshold as originally described by Wasserman *et al.* (1973). There is no direct evidence to support the notion that the muscle cell is hypoxic at this point. Consequently, in this thesis this point will be referred to as T_{VENT}.

of the exponential model used to describe the response of $\dot{V}O_2$ at the onset of exercise is variable and depends on the exercise protocol and intensity. It is difficult, and often inaccurate, to ascribe physiologic significance to the various model components.

Control of Oxygen Uptake at Exercise Onset

The response of $\dot{V}O_2$ at the onset of moderate intensity exercise, to below T_{VENT}, has been described to consist of three phases of response. The first phase involves an initial increase in $\dot{V}O_2$ at the onset of exercise and lasts for 10 -15 seconds. This initial increase has been attributed to increased blood flow through the lungs as well as a small increase in arteriovenous O_2 content difference (Casaburi, 1992; Cochrane and Hughson, 1992). From 15 seconds to approximately two minutes into exercise there is a further increase in $\dot{V}O_2$ due to continued increases in pulmonary blood flow as well as increases in arteriovenous O_2 content difference (a-v DO_2) at the level of the exercising muscle. These increases are followed by a plateau in $\dot{V}O_2$ for lower intensity exercise and a further drift in $\dot{V}O_2$ for exercise above T_{VENT} (Poole *et al.*, 1994).

Control of $\dot{V}O_2$ at the onset of exercise may vary depending on the exercise situation so that either O_2 transport or O_2 utilization is limiting the rate of increase in $\dot{V}O_2$ in a certain situation (Cochrane and Hughson, 1992; Hughson, 1990). Some of the factors which may determine the predominant limiting factor include intensity of exercise and muscle mass used. Above T_{VENT}, the response is further complicated by the appearance of a slow component which results in a delay or prevention of the achievement of a steady state O_2 uptake (Barstow, 1994; Camus *et al.*, 1988; Poole, 1994; Poole *et al.*, 1994; Whipp, 1994).

Supporting evidence for the theory that $\dot{V}O_2$ kinetics are limited by the acceleration of O_2

utilization have come from several experiments (Barstow *et al.*, 1994; Casaburi *et al.*, 1977; Mahler, 1985; Pendergast *et al.*, 1980). Mahler (1985) observed that the responses of creatine and $\dot{V}O_2$ followed similar time courses at the onset of electrically stimulated exercise in frog sartorius muscle. However, the exercising muscle was supplied with excess O_2 in this experimental model, thereby eliminating possible O_2 supply limitations. It is not clear if these findings can be applied to exercise in humans. Nuclear magnetic resonance (NMR) studies in which small muscles masses were exercised within the confines of the magnet have also shown that the time course for changes in potential controllers for oxygen utilization followed a similar time course to $\dot{V}O_2$ at the onset of exercise (Barstow *et al.*, 1994; McCully *et al.*, 1994; Yoshida and Watari, 1993). As well, several studies have shown that changes in blood flow occur prior to changes in $\dot{V}O_2$ at exercise onset (MacDonald *et al.*, 1997; Pendergast *et al.*, 1983; Pendergast *et al.*, 1980; Pendergast *et al.*, 1980; Shoemaker *et al.*, 1994). Although this would seem to indicate that oxygen supply is not limiting the response of $\dot{V}O_2$, the role of blood flow distribution within the exercising muscle is not known and there may exist several situations in which oxygen transport to the exercising muscle is contributing to the regulation of $\dot{V}O_2$ at the onset of exercise.

A decrease in the response rate of $\dot{V}O_2$ at the onset of exercise during several different experimental manipulations suggested that when oxygen supply to the working muscle was limited during the rest to exercise transition, the rate of $\dot{V}O_2$ increase was also reduced (Gausche *et al.*, 1989; Gerbino *et al.*, 1996; Hughson *et al.*, 1993; Hughson and Morrissey, 1983; Linnarsson, 1974; MacDonald *et al.*, 1997; Pedersen, 1987). As well, short term endurance training resulted

in faster $\dot{V}O_2$ kinetics prior to observed changes in oxidative potential of the muscle (Phillips *et al.*, 1995). This later observation provides counter evidence to that proposed to support an O_2 utilization limitation (Boning *et al.*, 1991; Cerretelli *et al.*, 1979; Hagberg *et al.*, 1980; Hickson *et al.*, 1978; Zhang *et al.*, 1991).

Several regulators and mechanisms have been suggested as key factors in the control of mitochondrial oxidative phosphorylation. A classical view of the regulation of oxidative phosphorylation had been based on studies of isolated mitochondria (Erecinska and Wilson, 1982). In these considerations of respiratory regulation it is assumed that the concentration of oxygen is high and constant. O_2 has not been included as a limiting factor in oxidative phosphorylation due to the low PO_2 (0.03 Torr) necessary to observe altered rates of metabolism in isolated mitochondria (Erecinska and Wilson, 1982). However, recently it has been observed that the affinity of cytochrome c, the terminal electron acceptor in oxidative phosphorylation, for O_2 varies with the energy state of the cell (Wilson and Rumsey, 1988). Changes in intracellular PO_2 could impact on the intracellular concentrations of metabolic substrates required to obtain a given ATP production rate. Intracellular PO_2 in higher, and more physiological ranges (10 Torr), may result in obligatory increases in the concentration of substrates required for ATP production according to the equation (Hogan *et al.*, 1992; Jones, 1986).

$$H^+ + NADH + 3Pi + 3ADP + \tfrac{1}{2} O_2 \rightarrow NAD^+ + 3ATP + H_2O$$

Studies involving hindlimb perfusion of dog working muscle showed a greater change in several of the proposed regulators of tissue respiration to achieve a given $\dot{V}O_2$ as PaO_2 decreased (Hogan *et al.*, 1992b). Experiments on isolated muscle (Wilson and Stainsby, 1978) and whole

body exercise (Katz and Salhin, 1988) resulted in similar conclusions. These observations suggest a mechanism for O_2 transport limitations at the onset of exercise when decreases in intracellular PO_2 might modulate the rate of increase in muscle $\dot{V}O_2$. Given this argument it is understandable that the intracellular PO_2 does not have to reach the previously suggested low values (Erecinska and Wilson, 1982) to limit the rate of oxidative phosphorylation. At the onset of exercise, a relative hypoxia might cause a temporary slowing of muscle $\dot{V}O_2$ while the intracellular environment adapts to this PO_2.

Due to the complexity of the O_2 delivery process, O_2 delivery to tissues is difficult to quantitatively evaluate. It is not known if O_2 delivery is sufficient for maximal respiration during all exercise conditions. In work rate transitions, at the onset of exercise, O_2 concentration at the mitochondria may not be constant (Arthur *et al.*, 1992; Hogan *et al.*, 1992). It is, however, difficult to measure or calculate O_2 concentration at the mitochondria during exercise transitions. Blood flow is one component of O_2 delivery that is altered at the onset of exercise and may be an important control mechanism for oxidative phosphorylation (Hogan *et al.*, 1992b).

Blood Flow at exercise onset

Measurement of Blood Flow

Blood flow in arteries is pulsatile in nature and blood vessels are relatively inaccessible for study in humans. Many methods have been utilized for measurement of blood flow to exercising muscle. Gaskell (1877) as cited in Anrep and von Saafeld (1935), determined that there were large increases in flow from muscle veins in dogs after the release of stimulated sustained contractions. This hyperemia was explained by peripheral vasodilation caused by local metabolites

produced during the contractions. As well it was observed that venous outflow from a muscle increased at the onset of a sustained contraction, followed by a decrease and sometimes cessation in outflow until the end of the contraction. The cause of the initial increase in venous outflow from the muscle was speculated to be due to compression of blood vessels in the muscle.

The first to produce estimates of blood flow during rhythmic contractions were Chaveau and Kaufmann (1887), as cited in Anrep *et al.* (1934). They studied blood flow in horse lips during chewing exercise by collecting venous outflow and observed that there was a large variability in blood flow to exercising muscle during rhythmic contractions. The methods used were incapable of providing quantitative estimates of the actual exercising muscle blood flows.

Anrep and von Saafeld, recognized that both arterial inflow and venous outflow measures were necessary to quantitatively determine blood flow through the muscle during contractions (Anrep and von Sallfeld, 1935). They used two hot wire anemometers to simultaneously record both inflow and outflow of blood in contracting dog gastrocnemius muscle and also noted that blood flow to exercising muscle was low during contraction and high during relaxation. They concluded that the average blood flow through muscle was increased due to rhythmic contraction. The magnitude of flow increases appeared to be dependent upon the strength and duration of the contraction and the contraction/relaxation duty cycle. The effect of a series of contractions was a summation in blood flow increases between contractions. One suggestion from these studies was that a vasodilator substance was released during contractions and could be detected in the venous effluent of the contracting muscle.

In 1949, Barcroft and Dornhorst attempted to measure the variations in blood flow during

rhythmic leg contractions in humans (Barcroft and Dornhorst, 1949). They used an air filled plethysmograph and venous occlusion cuffs along with manual femoral artery compression but were unable to achieve sufficient time resolution with their methodology to measure the rapid fluctuations in blood flow that they suspected occurred during exercise. However, they were able to confirm that contraction resulted in mechanical resistance to blood flow through muscle.

Since the time of these early experiments several methods have been employed in an effort to measure muscle blood flow during rhythmic exercise. Electromagnetic flowmeters were introduced in 1936 (Kolin, 1936) and first used on animals and humans in 1955 (Denison *et al.*, 1955). The electromagnet was wrapped around an exposed vessel. Blood flow through the vessel resulted in voltage changes, that were proportional to the flow, due to the electrical conductivity of blood. The obvious disadvantage of this technique is that the vessel being examined must be exposed and therefore, early experiments on humans were restricted to patients undergoing surgery (Gault *et al.*, 1966; Hall, 1997). This technique, however, did have a time resolution sufficient to follow the rapid blood flow fluctuations which occur during exercise. In spite of its limitations, electromagnetic flowmeters were used in studies in isolated cat muscle to confirm the earlier suggestion of rhythmic flow variations (Folkow *et al.*, 1970).

The ^{133}Xe clearance method has also been used to determine blood flow to exercising muscle. This method requires the injection of a radioactive label. The distribution of the label can be assessed before and after exercise as an indication of flow though the exercising muscle. It was first used by Tonnessen (1964) and provided measures of average flows at rest and exercise. Pendergast *et al.* (1980) studied ^{133}Xe clearance from exercising human muscle. They concluded

that changes in blood flow to exercising muscle precede changes in $\dot{V}O_2$. For quantitative measures of flow, however, the ^{133}Xe clearance technique seems to produce artificially low values for muscle blood flow due to tracer solubility and diffusion limitations (Cerretelli *et al.*, 1984). Muscle blood flow has also been measured in exercising animals with radiolabelled microspheres (Laughlin, 1987; Laughlin and Armstrong, 1983; Laughlin and Armstrong, 1982). Piiper *et al.*(1985), using labelled microspheres, determined that there is considerable blood flow in homogeneity in exercising muscle. This variability in blood distribution may have important implications in O_2 delivery to muscle that are not possible to determine from bulk flow measures.

Venous occlusion strain gauge plethysmography has been used to measure blood flow to exercising muscle (Formel and Doyle, 1957; Humphreys and Lind, 1963). Outflow from the examined vascular region is blocked by inflation of a cuff around the limb to sub-diastolic pressure levels, while the inflow is assumed to be unimpeded. The rate of volume increase of the limb is directly proportional to arterial blood inflow (Joyner *et al.*, 1990; Tschakovsky *et al.*, 1995). The restrictions in the application of this method include positioning of the limb above heart level. Motion artifacts make measurements during exercise unreliable and measures are traditionally taken during brief cessations in contraction or at the end of exercise. Tschakovsky *et al.* (1995) performed comparisons of venous occlusion strain gauge plethysmography versus Doppler and found that the former was only accurate for the first few beats after cuff occlusion.

Quantitative determination of muscle blood flow during exercise may be obtained through the use of thermodilution methods. This method was first used for exercising humans in 1964 (Ganz *et al.*, 1964) and requires the infusion of ice cold saline into the blood vessel and

measurement of the temperature of the blood several centimetres away. Based on the knowledge of the rate and temperature of saline infusion and the temperature of the blood downstream to the infusion, average blood flow can be calculated. There are several potential sources of error including inadequate mixing, inaccurate infusion rates and temperatures, mixing of flow from non-exercising muscle and flow fluctuations (Andersen and Saltin, 1985). This technique was originally used only for steady state flow situations (Andersen and Saltin, 1985; Poole *et al.*, 1992; Richardson *et al.*, 1995; Richardson *et al.*, 1993; Rowell *et al.*, 1986) but has recently been adapted for use in the non steady state transition at the onset of exercise (Grassi *et al.*, 1996). While thermodilution measures of blood flow have proven to be reliable and reproducible it has the disadvantage of being fairly invasive and is unable to describe the fluctuations in flow that occur with rhythmic exercise.

Doppler Ultrasound

In recent years Doppler ultrasound technology has been used to measure blood flow to exercising muscle (Hughson *et al.*, 1996; Radegran, 1997; Shoemaker *et al.*, 1994; Toska and Eriksen, 1994; Tschakovsky *et al.*, 1996; Tschakovsky *et al.*, 1995; Walloe and Wesche, 1988; Wesche, 1986). This method has the considerable advantage of being completely noninvasive and of sufficient time resolution for describing the fluctuations in muscle blood flow which occur during rhythmic exercise.

Satamura and Kaneko (1960) were the first to introduce Doppler for the measurement of blood flow of exposed vessels. Rushmer *et al.* (1966) advanced the technique to the point where blood flow could be measured in underlying vessels from the skin surface. The signal to noise ratio

was poor, however, and the technique required further development before it could be used for quantitative blood flow measures. Thoresen and Walloe (1980) were the first to apply the measures to skin blood flow. Doppler ultrasound was first used for measuring blood flow to exercising muscle during relatively small muscle mass contractions (Toska and Eriksen, 1994; Wesche, 1986). Shoemaker *et al.* (1996) used Doppler ultrasound to examine arterial blood flow to the legs at the onset of knee extension exercise and found that the blood flow responses at the onset of larger muscle mass exercise could be affected by short term training.

Using Doppler ultrasound technology, volumetric blood flow measures are derived from the product of conduit vessel area and blood velocity, averaged over the entire lumen of the vessel (Gill, 1985). In several early studies, only the blood velocity was measured and it was assumed that there was no change in vessel cross-sectional area during exercise (Shoemaker *et al.*, 1994; Toska and Eriksen, 1994; Tschakovsky *et al.*, 1996; Walloe and Wesche, 1988; Wesche, 1986). Development of Doppler technology now permits simultaneous determination of diameter and velocity during exercise.

Control of Blood Flow

At the level of the femoral artery the resting blood flow response has three characteristic phases with each heart cycle. During systole, there is a rapid increase in blood velocity, which peaks at peak systole, followed by a decrease in flow and negative or reverse flow, due to peripheral arteriolar resistance and arterial recoil. As the aortic valve closes the reverse flow is stopped and in late diastole there is a period of low forward or no flow as the blood passes out of the arteries and into the venous circulation.



Figure 1.1: *An example of the resting flow response through the femoral artery (top, left) and an image of the femoral artery (top right) obtained with pulsed and echo Doppler. The ECG tracing is shown (bottom left) to demonstrate the changes in flow with cardiac cycle.*

At the onset of exercise, during a contraction, the flow response depends upon the point during the cardiac cycle in which the contraction occurs, however the general response is one of reduced flow or reversed flow (Walloe and Wesche, 1988) during the contraction followed by an increase in flow during the relaxation phase. During exercise there are large fluctuations in the flow pattern with high flow during relaxation and low flow during contraction. The velocity pattern depends on the location of the artery in the vascular tree, and the tension, duration and timing of the contraction and relaxation during the cardiac cycle. As exercise continues, flow continues to increase to a steady state in an exponential fashion (Shoemaker *et al.*, 1994; Wesche, 1986).

Flow occurs between two points in a system due to differences in potential energy. In blood vessels, differences in energy are usually represented as differences in pressure. An adaptation of Ohm's law relates flow through a vessel to the change in pressure and the resistance to flow in the vessel.

$$Q = \Delta P \cdot 1/R$$

Q = blood flow through the vessel

ΔP = the pressure gradient between 2 points in the vascular bed

R = the resistance of the vasculature = 1/C, the conductance of the vessel

It has been shown that there is an immediate increase in blood flow to working muscle upon release of the initial contraction at the start of exercise (Honig, 1979; Shoemaker *et al.*, 1994; Tschakovsky *et al.*, 1996; Wesche, 1986). It has yet to be determined what causes this increase in blood flow.

There are many possible regulatory factors for blood flow at the onset of muscular contractions. To get an immediate increase in flow in conduit arteries there must be an immediate increase in the pressure gradient or conductance downstream. The possible regulating factors include changes in the pressure gradient across the exercising muscle, and changes in conductance due to neural influences, release of metabolites from the exercising muscle, or endothelial control (Shoemaker *et al.*, 1996).

A contributor to the increase in blood flow at the onset of exercise is thought to be the pumping action of rhythmically contracting muscle, known as the muscle pump effect (Laughlin, 1987; Leyk *et al.*, 1994; Sheriff *et al.*, 1993). Several authors have recently concluded that the muscle pump contributes to the rapid increase in flow to the muscle of exercising humans, but it does not account for all of the exercise hyperemia observed (Leyk *et al.*, 1996; Radegran, 1997; Toska and Eriksen, 1994; Tschakovsky *et al.*, 1995). Rapid vasodilation must occur at the onset of exercise. What causes this rapid vasodilation remains a critical area of research even 60 years after Anrep postulated that there must be some substance responsible for the rapid increase in flow they observed at the onset of exercise (Anrep and von Sallfeld, 1935).

Due to the rapid nature of the vasodilatory response, metabolites released from the exercising skeletal muscle are not believed to be important in the early blood flow response (Laughlin and Armstrong, 1983). They do, however, play a role in matching blood flow and metabolic demand during exercise after the initial rapid response. Possible metabolites from the exercising muscle include carbon dioxide, H^+, potassium, phosphate, lactate, O_2, adenosine, and adenosine nucleotides (Laughlin and Armstrong, 1983).

The time course of release of endothelial factors from the smooth muscle of the blood vessels in the active muscle is more compatible with a possible regulatory role in the early response of blood flow. Possible endothelial factors include nitric oxide (Ignarro *et al.*, 1987), prostaglandins (Koller *et al.*, 1993), and acetylcholine (Honig, 1979).

Withdrawal of sympathetic nervous system activity to the active tissue at the onset of exercise could rapidly increase perfusion. Research involving neurographic recording of the sympathetic nervous activity in muscle during exercise do not support this mechanism (Mitchell, 1990; Victor *et al.*, 1987), but further studies are necessary to fully determine the role of the nervous system in blood flow regulation at exercise onset. Indeed it has been observed that an early fall in arterial blood pressure at the onset of exercise is countered, presumably, by sympathetic vasoconstriction (Toska and Eriksen, 1994).

Coordination of the control of blood flow at the initiation of exercise is an area of ongoing study. It is not the aim of this thesis to examine the factors regulating blood flow, but to determine the time course of the blood flow response at the onset of large muscle mass exercise and the relationship of this response to the dynamics of $\dot{V}O_2$.

Aim of Studies

The central theme of the following studies is the examination of control mechanisms regulating oxygen utilization as the cardiovascular system adapts to meet the metabolic demands of exercising muscle at the onset of exercise. Specifically most of the studies have been designed to examine the control of blood flow during large muscle mass exercise in humans. The experimental objectives are to manipulate and quantify blood flow responses at the onset of knee extension exercise and infer relationships to cardiovascular control as it pertains to oxygen delivery.

The main hypothesis of these studies is that alterations in skeletal muscle blood flow at the onset of large muscle mass exercise will be associated with alterations in the rate of increase in oxidative phosphorylation at the onset of exercise.

Specific research questions:

1. To determine the relationship between the rate of increase in blood flow and the rate of increase of oxygen uptake at the onset of large muscle mass exercise through alterations in the rate of increase of limb blood flow at the onset of leg exercise. Can the blood flow response at the onset of exercise be manipulated by changing the perfusion pressure of the exercising vascular bed, changing the training status, changing the resting vascular conductance of active muscle with prior exercise, and changing the oxygen content of the arterial blood? Do these manipulations impact on the oxygen uptake kinetics at the onset of exercise? (Chapters II, III, IV)

2. To directly quantify the relationship between muscle oxygen uptake, leg blood flow and alveolar oxygen uptake at the onset of large muscle mass exercise using Doppler methodology for leg blood flow measures (Chapter IV).

3. To determine the ability of non-invasive reflectance near infrared spectroscopy to assess muscle O_2 availability at the onset of large muscle mass exercise through correlations with venous effluent O_2 saturation at the onset of large muscle mass exercise (Chapter V).

4. To determine the time course of blood flow redistribution from inactive skeletal muscle and non-active tissue at the onset of exercise. Can these measures be made at the onset of exercise? What role does redistribution of blood from inactive and non-active tissue play in the regulation of blood flow to active skeletal muscle at the onset of exercise? (Appendix 1 and 2).

This thesis contains four papers and two appendices originally written as separate manuscripts and presented as chapters. Below is a brief description of the contents of these chapters:

Summary of Papers

Paper I (Chapter II) Alveolar oxygen uptake and femoral artery blood flow dynamics in upright and supine leg exercise in humans

Transitions from rest to 40W leg kicking exercise were performed in both the upright and supine positions. In the supine, compared to the upright position, the mean arterial pressure assisting perfusion of the exercising leg was reduced. It was found that, at the onset of exercise, the reduction in mean perfusion pressure resulted in slowed blood flow and oxygen uptake kinetics. The blood flow kinetics were, however, faster than the oxygen uptake kinetics at all time points indicating that blood flow distribution, and leg oxygen extraction, in the exercising legs may play a role in oxygen transport regulation of oxidative phosphorylation.

Paper II (Chapter III) Effect of 16 Hours of intermittent heavy exercise on oxygen uptake and blood flow responses to submaximal leg exercise

The dynamic and steady-state responses of blood flow and oxygen uptake at the onset of leg kicking exercise were examined before and after 16 hours of intermittent high intensity exercise. It was found that this single day of intermittent exercise had no effect on the kinetic or steady state response of blood flow or oxygen uptake for transitions from 0W to 52 W kicking exercise. On the training day the effect of prior high intensity exercise, on blood flow responses, at the onset of subsequent kicking exercise transitions to below T_{VENT} was examined. It was found that prior high intensity exercise resulted in elevations of steady state blood flow both at 0W and after five minutes of 52 W kicking but did not affect the dynamic response of blood flow at exercise onset. The effect was similar after the first, eighth and sixteenth exercise bouts. These observations indicate that a single day of intermittent exercise has no lasting effects on the vascular conductance in the exercising muscle during steady state exercise or the response at the onset of exercise, while prior high intensity exercise increases steady state exercise vascular conductance and may explain previous observations of improved $\dot{V}O_2$ kinetics with prior high intensity exercise.

Paper III (Chapter IV) Effect of hyperoxia and hypoxia on oxygen uptake and leg blood flow responses to submaximal exercise

The effect of hyperoxia (F_IO2= 0.70) and hypoxia (F_IO_2= 0.14) on the dynamic response of alveolar and muscle oxygen uptake and leg blood flow and were examined at the onset of transitions in leg kicking exercise from rest to 50W. It was found that the oxygen delivery to the

exercising legs ($\dot{Q} \cdot CaO_2$) was not increased in hyperoxia compared to normoxia at rest or at any time point during exercise, due to adjustments in arterial oxygen content (CaO_2) and blood flow. There was also no change in the dynamic response of oxygen uptake with hyperoxic gas breathing. During hypoxic gas breathing, there were also adjustments in CaO_2 and blood flow which resulted in no change in oxygen delivery to the exercising legs. The dynamic response of oxygen uptake was similarly unaffected by the gas breathing. This study demonstrated the capacity of the cardiovascular system to regulate oxygen delivery to exercising muscle in kicking exercise below T_{VENT}.

<u>Paper IV (Chapter V)</u> Comparison of femoral blood gases and muscle near infrared spectroscopy at the onset of exercise in humans

The use of a near infrared spectroscopy (NIRS) for determination of muscle Hb/Mb O_2 saturation at the onset of exercise was evaluated through comparison to femoral venous blood oxygen saturation. Simultaneous measures were obtained both from the NIRS unit and blood gases at the onset of kicking exercise transitions from rest to 50 W in normoxia, hyperoxia and hypoxia. In all gas breathing conditions, both the NIRS and the blood gas values decreased at the onset of exercise. After one minute of exercise in normoxia and hyperoxia, the NIRS measures of oxygen saturation began to increase again while the femoral blood gas values continued to decrease. In hypoxia, the NIRS measures responded similarly to the blood gas values throughout the 6 minutes of exercise. These findings indicate that either the NIRS unit does not provide accurate estimations of muscle oxygen saturation at the onset of exercise or that the source of the

muscle oxygenation signal detected by the NIRS unit responds in a different manner than femoral venous oxygen saturation at the onset of exercise.

Appendices 1 and 2 Blood flow redistribution from inactive skeletal muscle and non-active tissue at the onset of exercise

It was determined that resting portal vein blood flow could be measured reproducibly with Doppler methodology (Appendix 1). It was intended that this technique would be further developed for the determination of the role of blood flow redistribution from the splanchnic region to the exercising muscle at the onset of exercise. However, there is significant respiratory interference with the Doppler signal during exercise. Further developments in the technique are necessary to continuously make these measures with validity at the onset of exercise

In a preliminary study (Appendix 2), the role of sympathetic nervous system innervation in the regulation of blood flow at the onset of exercise was examined in both able bodied (ABS) and spinal cord injured individuals (SCI). Single leg knee extension exercise was performed and blood flow to the inactive leg was measured. It was observed that in ABS but not in SCI, there was an increase in vascular conductance at the onset of exercise which resulted in transient increases in flow to inactive skeletal muscle. Due to the study design it was not clear if these decreases in resistance in ABS were due to transient reductions in sympathetic tone at the onset of exercise not possible in the SCI who lacked sympathetic as well as motor innervation to their legs.

Methodology

The papers in this thesis contain methods for providing information about several cardiovascular variables at the onset of exercise. Examination of the kinetic response of the cardiovascular system requires that these methods continuously provide accurate and valid information during the transition from rest to exercise. The techniques of measuring and analysing oxygen uptake, blood flow, blood pressure and blood gases at the onset of exercise have been used previously in our laboratory and will be described and discussed in this chapter. In addition, the technique of reflectance near infrared spectroscopy, for determination of muscle Hb/Mb O_2 saturation, which is new to our lab, will be described in detail.

Oxygen Uptake

Oxygen uptake was measured on a breath-by-breath gas exchange system (First Breath, St. Agatha, Ontario, Canada). This system consisted of either a digital volume turbine (VMM - 110, Alpha Technologies, Laguna Beach, CA) or an ultrasonic flow meter (UF 202, Kou and Assoc., Redmond, WA) for gas volume measurement, and a mass spectrometer (MGA-1100, Marquette Electronics Inc., Milwaukee, WI) for gas fraction measurement. Correction was made for lung gas stores by the nitrogen balance methods described by Beaver et al. (1981). This correction allowed for the calculation of alveolar oxygen uptake. The volume measurement system was calibrated prior to each test by pumping a gas through a known volume syringe at flow rates comparable to those observed during the exercise tests. The mass spectrometer was calibrated with known gases which spanned the gas concentrations observed during testing. The volume and flow were measured with no delay, however, the gas fractions were measured with delay due to

the transport time and mass spectrometer response time. A calibration procedure was performed to determine the delay or lag time.

The calculation of T_{VENT} was determined by a computer program which utilized the the V slope method (First Breath, St. Agatha, Ontario, Canada). This method uses a moving average of $\dot{V}CO_2$ plotted versus $\dot{V}O_2$ and the point of deflection is detected (Beaver *et al.*, 1986). T_{VENT} was also determined from plots of ventilatory equivalents for $\dot{V}O_2$ ($\dot{V}E/\dot{V}O_2$) and $\dot{V}CO_2$ ($\dot{V}E/\dot{V}CO_2$) as the point where $\dot{V}E/\dot{V}O_2$ began to increase while $\dot{V}E/\dot{V}CO_2$) was constant.

The analysis of the $\dot{V}O_2$ data involved linear interpolation of the calculated $\dot{V}O_2$ between the breaths to give values for $\dot{V}O_2$ at each second. The identical exercise test repetitions were then ensemble averaged to produce one data set per individual in each test condition. This averaging was used to minimize normal breath-by-breath variation and to make the basic response pattern stand out.

Blood Flow

The Doppler principle was first described by a Austrian physicist Christian Doppler (1803-1858) in a paper for the Royal Bohemian Society of Learning in 1842. The principle states that when an observer is moving relative to a wave source, the frequency measured is different from the emitted frequency.

Doppler ultrasound techniques make it possible to follow the time course of changes in blood flow at the onset of exercise. The Doppler ultrasound technique is based on the principle that stationary objects will reflect sound back at the same frequency as the transmitted sound while the sound reflected back from moving particles will be shifted in frequency. The magnitude of this

frequency shift will be proportional to the velocity of the moving particle according to the equation:

$$V = f_D \cdot c \cdot 2 \cdot f_t \cos(q)$$

v = velocity of the particles in cm./sec

f_D = shift frequency

f_t = transmitted frequency

q = angle of insonation

c = velocity of sound in tissue (blood) cm/sec

The sound reflected by the tissue/blood is in the auditory range and can be monitored continuously. The major advantage to the use of Doppler ultrasound techniques is that they are a non-invasive measure of blood flow on a beat to beat basis (Gill, 1985). Doppler ultrasound techniques have been used to examine blood flow in many vessels in the body and more recently to examine blood flow delivery to working muscle at the onset of exercise (Hughson *et al.*, 1996; Shoemaker *et al.*, 1994; Shoemaker *et al.*, 1996; Toska and Eriksen, 1994; Walloe and Wesche, 1988; Wesche, 1986).

To distinguish direction the method employed in the following experiments was quadrature phase detection. The received signal was mixed with two reference signals which were separated in phase by a 90° phase shift. This results in two audio frequency signals which both contain the Doppler information but are shifted by 90° positive or negative depending on the direction of flow. This feature is very important because in peripheral arteries flow may reverse directions once or more during each cardiac cycle.

In each experimental situation, blood flow at the onset of exercise was measured

continuously with a flat 4.0 MHz probe of a pulsed Doppler system (model 500V, Multigon Industries, Mt. Vernon NY) manually held on the skin surface approximately two cm distal to the inguinal ligament. The width of the ultrasound beam was set to insonate the whole artery and the positioning of the probe was manipulated prior to any tests and throughout testing to obtain good quality Doppler signals. These signals were continuously evaluated by the experimenter through both visual and auditory feedback. The spectra of frequency signals were processed by a mean velocity analyser which provided a generated average velocity signal weighted to the intensity of the signal at each frequency in the Doppler spectrum (Micco, 1989). This signal, which was taken to indicate the mean Doppler shift frequency at any given time point, was collected at 100 Hz on a dedicated computer system, along with simultaneous measures of heart rate and blood pressure. Prior to each test, calibration of the frequency signals was obtained through collection of a two point calibration from the mean velocity analyser. The mean velocity analyser produced appropriate frequency signals from an oscillator circuit to make 1 volt of output equal to the frequency which indicates 1 m/s blood velocity after application of the Doppler equation.

