Oxygen uptake and blood flow kinetics following the onset of exercise in trained humans

by

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Author's declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.
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Azmy Faisal

Abstract

The main hypothesis of this thesis was that the regulation of oxygen uptake ($\dot{V}O_2$) kinetics at the onset of exercise in trained young men is linked to cardiovascular adaptations. Two studies were conducted to investigate the interrelationships between oxygen (O_2) transport and O_2 utilization in accelerating $\dot{V}O_2$ kinetics at the onset of exercise. In the first study, simultaneous kinetics of $\dot{V}O_2$ and cardiac output ($\dot{Q}$) were studied during the transition to heavy and moderate cycling exercise (Chapter 2). The acceleration of $\dot{V}O_2$ kinetics during the heavy exercise that followed prior moderate or heavy exercise was enabled by the rapid increase in $\dot{Q}$; whereas, the acceleration of $\dot{V}O_2$ kinetics during moderate exercise that followed a heavy warm-up was associated with small changes in $\dot{Q}$ kinetics.

The objective of the second study was to determine, in a model of forearm exercise, if the elevation of forearm blood flow (FBF) prior to the onset of exercise by prior circulatory occlusion would accelerate FBF and muscle oxygen uptake ($\dot{V}O_{2mus}$) kinetics during subsequent exercise as demonstrated previously for prior exercise (Chapter 3). Prolonged ischemia (15 min occlusion) followed by 3 min recovery reduced FBF and impaired $\dot{V}O_{2mus}$ kinetics during subsequent heavy hand-grip exercise. However, prior heavy exercise confirmed the previous findings and resulted in a faster FBF and $\dot{V}O_{2mus}$ kinetics. There was a high positive correlation between the time course of change in FBF and $\dot{V}O_{2mus}$ at the onset heavy

exercise. In a follow up of the second study, to investigate a possible mechanism for the slower adaptation of $\dot{V}O_{2mus}$ following ischemia, the prior occlusion condition was repeated after ingesting a high dose of ibuprofen. Prostaglandin inhibition by ibuprofen augmented the FBF response during reactive hyperaemia and restored FBF during the heavy exercise that followed 15 min of circulatory occlusion to the control level.

These two studies provide evidence that O_2 delivery plays a dominant role in accelerating $\dot{V}O_2$ kinetics at the onset of heavy exercise in trained young men. The findings exposed differences in the mechanisms regulating pulmonary $\dot{V}O_2$ and $\dot{V}O_{2mus}$ with prior exercise resulting in higher $\dot{Q}$ and FBF, but no changes in O_2 extraction to yield the faster increase in pulmonary $\dot{V}O_2$ and $\dot{V}O_{2mus}$ at the onset of subsequent heavy exercise. In contrast, prior occlusion slightly retarded the increase in FBF and significantly reduced O_2 extraction thus delaying $\dot{V}O_{2mus}$ kinetics. The precise mechanisms impairing $\dot{V}O_{2mus}$ kinetics at the onset of heavy forearm hand-grip exercise that starts after a brief recovery from prolonged occlusion are still unknown, but this impairment may be partially due to a vasoconstrictor effect restricting blood flow during the adaptation to exercise and redistribution of the blood to the periphery.

In a third study, the influence of muscle activity on the $\dot{V}O_2$ slow component during heavy exercise and O_2 cost during moderate exercise that followed a heavy warm-up were examined (Chapter 4). The heavy exercise $\dot{V}O_2$ slow component was

attenuated in a graded fashion by prior moderate and heavy warm-ups, and the principal components analysis showed a moderate but significant correlation between the changes in the integrated electromyographic activity and the $\dot{V}O_2$ slow component amplitude. The higher O_2 cost of moderate exercise following a heavy warm-up was associated with higher mean power frequency. Changes in $\dot{V}O_2$ slow component and increased O_2 cost during moderate exercise after prior heavy warm-up appear to be related to some changes in surface electromyographic activity which may provide some evidence for increased muscle fibres recruitment.

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(a-v)DO_2 - Arterial-venous oxygen content difference

AUC - Area under the curve

C_2H_2 - Acetylene

COX - Cyclooxygenase

EMG - Electromyography

FBF - Forearm blood flow

Hb - Hemoglobin

He - Helium

HR - Heart Rate

iEMG - Integrated electromyography

MAP - Mean arterial pressure

MBV - Mean blood velocity

MPF - Mean power frequency

MRI - Magnetic resonance imaging

MRS - Magnetic resonance spectroscopy

MVC - Maximal voluntary contraction

NO - Nitric oxide

O_2 - Oxygen

PaO_2 - Intracellular partial pressure of oxygen

PCA - Principal components analysis

PCr - Phosphocreatine

PDHa - Pyruvate dehydrogenase

PGs – Prostaglandins

$\dot{Q}$ - Cardiac output

$\dot{Q}_{C2H2}$ - Acetylene rebreathing cardiac output

$\dot{Q}_{MF}$ - Finometer Modelflow cardiac output

SV - Stroke volume

SBF – Skin blood flow

τ_2 - Phase two time constant

$\dot{V}CO_2$ - Carbon dioxide output

$\dot{V}_E$ - Minute ventilation

$\dot{V}O_2$ - Oxygen uptake

$\dot{V}O_{2mus}$ - Muscle oxygen uptake

$\dot{V}O_{2peak}$ - Peak oxygen uptake

VT - Ventilatory threshold

Chapter 1
Literature Review

1.1 Introduction

Oxygen uptake ($\dot{V}O_2$) kinetics describe the time course of changes in muscle oxidative phosphorylation during work rate transitions. $\dot{V}O_2$ kinetics provide a unique window into understanding the efficiency of the metabolic system and the percentage contributions from both aerobic and non-aerobic sources. At the onset of exercise, $\dot{V}O_2$ increases in an exponential fashion (Henry, 1951; Henry & Demoor, 1956) with an error signal that is progressively reduced in proportion to the difference between the required and the actual $\dot{V}O_2$ at the new work rate (Hughson *et al.*, 2000; Whipp & Wasserman, 1972). A debate has developed surrounding the underlying physiological processes that regulate the rate of increase in oxidative metabolism during work rate transitions (Grassi, 2001; Hughson, 1990; Tschakovsky & Hughson, 1999; Whipp *et al.*, 2005). One position argues that a limitation of oxidative enzyme activity and mitochondrial substrate availability (metabolic inertia) restricts the rate at which oxygen (O_2) utilization can increase at the onset of exercise, independent of O_2 availability under most normal exercise conditions from rest to approximately 50-60% of maximal $\dot{V}O_2$ (Barstow *et al.*, 1994; Grassi, 2003; Hill *et al.*, 1924; Mahler, 1985). The second argument suggests that even under these normal conditions O_2 plays a critical role in regulating the adaptation of oxidative metabolism (Hughson & Morrissey, 1983; Linnarsson, 1974; MacPhee *et al.*, 2005). A consequence of the metabolic inertia hypothesis is that $\dot{V}O_2$ kinetics would be insensitive to increased or modest

reductions in O_2 delivery (Poole & Richardson, 1997). Whereas, the O_2 delivery hypothesis suggests that there is a rate limiting step in the O_2 cascade from the lungs to the mitochondria of the working muscle and a greater portion of ATP demand can be synthesized through the aerobic energy supply system with small perturbations of the metabolic controllers (phosphorylation and redox potentials), if more O_2 is made available (Hughson *et al.*, 2001; Wilson & Rumsey, 1988).

In spite of the disparate viewpoints mentioned above, it is generally accepted by both camps that there are conditions where altering O_2 delivery can impact $\dot{V}O_2$ kinetics. The debate remains; however, over the exact nature of these conditions. This debate can be distorted by a focus on the "either/or" nature of O_2 transport versus O_2 utilization limitations. Recently, the terms "regulation" or "modulation" of $\dot{V}O_2$ kinetics by O_2 transport have been used and these seem to be a more appropriate way to understand the interaction between the factors that determine the rate of increase in oxidative phosphorylation during work rate transitions (Hughson, 2005; Hughson, 2009; Hughson *et al.*, 2001; Tschakovsky & Hughson, 1999).

Several innovative experimental designs have been applied to examine the factors that control $\dot{V}O_2$ kinetics during work rate transitions. These designs centered mainly on alternating the metabolic environment and/or manipulating the rate of O_2 delivery prior to the exercise onset. The priming exercise model is the most common experimental paradigm used to address the role of O_2 delivery in regulating $\dot{V}O_2$ kinetics. Prior heavy exercise alters the local metabolic environment, increases muscle bed vasodilation, right-shifts the oxygen hemoglobin (HbO_2) dissociation curve and improves muscle perfusion to the

working muscles at the onset of a second exercise bout (Gerbino *et al.*, 1996; MacDonald *et al.*, 1997). However, prior heavy exercise also increases oxidative enzyme activity and elevates mitochondrial substrate availability at the onset of subsequent exercise (Gurd *et al.*, 2006).Thus, the role of O_2 delivery to enhance $\dot{V}O_2$ kinetics remains undefined due to the conflicting results and the interpretation of the findings using this model. Prior occlusion is an alternative experimental model that could elevate muscle blood flow for several minutes prior to a subsequent exercise bout (Carlsson *et al.*, 1987) without marked impact on the metabolic states (Mole *et al.*, 1985). Thus, this experimental paradigm may precisely address the role of O_2 delivery in regulating the $\dot{V}O_2$ kinetics during work rate transitions.

The studies applied in this thesis emphasize the influence of increased O_2 supply through the active (prior exercise) and passive (prior occlusion) warm-up, on accelerating pulmonary $\dot{V}O_2$ and muscle oxygen uptake ($\dot{V}O_{2mus}$)kinetics at the onset of dynamic exercise in trained humans. To help clarify the issue surrounding the role of O_2 delivery in controlling $\dot{V}O_2$ kinetics, it is important to monitor muscle blood flow in the exercising muscles or other surrogate markers of cardiovascular responses such as heart rate (HR) or cardiac output ($\dot{Q}$) kinetics. Relatively few studies have simultaneously examined the kinetics of $\dot{V}O_2$ and $\dot{Q}$ during exercise transitions (Perrey *et al.*, 2003a; Yoshida & Whipp, 1994). In the first study, simultaneous measurements of breath-by-breath $\dot{V}O_2$ and estimates of beat-by-beat $\dot{Q}$ have been applied to investigate the relative contributions of muscle metabolic and cardiovascular adaptations to $\dot{V}O_2$ kinetics during both moderate

and heavy cycling exercise (Chapter 2). In the second study, pulsed and echo Doppler ultrasound has been used to directly monitor muscle blood flow during rhythmic hand-grip exercise that follows identical bout of heavy hand-grip exercise or circulatory occlusion. The purpose of this study was to determine whether or not prior occlusion, followed by a brief recovery, will have similar effects as prior heavy exercise in accelerating forearm blood flow (FBF) and $\dot{V}O_{2mus}$ kinetics during subsequent heavy forearm exercise (Chapter 3). Also, the effect of inhibiting cyclooxygenase (COX) enzyme by a high dose of ibuprofen on FBF, $\dot{V}O_{2mus}$ and skin blood flow (SBF) during a heavy hand-grip exercise that follows prolonged ischemia was examined (Chapter 3). In the third study, electromyographic (EMG) activity of the lower limb muscles was measured simultaneously with breath-by-breath $\dot{V}O_2$ to examine whether or not changes in muscle activity are associated with the $\dot{V}O_2$ slow component during heavy exercise and the higher O_2 cost during moderate exercise that followed a heavy warm-up were examined (Chapter 4).

1.2 Oxygen uptake following exercise onset

1.2.1 Measurements and characteristics of oxygen uptake kinetics

The measurements of $\dot{V}O_2$ at the onset of exercise are first made at specific time intervals by the respirometer collection system (Krogh & Lindhard, 1913). The development of breath-by-breath measurements via the computerized mass spectrometer-based systems enabled the precise characteristics of $\dot{V}O_2$ dynamics during work rate transitions (Auchincloss *et al.*, 1966; Hughson, 1984a; Linnarsson & Lindborg, 1974; Whipp & Wasserman, 1972). At the onset of exercise, $\dot{V}O_2$ does not increase instantaneously, rather it rises in an exponential

fashion to reach the anticipated steady-state level and the rate of increase is directly proportional to the exercise intensity (Krogh & Lindhard, 1913; Linnarsson, 1974; Whipp & Wasserman, 1972). There is an initial rapid increase in $\dot{V}O_2$ lasting between 15 and 20 s (Phase I), that is attributed to a rapid increase in $\dot{Q}$ and pulmonary blood flow, due to the action of the muscle pump, and small increase in arterial-venous oxygen content difference [(a-v)DO_2] (Krogh & Lindhard, 1913; Whipp & Ward, 1982). This initial increase in Phase I is followed by a rapid exponential increase in $\dot{V}O_2$ (Phase II) that drives $\dot{V}O_2$ towards the essential steady state level. Phase II (primary phase) reflects the arrival of the venous blood from the exercising muscles to the lung (Whipp & Ward, 1982). Modeling studies of $\dot{Q}$ profiles and $\dot{V}O_2$ (Barstow *et al.*, 1990), direct experiential measurements of muscle $\dot{V}O_2$ (Grassi *et al.*, 1996; Koga *et al.*, 2005) and characterization of PCr degradation by magnetic resonance spectroscopy (MRS) (Rossiter *et al.*, 1999; Rossiter *et al.*, 2002a) have shown that the Phase II pulmonary $\dot{V}O_2$ kinetics largely reflect the kinetics of O_2 consumption in the exercising muscles following the onset of exercise. During heavy exercise, the attainment of steady-state $\dot{V}O_2$ is delayed or may be absent, and the slow component of $\dot{V}O_2$ (Phase III) is manifested (Whipp, 1994; Whipp & Wasserman, 1972). The majority of pulmonary $\dot{V}O_2$ during Phase III (86%) can be attributed to the exercising muscles (Poole *et al.*, 1991).

1.2.2 Control of oxygen uptake following exercise onset

The rate of increase in oxidative phosphorylation has been debated to be limited by the adaptations of O_2 transport and O_2 utilization mechanisms. The manipulation

of the muscle partial pressure of oxygen provides evidence to support the role of O_2 delivery in limiting the $\dot{V}O_2$ kinetics at the onset of exercise. Impairment of the mitochondrial O_2 supply in the following equation will restrict the rate of ATP synthesis through the oxidative energy supply system, and by consequence, the kinetics of $\dot{V}O_2$ through the transition to steady state will be slower (Poole *et al.*, 2008).

$$6\ ADP + 6\ Pi + 2(NADH + H+) + O_2 \rightarrow 6\ ATP + 2\ NAD + 2\ H_2O$$

Inspired hypoxic or hyperoxic gases modify the muscle energetic state (ATP + PCr) (Haseler *et al.*, 1998; Linnarsson *et al.*, 1974) and $\dot{V}O_2$ kinetics during submaximal work rates (Engelen *et al.*, 1996; Linnarsson, 1974; MacDonald *et al.*, 1997; Murphy *et al.*, 1989). Biochemical measurements of cellular metabolism have shown that O_2 delivery may adjust the phosphorylation and redox potentials needed to drive the oxidative metabolism (Wilson & Rumsey, 1988). Additionally, Hughson (2005; 2009) suggested theoretical models that illustrate the impact of dynamic changes in the intracellular partial pressure of oxygen (PO_2) on metabolic inertia (enzymes activity and metabolic substrates) and how the oxidative metabolism at the onset of exercise is modulated by a dynamic interaction between O_2 delivery and utilization mechanisms.

In addition, altering the O_2 availability to the working muscles by reducing HR dynamics using β-adrenergic receptor blockade drugs (Hughson, 1984b; Hughson & Smyth, 1983), or performing the exercise in supine posture (Hughson *et al.*, 1991a; Jones *et al.*, 2006; MacDonald *et al.*, 1998), above the heart level (Hughson *et al.*, 1996), where the blood flow to the working muscles is restricted, or transition from prior moderate exercise, where the rapid influence of the

parasympathetic system on O_2 delivery is limited,(Brittain *et al.*, 2001; Hughson & Morrissey, 1982; MacPhee *et al.*, 2005) has resulted in slower $\dot{V}O_2$ kinetics during the transition to moderate work rates, suggesting strong evidence to link $\dot{V}O_2$ kinetics to O_2 transport.

Conversely, researchers who argue against the potential role of O_2 availability in regulating the metabolic control and support the acceleration of O_2 utilization as a sole mechanism to control $\dot{V}O_2$ kinetics based their position on several experimental observations. Muscle O_2 transport dynamics as assessed from arterial blood flow (Bangsbo *et al.*, 2000; Grassi *et al.*, 1996; MacDonald *et al.*, 1998), $\dot{Q}$ (Perrey *et al.*, 2003a; Yoshida & Whipp, 1994) or HR (MacPhee *et al.*, 2005) have been shown to be faster than those of $\dot{V}O_2$; however, the assumption that microvascular perfusion can be extrapolated accurately from upstream arterial measurements or cardiovascular dynamics has been challenged. During upright exercise in healthy subjects, potentially reducing muscle O_2 supply following blood withdrawal (Burnley *et al.*, 2006) or increase O_2 transport through hemodilution (Berger *et al.*, 2006a) does not alter the $\dot{V}O_2$ kinetics. Yet, the blood flow distribution within the exercising muscle is not known in these studies. Moreover, alterations in O_2 supply by breathing hypoxic or hyperoxic gases have been shown to modify $\dot{V}O_2$ kinetics (Linnarsson, 1974; MacDonald *et al.*, 1997). Several studies have reported a similar time course for the reduction in muscle [PCr], estimated by MRS, and the increase in pulmonary $\dot{V}O_2$ during transition from rest to both moderate and heavy work rates (Rossiter *et al.*, 1999; Rossiter *et al.*, 2002a). The exponential nature of [PCr] and $\dot{V}O_2$ kinetics has led those researchers to assume that metabolic inertia is the only limiting step for $\dot{V}O_2$

kinetics (Bangsbo *et al.*, 2000; Grassi, 2003). However, altering the [PCr] and other metabolic intermediates by changing PO_2 challenge this argument (Haseler *et al.*, 1998; Hogan *et al.*, 1992; Linnarsson *et al.*, 1974). Pharmacological activation of pyruvate dehydrogenase (PDHa) with dichloroacetate (DCA) reduces substrate-level phosphorylation during subsequent exercise, suggesting an enhancement of the contribution of oxidative phosphorylation to energy turnover (Greenhaff *et al.*, 2002). Yet, this intervention has been shown not to speed $\dot{V}O_2$ or $\dot{V}O_{2mus}$ kinetics during heavy exercise (Bangsbo *et al.*, 2002; Grassi *et al.*, 2002; Jones *et al.*, 2004b; Rossiter *et al.*, 2003). Inhibition of nitric oxide (NO) synthesis with L-NAME resulted in faster $\dot{V}O_2$ kinetics which was thought to be associated with reduce O_2 avilability and enhance cytochrome c oxidase activity (Jones *et al.*, 2004a). However, NO synthesis inhibition elevates mean arterial blood pressure (MAP) and probably redistributes the blood during exercise (Frandsenn *et al.*, 2001). Furthermore, in the pump-perfused canine hind-limb model, the inhibition of NO synthesis with L-NAME did not alter muscle blood flow distribution to the gastrocnemius–plantaris–soleus muscle group (Krause *et al.*, 2005), and the direct inhibition of mitochondrial respiration by NO does not limit the kinetics of oxidative metabolism at exercise onset (Grassi *et al.*, 2005).

Effect of training on oxygen uptake kinetics

The sensitivity to changes in O_2 demand might be increased with training. Short periods of physical training can enhance both O_2 transport and O_2 utilization mechanisms and speed $\dot{V}O_2$ kinetics (Phillips *et al.*, 1995). Endurance exercise training has been shown to accelerate $\dot{V}O_2$ kinetics and reduce $\dot{V}O_2$ slow component amplitude (Berger *et al.*, 2006b; Carter *et al.*, 2000). However, it is not apparent what type of training program would be most advantageous to enhance

$\dot{V}O_2$ kinetics. Interestingly, repeated high intensity anaerobic training has been shown to be as effective as low intensity continuous aerobic training in enhancing the rate of oxidative metabolism at the onset of exercise (Bailey *et al.*, 2009a). Most recently, Bailey *et al.* (2010) have shown that 4 weeks of inspiratory muscle training increased blood flow to the exercising muscle and improved $\dot{V}O_2$ kinetics at the onset of high intensity exercise suggesting that enhanced O_2 delivery enabled the increase of oxidative phosphorylation during the transition to heavy exercise.

1.3 Cardiac output and blood flow at exercise onset

Blood circulation has been described by the Arab physician Ibn al-Nafis during the Islamic golden age in the early 13 th century. However, the quantitative methods to study the peripheral circulation of blood in human were not available until the 20th century. Both invasive and non-invasive methods for evaluating $\dot{Q}$ and blood flow in human during exercise have been used.

1.3.1 Measurements of cardiac output

$\dot{Q}$, the volume of blood being pumped by the heart in each minute, can be measured by several techniques. Each method has advantages and restrictions that may limit its application during exercise. The direct Fick method was first described by Adolf Eugen Fick in 1870. The Fick principle calculates the O_2 consumption from the measurement of the oxygen content of the venous, measured at the pulmonary artery, and arterial blood, which can be measured at the radial or femoral artery (Astrand *et al.*, 1964; Chapman *et al.*, 1950).Thus, $\dot{Q}$ can be calculated from the following equation: $\dot{Q} = \dot{V}O_2 / (a\text{-}v)DO_2$.

The thermodilution method was described initially by Fegler (1954) for measuring $\dot{Q}$ in animals. The method was then adapted for use in man by Branthwaite and Bradley (1968), and developed further by use of the Swan and Ganz catheter (Ganz & Swan, 1972). The thermodilution method requires the infusion of ice cold saline into the pulmonary artery and measurements of the temperature difference of blood upstream and downstream of the infusion point several centimetres away (Ganz & Swan, 1972). Cardiac output can then be determined based upon these measurements and knowledge of the rate and temperature at which the saline was infused. High $\dot{Q}$ will change the temperature rapidly, and low $\dot{Q}$ will change the temperature slowly (Runciman *et al.*, 1981; van Grondelle *et al.*, 1983). The Fick principle and thermodilution method are considered to be the gold standard techniques for measuring $\dot{Q}$.However, the invasive natures of these techniques and the need for a medical expertise limit their use during exercise (Warburton *et al.*, 1999). Also, these techniques are limited to study the rate of changes in $\dot{Q}$ during the transition from rest to higher work rates.

Rebreathing techniques have been employed in clinic settings (Hoeper *et al.*, 1999; Sackner *et al.*, 1980) and exercise (Hsia *et al.*, 1995; Johnson *et al.*, 2000; Reybrouck *et al.*, 1978; Simmons & Shephard, 1971) to determine $\dot{Q}$. Foreign gases such as acetylene (C_2H_2) and nitrous oxide (N_2O) are normally used in rebreathing techniques because they are inert, soluble, and enter the blood stream via pulmonary diffusion but do not bind with hemoglobin (Ayotte *et al.*, 1970; Triebwasser *et al.*, 1977; ZeidiFard *et al.*, 1976). Pulmonary blood flow is directly related to $\dot{Q}$ and can be estimated by the rate of disappearance of a soluble gas from the lungs into the blood stream, when the diffusion constant is known.

Rebreathing techniques are valid for determining $\dot{Q}$, but they are limited to be used during steady state exercise.

An accurate, continuous and non-invasive measurement of $\dot{Q}$ during exercise would be of great help to characterize the time course of changes in $\dot{Q}$ during work rate transitions. Beat by beat $\dot{Q}$ can be obtained by Doppler echocardiography (Ihlen *et al.*, 1987) and vascular impedance cardiography (Denniston *et al.*, 1976; Perrey *et al.*, 2003a) however, motion artifacts limit the use of these techniques to supine positions or low intensity exercise.

$\dot{Q}$ was also estimated from the finger cuff Modelflow technique that employs a non-linear, three-element windkessel equation (Wesseling *et al.*, 1993). Previous studies have reported that the Modelflow $\dot{Q}$ technique was valid in comparison to thermodilution (de Wilde *et al.*, 2007; Wesseling *et al.*, 1993) and Doppler echocardiography methods (Sugawara *et al.*, 2003), as well as CO_2 (Pitt *et al.*, 2004) and C_2H_2 rebreathing methods (Tam *et al.*, 2004).

Most recently, MRI techniques have been used to determine $\dot{Q}$. Simultaneous imaging of the aorta in combination with velocity encoded phase contrast MRI, which measures an average blood velocity across the vessel diameter, provides the most accurate measure of $\dot{Q}$ in large vessels (Groepenhoff *et al.*, 2007).

1.3.2 Cardiac output at exercise onset

To help clarify the issue surrounding the influence of O_2 delivery, it is important to monitor muscle blood flow. However, the direct measurement of leg

blood flow kinetics during cycling exercise is a challenge. Subsequently, $\dot{Q}$ kinetics (De Cort *et al.*, 1991; Perrey *et al.*, 2003a; Yoshida & Whipp, 1994) have been used as a surrogate marker. Recently, Lador *et al.* (2006) studied $\dot{Q}$ kinetics, using a Modelflow estimation technique (Wesseling *et al.*, 1993), during low (50W) and moderate (100W) cycling exercise bouts. The kinetics of $\dot{Q}$ have been shown to be faster than $\dot{V}O_2$ kinetics. The faster $\dot{Q}$ at the onset of exercise could be a consequence of a rapid increase in SV (Leyk *et al.*, 1995) resulting from the sudden increase in venous return with the onset of the higher work rate mediated primarily by the stronger muscle pump (Sheriff *et al.*, 1993; Tschakovsky *et al.*, 1996), and increased HR (Perrey *et al.*, 2003a) that reflects a greater reliance on parasympathetic activation and vagal withdrawal. Leyk *et al.* (1995) monitored the SV using Doppler ultrasound during upright cycling across exercise transitions to work rates of up to 200 W. They showed SV to increase rapidly and peak by 30 s following the onset of exercise.