In each of the studies presented in this thesis Doppler signals from at least two identical exercise repetitions were obtained. Post collection, the signals were converted to average velocity over each heart beat through application of the Doppler equation to the area under each mean blood velocity curve. This calculation used a constant Doppler angle of 45° to the femoral artery which was the internal angle of the pulsed Doppler probe as specified by the geometry of the probe. After the beat-by-beat mean blood velocity signals were obtained they were averaged over the time required for each contraction and relaxation cycle. This procedure was used to

include a contraction and relaxation phase in each velocity value. The average mean blood velocities from each separate exercise test were then time aligned and ensemble averaged to produce one data set for MBV for each subject in each testing condition.

During each of the experiments the femoral artery diameter was also continuously monitored using an echo Doppler and a hand held 7.5 MHz probe (model SSH140A, Toshiba Inc., Tochigi-Ken, Japan). The image of the artery was collected on videotape for further analysis. For femoral artery measures, the probe was positioned two cm distal to the inguinal ligament on the opposite leg from the pulsed Doppler measures. The estimates of diameter of the femoral artery were obtained from the average of three measurements at each time point. All measurements were made during diastole using an ECG trigger to update each arterial image at the same point of the cardiac cycle. The calipers used to mark the diameter were set to move in 0.1 mm increments. It has been previously observed that the day to day reproducibility of these measures ranges from 2- 4% (Shoemaker *et al.*, 1997). A continuous estimate of the diameter was obtained by a line fit to the values measured at specific time points throughout the exercise test. From the average MBV data sets and the lines best fit to the diameter response the leg blood flow for each contraction relaxation cycle was estimated as $LBF = MBV \cdot \pi r^2$.

Exponential Fitting

In order to quantify the dynamic response of LBF and $\dot{V}O_2$ at the onset of exercise , the average responses in each testing condition were fit to curves using an exponential fitting model. The curve fitting procedure for all experiments involved the calculation of a modelled exponential output for test values of the various parameters by using the least-squares error approach. The

actual data sets were then compared to the modelled outputs and the procedure was repeated until further changes in the parameters for the modelled output did not result in reductions in the mean squared error between the two data sets. The actual exponential model used for fitting the LBF and $\dot{V}O_2$ varied depending on the response of each variable and the test protocol used, however, the method used was similar in all cases.

In most cases, responses were fit to a two component exponential model, however, occasionally, an improved fit was obtained by fitting the LBF and $\dot{V}O_2$ responses to a three-component model. The three component model contained an extra amplitude (G_3), time delay (TD_3) and time constant (τ_3) to fit the slower adaptive phase observed in some tests.

The two component model had a baseline component (G_0), two amplitude terms (G_1 and G_2), two time constants (τ_1 and τ_2) and two time delays (TD_1 and TD_2)

$$Y(t) = G_0 + G_1 (1 - e^{-(t - TD1)/\tau 1}) \cdot u1 + G_2 (1 - e^{-(t - TD2)/\tau 2}) \cdot u2$$

where,

$$u1 = 0 \text{ for } t < TD_1 \text{ and } u1 = 1 \text{ for } t \geq TD_1$$

$$u2 = 0 \text{ for } t < TD_2 \text{ and } u2 = 1 \text{ for } t \geq TD_2$$

Y(t) is the time dependent variation in $\dot{V}O_2$ or LBF.

An estimate of the rate of change of each of these variables for each subject and condition was obtained by calculation of the mean response time (MRT). The MRT is the weighted mean of the time constant and time delay for each exponential term.

$$MRT = (G_1/(G_1 + G_2)) \cdot (TD_1 + \tau_1) + (G_2/(G_1 + G_2)) \cdot (TD_2 + \tau_2)$$

Blood Pressure

Blood pressure was measured continuously with a plethysmographic device (Finapres, Ohmeda, USA). This instrument is based on the volume clamp method of Penaz (1973) and measures blood pressure in the finger. During all experiments the cuff of the Finapres was placed around the middle finger on the subject's left hand and the finger was held at the level of the femoral artery at the same level as the Doppler probe. It has been shown that changes in intra-arterial pressure are accurately reflected by finger blood pressure measurements (Friedman 1990).

The blood pressure waveform was collected at 100 Hz on a dedicated computer along with simultaneous measurement of heart rate. The area under the blood pressure curve was then averaged for each heart beat to give mean arterial pressure on a beat-by-beat basis.

Blood Gases

The following is a description of the arterial and venous sampling and the blood analysis methods used in this thesis. Details of the methods can be found in Chapter IV.

Arterial blood samples were obtained from the radial artery and venous blood samples were obtained from the femoral vein. Samples (1 mL) were collected anaerobically in heparinized syringes for both venous and arterial blood. These samples were immediately but gently agitated and stored in an ice bath. Within one hour of collection, all whole blood samples were analysed for PO_2, PCO_2 and haematocrit by selective electrodes in a blood gas-electrolyte analyser (NovaStat Profile Plus 9, Waltham,MA). The analyser was calibrated at regular intervals during the analyses. O_2 saturation was obtained from the output of the analysis system. O_2 content was defined as the total amount of O_2 contained in a given volume of whole blood including dissolved and bound O_2 according to the equation:

$$O_2 \text{ content} = 1.39\ [Hb] \cdot (O_2 \text{ saturation} / 100) + 0.0031\ PO_2$$

where [Hb] = hemoglobin concentration

PO_2 = partial pressure of oxygen dissolved in the whole blood sample

Arteriovenous O_2 difference (a-vDO_2) was calculated from the difference in radial artery O_2 content (CaO_2) and femoral venous O_2 content (CvO_2). This difference was then divided by arterial O_2 content to give leg O_2 extraction. Leg $\dot{V}O_2$ mus was calculated as the product of the arterial venous O_2 difference and leg blood flow for each time point of blood sampling. Leg O_2 delivery was calculated as the product of LBF and CaO_2. LBF was estimated at the times of the blood sample from application of the exponential fitting equations for LBF (see methodology: exponential fitting).

One source of uncertainty in a-vDO_2 estimates, and subsequent calculations was the measure of venous and arterial O_2 content. Timing of the samples was monitored and samples were quickly mixed, placed in and ice bath and measured within one hour of collection. Previous studies have shown no significant decay of blood PO_2 over six hours after collection of samples from subjects breathing hypoxic and normoxic gas (Roca *et al.*, 1989). Knight *et al.* (1993) found arterial PO_2 in blood samples obtained from subjects during hyperoxic gas breathing fell rapidly over time despite immediate icing. As a precaution, they collected two samples at each time point and analysed one immediately for use in correction for the decay in PO_2 over time. This was not possible in the current thesis due to equipment limitations, however, the estimates of CvO_2 and CaO_2 obtained in each gas breathing condition were reasonable and consistent. Another possible source of error in the calculation of a-vDO_2 was the assumption that arterial O_2 content

was constant at the onset of exercise. Due to technical limitations, arterial blood samples were not obtained during the first minute after the onset of exercise. It was determined that CaO_2 was not different throughout exercise period and subsequently, the average exercise CaO_2 for each subject, in each gas condition, was used in calculation of a-vDO_2 at 20 and 40 seconds after exercise onset.

Near Infrared Spectroscopy

Near infrared spectroscopy is a technique which can be used for optical monitoring of oxygen saturation in biological tissues. The technique of NIRS relies on the fact that biological tissue (skin and bone) is relatively transparent to near infrared light (700 -1000 nm) and muscle tissue contains chromophores whose light absorption properties vary with oxygenation. The Lambert-Beer Law (Beer 1851) can be applied to relate the incident and transmitted radiation to the extinction coefficient of a chromophore. Although, technically this law is intended for use in a clear, non-scattering medium, it can be applied to biological tissue with some modifications. The major modification is that a correction factor which accounts for the increase in optical pathlength due to scattering in the tissue must be included. The modified Lambert Beer law is (Delpy *et al.*, 1988)

$$OD_\lambda = \text{Log}\ (\ I_o/I) = \epsilon_\lambda \cdot c \cdot L \cdot B + OD_{R,\lambda}$$

Where OD_λ is the optical density of the medium

λ is the wavelength of light used

I_0 is the incident radiation

I is the transmitted radiation

ϵ_λ is the extinction coefficient of the medium

c is the concentration of the chromophore

L is the distance between light entry and light exit point

B is the pathlength correction factor

$OD_{R,\lambda}$ represents the light losses due to scattering

If the light losses due to scattering in the tissue are a constant then optical density can be related to concentration and changes in optical density can be related to changes in chromophore concentration. However, if there is more than one chromophore present in the solution then more wavelengths will need to be measured. At least as many wavelengths as chromophores will have to be used to determine the change in concentration of each chromophore. In human muscle tissue there are at least five known chromophores present: oxyhemoglobin (HbO_2), hemoglobin (Hb), oxymyoglobin (MbO_2), myoglobin (Mb) and the oxidized form of cytochrome aa_3 (Cyt ox.). Near infrared light is absorbed by the iron-porphyrin complexes of HbO_2, Hb, MbO_2, and Mb and by the oxidized copper atoms of Cyt ox.(Hampson and Piantadosi, 1988).

In order to determine changes in muscle oxygenation (Hb+Mb) the spectral extinction coefficients must be known and incorporated into the algorithm used in the NIRS system. Unfortunately , the spectra for hemoglobin and myoglobin in muscle tissue are not sufficiently different to distinguish between them (Hardman *et al.*, 1966). Although it is not possible to distinguish between changes in concentration of the Mb and Hb, or MbO_2 and HbO_2, the contribution of myoglobin to the signal in human muscle has been estimated to be no greater than approximately 25% (Chance *et al.*, 1992). It has also been stated that no significant

deoxygenation of Mb occurs until complete deoxygenation of Hb, such as that which occurs after several minutes of cuff ischemia (Wang *et al.*, 1990).

The two possible methods for detecting the pathlength factor are pulse time and phase modulation. With the continuous dual wavelength NIRS system used in this thesis, neither the depth of light penetration or the pathlength factor to correct for scattering of light within the tissue concentration changes can be measured. The NIRS unit therefore, cannot be used to determine absolute oxy-deoxy Hb/Mb changes. Inter-individual variation in pathlength factor (Delpy *et al.*, 1988) and differences in fat/muscle ratio can account for individual differences in results. The amount of muscle tissue contributing to the NIRS signals can also vary considerably. As well the sensitivity of the measurement varies as a function of the intensity of NIR light emitted.

In the final paper of this thesis (Paper IV) tissue O_2 saturation (SO_2) was estimated via NIRS with a commercially available unit (Runman™, NIM Incorporated, Philadelphia). Reflected light was measured percutaneously at two specific wavelengths (760 and 850 nm). The lamp intensity was set at 6 volts for all tests and the time constant for the unit set to the shortest response time (15 sec). The sensor was positioned lengthwise 10 to 12 cm above the knee over the vastus lateralis and protected from skin moisture by a clear plastic wrap. An elastic strap was placed around the thigh, over the sensor to prevent displacement and the detection of ambient room light. Two separate outputs were obtained from the NIRS unit and sampled at 100 Hz on a dedicated computer system. The output containing the difference in the two received wavelengths (760-850 nm) was monitored as an index of relative hemoglobin (Hb) and myoglobin (Mb) deoxygenation and the output containing the sum of the two received wavelengths (760+850) was monitored as

an index of changes in tissue blood volume (Chance *et al.*, 1992).

The Runman™ unit was calibrated before each exercise transition with the probe in place on the vastus lateralis. The electrical output of the Runman™ unit was adjusted to 0 mV using the balance control and then the gain of the unit was adjusted to provide signal deflections in the range of 600 to 1000 mV. For the difference channel (760-850 nm) NIR-SO_{2mus} was expressed on a relative scale as a percent of individual calibration under each gas condition with 100 % saturation equal to the resting saturation and 0% saturation equal to the full scale deflection between -600 and -1000 mV. For the sum channel (760+850 nm) tissue blood volume was expressed on a relative scale as a percent of individual calibration with 0% change in blood volume equal to the resting saturation and 100% increase in blood volume equal to the full scale deflection between -600 and -1000 mV.

General Discussion

Previous investigations have determined that Doppler methodology can be used to measure blood flow non-invasively and continuously at the onset of small muscle mass (Hughson *et al.*, 1996) and large muscle mass exercise (Eriksen *et al.*, 1990; Shoemaker *et al.*, 1994; Shoemaker *et al.*, 1996; Walloe and Wesche, 1988). Other recent publications indicate that blood flow values obtained through the use of Doppler ultrasound are reproducible (Shoemaker *et al.*, 1997) and of similar magnitude to venous outflow measures obtained through thermodilution techniques (Radegran, 1997). Much research has been devoted to determinations of the mechanisms regulating the initial hyperemia at the onset of exercise, however it was not the focus of this thesis to examine these possible regulatory factors. The major observation of this thesis is that simultaneous measurements of blood flow and O_2 uptake can provide information about the regulation of oxygen utilization at the onset of exercise.

The results of this thesis show that continuous measurement of both blood flow and O_2 uptake at the onset of exercise can provide insight into the regulation of O_2 delivery to and O_2 utilization by exercising skeletal muscle. In Chapter II the time course of the increase in blood flow and O_2 uptake at the onset of dynamic kicking exercise in both the upright and supine positions was characterized. As observed previously, blood flow increased with a biphasic response at the onset of exercise (Leyk *et al.*, 1994; Sheriff *et al.*, 1993; Shoemaker *et al.*, 1996). Faster increases in femoral artery blood flow and $\dot{V}O_2$ were observed in the upright versus supine position. Although the response of blood flow was observed to precede the $\dot{V}O_2$ response in both body positions, this acceleration in upright versus supine position suggests that the response

of blood flow at the onset of exercise can impact on the regulation of O_2 uptake. One of the most interesting findings from this study was that the temporal responses of leg blood flow and alveolar O_2 uptake seemed to parallel each other at the onset of kicking exercise in the supine position, and to a lesser extent in the upright position. These observations led to further investigations into the impact of alterations in blood flow dynamic responses and the consequences for O_2 uptake at the onset of large muscle mass exercise.

Chapters III and IV of this thesis were investigations into the dynamic response of blood flow and O_2 uptake at the onset of exercise in a variety of exercise situations. To examine whether prior high intensity exercise would result in a more rapid blood flow response, high intensity cycle exercise was performed prior to leg kicking exercise. This manoeuver resulted in elevated steady state flows but no change in the dynamic response time of LBF. A single day of intermittent exercise training was found to have no impact on the time course of increase of either LBF and $\dot{V}O_2$. Altered gas breathing was used to determine if alterations in inspired O_2 would result in differences in O_2 delivery to the exercising limbs and therefore differences in O_2 utilization at exercise onset. It was found that, for leg kicking exercise below T_{VENT}, adjustments in LBF and vascular conductance result in unchanged O_2 delivery to the exercising legs in response to alterations in CaO_2.

Despite the non-invasive nature of Doppler ultrasound technology, in order to accurately determine O_2 utilization in the exercising limb, it is necessary to obtain O_2 content values from venous and arterial blood samples. It would be advantageous to have a method of non-invasively determining the oxygenation of the exercising limb at the onset of exercise and in the subsequent

steady state. In Chapter V a method of non-invasively determining the Hb/Mb O_2 saturation in muscle at the onset of exercise was compared to femoral venous blood O_2 saturation. Unfortunately, the signal obtained from the NIRS instrument did not account for all of the variation observed in the femoral venous oxygen saturation signal at the onset of exercise. It would appear that this technique is not appropriate for tissue O_2 saturation monitoring at the onset of large muscle mass exercise.

Validation of Blood Flow via Doppler

Most of the studies conducted in our laboratory do not involve direct measures of muscle $\dot{V}O_2$. It is difficult to compare measures of leg blood flow during kicking exercise, which involves both flexion and extension, to previous literature because most previous studies have measured LBF during knee extension only. Two studies have compared one and two leg knee extension only exercise and determined that the LBF in one leg during two legged knee extension exercise is roughly equivalent to the LBF during one leg knee extension exercise at half of the two legged work rate (Magnusson *et al.*, 1993; Magnusson *et al.*, 1997). This relationship has been used in an effort to compare the LBF measures found in this thesis to previous literature.

Radegran (1997), using Doppler methods, determined that for 1 legged knee extension exercise, LBF was related to WR by the equation:

$$LBF = 0.084\ WR\ (W) + 1.3171\ L/min$$

The values found in this thesis are similar in magnitude to those calculated by this relationship, however, the exercise values found in Chapters II and III are slightly lower than expected from the equation. In Chapter IV, the values obtained at 48W kicking are similar to the

calculated values. The possible reasons for these differences include Doppler methodological errors, exercise variability, subject efficiency, and variability in LBF responses. Indeed, Radegran reported a large variability in LBF between four subjects exercising at 30 W, with LBF reported to range between 3.5 and 5.4 L/min (Radegran, 1997).

A comparison of the studies included in the current thesis as well as a number of previous studies indicates that the LBF and $\dot{V}O_2$ measures from the current thesis are similar in magnitude to what has been previously reported (Table 1.1). Most previous studies have utilized thermodilution techniques for measurement of LBF and leg$\dot{V}O_2$ (Andersen and Saltin, 1985; Kim *et al.*, 1995; Koskolou *et al.*, 1997; Magnusson *et al.*, 1997; Richardson *et al.*, 1995; Richardson *et al.*, 1993; Rowell *et al.*, 1986; Savard *et al.*, 1988). In general the LBF values obtained from these studies are slightly larger in magnitude than the LBF values from the current thesis, when one versus two leg exercise is taken into account (Table 1.1). As stated previously, though, the current studies involved leg kicking exercise (extension and flexion) as opposed to knee extension exercise, and direct comparisons are difficult. Studies by both Borkoff *et al.* (1997) and Radegran (1997) also utilized Doppler technology. The validity of the current measures of LBF and $\dot{V}O_2$ have been discussed in detail in the methods section of Chapter I as well as the Discussion of Chapters II, III and IV.

Table 1.1 *Comparison of leg blood flow and oxygen uptake measures in voluntary leg exercise.*

Study	Method	exercise	WR	LBF mL/min	$\dot{V}O_2$alv mL/min	HR bt/min	$\dot{V}O_2$leg mL/min	a-vDO_2 mL/dl
MacDonald Chapter II	Doppler	2leg flex/ext	rest	354	353	64		
			40W	2043	907	82	277	136*
MacDonald Chapter III	Doppler	2 leg flex/ext	0W	1487	672	90	156	105*
			52W	2700	1140	108	390	144*
MacDonald Chapter IV	Doppler	2leg flex/ext	rest	365	345	64	22.5	61.4121
			48W	3658	1280	108	442.5	
Borkoff *et al.* (1997)	Doppler	2 leg flex/ext	rest	350	369	51		
			10W	1250	660	58	146	118*
			30W	2050	847	69	240	120*
			50W	2525	1033	77	332	134*
Magnusson *et al.* (1997)	thermo-dilution	2leg ext	rest	400	280	61	25	62
			0W	2000	480	143	410	125
			40W	3000	1000		250	137
			86W	4800	1650		630	138
		1 leg ext	rest	400	280	61	25	62
			0W	2000	480	122	250	125
			20W	3000	730		410	137
			43W	4600	1290		630	136
Koskolou *et al.*(1997)	thermo-dilution	2 leg ext	rest	300	300	80	27	90
			30W	2900	800	100	350	125
			~70W	4200	1400	120	600	132
			~140W	6500	2500	157	980	142
Radegran & Saltin (1997)	Doppler	1 leg ext	rest	310				
			20W	3100				
			40W	5120				
			70W	7220				
	thermo-dilution	1 leg ext	rest	260				
			20W					
			40W	4960				
			70W	7070				
Andersen & Saltin (1985)	thermo-dilution	1 leg ext	10W	2600	560	83	280	54
			20W	3270	680		410	114
			30W	3940	830		510	129*
			40W	4720	990		650	138*
			55W	5580	139	146	820	140
Kim *et al.* (1995)	thermo-dilution	1 leg ext	0W	1600	550	80	140	88*
			10W	2500	610	95	260	104*
			20W	3100	700	110	300	97*
			30W	5000	900	118	450	90*
			40W	5300	1300	126	640	121*
Rowell *et al.* (1986)	thermo-dilution	1 leg ext	rest	250			25	95(100*)
			20W	3280		83	388	120(118*)
			38W	4270		95	556	130
			52W	5810		123	771	132
Richardson *et al.* (1995; 1993)	thermo-dilution	1 leg ext	25W	3720	790	93	460	122.1
			50W	5870	1090	117	810	137.1
			78W	7520	1510	139	1110	146.9
			93W	8770	1890	158	1340	152.6
			100W	9100	2270	167	1420	154.4

WR, work rate; LBF, leg blood flow; $\dot{V}O_2$alv, alveolar oxygen uptake; HR, heart rate; $\dot{V}O_2$leg, leg oxygen uptake; a-vDO_2, arteriovenous oxygen content difference * indicates that a-vDO_2 was calculated from $\dot{V}O_2$leg/LBF.

The Dynamic Response of Blood Flow at Exercise Onset

In all studies there was rapid increase in LBF at the onset of exercise from a baseline of rest or unloaded kicking exercise. For exercise transitions to below T_{VENT}, the response at the onset of exercise is rapid and follows a biphasic response pattern. The initial rapid increase is observed within the first few seconds after exercise onset and is followed by a slower increase to a plateau or further drift as exercise continues. This drift in LBF later in exercise was observed with higher work rate and occasionally, variability in the LBF response was observed. These variations may be due to changes in kicking pattern or an initial overshoot in LBF followed by adjustments to a steady state. The response of blood flow was slower in supine versus upright exercise but was unchanged by a single day of intermittent exercise training or breathing 70% or 14 % O_2 compared to breathing room air.

It is interesting to note that minor changes in MBV and diameter combined to regulate LBF to the same level at rest and during steady state exercise in each exercise situation despite variations in HR, MAP, and the dynamic response of LBF. These adaptations indicate that regulatory mechanisms exist to maintain the LBF to skeletal muscle based on the metabolic demands during rest and exercise below T_{VENT}. The exact nature of these regulatory mechanisms is not known but it has been speculated that metabolites released from active skeletal muscle play an important role (Laughlin and Armstrong, 1983).

The Relationship of Blood Flow to Oxygen Uptake at Exercise Onset

Faster kinetics for the $\dot{V}O_2$ and LBF on transient of step changes in submaximal kicking exercise in the upright versus supine position were observed. These results provide evidence that

the supply of oxygen contributes to the control of tissue metabolism. In addition, a lack of acceleration of both LBF and $\dot{V}O_2$ kinetics with a single day of intermittent exercise training and with hyperoxic and hypoxic gas breathing were observed. These results do not rule out the possibility that the process of utilization of O_2 by the working muscle contributes to the regulation of $\dot{V}O_2$ kinetics at the onset of exercise below T_{VENT}. These observations do, however, characterize the relationship between O_2 delivery and O_2 utilization in a number of exercise situations.

The response pattern of LBF and $\dot{V}O_2$ followed a similar time course at the onset of exercise in a variety of exercise situations. This observation suggests a close relationship between oxygen delivery and oxygen utilization at the onset of moderate intensity kicking exercise. This relationship was especially evident for exercise in the supine position where both the $\dot{V}O_2$ and LBF responses were slowed relative to upright exercise. Previous research by Hogan *et al.* (1992b), has shown that the degree of tissue oxygenation may be important in modulating the levels of other regulators of oxidative phosphorylation such that " a greater change in any of the proposed regulators of tissue respiration (e.g. phosphocreatine, ADP) was required to achieve a given $\dot{V}O_2$ as PaO_2 was decreased". However, further research has shown that changes in oxygen delivery may not result in increased levels of the proposed effectors of muscle $\dot{V}O_2$, if the step changes in O_2 delivery are relatively small (Hogan *et al.*, 1996). In this situation, the gradual changes in O_2 delivery resulted in decreases in force development and $\dot{V}O_2$ which may be due to adjustments to the O_2 delivery changes or due to the low contractile intensity used the experiments (Hogan *et al.*, 1996). At the onset of step changes in large muscle mass exercise, such as those transitions

examined in this thesis, the muscle blood flow response and the availability of O_2 at the level of the working muscle appears to be an important modulator in the rate of increase in $\dot{V}O_2$.

Blood flow to tissues may be regulated by an O_2 sensing mechanism in which O_2 delivery to exercising muscle is regulated and oxidative phosphorylation is therefore sensitive to tissue oxygenation. This tissue oxygen sensitivity would, thereby, factor in the regulation of local vasculature and muscle microcirculation. In agreement with this theory, it was observed in Chapter IV that when the CaO_2 was altered, with changes in inspired oxygen concentration, regulatory mechanisms were able to adapt and continue to deliver oxygen to the exercising tissue at the same rate as during normoxic gas breathing. The mechanism for this regulation is not known, however, it does seem that there are important interactions between cellular oxidative phosphorylation and oxygen delivery.

Conclusions

In summary, the results of the studies presented in this thesis demonstrate that the responses of LBF and $\dot{V}O_2$ can be quantified at the onset of large muscle mass dynamic exercise. As hypothesized, when skeletal muscle blood flow was slowed at the onset of supine exercise, the rate of increase in $\dot{V}O_2$ was also slowed. The other experimental manipulations of changing training status, prior exercise and altered gas breathing did not impact on the dynamic response of either LBF or $\dot{V}O_2$ at the onset of moderate intensity kicking exercise. The regulatory mechanisms controlling these responses remains undetermined.

It would be advantageous to know the O_2 availability as well as the distribution of blood at the level of the working muscle, however, techniques for determining these factors are not

available. In addition, the role of blood redistribution from non-exercising tissues to exercising skeletal muscle in the control of blood flow and $\dot{V}O_2$ at the onset of exercise has not been determined. These are some issues which are addressed in the following recommendations for future studies.

Future Considerations

The studies contained in this thesis address issues surrounding the responses of blood flow and O_2 uptake at the onset of large muscle mass dynamic exercise. Perfusion pressure, training status, vascular conductance, and arterial oxygen content were altered and the effects on the dynamic response of blood flow and $\dot{V}O_2$ were examined.

1. This thesis did not deal with possible mechanisms regulating the increase in blood flow at the onset of exercise. Further examinations into control of the immediate increase in blood flow at the onset of exercise as related to the roles of vasodilation and the muscle pump as well as examination of steady state blood flow regulation are needed

2. Prior high intensity exercise was found to elevate the vascular conductance of the legs at 0W and 52W but did not impact the kinetic response of blood flow at the onset of exercise. Further examinations including simultaneous measures of blood flow and O_2 uptake are necessary to determine if these alterations in vascular conductance affect the $\dot{V}O_2$ kinetics and regulation of O_2 utilization at exercise onset. As well, previous research has indicated that prior high intensity exercise results in elevated $\dot{V}O_2$ kinetics for a subsequent transition to above, but not below, T_{VENT}. Further studies should include exercise transitions to both intensity domains.

3. It was found that a single day of intermittent high intensity exercise did not accelerate the kinetics of blood flow and $\dot{V}O_2$, although previous examinations have shown accelerated blood flow responses after ten days of endurance training (Shoemaker *et al.*, 1996). Cross sectional studies have also shown , increases in conductance vessel diameter with exercise training (Huonker *et al.*, 1996; Zeppilli *et al.*, 1995). Further, detailed examinations into blood flow and vessel

diameter responses may provide insight into the time course of these changes.

4. Chapter IV focussed on the effect of inspired oxygen concentration and it was found that in spite of alterations in CaO_2 with 14% and 70% O_2 gas breathing, oxygen delivery to and $\dot{V}O_2$ in the legs was not altered at rest, exercise or during the transition between the two states. It is possible that further decreases in F_IO_2 could result in decreases in O_2 delivery at the onset of exercise. As well, step increases in exercise to a higher intensity exercise level might result in alterations in O_2 delivery and O_2 uptake at the onset of exercise. Adaptations which maintained O_2 delivery in both hyperoxia and hypoxia similar to normoxia were observed, however, it is not known what regulates the O_2 delivery to skeletal muscle. Further examination into the effect of altered gas breathing on microvasculature may provide insights into these mechanisms.

5. The results of Chapter V indicated that NIRS does not provide the same information as femoral venous blood samples at the onset of exercise. Further determinations into the origin of the NIRS signals, the contribution of myoglobin, and the response at the onset of small muscle mass exercise are necessary to determine the validity and applicability of NIRS measures of muscle oxygenation at exercise onset.

6. One of the aims of this thesis was to determine the role of blood redistribution from non exercising tissues to active muscle vascular beds at the onset of exercise. The results of the preliminary study located in the Appendix I indicate that further advancements in Doppler techniques, including correction for loss of signal due to respiratory interference, are necessary to measure splanchnic blood flow contributions at exercise onset. Further examinations into the role of the sympathetic system at the onset of exercise should include controlled situations in which

blood flow signals are collected continuously and EMG activity of the non exercising leg is monitored.

7. With respect to specifics of utilizing the techniques applied to oxygen uptake and blood flow measurements in this thesis, future studies should take a number of issues under consideration. In order to determine kinetic responses, the error associated with the exponential fitting procedure is reduced when examining responses with larger amplitudes. For this reason, any attempt to increase the response amplitude, such as transitions from a baseline of rest rather than from unloaded exercise and recruitment of subjects capable of generating higher power outputs on the kicking ergometer are recommended. In addition, now that the responses of blood flow and oxygen uptake during transitions in kicking exercise have been characterized, future studies should include power calculations for determination of the number of subjects needed for reliable statistical measures.

CHAPTER II

Alveolar oxygen uptake and femoral artery blood flow dynamics in upright and supine leg exercise in humans

(Submitted to Journal of Applied Physiology June 1997)

ABSTRACT

We tested the hypothesis that the slower increase in alveolar oxygen uptake ($\dot{V}O_2$) at the onset of supine, compared to upright, exercise would be accompanied by a slower rate of increase in leg blood flow (LBF). Seven healthy subjects performed transitions from rest to 40 W knee extension exercise in the upright and supine positions. Blood flow was measured continuously with pulsed and echo Doppler methods and $\dot{V}O_2$ was measured breath-by-breath at the mouth. At rest, a smaller diameter of the femoral artery in the supine position ($p<0.05$) was compensated by a greater mean blood flow velocity (MBV) ($p<0.05$) so that leg blood flow was not different in the two positions. At the end of 6 minutes of exercise, femoral artery diameter was larger in the upright position and there were no differences in any of $\dot{V}O_2$, MBV or LBF between upright and supine positions. The rates of increase of $\dot{V}O_2$ and LBF in the transition between rest and 40 W exercise, as evaluated by the mean response time (time to 63% of the increase), were slower in the supine ($\dot{V}O_2$ = 39.7± 3.8 s, LBF = 27.6±3.9 s) than in the upright positions ($\dot{V}O_2$ =29.3±3.0 s, LBF=17.3±4.0 s, $p<0.05$). These data support our hypothesis that oxidative metabolism at the onset of exercise is modulated in the supine position by the supply of O_2 due to a slower rate of increase in LBF. The potential link between blood flow redistribution and metabolism is discussed.

Key words: kicking exercise, Doppler velocimetry, echo-Doppler, leg blood flow, O_2 uptake

INTRODUCTION

At the onset of submaximal exercise in the supine position, the rate of increase in alveolar oxygen uptake ($\dot{V}O_2$) is slower than when the same exercise work rate is completed in the upright position (Cerretelli *et al.*, 1977; Convertino *et al.*, 1984; Hughson *et al.*, 1993; Karlsson *et al.*, 1975). It has been speculated that this slower rate of increase in $\dot{V}O_2$ might be due to a slower rate of increase in the supply of O_2 to the working muscles (Eiken, 1988a; Hughson *et al.*, 1993a). Blood flow is one component of oxygen delivery that is altered at the onset of exercise and may be an important modulator for oxidative phosphorylation in this situation (Hogan *et al.*, 1992b). Information on the dynamics of the muscle blood flow response at the onset of large muscle mass exercise is lacking.

Recently, it has become possible to monitor the changes in skeletal muscle blood flow in the transition from rest to exercise, and to determine the relationship between O_2 delivery and O_2 utilization (Shoemaker *et al.*, 1994; Shoemaker *et al.*, 1996). Grassi et al. (1996) used thermodilution techniques to monitor blood flow in combination with direct femoral vein samples to determine O_2 extraction and found that the time course of increase in muscle blood flow and muscle $\dot{V}O_2$ were similar during upright cycle ergometry. Hughson et al.(1996), using Doppler ultrasound technology, observed a close correlation between the rate of increase in blood flow to the forearm muscles and the muscle $\dot{V}O_2$. They further noted that both flow and muscle $\dot{V}O_2$ adapted more slowly when the exercising forearm was above, rather than below the heart.

The rationale for this study was to incorporate manipulations in perfusion pressure in a leg exercise model to examine the effect of blood flow delivery at the onset of large muscle mass

exercise. To study this, we have used combined pulsed and echo Doppler methods to continuously quantify the femoral artery blood flow in the transition from rest to submaximal knee extension/flexion exercise in both the supine and the upright positions. We tested the hypothesis that the slower rate of increase in alveolar $\dot{V}O_2$ at the onset of supine exercise would be parallelled by slower increases in leg blood flow (LBF) in the same posture.

METHODS

The experiments were carried out on seven healthy young volunteers (5 men and 2 women, age 27 ± 5, height 180 ± 5 cm, and weight 75 ± 9 kg; mean ± SE). After reading a description of the methods and possible risks, each signed a consent form approved by the Office of Human Research at the University of Waterloo. Each subject practised the exercise to become familiar with the activity so that high quality cardiorespiratory and Doppler signals could be obtained in both positions at rest and during exercise. This was important as complete relaxation of knee extensors during knee flexion was necessary to achieve optimal blood flow between extension periods, and to reduce motion artifact in the Doppler signals. On the data collection day, subjects reported to the laboratory at least two hours after their last meal. They were asked to avoid caffeine and alcohol ingestion and strenuous exercise for 24 hours prior to the test.

Experimental Design: Testing was performed on an electrically braked knee extension/flexion ergometer. In this study the ergometer was configured so that subjects worked against a resistance on both extension and flexion and maintained a knee extension/flexion cadence of approximately 44 cycles per leg/min at a work rate of 40 W. The ergometer had an adjustable back rest that allowed the subjects to sit with a hip angle of 120° during the upright tests, while the hips were completely extended (180°) during the supine tests. Aside from the hip angle, the exercise task was identical in both positions. The exercise intensity of 40 W was selected because it provided a signal ($\dot{V}O_2$ and LBF) of sufficient amplitude that curve fitting to the time course could be accomplished with some confidence.

The test protocol consisted of at least 5 minutes of baseline rest. To begin a trial, the

flywheel of the ergometer was hand cranked by an assistant and the subjects were issued a verbal command to start 6 minutes of exercise at 40 W. Each subject performed 2 trials in each posture, separated by at least 15-20 minutes of rest. The order of postures was assigned randomly.

Data Acquisition: Breath-by-breath ventilation and gas exchange were measured with a computerized system (First Breath, St. Agatha, Ontario, Canada). Fractional concentration of O_2, CO_2, and N_2 were measured with a mass spectrometer (MGA-1100, Marquette Electronics Inc., Milwaukee, WI), and inspired and expired volumes were measured with a volume turbine (VMM-110, Alpha Technologies Inc., Laguna Beach, CA). Calibration of the mass spectrometer was performed prior to each test using two gas tanks of known concentration. Volume was calibrated by manually pumping a 3 litre syringe at a flow rate similar to that of respiration during the exercise test. $\dot{V}O_2$ was corrected on a breath-by-breath basis for changes in lung gas stores due to altered lung volume or alveolar composition, as described previously (Hughson *et al.*, 1991). Matching of fractional gas concentrations with the appropriate volume was done by accounting for the sum of the transport lag plus the instrument response time.

Blood flow to the exercising legs was obtained with combined pulsed and echo Doppler ultrasound to measure mean blood velocity (MBV) and femoral artery diameter, respectively, from a site approximately 2-3 cm distal to the inguinal ligament. MBV and diameter measured at this site do not reflect MBV and diameter at the level of the resistance vessels in the exercising muscles. However, LBF calculated from femoral MBV and diameter, reflects total leg blood flow. Blood velocity was obtained on a beat-by-beat basis from the left leg with the pulsed Doppler system (model 500V, Multigon Industries, Mt. Vernon, NY) using a flat 4 MHz probe with a fixed

angle of insonation of 45°. The spectrum of audio range signals reflected from the moving blood cells was processed on line by a Doppler signal processor to yield instantaneous mean blood velocity (Micco, 1989). The velocity signal was recorded at 100 Hz on a computer system along with the ECG so that the data could be analysed beat-by-beat. Calibration signals in the Doppler shift frequency range were generated from the Doppler signal processor. A hand held linear array 7.5 MHz probe was used to obtain B-mode echo images of the right femoral artery using a medical diagnostic ultrasound system (model SSH140A, Toshiba Inc., Tochigi-Ken, Japan). The images of the artery were recorded on VHS videotape and analysed for artery diameter with on-screen calipers. Vessel diameter was measured 3 times during rest, each 10 seconds during the first minute of exercise, then at 1 minute intervals to the end of exercise. The diameter data were fit with a linear or exponential regression to obtain an average response and reduce random error. Mean blood flow to the leg (LBF) was calculated on a beat-by-beat basis by multiplying the mean blood velocity with the estimated diameter from the regression equation for each time point.

Mean arterial pressure was measured with a pneumatic finger cuff (Ohmeda 2300, Finapres, Englewood, CO). The hand was placed at the level of the femoral artery to give an estimate of perfusion pressure.

Kinetic Analysis: Breath-by-breath values for $\dot{V}O_2$ were linearly interpolated between breaths to give values at 1 second intervals. The time course data for $\dot{V}O_2$ and LBF collected from both trials in each condition, were ensemble averaged to produce a single data set for each variable, for each subject, in each test condition. The time course of changes in $\dot{V}O_2$ and LBF were analysed by fitting an exponential curve to the average results of the trials. A two component

exponential model was fit to the data using a least square procedure. As previously described (Hughson *et al.*, 1988), the model had a baseline component (G0), two amplitude terms (G1 and G2), two time constants (τ1 and τ2) and two time delays (TD1 and TD2)

$$Y(t) = G0 + G1\,(1 - e^{-(t - TD1)/\tau 1}) \cdot u1 + G2\,(1 - e^{-(t - TD2)/\tau 2}) \cdot u2$$

where,

$$u1 = 0 \text{ for } t < TD1 \text{ and } u1 = 1 \text{ for } t \geq TD1$$

$$u2 = 0 \text{ for } t < TD2 \text{ and } u2 = 1 \text{ for } t \geq TD2$$

Y(t) is the time dependent variation in $\dot{V}O_2$ or LBF.

An indicator of the rate of change of each of these variables for each subject and condition was obtained by calculation of the mean response time (MRT). The MRT is the time required to achieve approximately 63% of the difference between baseline and the exercise plateau. It is determined from the weighted mean of the time constant and time delay for each exponential term (Hughson *et al.*, 1988).

$$MRT = (G1/(G1 + G2)) \cdot (TD1 + \tau 1) + (G2/(G1 + G2)) \cdot (TD2 + \tau 2)$$

Statistical Analysis: The effects of body position (upright *vs.* supine) on the steady-state values of MBV, LBF, MAP, $\dot{V}O_2$, and HR and on the kinetic fitting parameters for LBF and $\dot{V}O_2$ were analysed by a repeated measures one way ANOVA. The combined effects of body position (upright *vs.* supine) and intensity (rest *vs.* exercise) were determined for steady-state diameter measures by a repeated measures two way ANOVA. A 2 way ANOVA was performed on the MRT's for LBF and $\dot{V}O_2$ with position (upright *vs.* supine) and variable (LBF *vs.* $\dot{V}O_2$) forming

the dependent variables. The level of significance for main effects and interactions was set at $p<0.05$ and significant differences were analysed with Student Neuman-Keuls post hoc test.

RESULTS

As anticipated, the mean arterial pressure measured at the level of the legs was less in the supine position than during the upright exercise at each of rest and 40W exercise (Figure 2.1, Table 2.1). Resting HR was lower in the supine versus upright position and there was no difference in HR during exercise between positions (Table 2.1, Figure 2.1). At rest, the MBV was lower in the upright versus supine position (Table 2.1, Figure 2.2). By the end of 6 minutes of exercise, the MBV was not different between upright and supine positions (Table 2.1, Figure 2.2). Resting and exercise femoral artery diameter was smaller in the supine position compared to the upright position (Table 2.1, Figure 2.2).

Baseline and the end exercise values for LBF and $\dot{V}O_2$ did not differ between positions (Table 2.1, Figure 2.2). Immediately on starting exercise there was a rapid increase in both LBF and $\dot{V}O_2$ in the upright and supine positions (Table 2.2, Figure 2.3). The rate of increase in $\dot{V}O_2$ was faster in the upright than the supine position as indicated by both $\tau 2$ and MRT (Table 2.2, Figure 2.3). There were no differences in the gain terms (G1 and G2) for $\dot{V}O_2$. The rate of increase in LBF was also faster in the upright than the supine position as indicated by the MRT. For LBF, there was a greater contribution of the first phase response (G1) and a smaller contribution of the second phase response (G2) in the upright compared to the supine positions (Table 2.2).

The MRT of the LBF response was faster than that of the $\dot{V}O_2$ in both upright and supine positions (Table 2.2). The clear relationship between the slower LBF and the slower $\dot{V}O_2$ was evident in supine position (Figure 2.3).

Table 2.1. *Cardiorespiratory responses at rest and during steady-state of 40 W exercise*

	Rest		Exercise	
	Upright	Supine	Upright	Supine
MBV (cm/s)	7.70±0.75	9.14±0.93*	42.7±4.8	47.5±5.8
Diameter(mm)	10.1±0.4	9.0±0.4*	10.2±0.4	9.7±0.3*
LBF (mL/min)	354±36	339±29	2043±186	2042±226
MAP (mmHg)	116±3	92±4*	122±4	100±4*
$\dot{V}O_2$ (mL/min)	353±13	369±25	907±28	930±38
HR (beats/min)	64 ± 3	59 ± 3*	82 ± 4	86 ± 5

Values are means ±SE for 7 subjects. MBV, mean blood velocity in femoral artery; Diameter, diameter of femoral artery; LBF, leg blood flow in one leg; MAP, mean arterial pressure at the level of Doppler flow measurements; $\dot{V}O_2$, total body oxygen uptake; HR, heart rate.

*Significantly different from upright, all $p<0.05$.

Table 2.2. *Steady state and dynamic responses of LBF in one leg and $\dot{V}O_2$ at rest and during 40 W knee extension/flexion exercise.*

	TG (mL/min)	G0 (mL/min)	G1 (mL/min)	TD1 (s)	τ1 (s)	G2 (mL/min)	TD2 (s)	τ2 (s)	MRT (s)
Upright									
LBF	1662±198	348±34	1439±237	1.7±0.5	5.5±1.8	259±260	18.8±3.1	39.7±13.2	17.3±4.0
$\dot{V}O_2$	543±32	385±44	293±34	1.3±0.8	9.1±3.1	250±51	25.3±3.5	23.1±4.1	29.3±3.0†
Supine									
LBF	1694±245	330±29	835±129*	2.6±0.8	2.3±0.3	858±144*	21.4±1.4	28.3±6.6	27.6±3.9*
$\dot{V}O_2$	563±44	396±53	273±24	2.2±1.1	10.8±1.8	289±55	28.5±2.8	38.7±5.1*	39.7±3.8*†

Values are means ±SE for 7 subjects. TG, total gain; G0, baseline resting value; G*n*, gain; TD *n*, time delay; τ*n*, time constant; MRT, mean response time;. *Significantly different from upright. †Significantly different from MRT of LBF within the same position, all $p<0.05$.

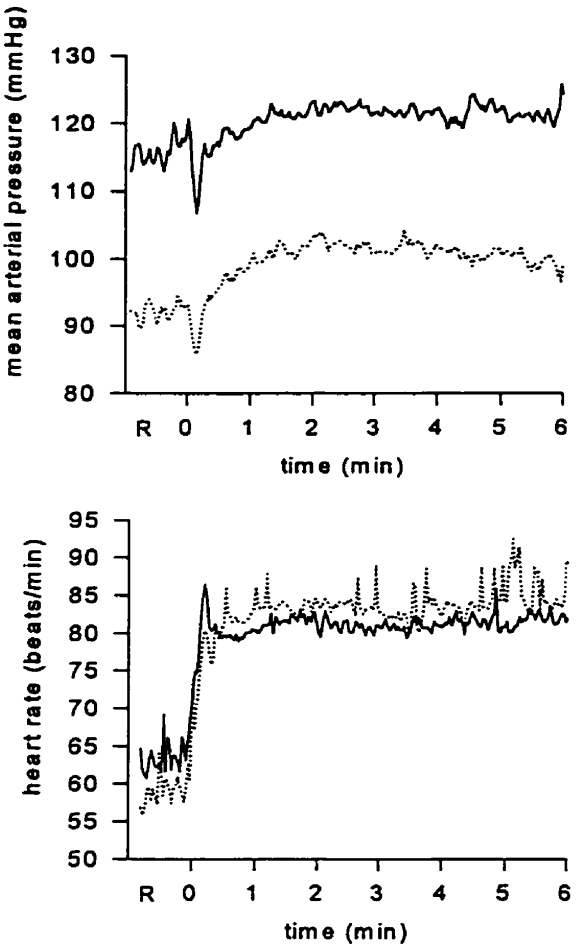


Figure 2.1 *Mean arterial pressure at the level of the femoral artery and heart rate during rest (R) and 6 minutes of 40W kicking exercise. Values are average of 7 subjects in upright (____) and supine (.....) body positions. For representative estimates of SE refer to Table 2.1.*

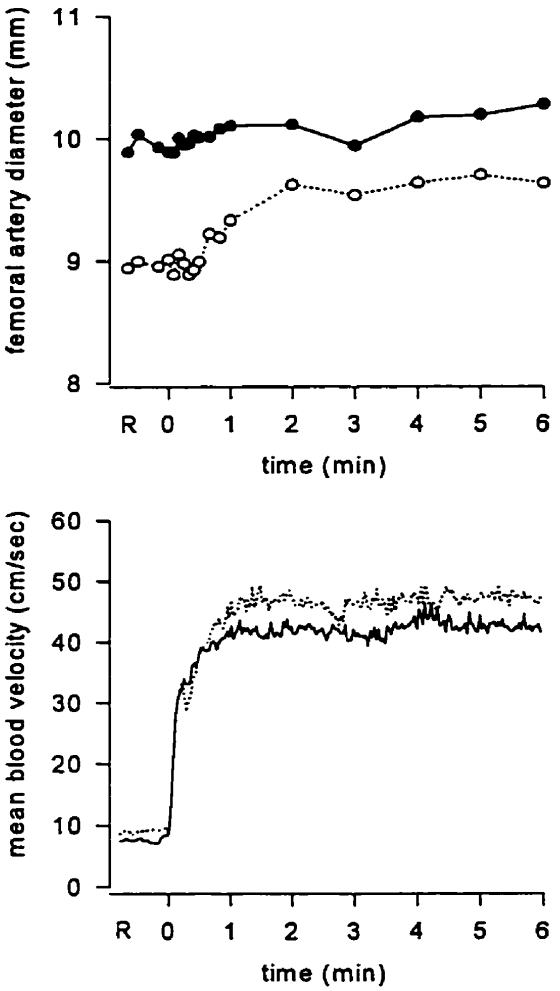


Figure 2.2 *Femoral artery diameter and MBV for 1 leg during rest (R) and 6 minutes of exercise. Values are average of 7 subjects in upright (____) and supine (.....) body positions. For representative estimates of SE refer to Table 2.1.*

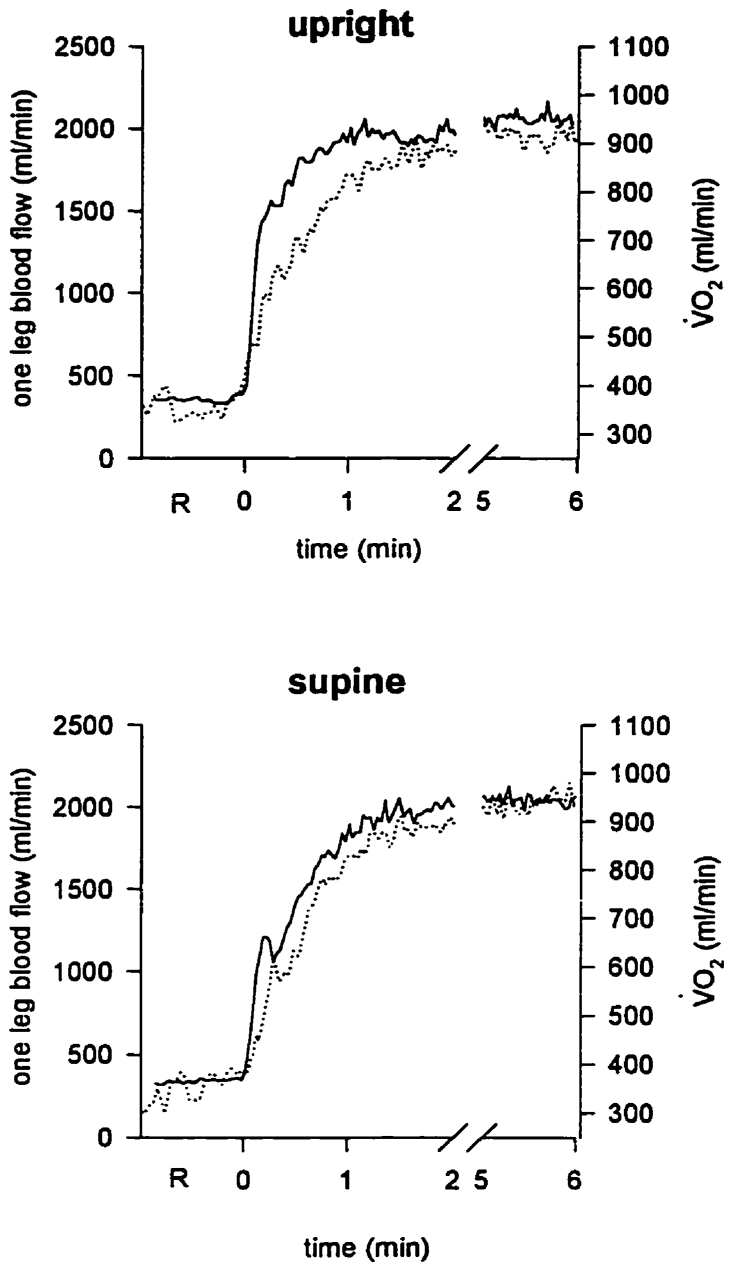


Figure 2.3 *LBF for 1 leg (____) and $\dot{V}O_2$ (.....) during rest (R) and 6 minutes of exercise in upright (top) and supine (bottom) body positions. Values are average of 7 subjects. For representative estimates of SE refer to Table 1.*

DISCUSSION

The primary finding of this study was that both blood flow to the exercising leg muscles and alveolar $\dot{V}O_2$ increased more slowly at the onset of exercise in the supine compared to the upright posture. The time course of change, given by the mean response time, was 35% slower for $\dot{V}O_2$ and 60% slower for LBF in supine exercise compared with the same exercise performed in the upright position. These data suggest that O_2 supply at the onset of exercise might alter metabolic control and limit the rate of increase in muscle $\dot{V}O_2$. Thus, these data support our hypothesis that in the supine position, the slower rate of increase in alveolar $\dot{V}O_2$ observed frequently at the onset of exercise (Cerretelli *et al.*, 1977; Convertino *et al.*, 1984; Hughson *et al.*, 1993; Karlsson *et al.*, 1975; Leyk *et al.*, 1994) was a consequence of a delayed adaptation in LBF.

The exercise model. Exercise required contractions of both the knee extensor and flexor muscles of both legs. This is different from the knee extension only exercise employed by other researchers (Radegran, 1997; Richardson *et al.*, 1995; Richardson *et al.*, 1993). We use this mode of exercise to involve a relatively large muscle mass while focussing the activity within muscles served by the femoral artery. There is very little, or no, involvement of muscles of the hips or above in both the upright and supine positions. Although it is possible that slightly different recruitment of muscles in the two postures might have accounted for the differences in LBF and $\dot{V}O_2$ kinetics, we do not believe this to be the case. Rather, the legs were exercised in exactly the same manner in both postures.

The work rate was clearly of light to moderate intensity for all subjects. The heart rate response in the upright position showed a slight overshoot in the first seconds after the onset of

exercise (Figure 2.1), and for both upright and supine positions, exercise heart rate was only 82-86 beats/min. Further, the alveolar $\dot{V}O_2$ and LBF had MRT values of less than 40 s, even in supine exercise, indicating that the steady state was achieved in less than 4 minutes, in all tests. Richardson *et al.* (Richardson *et al.*, 1995; Richardson *et al.*, 1993) have shown that the peak work rate for single leg, knee extension only exercise, was in excess of 100 W in trained cyclists. Our subjects were not trained, but used flexor and extensor muscles of both legs during exercise at 40 W. Further studies in our laboratory have confirmed that 40 W represents only about 1/3 of the peak work rate reached in incremental exercise on this ergometer (150 W) in typical female and male subjects.

Doppler blood flow measurements have only recently been applied to the study of blood flow during quadriceps muscle exercise (Radegran, 1997; Shoemaker *et al.*, 1994; Walloe and Wesche, 1988). In a recent comparison between Doppler and the thermodilution technique during 1 leg knee extension exercise, similar blood flow values were observed with the two methods across a wide range of work rates (Radegran, 1997).

Steady-state exercise. There were no differences, in the steady state values of $\dot{V}O_2$ or LBF at rest, or at 40 W, between the upright and supine postures in this study. Similar levels of $\dot{V}O_2$ are expected during exercise at the same absolute power output if, as in our study, modifying body position does not alter the work done against gravity (Leyk *et al.*, 1994). It is well established that cardiac output is greater in the supine compared to the upright position at rest and during lighter intensities of exercise (Karlsson *et al.*, 1975; Leyk *et al.*, 1994). There have been few studies of leg muscle blood flow in the different body positions. We found that the LBF at rest

was not different between body positions. But a significantly smaller femoral arterial diameter in the supine position combined with a greater MBV to achieve this similar flow (Table 1). Leyk *et al.* (1994) also found a significantly smaller diameter and a greater peak systolic blood velocity in the supine position. However, their calculated resting LBF was significantly greater in the supine than the upright posture. Leyk *et al.* (1994) kept their subjects in a seated posture so that the legs were above heart level, while our subjects were supine with the thigh muscles at heart level and the calf muscles below the heart. Consistent with our observations of no differences in blood flow to the exercising muscle with body position, both Van Leeuwen *et al.* (1992) and Leyk *et al.* (1994) observed no difference in leg blood flow in upright compared to supine seated positions during calf muscle exercise. The similar levels of LBF were achieved in the face of significantly lower MAP at the level of the exercising muscle in the supine exercise. This must mean that greater local vasodilation occurred in the exercising muscles in the supine position.

Cardiovascular responses at the onset of exercise. In selecting knee extension/flexion exercise, we realized that it was not possible to isolate the blood flow to only the working muscles. The femoral artery flow supplies the active quadriceps and hamstring muscle groups, as well as inactive lower leg muscles and skin. Therefore, at the onset of exercise, it is not clear how blood flow is distributed between working and non-working muscles. Most of the initial increase in blood flow would be expected to go to working muscles as the mechanical effects of the muscle pump and local vasodilation combine to increase vascular conductance (Leyk *et al.*, 1994; Sheriff *et al.*, 1993; Tschakovsky *et al.*, 1996). As evident in the bi-phasic response of the LBF (see Figure 2.3) in both the supine and upright postures, further dilation occurs after 15-20 s so that

steady state blood flow for light to moderate intensity exercise is achieved within 1-3 minutes (Hughson *et al.*, 1996). This progressive dilation is consistent with negative feedback control required to supply appropriate flow for the metabolic demands.

The distribution of blood flow within active muscles is also influenced by the interactions between the muscle pump and local, metabolically induced vasodilation. During submaximal exercise, only a fraction of the fibres contract, yet these fibres are distributed so that tension developed throughout the muscle will act as the pump to eject blood from the venules and veins. On muscle relaxation, the pressure gradient across the capillary bed is increased so that blood flow increases. But flow within the muscle would be increased by the muscle pump mechanism, without regard to the local distribution of active versus non-active muscle fibres. In the upright position, the greater initial increase in flow (G1 of the exponential fitting model, see Table 2.2) was a consequence of a greater increase in arterial to venous pressure gradient as gravity would have filled the veins at rest. As activity continues, local vasodilation will occur in the vicinity of the active fibres, while vasoconstriction will probably occur near inactive fibres where blood flow had increased out of proportion to the metabolic demands.

Recently, Grassi *et al.* (1996) stated that their observation of no immediate decline in femoral venous PO_2 was evidence that leg muscle $\dot{V}O_2$ was not constrained by bulk delivery of O_2. However, in the absence of information about the potential transient mismatching of blood O_2 supply to O_2 demand or of the effect of blood pooled in the leg veins while at rest, this interpretation needs to be viewed with caution. It is unlikely that the complex pattern of flow distribution will be resolved in studies of human muscle with currently available techniques.

The central cardiovascular responses at the onset of exercise appeared to be similar in each of the upright and supine positions. Heart rate increased very rapidly, with an overshoot in some tests. This overshoot is a consequence of very rapid vagal withdrawal in excess of that required to achieve the steady state heart rate (Toska and Eriksen, 1994). In Fig. 2.1, it is apparent that MAP decreased by about 10 mmHg at the onset of exercise, presumably because of a rapid reduction in peripheral resistance (Toska and Eriksen, 1994). MAP recovered rapidly as a consequence of the increase in cardiac output, and modifications in total peripheral resistance.

Blood flow and metabolism. The slower alveolar $\dot{V}O_2$ response observed when femoral artery blood flow also adapted more slowly at the onset of supine exercise suggests a link between O_2 transport and utilization. At least for the supine position, the rate of increase in O_2 supply (LBF) appeared to limit the rate at which $\dot{V}O_2$ increased. The consequence of this was that energy not supplied by oxidative metabolism must be supplied by anaerobic glycolysis with net accumulation of lactate and utilization of stored high-energy phosphates. However, in both body positions, the MRT for LBF was faster by about 10-12 s than for $\dot{V}O_2$ (Table 2.2). There are two possible explanations for these findings. First, it might be that the change in body position induced a change in the dynamics of both $\dot{V}O_2$ and LBF. As we discussed previously, this is unlikely, as the position of the legs remained constant while the body rotated at the hips in the two different postures. A second explanation could be related to the issue of within muscle blood flow distribution as considered above. That is, the muscle pump increases flow within a muscle without regard for metabolic requirements. It is only with continued exercise and the effects of negative feedback on local vascular responses that this flow is redistributed to achieve optimal matching of

O_2 supply to metabolic demand.

Classically, O_2 has not been viewed as a limiting factor in the process of oxidative phosphorylation because one-half maximal rate occurs at an intracellular PO_2 of 0.03 Torr in studies of isolated mitochondria (Erecinska and Wilson, 1982). However, the affinity of cytochrome c for O_2 varies with the energy state of the cell and intracellular PO_2 values of 10 Torr or above could have an impact on the intracellular concentrations of metabolic substrates required to drive the oxidative mechanisms at a given rate of ATP production (Hogan *et al.*, 1992; Jones, 1986). Given this argument it is understandable that the intracellular PO_2 does not have to reach zero to contribute to the rate limitation of oxidative phosphorylation. Rather, a relative hypoxia in the rest to exercise transition might cause a temporary slowing of muscle $\dot{V}O_2$ while the intracellular environment adapts to this PO_2.

The consequences of a slow adaptation of the oxidative metabolic processes at the onset of exercise are now more apparent. When the blood flow (Shoemaker *et al.*, 1996) and alveolar $\dot{V}O_2$ (Phillips *et al.*, 1995) increased more rapidly at the onset of submaximal exercise after a short-term period of exercise training, there were less marked reductions in intracellular phosphocreatine, and smaller increases in blood and muscle lactate (Phillips *et al.*, 1995).

Conclusions. These are the first estimations of leg blood flow dynamics that have been measured simultaneously with alveolar oxygen uptake during transients in work rate in upright and supine leg exercise. A reduction in perfusion pressure in the supine position appears to have been responsible for the slower increase in LBF and therefore the O_2 delivery response at the onset of exercise. These findings are in support of the hypothesis that O_2 availability can play a limiting role

in the adaptation of muscle $\dot{V}O_2$. The results further suggest that local vascular regulatory factors might be important in determining the time dependent distribution of blood flow within exercising muscles.

CHAPTER III

Effect of 16 hours of intermittent heavy exercise on oxygen uptake and leg blood flow responses to submaximal leg exercise

ABSTRACT

The objective of this study was to determine the effect of 16 hours of intermittent heavy exercise on the responses of oxygen uptake ($\dot{V}O_2$) and leg blood flow (LBF) during two legged kicking exercise. Six healthy, untrained subjects exercised for six minutes each hour at 85% of peak $\dot{V}O_2$ for 16 consecutive hours. Prior to and three days post-training alveolar $\dot{V}O_2$ was determined breath-by-breath and LBF was determined via Doppler methods during transitions from 0W to 50W kicking exercise. In an effort to determine whether changes occurred during the day of training, blood flow responses to submaximal kicking transitions were determined 15 minutes after high intensity exercise at hours 1 , 8 and 16. The single day of training had no effect on either the kinetic response of $\dot{V}O_2$ or LBF at the onset of exercise or the steady state LBF or $\dot{V}O_2$ at 0W or 52 W exercise measured three days post-training. On the day of training, the kinetic response of LBF was not altered by prior high intensity cycling exercise, however, the steady state LBF was elevated at both 0W and at 52 W compared to pre- and post- training. The vascular conductance was elevated on the training day compared to both pre- and post- training. These results indicate that a single day of intermittent heavy exercise did not result in adaptations in blood flow or $\dot{V}O_2$ kinetics compared to pre-training. However, in tests conducted 15 minutes after the intermittent high intensity exercise, vascular conductance in the exercising muscle was elevated. Due to equipment limitations, it was not known if the $\dot{V}O_2$ was altered in parallel with the change in LBF after prior high intensity exercise.