1.3.3 Measurements of blood flow

Early measurements of blood flow in humans during exercise were obtained by venous occlusion plethsmography (Humphreys & Lind, 1963). The basis of this technique lies in the fact that an external pressure (sub-diastolic pressure not more than 50 mmHg) suppresses only the venous outflow, while the arterial inflow remains unaffected. Joyner *et al.*(1990) have shown a fast adaption in exercise blood flow measured by venous occlusion plethsmography. However, the method has certain disadvantages. First, measures taken during muscular work are not good, measures are usually taken during short pauses in contraction or at the end of exercise, and therefore the obtained blood flow data are a combination of exercise and reactive

hypermia. Second, the limb must be placed above the level of the heart; which negatively affects the blood flow and oxygen uptake kinetics (Hughson *et al.*, 1996). In comparison to Doppler ultrasound, the venous occlusion plethsmography technique has been shown to be accurate only in the first beats after the release from circulatory occlusion (Tschakovsky *et al.*, 1995).

The local injection technique with a radioactive label has also been used to determine the blood flow to the working muscles (Grimby *et al.*, 1967; Holzman *et al.*, 1964; Tonnessen, 1964). The distribution of the label can be assessed before and after exercise as an indication of the flow through the exercising muscle. Using the ^{133}Xe clearance methods, Tonnessen (1964) reported that there is a gradual increase in the blood flow to the calf muscle with the increase in work rates up to 70 % of the maximal. Pendergast *et al.*(1980) concluded that changes in muscle blood flow kinetics are faster than those of $\dot{V}O_2$. However, the ^{133}Xe clearance method seems to have qualitative rather than quantitative significances due to the considerable underestimation of blood flow in the working muscles (Cerretelli *et al.*, 1984).

Quantitative determination of muscle blood flow during exercise may be obtained by the thermodilution method (Andersen & Saltin, 1985; Ganz *et al.*, 1964; Richardson *et al.*, 1993; Rowell *et al.*, 1986). Ganz (1964) was the first to use the thermodilution method to measure the blood flow in the femoral artery during exercise. thermodilution method is used to assess blood flow during steady state exercise (Andersen & Saltin, 1985). However, attempts have been made to use it to characterize the time course of changes in blood flow at the onset of exercise (Grassi *et al.*, 1996; Knight *et al.*, 1993). The invasive nature of the thermodilution methods reduces the ability to employ it during exercise.

Doppler ultrasound technology has been extensively used in the measurement of blood flow in the last two decades. It has been used to measure blood flow non-invasively and continuously at the onset of forearm exercise (Hughson *et al.*, 1996; Tschakovsky *et al.*, 1995; van Beekvelt *et al.*, 2001) and kicking exercise (Nyberg *et al.*, 2010; Paterson *et al.*, 2005; Shoemaker *et al.*, 1994). Doppler ultrasound was shown to be reproducible in the measurements of arterial mean blood velocity (MBV) and diameter during both rest and exercise across different days (Shoemaker *et al.*, 1996a). Previous studies have reported a strong relationship between Doppler ultrasound and strain-gauge plethysmography for MBV measurements (Tschakovsky *et al.*, 1995; Van Leeuwen *et al.*, 1992). Radegran (1997)has reported that ultrasound Doppler estimates of blood flow during dynamic knee extensor exercise was valid in comparison to thermodilution techniques. Figure 1.1 is an example of the output of Doppler ultrasound which allows to non-invasively measuring the blood velocity to the exercising muscles.

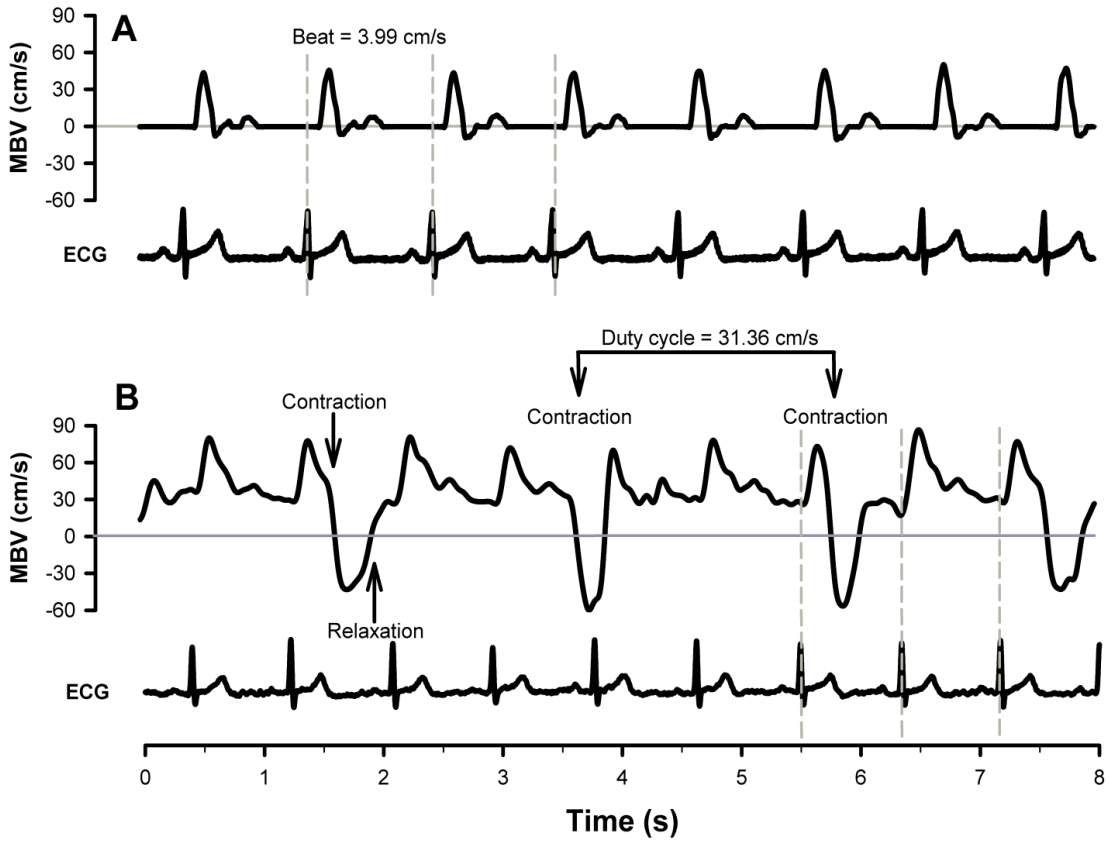

Figure 1.1: Representative tracing of mean blood velocity by Doppler ultrasound in the biracial artery.

At the resting state (A), the blood flow response has three distinguish phases within each heart cycle. During systole, there is rapid increase in blood velocity, which peaks at peak systole. This initial increase in blood velocity is followed by a decrease in flow and reverse flow that stops as the aortic valve closes. In late diastole, there is a period of low forward flow or no flow as the blood passes out of the arteries and into the venous circulation. During exercise (B), there are large oscillations in the flow pattern with a reverse flow during contraction followed by an increase in flow during the relaxation phase.

1.3.4 Blood flow following exercise onset

Following the onset of exercise, muscle blood flow increases rapidly in a biphasic manner (Shoemaker *et al.*, 1994; Shoemaker *et al.*, 1996a), with initial rapid adaptation followed by slower adaption to match the changes in metabolic demand of the muscles (Hughson *et al.*, 2003; Radegran & Saltin, 1998). Both neural vasoconstrictor activity and locally derived vasodilator substances contribute to this precise blood flow regulation (Clifford & Hellsten, 2004). The initial rapid increase in blood flow, which occurs within the first 5-10 s of exercise, has been attributed to the muscle pump (Laughlin, 1987; Sheriff *et al.*, 1993), rapid vasodilation due to mechanical factors (Kirby *et al.*, 2007; Shoemaker *et al.*, 1998), and instantaneous increases in adenosine (Saltin *et al.*, 1998), acetylcholine spillover from neuromuscular junctions (Segal & Kurjiaka, 1995) and potassium concentrations (Armstrong *et al.*, 2007; Clifford, 2007; Hilton *et al.*, 1978; Murrant & Sarelius, 2002). A second, slower increase in blood flow is characterized by the release of endothelium mediated vasodilator substances such as NO (Dyke *et al.*, 1995) and prostaglandins (PGs) (Kilbom & Wennmalm, 1976; Nyberg *et al.*, 2010; Wilson & Kapoor, 1993) that influence the vascular tone and initiate further increases in exercise hyperemia. Following the first minute of exercise, further vasodilation arises due to the release of intramuscular vasoactive metabolites and thereby matches blood flow to the metabolic demands of the exercising muscles (Boushel, 2003; Rowell, 1997; Rowell, 2004). It is not the aim of this thesis to examine the factors controlling blood flow at the onset of exercise. Rather, the aim is to determine the interrelationship between the kinetics of blood flow and those of $\dot{V}O_{2mus}$ during work rate transitions.

1.4 Methodology

The applications of non-invasive technologies which precisely measure/estimate the blood flow response in a beat by beat basis can provide an accurate assessment to the role of O_2 delivery in controlling $\dot{V}O_2$ kinetics at the onset of exercise. In the studies applied in this thesis, several cardiovascular variables have been measured during the transition from rest to higher work rates. The techniques of measuring and analyzing $\dot{V}O_2$, blood flow, blood pressure (BP), $\dot{Q}$, and blood gases during work rate transitions have been used extensively in our lab over the past three decades. In addition, measurements of the muscle activity using surface EMG have been applied in a few studies (Perrey *et al.*, 2001; Perrey *et al.*, 2003b; Tordi *et al.*, 2003). This section will illustrate the principles behind these techniques and the measurements will be described in more details in the methods sections of the following chapters.

1.4.1 Oxygen uptake

Oxygen uptake was measured on a breath-by-breath basis (First Breath, Waterloo, Ontario, Canada). This system consisted of a digital volume turbine (UVM-17125, VacuMed, Ventura, CA) for gas volume measurement, and a mass spectrometer (Innovision, Amis 2000, Odense, Denmark) 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 an automated 3 L syringe (Vacumed, Ventura, CA) at flow rates comparable to those observed during exercise. The mass spectrometer was calibrated with precision medical gases which

spanned the gas concentrations observed during testing. The volume and flow were measured with no time delay; however, the gas fractions were measured with delay due to the difference between the transport time and mass spectrometer response time.

1.4.2 Blood flow

Blood flow was obtained by pulsed and echo Doppler ultrasound. The technique of Doppler ultrasound applied in measuring blood flow is based on the principle that immobile 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 directly proportional to the velocity of the moving element according to the following equation:

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

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.

1.4.3 Blood pressure

A finger plethysmograph (Finometer, Finapres Medical System, Arnhem, Netherlands) was used to measure arterial blood pressure on a beat-by-beat basis. The system has an infrared emitting diode in the finger cuff and a detector

immediately opposite, such that when the cuff wrapped around the finger, light from the diode travels through the finger and the amount reaching the other side can be detected. Absorption of the light is proportional to the distance through the finger that it must travel. With each heart beat, the change in vessel transmural pressure causes the finger to "swell" proportionally. Matching the changes in transmural pressure via instantaneous, equal increases in cuff pressure therefore maintains finger volume and provides continuous estimates of arterial pressure. Comparisons with direct arterial blood pressure measures indicate a good agreement (Imholz *et al.*, 1990).

1.4.4 Cardiac Output

In this thesis, $\dot{Q}$ has been measured non-invasively using Finometer finger cuff technique and validated using C_2H_2 rebreathing methods.

Finometer Modelflow cardiac output

The Modelflow algorithm implemented in the Finometer uses aortic characteristic impedance, windkessel compliance of the arterial system and peripheral vascular resistance to compute flow pulses from an arterial pressure pulses (Wesseling *et al.*, 1993), and has been shown to estimate changes in $\dot{Q}$ during moderate and heavy exercise with high precision. Moreover, the reconstruction of brachial artery waveform employed by Finometer had shown to improve the accuracy and sensitivity to detect the changes in cardiovascular measurements (Guelen *et al.*, 2003; Guelen *et al.*, 2008; Maestri *et al.*, 2005; Schutte *et al.*, 2004).

Acetylene rebreathing cardiac output

C_2H_2 rebreathing was used as a reference method in this study, because it has been shown to be valid (Bell *et al.*, 2003; Dibski *et al.*, 2005; Hunt *et al.*, 1997; Warburton *et al.*, 1998) and in fairly high agreement with dye-dilution and direct Fick methods when measuring $\dot{Q}$ at rest and during exercise up to 90% of the maximal $\dot{V}O_2$ (Liu *et al.*, 1997; Triebwasser *et al.*, 1977).

C_2H_2 is used to measure blood flow through the lungs since it is soluble gas and enters the blood stream with a known diffusion constant. An inert, insoluble gas (e.g., He, Ar, or CH_4) is also added to the gas mixture to ensure full equilibration of the system (bag/lung) before the disappearance rate of the acetylene is measured (Triebwasser *et al.*, 1977). The decay curves of the soluble and inert gas mixture (C_2H_2/He) can be used to compute $\dot{Q}$ as it is directly related to the rate of blood flow through the lungs.

1.5 Exponential characteristics of $\dot{V}O_2$ kinetics

The exponential function has an amplitude (A) and time constant (τ) which reflects the time required to reach 63% of the total amplitude or 63% of each distinct phase of the $\dot{V}O_2$ response. Generally, the steady-state is attained and the response amplitude is completed after 4 time constants (Hughson, 2005).

The curve fitting procedure calculates the various parameters of the exponential model by using the least-squares error approach. The best fit is defined by the minimum residual sum of squares between the model parameters and the actual data set with a symmetrical distribution around the zero-line as seen in Fig. 1.2.

$\dot{V}O_2$ kinetics have been described using either mono or higher order exponential models. A mono-exponential model quantifies the overall response of the exercise bout using the effective time constant (Gerbino *et al.*, 1996) that estimates the time required to achieve 63% of the total amplitude through the following equation

$$\dot{V}O_2 \text{ (at any time point)} = \dot{V}O_2 \text{ (baseline)} + A\ (1 - e^{-(t/\tau)}).$$

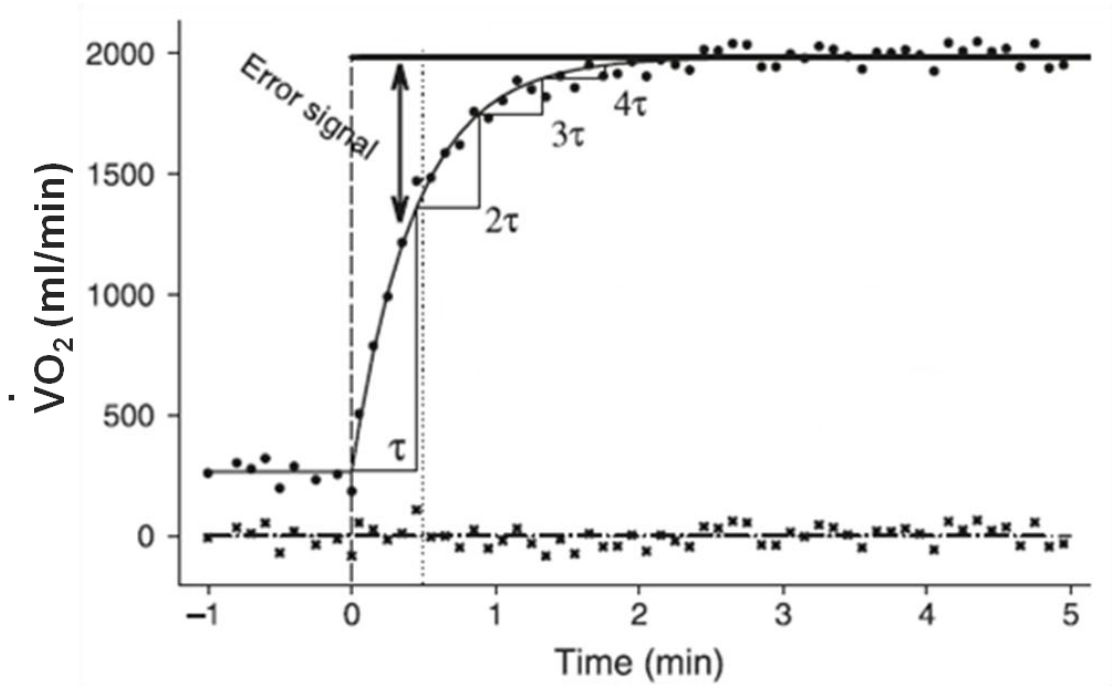

Figure 1.2: A schematic illustration of the exponential characteristics of $\dot{V}O_2$.

However, the low sampling frequency of blood during exercise did not enable a precise characterization of $\dot{V}O_{2mus}$ kinetics using the effective time constant equation. Therefore, to estimate $\dot{V}O_{2mus}$ kinetics in this study, the area under the curve (AUC) of $\dot{V}O_{2mus}$ was calculated using the first order hold method. $\dot{V}O_{2mus}$ kinetics were quantified from the rise time (τ) based on the calculation of AUC and

the given amplitude assuming a first order linear dynamic system. The rise time for $\dot{V}O_{2mus}$ was then estimated from the following equation:

$$AUC\ (\ \dot{V}O_{2mus}\ ) = A\ [(t_f - t_s) + \tau\ (e^{-(\ t_f\ /\ \tau)} - e^{-(\ t_s\ /\ \tau)})]$$

where, AUC ($\dot{V}O_{2mus}$) is the numerical value calculated from the first order hold method for $\dot{V}O_{2mus}$ curve, A is the end exercise amplitude, t_f is the final time of exercise, t_s is the start time to calculate AUC and τ is the rise time of $\dot{V}O_{2mus}$. Same procedures to calculate FBF kinetics were used in Chapter 3.

A higher order mathematical model partitions the $\dot{V}O_2$ response into distinct phases, where the Phase II time constant (τ_2; time to reach 63% of the primary phase) characterizes the rate of increase in oxidative phosporylation (Hughson *et al.*, 2001). The moderate and heavy bouts were curve fitted by two- (phases I and II) and three-component (phases I, II and III) exponential models respectively, according to the following equation. The two component model has a baseline, two amplitude terms (A_1 and A_2), two time constants (τ_1 and τ_2) and two time delays (TD_1 and TD_2). The three component model has an extra amplitude (A_3), time constant (τ_3), and time delay (TD_3) to fit the slower adaptive phase manifesting during heavy exercise.

$$+ A_1 (1-e^{-(t-TD1)/\tau_1}) U_1 \qquad \text{Phase I}$$

$$\dot{V}O_2(t) = \dot{V}O_2(\text{baseline}) + A_2 (1-e^{-(t-TD2)/\tau_2}) U_2 \qquad \text{Phase II}$$

$$+ A_3 (1-e^{-(t-TD3)/\tau_3}) U_3 \qquad \text{Phase III}$$

Where,

$U_1 = 0$ for $t < TD_1$ and $U_1 = 1$ for $t > TD_1$

$U_2 = 0$ for $t < TD_2$ and $U_2 = 1$ for $t > TD_2$

$U_3 = 0$ for $t < TD_3$ and $U_3 = 1$ for $t > TD_3$

Same procedures to describe $\dot{Q}$ kinetics where used in Chapter 2.

1.6 Aim of studies

The fundamental idea of the following studies is to examine the control mechanisms of oxygen uptake following the onset of exercise. Specifically, the studies have been designed to investigate the interrelationships between cardiovascular adaptations and O_2 utilization in accelerating $\dot{V}O_2$ kinetics in trained humans. The experimental objectives were to manipulate and quantify $\dot{Q}$ and FBF responses at the onset of dynamic exercise and infer relationships between O_2 transport and O_2 utilization in accelerating $\dot{V}O_2$ kinetics. The main hypothesis of these studies is that alterations in cardiovascular responses "$\dot{Q}$ and FBF" at the onset of exercise will be associated with alterations in the rate of increase in oxidative phosphorylation.

Specific research questions:

I. To examine the effects of prior moderate and heavy warm-up on pulmonary $\dot{V}O_2$ and $\dot{Q}$ kinetics at the onset of subsequent moderate and heavy cycling exercise (Chapters 2).

II. To determine in a model of forearm exercise, if the increase of blood flow at exercise onset following prolonged ischemia would accelerate the blood flow and $\dot{V}O_{2mus}$ kinetics during subsequent heavy exercise as demonstrated previously for prior heavy exercise (Chapter 3).

III. To quantify the role of muscle activity, as assessed by EMG, on the development of $\dot{V}O_2$ slow component during heavy exercise and increased O_2 cost during moderate exercise that followed a heavy warm-up (Chapter 4).

IV. To study the effect of circadian rhythm on $\dot{V}O_2$ kinetics and BP regulations at the onset of moderate a heavy exercise (Appendix A).

Chapter 2

Prior moderate and heavy exercise accelerate oxygen uptake and cardiac output kinetics in endurance athletes

This chapter is the basis for the published paper:

Faisal A, Beavers KR, Robertson AD, & Hughson RL (2009). Prior moderate and heavy exercise accelerate oxygen uptake and cardiac output kinetics in endurance athletes. *J Appl Physiol* **106**, 1553-1563.

2.1 Overview

Cardiorespiratory interactions at the onset of dynamic cycling exercise are modified by warm-up exercises. We tested the hypotheses that $\dot{V}O_2$ and $\dot{Q}$ kinetics would be accelerated at the onset of heavy and moderate cycling exercise by prior warm-up. Nine male endurance athletes ($\dot{V}O_{2peak}$: 60.5 ± 3.2 ml/min/kg) performed multiple rides of two different 36-minute cycling protocols involving 6-minute bouts at moderate and heavy intensities. Breath-by-breath $\dot{V}O_2$ and beat-by-beat stroke volume (SV) and $\dot{Q}$ estimated by Modelflow from the finger pulse were measured simultaneously with kinetics quantified from the phase two time constant (τ_2). One novel finding was that both moderate (M) and heavy (H) warm-up bouts accelerated phase two $\dot{V}O_2$ kinetics during a subsequent bout of heavy exercise (τ_2: after M=22.5 ± 2.7 s, after H=22.1 ± 2.9 vs. 26.2 ± 3.2 s; $P < 0.01$). $\dot{Q}$ kinetics in heavy exercise were accelerated by both warm-up intensities (τ_2: M=22.0 ± 4.1 s, H=23.8 ± 5.6 s vs. 27.4 ± 7.2 s; $P < 0.05$). During moderate exercise, prior heavy intensity warm-up (one or two bouts) accelerated $\dot{V}O_2$ kinetics and elevated $\dot{Q}$ at exercise onset, with no changes in $\dot{Q}$ kinetics. A second novel finding was a significant overshoot in the estimate of SV from Modelflow in the first minutes of each moderate and heavy exercise bout. These findings suggest that the acceleration of $\dot{V}O_2$ kinetics during heavy exercise was enabled by the acceleration of $\dot{Q}$ kinetics, and that rapid increases in $\dot{Q}$ at the onset of moderate and heavy exercise might result in part from an overshoot of SV.

2.2 Introduction

$\dot{V}O_2$ kinetics reflect the oxidative energy supply at the onset of work rate transitions. Prior exercise might alter the rate of increase of $\dot{V}O_2$ and $\dot{Q}$ during a subsequent bout of exercise, but research findings utilizing this model are controversial. The first systematic investigation of prior exercise on $\dot{V}O_2$ kinetics by Gerbino *et al.* (1996) reported faster $\dot{V}O_2$ kinetics (shown by smaller effective time constant $\tau\dot{V}O_2$) in a second heavy exercise bout. However, they observed no effects of prior heavy exercise on ensuing moderate exercise bouts or of prior moderate exercise on following bouts of either moderate or heavy exercise (Gerbino *et al.*, 1996). Since then, many supportive and contradictory findings have been reported for $\dot{V}O_2$ kinetics while observations of $\dot{Q}$ have been limited.

Faster $\dot{V}O_2$ kinetics have been found in a bout of heavy exercise that followed an identical heavy exercise bout (MacDonald *et al.*, 1997; Perrey *et al.*, 2003a) or multiple sprint cycling (Tordi *et al.*, 2003), but contrary findings of no change in kinetics have been reported (Burnley *et al.*, 2000; Wilkerson *et al.*, 2004). The reasons for these discrepancies in the responses to heavy exercise after a heavy warm-up could be a consequence of varying signal-to-noise relationships related to single or multiple test repetitions, to method of data analysis with overall kinetics versus a focus on the phase II kinetics, to differences in recovery intensity between exercise bouts, or to differences in physical fitness of the subjects studied. Likewise, varying results of accelerated or no change in kinetics have been reported for the effects of lower intensities of warm-up on $\dot{V}O_2$ kinetics on subsequent heavy (Burnley *et al.*, 2000; Campbell-O'Sullivan *et al.*, 2002; Gerbino *et al.*, 1996;

MacDonald *et al.*, 1997) or moderate (Ferreira *et al.*, 2005b; Gerbino *et al.*, 1996) exercise. Several studies have reported no change in $\dot{V}O_2$ kinetics in moderate exercise that follows a bout of heavy warm-up exercise (Burnley *et al.*, 2000; Gerbino *et al.*, 1996; MacDonald *et al.*, 1997). However, Gurd *et al.*(2006) recently reported faster phase II time constant (τ_2) for $\dot{V}O_2$ during moderate exercise that followed a heavy warm-up. They observed elevated PDHa activity at baseline after the heavy bout and further activation during the second moderate exercise bout.