INTRODUCTION

Previous studies have shown more rapid transitions to steady state oxygen uptake ($\dot{V}O_2$) after endurance training (Cerretelli *et al.*, 1979; Hagberg *et al.*, 1980; Hickson *et al.*, 1978; Phillips *et al.*, 1995; Zhang *et al.*, 1991) and with prior high intensity exercise (Gausche *et al.*, 1989; Gerbino *et al.*, 1996; MacDonald *et al.*, 1997; Pendergast *et al.*, 1983). Increases in oxygen delivery to the exercising muscle have been suggested as one possible mechanism for these altered rates of increase in $\dot{V}O_2$ at exercise onset.

Shoemaker *et al.* (1996) observed an acceleration in the adaptation of mean blood velocity supplying the exercising muscle at the onset of knee extension exercise following short term endurance training. These adjustments in blood flow responses were observed prior to changes in mitochondrial potential of the muscle (Phillips *et al.*, 1995). Possible mechanisms include increases in stroke volume and improvements in functional vasodilation at the onset of exercise. The complete time course of changes in blood flow after the initiation of an endurance training protocol is not known.

The mechanism by which prior high intensity exercise affects $\dot{V}O_2$ kinetics is unclear but metabolic acidosis may play a role (Gausche *et al.*, 1989; Gerbino *et al.*, 1996; MacDonald *et al.*, 1997). Changes in lactic acid and acidity in the working muscle after prior high intensity exercise have been postulated to elevate muscle blood flow and improve O_2 diffusion gradients at the onset of exercise. It has been proposed that acceleration in the delivery of O_2 to the working muscle at the onset of exercise may result in faster adjustments to steady state (Linnarsson, 1974; Wagner, 1991). The limitation of studies on prior high intensity exercise with respect to regulation

of oxygen utilization is that blood flow, and therefore oxygen delivery, at the onset of exercise was not determined.

In the study presented here we have employed a combination of intermittent exercise training and prior high intensity exercise to determine the regulation of blood flow and oxygen uptake responses at the onset of exercise. In an effort to determine the time course of changes in blood flow and oxygen uptake during training, the blood flow and oxygen uptake adaptations at the onset of leg kicking exercise were examined prior to and after a single day of intermittent exercise training. We hypothesized that a single day of intermittent high intensity exercise may accelerate the transitions of leg blood flow and oxygen uptake during submaximal kicking exercise performed post training versus pre training as previously observed after endurance exercise training. The effects of prior high intensity cycling exercise on leg blood flow responses to submaximal kicking exercise were also examined throughout the training day. It was hypothesized that prior high intensity exercise would elevate leg blood flow during the steady state and possibly during transitions from one work rate to another.

METHODS

6 healthy male volunteers (age 22±1 years, height 178±3 cm, and weight 82±5 kg, mean ■ SE) participated in this study. They were not specifically trained and all gave written consent on a form approved by the Office of Human Research and Animal Care at the University of Waterloo.

Exercise Protocol

Subjects reported to the laboratory on two occasions for training on the kicking ergometer to assist in learning the exercise mode. The kicking ergometer is a specially designed piece of exercise equipment which allows electrically braked exercise of both the quadriceps and hamstrings muscle in an alternating "kicking fashion". Subjects remained in the seated position for all tests with a hip angle of 120° and knee extension and flexion between 90° and 135°. The kicking frequency, between 44 and 50 kicks per leg per minute, was maintained via visual feedback. The third visit to the laboratory involved a progressive kicking test to fatigue or functional limitation. The work rate protocol for this progressive test was a 15 W/min ramp. The progressive test was stopped when the subject could no longer maintain the kicking frequency or needed accessory muscle assistance. The gas exchange and work rate data from the ramp test were used to estimate the T_{VENT} and peak $\dot{V}O_2$. These values were then used in choosing the individual work rates for the step tests. Subjects also performed progressive tests to exhaustion on the cycle ergometer. The work rate data from these ramp tests were used to estimate the work rate for cycling exercise on the training day.

The next five testing sessions involved the step test protocol which consisted of two

identical step transitions in work rate performed with no resting period between them. A typical step test involved unloaded kicking at 0W for four minutes followed six minutes of kicking at a work rate at 85% of T_{VENT}. No warning was given to the subject prior to transitions in work rate although they were aware of the protocol before the test. Subjects were tested prior to training, after one, eight and sixteen hours of training and three days post- training.

The training protocol consisted of exercise at 85% of peak cycling $\dot{V}O_2$ for six minutes each hour for sixteen consecutive hours. During the pre- and post-training day testing leg blood flow as well as cardiorespiratory dynamics were measured. On the day of training only leg blood flow and not cardiorespiratory dynamics were measured during the leg kicking exercise sessions. On the training day, measures of blood flow responses were made approximately fifteen minutes after the high intensity cycling exercise task.

Data Collection:

During the pre- and post- training breath by breath ventilation and gas exchange were measured on a computerized system (First Breath, St. Agatha, ON) with a mass spectrometer (MGA-1100A, Marquette, Milwaukee, WI) and a digital volume turbine (VMM-110, Alpha Technologies, Laguna Beach, CA) or ultrasonic flow meter (UF202, Kou & Assoc. Redmond,WA). Matching of fractional gas concentrations with the appropriate volume was done by accounting for the sum of the transport lag plus the instrument response time. The mass spectrometer and the digital volume turbine or ultrasonic flow meter were calibrated separately for each test. The mass spectrometer was calibrated using two precision gas mixtures that spanned the anticipated fractional gas concentrations. Volume was calibrated by manually pumping a 3 litre

syringe at a flow rate similar to that of respiration during the exercise test. $\dot{V}O_2$ was corrected on a breath-by-breath basis for changes in lung gas stores due to altered lung volume or alveolar composition, as described previously (Hughson *et al.*, 1991).

Leg blood flow was determined from measures of femoral artery diameter by echo Doppler ultrasound (model SSH-140A, Toshiba, Tochigi-Ken, Japan) and femoral artery mean blood velocity via pulsed Doppler ultrasound (model 500V, Multigon Industries Inc., Mt. Vernon, NY) during of the kicking exercise step tests. A 7.5 MHz hand held linear array probe was held approximately 2 cm distal to the inguinal ligament during the kicking tests to continuously monitor the femoral artery diameter. The imaged data were stored on videotape. Measures of femoral artery diameter were obtained from the video recordings of the arterial image. These measures were obtained five times at rest, at ten second intervals during the first minute of exercise and each minute thereafter. A 4MHz pulsed Doppler probe was held on the opposite leg for beat-by-beat measurements of femoral artery velocity spectra. The spectra was processed continuously via a mean velocity processor (Micco, 1989) and collected on a computer based system at 100 Hz along with heart rate and blood pressure. Calibration signals in the Doppler shift frequency range were generated from the Doppler signal processor. The mean blood velocity (MBV) for each trial was obtained through integration of the area under the curve for each heart beat. The two trials at each training time point were then time aligned and ensemble averaged to yield a single MBV data set for each subject at each training point. The average diameter data for each time point were fit with a linear or exponential regression to obtain an average response and reduce random error. Mean blood flow to the leg (LBF) was calculated on a beat-by-beat basis by multiplying the

average mean blood velocity with the estimated diameter from the regression equation for each time point.

Blood pressure (BP) was obtained by using a pneumatic finger cuff (Ohmeda 2300, Finapres, Englewood, CO). The hand from which the BP was monitored was positioned so that the finger cuff was at the level of the Doppler probe to indicate leg perfusion pressure. An estimate of femoral artery vascular conductance (VC_{fa}) was calculated as the quotient of LBF/MAP at the level of the femoral artery (Shoemaker *et al.*, 1996).

Breath-by-breath values for $\dot{V}O_2$ were linearly interpolated between breaths to give values at 1 second intervals. The time course data for $\dot{V}O_2$ collected from two trials in each during pre- and post-training, were ensemble averaged to produce a single data set for each variable, for each subject, in each training time point.

Kinetic Analysis: The time course of changes in $\dot{V}O_2$ and LBF were analysed by fitting an exponential curve to the average results of the trials. A two or three component exponential model was fit to the data using a least square procedure. As previously described (Hughson *et al.*, 1988), the two component model had a baseline component (G0), two amplitude terms (G1 and G2), two time constants (τ1 and τ2) and two time delays (TD1 and TD2)

$$Y(t) = G0 + G1\,(1 - e^{-(t - TD1)/\tau 1}) \cdot u1 + G2\,(1 - e^{-(t - TD2)/\tau 2}) \cdot u2$$

where,

$u1 = 0$ for $t < TD1$ and $u1 = 1$ for $t \geq TD1$

$u2 = 0$ for $t < TD2$ and $u2 = 1$ for $t \geq TD2$

Y(t) is the time dependent variation in $\dot{V}O_2$ or LBF.

Some of the responses were fit to a three component model. The three component model contained an extra amplitude term (G3) and time constant ($\tau 3$) in order to fit the slower adaptive phase in these tests.

$$\dot{V}O_2(t) = G0 + G1\,(1 - e^{-(t - TD1)/\tau 1}) \cdot u1 + G2\,(1 - e^{-(t - TD2)/\tau 2}) \cdot u2 + G3\,(1 - e^{-(t - TD3)/\tau 3}) \cdot u2$$

where, u1 and u2 were as defined above and

$$u3 = 0 \text{ for } t < TD3 \text{ and } u3 = 1 \text{ for } t \geq TD3$$

The overall time course of the response was determined from mean response time (MRT). The MRT is the time it takes to reach approximately 63% of the total amplitude of the response from the baseline to the final plateau value. It was calculated as a weighted sum of the time delay and time constant for each component (Hughson *et al.*, 1993a).

$$MRT = (G1/(G1 + G2 + G3)) \cdot (TD1 + \tau 1) +$$
$$(G2/(G1 + G2 + G3)) \cdot (TD2 + \tau 2) +$$
$$(G3/(G1 + G2 + G3)) \cdot (TD3 + \tau 3)$$

Steady-state analysis

Rest and exercise average values were obtained for each of LBF, $\dot{V}O_2$, diameter, HR, MAP and MBV. These values were averages of 1 minute of data at each training time point, at rest and during the last minute of exercise.

Statistical analysis

The effect of training status on the rest and exercise steady state values for LBF, $\dot{V}O_2$, HR, MAP and MBV and the kinetics of LBF and $\dot{V}O_2$ responses were analysed by a one-way

repeated measures analysis of variance. The effect of training status on the time course data for femoral artery diameter was analysed by a one way repeated measures analysis of variance. The level of significance for the main effects were set at $P<0.05$. Any differences were further analysed with Student-Neumann-Keuls post hoc test. All data are presented as mean ± standard error (SE).

RESULTS

A peak work rate of 172±13W was attained during the incremental ramp kicking test prior to training. This work rate resulted in a $\dot{V}O_2$peak of 2153±104 mL/min and HR of 155±65 beats/min. The TVENT was 1434±81 mL/min and all subsequent kicking exercise consisted of step changes from 0W to 52±2 W (Table 3.1).

Effect of Single Day of Training

The responses measured three days post-training showed that 16 hours of intermittent high intensity exercise had no effect on steady state $\dot{V}O_2$, LBF, MBV, HR, MAP, VC_{fa} or femoral artery diameter at 0W or at 52W (Table 3.2, Figure 3.1). The dynamic adjustment of LBF and $\dot{V}O_2$ at the onset of exercise was also not different after the single day of intermittent exercise training compared to pre-training (Table 3.3, Figure 3.2).

Effect of Prior Exercise

At all testing times on the training day, prior high intensity exercise resulted in elevated LBF at both 0W and 52W kicking exercise compared to kicking transitions prior to and post-training when kicking exercise was not preceded by high intensity cycling exercise (Figure 3.3). Prior high intensity cycling exercise elevated VC_{fa} during 52 W kicking at all time points and elevated VC_{fa} during 0 W kicking at hours 1 and 16. The kinetic response of LBF at the onset of kicking exercise was, however, not affected by prior high intensity exercise as evidenced by no change in the MRT on training day versus both pre- and post-training (Figure 3.4, Table 3.3).

Table 3.1. *Ramp kicking test results and step test work rates, oxygen uptakes and percentage of ventilatory threshold for each subject.*

subject	Ramp Kicking Test				Kicking tests		
	WR_{peak}	$\dot{V}O_{2peak}$	HR_{peak}	TVENT	WR	$\dot{V}O_2$	%TVENT
	(W)	(mL/min)	(bt/min)	(mL/min)	(W)	(mL/min)	(%)
1	195	2321	153	1409	55	1039	74
2	135	1730	155	1234	50	1129	91
3	165	2327	144	1500	50	1219	81
4	225	2416	163	1795	60	1186	66
5	165	2082	142	1329	45	1062	80
6	150	2044	175	1341	50	1200	89
avg	172	2153	155	1434	52	1139	80
±SE	±13	±104	±65	±81	±2	±31	±4

Average values are means ± SE for 6 subjects. WR, work rate; $\dot{V}O_2$, oxygen uptake, HR, heart rate and TVENT, ventilatory threshold.

Table 3.2. *Average responses during loadless kicking and during the last minute of loaded kicking exercise pre- training, at hours one, eight and sixteen of training and post- training.*

Variable	pre- training	hour 1	hour 8	hour 16	post- training
$\dot{V}O_2$ (mL/min)					
0W	672±12				660±13.8
52W	1140±29				1138±34
HR (bt/min)					
0W	90±3^{a}	106±4^{b}	107±2^{b}	109±5^{b}	88±3^{a}
52W	108±4^{a}	123±5^{b}	128±5^{c}	130±6^{c}	109±6^{a}
MAP (mmHg)					
0W	116±2^{a}	97±9^{b}	113±7^{a}	104±4^{ab}	109±3^{ab}
52W	127±2^{a}	112±4^{c}	123±3^{ab}	114±4^{bc}	118±2^{abc}
MBV (cm/sec)					
0W	33.2±2.4^{a}	39.8±2.4^{b}	41.2±1.5^{b}	45.0±2.0^{c}	34.6±1.6^{a}
52W	60.5±2.7^{a}	66.7±1.4^{a}	76.7±3.6^{b}	74.8±3.8^{b}	59.6±2.7^{a}
diameter (mm)					
0W	9.74±0.23	10.10±0.20	9.95±0.16	9.62±0.24	9.91±0.16
52W	9.74±0.22	9.98±0.17	9.97±0.15	9.69±0.23	9.92±0.16
LBF (mL/min)					
0W	1487±132^{a}	1902±104^{b}	1920±64^{b}	1958±91^{b}	1607±80^{a}
52W	2700±141^{a}	3142±71^{b}	3609±210^{c}	3300±169^{bc}	2764±146^{a}
VC_{fa} (mL/min/mmHg) 0W	12.9±1.3^{a}	21.1±3.2^{c}	17.3±1.3^{abc}	19.0±1.2^{bc}	14.9±1.1^{ab}
52W	21.3±1.1^{a}	28.2±1.1^{b}	29.3±1.8^{b}	28.9±0.7^{b}	23.6±1.5^{a}

$\dot{V}O_2$, oxygen uptake; HR, heart rate; MAP, mean arterial pressure; MBV, mean blood velocity; diameter, femoral artery diameter; LBF, leg blood flow, VC_{fa}, femoral artery vascular conductance. Values are mean ± SE for 6 subjects. Different letter subscripts indicate differences throughout training $p \leq 0.05$.

Table 3.3. *Kinetic fitting parameters for alveolar $\dot{V}O_2$ and LBF during kicking exercise pre- and post- training and for LBF during kicking exercise on the training day*

		MRT	TG	G0	G1	TD1	τ1	G2	TD2	τ2	G3	TD3	τ3
LBF													
	pre - training	70.8 ±28.8	1302 ±133	1497 ±128	4342 ±2391	2.9 ±1.9	16.1 ±5.4	-3292 ±2456	24.4 ±7.6	39.9 ±8.7	379 ±200 (n=4)	191.8 ±23.2	80.6 ±50.6
	post-training	53.2 ±29.7	1208 ±111	1593 ±79	1395 ±519	0.8 ±0.5	25.0 ±5.7	-330 ±595	35.2 ±8.3	44.9 ±11.6	288 ±230 (n=3)	162.3 ±13.8	104.9 ±53.3
	Hr 1	56.2 ±29.7	1342 ±107	1890 ±98*	957 ±343	1.5 ±0.5	13.6 ±5.1	253 ±278	37.9 ±13.8	18.4 ±3.6			
	Hr 8	73.3 ±38.9	1808 ±327	1902 ±70*	1070 ±279	2.5 ±0.7	14.5 ±4.5	739 ±300	65.1 ±26.9	95.0 ±60.4			
	Hr 16	64.4 ±40.0	1575 ±211	1987 ±123*	969 ±239	4.9 ±4.6	14.3 ±4.0	606 ±262	60.9 ±18.4	113.7 ±68.6			
$\dot{V}O_2$													
	pre	65.1 ±24.8	477 ±41	672 ±10	185 ±41	2.7 ±1.2	14.5 ±5.4	292 ±31	26.1 ±3.2	83.5 ±54.5			
	post	52.9 ±13.9	480 ±30	661 ±12	300 ±54	1.7 ±1.4	19.3 ±4.6	182 ±41	27.2 ±2.3	78.4 ±40.9			

Values are means ± SE for 6 subjects. HR 1 , hour 1; HR 8, hour 8; HR 16, hour 16; MRT, mean response time; TG, total gain; G0 baseline resting value; G*n*, gain; TD*n*, time delay; τ*n*, time constant, LBF, one leg blood flow; $\dot{V}O_2$, alveolar oxygen uptake. *significant difference from pre- training $p<0.05$.

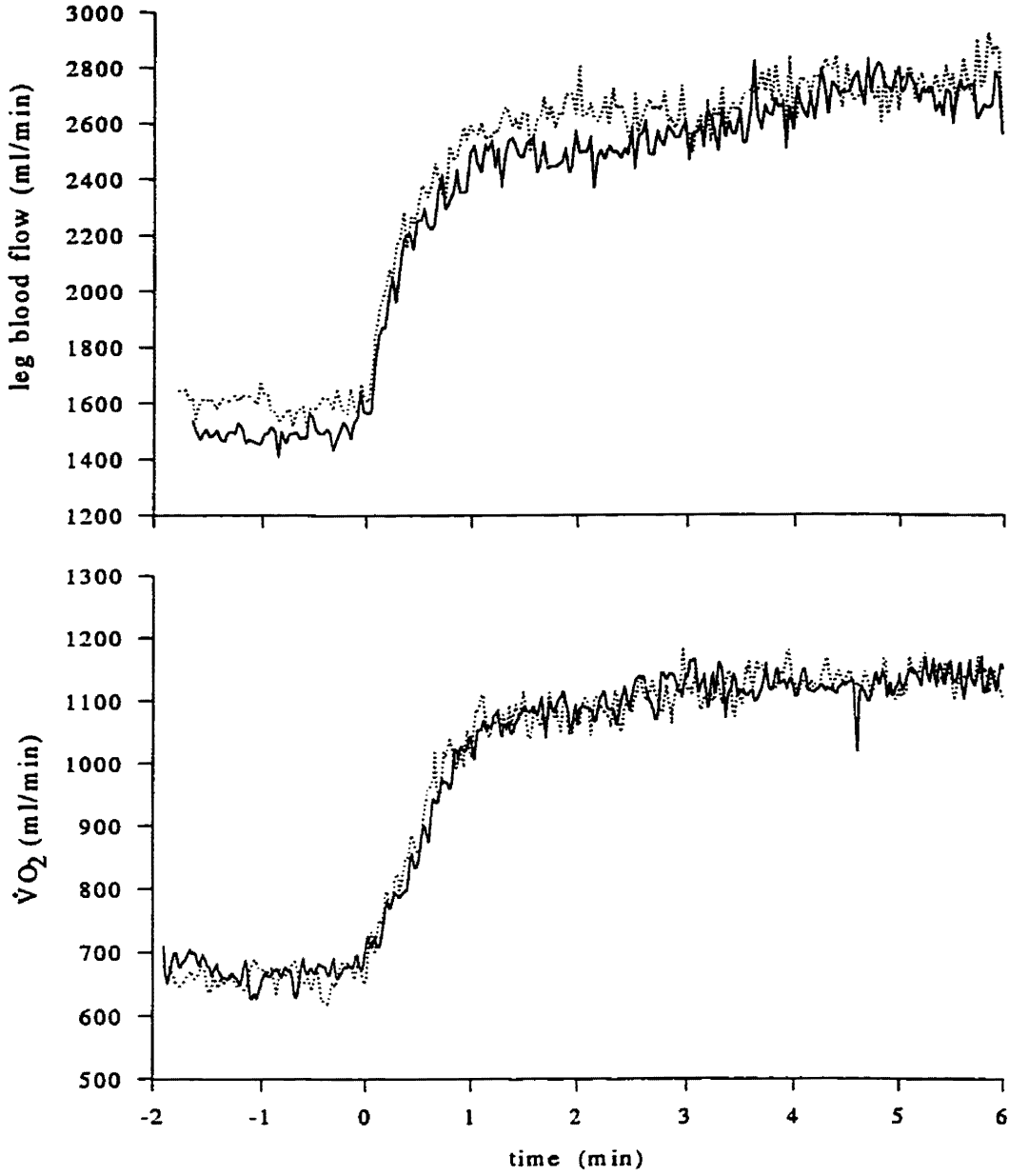


Figure 3.1 *Time course of changes in leg blood flow (LBF, top) and O_2 uptake ($\dot{V}O_2$, bottom) during kicking exercise prior to (____) and post- (_ _ _) training day. Time 0 indicates the onset of loaded kicking exercise. Values are means for 6 subjects.*

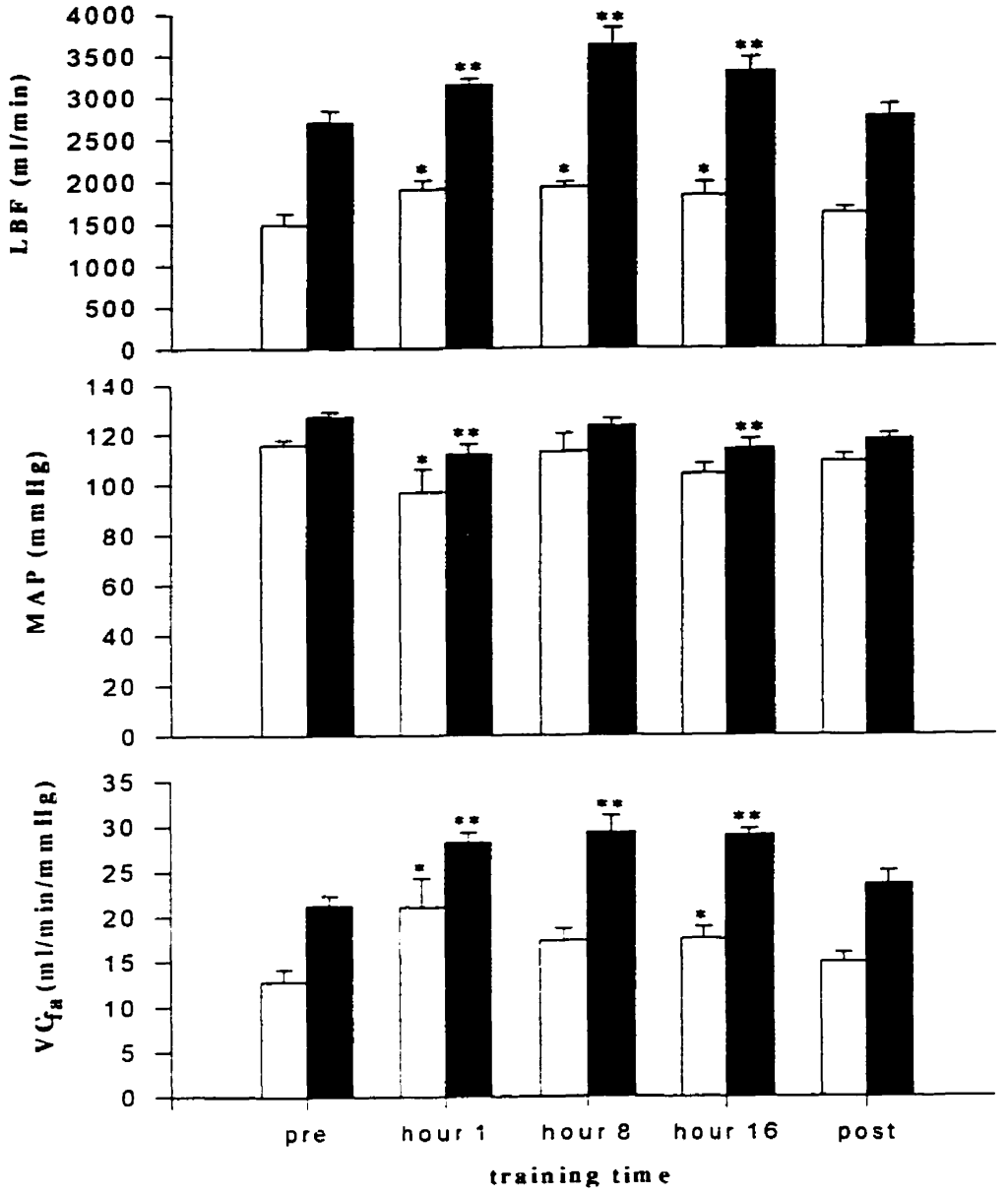


Figure 3.2 *Steady state exercise response of leg blood flow (LBF, top), mean arterial pressure (MAP, middle), and femoral artery vascular conductance (VC_{fa}, bottom) during 0W (open) and 52 W (filled) kicking exercise, pre-training (pre), post-training (post), and after hours 1,8, and 16 or intermittent cycle exercise. Values are means ± SE for 6 subjects. * significantly different from pre at 0W and ** significantly different from pre at 52 W, $p < 0.05$.*

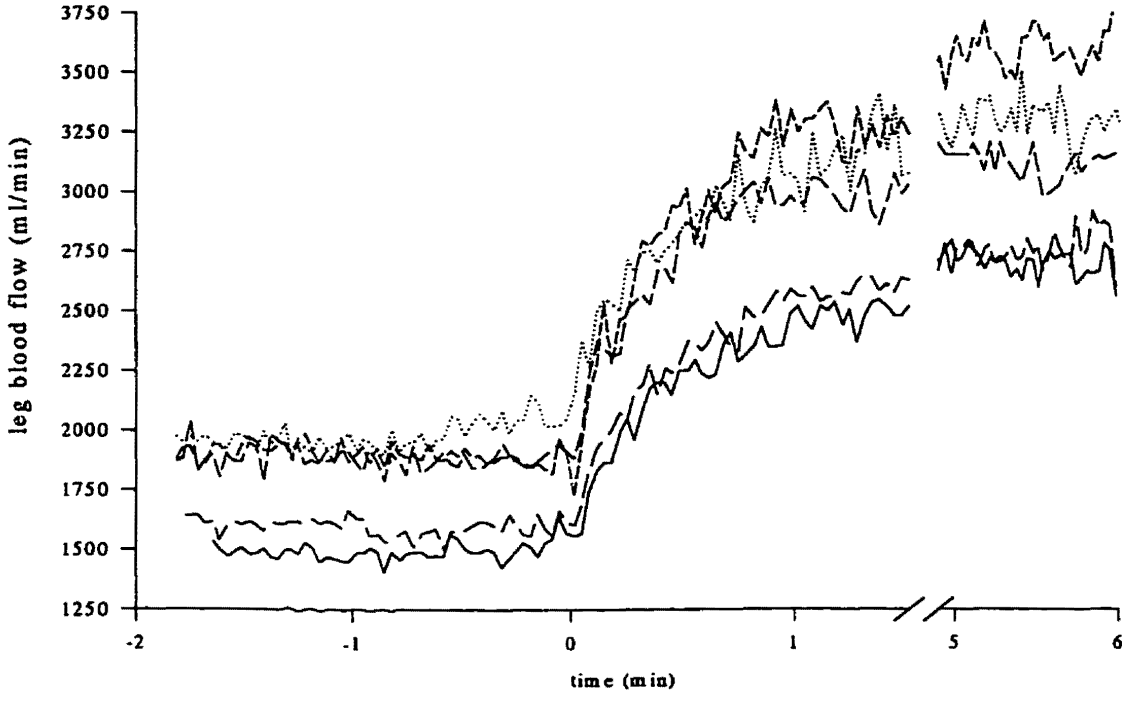


Figure 3.4 *Time course of changes in leg blood flow during kicking exercise prior to training (____), post- training (_ _ _), and on the training day after high intensity cycling exercise at hours 1 (— . —), 8 (—..—), and 16 (.....). Time 0 indicates the onset of loaded kicking exercise. Values are means for 6 subjects.*

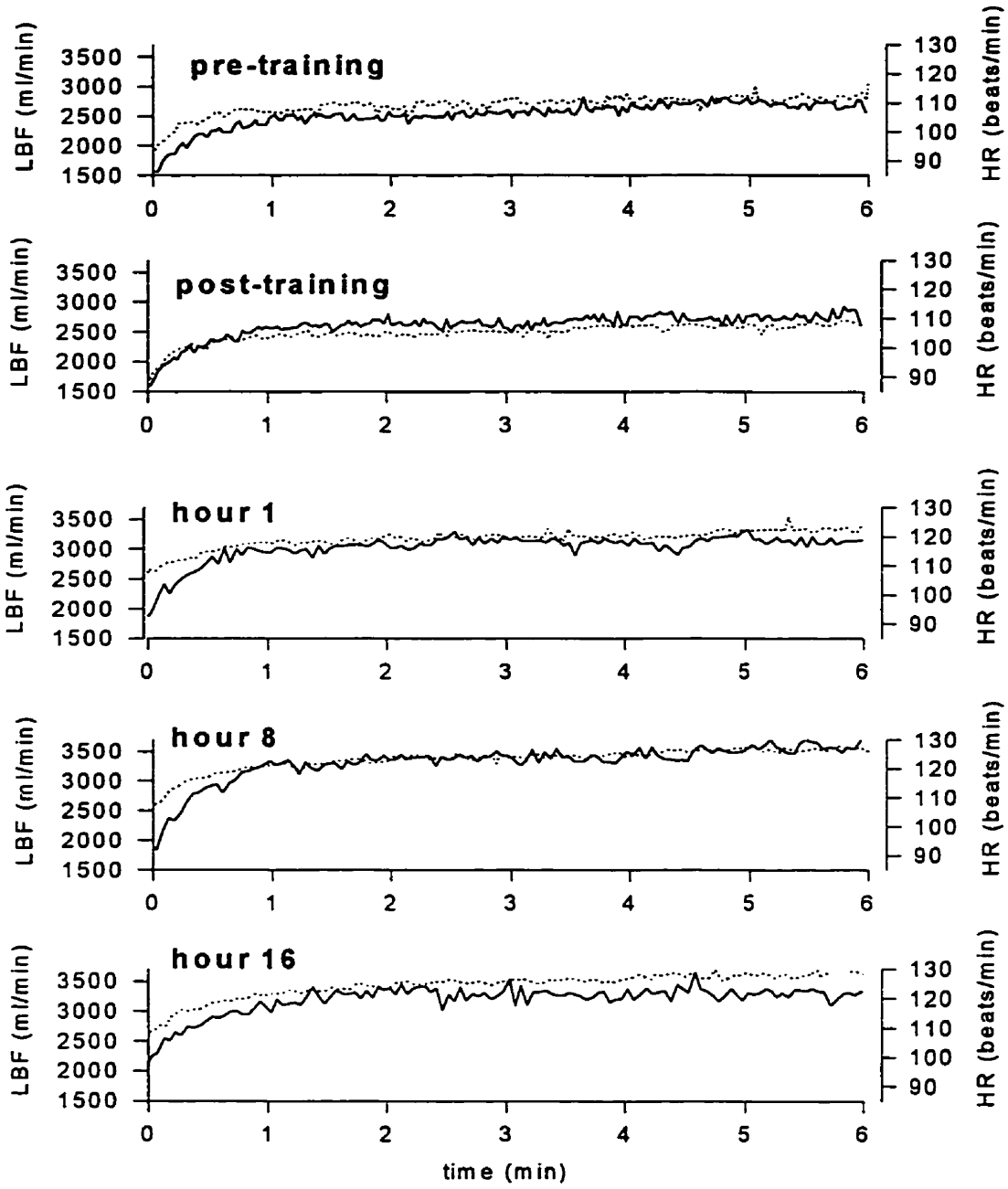


Figure 3.5 *Relationship of leg blood flow (LBF, ____) to heart rate (HR, ...) during kicking exercise prior to training, post- training, and on the training day after high intensity cycling exercise at hours 1, 8, and 16. Time 0 indicates the onset of loaded kicking exercise. Values are means for 6 subjects.*

DISCUSSION

The major finding of this study was that prior high intensity cycling exercise resulted in an increase in the blood flow and vascular conductance in the exercising legs during 0W and 50W kicking exercise. The single day of intermittent exercise training performed in this study did not affect the steady state or transient leg blood flow and $\dot{V}O_2$ responses during step increases in kicking exercise post- training compared to prior training.

Effect of Training

It has previously been observed that endurance training results in faster adaptations to steady state in blood flow (Shoemaker *et al.*, 1996) and $\dot{V}O_2$ (Cerretelli *et al.*, 1979; Hagberg *et al.*, 1980; Hickson *et al.*, 1978; Phillips *et al.*, 1995; Zhang *et al.*, 1991). It is likely that the single day of intermittent exercise performed in this study was not sufficient to elicit effects on either oxygen delivery or muscle metabolic potential. Previous examinations which have shown alterations in $\dot{V}O_2$ and LBF adaptations at the onset of exercise, involved considerably longer training protocols of 5 days to several weeks.

The mean response times obtained from the LBF and $\dot{V}O_2$ kinetic fitting procedure resulted in slower kinetics compared to previous studies of exercise below TVENT. Two and sometimes three phases of the blood flow response have been described previously (Eriksen *et al.*, 1990; Shoemaker *et al.*, 1996) and several of the LBF responses obtained in the present study resulted in long time constants for the second and third phases of the response.

The measurement of LBF could be influenced by any number of potential methodological sources of error. One possible source of error is variability in subjects performance of the kicking

exercise. To counter this source of variability, subjects were familiarized with the exercise twice before any testing and were continually provided visual feedback with respect to kicking frequency. There was no apparent reduction in HR during the kicking exercise (Figure 3.5) so variability in kicking performance seems unlikely. Another source of error is measurement variability. However, this method of blood flow measurement has been used previously in our lab with repeatable results (Shoemaker *et al.*, 1994; Shoemaker *et al.*, 1996). Probe placement was adjusted to optimize the signal at all times and the quality of the signal during each heart beat was also assessed during data analysis.

Prior High Intensity Exercise

Previous studies have reported that prior high intensity exercise results in accelerated adaptations of O_2 uptake at the onset of a subsequent increase in work rate to above, but not below TVENT (GERBINO *ET AL.*, 1996; WILSON *ET AL.*, 1988). The authors suggested that the residual metabolic acidemia from the high intensity warm-up resulted in vasodilatory effects on the exercising muscle and consequently increased muscle perfusion during exercise (Gerbino *et al.*, 1996). Another possible effect of prior exercise includes an improvement in the diffusional gradient between capillary and muscle (Boning *et al.*, 1991; Gerbino *et al.*, 1996). The ability to accelerate above TVENT $\dot{V}O_2$ kinetics with prior exercise was interpreted as support for an O_2 delivery limitation at the onset of exercise above TVENT.

In the present study prior high intensity exercise resulted in an increase in VC_{f_a} at all time points on the training day, confirming previous speculation about possible increases in muscle perfusion to exercising muscle after prior exercise. The mechanism for these increases in LBF is

not known. Metabolites from the active skeletal muscle may play a role in regulation of blood flow during exercise (Laughlin and Armstrong, 1983). Although prior high intensity exercise increased the vascular conductance during steady state exercise there was no effect on the rate of adjustment to steady state after a step transition in work rate. Increases in blood flow suggest increased O_2 delivery to the exercising muscle, however, the potential impact on $\dot{V}O_2$ kinetics was not determined because the equipment was not available to make these measurements.

The effect of prior exercise on $\dot{V}O_2$ kinetics is not limited to exercise performed within the muscle which performed the initial exercise bout. Studies have shown that there is a remote site effect, which results in accelerated $\dot{V}O_2$ kinetics with prior exercise (Bohnert, Ward, & Whipp. 1993; Pendergast *et al.*, 1983). This supports the hypothesis that the prior exercise results in increases in O_2 delivery and subsequently increases in the rate of O_2 utilization at the onset of exercise.

O_2 availability may not be a regulating factor in some exercise situations, such as normoxic cycling exercise below TVENT (Gerbino *et al.*, 1996; Hughson, 1990). The design of this study did not permit determination of the $\dot{V}O_2$ on the training day. Therefore we were restricted to observations of the effect of prior exercise on LBF and VC_{fa}. Further examinations are necessary to determine if alterations in LBF and VC_{fa} due to prior high intensity exercise result in changes in $\dot{V}O_2$ kinetics for kicking exercise below TVENT. Therefore it is not known if the increase in blood flow observed in the kicking exercise resulted in increases in $\dot{V}O_2$ kinetics during the exercise transition.

We have demonstrated that prior heavy exercise increased VC_{fa} and LBF in steady state

kicking exercise below TVENT. However, a single day of intermittent exercise had no effect on the responses at the onset of step increases in work rate to below TVENT. These data support the hypothesis that prior high intensity exercise resulted in elevations in muscle perfusion and possibly oxygen delivery. These increases in vascular conductance and muscle perfusion appear to be mediated by the metabolic acidosis of the prior exercise bout and may have important consequences for regulation of oxygen utilization at the onset of exercise.

CHAPTER IV

Effect of hyperoxia and hypoxia on oxygen uptake and leg blood flow responses to submaximal leg exercise

ABSTRACT

The purpose of this study was to determine the effect of hyperoxic and hypoxic gas breathing on the responses of alveolar ($\dot{V}O_2$alv) and leg oxygen uptake ($\dot{V}O_2$ mus) and leg blood flow (LBF) at the onset of submaximal kicking exercise. $\dot{V}O_2$alv, $\dot{V}O_2$ mus and LBF responses were determined in normoxic ($FIO_2 = 0.21$), hypoxic ($FIO_2 = 0.14$) and hyperoxic ($FIO_2 = 0.70$) gas breathing conditions. Eight healthy subjects performed transitions in leg kicking exercise from rest to 50 ± 3W. Blood flow was measured continuously with pulsed and echo Doppler methods, $\dot{V}O_2$alv was measured breath-by-breath at the mouth and, in 6 subjects, $\dot{V}O_2$mus was determined from LBF and radial artery and femoral vein blood samples. Although there were significant between condition differences in arterial and venous O_2 contents, none of O_2 delivery, $\dot{V}O_2$alv, $\dot{V}O_2$mus or LBF was affected by altered gas breathing at rest or at any of the exercise time points. Similarly, the dynamic response of $\dot{V}O_2$alv and LBF did not differ in the different gas conditions. Hyperoxia or hypoxia did not affect the dynamic responses at the onset of leg kicking exercise below TVENT within the range of altered gas breathing conditions used in this study. During rest and exercise, the arterial O_2 content (CaO_2) was reduced by hypoxia and increased by hyperoxia relative to normoxia, but these changes were combined with small, non-significant, changes in LBF such that oxygen (O_2) delivery to the legs was not different between gas conditions. The finding of similar $\dot{V}O_2$mus responses at the onset of exercise for all gas conditions demonstrated that physiological adaptations in blood flow and O_2 extraction were possible, in this study of submaximal exercise, to counter significant alterations in arterial O_2 content.

INTRODUCTION

Alterations in arterial partial pressure of oxygen (PO_2) have been used extensively in exercise physiology as a method of attempting to alter the O_2 delivery to the working muscle during exercise (Bredle *et al.*, 1988; Crawford *et al.*, 1997; Hughson *et al.*, 1993; Knight *et al.*, 1996; Knight *et al.*, 1993; Koskolou *et al.*, 1997; Linnarsson, 1974; Linnarsson *et al.*, 1974; Richardson *et al.*, 1995; Rowell *et al.*, 1986; Welch *et al.*, 1977; Wilson *et al.*, 1975). Many of studies have focussed on the role of O_2 delivery at maximal exercise (Knight *et al.*, 1996; Knight *et al.*, 1993; Koskolou *et al.*, 1997; Richardson *et al.*, 1995; Rowell *et al.*, 1986). However, it has been suggested that the effects of altered gas breathing may be most significant in the non-steady state phase at the onset of exercise (Hughson, 1990). If the limit to oxygen uptake ($\dot{V}O_2$) at the onset of exercise is the body's ability to deliver O_2 to the working muscle, then alterations in the O_2 delivery should affect the observed rate of increase of $\dot{V}O_2$ at the onset of exercise.

It was found previously that hyperoxia was unable to accelerate $\dot{V}O_2$ kinetics at the onset of a step change in work rate below the ventilatory threshold (T_{VENT}) (Hughson and Kowalchuk, 1995; Linnarsson, 1974) but hyperoxic gas breathing accelerated the $\dot{V}O_2$ kinetics at the onset of exercise transitions to work rates above T_{VENT} (MacDonald *et al.*, 1997; Pedersen, 1987). Hypoxic gas breathing was found to either slow (Hughson and Kowalchuk, 1995; Linnarsson, 1974; Linnarsson *et al.*, 1974; Murphy *et al.*, 1989) or not change (Griffiths *et al.*, 1986) $\dot{V}O_2$ kinetics at the onset of step increases in WR below T_{VENT}.

Some researchers have suggested that oxygen delivery to the exercising muscle is not actually increased with hyperoxic gas breathing due the combined effects of increases in arterial

oxygen content and decreases in muscle blood flow (Bredle *et al.*, 1988; Crawford *et al.*, 1997; Welch *et al.*, 1977). Previous research has shown oxygen delivery to be unchanged (Bredle *et al.*, 1988; Hermiston and Bonde-Petersen, 1975; Welch *et al.*, 1977; Wilson and Stainsby, 1978) or increased(Knight *et al.*, 1993; Wolfe *et al.*, 1987) with hyperoxia and decreased (Richardson *et al.*, 1995) or unchanged (Welch *et al.*, 1977) with hypoxia at submaximal exercise levels.

In light of these observations, determinations of the dynamic response of $\dot{V}O_2$alv , leg blood flow (LBF), leg $\dot{V}O_2$ ($\dot{V}O_2$mus) and O_2 delivery at the onset of exercise will further the understanding of cardiovascular regulatory mechanisms during exercise. We hypothesized that oxygen delivery in the non- steady state adjustment to the onset of exercise below T_{VENT} will be less in hypoxia and unchanged in hyperoxia. In hypoxia, these alterations in oxygen delivery will be reflected in slower muscle and whole body $\dot{V}O_2$ kinetics. The lack of increase in O_2 delivery in hyperoxic gas breathing for exercise below T_{VENT} will be accompanied by no difference in the kinetics of $\dot{V}O_2$ relative to tests in normoxia.

METHODS

Eight healthy male and female volunteers (4 men and 4 women, age 24±1 years, height 176±3 cm, and weight 71±3 kg, mean ● SE) participated in this study. They were not specifically exercise trained and all gave written consent on a form approved by the Office of Human Research and Animal Care at the University of Waterloo.

Exercise Protocol

Subjects reported to the laboratory on three occasions for training on the kicking ergometer to assist in learning the exercise mode. The kicking ergometer is a specially designed piece of exercise equipment that allows electrically braked exercise of both the quadriceps and hamstrings muscle in an alternating "kicking fashion". Subjects remained in the seated position for all tests with a hip angle of 120° and knee extension and flexion between 90° and 135°. The kicking frequency, between 44 and 50 kicks per leg per minute, was maintained via feedback from a visual display metre. The fourth visit to the laboratory involved a progressive kicking test to exhaustion, or functional limit, in hypoxic conditions. The work rate protocol for this progressive test was a 15W/min ramp. The progressive test was stopped when the subject could no longer maintain the kicking frequency or needed accessory muscle assistance. That is, peak work rate was limited by the ability of the knee flexor/extensor muscles. The gas exchange and work rate data from the ramp test were used to estimate the T_{VENT} and peak $\dot{V}O_2$. These values were then used in choosing the individual work rates for the step tests.

The next 4 testing sessions involved the step test protocol which consisted of three identical step transitions in work rate separated by ten minutes of rest and gas accommodation. A

typical step test involved gas accommodation at rest for five minutes followed by periods of data collection at rest for four minutes and during six minutes of kicking at a work rate set to be equivalent to the work rate at 85% of hypoxic T_{VENT}. The flywheel of the kicking ergometer was manually accelerated, by a laboratory assistant, for 30 seconds prior to the onset of exercise to allow the subjects to begin working at the appropriate work rate. Otherwise, no warning was given to the subject prior to the start of exercise although they were aware of the protocol before the test.

The three possible gas breathing conditions were determined by the gas inhaled during each gas transition. One of the experimental gas mixtures of normoxia (room air), hyperoxia (70% O_2, balance N_2) and hypoxia (14% O_2, balance N_2) was breathed on each testing day in a randomized order with each subject performing a step test three times in each gas breathing condition.

Data Collection:

Breath by breath ventilation and gas exchange was measured on a computerized system (First Breath, St. Agatha, Ontario, Canada) with a mass spectrometer (MGA-1100A, Marquette, WI) and the digital volume turbine (VMM-110, Alpha Technologies, Laguna Beach, CA) or ultrasonic flowmeter (UF202, Kou & Assoc. Redmond,WA). For the hyperoxic and hypoxic tests a large Tissot tank was filled with inspiratory gases from cylinders containing the 70% and 14% O_2 mixtures respectively. The Tissot tank was connected to a Y-valve to permit inspiration from the tank. The barometric pressure, temperature and water vapour pressure were measured

before each test. Matching of fractional gas concentrations with the appropriate volume was done by accounting for the sum of the transport lag plus the instrument response time. The mass spectrometer was calibrated for normoxia, hypoxia and hyperoxia using 2 precision gas mixtures that spanned the anticipated fractional gas concentrations in each of the gas conditions. Volume was calibrated by manually pumping a 3 litre syringe at a flow rate similar to that of respiration during the exercise test. $\dot{V}O_2$ was corrected on a breath-by-breath basis for changes in lung gas stores due to altered lung volume or alveolar composition, as described previously (Hughson *et al.*, 1991).

Leg blood flow was determined from measures of femoral artery diameter by echo Doppler ultrasound (model SSH-140A, Toshiba Inc., Tochigi-Ken, Japan) and femoral artery mean blood velocity via pulsed Doppler ultrasound (model 500V, Multigon Industries, Mt. Vernon, NY) during the progressive exercise test as well as the 3 of the step tests in each gas breathing conditions. A 7.5 MHZ hand held Linear array probe was held approximately 2 cm distal to the inguinal ligament during the kicking tests to continuously monitor the femoral artery diameter. The imaged data were stored on videotape. Measures of femoral artery diameter were obtained from the video recordings of the arterial image. These measures were obtained 5 times at rest, at 10 second intervals during the first minute of exercise and each minute thereafter. A 4MHz pulsed Doppler probe was be held on the opposite leg for beat by beat measurements of femoral artery velocity spectra. The spectrum was processed continuously via a mean velocity processor (Micco, 1989) and collected on a computer based system at 100 Hz along with heart rate and blood pressure. Calibration signals in the Doppler shift frequency range were generated

from the Doppler signal processor. The mean blood velocity (MBV) for each trial was obtained through integration of the area under the curve for each heart beat. The three trials in each gas condition were then ensemble averaged to yield a single MBV data set for each subject in each gas condition. The average diameter data for each gas condition were fit with a linear or exponential regression to obtain an average response and reduce random error. Mean blood flow to the leg (LBF) was calculated on a beat-by-beat basis by multiplying the average mean blood velocity with the estimated diameter from the regression equation for each time point.

Blood pressure was obtained by using a pneumatic finger cuff (Ohmeda 2300, Finapres, Longwood, CO). The hand was positioned so that the finger cuff was at the level of the Doppler probe to indicate perfusion pressure.

Kinetic Analysis: Breath-by-breath values for $\dot{V}O_2$ were linearly interpolated between breaths to give values at 1 second intervals. The time course data for $\dot{V}O_2$ collected from three trials in each gas breathing condition, were ensemble averaged to produce a single data set for each variable, for each subject, in each gas condition.

The time course of changes in $\dot{V}O_2$ and LBF were analysed by fitting an exponential curve to the average results of the trials. A two or three component exponential model was fit to the data using a least square procedure. As previously described (Hughson and Kowalchuk, 1995), the two component model had a baseline component (G0), two amplitude terms (G1 and G2), two time constants (τ1 and τ2) and two time delays (TD1 and TD2)

$$Y(t) = G0 + G1\,(1 - e^{-(t - TD1)/\tau 1}) \cdot u1 + G2\,(1 - e^{-(t - TD2)/\tau 2}) \cdot u2$$

where,

$u1 = 0$ for $t < TD1$ and $u1 = 1$ for $t \geq TD1$

$u2 = 0$ for $t < TD2$ and $u2 = 1$ for $t \geq TD2$

Y(t) is the time dependent variation in $\dot{V}O_2$ or LBF.

Some of the responses were fit to a three component model. The three component model contained an extra amplitude term (G3) and time constant ($\tau 3$) in order to fit the slower adaptive phase in these tests.

$$\dot{V}O_2(t) = G0 + G1\,(1 - e^{-(t - TD1)/\tau 1}) \cdot u1 + G2\,(1 - e^{-(t - TD2)/\tau 2}) \cdot u2 + G3\,(1 - e^{-(t - TD3)/\tau 3}) \cdot u3$$

where, u1 and u2 were as defined above and

$u3 = 0$ for $t < TD3$ and $u3 = 1$ for $t \geq TD3$

The overall time course of the response was determined from mean response time (MRT). The MRT is the time it takes to reach approximately 63% of the total amplitude of the response from the baseline to the final plateau value. It was calculated as a weighted sum of the time delay and time constant for each component (Hughson *et al.*, 1993b).

$$MRT = (G1/(G1 + G2 + G3)) \cdot (TD1 + \tau 1) +$$
$$(G2/(G1 + G2 + G3)) \cdot (TD2 + \tau 2) +$$
$$(G3/(G1 + G2 + G3)) \cdot (TD3 + \tau 3)$$

Steady-state analysis

Rest and exercise average values were obtained for each of LBF, $\dot{V}O_2$, diameter, HR, MAP and MBV. These values were averages of one minute of data in each gas condition at rest and during the last minute of exercise.

Blood Samples

Six of the eight subjects also completed testing sessions which involved femoral venous and radial arterial blood sampling during the exercise transitions. For each of these subjects one exercise transition in each gas condition was performed to obtain measurements of blood gas and metabolic responses.

On one testing day subjects reported to the laboratory 30 minutes prior to testing and had catheters inserted. The order of the conditions was randomized.

A 1.5 inch, plastic, radial artery catheter (20Ga, Angiocath, Becton-Dickinson, Sandy, Utah) was inserted into the left radial artery under local anaesthetic (Lidocaine HCL, 2%, Astra, Mississauga, ON). Patency was maintained with a pressurized flush system (0.9%NaCl with sodium heparin 500I.U./500mL NaCl; approximately 15mL/hour). Subjects lay supine and a 16cm plastic catheter was inserted two cm below the inguinal ligament into the left femoral vein under local anaesthetic (Lidocaine HCL, 2%, Astra, Mississauga, ON). In one subject the catheter was inserted in the right femoral vein. The catheter was fixed to the skin and flushed regularly with normal saline. The position of the catheter was confirmed to be within the femoral vein approximately three cm proximal to the inguinal ligament in a subset of the subjects with ultrasound imaging. The catheter was kept patent with a continuous drip of saline (0.9%NaCl).

After the usual gas accommodation period, venous blood samples were obtained two times during rest, at twenty and forty seconds during the first minute of exercise and at the first, third and fifth minutes of the exercise transition. Arterial blood samples were obtained two times during rest, and during the first, third and fifth minutes of the exercise transition. During these tests, femoral artery mean blood velocity, heart rate and blood pressure, but not femoral artery

diameter were monitored.

In each experimental condition one mL samples were collected in heparinized syringes. These samples were immediately but gently agitated and stored in an ice bath. Within one hour of collection, all whole blood samples were analysed for PO_2, PCO_2, haematocrit by selective electrodes in a blood gas-electrolyte analyser (NovaStat Profile Plus 9, Waltham,MA). The analyser was calibrated at regular intervals during the analyses. O_2 saturation and content were obtained from the output of the analysis system after application of standard equations.

Blood Data Analysis

Arteriovenous O_2 difference (a-vDO_2) was calculated from the difference in radial artery O_2 content (CaO_2) and femoral venous O_2 content (CvO_2). This difference was then divided by arterial O_2 content to give leg O_2 extraction. Arterial blood samples were not obtained during the first minute of exercise. It was determined that the exercise CaO_2 values were not different so the average exercise CaO_2 was used as the arterial value in the calculations for 20 and 40 seconds after the onset of exercise.

Leg $\dot{V}O_2$ mus was calculated as the product of the arterial venous O_2 difference and leg blood flow for each time point of blood sampling. To obtain estimates of blood flow at the same time as the blood samples, the best fit of the LBF response was used to calculate blood flow at the time points corresponding to the times of the blood samples. These best fits were obtained from the average of 3 trials from the separate testing day as described above. It was necessary to use these flow values due to the fact that only MBV and not diameter was monitored on the day of the blood sampling. In order to determine that the exercise responses were the same on the two

different testing days the MBV response and the heart rate and $\dot{V}O_2$ alv responses at rest and end exercise were compared on the regular testing day to the blood sample testing day. No differences were found and therefore it was found appropriate to use the previous LBF values. Leg O_2 delivery was calculated as the product of LBF and CaO_2.

Statistical analysis

The effect of different gas breathing conditions on the rest and exercise steady state values for LBF, $\dot{V}O_2$, HR, MAP and MBV and the kinetics of the blood flow and $\dot{V}O_2$ alv responses were analysed by a one-way repeated measures analysis of variance. The main effects of gas breathing (three levels of the variable) and time (seven levels of the variable) on the time course data for femoral artery diameter, LBF, $\dot{V}O_2$ mus, $\dot{V}O_2$ alv, leg O_2 delivery, CaO_2, CvO_2, a-vDO_2, and leg O_2 extraction were analysed by a two way repeated measures analysis of variance with gas condition and time forming the 2 dependent variables. The level of significance for the main effects and interactions was set at $P< 0.05$. If a significant interaction was present (gas x time) then pairwise comparisons were performed by examining the simple effects. Any differences were further analysed with Student Neuman-Keuls post hoc test. All data are presented as mean ± standard error (SE).

RESULTS

The peak work rate obtained in the hypoxic ramp kicking tests was 131±10W, the peak hypoxic $\dot{V}O_2$ was 1913±109 and TVENT 1394±75 was (Table 4.1). The exercise work rate used for all other tests was 50±3W and corresponded to 93% of TVENT in normoxia, 97% in hyperoxia and 93% in hypoxia (mean ± SE for 8 subjects) (Table 4.1).

Dynamic responses of $\dot{V}O_2$alv and LBF

No difference was found between any of the gas breathing conditions for MRT or any of the kinetic fitting parameters for either $\dot{V}O_2$alv or LBF (Table 4.2, Figure 4.1).

Steady state responses

There were no differences in any of LBF, MBV or $\dot{V}O_2$alv at rest or during the last minute of exercise between any of the gas breathing conditions (Table 4.3, Figure 4.2). Rest and exercise HR was higher for hypoxia than for normoxia or hyperoxia (Table 4.3, Figure 4.2). Resting MAP was higher for hypoxia compared to hyperoxia and exercise MAP was higher in hypoxia versus both normoxia and hyperoxia. Resting and exercise femoral artery diameters were smaller in hyperoxia compared to normoxia and hypoxia and there was no difference between rest and end exercise diameter for any of the gas conditions (Figure 4.2, Table 4.3).

Blood ride analysis

The response of MBV, HR and $\dot{V}O_2$ alv was similar on the day of the blood collection trials versus the regular testing days (Figure 4.3).

CaO_2 was elevated in hyperoxia and attenuated in hypoxia at all time points during rest and exercise and did not change throughout the testing protocol (Table 4.4, Figure 4.4). CvO_2

was lower in hypoxia and higher in hyperoxia compared to normoxia. CvO_2 was also significantly lower than resting levels for all trials at all exercise time points. A-vDO_2 was elevated from resting levels at all exercise times for all gas conditions and a-vDO_2 in hypoxia was lower than normoxia or hyperoxia at all time points (Table 4.5, Figure 4.4). Leg O_2 extraction ,an indicator of % extraction, was not different between normoxic and hypoxic gas conditions but was lower in hyperoxia versus normoxia and hypoxia at 20 seconds and five minutes after the onset of exercise. Leg O_2 extraction was elevated from resting levels from 40 seconds after the onset of exercise to end exercise for all gas breathing conditions.

LBF, VO_2mus, $\dot{V}O_2$alv, and O_2 delivery were elevated from resting levels at all exercise times for all gas conditions (Table 4.5, Figure 4.5). There was no effect of gas on LBF, $\dot{V}O_2$mus, $\dot{V}O_2$alv or O_2 delivery at any time point. (Table 4.5, Figure 4.6).

Table 4.1 *Ramp kicking test results and step test work rates, oxygen uptakes and percentage of ventilatory threshold for each subject.*

	hypoxic ramp test				step tests						
subject	peak WR	peak $\dot{V}O_2$	peak HR	hypoxic TVENT	WR	Normoxia (21% O_2)		Hyperoxia (70%O_2)		Hypoxia (14%O_2)	
	(W)	(mL/min)	(bt/min)	(mL/min)	(W)	$\dot{V}O_2$ (mL/min)	%TVENT (%)	$\dot{V}O_2$ (mL/min)	%TVENT (%)	$\dot{V}O_2$ (mL/min)	%TVENT (%)
1	150	2135	156	1549	55	1333	86	1347	87	1278	83
2	120	1578	130	1121	40	1041	93	1136	101	1075	96
3	105	1953	160	1404	45	1347	96	1427	102	1300	93
4	165	1904	138	1392	60	1428	103	1494	107	1375	99
5	90	1434	172	1110	40	1228	111	1225	110	1134	102
6	120	2072	144	1435	50	1217	85	1208	85	1194	83
7	135	1829	168	1382	50	1349	98	1378	100	1417	103
8	165	2402	126	1756	60	1296	74	1420	81	1448	82
avg	131	1913	149	1394	50	1280	93	1329	97	1278	93
±SE	±10	±109	±6	±75	±3	±42	±4	±44	±4	±48	±3

Average values are means ± SE for 8 subjects. WR, work rate; $\dot{V}O_2$, oxygen uptake, HR, heart rate and TVENT, ventilatory threshold.

Table 4.2 *Kinetic fitting parameters for alveolar $\dot{V}O_2$ and LBF in one leg during kicking exercise in normoxic, hyperoxic and hypoxic conditions.*

	FIO_2	MRT	TG	G0	G1	TD1	τ1	G2	TD2	τ2	G3	TD3	τ3
LBF													
	0.21	43.9 ±15.4	3438 ±257	359 ±31	2609 ±596	0.5 ±0.2	6.7 ±3.1	-336 ±1366	16.5 ±2.5	42.5 ±14.0	3106 ±2476 (n=3)	63.5 ±18.9	118.9 ±29.1
	0.70	64.7 ±34.6	3830 ±548	329 ±43	4858 ±2907	0.6 ±0.4	14.8 ±8.1	-3012 ±4165	15.4 ±3.7	42.6 ±13.8	5292 ±4654 (n=2)	68.5 ±22.9	128.8 ±66.6
	0.14	58.3 ±11.4	3860 ±277	367 ±31	2389 ±654	0.9 ±0.3	5.5 ±1.5	250 ±1218	16.1 ±3.2	27.2 ±5.7	1953 ±977 (n=5)	93.8 ±38.4	164.9 ±36.1
$\dot{V}O_2$													
	0.21	51.8 ±7.5	957 ±33	345 ±21	445 ±72	0.4 ±0.2	8.6 ±1.6	363 ±94	21.8 ±3.0	35.9 ±9.0	239 ±51 (n=5)	103.5 ±27.5	115.9 ±21.4
	0.70	58.5 ±13.7	1039 ±54	332 ±14	605 ±120	0.9 ±0.7	11.3 ±3.3	278 ±133	18.5 ±2.7	24.5 ±3.5	212 ±55 (n=6)	117.6 ±40.6	169.9 ±30.2
	0.14	74.5 ±12.6	995 ±36	344 ±12	433 ±54.5	0.2 ±0.1	11.9 ±5.6	399 ±67	20.9 ±3.2	36.8 ±6.1	218 ±38 (n=6)	115.6 ±42.7	191.0 ±32.0

Values are means ± SE for 8 subjects. FIO_2, inspired fraction of O_2; MRT, mean response time; TG, total gain; G0 baseline resting value; G*n*, gain; TD*n*, time delay; τ*n*, time constant, LBF, one leg blood flow; $\dot{V}O_2$, alveolar oxygen uptake.

Table 4.3 *Average values of leg blood flow, alveolar oxygen uptake, heart rate, mean arterial pressure , femoral artery diameter and femoral artery mean blood velocity for 1 minute of rest and the last minute of kicking exercise.*

	rest			exercise		
FIO_2	0.21	0.70	0.14	0.21	0.70	0.14
$\dot{V}O_2$alv (mL/min)	345±21	329±12	345±13	1280±42	1329±45	1278±48
HR (beats/min)	64±3	61±4	72±3*†	108±7	102±6	121±7*†
MAP (mmHg)	112±3	106±3	114±3†	130±3	127±2	139±3*†
LBF (mL/min)	365±34	324±47	365±27	3658±178	3740±169	4026±243
diameter (mm)	9.87±0.52	9.49±0.51*	10.01±0.50†	9.96±0.48†	9.89±0.49	10.18±0.47†
MBV (cm/sec)	8.7±1.4	7.9±1.2	8.2±1.0	80.9±7.0	83.6±6.6	84.4±6.8

Values are mean±SE for 8 subjects. LBF, leg blood flow; $\dot{V}O_2$alv, alveolar oxygen uptake; HR, heart rate; MAP, mean arterial pressure at the level of the femoral artery; diameter, femoral artery diameter; MBV, mean blood velocity. * significantly different from normoxia for the same time (rest or exercise); † significantly different from hyperoxia for the same time.