Kinetics of $\dot{Q}$ have been investigated primarily in light or moderate exercise where a rapid increase in HR attributed to vagal withdrawal was largely responsible for the adaptation of $\dot{Q}$ to steady state (Fagraeus & Linnarsson, 1976; Inman *et al.*, 1987; Lador *et al.*, 2006; Linnarsson, 1974; Yoshida & Whipp, 1994). When the metabolic demand was elevated and sympathetic contributions were required to increase HR the kinetics of both $\dot{Q}$ and $\dot{V}O_2$ were slower (Inman *et al.*, 1987) but prior heavy exercise accelerated $\dot{Q}$ and $\dot{V}O_2$ in subsequent heavy cycling exercise (Perrey *et al.*, 2003a).

Relatively few studies have examined simultaneous kinetics of $\dot{V}O_2$ and $\dot{Q}$ during exercise transitions (Perrey *et al.*, 2003a; Yoshida & Whipp, 1994). In the current study, we examined the relationship between $\dot{V}O_2$ and $\dot{Q}$ kinetics to investigate the relative contributions of muscle metabolic and cardiovascular adaptations to $\dot{V}O_2$ kinetics at the onset of both moderate and heavy exercise. As well, we investigated the effect of prior warm-up in very fit young men who were able to perform high work rates in order to generate a large amplitude signal

during multiple repetitions to enhance the signal-to-noise ratio in the subsequent response kinetics.

Two primary hypotheses were tested: first, that both heavy and moderate prior exercise would accelerate the kinetics of $\dot{V}O_2$ and $\dot{Q}$ in subsequent heavy exercise; and second, that prior heavy exercise would accelerate the kinetics of $\dot{V}O_2$ and $\dot{Q}$ in subsequent moderate exercise.

2.3 Methods

2.3.1 Subjects

Nine male endurance athletes (age: 22.3 ± 2.7 years, height: 178.6 ± 7.8 cm, weight: 71.9 ± 6.5 kg, peak oxygen uptake ($\dot{V}O_{2peak}$): 60.5 ± 3.2 ml/min/kg; mean ± SD) consented to participate in this study. None of the participants was a cyclist, but all included some cycling in their fitness regimes and all were pre-screened with $\dot{V}O_{2peak}$ > 55 ml/min/kg. This study was approved by the Office of Research Ethics at the University of Waterloo and all subjects provided written informed consent following full description of the protocols.

2.3.2 Experimental design

$\dot{V}O_{2peak}$ and ventilatory threshold (VT) were determined using an incremental exercise test to volitional exhaustion on an electrically-braked cycle ergometer (Excalibur, Lode, Groningen, Netherlands). After an initial 4-min period of cycling at 20 W, the work rate increased as a ramp of 30 W/min. The pedaling cadence was maintained at ~ 80 rpm for the duration of the test. The test was stopped when the subject was unable to continue or was unable to maintain a

cadence >75 rpm. The $\dot{V}O_{2peak}$ was calculated as the average of the last 15 seconds of the test. The VT was estimated from the breakpoint in the curve of carbon dioxide output ($\dot{V}CO_2$) as a function of $\dot{V}O_2$ (V-slope method) (Beaver *et al.*, 1986) and confirmed by the point at which minute ventilation ($\dot{V}_E$) to $\dot{V}O_2$ ratio increased without an increase in $\dot{V}_E$ to CO_2 output ($\dot{V}CO_2$) ratio.

Each subject, over a 3-month period, performed multiple rides of two different 36-minute cycling protocols involving 6-minute bouts at moderate (M) and heavy (H) intensities (power output requiring $\dot{V}O_2$ equivalent to 80% of the $\dot{V}O_2$ at VT and 85% of $\dot{V}O_{2peak}$, respectively) interspersed with 6-minute bouts at 20 W to achieve similar protocols to other studies of prior exercise that have used unloaded cycling (Gerbino *et al.*, 1996) , 20W (Burnley *et al.*, 2000; Gurd *et al.*, 2006), 25W (MacDonald *et al.*, 1997) or 35% $\dot{V}O_{2peak}$ (Perrey *et al.*, 2003a). The exercises were named by the intensity, bout number and protocol letter as follows: Protocol A = moderate ($M1_A$) followed by heavy ($H2_A$) followed by moderate ($M3_A$); Protocol B = heavy ($H1_B$) followed by heavy ($H2_B$) followed by moderate ($M3_B$); (Fig. 2.1). The pedaling frequency was maintained at 80 rpm throughout each protocol. The same cycle ergometer and individual set up (handle bar and seat position allowing nearly full knee extension) was used for each ride in the study.

The competitive athletes were in the maintenance phase of their training regimen. To ensure that there were no changes in their physical fitness during the testing period, an incremental exercise test was performed by each participant within one week of finishing his testing series.

On testing days, subjects reported to the laboratory in a rested, hydrated state. They were asked to abstain from caffeine 12 h and alcohol 24 h before testing. No more than two testing sessions per week were arranged; rides were performed at least 24 h following the participants' last exercise regimen, 72 h following a heavy training session, and 48 h following their last study ride. The tests were completed in a quiet, air-conditioned laboratory at a temperature of ~23°C.

Breath-by-breath oxygen uptake

Ventilation and gas exchange were measured continuously in at least 8 rides (minimum 4 of each protocol) on a breath-by-breath basis (First Breath, Waterloo, ON) measuring inspired and expired concentrations of O_2, CO_2, and N_2 by mass spectrometry (Innovision, Amis 2000, Odense, Denmark) and gas volumes by a bidirectional, low resistance, low dead space (90 ml) turbine (UVM-17125, VacuMed, Ventura, CA, modified to remove the signal delay on transition of flow direction). This system uses a modification of the Auchincloss algorithm incorporating an estimate of the effective lung volume to determine breath-by-breath changes in lung gas stores (Hughson *et al.*, 1991b).Calibrations with precision medical gases and an automated 3 L syringe (Vacumed, Ventura, CA) and measurement of the delay time to match the volume and gas signals were performed prior to each test.

Cardiac output

Throughout each exercise test $\dot{Q}$ was continuously estimated beat-by-beat from the pulse wave of the finger arterial pressure (Finometer, Finapres Medical System, Arnhem, Netherlands) utilizing the Modelflow algorithm to estimate SV (Wesseling *et al.*, 1993) incorporating factors of age, sex, height and weight to estimate the aortic cross-sectional area (Langewouters *et al.*, 1984). The left arm was supported slightly below heart level by a sling to minimize arm and hand movement. The support of one arm did not affect cycling mechanics or require additional activity in the other arm for these highly fit subjects. The $\dot{Q}_{MF}$ signal was shifted -1s and 1 beat back to compensate for Finometer's internal digital signal processing delay. The signals were linearly interpolated at 1-s intervals, the rides were time aligned and averaged together to yield a single data set for each subject in each protocol. The Modelflow algorithm follows changes in $\dot{Q}$ (Tam *et al.*, 2004) but might exhibit bias relative to a standard method, especially when applied over a wide range of metabolic demand as in the current study. Therefore, we compared $\dot{Q}_{MF}$ with those from acetylene rebreathing ($\dot{Q}_{C2H2}$) (Triebwasser *et al.*, 1977) using a gas mixture containing 7% helium (He), 0.7% C_2H_2, 30% O_2 and balance N_2 from a 5-L rebreathing bag filled to 1.5 times each person's tidal volume (Bell *et al.*, 2003). Each subject performed multiple repetitions of $\dot{Q}_{C2H2}$ at the three work rates in 8 separate rides (total: 24 at 20 W, 12 at moderate and 12 at heavy work rates). Calibrations could not be performed in the exercise transients as the C_2H_2 method requires constant $\dot{Q}$ during the measurement period. Therefore, $\dot{Q}_{C2H2}$ was calculated between 3.5 and 4 minutes (2 rides of each protocol), and 5 and 5.5

minutes (2 rides of each protocol) and these were compared to corresponding values for $\dot{Q}_{MF}$ at each work rate. Given the small overall bias (see Results) no adjustment was applied to the $\dot{Q}_{MF}$ as an estimate of $\dot{Q}$ during exercise. BP, $\dot{Q}_{MF}$ and electrocardiogram (ECG) (Pilot 9200, Colin Medical Instruments, San Antonio, TX) signals were sampled at 1 kHz (PowerLab, AD Instruments, Colorado Springs, CO).

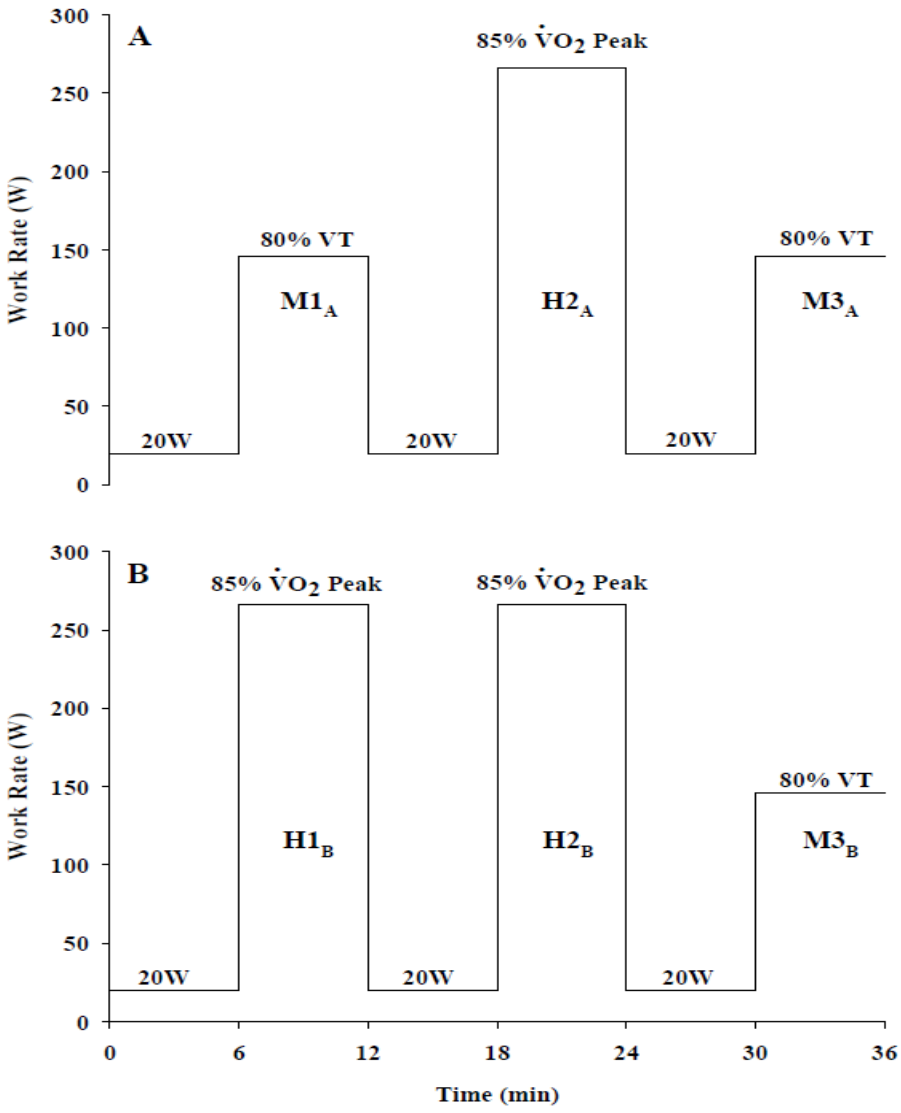

Figure 2.1: Two different testing protocols employed to examine the influence of prior exercise on $\dot{V}O_2$ and $\dot{Q}_{MF}$ kinetics.

For each exercise bout, the M or H indicates moderate or heavy work rate; 1, 2 or 3 indicates the order of the exercise bout within the sequence; and, A or B indicates the two different protocols. The specific bouts are $M1_A$ and $H1_B$ (moderate and heavy control bouts respectively), $H2_A$ and $H2_B$ (heavy exercise after a moderate or heavy prior exercise respectively), $M3_A$ and $M3_B$ (moderate exercise after one or two heavy prior exercise bouts).

2.3.3 Data Analysis

Breath-by-breath $\dot{V}O_2$ and beat-by-beat $\dot{Q}_{MF}$ analysis

Breath-by-breath $\dot{V}O_2$ and beat-by-beat $\dot{Q}_{MF}$ data were obtained from at least 4 repetitions of each of protocols A and B, linearly interpolated at 1-s intervals, time-aligned to the beginning of exercise, and averaged together to yield a single data set for each subject. The moderate and heavy bouts were curve fitted by two- (phases I and II) and three-component (phases I, II and III) exponential models respectively, according to the following equation:

$$
\begin{aligned}
Y(t) = {} & A_0 + A_1 (1-e^{-(t-TD1)/\tau_1}) U_1 \quad \text{Phase I} \\
& + A_2 (1-e^{-(t-TD2)/\tau_2}) U_2 \quad \text{Phase II} \\
& + A_3 (1-e^{-(t-TD3)/\tau_3}) U_3 \quad \text{Phase III}
\end{aligned}
$$

Where,

$U_1 = 0$ for $t < TD_1$ and $U_1 = 1$ for $t > TD_1$

$U_2 = 0$ for $t < TD_2$ and $U_2 = 1$ for $t > TD_2$

$U_3 = 0$ for $t < TD_3$ and $U_3 = 1$ for $t > TD_3$

Y(t) is the absolute $\dot{V}O_2$ or $\dot{Q}_{MF}$ at a given time t; A_0 is the amplitude of the baseline for $\dot{V}O_2$ or $\dot{Q}_{MF}$ measured in the 2 minutes preceding the onset of exercise while cycling at 20 W; A_1, A_2 and A_3 are the amplitudes, TD_1, TD_2 and TD_3 are the time delays, and τ_1, τ_2 and τ_3 are the time constants of phases I, II and III respectively of the $\dot{V}O_2$ and $\dot{Q}_{MF}$ kinetic responses.

Phase I was fit with parameters that insured a completed response before the start of phase II (i.e. 4 x τ_1 < TD_2) so there was no impact of phase I on the phase II kinetics (Hughson *et al.*, 2000) allowing comparison with studies which omitted the phase I from the fitting analysis (Barstow *et al.*, 1996; Burnley *et al.*, 2000). Curve-fitting utilized an iterative non-linear regression process in which the best fit was defined by minimizing the residual sum of squares with a symmetrical distribution around the zero-line (see Fig.2.2). The rate of activation of the aerobic system during the exercise transition was characterized by τ_2 for $\dot{V}O_2$.

Acetylene cardiac output analysis

In order to reduce signal noise and improve reliability in the rebreathing data, a 5-point moving average was applied to every rebreathe trial. Complete mixing of the lung-bag system was indicated by the equilibration of He levels. After complete mixing was reached, 4 end-expiration points were selected from the C_2H_2 decay curve. End-tidal gas measurements were taken from the first 12s during the heavy bouts, and 15s during the 20 W and moderate bouts to avoid recirculation errors (Alves *et al.*, 1985; Liu *et al.*, 1997). Rebreathing trials were excluded from the analysis if the initial measured He and C_2H_2 values during the transition to rebreathing were disparate from the known concentrations in the tank, which may result from subjects being turned onto the rebreathing bag prior to complete exhalation. Linear regression was performed to calculate $\dot{Q}$ according to the following equation:

$$Q = -B_S \left[\frac{V_A \left[\frac{760}{P_B - 47} \right] + \alpha_t V_t}{\alpha_B} \right]$$

where Bs is the slope of the C_2H_2 disappearance , V_A(STPD) is the bag volume(mL), P_B is the barometric pressure (mmHg), Vt is the lung tissue volume (mL), α_τ and α_B are the Bunsen solubility coefficients for acetylene in the lung tissue (0.768) and blood (0.71) respectively (Petrini *et al.*, 1978; Smyth *et al.*, 1984; Triebwasser *et al.*, 1977).

Arterial-venous oxygen content difference

To account for the lower sampling rate for $\dot{V}O_2$ (breath-by-breath) compared to the estimates of $\dot{Q}_{MF}$ (beat-by-beat), the latter were averaged over each breath cycle to yield a single data point to match $\dot{V}O_2$ data. (A-v)DO_2 was then calculated using the Fick equation: (a-v)DO_2 = $\dot{V}O_2$ / $\dot{Q}$

Baseline values for $\dot{V}O_2$, $\dot{Q}_{MF}$ and (a-v)DO_2 were the average of the 30 s preceding the onset of moderate and heavy cycling bouts. The non-interpolated values of $\dot{V}O_2$, $\dot{Q}_{MF}$ and (a-v)DO_2 values were averaged in 10 s windows during each exercise bout and the relative contribution of $\dot{Q}$ and (a-v)DO_2 to the overall changes in $\dot{V}O_2$ (expressed in %) were approximated through the differentiation of the Fick equation as shown by Fukuba *et al.* (2007):

$$\Delta \dot{V}O_2 \approx (a\text{-}v)DO_2 \cdot \Delta \dot{Q} + \dot{Q} \cdot \Delta (a\text{-}v)DO_2$$

Dividing the differentiated equation by $\dot{V}O_2$ shows that the relative change in $\dot{V}O_2$ is approximately equal to the sum of the relative changes in $\dot{Q}$ and (a-v)DO_2:

$$\frac{\Delta \dot{V}O_2}{\dot{V}O_2} \approx \frac{\Delta \dot{Q}}{\dot{Q}} + \frac{\Delta (a-v)DO_2}{(a-v)DO_2}$$

2.3.4 Statistical analysis

Regression and Bland-Altman analyses (Mantha *et al.*, 2000) were used to examine the accuracy and reliability of the Finometer $\dot{Q}$ method. Additionally, a one-way repeated measures analysis of variance (ANOVA) and coefficient of variation (CV) analysis were completed to demonstrate the variance of the $\dot{Q}$ across the multiple bouts. The effect of prior exercise on $\dot{V}O_2$ and $\dot{Q}$ kinetics as well as selected time values of HR and SV were analyzed using one-way repeated measures ANOVA. When significant effects were observed, the Bonferroni post hoc test was used for comparisons. Regression analyses compared the $\dot{Q}$ and $\dot{V}O_2$ kinetics to each other. All data are expressed as mean ± SD. The data were analyzed using Statistical Analysis Software (SAS) package 9.1 (SAS Institute, Cary, NC).

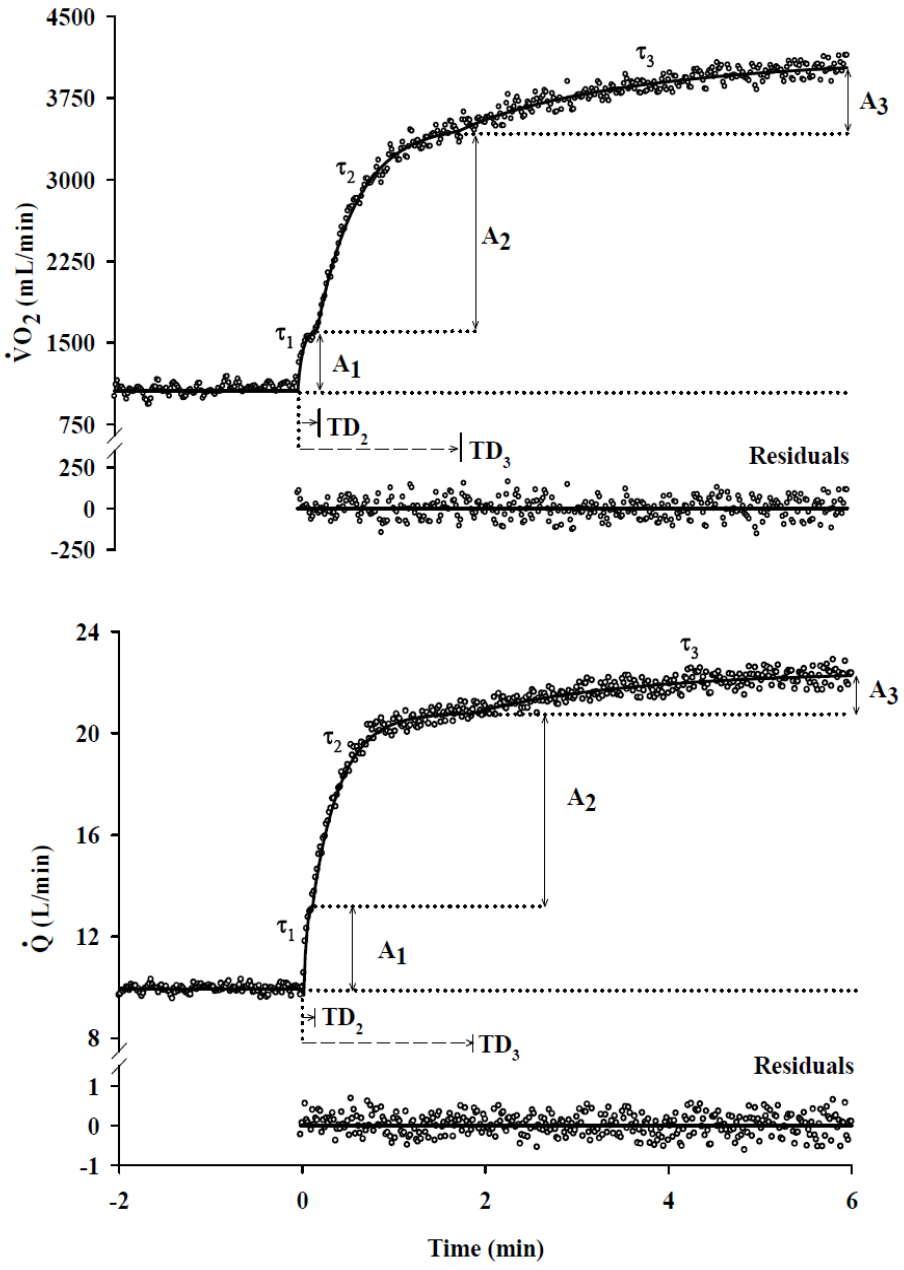

Figure 2.2: Representative $\dot{V}O_2$ (top) and $\dot{Q}_{MF}$ (bottom) data from one participant during heavy exercise.

The three-component model has been used to estimate the fitting parameters for both curves. The quality of fit is shown in the small residuals, which are evenly distributed around the zero line in both plots.

2.4 Results

2.4.1 Incremental exercise test

The pre-study $\dot{V}O_{2peak}$ (4325 ± 516 ml/min, 60.5 ± 3.2 ml/min/kg) was reached at a work rate of 390 ± 44 W during the 30 W/min incremental exercise test. VT occurred at 65 ± 5.7 % of $\dot{V}O_{2peak}$. The work rates during the moderate and heavy bouts were 146 ± 33 W and 266 ± 35 W, respectively. At the completion of the study $\dot{V}O_{2peak}$ (4328 ± 537 ml/min, 60.6 ± 3.9 ml/min/kg) and work rate (396 ± 41 W) did not differ from the pre-study values (P = 0.89).

2.4.2 Modelflow cardiac output validation

The $\dot{Q}_{MF}$ and $\dot{Q}_{C2H2}$ were highly correlated both on a subject-subject basis and overall. Individually, the average correlation coefficient was 0.93 ± 0.03 and ranged from 0.90 to 0.96 (Fig. 2.3). The overall regression equation between methods was $\dot{Q}_{MF}$ = 0.94 $\dot{Q}_{C2H2}$ + 1.09 with r = 0.92 (P < 0.001). The results of the Bland –Altman analysis (Fig. 2.4) showed small bias at the three different work rates 20W, 80% VT and 85% $\dot{V}O_{2peak}$ (0.01, 0.05,-0.97; respectively). The overall bias (mean $\dot{Q}_{C2H2}$ - $\dot{Q}_{MF}$) was low at -0.20 l/min with 95% limits of agreement from -4.16 to 3.76 l/min. $\dot{Q}_{MF}$ variability during repeated measurements was low. There were no significant differences in $\dot{Q}_{MF}$ across the multiple repetitions of each protocol (P > 0.41 for moderate bouts; P > 0.67 for heavy bouts). The mean CV in all subjects over all rides was 6.7 ± 2.4 % during moderate bouts (ranging from 3.7 to 11.6) and 7 ± 2.1 % during heavy bouts (ranging from 4.8 to 11.7). The

regression equations for both $\dot{Q}_{MF}$ and $\dot{Q}_{C2H2}$ with $\dot{V}O_2$ in all subjects were similar [$\dot{Q}_{MF}$ = 0.005 $\dot{V}O_2$ + 5.58 with r = 0.97 (P < 0.001); $\dot{Q}_{C2H2}$ = 0.004 $\dot{V}O_2$ + 5.89 with r = 0.96 (P < 0.001)]. Overall, the Modelflow method provided a valid estimate of $\dot{Q}$ during exercise at the three different intensities of cycling exercise.

2.4.3 Oxygen uptake and cardiac output kinetics

During a heavy exercise bout that followed either prior moderate or heavy exercise τ_2 values for both $\dot{V}O_2$ and $\dot{Q}_{MF}$ were less than when heavy exercise was not preceded by warm-up (Fig. 2.5, Table 2.1). $\dot{V}O_2$ responses were normalized to the amplitude at the end of the primary phase to eliminate the effect of elevated baseline $\dot{V}O_2$ (Fig. 2.6). The moderate exercise bouts that followed prior heavy exercise (one and two bouts) had smaller τ_2 for $\dot{V}O_2$ than in the no warm-up condition. The reduction in τ_2 for $\dot{Q}_{MF}$ was not significantly different compared to the control (Fig. 2.7, Table 2.2). There was a significant relationship between the time constants for $\dot{V}O_2$ and $\dot{Q}_{MF}$ determined for all heavy bouts (r = 0.47, P = 0.01). However, the overall relationship between $\dot{V}O_2$ τ_2 and $\dot{Q}_{MF}$ τ_2 during moderate bouts did not reach the significant level (r = 0.14, P = 0.5, Fig. 2.8).

Baseline (A_0) values for $\dot{V}O_2$ were significantly elevated when either moderate or heavy exercise was preceded by one bout of heavy exercise and there was further small, but significant elevation when moderate exercise was preceded by two heavy bouts (Table 2.1 and 2.2). Prior moderate exercise had no effect on the A_0 for $\dot{V}O_2$. Neither moderate nor heavy prior exercise affected the amplitude of $\dot{V}O_2$ during phase I (A_1) or phase II (A_2) in the following bouts of moderate or heavy exercise

(Table 2.1 and 2.2). Prior warm-up had a graded effect on the amplitude of the $\dot{V}O_2$ slow component (A_3), such that the prior heavy exercise resulted in a greater attenuation than moderate exercise (Table 2.1).

A_0 for $\dot{Q}_{MF}$ was significantly elevated by prior heavy exercise (Table 2.1 and 2.2). There was no impact of prior exercise on the $\dot{Q}_{MF}$ amplitudes A_1 or A_2 in the heavy exercise bouts (Table 2.1). During the moderate exercises both A_1 and A_2 were significantly reduced by one or two bouts of prior heavy exercise (Table 2.2); however, due to the higher starting values the absolute average $\dot{Q}_{MF}$ from 15-45 s after exercise onset was greater after two prior heavy bouts ($M3_B$ = 15.5 ± 4.5 l/min, $P < 0.01$) compared to no prior exercise ($M1_A$ = 14.5 ± 3.3 l/min) but was not different after one bout of heavy prior exercise ($M3_A$ = 14.9 ± 4.2 l/min).

The HR and SV contributions to $\dot{Q}_{MF}$ were markedly impacted by the prior exercise condition (Table 3, Fig. 2.9 and 2.10). During both moderate and heavy exercise, there was a significant overshoot of the SV within the first two minutes of exercise (Fig. 2.9 and 2.10, Table 2.3). During moderate bouts, one prior heavy exercise bout elevated HR and reduced the baseline, peak, and end bout SV, and these responses were further diminished by two prior heavy exercise bouts. During heavy bouts, baseline and end bout HR were elevated by prior heavy exercise, but there were no differences in the baseline, peak or end exercise SV between bouts with or without warm-up exercise.

2.4.4 Arterial-venous oxygen content difference

Prior exercise elicited changes in $\dot{Q}$ and (a-v)DO_2, combining to accelerate $\dot{V}O_2$ kinetics of a subsequent moderate or heavy exercise bout. In a moderate bout following one heavy bout ($M3_A$), increases in (a-v)DO_2 mainly contributed to the speeding of $\dot{V}O_2$ kinetics. Relative to the bout without prior exercise ($M1_A$), there were increases in the (a-v)DO_2 at 30 s (129.8 ± 12.5 vs. 117.0 ± 10.5 mlO_2/l blood) and at 90 s (145.0 ± 13.9 vs. 134.4 ± 12.7 mlO_2/l blood). These increases in (a-v)DO_2 contributed to 75 % of the change in $\dot{V}O_2$ kinetics [(Δ(a-v)DO_2 /(a-v)DO_2 / ($\Delta\dot{V}O_2 / \dot{V}O_2$) = 10.95% / 14.62%] at 30 s, and reached 100 % by 90 s. In the moderate bout that followed two heavy bouts ($M3_B$) both (a-v)DO_2 and $\dot{Q}$ contributed to the change in $\dot{V}O_2$ (Fig. 2.11 - top). $\dot{Q}$ contributed to 52 % [($\Delta\dot{Q} / \dot{Q}$) / ($\Delta\dot{V}O_2 / \dot{V}O_2$) = 8.26% / 15.93%] and 23 % of the changes in $\dot{V}O_2$ vs. $M1_A$ at 30 and 90 s, respectively. For the heavy exercise bouts that followed either moderate or heavy exercise, $\dot{Q}$ appeared to be the major contributor to changes in $\dot{V}O_2$ kinetics (Fig. 2.11 - bottom).

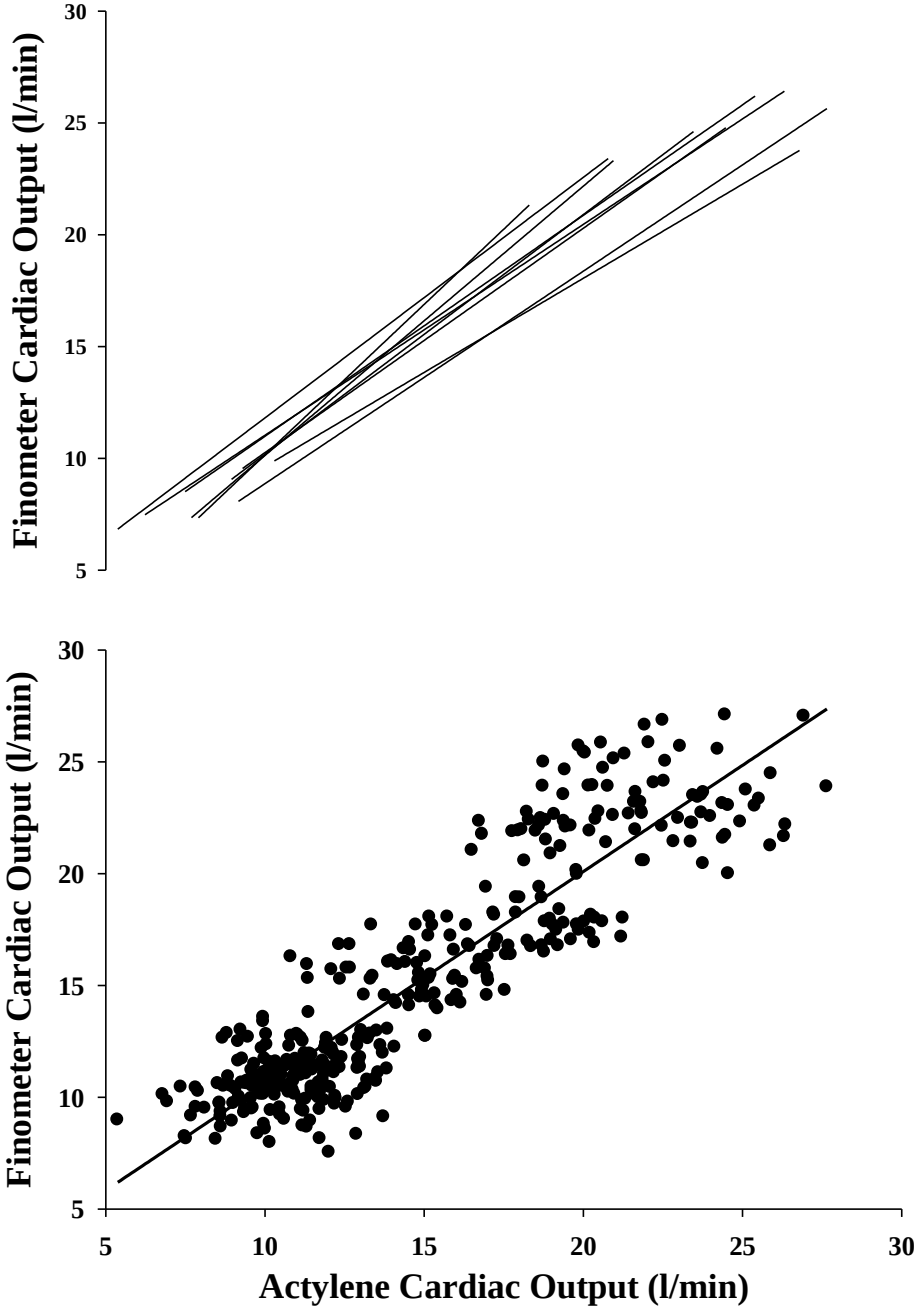

Figure 2.3: Linear regressions comparing the Finometer Modelflow estimated cardiac output and acetylene rebreathing cardiac output.

Individual regressions (above, range: r = 0.90-0.96, P <0.001) and overall regression (below, r = 0.92, P <0.001).

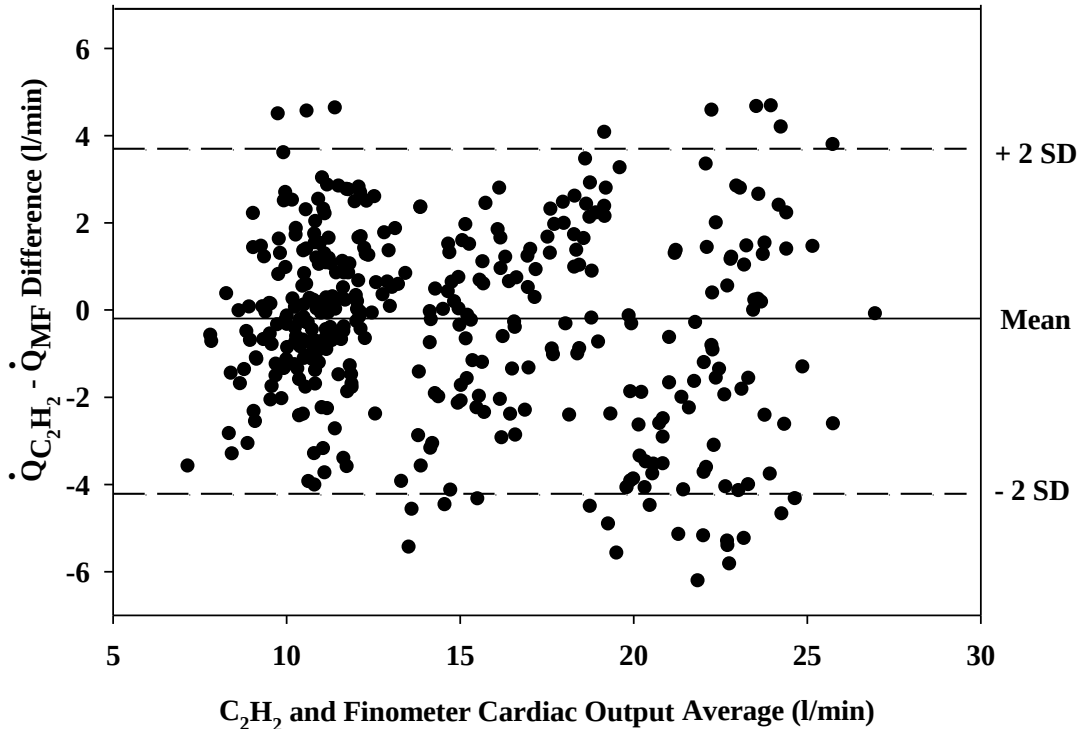

Figure 2.4: Bland-Altman analysis of $\dot{Q}_{MF}$ and $\dot{Q}_{C2H2}$.

Overall bias was -0.20 l/min and 95% limits of agreement ranged from -4.16 to 3.76 l/min. This figure reflects a maximum of 24 measures taken at 20W, 12 measures taken at 80%VT and 12 measures taken at 85% $\dot{V}O_{2peak}$ for each subject (n = 9).

Table 2.1: Fitting parameters of oxygen uptake and cardiac output kinetics during heavy bouts

Parameters	Oxygen Uptake			Cardiac Output		
	$H1_B$	$H2_A$	$H2_B$	$H1_B$	$H2_A$	$H2_B$
A_0, l/min	0.99 ± 0.05	1.02 ± 0.07	1.17 ± 0.05 *‡	10.16 ± 0.97	10.25 ± 0.94	11.90 ± 0.75 *‡
A_1, l/min	0.47 ± 0.16	0.49 ± 0.13	0.44 ± 0.13	3.19 ± 0.78	2.86 ± 0.63	2.99 ± 0.45
A_2, l/min	1.71 ± 0.23	1.68 ± 0.24	1.79 ± 0.26	8.18 ± 1.82	8.01 ± 1.30	8.16 ± 1.47
A_1+A_2, l/min	2.18 ± 0.33	2.16 ± 0.34	2.23 ± 0.34	11.37 ± 1.68	10.87 ± 1.48	11.15 ± 1.59
A_0+A_1+A_2, l/min	3.17 ± 0.35	3.18 ± 0.34	3.40 ±0.36 *‡	21.53 ± 1.88	21.12 ± 1.33	23. 05 ± 1.76 †‡
A_3, l/min	0.64 ± 0.15	0.55 ± 0.11†	0.39 ±0.07 *‡	1.51 ± 0.61	1.59 ± 0.74	1.40 ± 0.70
τ_2, s	26.15 ± 3.16	22.47 ± 2.71 *	22.05 ± 2.91 *	27.43 ± 7.22	22.0 ± 4.08 †	23.77 ± 5.58 †
τ_3, s	156.6 ± 34.6	141.2 ± 34.9	137.1 ± 29.3	98.3 ± 13.5	103.2 ± 8.8	97.1 ± 16.6
TD_2, s	11.83 ± 2.02	11.9 ± 1.47	10.29 ± 1.06 †§	7.3 ± 1.59	7.85 ± 2.26	9.01 ± 2.86
TD_3, s	98.9 ± 15.2	81.9 ± 10.4 *	75.5 ± 7.3 *	115.4 ± 18.3	98.1 ± 7.9 †	97.8 ± 10.5 †
End bout, l/min	3.7 ± 0.39	3.64 ± 0.37	3.73 ± 0.36	23.0 ± 1.38	22.68 ± 1.05	24.3 ±1.29 †‡

Mean ± SD, n = 9

* $P < 0.01$ $H1_B$ vs. $H2_A$-$H2_B$; † $P < 0.05$ $H1_B$ vs. $H2_A$-$H2_B$; ‡ $P < 0.01$ $H2_A$ vs. $H2_B$; § $P < 0.05$ $H2_A$ vs. $H2_B$

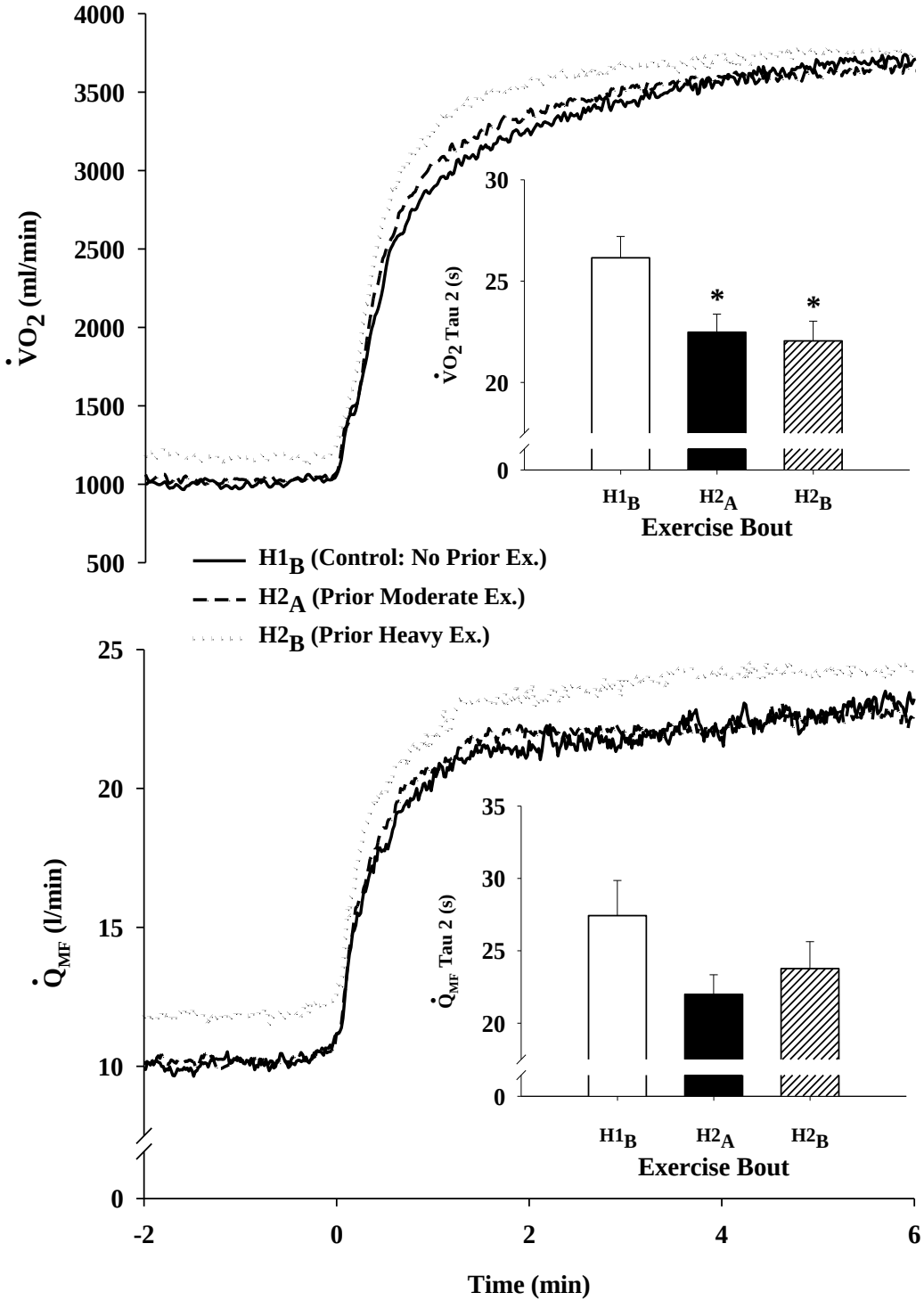

Figure 2.5: $\dot{V}O_2$ (top) and $\dot{Q}_{MF}$ (bottom) time series data for heavy bouts.

Insets: Tau_2 (mean ± SE); * $P < 0.01$; † $P < 0.05$ compared to $H1_B$. Data lines are the average of 9 subjects with ≥ 4 repetitions per each exercise condition.

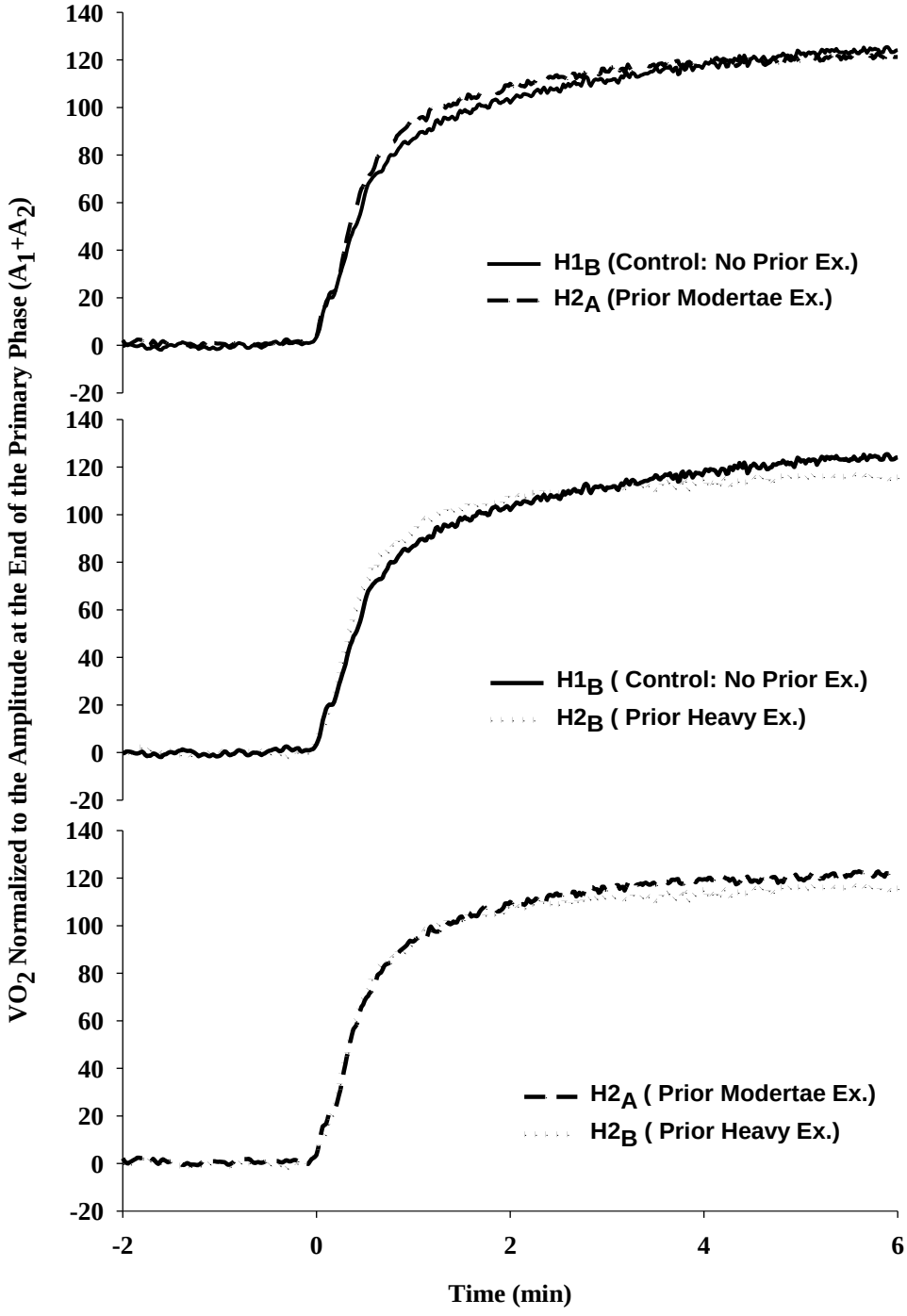

Figure 2.6: Comparison of the normalized $\dot{V}O_2$ to the amplitude at the end of primary phase during heavy bouts.

Differences in primary phase kinetics are clear between $H2_A$ and $H2_B$ vs. $H1_B$.

Table 2.2: Fitting parameters of oxygen uptake and cardiac output kinetics during moderate bouts

Parameters	Oxygen Uptake			Cardiac Output		
	$M1_A$	**$M3_A$**	**$M3_B$**	**$M1_A$**	**$M3_A$**	**$M3_B$**
A_0, l/min	0.99 ± 0.05	1.15 ± 0.07 *	1.19 ± 0.06 *§	9.36 ± 0.98	11.08 ± 0.80 *	11.13 ± 0.72 *
A_1, l/min	0.33 ± 0.14	0.28 ± 0.09	0.31 ± 0.11	1.82 ± 0.42	1.24 ± 0.39 *	1.38 ± 0.31 *
A_2, l/min	0.92 ± 0.23	0.9 ± 0.26	0.89 ± 0.25	5.02 ± 1.36	3.95 ± 1015 *	4.21 ± 1.21 *
τ_2, s	22.85 ± 4.81	18.91 ± 3.38 †	16.92 ± 2.67 *	20.15 ±5.22	19.13 ± 5.72	18.82 ± 4.29
TD_2, s	13.6 ± 1.67	12.8 ± 1.79	14.2 ± 1.57	7.87 ± 2.65	9.65 ± 2.83	8.51 ± 3.37
End bout, l/min	2.25 ± 0.37	2.34 ± 0.36 *	2.39 ± 0.36 *§	16.1 ± 1.47	16.37 ± 1.49	16.81 ±1.47 †

Mean ± SD, n = 9

* $P < 0.01$ $M1_A$ vs. $M3_A$-$M3_B$; † $P < 0.05$ $M1_A$ vs. $M3_A$-$M3_B$; § $P < 0.05$ $M3_A$ vs. $M3_B$

Amplitude parameters A_0, A_1 and A_2 for the baseline, phase 1 and phase 2 respectively; time constant (τ_2) and time delay (TD_2) for the primary phase; end bout is the average $\dot{V}O_2$ or $\dot{Q}_{MF}$ in the last minute of each exercise bout. The moderate bouts for protocols A and B are shown in Fig. 2.1.

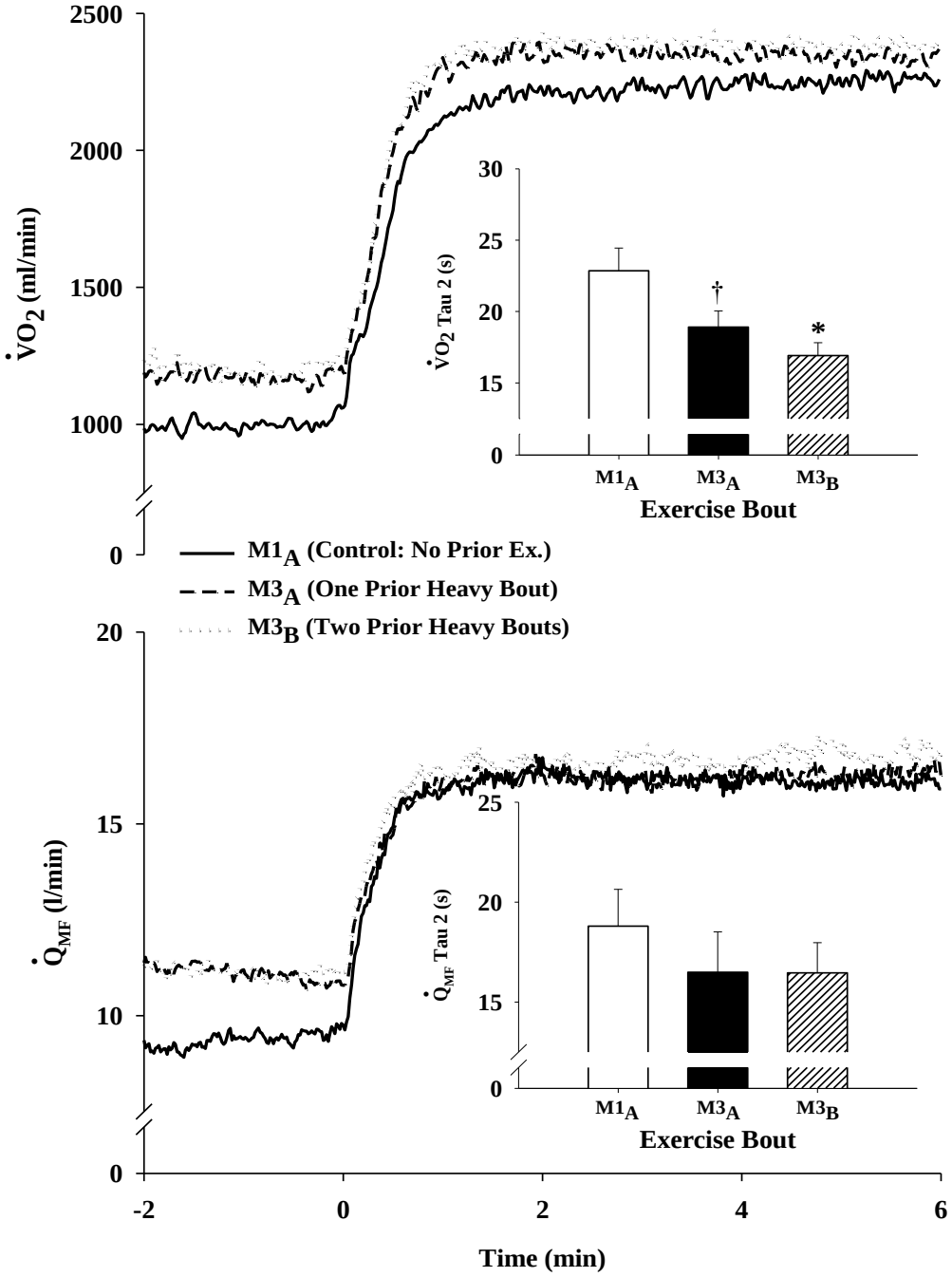

Figure 2.7: $\dot{V}O_2$ (top) and $\dot{Q}_{MF}$ (bottom) time series data for moderate bouts.