Table 4.4 *Arterial and venous O_2 content, arteriovenous O_2 content difference and leg O_2 extraction during kicking exercise in normoxic, hyperoxic and hypoxic conditions.*

		Time					
	FIO_2	rest	20s	40s	1min	3min	5min
CaO_2(mL/L)	0.21 †	193.2±5.0	196.8±4.1	196.8±4.1	195.7±4.0	197.3±4.2	197.0±4.2
	0.70 *	205.5±4.1	209.6±5.8	208.3±4.5	208.5±4.6	208.5±5.3	209.9±4.3
	0.14 †*	175.9±5.0	172.3±4.0	172.3±4.0	172.0±5.1	172.7±3.6	172.7±3.4
CvO_2 (mL/L)	0.21 †	131.8±6.7^{a}	113.5±8.3^{b}	96.8±4.3^{c}	91.7±4.6^{c,d}	80.7±4.2^{d}	76.0±4.0^{d}
	0.70 *	142.7±7.8^{a}	137.6±4.3^{b}	110.3±4.6^{c}	106.8±4.1^{c,d}	90.6±5.0^{d}	97.8±4.1^{d}
	0.14 *†	122.7±4.3^{a}	100.0±4.0^{b}	82.0±3.0^{c}	76.2±1.2^{c,d}	68.2±2.0^{d}	64.4±3.2^{d}
a-vDO_2(mL O_2/l)	0.21	61.4±10.2^{a}	83.3±11.7^{b}	100.0±7.6^{c}	104.0±6.8^{c,d}	116.7±5.9^{d}	121.0±5.0^{d}
	0.70	62.8±11.5^{a}	72.0±6.0^{b}	98.0±5.2^{c}	103.2±4.4^{c,d}	117.8±4.0^{d}	112.1±2.8^{d}
	0.14 *†	53.2±7.3^{a}	72.2±6.6^{b}	90.3±6.7^{c}	95.8±5.6^{c,d}	104.5±3.9^{d}	108.2±4.1^{d}
leg O_2 extraction (%)	0.21	31.4±4.6 ^{a}	41.9±5.0 ^{a,b,†}	50.6±2.8 ^{b,c}	53.0±2.7 ^{c}	59.0±2.3 ^{c}	61.4±2.0 ^{c,†}
	0.70	30.1±4.9 ^{a}	34.2±2.3 ^{a,*}	47.0±2.2 ^{b}	49.1±1.6 ^{b}	59.6±1.8 ^{b}	53.5±1.3 ^{b,*}
	0.14	29.9±3.4 ^{a}	41.7±3.0 ^{b,†}	52.2±2.6 ^{c}	55.5±1.6 ^{c,d}	60.4±1.4 ^{d}	62.7±1.9 ^{d,†}

Values are means ±SE for 6 subjects. CaO_2, arterial oxygen content; CvO_2, venous oxygen content; a-vDO_2, arteriovenous oxygen content difference. Different letter superscipts indicate differences between times within a gas breathing condition. * indicates different from normoxia and † indicates different from hyperoxia. When no interactions between gas breathing and time were observed, the main effects for time were indicated at the beginning of the row for each gas breathing condition.

Table 4.5 *Leg blood flow, leg oxygen uptake, alveolar oxygen uptake and leg O_2 delivery during kicking exercise in normoxic, hyperoxic and hypoxic conditions.*

		Time					
	FIO_2	rest	20s	40s	1min	3min	5min
LBF (L/min)	0.21	0.74±0.08[a]	4.86±0.33[b]	6.21±0.39[c]	6.65±0.43[c,d]	7.14±0.38[d]	7.36±0.41[d]
	0.70	0.63±0.10[a]	4.52±0.55[b]	5.99±0.40[c]	6.53±0.39[c,d]	7.19±0.35[d,e]	7.38±0.42[e]
	0.14	0.75±0.08[a]	4.42±0.50[b]	5.98±0.58[c]	6.63±0.60[d]	7.69±0.56[e]	8.02±0.53[e]
$\dot{V}O_2$mus (mL/min)	0.21	45±8[a]	395±44[b]	609±26[c]	678±51[d]	826±35[e]	885±42[e]
	0.70	35±4[a]	302±48[b]	581±32[c]	661±34[d]	844±43[e]	823±35[e]
	0.14	39±5[a]	314±35[b]	530±41[c]	627±48[d]	797±47[e]	862±48[e]
$\dot{V}O_2$alv (mL/min)	0.21	330±22[a]	718±43[b]	962±42[c]	1067±52[d]	1220±53[e]	1243±49[e]
	0.70	318±14[a]	738±35[b]	980±37[c]	1098±44[d]	1257±45[e]	1293±50[e]
	0.14	342±16[a]	703±33[b]	933±39[c]	1052±49[d]	1222±61[e]	1262±65[e]
O_2 delivery(L/min)	0.21	0.14±0.02[a]	0.96±0.06[b]	1.22±0.06[c]	1.29±0.07[c]	1.41±0.06[d]	1.44±0.07[d]
	0.70	0.13±0.02[a]	0.88±0.12[b]	1.24±0.07[c]	1.36±0.07[c,d]	1.49±0.06[d,e]	1.54±0.08[e]
	0.14	0.13±0.01[a]	0.76±0.08[b]	1.02±0.09[c]	1.13±0.09[c]	1.32±0.09[d]	1.38±0.08[d]

Data are means ±SE for 6 subjects; LBF, two leg blood flow ; $\dot{V}O_2$mus, two leg oxygen uptake; $\dot{V}O_2$alv, alveolar oxygen uptake. Times indicate time after the onset of exercise. Different letter superscripts indicate differences between times within a gas breathing condition.

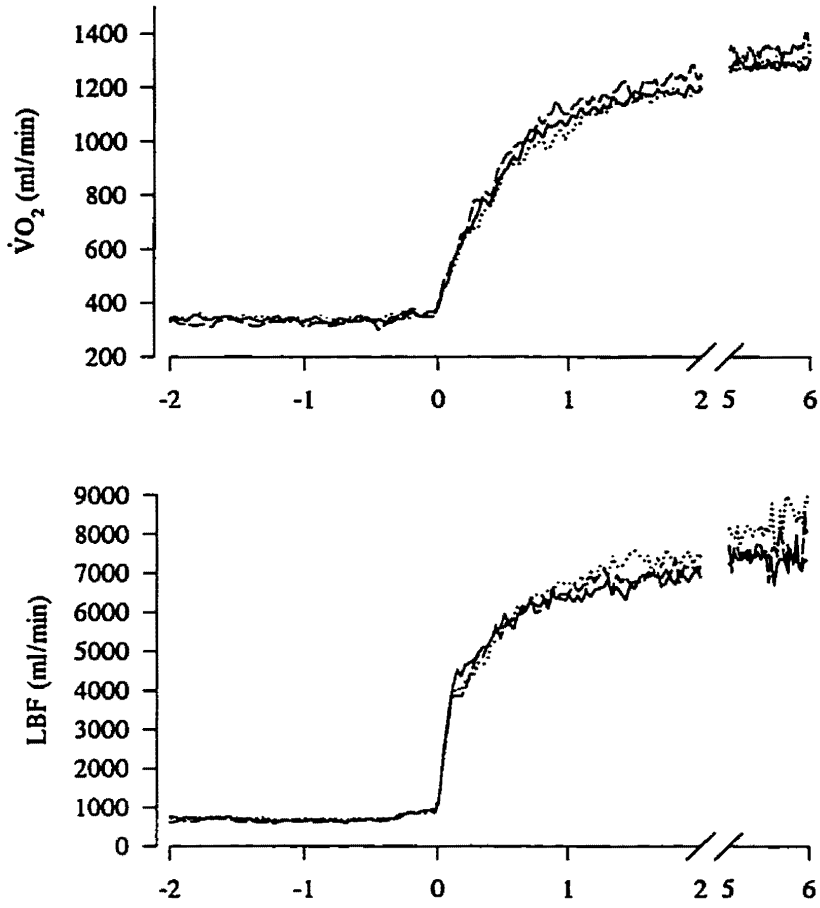


Figure 4.1 *LBF (top) and $\dot{V}O_2$ (bottom) during rest and exercise in normoxia (___), hyperoxia (_ _ _) and hypoxia (....). Time 0 indicates the onset of exercise. Values shown are means for 8 subjects.*

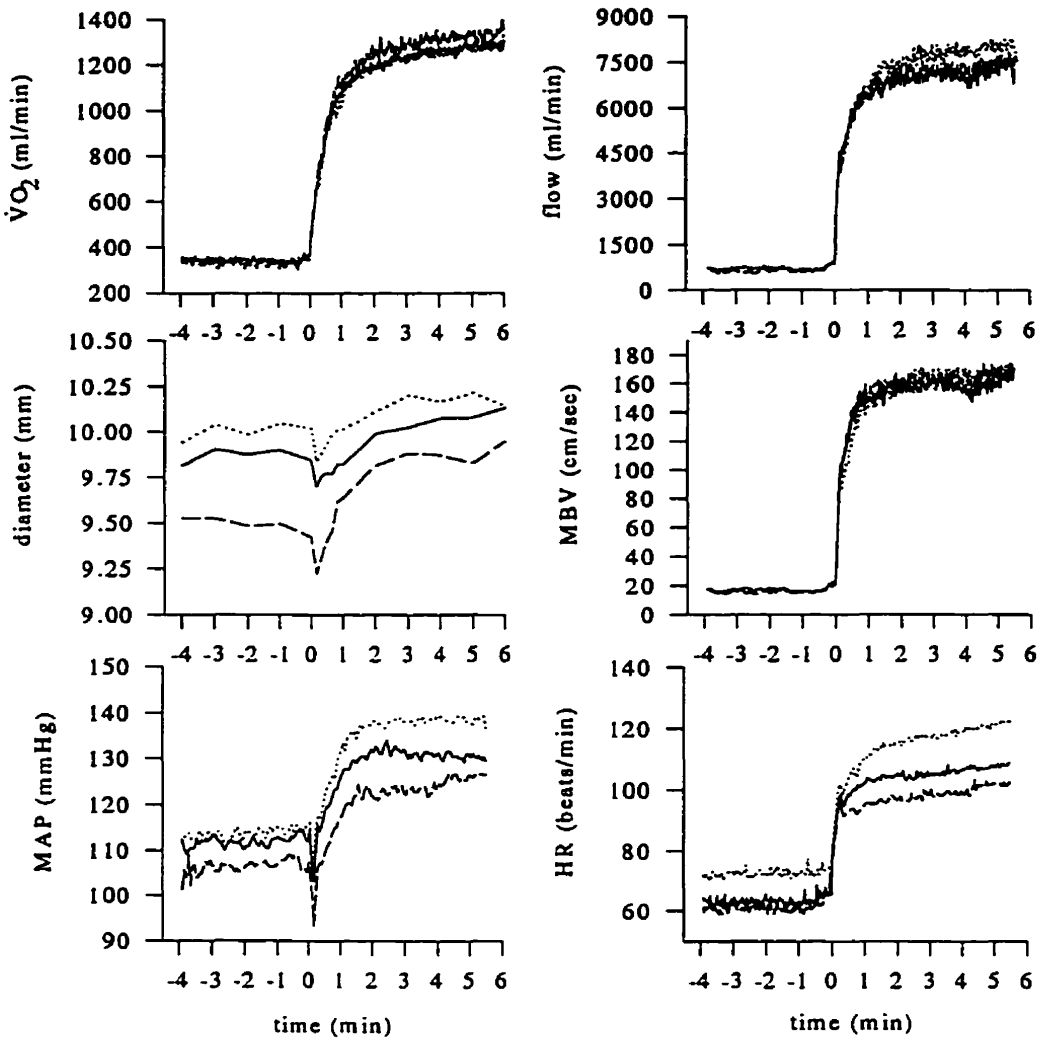


Figure 4.2 *Time course of changes in alveolar O_2 uptake ($\dot{V}O_2$alv; top left), leg blood flow(LBF; top right), femoral artery diameter (diameter; middle left), femoral artery mean blood velocity (MBV; middle right), mean arterial pressure at the level of the femoral artery (MAP; bottom left), and heart rate (HR; bottom right) are shown for normoxic (___), hyperoxic(_ _ _) and hypoxic (....) gas breathing conditions. Time 0 indicates the onset of exercise. Values shown are mean for 8 subjects during the regular testing sessions.*

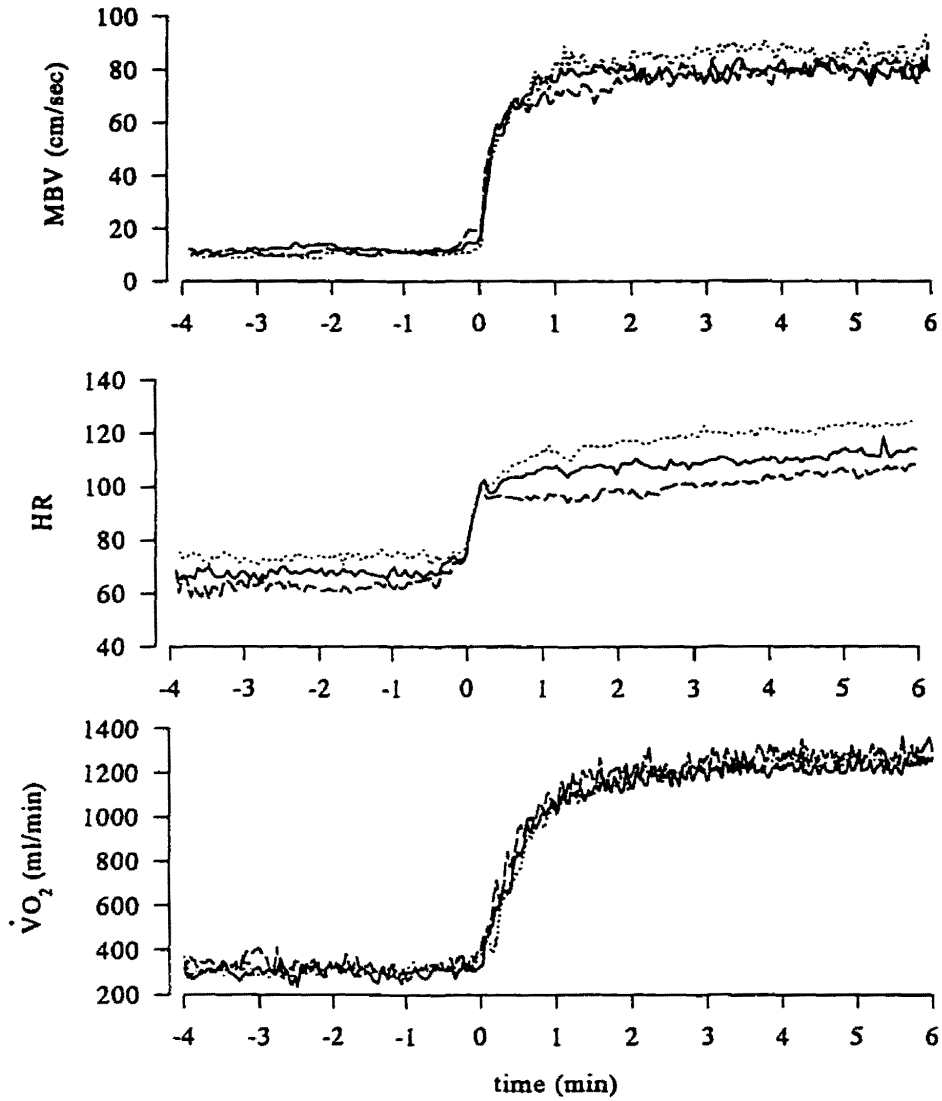


Figure 4.3 *Time course of changes in femoral artery mean blood velocity (MBV; top), heart rate (HR; middle)and alveolar O_2 uptake ($\dot{V}O_2$alv; bottom) are shown for normoxic (___), hyperoxic(_ _ _) and hypoxic (....) gas breathing conditions during the blood sample rides. Time 0 indicates the onset of exercise. Values shown are mean for 6 subjects.*

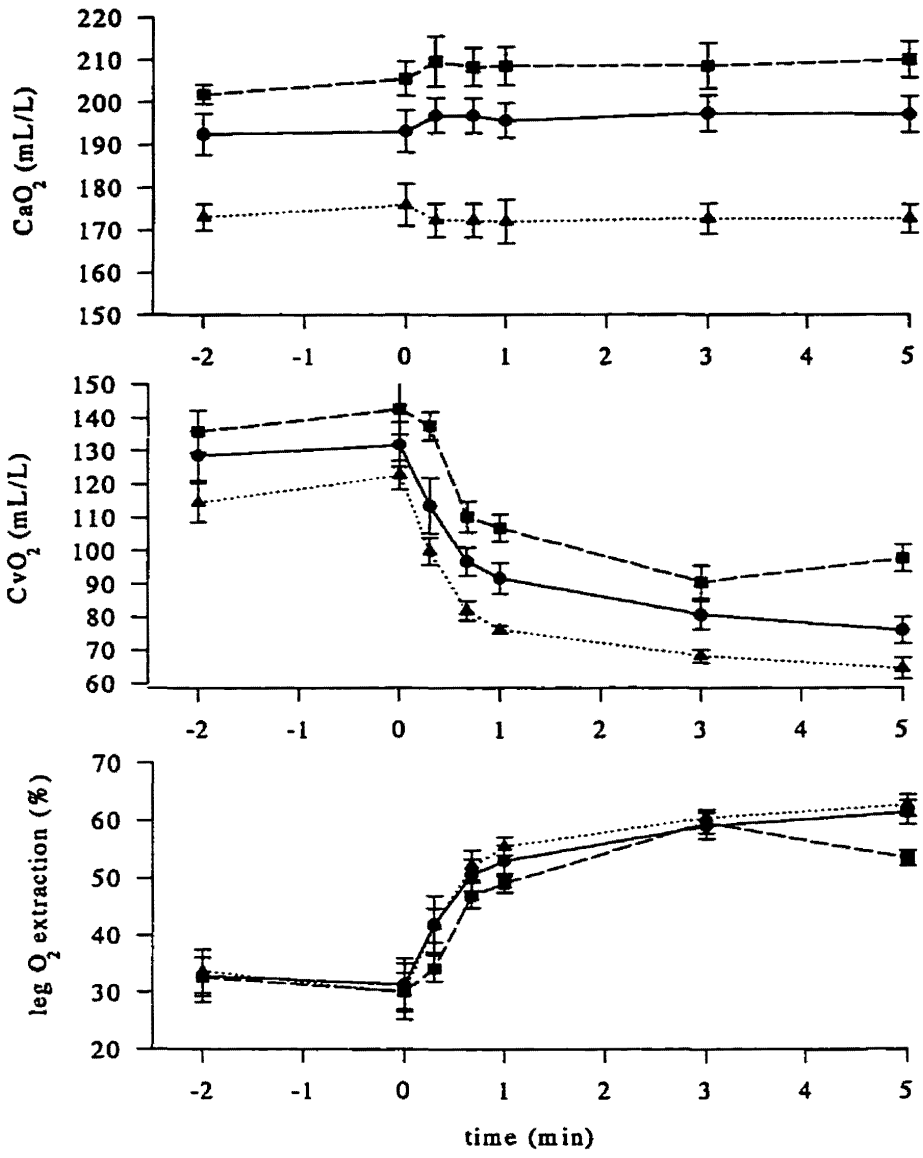


Figure 4.4 *Time course of change in arterial oxygen content (CaO_2 ; top), venous O_2 content (CvO_2; middle) and leg O_2 extraction (% extraction; bottom) are shown for normoxic (__●__), hyperoxic(- -■- -) and hypoxic (..▲..) gas breathing conditions. Time 0 indicates the onset of exercise. Values are means ± SE for 6 subjects.*

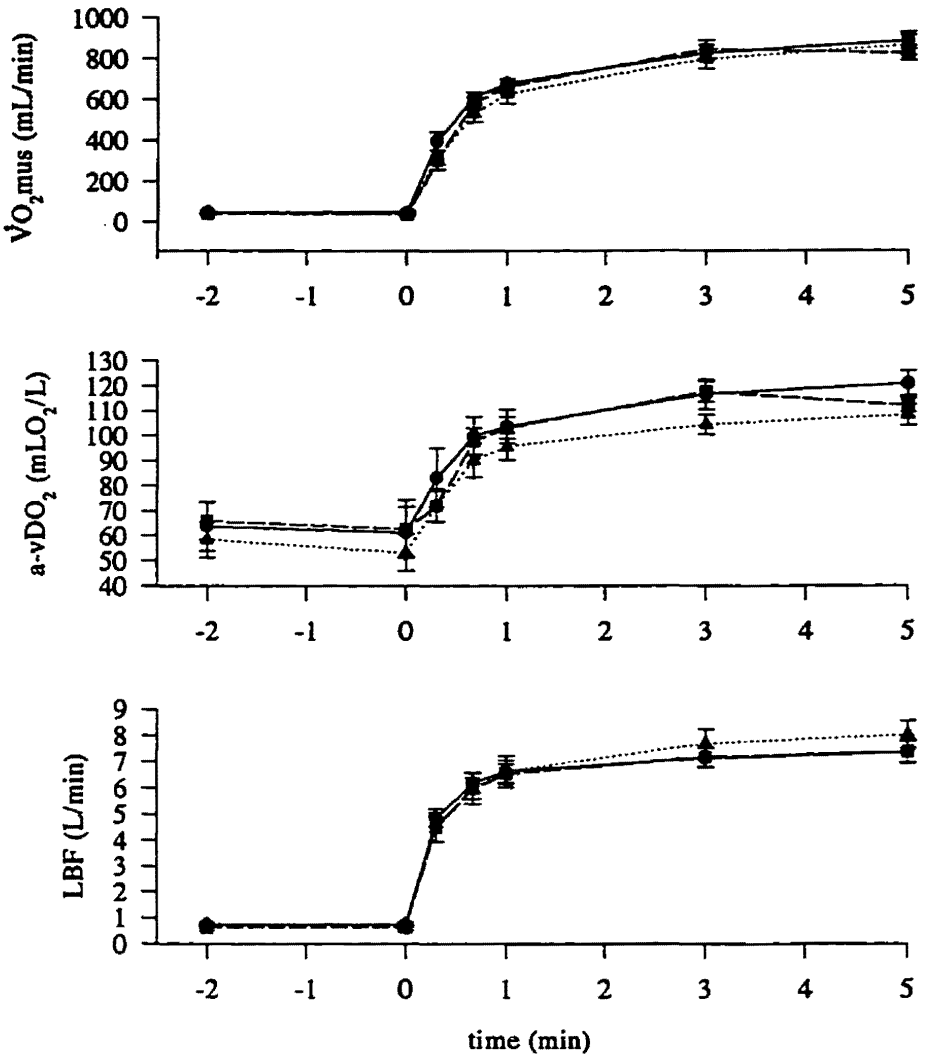


Figure 4.5 *Time course of change in muscle oxygen uptake ($\dot{V}O_2$ mus; top), arteriovenous O_2 content difference (a-vDO_2; middle) and 2 leg blood flow (LBF; bottom) are shown for normoxic (__●__) hyperoxic (- -■- -) and hypoxic (..▲..) gas breathing conditions. Time 0 indicates the onset of exercise. Values are means ± SE for 6 subjects.*

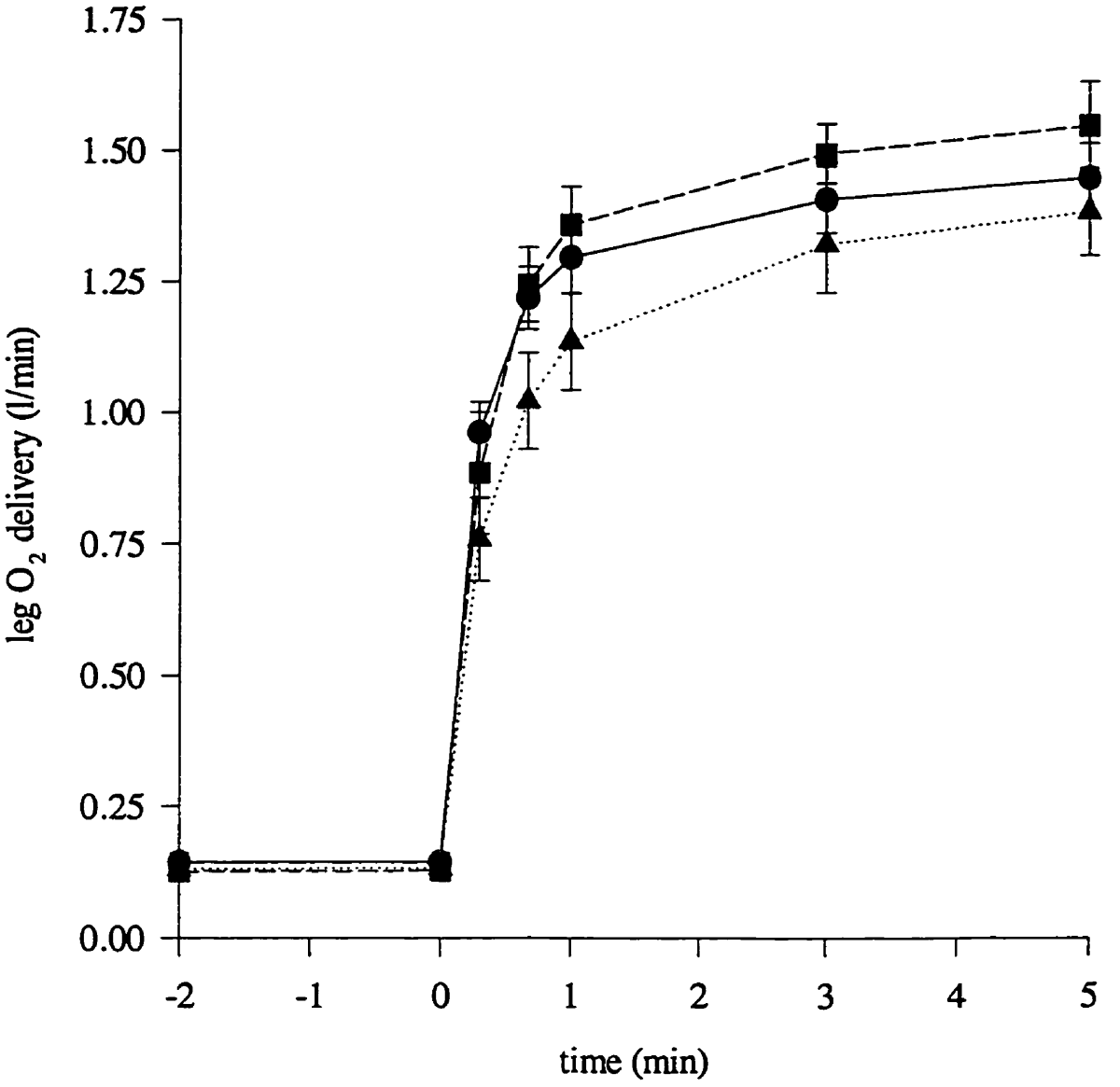


Figure 4.6 *Time course of change in leg oxygen delivery (leg O_2 delivery) is shown for normoxic (__●__) hyperoxic (- -■- -) and hypoxic (..▲..) gas breathing conditions. Time 0 indicates the onset of exercise. Values are means ± SE for 6 subjects.*

DISCUSSION

The main finding of this study was that physiological adaptations took place to counter significant alterations in arterial blood O_2 content. As a result of these adaptations, no differences were seen in $\dot{V}O_2$mus at the onset of exercise under the three gas breathing conditions. This outcome was consistent with our hypothesis that O_2 delivery during hyperoxia would be maintained near control levels for exercise below TVENT. These observations were, however, counter to the hypothesis that hypoxia would cause sufficient reduction in O_2 transport at the start of exercise to cause delayed adaptation of $\dot{V}O_2$alv and $\dot{V}O_2$mus. Our findings, with 14% inspired O_2, were consistent with the recent study by Koskoulou *et al.* (1997) in which 11%, but not 16%, inspired O_2 caused a decrease in O_2 delivery to the exercising legs.

Kinetics of LBF and VO2

The observation of no change in $\dot{V}O_2$ kinetics with hypoxic gas breathing is in contrast to several previous studies (Hughson and Kowalchuk, 1995; Linnarsson, 1974; Linnarsson *et al.*, 1974; Murphy *et al.*, 1989). Hyperoxic gas breathing has been found to result in unchanged (Hughson and Kowalchuk, 1995; Linnarsson, 1974) or faster (Linnarsson *et al.*, 1974; MacDonald *et al.*, 1997; Pedersen, 1987) $\dot{V}O_2$ kinetics, depending in part, on the exercise intensity. The current findings of no change in O_2 delivery with hyperoxic gas breathing may provide some explanation for these findings of unchanged kinetics. It cannot be determined from the current experiments whether hyperoxic gas breathing might result in an increase in O_2 delivery for exercise at higher work rates and therefore explain the findings of faster kinetics for these work rates (Linnarsson *et al.*, 1974; MacDonald *et al.*,

1997; Pedersen, 1987).

The kinetics of both the $\dot{V}O_2$ and LBF for some subjects were slow in comparison to those observed previously for below T_{VENT} exercise (Hughson and Kowalchuk, 1995; MacDonald *et al.*, 1997; Murphy *et al.*, 1989; Shoemaker *et al.*, 1994; Shoemaker *et al.*, 1996). The considerable drift observed in the $\dot{V}O_2$ and HR responses for some subjects confirms that some subjects were not exercising below Tvent (Table 4.1). In spite of these challenges to the O_2 delivery system the response of $\dot{V}O_2$ was maintained at rest and during exercise.

The application of different gas breathing conditions was used in an effort to alter O_2 delivery at the onset of exercise. Neither the hypoxic nor hyperoxic gas concentrations used in this study resulted in an alteration of O_2 delivery (Figure 5, Table 4). This may account for the lack of difference in kinetic responses observed here and observed previously for low to moderate intensity exercise with hyperoxic gas breathing (Hughson and Kowalchuk, 1995; Linnarsson, 1974). . There are two possible explanations for this observation. The first is that for the exercise challenge used in this study, O_2 delivery was not limiting for this exercise transition. The second explanation is that with the gas breathing conditions used here the changes in the O_2 delivery were too small to effect any alterations in $\dot{V}O_2$ kinetics. With the relatively small muscle mass used in kicking exercise compared to cycling, adaptations in O_2 delivery were able to compensate for changes in CaO_2 at the onset of exercise.

$\dot{V}O_2$ muscle

Koskolou *et.al.* (1997) found that 11% O_2 but not 16%O_2 gas breathing in steady-state submaximal 2 legged kicking exercise resulted in increased LBF and decreased CaO_2 which combined

to maintain O_2 delivery to the exercising legs. The results at 11 % O_2 were in support of previous findings by Rowell (1986) and in contrast to those of Richardson *et al.* (1995).

Rowell *et al.* (1986), using normal untrained male subjects performing 1 leg knee extensor exercise found that reduced FIO_2 resulted in an increased flow and decreased arterial and venous O_2 content. The elevated flow resulted in a maintained O_2 delivery. In contrast, Richardson *et al.* (1995), using highly trained cyclists performing a similar type of exercise, found that reduced inspired O_2 resulted in a decrease in CaO_2 and no change in flow at submaximal levels . These subjects were able to maintain the submaximal $\dot{V}O_2$ at normoxic levels by increasing O_2 extraction.

We found, in untrained male and female subjects, during hypoxic gas breathing non-significant increases in flow at rest and exercise, combined with a reduced CaO_2 resulted in unchanged O_2 delivery compared to normoxia and hyperoxia. $\dot{V}O_2$ mus was maintained through slight, but not significant, alterations in LBF and significant changes in arteriovenous oxygen content difference.

O_2 breathing has been suggested as a vasoconstrictor in a number of studies (Bredle *et al.*, 1988; Lindbom and Arfors, 1985; Rubanyi and Vanhoutte, 1986; Seals *et al.*, 1991; Stuart *et al.*, 1984; Welch *et al.*, 1977; Wilson and Stainsby, 1978). Previous studies in rat hindlimb, using a pump perfused model and constant pressure and flow protocols, demonstrated that hyperoxic perfusion of resting muscle resulted in increased leg hindrance, which is an index of resistance attributable to vessel diameter (Bredle *et al.*, 1988). In order to maintain limb blood flow in hyperoxia, perfusion pressure was elevated above normoxic mean systemic pressure and $\dot{V}O_2$ increased slightly. When perfusion pressure was held at normoxic levels, limb blood flow and $\dot{V}O_2$ decreased. These findings were not

previous observations of reduced steady-state heart rate in hyperoxia (Ekblom *et al.*, 1975) and elevated steady state heart rate in hypoxia (Hughson and Kowalchuk, 1995; Linnarsson *et al.*, 1974; Murphy *et al.*, 1989) compared to normoxia. Cardiac output has previously been found to be elevated in hypoxic gas breathing at rest and submaximal exercise (Koskolou *et al.*, 1997). The observed elevation in MAP in hypoxia, might indicate that there was a baroreflex vasoconstriction in order to oppose a relative vasodilation in the exercising legs. The mechanism for hypoxic vasodilation has been postulated to be either central or peripheral in origin (Marshall, 1995; Skinner and Marshall, 1996).

Conclusion

For kicking exercise below TVENT the current findings indicate that there is a mechanism in place for regulating O_2 delivery in altered gas breathing. It is possible that the level of hypoxia used here was not severe enough to produce sufficient alteration in O_2 delivery to assist in determining the limitation to O_2 uptake at the onset of kicking exercise. Further studies examining the role of altered gas breathing on blood flow and O_2 delivery at a wider range of work rates and inspired O_2 concentrations would be useful. The results of this study are unable to distinguish between potential mechanisms for O_2 uptake regulation at exercise onset but show that under these conditions small, physiologically important, changes can occur to maintain appropriate O_2 delivery and utilization at the level of the working muscle.

CHAPTER V

Comparison of femoral blood gases and muscle near infrared spectroscopy at the onset of exercise in humans

ABSTRACT

We hypothesized that near infrared spectroscopy (NIRS) measures of haemoglobin/myoglobin oxygen saturation (IR-SO_2) in the capillary bed of exercising muscle would parallel changes in femoral venous O_2 saturation ($SfvO_2$), at the onset of leg kicking exercise in humans. Six healthy subjects performed transitions from rest to 48 ±3 W (mean ±SE) two legged kicking exercise while breathing 14%, 21% or 70% inspired O_2. IR-SO_2 was measured in the vastus lateralis muscle continuously during all tests and femoral venous and radial artery blood samples were drawn simultaneously during rest and 5 minutes of exercise. In all gas breathing conditions there was a rapid decrease in both IR-SO_2mus and $SfvO_2$ at the onset of moderate intensity leg kicking exercise. The $SfvO_2$ remained lower than resting values for all exercise time points in all gas breathing conditions. However, in normoxia and hyperoxia, the IR-SO_2 increased in steady state exercise after reaching a minimum value one minute after the onset of exercise. Femoral venous O_2 saturation was significantly correlated with IR-SO_2 in all gas breathing conditions when considering the time range from rest to the first minute of exercise, however, the correlation was only significant in the hypoxic condition during all exercise time points . These data indicate a marked separation between the measured $SfvO_2$ and the indirect estimates of Hb/Mb desaturation from NIRS. Further investigations into the origin of the NIRS signal and the saturation of myoglobin and its contribution to the NIRS signal at exercise onset are necessary.

INTRODUCTION

The availability of oxygen to skeletal muscle at the onset of exercise is difficult to measure in humans. Current methods in use include venous and arterial blood sampling combined with measures of blood flow via Doppler (Hughson *et al.*, 1996) or thermodilution (Grassi *et al.*, 1996). These methods are invasive and difficult to obtain on a continuous time frame during exercise transitions.

For this reason, it would be beneficial to have a non-invasive method of continuously determining the relative O_2 saturation in exercising muscle at the onset of exercise. Near infrared spectroscopy (NIRS) utilizes the principle that the absorbance of near infrared light by haemoglobin and myoglobin differs at different wavelengths (Chance *et al.*, 1988) and has been used to estimate relative O_2 saturation in human muscle (Belardinelli *et al.*, 1995; Chance *et al.*, 1992; Chance *et al.*, 1988; Costes *et al.*, 1996; Hampson and Piantadosi, 1988; Mancini *et al.*, 1994; Sahlin, 1992; Wilson *et al.*, 1989) . Previous research in both animals (Wilson *et al.*, 1989) and humans (Mancini *et al.*, 1994) has shown close correlations between trends in measures of tissue oxygenation determined *via* NIRS and venous O_2 saturation draining the exercising muscle. These studies examined the correlation between the two measures during incremental exercise, where it was shown that skeletal muscle oxygenation, measured by NIRS, progressively decreased as exercise work rate increased (Belardinelli *et al.*, 1995; Wilson *et al.*, 1989). Costes *et al* . (1996), however, showed that for steady-state cycling exercise in humans, femoral venous O_2 saturation parallelled relative oxygenation changes measured via NIRS in hypoxic gas breathing but

not in normoxic gas breathing. The temporal response of NIRS measurements at exercise onset has not been validated by measures of venous effluent O_2 saturation.

The purpose of the present study was to assess the oxygenation in human skeletal muscle at the onset of leg kicking in normoxia, hyperoxia and hypoxia using non- invasive reflectance NIRS. The results in each gas breathing condition were compared with simultaneous measures of O_2 saturation of venous blood draining the exercising leg to determine correlations between the two techniques for estimating muscle oxygen availability. We hypothesized that the non-invasive NIRS measures would parallel those from direct blood sampling.

METHODS

Six healthy male and female volunteers (2 men and 4 women, age 25±1 years, height 175±3 cm, and weight 68±4 kg, all mean ± SE) participated in this study. They were not engaging in any regular endurance training exercise. All subjects gave written consent, on forms approved by the University of Waterloo Office of Human and Animal Research, after receiving a full written and oral description of the experimental protocol.

Exercise Protocol

Subjects reported to the laboratory on 3 occasions for training on the kicking ergometer to assist in learning the exercise mode. The kicking ergometer is a specially designed piece of exercise equipment which allows electrically braked exercise of both the quadriceps and hamstrings muscle in an alternating "kicking fashion". Subjects remained in the seated position for all tests with a hip angle of 120° and knee extension and flexion between 90° and 135°. The kicking frequency, between 44 and 50 kicks per leg per minute, was maintained via feedback from a meter attached to the ergometer. The fourth visit to the laboratory involved a progressive kicking test to exhaustion or functional limitation in hypoxic conditions. The work rate protocol for this progressive test was a 15W/min ramp. The progressive test was stopped when the subject could no longer maintain the kicking frequency or needed accessory muscle assistance as assessed by the investigator. The gas exchange and work rate data from the ramp test were used to estimate the subject's T_{VENT} and peak $\dot{V}O_2$. These values were then used in choosing the individual work rates for the step tests.

The step test protocol consisted of three identical step transitions in work rate separated by

ten minutes of rest and gas accommodation. A typical step test involved gas accommodation at rest for five minutes followed by rest for four minutes and six minutes of kicking at a work rate between 89 and 94% of hypoxic T_{VENT}. The flywheel of the kicking ergometer was manually accelerated for 30 seconds prior to the onset of exercise to allow the subjects to begin working at the appropriate work rate. Otherwise, no warning was given to the subject prior to the start of exercise although they were aware of the protocol before the test.

The three possible gas breathing conditions were determined by the gas inhaled during each gas transition. Each of the experimental gas mixtures of normoxia (room air), hyperoxia (70% O_2, balance N_2) and hypoxia (14% O_2, balance N_2) was breathed on each testing day in a randomized order with each subject performing one step test in each gas breathing condition on a single testing day.

Data Collection:

Breath by breath ventilation and gas exchange was measured on a computerized system (First Breath, St. Agatha, ON) with a mass spectrometer (MGA-1100A, Marquette electronics Inc., Milwaukee, WI) and the digital volume turbine (VMM-110,Alpha, Technologies, Laguana Beach, CA) or ultrasonic flowmeter (UF202, Kou and Assoc., Redmond, WA). For the hyperoxic and hypoxic tests a large Tissot tank was filled with inspiratory gases from cylinders containing the 70% and 14% O_2 mixtures respectively. The Tissot tank was connected to a Y-valve to permit inspiration from the tank. The barometric pressure, temperature and water vapour pressure were measured before each test. Matching of fractional gas concentrations with the

appropriate volume was done by accounting for the sum of the transport lag plus the instrument response time. The mass spectrometer and the digital volume turbine or ultrasonic flow meter were calibrated separately for normoxia, hyperoxia and hypoxia and appropriate lag times utilized. The mass spectrometer was calibrated for normoxia, hypoxia and hyperoxia using 2 precision gas mixtures that spanned the anticipated fractional gas concentrations in each of the gas conditions. Volume was calibrated by manually pumping a 3 litre syringe at a flow rate similar to that of respiration during the exercise test. $\dot{V}O_2$ was corrected on a breath-by-breath basis for changes in lung gas stores due to altered lung volume or alveolar gas composition, as described previously (Hughson *et al.*, 1991).

Blood Samples

Femoral venous and radial arterial blood was sampled during the exercise transitions to obtain measurements of blood gas and metabolic responses. On one testing day subjects reported to the laboratory one-half hour prior to testing and had catheters inserted in the femoral vein and radial artery.

A 1 ½ " plastic radial artery catheter (20 gauge, Angiocath, Becton-Dickenson, Sandy, UT) was inserted into the left radial artery under local anaesthetic (Lidocaine HCl, 2%, Astra, Mississauga, ON). Patency was maintained with a pressurized flush system (0.9% NaCl + Sodium heparin 500 I.U./500mL NaCl; approximately 15 mL/hour). Subjects lay supine and a 16cm plastic catheter (16 gauge, Arrow, Redding, PA) was inserted two cm below the inguinal ligament into the left femoral vein under local anaesthetic (see above). In one subject the femoral catheter

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and the time constant for the unit set to the shortest response time (15 sec). The sensor was positioned lengthwise 10 to 12 cm above the knee over the right vastus lateralis in 5 subjects and over the left vastus lateralis in one subject and protected from skin moisture by a clear plastic wrap. An elastic strap was placed around the thigh, over the sensor to prevent displacement and the detection of ambient room light. The average depth of penetration of NIR light in skeletal muscle is estimated to be 2.5 to 3.0 cm (Chance *et al.*, 1988). Two separate outputs were obtained from the NIRS unit and sampled at 100 Hz on a dedicated computer system. The output containing the difference in the two received wavelengths (760-850 nm) was monitored as an index of relative haemoglobin (Hb) and myoglobin (Mb) deoxygenation and the output containing the sum of the two received wavelengths (760+850) was monitored as an index of changes in tissue blood volume (Chance *et al.*, 1992). The signals were averaged over twenty seconds preceding the venous blood sample times to obtain values for both channels twice at rest, at twenty and forty seconds after the onset of exercise and during minutes one, three and five thereafter.

The Runman™ unit was calibrated before each exercise transition with the probe in place on the vastus lateralis and the subjects seated at rest and breathing the gas concentration to be used during the exercise trial. The electrical output of the Runman™ unit was adjusted to 0 mV using the balance control and then the gain of the unit was adjusted to provide signal deflections in the range of 600 to 1000 mV. For the difference channel (760-850 nm) IR-SO_2 was expressed on a relative scale as a percent of individual calibration under each gas condition with 100 % saturation equal to the resting saturation in each gas condition and 0% saturation arbitrarily set to the full scale

negative deflection used in instrument calibration. For the sum channel (760+850 nm) tissue blood volume was expressed on a relative scale as a percent of individual calibration under each gas condition with 0 % change in blood volume equal to the resting saturation in each gas condition and 100% increase in blood volume equal to the full scale negative deflection. Since the calibration was relative to rest in each gas, it was not possible to compare IR-SO_2 measures between gas conditions but correlations between IR-SO_2 and $SfvO_2$ were possible. The results obtained with respect to tissue O_2 saturation were obtained during experiments as part of a larger study and due to technical limitations the NIRS signals were obtained on the opposite leg from the femoral venous blood samples.

Statistical analysis

The main effects of gas breathing (three levels of the variable) and time (seven levels of the variable) on the $SfvO_2$ and SaO_2 responses were analysed by a two-way analysis of variance (ANOVA) due to the fact that comparisons between gas breathing conditions were possible for blood sample data. Any significant ($p \leq 0.05$) interactions from the 2-way ANOVA were further analysed with Student-Neumann-Keuls post hoc test. Since all NIRS measures were expressed as relative values, the effect of gas breathing on IR-SO_2 and changes in tissue blood volume were analysed by a one way analysis of variance. Correlations between IR-SO_2 and $SfvO_2$ were analysed by linear regression. The correlation coefficients (r) were determined. The level of significance for the main effects and interactions were set at $P<0.05$. All data are presented as mean ± standard error (SE).

RESULTS

During the incremental kicking test subjects reached a peak kicking workload of 122±11 watts which corresponded to a $\dot{V}O_2$ of 1878±142 mL/L and a heart rate of 150±8 beats/min. The T_{VENT} during the incremental kicking tests was determined to be 1368±94 mL/min. The work rates, $\dot{V}O_2$ and heart rate during the step test sessions are shown in Table 5.1.

Arterial oxygen saturation (SaO_2) was significantly elevated in hyperoxic gas breathing and reduced in hypoxic gas breathing at all time points during the test. There was no change from rest to exercise in normoxia and hyperoxia. The SaO_2 during the hypoxic gas breathing condition was lower during exercise than rest for all time points (Table 5.2). The femoral venous oxygen saturation ($SfvO_2$) was lower in hypoxia and higher in hyperoxia relative to normoxia at all time points during the test. At the onset of exercise there was a significant decrease in $SfvO_2$ relative to rest by 20 seconds in normoxia and hypoxia and by 40 seconds of exercise in hyperoxia (Table 5.2 Figure 5.1).

Due to the calibration method used here it was not possible to compare near-infrared muscle oxygen saturation (IR-SO_2) between gases. IR-SO_2 remained constant over the four minutes of rest and decreased by 20 seconds after the onset of exercise in all gas breathing conditions. In hypoxia IR-SO_2 reached a minimum value by 40 seconds into the exercise and remained at a lower level throughout the exercise. In hyperoxia and normoxia IR-SO_2 reached a minimum level at 40 seconds after the onset of exercise and increased again at 3 and 5 minutes (Table 5.2, Figure 5.1). In hyperoxia the IR-SO_2 was not different from rest by 5 minutes of

exercise.

In all gas breathing conditions measures of IR-SO_2 were significantly correlated with $SfvO_2$ when considering the time period from rest to 40 seconds after the onset of exercise (normoxia r=0.53, hyperoxia r=0.57, hypoxia r=0.61). If the total exercise time period was considered measures of NIR-SO_2 was significantly correlated with $SfvO_2$ in hypoxia (r=0.42) but not in hyperoxia (r=0.05) or in normoxia (r=0.02) (Figure 5.2).

There was a decrease in the tissue blood volume channel at the onset of exercise in all gas breathing conditions. This initial decrease was followed by an increase as the exercise continued (Table 5.2 , Figure 5.3) so that at 5 minutes, tissue blood volume was elevated with respect to rest in normoxia and equal to rest in hyperoxia and hypoxia.

Table 5.1. *Work load, alveolar O_2 uptake, percentage of ventilatory threshold and heart rate during kicking exercise in normoxia, hyperoxia and hypoxia.*

Gas Condition	WL (watts)	$\dot{V}O_2$ (mL/min)	$\%T_{VENT}$ (%)	HR (beats/min)
normoxia (FIO_2=0.21)	48±3	1224±38	89±6	112±10
hyperoxia (FIO_2=0.70)	48±3	1280±33	94±6	106±10
hypoxia (FIO_2=0.14)	48±3	1251±53	91±5	122±10

Values are means ± SE for 6 subjects. WL, work load; $\dot{V}O_2$, alveolar oxygen uptake; T_{VENT}, ventilatory threshold; HR, heart rate; FIO_2= fractional oxygen concentration.

Table 5.2. *Arterial and venous blood and near-infrared muscle O_2saturation in normoxic, hyperoxic and hypoxic conditions.*

		Time						
		rest	rest	20s	40s	1min	3min	5min
SaO_2(%)	normoxic ‡	97.9±0.4	98.0±0.2			97.7±0.3	97.8±0.2	97.6±0.3
	hyperoxic †	100.0±0.0	100.0±0.0			100.0±0.0	100.0±0.0	99.9±0.2
	hypoxic† ‡	89.5±0.8^a	88.8±0.6^a			85.7±0.5^b	85.9±0.4^b	85.1±0.4^b
$SfvO_2$ (%)	normoxic ‡	69.9±4.7^a	68.2±5.8^a	59.2±4.6^b	48.7±2.3^c	46.0±2.3^c	41.9±2.0^c	40.1±2.2^c
	hyperoxic †	75.1±5.5^a	75.4±5.7^a	71.5±2.9^a	54.9±2.0^b	53.0±1.8^b	47.7±2.4^b	50.8±1.8^b
	hypoxic †‡	64.1±4.2^a	63.9±4.4^a	51.5±3.2^b	41.8±2.0^c	38.6±1.0^c	34.7±1.2^c	33.9±1.8^c
IR-SO_2(%)	normoxic	99.6±0.3^a	99.6±0.4^a	95.7±1.6^b	88.6±2.0^c	89.1±1.6^c	92.7±1.6^b	94.3±1.7^b
	hyperoxic	100.3±0.5^a	100.0±0.4^a	92.9±1.9^b	86.1±2.5^c	87.9±2.1^c	94.5±1.2^b	96.3±1.4^{a,b}
	hypoxic	99.7±0.4^a	99.6±0.6^a	94.8±1.3^b	88.6±2.3^c	88.0±2.2^c	88.3±1.3^c	89.3±1.3^c
IR TBV (%)	normoxia	-0.4±1.2^{a,b}	0.5±0.5^{a,b}	-2.9±2.0^b	-4.1±2.5^b	0.6±4.0^{a,b}	9.2±4.8^{a,c}	14.1±4.8^c
	hyperoxia	1.5±1.3^a	0.2±1.4^a	-18.3±6.5^b	-14.6±6.1^b	-9.6±6.0^b	3.1±4.2^a	9.4±2.6^a
	hypoxia	3.2±1.1^{a,b}	3.0±0.9^{a,b}	-8.3±6.9^b	-5.6±8.0^{a,b}	-3.5±7.2^{a,b}	4.0±4.8^{a,b}	10.1±3.8^a

Values are means ±SE for 6 subjects. SaO_2, arterial oxygen saturation; $SfvO_2$, femoral venous oxygen saturation; IR-SO_2, near infrared muscle oxygen saturation; IR TBV, near infrared tissue blood volume. Different letter superscripts indicate differences between times within the same gas condition. † significantly different from normoxia and ‡ significantly different from hyperoxia for all time points, $p \leq 0.05$.

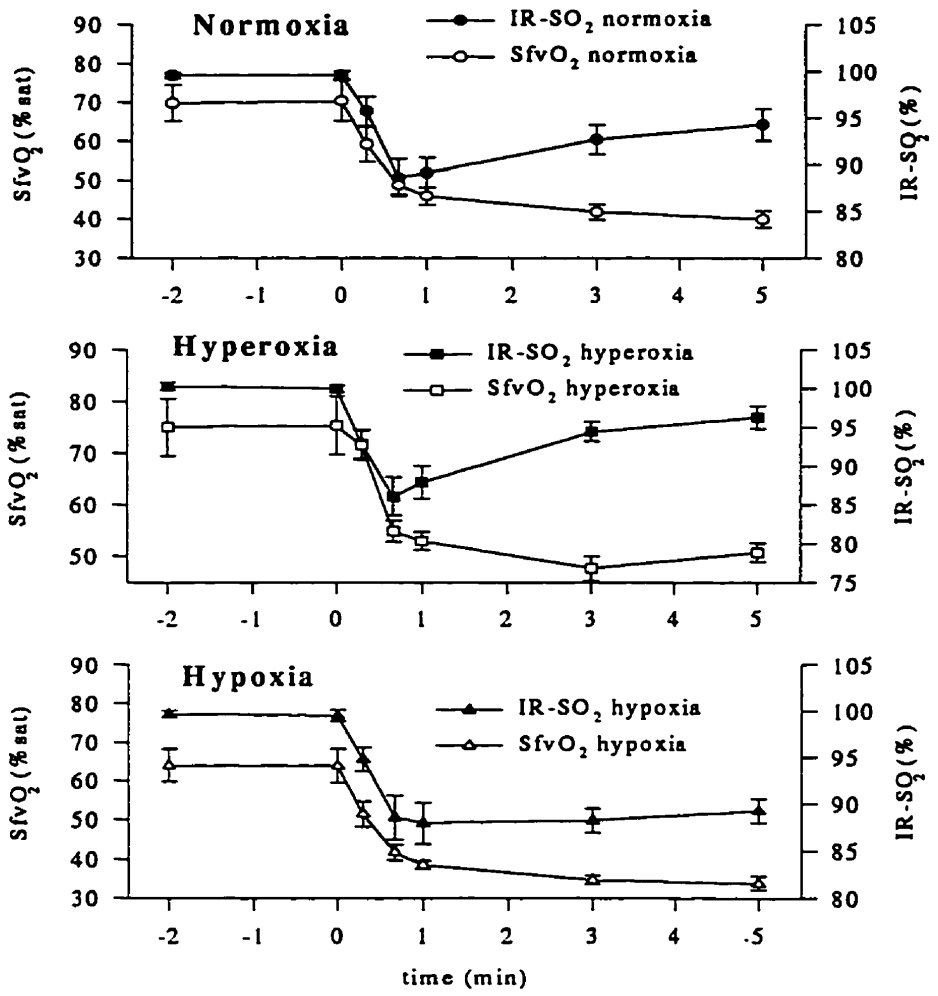


Figure 5.1 *Temporal variations in IR-SO_2 (filled) and $SfvO_2$ (open) in normoxic (circles, top), hyperoxic (squares, middle) and hypoxic (triangles, bottom)gas breathing conditions. Time 0 indicates the onset of exercise . Values shown are means for 6 subjects.*

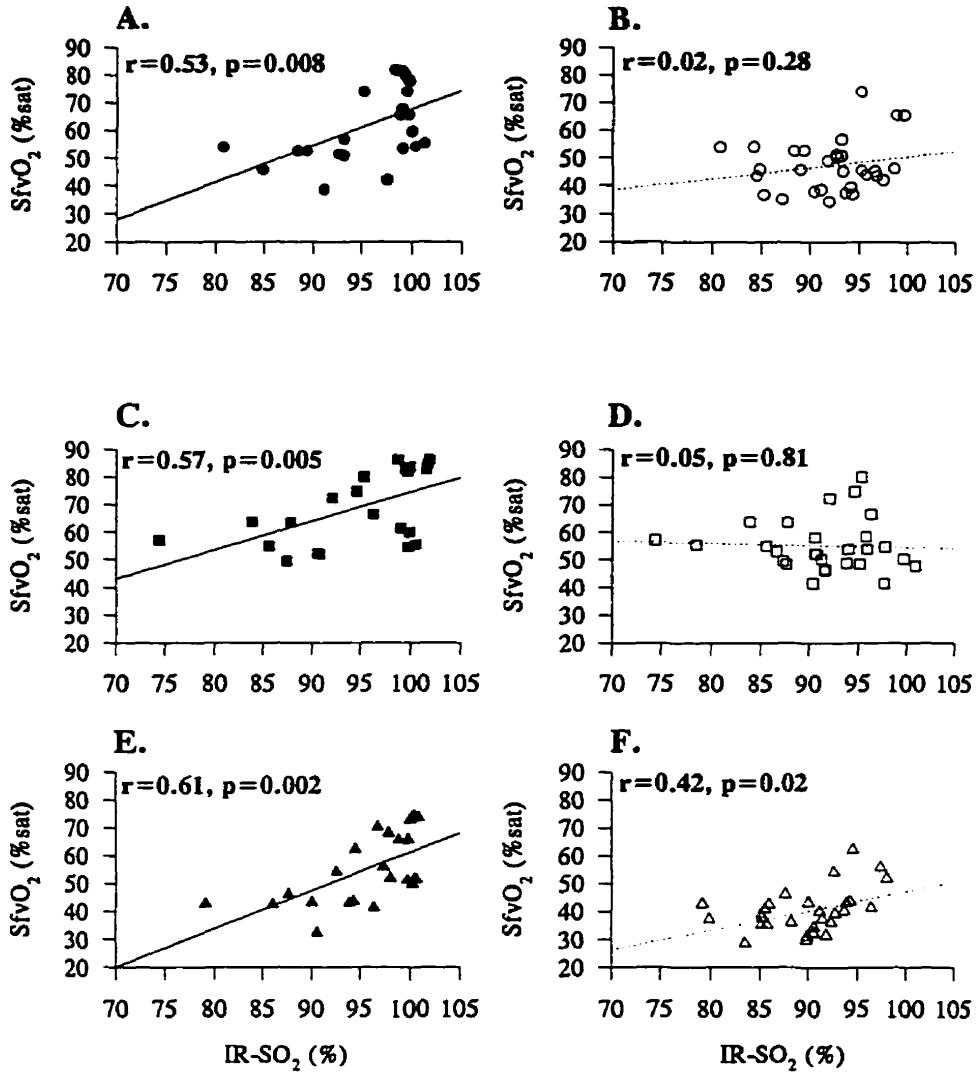


Figure 5.2 *Correlations of IR-SO_2 and $SfvO_2$ for normoxia (●, ○, top), hyperoxia (■, □, middle) and hypoxia (▲, △, bottom). A. Correlations for rest to 0.67 minutes in normoxia; B. Correlations for all exercise time points in normoxia; C. Correlation for rest to 0.67 minutes in hyperoxia; D. Correlations for all exercise time points in hyperoxia; E. Correlation for rest to 0.67 minutes in hypoxia; F. Correlations for all exercise time points in hypoxia. Lines indicate first order regressions for all data points on each graph. Values shown are for 6 subjects. r, regression coefficient.*

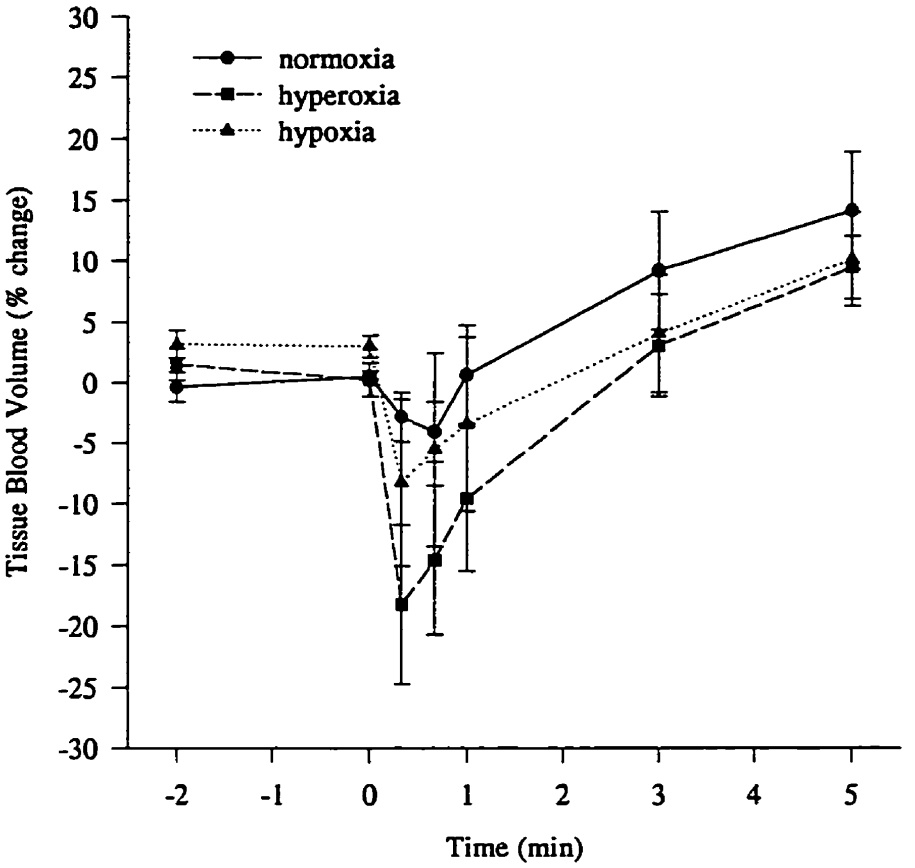


Figure 5.3 *Changes in tissue blood volume at the onset of exercise in normoxia (—●—), hyperoxia (- -■- -) and hypoxia(···▲···). Values are means ±SE for 6 subjects. Time 0 indicates the start of kicking exercise.*

DISCUSSION

IR-SO_2 and $SfvO_2$ both decreased at the onset of exercise in all gas breathing conditions. After the initial onset of exercise there was a marked separation in the response of the two measures with IR-SO_2 increasing again in hyperoxia and normoxia while $SfvO_2$ continued to decrease to a plateau in all three gas breathing conditions. These findings concur with those of Costes *et al.* (1996) who studied steady-state exercise in humans and are in contrast to validation studies in humans with ramp forearm exercise (Mancini *et al.*, 1994) and in animals with stimulated contractions (Wilson *et al.*, 1989).

Validity of IR-SO_2

This study employed reflectance spectroscopy and, because we do not know the photon pathways, only trends in muscle O_2 saturation, not absolute changes, could be determined. The correlation coefficients between IR-SO_2 and venous effluent O_2 saturation were lower ($r=0.02$ to $r=0.61$) than those reported in two previous studies ($r=0.97$) (Wilson *et al.*, 1989) and ($r=0.92$) (Mancini *et al.*, 1994) indicating that femoral venous SO_2 did not account for all of the NIRS signal observed at the onset of leg kicking exercise in humans. Both in this study and that of Costes *et al.* (1996), leg exercise was used to evaluate the measurement of tissue oxygen saturation. It is possible that NIRS spectrophotometry is only valid for small muscle mass exercise, such as that used in previous validation studies (Mancini *et al.*, 1994; Wilson *et al.*, 1989). The choice of protocol may have also influenced the results. In previous studies (Mancini *et al.*, 1994; Wilson *et al.*, 1989) ramp protocols were employed and good correlations between venous effluent and IR-

SO_2 were obtained.