Insets: Tau_2 (mean ± SE); * $P < 0.01$; † $P < 0.05$ compared to $M1_A$. Data lines are the average of 9 subjects with ≥ 4 repetitions per each exercise condition.

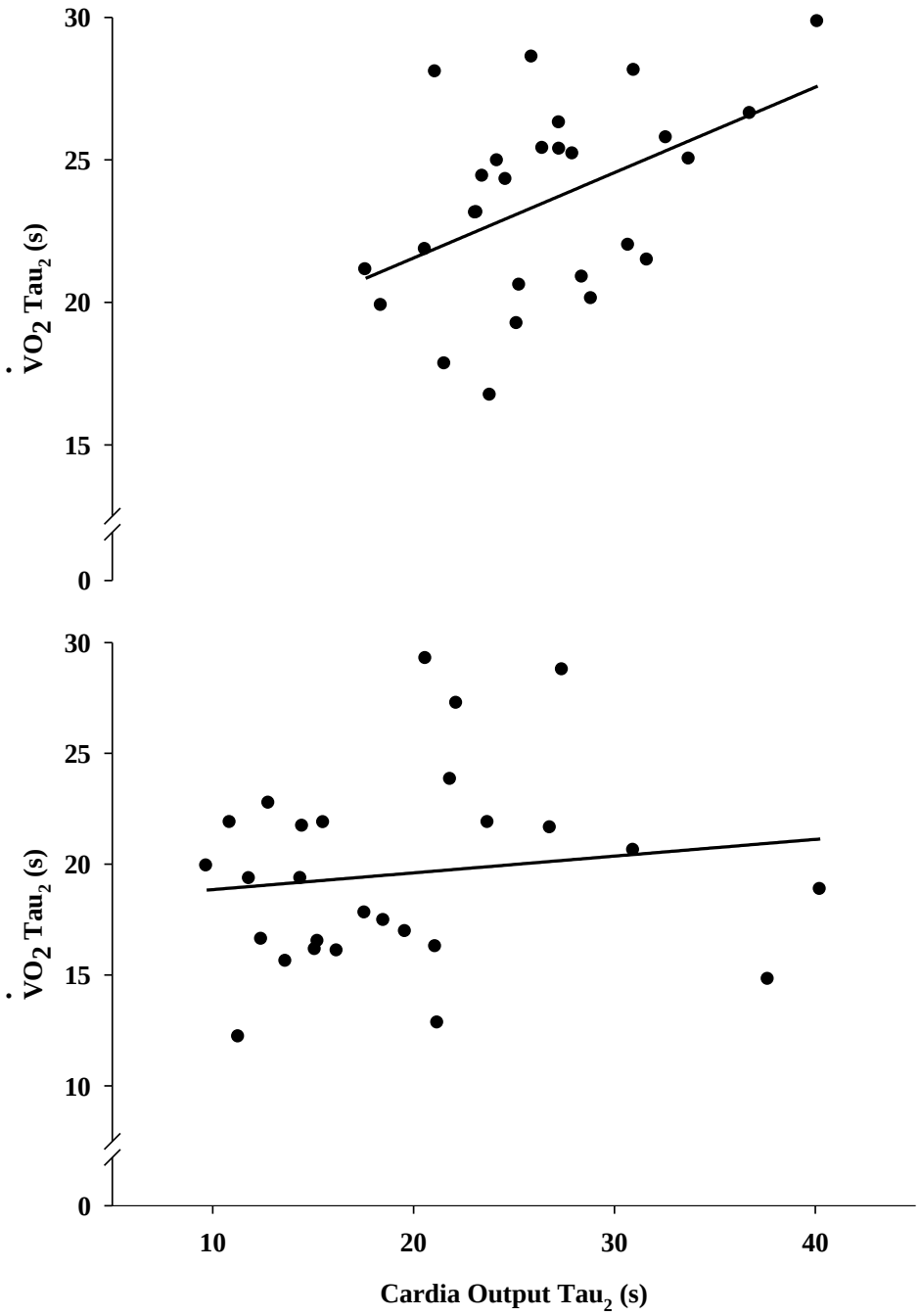

Figure 2.8: Linear regressions comparing $\dot{V}O_2\,Tau_2$ to $\dot{Q}_{MF}\,Tau_2$ for heavy and moderate bouts.

Heavy bouts (top, r = 0.47, P <0.05), moderate bouts (bottom, r = 0.135, P = 0.50).

Table 2.3: Cardiac output variability, heart rate and stroke volume during heavy and moderate bouts

Variables	Heavy Bouts			Moderate Bouts		
	$H1_B$	$H2_A$	$H2_B$	$M1_A$	$M3_A$	$M3_B$
$\dot{Q}_{cv}$, %	7.7 ± 3.1	7.4 ± 2.6	5.8 ± 2.6	6.9 ± 3.2	7.2 ± 2.9	6.2 ±2.1
$\dot{Q}_{C2H2}$ - $\dot{Q}_{MF}$ Bias, l/min	- 0.8 ± 2.5	-0.5 ± 2.7	- 1.6 ± 2.1	0.4 ± 1.5	0.5 ± 1.4	-0.3 ± 1.9
Baseline HR, bpm	87.8 ± 7.0	91.0 ± 8.2	108.9 ± 8.7 *‡	82.2 ± 7.2	104.9± 10.7 *	111.9 ± 10.3 *‡
End Bout HR, bpm	169.4 ± 5.3	169.8 ± 6.7	177.1 ± 6.2 *‡	124.5 ± 10.3	138.3± 10.8 *	146.9 ± 9.2 *‡
Baseline SV, ml	115.8 ± 13.2	116.6 ± 11.5	108.0 ± 13.0	118.8 ± 13.1	106.8± 10.4†	96.1 ± 9.4 §*
Peak SV, ml	151.8 ± 13.7	154.5 ± 11.1	151.5 ± 14.0	141.4 ± 12.5	130.1± 10.4 *	126.2 ± 12.7 *
End Bout SV, ml	132.0 ± 8.8	137.8 ± 11.4	135.7 ± 6.7	130.9 ± 11.8	119.8± 10.5 *	115.4 ± 8.7 *
Peak - Baseline SV, ml	36.0 ± 9.9	37.9 ± 12.8	43.5 ±8.7	22.6 ± 8.7	23.3 ± 7.3	30.9 ± 8.7 †
Peak - End SV, ml	19.8 ± 9.1	16.7 ± 6.9	15.8 ± 8.9	10.6 ± 5.1	10.3 ± 4.6	11.6 ± 5.4

Mean ± SD, n = 9

* $P < 0.01$ and † $P < 0.05$ compared to the control conditions ($H1_B$, $M1_A$); ‡ $P < 0.01$ $H2_A$ vs. $H2_B$ and $M3_A$ vs. $M3_B$; § $P < 0.05$ $H2_A$ vs. $H2_B$ and $M3_A$ vs. $M3_B$.

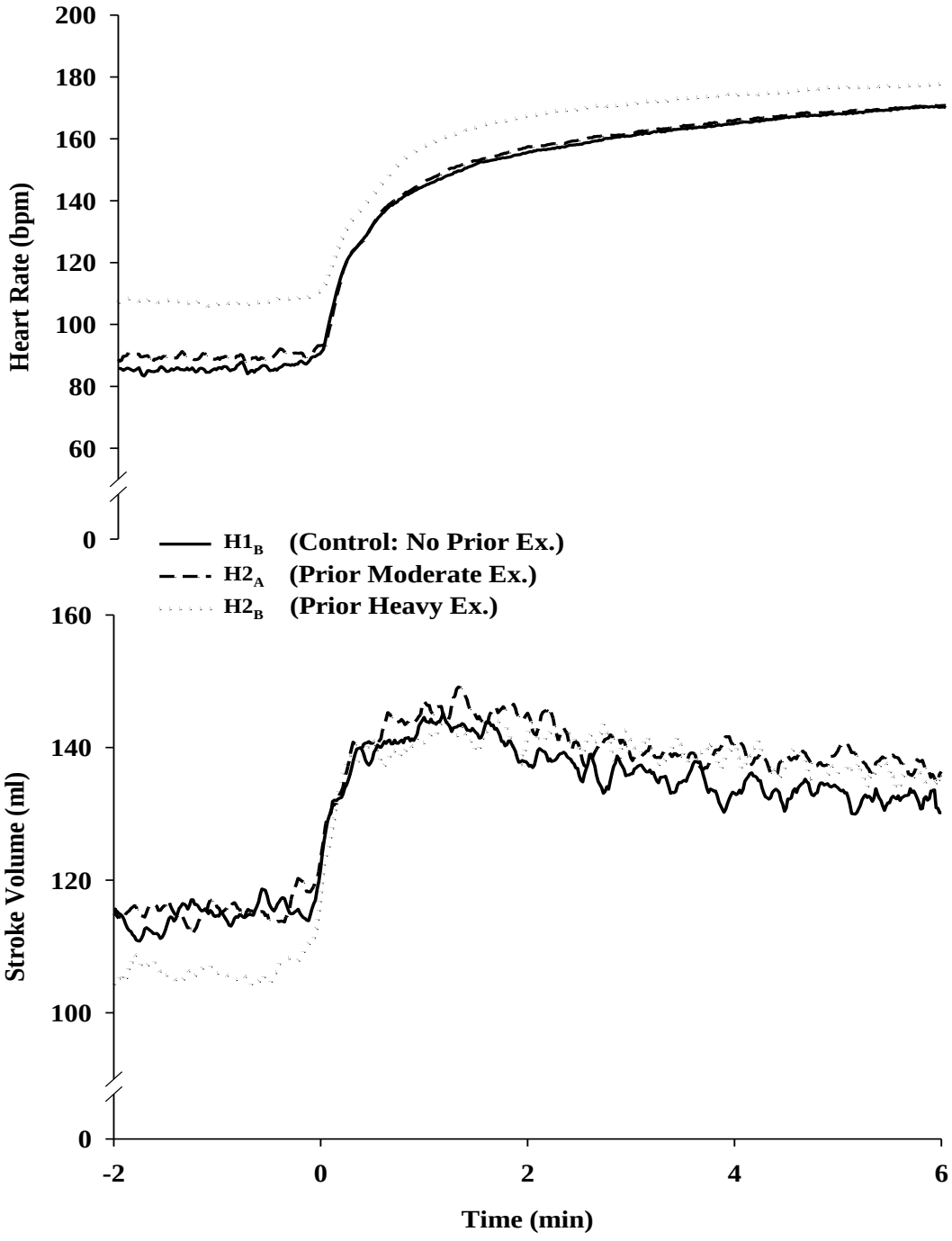

Figure 2.9: HR (top) and SV (bottom) during heavy work rate transitions.

SV is presented as a 5-second moving average. Data lines are the average of 9 subjects with ≥ 4 repetitions per each exercise condition.

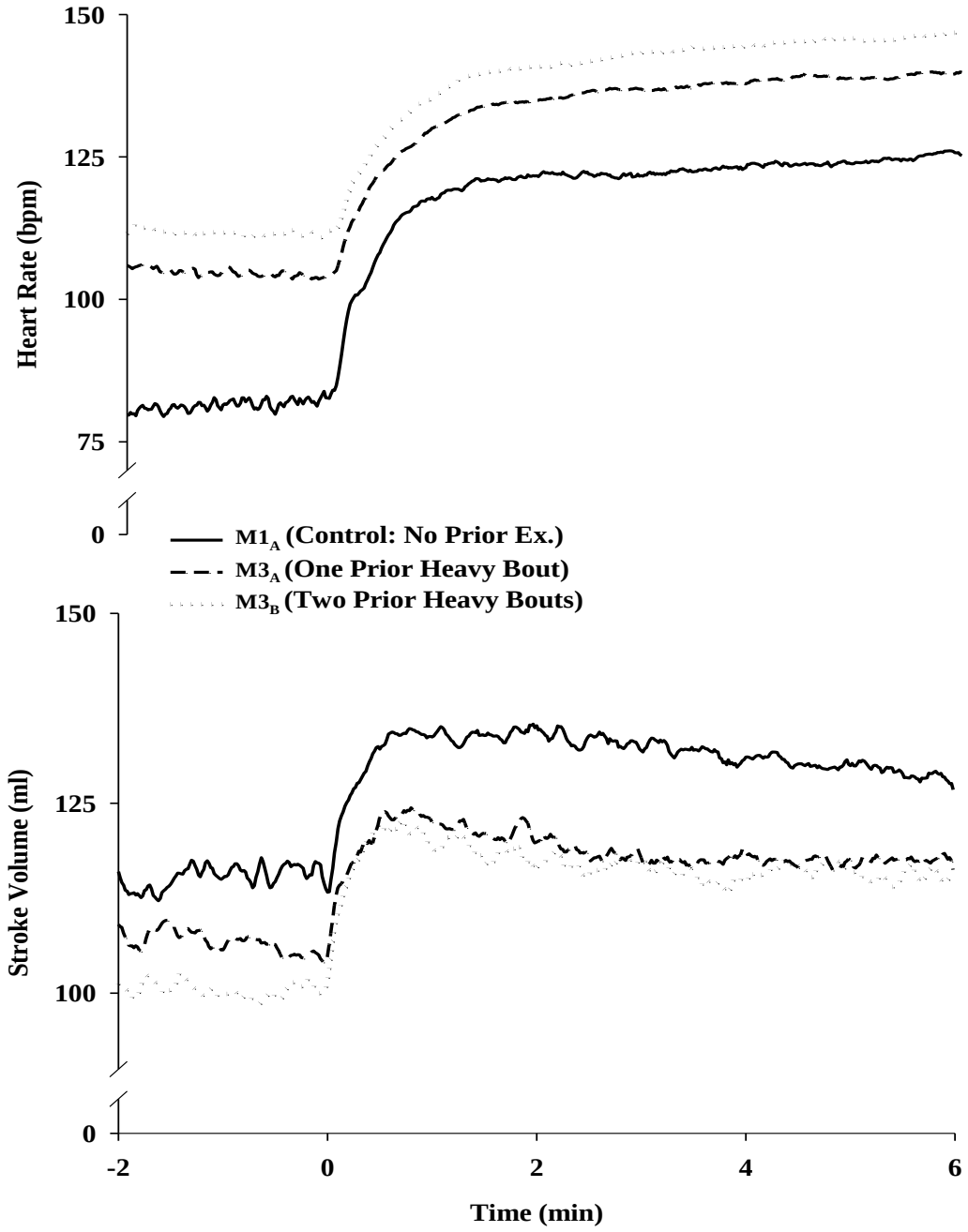

Figure 2.10: HR (top) and SV (bottom) during moderate work rate transitions. SV is presented as a 5-second moving average. Data lines are the average of 9 subjects with ≥ 4 repetitions per each exercise condition.

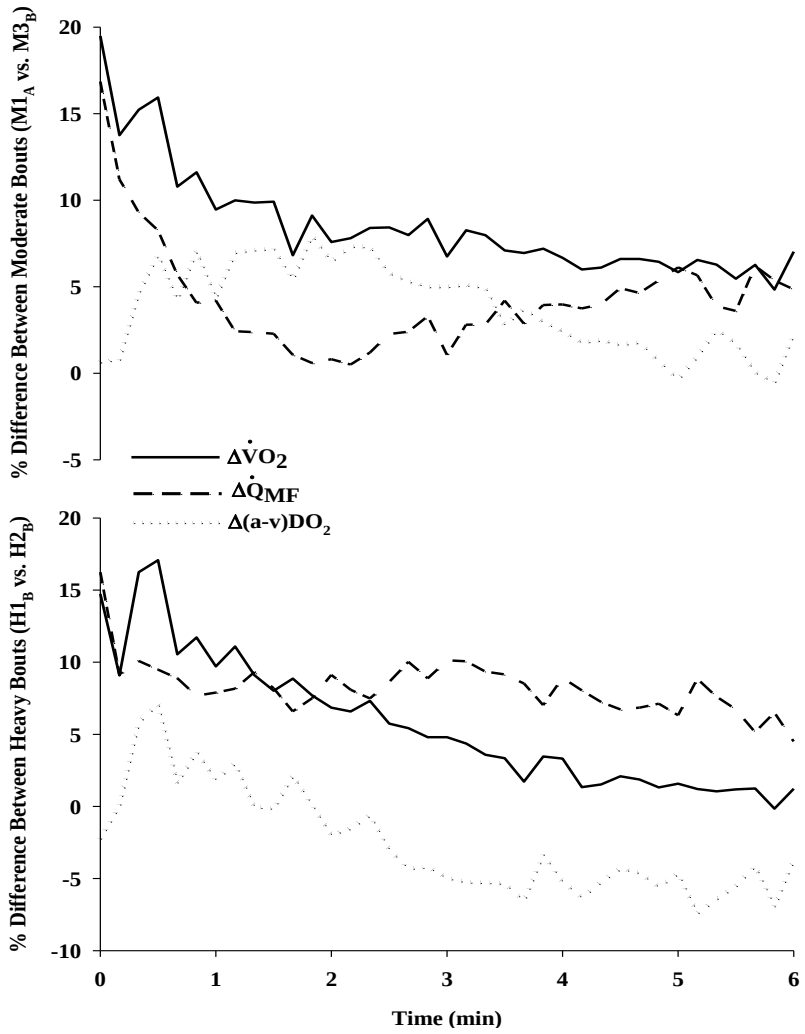

Figure 2.11: Percent differences of $\dot{V}O_2$, $\dot{Q}$, and (a-v)DO_2 within moderate and heavy bouts.

Top panel: $M1_A$ (control; no prior exercise) and $M3_B$ (prior one heavy bout) and heavy bouts .Bottom panel: $H1_B$ (control; no prior exercise) and $H2_B$ (prior heavy bout) Data lines are the average of 10 s window for 9 subjects with ≥ 4 repetitions per each exercise condition. Changes in $\dot{V}O_2$ are approximately equal to changes in $\dot{Q}$ plus changes in (a-v)DO_2 (see text for details).

2.5 Discussion

Our results provided several unique observations concerning $\dot{V}O_2$ and $\dot{Q}$ kinetics during moderate and heavy cycling exercise in endurance athletes. In support of our first hypothesis, we observed for the first time that a 6-minute moderate warm-up bout, as well as prior heavy exercise, was capable of reducing τ_2 for both $\dot{V}O_2$ and $\dot{Q}$ during a subsequent heavy exercise bout. In partial support of our second hypothesis, we observed lower τ_2 for $\dot{V}O_2$ when moderate exercise followed one or two bouts of heavy exercise; but, the τ_2 for $\dot{Q}$ was not significantly altered although absolute $\dot{Q}$ was elevated early in exercise that followed two prior bouts of heavy exercise. Our data also revealed novel responses for SV with a highly significant overshoot during the early phase of heavy and moderate exercise bouts. As well, SV recovered to a common plateau in all heavy exercise bouts regardless of the prior exercise condition, but remained below the no-prior exercise condition during moderate exercise that followed one or two bouts of heavy exercise.

2.5.1 Methodological considerations and limitations

The current data should be put in the perspective that they were obtained in highly fit young men who performed multiple repetitions of exercise tests to enhance the ability to resolve effects of prior exercise on $\dot{V}O_2$ and $\dot{Q}$ responses. The data then might not reflect the general population, but rather the extreme with well adapted cardiovascular and metabolic responses.

Continuous estimates of $\dot{Q}$ were obtained in the current study by Modelflow analysis of the finger arterial pressure waveform (Wesseling *et al.*, 1993). Changes

in $\dot{Q}$ during transitions to higher work rates have been examined by various non-invasive techniques (Inman *et al.*, 1987; Leyk *et al.*, 1995; Perrey *et al.*, 2003a; Yoshida & Whipp, 1994), including Modelflow (Lador *et al.*, 2006); although, these studies involved less intense exercise than the current study. Previous studies have reported that the Finometer Modelflow $\dot{Q}$ technique was valid in comparison to thermodilution (de Wilde *et al.*, 2007; Wesseling *et al.*, 1993) and Doppler echocardiography methods (Sugawara *et al.*, 2003), as well as CO_2 (Pitt *et al.*, 2004) and C_2H_2 rebreathing methods (Tam *et al.*, 2004). Since Modelflow has not been investigated under the conditions of our experiments we conducted a comparison with acetylene rebreathing. There was a high correlation between the $\dot{Q}_{MF}$ and $\dot{Q}_{C2H2}$ from steady state baseline cycling at 20 W to steady state exercise at 85% $\dot{V}O_{2peak}$ with only a small bias and a reasonably small 95% confidence interval for the differences between $\dot{Q}_{C2H2}$ and $\dot{Q}_{MF}$ (Fig. 2.3 and 2.4). As well, the bias was not affected by the protocol, since data from both moderate and heavy bouts confirm that there was no significant bias introduced by the type of prior exercise (Table 2.3). Thus, in the steady state of exercise; there were no systematic errors in estimates of $\dot{Q}$ introduced by the non-invasive Modelflow approach. Methodologically, it was not possible to confirm that there was no bias during the non-steady state phase when we observed differences in $\dot{Q}_{MF}$ kinetics and the overshoot of SV. It is possible that rapid changes in aortic impedance in the transitions to higher work rates affected the accuracy of the Modelflow calculation during exercise onset so caution should be exercised in the interpretation of the

$\dot{Q}_{MF}$ and SV. Notably, Harms *et al.*(1999) reported that, during a dynamic postural shift, changes in $\dot{Q}_{MF}$ did track estimates from Doppler ultrasound.

The breath-by-breath measurements in the current study utilized a modification of the Auchincloss algorithm and estimates of a nominal lung volume (NLV) to follow changes in lung gas stores and adjust the gas exchange measured at the mouth (Hughson *et al.*, 1991b). Based on previous observations that minimal breath-by-breath variation in estimates of $\dot{V}O_2$ was obtained with a lung volume smaller than functional residual capacity (FRC), we arbitrarily chose NLV to equal 50% of FRC (Hughson *et al.*, 1991b). Recently, Cautero *et al.* (2002) suggested the Grønlund algorithm, which defines a respiratory cycle by equal fractions of O_2 in expired air, might provide a more accurate measure of breath-by-breath alveolar $\dot{V}O_2$ and that the phase II time constant was faster than when compared to the Auchincloss algorithm. In the current study, the absolute value of τ_2 might differ if $\dot{V}O_2$ had been calculated with the Grønlund algorithm but our comparisons within subjects would not be biased by the method of calculation.

Characterization of $\dot{V}O_2$ kinetics has normally been approached through multi-component exponential modeling with the major emphasis on the phase II response that might reflect changes in muscle oxidative metabolism (Linnarsson, 1974; Whipp & Ward, 1982). We retained this convention even though nonlinearities during phase II (Hughson *et al.*, 2001; Rossiter *et al.*, 2001) might question the validity of this approach because current techniques are unable to resolve this effect in breath-by-breath data for $\dot{V}O_2$. We chose to fit the cardiodynamic phase (Whipp & Ward, 1982), by an exponential model with a tau1

(τ_1) value fast enough to achieve a complete response before the start of phase II (i.e. 4 * τ_1 < TD_2) (Hughson *et al.*, 2000).

There have been fewer studies of the kinetics of $\dot{Q}$ than $\dot{V}O_2$, but it is obvious from the HR and SV data (Fig. 2.9 and 2.10) that presenting the kinetics of $\dot{Q}$ by a simple two or three component exponential model is at best an approximation even though residuals appear to be evenly distributed (see Fig.2.2 - bottom). The initial overshoot of SV while HR progressively increased violates the concept that one exponential term corresponds to a single physiological control mechanism for $\dot{Q}$. However, using the same model for $\dot{V}O_2$ and $\dot{Q}_{MF}$ simplified comparisons of these variables within our study and with other research.

An important limitation in the current study is that our measurement of $\dot{Q}_{MF}$ does not provide insight into the site-specific delivery of oxygenated blood to the exercising muscles. Recent data from investigation of multiple muscle sites with near infrared spectroscopy (Koga *et al.*, 2007) revealed considerable heterogeneity of tissue oxygenation suggesting that even studies that measure blood flow and O_2 extraction across working muscles (Grassi *et al.*, 1996; Hughson *et al.*, 1996) have limitations with regard to determining the matching of perfusion with metabolic demand.

2.5.2 Oxygen Uptake and Cardiac Output Kinetics during Heavy Exercise

In the current study we observed faster $\dot{V}O_2$ and $\dot{Q}_{MF}$ kinetics in a second heavy bout following six minutes of prior heavy or moderate warm-up exercise. The effect of prior heavy exercise will be considered first, as there are more previous data on this experimental model; then, our novel finding that a moderate warm-up

was capable of accelerating $\dot{V}O_2$ and $\dot{Q}_{MF}$ kinetics during a subsequent heavy exercise will be examined.

Almost universally in previous research, $\dot{V}O_2$ was found to be elevated in the first minutes of heavy exercise that followed soon after previous single or multiple bouts of heavy exercise (Burnley *et al.*, 2000; Gerbino *et al.*, 1996; MacDonald *et al.*, 1997; Tordi *et al.*, 2003). However, what is far from universal is agreement on whether the $\dot{V}O_2$ is simply elevated without a change in kinetics (Burnley *et al.*, 2000; Wilkerson *et al.*, 2004) or whether $\dot{V}O_2$ actually increases more rapidly toward the required level (MacDonald *et al.*, 1997; Rossiter *et al.*, 2001; Tordi *et al.*, 2003).

The current study and that of Tordi *et al.* (2003), where there was clear evidence of faster $\tau_{2,}$ included only subjects who were well trained athletes. There is some evidence that O_2 delivery may limit maximal oxygen consumption (Knight *et al.*, 1993; Richardson, 2000; Richardson *et al.*, 1999). Greater O_2 delivery with increased leg blood flow during heavy exercise following prior heavy exercise (Fukuba *et al.*, 2007; Hughson *et al.*, 2003), might contribute to an enhanced intracellular PaO_2 (Hughson, 2005; Tschakovsky & Hughson, 1999) and lessen PCr degradation (Rossiter *et al.*, 2001). Physical training induces tighter metabolic coupling, such that smaller changes in the energy state of the muscle are needed to stimulate enzymes of oxidative phosphorylation (Phillips *et al.*, 1996). This tighter coupling is consistent with the hypothesis that elite athletes would be more susceptible to limitations in oxygen delivery at the onset of heavy exercise.

Similar to Perrey *et al.*(2003a), we observed that prior heavy exercise accelerated $\dot{Q}$ in subsequent heavy cycling exercise. The increase in $\dot{Q}_{MF}$ that we observed during heavy exercise was a consequence of a rapid increase in SV resulting from increased ejection fraction and a somewhat slower increase in HR that probably reflects a relatively greater reliance on sympathetic activation than on vagal withdrawal (see Fig. 2.9). To the best of our knowledge, this is the first study to report an overshoot of SV in the early phase of heavy exercise; although our validation of Modelflow SV against C_2H_2 rebreathing was not specifically tested in this transient phase. We found that SV reached values within the first minute of exercise that were 15-20 mL greater than the steady state values at the end of the exercise bouts. Leyk *et al.* (1995) monitored the SV using Doppler ultrasound during upright cycling across exercise transitions to work rates of up to 200 W. They showed SV to increase rapidly and peak by 30 s of exercise onset but the resolution of their data was not sufficient to address the possibility of the SV overshoot that has been observed in the first minute of exercise transitions in this study (Fig. 2.9 and 2.10). It seems probable that the very rapid increase in SV reflects the sudden increase in venous return with the onset of the higher work rate mediated primarily by the stronger muscle pump (Sheriff *et al.*, 1993; Tschakovsky *et al.*, 1996). Although SV was lower in the baseline period after prior heavy exercise, there were no differences in peak values between the exercise conditions. The elevated HR after the prior heavy exercise bout was sustained during the subsequent bout of heavy exercise, resulting in a significantly higher $\dot{Q}$ at the end of exercise in this condition. The higher $\dot{Q}$ might have been required as a mechanism to assist with thermoregulation.

Several interacting mechanisms are likely responsible for improved O_2 delivery following a heavy warm-up and contribute to accelerated $\dot{V}O_2$ kinetics during a subsequent heavy bout: (i) an accumulation of vasoactive metabolites enhances vasodilation and increases blood flow to exercising muscles (Krustrup *et al.*, 2001; MacDonald *et al.*, 2001b); (ii) a right-shift of the oxy-hemoglobin dissociation curve resulting from the accumulation of H^+ and increased temperature promotes O_2 offloading at the muscle (Gerbino *et al.*, 1996; MacDonald *et al.*, 2001b); and (iii) a reduction in regional perfusion heterogeneities in the muscle microvasculature improves the matching between O_2 delivery and oxygen utilization (DeLorey *et al.*, 2007; Fukuba *et al.*, 2002).

While we have shown that enhanced O_2 delivery plays an overriding part in accelerating $\dot{V}O_2$ kinetics following heavy exercise, others have argued that the overall $\dot{V}O_2$ kinetics acceleration are due to an increase in the primary phase amplitude and attenuation of the slow component amplitude (Burnley *et al.*, 2002b; Burnley *et al.*, 2000; Fukuba *et al.*, 2002; Koppo & Bouckaert, 2001; Perrey *et al.*, 2003a). In agreement with these previous studies, we observed an increase in the absolute $\dot{V}O_2$ at the end of primary phase. However a higher end- primary phase $\dot{V}O_2$ showed in this study and others (Burnley *et al.*, 2002b; Tordi *et al.*, 2003; Wilkerson *et al.*, 2004) results from a higher baseline, not a real increase in the primary phase amplitude (Burnley *et al.*, 2000; Jones *et al.*, 2006; Koppo & Bouckaert, 2001; Marles *et al.*, 2007; Tordi *et al.*, 2003). To eliminate the effect of the elevated baseline $\dot{V}O_2$ following the heavy bout, Jones *et al.* (2003) showed the normalized $\dot{V}O_2$ responses to the amplitude of the primary phase and reported

that the kinetics of the primary component are unaffected by a prior heavy bout. Contrary to their conclusion, our data (Fig. 2.6) showed that prior moderate and heavy bouts accelerated the primary component of $\dot{V}O_2$ kinetics of a subsequent heavy bout even after normalization to the amplitude ($A_1 + A_2$) at the end of the primary phase. Recently, Hughson *et al.* (2001) used a computer simulation model to demonstrate the difficulty in detecting either dynamic nonlinearities or differences in time constant even when they are present. Therefore, optimizing the experimental conditions is essential to determine altered τ_2 (Rossiter *et al.*, 2001). In this study, a well-controlled experimental design was developed by selecting a homogeneous group of endurance athletes and having them complete multiple trials to increase the $\dot{V}O_2$ signal to noise ratio enabled us to detect a significant effect of prior exercise on the primary component $\dot{V}O_2$ kinetics.

A unique finding of this study was that a prior bout of moderate cycling exercise accelerated $\dot{V}O_2$ kinetics (reduced τ_2) in a subsequent heavy bout. The similar responses shown in the acceleration of $\dot{Q}_{MF}$ and $\dot{V}O_2$ kinetics following both prior heavy and moderate warm-ups —despite the expected higher baseline [La^-] following the heavy warm-up, as shown in Chapter 4 – provide evidence that accumulation of H^+ during the heavy warm-up is not the sole factor contributing to acceleration of the $\dot{V}O_2$ kinetics (Burnley *et al.*, 2002a; Koppo & Bouckaert, 2000; Koppo & Bouckaert, 2002). This conclusion is in agreement with Fukuba *et al.* (2002), who showed a significant difference in mean response time of the second heavy cycling bout after an identical heavy cycling bout, but not after a heavy arm cranking exercise bout, despite similar baseline plasma La^- concentrations. These

findings support the idea that changes in $\dot{V}O_2$ kinetics are not simply due to systemic lactic acidosis. Highly trained athletes might exhibit an improved sensitivity to the local metabolic environment and a more tightly regulated O_2 delivery system to the working muscles. As a consequence of the prior moderate exercise, we observed slight increases in HR, $\dot{Q}_{MF}$, and [La^-] during the subsequent baseline period. These factors might reflect conditions compatible with enhanced vasodilation and O_2 offloading at the working muscle and speeding the kinetics of $\dot{Q}_{MF}$ and $\dot{V}O_2$ during subsequent heavy bouts.

2.5.3 Oxygen uptake and cardiac output kinetics during moderate exercise

Our second hypothesis proposed that both $\dot{V}O_2$ and $\dot{Q}$ kinetics would also be accelerated when moderate exercise followed heavy warm-up exercise. There were faster $\dot{V}O_2$ kinetics after heavy warm-up (one or two bouts) exercise, but $\dot{Q}_{MF}$ kinetics were not significantly affected. The observation of faster $\dot{V}O_2$ kinetics during moderate exercise after a heavy warm-up confirmed the recent finding by Gurd *et al.* (2006), but contrasts with the results of others (Burnley *et al.*, 2000; Gerbino *et al.*, 1996; MacDonald *et al.*, 1997).

The mechanism(s) that made the elevated $\dot{V}O_2$ possible during moderate exercise after heavy exercise has (have) not been investigated in detail since only this study and that of Gurd *et al.* (2006) have reported this observation in young men. Gurd *et al.* (2006) found that the intramuscular environment was enhanced PDHa activity and substrate concentrations (e.g. acetyl CoA and NADH) were elevated by a prior heavy exercise bout, but they also found greater tissue oxygenation by near infrared spectroscopy suggesting elevated blood flow. This

latter observation might appear in conflict with the current study where the kinetics of $\dot{Q}_{MF}$ were not changed but several factors need to be considered. $\dot{Q}_{MF}$ was elevated in the baseline period before the moderate exercise bout by both one and two prior heavy exercise bouts. As well, $\dot{Q}_{MF}$ was significantly elevated during the first 45 s by the two bouts of prior heavy exercise ($M3_B$). It is possible that perfusion of the exercising muscles was elevated in the transition to the moderate exercise, but data are not available in the current study to confirm the blood flow distribution or to indicate how the change in whole body (a-v)DO_2 reflected the O_2 extraction across the working muscle. The absence of effect of prior exercise on $\dot{Q}_{MF}$ kinetics was similar to the report of Yoshida *et al.* (1995) who saw no effect during moderate one-legged cycling. In a more controlled assessment of the role of O_2 extraction in accelerating $\dot{V}O_2$ kinetics, DCA was infused directly into the exercising muscle to stimulate PDHa activity (Howlett *et al.*, 1999b). Oxidative metabolism was increased, as determined by PCr utilization and cellular energetic, at the onset of moderate (Howlett *et al.*, 1999b) but not sprint exercise (Howlett *et al.*, 1999a). It could be concluded that the muscles' inability to increase metabolism during the heavy exercise was due to O_2 delivery and/or other metabolic limitations, supporting the results shown in our study.

2.5.4 Heart rate and stroke volume at the onset of exercise

The contribution of rapid increases in HR and SV to the kinetics of oxidative metabolism at the onset of exercise was recognized by Linnarrson (1974), but it was the observation of Hughson and Morrissey (1982) that kinetics of $\dot{V}O_2$ were markedly slowed when HR increased more slowly that emphasized the importance of the O_2 transport mechanism to metabolic regulation. This previous work

demonstrated that the HR response is considerably faster if HR starts from a low level that allows parasympathetic withdrawal to rapidly increase HR to ~100 beats/min.

The magnitude and rate of increase in HR at the onset of exercise is determined by the interplay of several factors. First, the baseline HR is extremely important as it determines whether parasympathetic and sympathetic nervous systems can contribute to the rate of increase in HR. Second, the magnitude of increase in HR is proportional to the magnitude of the step increase in metabolic demand. Third, other factors such as requirements for thermoregulation can influence the HR and SV.

In the moderate exercise bouts, HR was relatively stable by 2-min of exercise and increased by less than 5 beats/min over the next 4-min of exercise. However, the absolute HR did differ between each exercise condition with higher absolute HR after one or two bouts of prior heavy exercise. The elevated baseline HR after prior heavy exercise to over 100 bpm in $M3_A$ and $M3_B$ (Fig. 2.10) probably contributed to a slower increase with the start of moderate exercise as vagal activity was already withdrawn and increased sympathetic activity was required to increase HR (Fagraeus & Linnarsson, 1976). In the heavy exercise bouts, HR was elevated by prior heavy but not moderate exercise, and HR was significantly elevated throughout the heavy exercise bout that was preceded by the heavy warm-up exercise. HR had not stabilized by 2-min but continued to increase through 6-min by 10 to 20-beats/min in each heavy exercise bout. The elevated HR after previous heavy exercise and the progressive rise in HR in the heavy exercise bouts might reflect the elevated metabolic demand as well as the increased thermoregulatory stress.

There have been few studies employing techniques that determine beat-to-beat SV, but to the best of my knowledge this is the first study to describe a significant overshoot in the first minutes of moderate and heavy exercise. At the onset of 50 or 100 W cycling exercise, Lador *et al.* (2006) displayed the first 45-s of SV but this was not sufficient to see the time course of the overshoot that described in this study. The overshoot and overall elevation in SV contrasts with the finding of a reduction in SV during mild supine exercise (Elstad *et al.*, 2009). The very rapid increase in SV reflects the sudden increase in venous return with the onset of the higher work rate mediated primarily by the stronger muscle pump during upright cycling exercise (Sheriff *et al.*, 1993; Tschakovsky *et al.*, 1996). SV was not different between the moderate and heavy exercise bouts except when heavy exercise preceded the moderate exercise bouts. This suggests that the prior exercise probably induced the requirement for an increased thermoregulatory response with greater SBF (*see Appendix A*) and impaired venous return. It will be of interest to determine if the SV overshoot is a characteristic of well trained athletes or common to healthy people.

2.6 Conclusions

In the current study, we combined estimates of continues $\dot{Q}_{MF}$, validated in steady state against acetylene rebreathing, with breath-by-breath measurements of $\dot{V}O_2$ to study the dynamic aspects of the cardiorespiratory responses at the onset of moderate and heavy exercise with prior warm-up. It was observed that the estimates of SV had a significant overshoot in the first minute of both moderate and heavy exercise that contributed to the rapid increase in $\dot{Q}_{MF}$ at the onset of exercise. In support of the hypotheses, the $\dot{V}O_2$ response during moderate exercise was significantly accelerated by prior heavy exercise. As well, in heavy exercise both the $\dot{V}O_2$ and $\dot{Q}_{MF}$ responses were accelerated not only by prior heavy but also by moderate warm-up exercise. Overall, these data showed that prior exercise can accelerate the phase II kinetics of $\dot{V}O_2$ at least in a population of very fit young men. The relatively large amplitude of Q and $\dot{V}O_2$ in the heavy exercise enabled discovery of significant links between O_2 transport and O_2 utilization. In moderate exercise, the amplitude of the Q and $\dot{V}O_2$ signals was smaller. A consequence of this was that alternative means of supplying O_2 were available so that clear concluding statements cannot be made about the link between O_2 transport and O_2 utilization. Thus, at least for heavy exercise the data suggest that enhanced O_2 delivery contributed along with greater metabolic activation (Tschakovsky & Hughson, 1999) to accelerate $\dot{V}O_2$ kinetics in heavy exercise following priming exercise.

Chapter 3

Prolonged ischemia impairs blood flow and muscle oxygen uptake Dynamics during subsequent heavy exercise

This chapter is the basis for the published paper*:*

Faisal A, Dyson KS and Hughson RL. Prolonged ischemia impairs muscle blood flow and oxygen uptake dynamics during subsequent heavy exercise. *J Physiol.*588. 19 (2010) pp 3785-3797.

3.1 Overview

Muscle oxygen uptake dynamics at the onset of exercise can be affected by prior heavy exercise. We tested the hypothesis that elevated FBF following prior circulatory occlusion would also be associated with accelerated $\dot{V}O_{2mus}$ dynamics during subsequent heavy hand-grip exercise. Ten trained young men performed 5 minutes of heavy hand-grip exercise at 30% MVC as a control [CON], and 4 additional heavy bouts after brief recovery from: I) prior heavy exercise [Heavy A], II) heavy exercise followed by 2 minutes occlusion [Heavy B], III) 15 minutes occlusion [Heavy C], and IV) 5 minutes occlusion with1 minute of moderate exercise during occlusion [Heavy D]. FBF was measured by ultrasound and arterial venous oxygen content difference was calculated from venous blood samples to estimate $\dot{V}O_{2mus}$. FBF and $\dot{V}O_{2mus}$ dynamics were quantified from the rise time. All priming conditions elevated FBF immediately before the start of subsequent heavy bout (Heavy A: 207.4 ± 92.8, B: 207.8 ± 75.8, C: 135.8 ± 59.2, D: 199.5 ± 59.0 vs. CON: 57.4 ± 16.6 ml min^{-1}, $P < 0.01$). Unexpectedly, prior occlusion reduced FBF and O_2 extraction at the onset of subsequent heavy exercise and consequently slowed $\dot{V}O_{2mus}$ dynamics (Heavy C: rise time = 95.9 ± 28.9 vs. CON: 58.6 ± 14.3 s, $P < 0.01$). FBF and $\dot{V}O_{2mus}$ dynamics were faster in Heavy A, B and D compared to CON ($P < 0.05$). Overall, there was a positive correlation between the rise times for $\dot{V}O_{2mus}$ and FBF ($r^2 = 0.75$) indicating that $\dot{V}O_{2mus}$ dynamics during heavy forearm exercise are linked to O_2 delivery in trained young men. To investigate a possible mechanism for slower adaptation of $\dot{V}O_{2mus}$ following ischemia, the prior occlusion condition was repeated after ingesting a high dose of ibuprofen. This resulted in restoration of the FBF and $\dot{V}O_{2mus}$ to control levels suggesting that a prostaglandin-

mediated mechanism after occlusion retarded the adaptation of blood flow and O_2 consumption at the onset of subsequent heavy exercise.

3.2 Introduction

During transitions of exercise to higher power output, faster activation of the aerobic energy supply system will result in a smaller O_2 deficit and reduced homeostatic disturbance. Prior heavy exercise is an experimental paradigm that accelerates $\dot{V}O_2$ kinetics at the onset of subsequent exercise, potentially through several mechanisms that affect both O_2 transport and utilization. These mechanisms include increased oxidative enzyme activity and elevated mitochondrial substrate availability (Gurd *et al.*, 2006), increased muscle blood flow and right-shifting of the oxygen hemoglobin (HbO_2) dissociation curve (Gerbino *et al.*, 1996; MacDonald *et al.*, 1997) and improved distribution of O_2 delivery to O_2 requirement within the exercising muscle (DeLorey *et al.*, 2007; Fukuba *et al.*, 2002).

Post circulatory occlusion is an alternative experimental model that could elevate muscle blood flow for several minutes prior to subsequent exercise bout (Carlsson *et al.*, 1987). However, the changes in the metabolic environment during occlusion are much less pronounced than during prior heavy forearm exercise (Mole *et al.*, 1985). Few studies have used the prior occlusion model to examine the factors that affect pulmonary $\dot{V}O_2$ kinetics during a subsequent exercise transition (Paganelli *et al.*, 1989; Walsh *et al.*, 2002), while no study has looked at blood flow and $\dot{V}O_{2mus}$ responses at the onset of exercise that followed circulatory occlusion. Paganelli *et al.* (1989) reported that prior 5-10 min of forearm occlusion or 3 min of occlusion with moderate exercise reduced the half-time of pulmonary $\dot{V}O_2$ on-response kinetics during subsequent heavy arm-cranking exercise by 15 and 50%, respectively. In another study, occlusion of resting thigh muscles for 5 and 10 min

accelerated the overall pulmonary $\dot{V}O_2$ kinetics during subsequent heavy cycling exercise with no effects on the distinct primary phase kinetics (Walsh *et al.*, 2002). But, in this latter study the circulatory occlusion was terminated within 5 s prior to the onset of the criterion exercise and the potential replenishment of O_2 stores on the $\dot{V}O_2$ response and its impact on kinetics was not considered.

It has been shown previously that prior heavy exercise elevated FBF and $\dot{V}O_{2mus}$ at the onset of subsequent heavy dynamic forearm exercise (MacDonald *et al.*, 2001b), and that the kinetics of both leg blood flow and $\dot{V}O_{2mus}$ were accelerated in the second of two heavy knee-extension exercise bouts (Fukuba *et al.*, 2007). The purpose of the current study was to determine whether prior occlusion, with or without exercise, followed by brief recovery had similar effects as prior exercise on FBF and $\dot{V}O_{2mus}$ kinetics during subsequent heavy forearm exercise in fit young men. We tested the hypothesis that all priming conditions (heavy exercise, heavy exercise followed immediately by occlusion, occlusion alone and occlusion with moderate exercise) would enhance FBF and $\dot{V}O_{2mus}$ dynamics during the adaptive phase of subsequent heavy forearm hand-grip exercise.

3.3 Methods

Twenty four men who regularly exercised their forearm muscles were recruited for this study. The participants were divided into three groups: group A (10 varsity hockey players, age: 23.0 ± 1.4 years; height: 180.5 ± 7.1 cm, weight: 85.1 ± 8.5 kg, maximal voluntary isometric contraction strength [MVC]: 54.0 ± 6.2 kg), group B (8 varsity athletes, age: 24.6 ± 3.6 years; height: 186.1 ± 8.9 cm, weight: 86.4 ± 11.3 kg, MVC: 49.6 ± 4.0 kg) and group C (8 varsity athletes, age:

24.3 ± 2.7 years; height: 179.5 ± 7.9 cm, weight: 82.5 ± 12.0 kg, MVC: 47.0 ± 3.6 kg). One subject participated in both group A and B, and another subject participated in both group B and C. MVC was determined from the best of three attempts taken in the supine position. The study was approved by the Office of Research Ethics at the University of Waterloo, and all subjects provided written, informed consent following full description of the protocols.

3.3.1 Experimental design

Group **A** reported to the laboratory on 4 non-consecutive days to perform the different exercise protocols (Fig.3.1). The protocols were designed to test the effect of the prior exercise, occlusion, or exercise and occlusion on FBF, $(a\text{-}v)DO_2$ and $\dot{V}O_{2mus}$ responses during subsequent heavy rhythmic hand-grip exercise. Protocol A consisted of 2 heavy hand-grip exercise bouts separated by 6 minutes of passive recovery. Protocol B consisted of a bout of heavy hand-grip exercise immediately followed by 2 minutes of forearm circulatory occlusion, 6 minutes of recovery then another bout of heavy hand-grip exercise. Protocol C consisted of a 15 minutes period of forearm circulatory occlusion followed by 3 minutes of recovery then a bout of heavy hand-grip exercise. Protocol D consisted of a 5 minutes period of forearm circulatory occlusion that included one minute of moderate (15% MVC, 7.6 ± 1.0 kg) dynamic hand-grip exercise followed by 3 minutes of recovery then a bout of heavy hand-grip exercise. The protocols were applied in a supine position using the dominant arm extended at the heart level. The first heavy bout in protocol A served as a control (CON) and the testing heavy bouts were named by the protocol letters as described in Fig.3.1.

Group **B** completed a control heavy exercise trial (the first bout in protocol A), and 2 trials of the prior 15 minutes occlusion protocol (protocol C) on 3 non-consecutive days. One of the prior occlusion trials was performed after ingesting 800 mg of ibuprofen (IB) (2 doses of 400 mg, one 3 hours prior, and one 1 hour prior to the start of the protocol), while the other was performed after a placebo (PL)(same capsule as ibuprofen, filled with sodium bicarbonate).

Group **C** completed protocol A, and 2 trials of protocol C on 3 non-consecutive days. The 2 occlusion trials (protocol C) were performed after ingesting ibuprofen and placebo exactly as described above in group B. All groups visited the laboratory for a familiarization session before the start of the real study.
All the rhythmic hand-grip exercise bouts lasted for 5 minutes at ≈ 30% MVC (group A: 13.7 ± 1.7 kg, group B: 12.5 ± 1.1 kg, group C: 11.8 ± 1.0 kg). The participants were required to raise the weight for 0.5 s and lower it for 0.5 s through a distance of 5 cm with a 1 s pause before the next contraction.

The protocols were assigned in randomized order and the sessions were separated by at least 48 hours. Testing was performed at least 24 h following the participants' last exercise regimen and 72 h following an intense strength training session. The participants arrived in the laboratory in a rested state at least 2 h after eating and they were asked to abstain from caffeine 12 h and alcohol 24 h before testing. The participants were rested in supine position for 30 minutes before initiation of the protocols. The tests were completed in a quiet, air-conditioned laboratory at a temperature of ~ 22°C

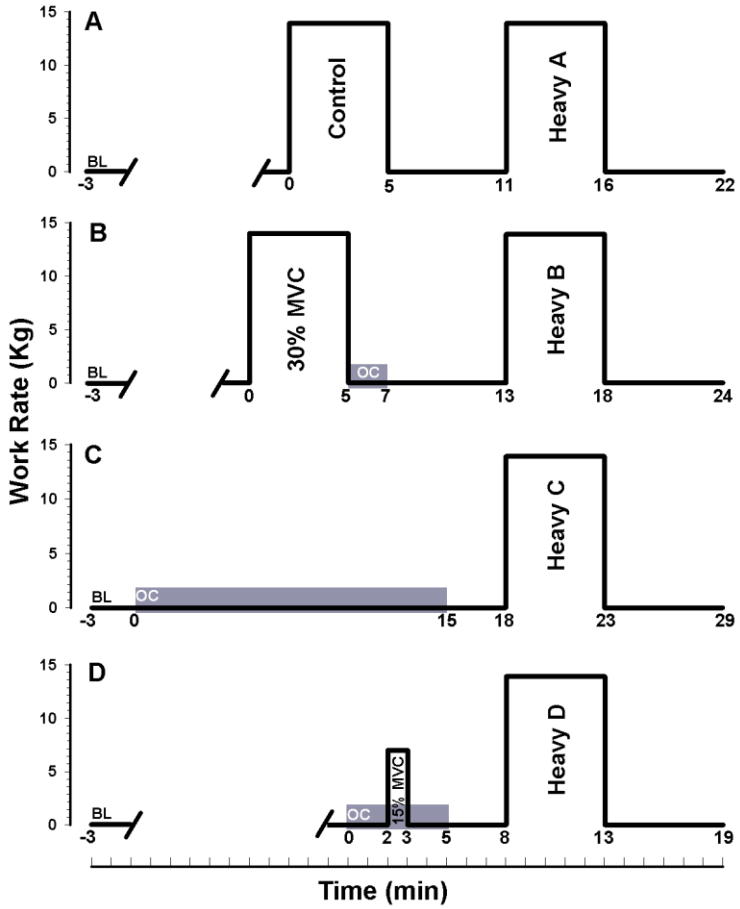

Figure 3.1: Prior exercise and occlusion testing protocols.