The IR-SO_2 signal represents a weighted average of arterial, capillary and venous Hb O_2 saturation as well as contributions from intracellular Mb O_2 saturation (Chance *et al.*, 1988). In the present study we know that the arterial oxygen saturation did not change at the onset of exercise in normoxia and hyperoxia and decreased in hypoxia. These differences in arterial oxygen saturation may account for some of the NIRS signal changes.

Another possible contributor to the NIRS signal is Mb O_2 saturation. In the present study we have no determinations of changes in Mb O_2 saturation. Bellardini *et al.* (1995b) suggested that O_2 desaturation, measured with NIRS, was due to loss of O_2 from Hb, for steady state exercise below the lactate threshold, and was due to O_2 loss from Mb, for exercise above the lactate threshold. Several other studies have also suggested that changes in Hb O_2 saturation are primarily responsible for absorption changes observed with NIRS (Chance *et al.*, 1992; Mancini *et al.*, 1994; Wilson *et al.*, 1989). In the present study, subjects were working at or below their ventilatory threshold by the end of six minutes of kicking exercise in each gas condition (Table 5.1) and MbO_2 saturation would not be expected to have a major influence on IR-SO_2 signal changes. However, the possibility of Mb desaturation at the onset of exercise has not been determined and it is impossible to distinguish between Mb and Hb signals based on their light absorption characteristics.

It is possible that the venous blood sampled in this study did not accurately represent exercising muscle oxygen saturation due to contamination by blood originating from tissue other

than exercising muscle. Femoral venous O_2 saturation represents the sum of all blood returning from the exercising leg, while the NIRS signal originates in the exercising muscle only (Chance *et al.*, 1992). In previous studies, which have shown a significant correlation between IR-SO_2 and venous SO_2, the venous effluent was obtained from a deep forearm vein draining the exercising muscle (Mancini *et al.*, 1994) and from a vein draining the exercising muscle only (Wilson *et al.*, 1989). In both this study and the work of Costes *et al.* (1996), in which the correlation coefficient was $r = 0.55$ in hypoxia, the venous effluent was obtained in the femoral vein more distal to the exercising muscle. However, the $SfvO_2$ showed a decrease in saturation to a plateau in all gas conditions and contamination from non exercising muscle would be expected to increase, not decrease, the O_2 saturation of the effluent blood. For this reason we conclude that $SfvO_2$ accurately represented the venous SO_2 in the exercising muscle in the present experiments and cannot account for the temporal differences in $SfvO_2$ and IR-SO_2.

The type of exercise used in the present study may have influenced the results. IR-SO_2 and venous blood samples were obtained from opposite legs and it is possible that there was a systematic difference in the O_2 availability in the two legs. However, this is highly unlikely because the subjects had practised the kicking exercise and were provided feedback to maintain kicking frequency and work by both exercising legs.

Hypoxia versus normoxia and hyperoxia

In hypoxia IR-SO_2 was significantly correlated with $SfvO_2$ at all time points, while this correlation was only significant during rest and the initial onset of exercise in hyperoxia and

normoxia (Table 2, Figure 2). These differences in correlation observed in hypoxia versus normoxia and hyperoxia could be due to several factors. It is possible that there was a systematic error in the placement and operation of the probe in different gas breathing conditions. However, the order of the trials was randomized and all were performed on the same day with no movement of the probe between trials. It is, therefore, very unlikely that such an error could account for the observed differences between gas conditions.

Changes in tissue blood volume monitored via NIRS in this study indicate that there is a transient decrease in tissue blood volume at the onset of exercise, which reached significance in hyperoxia. This decrease was followed by increases over time to result in tissue blood volume greater than rest at end exercise in normoxia and equal to rest in hypoxia and hyperoxia. These changes in the tissue blood volume signal were observed in all gas breathing conditions and may represent a redistribution of blood in the exercising leg or changes in penetration depth or muscle geometry at the onset of exercise. However, that these trends were observed in all gas breathing conditions suggests that changes in blood volume cannot explain the differences in correlation observed between IR-SO_2 and $SfvO_2$ between the gas conditions.

The lower saturation of the arterial blood supplying the muscle and the possible desaturation of Mb in hypoxia may have contributed to the differences in correlations between gas conditions (Costes *et al.*, 1996). These workloads were relatively light, however, and it has been previously suggested that myoglobin does not contribute to the NIRS signal until more severe desaturation in the venous effluent is observed (Mancini *et al.*, 1994; Wilson *et al.*, 1989). In spite of these

findings, previous determinations of Mb contribution to NIRS signals have not examined Mb desaturation at the onset of exercise.

Variations in skin or muscle blood flow may have contributed to the different correlations observed in different gas conditions. However, skin blood flow did not influence the NIRS difference signal in previous research (Hampson and Piantadosi, 1988; Mancini *et al.*, 1994). In a previous study by Costes *et al.*, (1996) a gradual drift towards reoxygenation was also observed in normoxic but not hypoxic exercise and it was postulated that differences in blood flow may account for the variability of results in different gas breathing conditions . In the present study total leg blood flow was measured simultaneously with NIRS measurements via Doppler ultrasound technology and it was determined that the leg blood flow increased similarly at exercise onset in all conditions and therefore cannot explain the IR-SO_2 signal differences observed here.

Others have also observed a time dependent, relative reoxygenation measured by NIRS techniques, during steady state exercise in normoxia following a minimum saturation reached by 60 to 120 seconds (Belardinelli *et al.*, 1995a; Mancini *et al.*, 1994a). It was suggested that the resaturation may be due to muscle fatigue and a possible reoxygenation of Mb (Mancini *et al.*, 1994) or improved tissue oxygenation with time (Belardinelli *et al.*, 1995a). The present observations indicate that either these observations are a misinterpretation based on the absence of $SfvO_2$ values or that the IR-SO_2 signal represents changes in tissue oxygenation which are not detectable with venous blood sampling. The IR-SO_2 signal in hyperoxia and normoxia may represent a relative Mb and tissue desaturation at the onset of exercise followed by reoxygenation

in the steady state exercise with no detectable change in venous effluent SO_2. In hypoxia, the lowered exercise arterial oxygenation may have prevented this reoxygenation or contributed to the plateau in the IR-SO_2.

Conclusions

Changes in femoral venous effluent cannot account for all of the NIRS oxygen saturation signal response at the onset of exercise or during the subsequent steady state. The correlation between these two measures improved in hypoxic gas breathing and worsened with hyperoxic gas breathing. It is possible that either the NIRS signal does not accurately reflect Hb/Mb desaturation at the onset of leg exercise or that the origin of the NIRS signal is different from the source of $SfvO_2$ measures. Further determinations of tissue Mb O_2 saturation at the onset of exercise are necessary to validate the use of NIRS and to determine the role of O_2 availability at the onset of large muscle mass exercise.

APPENDIX 1

Portal vein blood flow by echo-Doppler ultrasound: day-to day repeatability

This appendix shows the between day repeatability of simultaneous measures of portal vein cross-sectional area and mean blood velocity (MBV) during rest using an echo Doppler for B-mode images and pulsed Doppler ultrasound. On 3 separate days, 5 volunteers were tested at the same time of day. On each day 3 separate measures were made. Cross sectional area and MBV of the portal vein were estimated from a frozen image corresponding to one respiratory cycle. The portal vein area and MBV did not differ between measures within one day or between measures on separate days. The mean between day coefficient of variation for area was 18% and the mean between day coefficient of variation for MBV was 18.3%. The portal vein blood flow was calculated from the MBV cross-sectional area assuming plug flow characteristics. The average area for 5 subjects across all three days was 53.8mm^2 , the average MBV was 0.21 m/sec and the average blood flow was 1.31 L/min. The data indicate that Doppler ultrasound measures of portal vein MBV and area during rest were reproducible across different test days and can be used as a reliable and non-invasive means of examining blood flow distribution and control at rest and response to exercise.

INTRODUCTION

A central question in exercise physiology is how adjustments in circulation meet the demands for oxygen transport to working muscles. The distribution of cardiac output during exercise and the significance of alterations in regional blood flow in response to exercise have been extensively studied (Rowell, 1993).

At rest, the splanchnic vascular bed receives approximately 25% of cardiac output and extracts 15- 20% of the oxygen available in that blood (Rowell, 1993). It has, therefore, been traditionally looked at as a major site for redistribution of blood and blood pressure regulation. Previous methods of measuring and estimating splanchnic blood flow include splanchnic (a-v)O_2 (Quamar and Read, 1987), peripheral clearance of indocyanine green dye (Rowell *et al.*, 1965), as well as isolated organ preparations performed in animals (Fronek and Fronek, 1970; Laughlin and Armstrong, 1982). All of these techniques are fairly invasive and there is questionable relevance of the animal studies to the human vascular response (Eriksen and Waaler, 1994). The use of Doppler ultrasound techniques to quantify blood flow in the portal vein represents a significant advance in research in blood flow redistribution (Ackroyd *et al.*, 1985; Eriksen and Waaler, 1994; Ohnishi *et al.*, 1985).

The purpose of this study was to determine the day-to-day repeatability of measures of portal vein blood flow as an indication of splanchnic blood flow using echo-Doppler for measure of vessel diameter and blood velocity and calculation of blood flow.

METHODS

Five subjects (age 26 ± 2 years , height 178 ± 4 cm, weight 70 ± 6 kg) volunteered for this study and were tested on three separate days. Subjects arrived for testing at the same time of day and were requested to refrain from eating two hours prior to testing on each testing day. All subjects gave written consent on a form approved by the Faculty Ethics Committee at the university of Nijmegen, the Netherlands.

On each testing day three consecutive measures of portal vein cross sectional area and mean blood velocity were made. All measures were made by the same experimenter while the subjects was in a seated, upright position at rest. Portal vein area and mean blood velocity was measured from a frozen screen image (Figure 6.1) using an echo Doppler (Toshiba Inc. Tochigi-Ken, Japan) operating in duplex mode and with a 3.75 MHZ phased array sector scanning probe. The velocity was recorded distal to the entry of the portal vein in the liver and proximal to its bifurcation. Flow was calculated as the product of velocity and cross sectional area.

The between day variability for cross sectional area and MBV was determined by comparing the absolute value through a 2 way analysis of variance (ANOVA) for repeated measures (SAS). The level of significance was set at $p \leq 0.05$. Data are presented as mean ± standard error. A coefficient of variation was calculated for each subject at each of the selected times across the three days.

RESULTS

Average portal vein cross-sectional area, MBV, and portal vein blood flow did not differ between testing days (Table 6.1, Figure 6.2). The coefficient of variation s for area and MBV were 18.0% and 18.3% respectively.

Table 6.1 *Average portal vein cross sectional area, mean blood velocity and blood flow during each testing day and between day coefficients of variation.*

	Area (mm^2)	MBV (m/sec)	Flow (L/min)
Day 1	52.9±4.4	0.19±0.01	1.20±.10
Day 2	51.1±3.3	0.21±0.01	1.22±0.06
Day 3	57.4±3.7	0.23±0.01	1.52±0.10
coefficient of variation (%)	18.0%	18.3%	

Values are mean ± SE for 5 subjects. Area, cross sectional area of the portal vein; MBV, mean blood velocity through the portal vein; Flow , blood flow through the portal vein.



Figure 6.1 An example of blood flow through the portal vein at rest (left) and an image of the portal vein (right) obtained with pulsed and echo Doppler.

CONCLUSIONS

It has been demonstrated that measures of portal vein blood flow using echo-Doppler ultrasound imaging to determine vessel diameter and pulsed Doppler to determine blood flow is a repeatable measure. Doppler ultrasound is a noninvasive technique which may have applications in measuring blood flow redistribution during rest, exercise and cardiovascular stress. At present its repeatability during rest has been demonstrated but more investigations must include measures during exercise as well as comparisons to the more invasive techniques currently in use.

APPENDIX II

Control of blood flow to inactive muscle at the onset of leg exercise

This appendix shows the results of a study examining the effect of sympathetic innervation on the adaptation to submaximal knee extension exercise in 7 able bodied (ABS) and 7 spinal cord injured (SCI) subjects with lesions between T12 and C5. ABS performed moderate intensity voluntary single leg knee extension exercise (VOL). ABS and SCI performed involuntary single leg knee extension exercise with electrical stimulation (STIM). At the onset of both VOL and STIM exercise in ABS but not SCI, there was a transient increase in leg blood flow (LBF) to the inactive leg as determined by Doppler ultrasound (ABS(vol): LBF(rest)=232±16, LBF (15sec) = 788±102, LBF (4min) = 466±61; ABS (stim): LBF(rest) = 260±33, LBF(15sec) = 406±104, LBF(4min)=277±26; SCI : LBF(rest) = 116±16, LBF(15sec) = 126±22, LBF(4min) = 127±21; all values mL/min±SE). Femoral artery diameter (D) and thigh volume (V) were less in SCI (D=6.23±0.37mm, V=4.39±0.78 l) compared to ABS (D=10.66±0.48, V=7.75±0.66 l). HR and MAP were elevated with VOL but not with STIM exercise. The transient increase in flow at the onset of exercise in ABS results from a decrease in vascular resistance at the onset of exercise.

INTRODUCTION

It has been established that at the onset of exercise, blood flow to the exercising muscle increases rapidly (Honig, 1979; Shoemaker *et al.*, 1994; Tschakovsky *et al.*, 1996; Wesche, 1986). The control mechanisms responsible for this response have not been fully determined. However, for large muscle mass exercise, both central and peripheral mechanisms have been suggested as regulating factors (Shoemaker *et al.*, 1996). The role of the sympathetic nervous system in regulating blood flow at the onset of exercise is not known (Mitchell, 1990; Victor *et al.*, 1987).

Hopman *et al.* (1993) observed a transient increase in volume of inactive legs at the onset of arm exercise in able bodied subjects. This increase was not observed in spinal cord injured subjects, who were lacking not only motor but also sympathetic innervation to the legs. It was suggested that this transient increase may be due to a brief withdrawal of sympathetic vascular tone in the inactive muscle at the onset of exercise that allowed an increase in blood flow. This sympathetic control was only possible in the able bodied subjects and was not observed in the spinal cord injured subjects due to their lack of sympathetic innervation.

The purpose of this study was, therefore to examine the effect of sympathetic innervation on the blood flow adaptation at the onset of exercise by determining the blood flow responses in the inactive leg during 1 leg knee extension in both ABS and SCI subjects. We hypothesized that lack of sympathetic innervation in SCI may prevent transient increases in leg blood flow in contralateral inactive legs in comparison to ABS at the onset of leg exercise.

METHODS

Seven able bodied subjects (age 25 ● 1 years, height 184 ± 2 cm, weight 82 ± 3 kg) and seven spinal cord injured subjects (age 37 ± 3 years, height 183 ± 3 cm, weight 77 ± 7 kg) volunteered for this study. Subjects were requested to refrain from eating and smoking two hours prior to testing. All subjects gave written consent on forms approved by the Faculty Ethics Committee at the University of Nijmegen, the Netherlands.

ABS performed both one leg dynamic knee extension and one leg stimulated knee extension exercise and SCI performed one leg stimulated knee extension exercise. Tests consisted of four minutes of rest followed by six minutes of exercise. Exercise was performed on a modified Lode ergometer for knee extension. Exercise was performed with the left leg while the right leg was elevated on a stool approximately 50 cm below heart level. Blood flow was measured on the right leg during all tests.

Electrical stimulation was achieved using a Danica Elpha 200 pain and muscle stimulator. The area under the electrodes was shaved and cleaned prior to electrode application . The stimulating electrode (4 cm x 3 cm rubber) was placed on the vastus medialis motor point and the ground electrode was placed further laterally on the thigh. The stimulation current used was twice the threshold stimulation and the stimulation parameters were 50 Hz stimulation, pulse width = 0.1 msec, on time = two sec and off time = two sec.

Blood flow was calculated as the product of femoral artery mean blood velocity and diameter measures with Doppler ultrasound (Toshiba, Tochigi-Ken, Japan) using a 7.5 MHZ

probe, operating in pulsed duplex mode at a depth of four cm. The audio frequency signal of the velocity spectra was recorded continuously on tape. The velocity was then further analysed by fast Fourier transform using a spectrum analyser (Angiodyne, Nijmegen, the Netherlands) to yield the mean blood velocity over each heart beat. During each minute of the test one heart beat was analysed and during the first minute of exercise the analyses was performed once every ten seconds for a total of 15 measures throughout the test. Femoral artery diameter measures were made each minute throughout the test on a frozen screen and the values were then recorded during playback of the video tape.

Thigh volume was determined from circumference, length and skinfold measures according to the method of Jones and Pearson (1969). Quadriceps femoris muscle mass was estimated according to the equation (Andersen and Saltin, 1985)

$$\text{mass} = 0.307V + 0.353$$

where mass = quadriceps muscle mass

V = thigh volume

Mean arterial pressure (MAP) at the level of the femoral artery was collected continuously during rest and exercise (Finapress, Ohmeda 2300).

The main effects of testing condition (ABS voluntary, ABS stimulated and SCI stimulated) and time (8 levels), on femoral artery diameter were analysed by a 2 way ANOVA. The main effects of testing condition (ABS voluntary, ABS stimulated and SCI stimulated) and time (15 levels) on femoral artery blood flow were analysed by a 2 way ANOVA. The main effects of

testing condition (ABS voluntary, ABS stimulated and SCI stimulated) and time (rest and end exercise on heart rate and MAP were analysed by a 2 way ANOVA. The effect of condition (ABS vs. SCI) on thigh volume was analysed by a 1 way ANOVA. The level of significance for the main effects was set at $P < 0.05$. Any differences were further analysed with Student- Neuman Keuls post hoc test. All data are presented as mean ± standard error (SE).

RESULTS

Femoral artery diameters and thigh volume were less in SCI (D = 6.23±0.37 mm, V = 4.39 ± 0.78 l) compared to ABS (D = 10.66 ± 0.48, V = 7.75 ± 0.66 l) (Figure 6.4) Femoral artery diameter did not change during rest or exercise for any condition. HR and MAP were both elevated with VOL but not STIM exercise (Figure 6.3). Leg blood flows were higher in ABS than SCI for all time points . At the onset of exercise there was an increase in flow in the ABS for voluntary and stimulated exercise compared to rest in the same condition, but no change in flow in SCI (Figure 6.4).

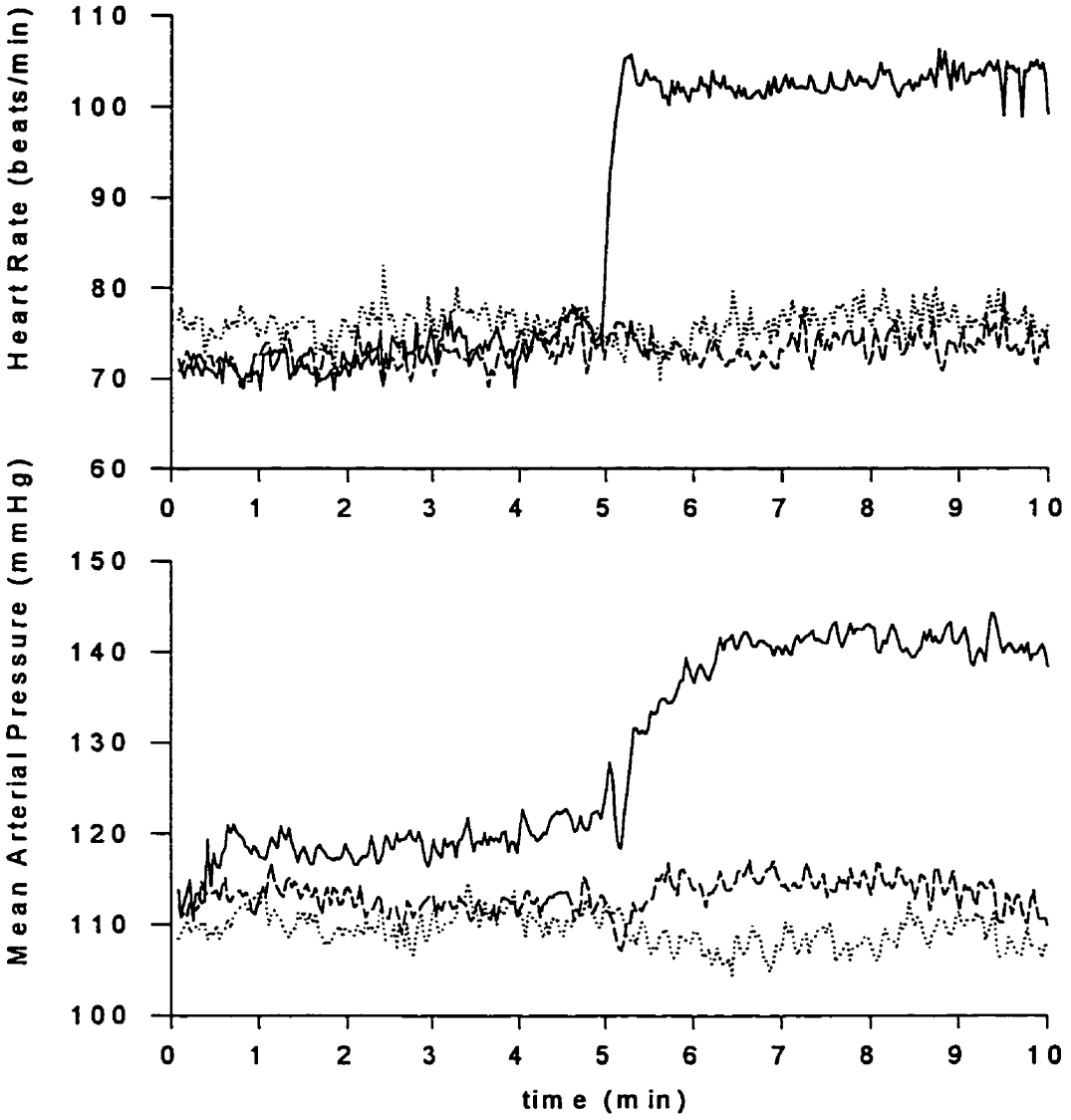


Figure 6.3 *Heart rate (HR, top) and mean arterial pressure (MAP, bottom) at rest and during exercise for ABS voluntary exercise (____), ABS stimulated exercise (_ _ _) and for SCI stimulated exercise (.....). Time 4 minutes indicates the onset of exercise. Values are mean for 7 subjects.*

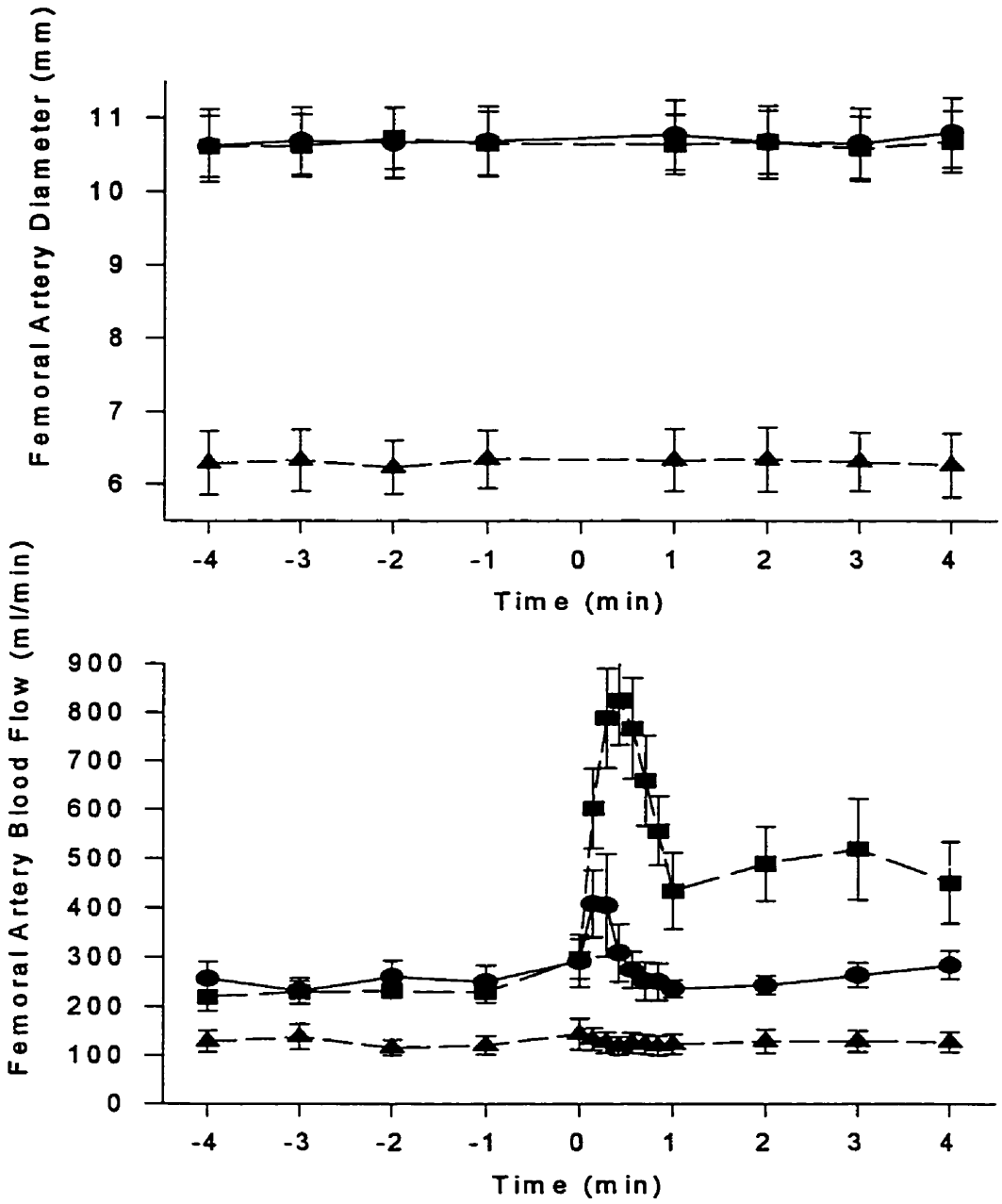


Figure 6.4 *Femoral artery diameter (top) and flow (bottom) for ABS voluntary exercise (■) ABS stimulated exercise (●) and SCI stimulated exercise (▲) all values are mean ± SE for 7 subjects.*

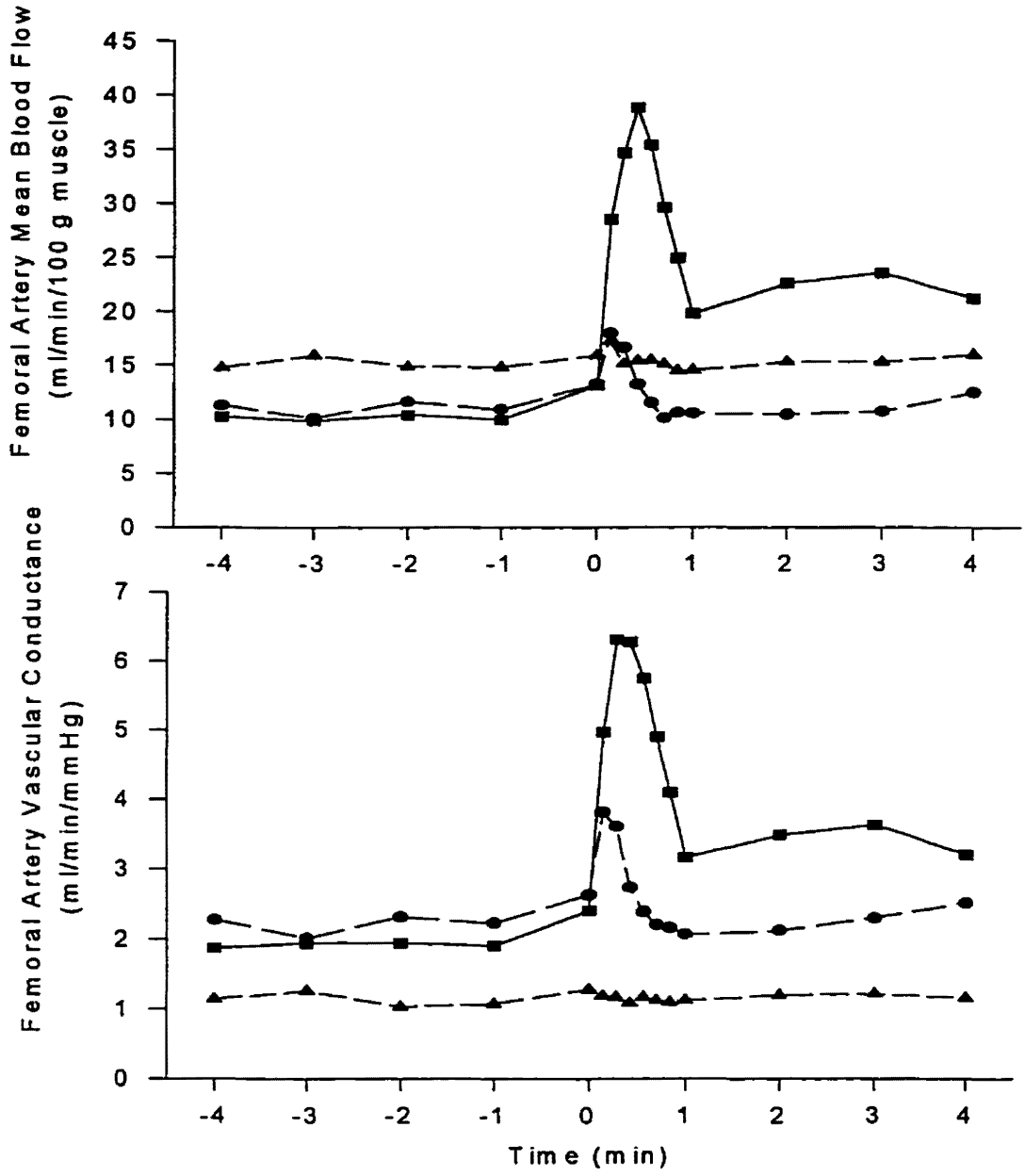


Figure 6.5 *Femoral artery blood flow normalized for quadriceps muscle mass (top) and leg vascular conductance (bottom) for ABS voluntary exercise (■) ABS stimulated exercise (●) and SCI stimulated exercise (▲) all values are mean for 7 subjects*

CONCLUSIONS

It was observed that SCI individuals had significantly smaller femoral arteries, thigh volumes and leg blood flows compared to ABS. In the ABS, but not in the SCI there was an increase in vascular conductance at the onset of leg exercise which resulted in a transient increase in flow to the inactive leg. It is not, however, clear if this decrease in resistance in the ABS was due to a transient reduction in sympathetic tone at the onset of exercise.

In this study, continuous blood flow measures were not obtained throughout the tests due to technical limitations. The blood flow was obtained for a representative heart beat during each time point and the exercise protocol was performed three times on each subject to reduce random error. We have no confirmation that the non-exercising leg was truly inactive. If the leg was active at the onset of contralateral leg exercise this may have contributed to the transient flow increases seen in the ABS at the onset of both voluntary and stimulated leg exercise. A primary focus of this study was the comparison of ABS and SCI, however, qualitatively, the force of contraction generated with stimulation of the SCI appeared to be less than in ABS. An effort had been made to normalize the LBF values for active muscle mass (Figure 6.5), however, the equation used to estimate quadriceps muscle mass has not been validated in SCI individuals where the ratio of muscle to leg volume may be different than in ABS (Andersen and Saltin, 1985). This difference may also explain the lack of flow response at the onset of stimulated exercise in the SCI.

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