Four different testing protocols employed to examine the influence of (A) prior heavy exercise, (B) prior heavy exercise followed by 2 min occlusion, (C) 15 min of occlusion and (D) 5 min of occlusion including 1 min of moderate exercise on $\dot{V}O_{2mus}$ and FBF responses during a subsequent heavy exercise bout. The specific heavy bouts are named based on the testing protocol, Heavy A, Heavy B, Heavy C, and Heavy D. The moderate and heavy exercise bouts were set at 15% and 30% MVC, respectively.

3.3.2 Forearm circulatory occlusion

In the occlusion protocols, brachial artery blood flow was arrested by rapid inflation (E-20 Rapid Cuff Inflator, D. E. Hokanson, Issaquah, USA) of a standard BP cuff to ~ 250 mmHg. The occlusion cuff was placed just distal to the elbow. The occlusion period was terminated by rapid deflation of the occlusion cuff.

3.3.3 Data acquisition

Brachial artery MBV, BP and HR were measured beat by beat. Brachial artery MBV responses were determined by pulsed- Doppler ultrasound (500V, Multigon Industries, Yonkers, N.Y.). A 4-MHz Doppler probe was fastened above the brachial artery proximal to the cubital fossa with strips of surgical tape. The signal was directed 45° relative to the skin and the ultrasound gate was adjusted to encompass the total width of the artery. MBV, BP measured by photoplethysmograph finger blood pressure cuff in the non-exercising hand (Finometer, Finapres Medical System, Arnhem, the Netherlands) and electrocardiogram (Pilot 9200, Colin Medical Instruments, San Antonio, TX) were sampled at 1 kHz (PowerLab, AD Instruments, Colorado Springs, CO). The brachial artery was imaged continuously approximately 5 cm proximal to the 4 MHz probe during rest and exercise using a linear 7.5-MHz probe (Micromaxx, Sonosite, Seattle, WA) operating in M mode. The imaged data were stored on videotape then digitized and vessel diameters were measured by custom edge-detection software.

Venous blood sampling

A catheter was inserted in an antecubital vein in a retrograde fashion (Mottram, 1955) to maximize collection of blood from deep veins draining the finger flexor muscles. Blood samples (1 ml) were drawn in heparinized syringes at baseline and during exercise (each 15 s from 0 to 90 s and each minute from the

second minute to end exercise). The samples were immediately put in an ice bath and analyzed by CO-oximeter (Stat Profile pHOx, Nova Biomedical, Waltham, MA) for O_2 saturation and hemoglobin content from which O_2 content was calculated. The CO-oximeter was calibrated at regular intervals during the analyses. Blood samples (3 ml) were drawn in non- heparinized syringes at rest and during the last 30 s of each exercise and recovery period for immediate lactate [La^-] analysis (Lactate Pro, Hurstville, Australia).

Skin blood flow

Relative forearm SBF was estimated continuously by a laser Doppler probe (MoorLAB, Moor Instruments Ltd, Devon, UK) placed 5cm distal to the occlusion cuff , on the volar aspect of the exercising forearm. SBF was measured only in group C. Baseline SBF was measured for at least 5 minutes before the actual start of the protocols. SBF data were represented as a percentage of baselines.

3.3.4 Data analysis

FBF was calculated from brachial artery MBV and cross sectional area as $FBF = MBV \times \pi r^2$, where r is the vessel radius. MBV was calculated beat by beat at rest and was averaged over the contraction to contraction cycle (duty cycle ~ 2 s) during exercise (Fig. 3.2). Brachial artery diameters were obtained by automated edge detection software from M-mode images, averaged over 3 full cardiac cycles matching the time points of blood sampling. Exercise FBF values were then calculated from the brachial artery diameter and the average MBV over 3 contraction/relaxation cycles (~ 6 s window) centered on the time points of blood withdrawal. Forearm $\dot{V}O_{2mus}$ was determined from the quantitative estimates of FBF and (a-v)DO_2 using the Fick equation, $\dot{V}O_{2mus} = FBF \times (a\text{-}v)DO_2$. O_2 extraction was

calculated based on the assumption that arterial hemoglobin was equivalent to that of the venous sample and arterial O_2 saturation remained constant at 97%, as shown by pulse oximetry (Hampson & Piantadosi, 1988) and blood gas analyzer (Wilkins *et al.*, 2008). Intracellular partial pressure of oxygen (PaO_2) was estimated at 90 mmHg based on the HbO_2 dissociation curve with estimated arterial O_2 content ($mlO_2\ l^{-1}$ blood) = (1.38 [hemoglobin (g/l)] × O_2 saturation) + 0.0031 × PaO_2 (mmHg) (Nordmeyer *et al.*, 2007). (A-v)DO_2 was calculated by subtracting the measured venous O_2 content from estimated arterial O_2 content (Hughson *et al.*, 1996; MacDonald *et al.*, 2001b; van Beekvelt *et al.*, 2001). Measures taken to represent the value at the start of exercise were obtained during the 15 s immediately prior to exercise onset.

In addition to the comparison between the absolute values for $\dot{V}O_{2mus}$ and FBF at the time points of blood sampling through the exercise bouts, we characterized $\dot{V}O_{2mus}$ and FBF kinetics responses during exercise transitions. To estimate the kinetics, the areas under the curves (AUC) of $\dot{V}O_{2mus}$ and FBF were calculated using the first order hold method. $\dot{V}O_{2mus}$ and FBF kinetics were quantified from the rise time (τ) based on the calculation of AUC and the given amplitude assuming a first order linear dynamic system. The rise time for $\dot{V}O_{2mus}$ and FBF were estimated from the following equation

$$AUC(Y) = A\ [(t_f - t_s) + \tau\ (e^{-(t_f / \tau)} - e^{-(t_s / \tau)})]$$

where, AUC(Y) is the numerical value calculated from the first order hold method for the $\dot{V}O_{2mus}$ and FBF curves, A is the end exercise amplitude, t_f is the final time (300s), t_s is the start time to calculate AUC and τ is the rise time of $\dot{V}O_{2mus}$ and

FBF. For $\dot{V}O_{2mus}$, values were obtained at the blood sampling points, with $t_s = 0$. FBF values were the 6 s windows as described above, and kinetics were calculated starting from $t_s = 12$ s of exercise to eliminate the impact of the rapid increase in blood flow due to mechanical factors during the first seconds of exercise onset (Shoemaker *et al.*, 1998).

3.3.5 Statistical analysis

A two-way ANOVA with repeated measures was used to determine significant differences over all the heavy bouts across the time points of blood sampling. When significant effects were observed, the contrast adjusted test was used for comparisons. A one-way ANOVA with repeated measures was used to define significant differences in the rise times for $\dot{V}O_{2mus}$ and FBF, baseline and end exercise [La^-] across the heavy bouts. When significant effects were observed, the Tukey post hoc test was used for comparisons. Regression analyses compared FBF and $\dot{V}O_{2mus}$ kinetics to each other. All data are expressed as means ± SD and a probability of $P < 0.05$ was accepted as statistically significant. The data were analyzed using Statistical Analysis Software package 9.1 (SAS Institute, Cary, NC).

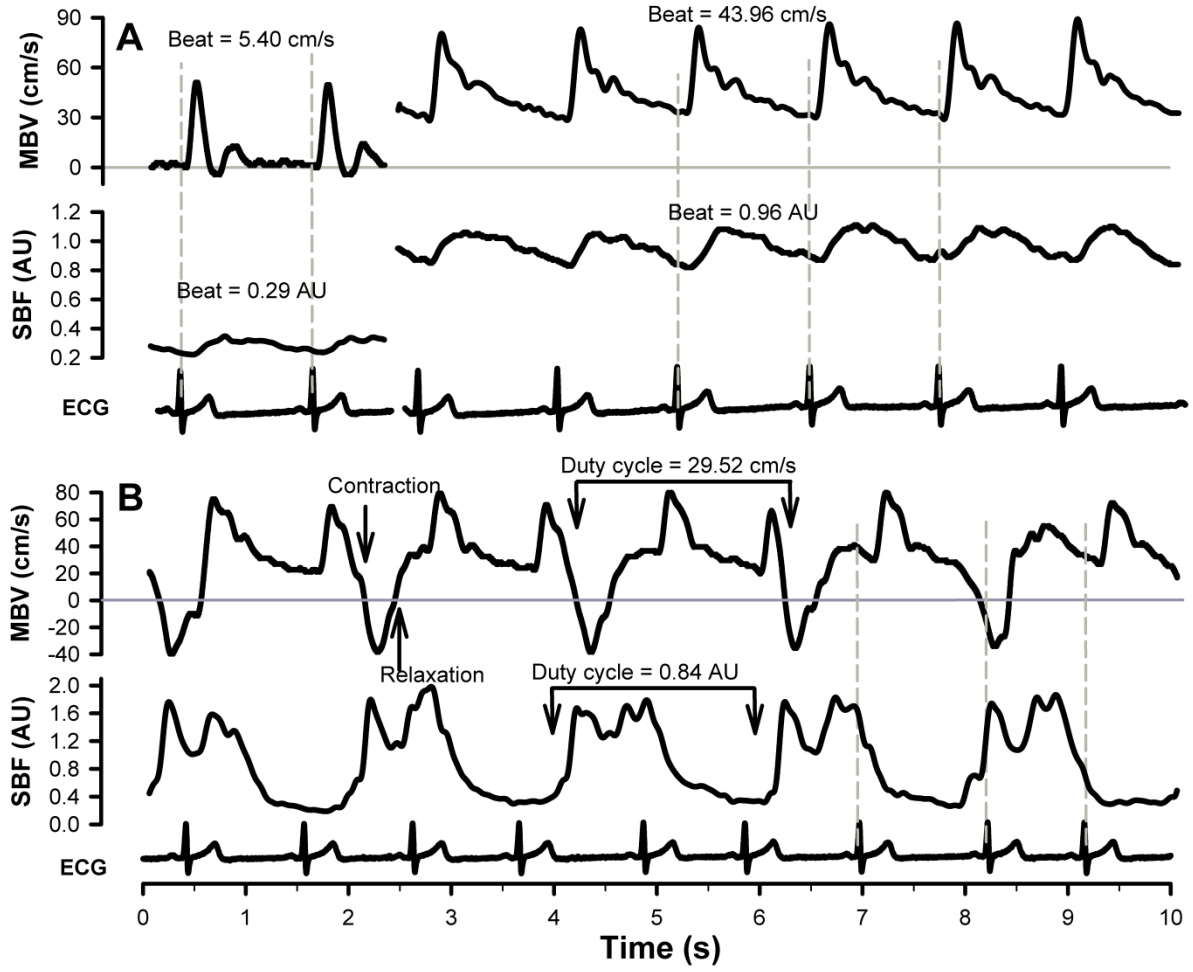

Figure 3.2: Representative tracing of MBV and SBF during resting and reactive hyperemia (A) and heavy dynamic handgrip exercise (B).

During resting the MBV and SBF were averaged beat to beat synchronized with the ECG signal. During exercise MBV was averaged over the duty cycle (contraction to contraction). Beat to beat averages during exercise would not be representative of the true MBV, as the duty cycles were not synchronized to the ECG. SBF was averaged over 2 s cycles.

3.4 Results

3.4.1 Group A

There were no differences in any physiological variable between the first heavy bout in protocol A and the first heavy bout in protocol B across all the time points (*P* ranged from 0.08 to 0.96); therefore we chose the first heavy bout in protocol A to serve as a control condition to the heavy testing bouts (Heavy A-D). Across the 4 testing days there were no significant differences at baseline values of any testing variable (Table 3.1).

Forearm Blood Flow

All priming conditions (exercise, exercise followed by occlusion, occlusion, occlusion with exercise) resulted in a significantly higher FBF at the start of subsequent testing bouts (Heavy A, B, C, D) compared to Con (Heavy A: 207.4 ± 92.8, B: 207.8 ± 75.8, C: 135.8 ± 59.2, D: 199.5 ± 59.0 vs. Con: 57.4 ± 16.6 ml/min, $P < 0.01$ for all). FBF was significantly higher at the start of Heavy A, B and D compared to Heavy C ($P < 0.01$ for all). During heavy exercise bouts, FBF progressively increased to steady state. There was significantly higher FBF through the first 2 min of exercise in Heavy A, B and D compared to CON and Heavy C (Fig. 3.3). By the end of exercise there were no significant differences between all bouts (Con: 404.6 ± 104.9, A: 445.1 ± 122.2, B: 416.2 ± 142.8, C: 410.7 ± 109.7, D: 435.0 ± 109.5 ml/min, $P > 0.05$). FBF kinetics were faster in Heavy A, B, and D compared to CON as shown by a shorter rise time (τ)(Heavy A: 47.6 ± 14.2, B: 39.6 ± 18.4, D: 50.4 ± 15.8 vs. Con: 65.6 ± 13.0 s, $P < 0.05$). Prior occlusion resulted in slower FBF compared to other conditions (Heavy C: 88.9 ± 29.1 s, $P < 0.05$).

Arterial-venous oxygen content difference

Each of the prior conditions elicited a significantly lower (a-v)DO_2 at the start of subsequent heavy exercise bouts compared to Con (Heavy A: 31.5 ± 10.5, B: 37.7 ± 22.3, C: 13.8 ± 7.1, D: 31.2 ± 7.2 vs. Con: 57.2 ± 15.1 mlO_2/l blood, P < 0.01 for all). With the initiation of exercise, (a-v)DO_2 increased rapidly and by 30s there were no differences between Heavy A, B and D and Con. Only prior occlusion resulted in a lower (a-v)DO_2 through the first 90 s of exercise in Heavy C compared to Con (Fig. 3.4). There was an overshoot response in (a-v)DO_2 during CON and Heavy A bouts with a significantly greater value at 90s than end exercise (P = 0.05). This overshoot response was not present following the prior conditions that included occlusion.

Muscle oxygen uptake

Immediately prior to the start of exercise $\dot{V}O_{2mus}$ tended to be greater in the conditions that included prior exercise but individual variability precluded significance (Con: 3.3 ± 1.3, A: 6.1 ± 2.1, B: 7.5 ± 4.8, C: 2.0 ± 1.4, D: 6.3 ± 2.6 ml/min, P range: 0.16 - 0.66). Within the first minutes of exercise, $\dot{V}O_{2mus}$ was higher in Heavy A and Heavy B compared to Con, however prior occlusion depressed the $\dot{V}O_{2mus}$ response in Heavy C (Fig.3.5). By the end of exercise, $\dot{V}O_{2mus}$ was not different between Heavy B, Heavy C and Heavy D and Con. However, Heavy A was slightly higher than Con and Heavy C (P = 0.04). Prior heavy exercise or occlusion plus exercise resulted in faster rise time for $\dot{V}O_{2mus}$ in Heavy A, B and D compared to Con (Heavy A: 41.6 ± 11.5, B: 40.7 ± 10.5, D: 43.6 ± 13.4 s vs. Con: 58.6 ± 14.3 s, P < 0.05). Prior occlusion resulted in a slower $\dot{V}O_{2mus}$ kinetics in Heavy C compared to all other conditions (Heavy C: 95.9 ± 28.9 s, P < 0.01). Over

all the heavy bouts (Con, Heavy A, B, C, D), there was a positive correlation between the rise time of FBF and $\dot{V}O_{2mus}$ (Fig.3.6; $r^2 = 0.75$, $P < 0.001$).

Mean arterial pressure (MAP)

The extent of MAP elevation at the start of exercise was dependent on the priming condition. In Heavy A, 6 min resting was able to recover MAP to prior Con level (Con: 88.9 ± 5.8 vs. Heavy A: 91.2 ± 4.8 mmHg, $P > 0.05$). With ischemic loading during the priming condition, MAP was elevated at the start of subsequent heavy exercise compared to Con (Heavy B: 99.3 ± 6.2, C 100.8 ± 8.3, D: 101.0 ± 13.8 mmHg, $P < 0.01$ for all compared to Con). Throughout all testing bouts (Heavy A-D) MAP was significantly higher than Con; by the end of exercise MAP was (Heavy A: 119.7 ± 12.9, B: 124.7 ± 8.6, C: 123.5 ± 9.7, D: 126.9 ± 10.2 vs. Con: 112.5 ± 11.1 mmHg, $P < 0.01$ for all) (Fig.3.7).

Lactate

The baseline [La^-] immediately prior to exercise was higher in the heavy exercise bouts Heavy A: 3.0 ± 1.0, Heavy B: 3.6 ± 0.7 and Heavy D: 3.4 ± 0.9 than CON: 1.3 ± 0.4 and Heavy C: 1.5 ± 0.3 mmol/l; $P < 0.01$ for all. At the end of exercise, there were no significant differences between all the heavy bouts (Con: 4.4 ± 1.3, A: 4.3 ± 1.5, B: 4.6 ± 1.3, C: 4.5 ± 1.1 and D: 4.2 ± 1.7 mmol/l; $P > 0.05$).

Table 3.1: Baselines values in the four testing days

Parameters	Heavy A	Heavy B	Heavy C	Heavy D	*P* Values
HR, bpm	61.4 ± 3.7	65.4 ± 9.2	60.5 ± 9.2	61.9 ± 8.1	0.10 – 0.86
MAP, mmHg	89.1 ± 5.6	91.1 ± 6.1	93.0 ± 6.6	92.0 ± 7.0	0.09 – 0.65
$\dot{V}O_{2mus}$ **, ml/min**	3.2 ± 1.3	3.1 ± 1.8	3.0 ± 0.9	3.1 ± 1.4	0.70 – 0.96
FBF, ml/min	57.4 ± 16.6	48.1 ± 18.3	50.3 ± 17.4	47.8 ± 21.3	0.11 – 0.96
(A-v)DO_2, ml/l	56.4 ± 15.6	62.8 ± 18.3	61.6 ± 11.3	65.3 ± 13.5	0.16 – 0.84
[La^-], m.mol/l	1.3 ± 0.4	1.3 ± 0.3	1.2 ± 0.4	1.3 ± 0.4	0.36 – 1.00

Mean ± SD, n = 10

P values are the range of the contrasts between the protocols A, B, C and D.

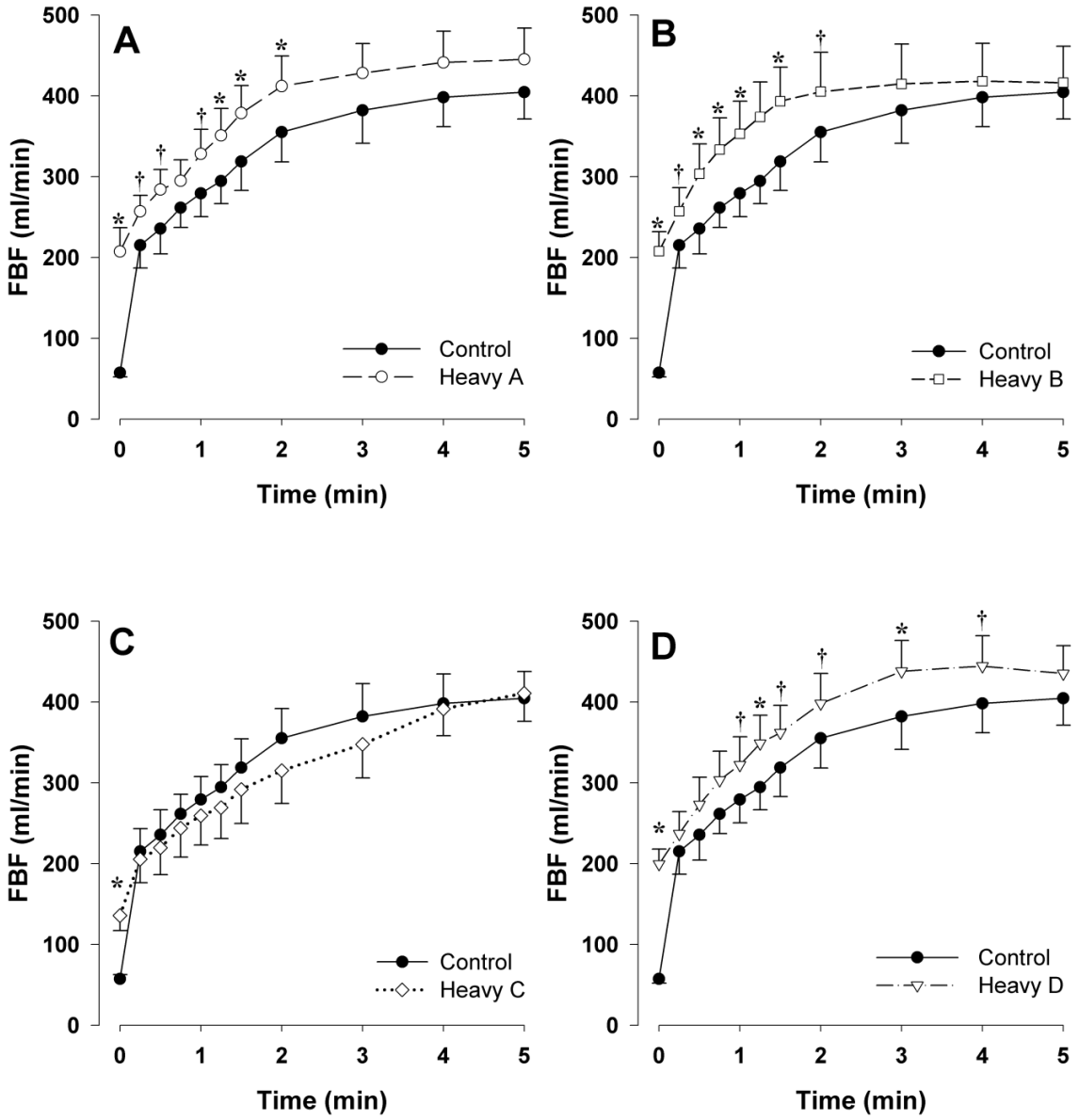

Figure 3.3: FBF responses during exercise in the four Heavy bouts (Heavy A-D) compared to the Control.

All priming conditions resulted in higher FBF at the start of subsequent heavy exercise (bouts Heavy A-D) compared to Con, however by the end of exercise there were no differences. During exercise, Heavy A, B, and D all showed higher FBF compared to the Con. Data points are the average responses of 10 subjects. (Means ± SE) * $P < 0.01$, † $P < 0.05$.

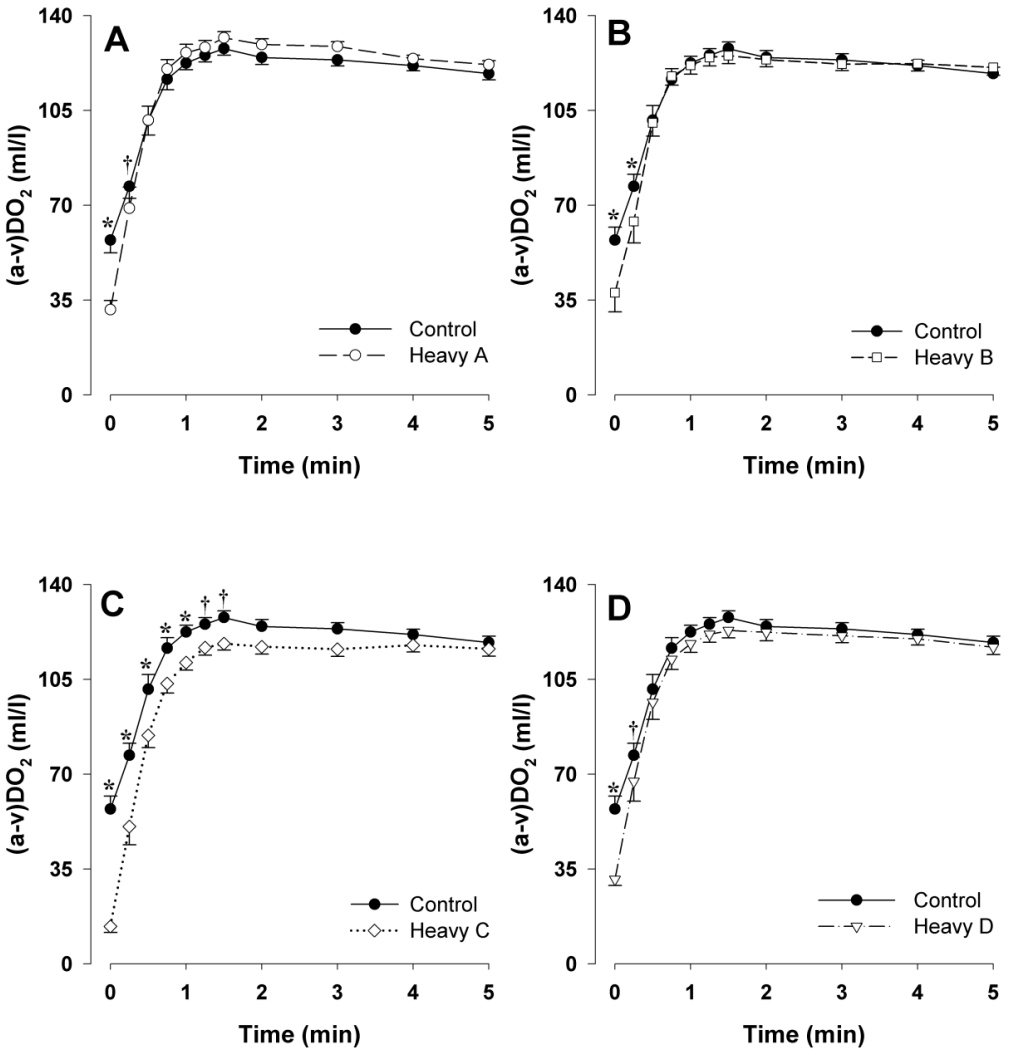

Figure 3.4: (A-v)DO_2 responses during exercise in the four Heavy bouts (A-D) compared to the Control.

All priming conditions resulted in lower (a-v)DO_2 at the start of the subsequent heavy exercise (bouts Heavy A-D) compared to the Con. By 45 s of exercise there were no significant differences in (a-v)DO_2 between Con and Heavy A, B, and D. However (a-v)DO_2 was lower up to 90 s of exercise in Heavy C compared to Con. There was an overshoot of (a-v)DO_2 at 90 s of exercise in Con and Heavy A compared to end exercise. Data points are the average responses of 10 subjects. (Means ± SE) * P <0.01, † P < 0.05.

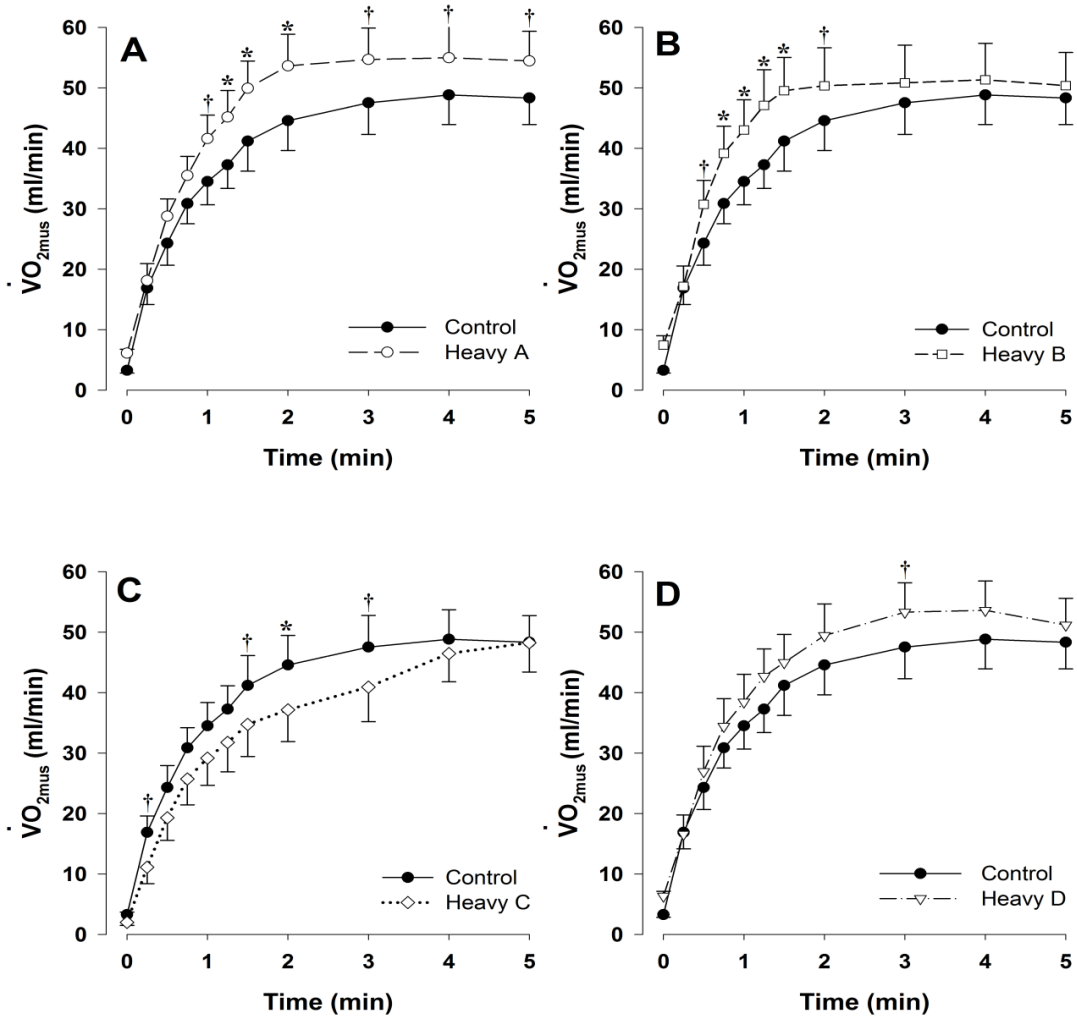

Figure 3.5: $\dot{V}O_{2mus}$ responses during exercise in the four Heavy bouts (A-D) compared to the Control.

At the start of exercise, $\dot{V}O_{2mus}$ in all conditions was not different compared to the Con. $\dot{V}O_{2mus}$ in Heavy A was significantly higher than Con from the 1st minute through the end of exercise. $\dot{V}O_{2mus}$ in Heavy B was significantly higher during exercise onset (from 30 s through 2 minutes). $\dot{V}O_{2mus}$ in Heavy C was depressed during exercise onset, but reached the same level as Con by the 4th minute of exercise. Heavy D was not different from the Con condition. Data points are the average of 10 subjects. (Means ± SE) * P <0.01, † P < 0.05.

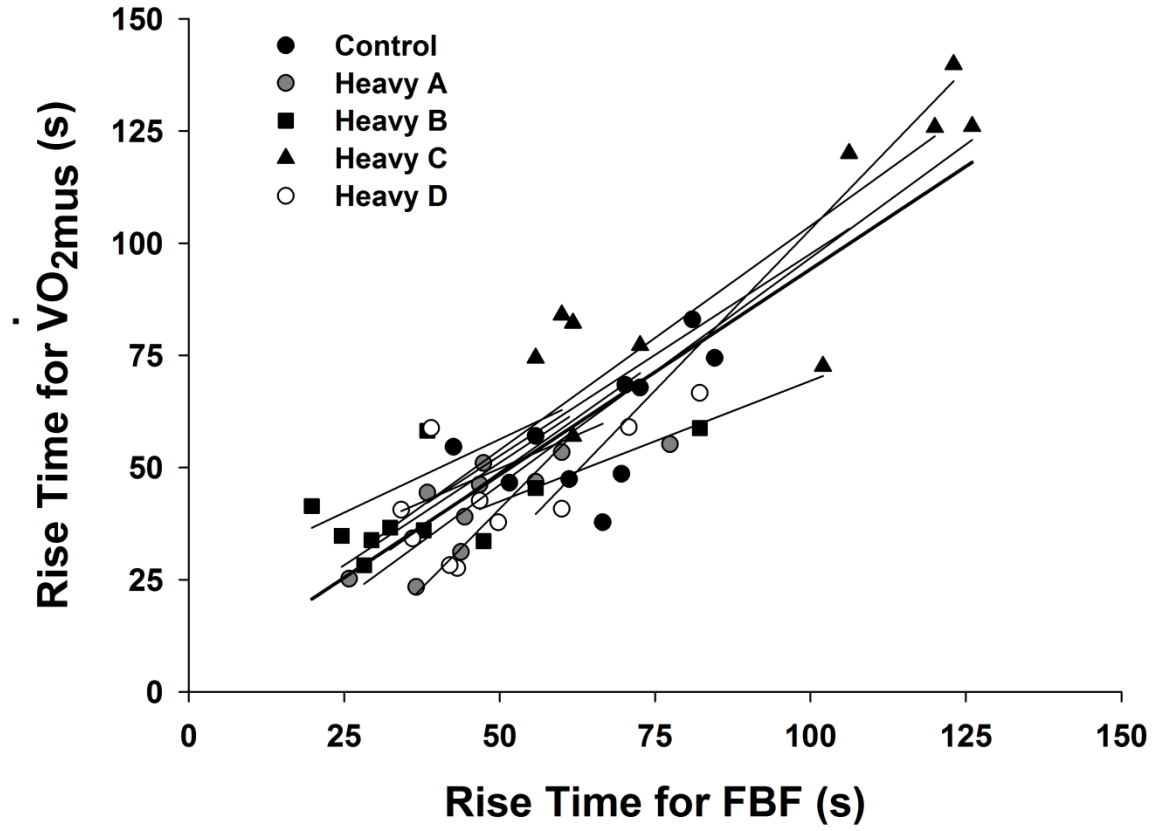

Figure 3.6: Rise time correlation for $\dot{V}O_{2mus}$ and FBF.

Across all conditions, the rise time (τ) for $\dot{V}O_{2mus}$ was significantly correlated to the rise time (τ) for FBF ($\tau \dot{V}O_{2mus}$ = 2.5 + 0.92 * τ FBF; r^2 = 0.75, P < 0.001).

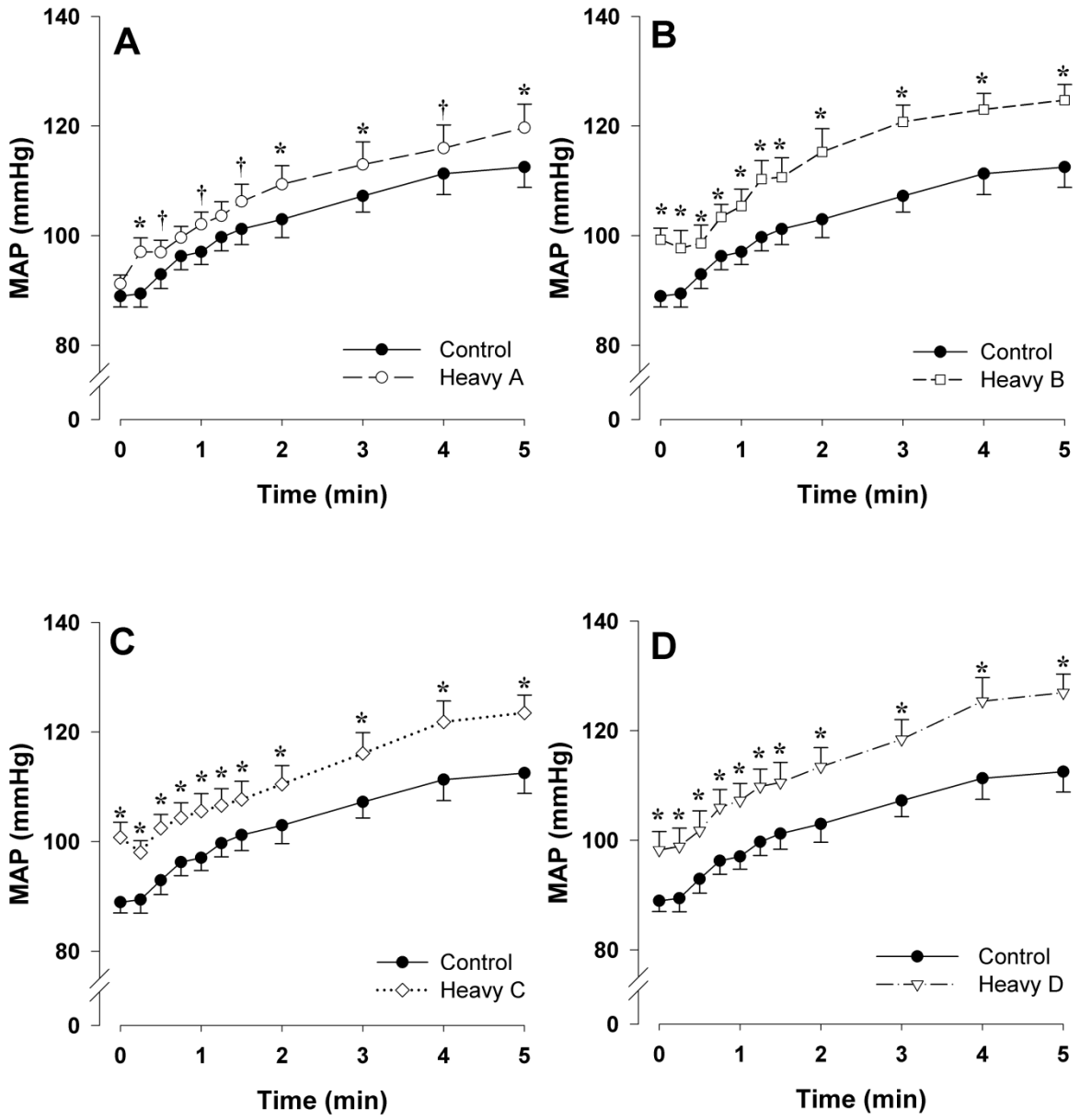

Figure 3.7: MAP responses during exercise in the four Heavy bouts (A-D) compared to the Control.

MAP was significantly higher in all Heavy conditions, both prior to and throughout exercise, except for the baseline of Heavy A. Data points are the average responses of 9 subjects. (Means ± SE) * P <0.01, † P < 0.05.

3.4.2 Group B

Post occlusion responses with ibuprofen and placebo

Following 15 min of occlusion, ibuprofen resulted in a significantly higher FBF between 30 and 60s of hyperemia compared to placebo (Fig.3.8 A). At 30 and 60 s, FBF with IB were 621.34 ± 169.55, 539.58 ± 157.07 vs. PL 545.04 ± 178.26, 440.77 ± 160.38 ml/min, respectively, $P < 0.05$). No differences were seen between the effect of IB and PL on (a-v)DO_2 during post occlusion period. Although there were significant differences in FBF between the 30 and 60 s, the slightly lower (a-v)DO_2 in IB at these time points negate the ability to detect any significant differences in $\dot{V}O_{2mus}$ (Fig. 3.8 B and C). The effect of IB on MAP was maintained lower following release of the occlusion cuff. MAP was significantly lower at all time points of hyperemia compared to PL (Fig.3.9 A). After cuff release MAP with IB was 91.86 ± 4.18 vs. PL 97.49 ± 4.64 mmHg, $P < 0.05$. By 3 min of hyperemia, MAP with IB was 93.88 ± 3.49 vs. 99.83 ± 5.32 mmHg, $P < 0.05$. As a result of higher FBF and lower MAP with ibuprofen, FVC was significantly elevated from cuff release through to 90s of the reactive hyperemia with IB (Fig.3.9 B). At 0 and 90 s of hyperemia, FVC with ibuprofen were 6.07 ± 1.36, 3.96 ± 1.12 vs. placebo 5.09 ± 1.21, 3.03 ± 1.07 ml/min/mmHg, respectively, $P < 0.05$). Over the 3 min of reactive hyperemia, there were a significantly greater FBF and FVC areas with IB compared to PL (FBF: 1160.58 ± 289.69 vs. 998.55 ± 284.66 ml/min; FVC: 12.71 ± 3.18 vs. 10.49 ± 3.23 ml/min/mmHg, $P < 0.05$) but no changes in $\dot{V}O_{2mus}$ were shown.

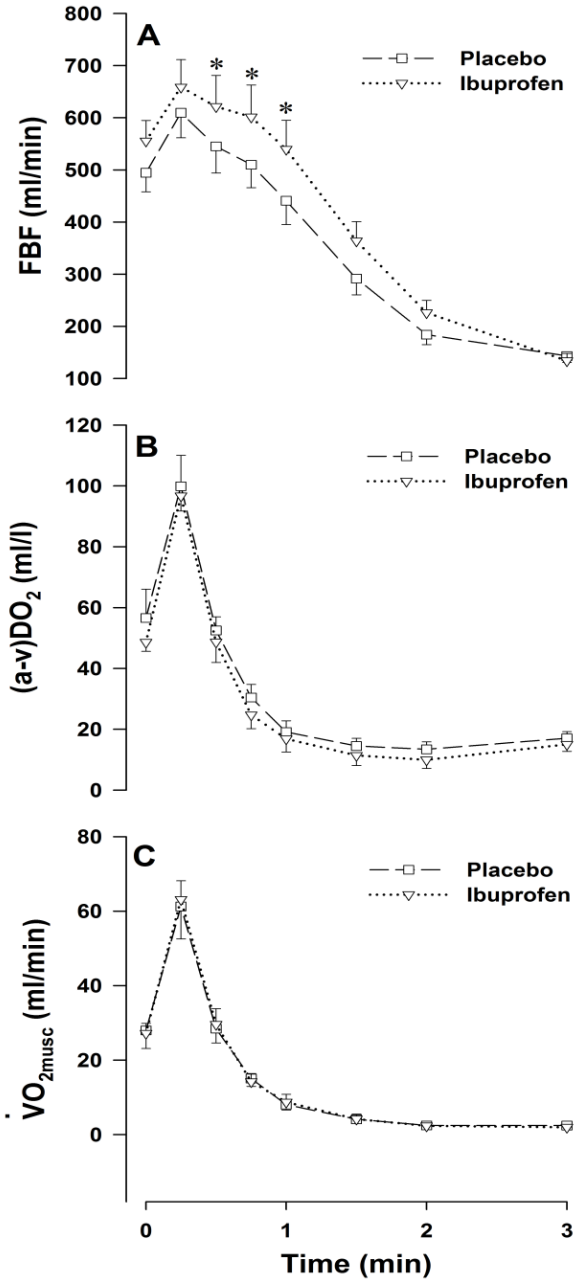

Figure 3.8: FBF (A), (a-v)DO_2 (B) and $\dot{V}O_{2mus}$ (C) responses during the reactive hyperemia in placebo and ibuprofen trials.

FBF was significantly higher from 30 s to 60 s in ibuprofen trial compared to placebo, but there were no differences between ibuprofen and placebo trials in (a-v)DO_2 or $\dot{V}O_{2mus}$ throughout the 3 min that followed prior occlusion. Data points are the average responses of 8 subjects. (Means ± SE) * P <0.01.

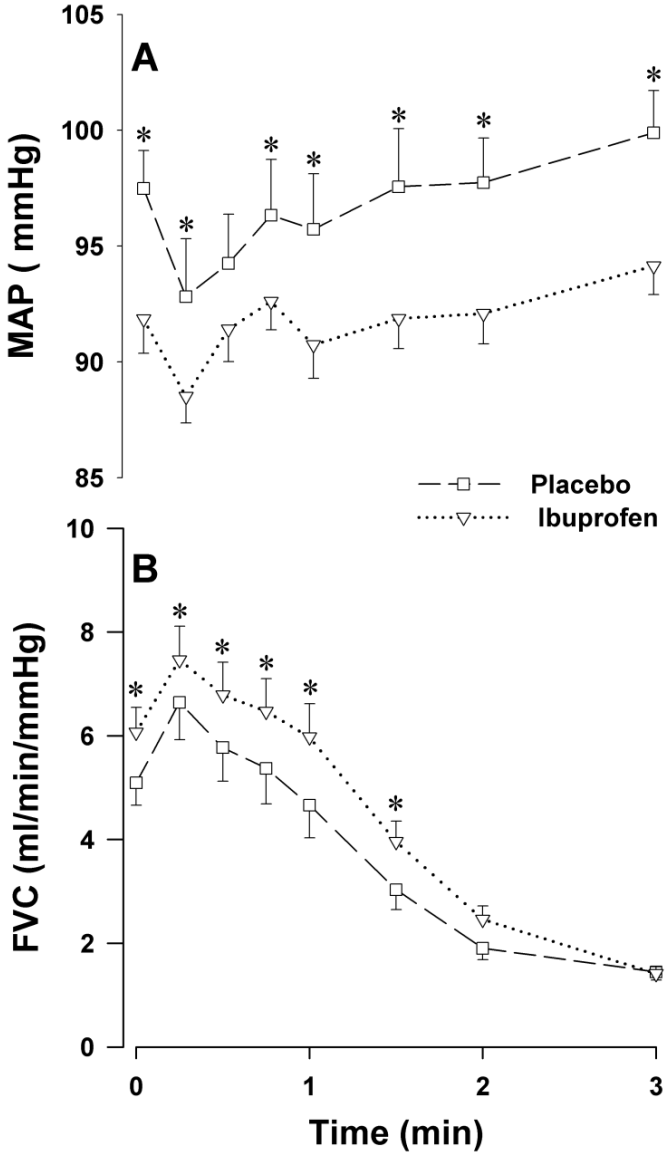

Figure 3.9: MAP (A) and FVC (B) responses during the reactive hyperemia in placebo and ibuprofen trials.

MAP was significantly higher throughout the 3 min of recovery following occlusion in the placebo trial compared to ibuprofen trial. FVC was significantly higher within the first 90 s following occlusion in the ibuprofen trial than placebo. Data points are the average responses of 8 subjects. (Means ± SE) * P <0.01.

Exercise responses

Priming occlusion with IB or PL resulted in a significantly higher FBF at the start of subsequent heavy exercise bouts compared to Control (IB: 134.05 ± 39.03, PL: 143.17 ± 39.82 vs. Control: 54.82 ± 11.31 ml/min, $P < 0.01$ for both) (Fig.3.10 A). During exercise, FBF response in Control was in between the IB and PL curves and was not different from either of them. However, FBF with IB was significantly higher through 1 to 3 min of exercise compared to PL. At 1 and 3 min of exercise, FBF with IB were 378.96 ± 96.84, 479.70 ± 103.60 vs. PL 312.16 ± 88.46, 425.82 ± 98.36 ml/min, $P < 0.05$.

Priming occlusion with IB or PL elicited a significantly lower (a-v)DO_2 compared to control at the start of exercise (IB: 15.04 ± 6.48, PL: 17.01 ± 6.21.vs. Con: 51.30 ± 10.39 mlO_2/l blood, $P < 0.01$) as well through the first 3 min of subsequent heavy exercise bouts (at 3 min; IB: 105.38 ± 6.87, PL 106.24 ± 8.19 vs. Control: 115.98 ± 5.14 mlO_2/l blood, $P < 0.01$ for all points) (Fig.3.10 B). There were no differences between IB and PL at any time point. There was an overshoot response in (a-v)DO_2 during the Control bout with a significant difference between 90s and end exercise ($P = 0.05$). This overshoot response was not present following occlusion.

$\dot{V}O_{2mus}$ was calculated from FBF and (a-v)DO_2. Only prior occlusion with PL resulted in a depressed $\dot{V}O_{2mus}$ from 15s through 3 min of exercise compared to control (Fig 3.10 C).

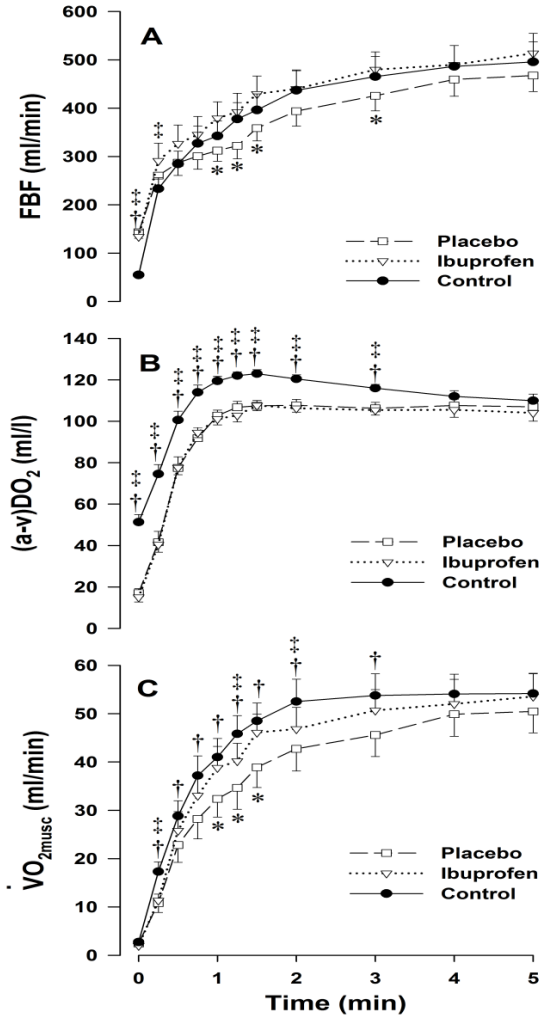

Figure 3.10: FBF (A), (a-v)DO2 (B) and $\dot{V}O_{2mus}$ (C) responses during exercise in placebo and ibuprofen trials compared to control.

Prior occlusion with or without ibuprofen resulted in higher FBF and lower (a-v)DO2 at the start of the subsequent heavy exercise (Placebo and Ibuprofen) compared to Control. During exercise in the placebo condition FBF, (a-v)DO2 and $\dot{V}O_{2mus}$ responses were lower than Control up to 3 min, while with ibuprofen (a-v)DO2 was lower than control through the first 3 min with no differences in $\dot{V}O_{2mus}$. Data points are the average responses of 8 subjects. (Means ± SE) * P <0.01, † $P < 0.05$ (Placebo vs. Control), ‡ $P < 0.05$ (Ibuprofen vs. Control).

At 15 s and 3 min of exercise, $\dot{V}O_{2mus}$ following occlusion with PL was 10.80 ± 5.49, 45.58 ± 12.54 vs. Con: 17.31 ± 5.69, 53.77 ± 12.67 ml/min, respectively, $P < 0.05$). There were no differences between the 3 conditions in FBF, (a-v)DO_2 and $\dot{V}O_{2mus}$ by the end of exercise.

MAP was significantly higher following the occlusion with PL compared to CON and occlusion with IB at the start of exercise (PL: 99.89 ± 5.17 vs. Con: 93.08 ± 8.44; IB: 94.14 ± 3.51, $P < 0.05$) . The changes over the course of exercise were similar between the 3 conditions; MAP with occlusion and PL was maintained at an elevated level compared to CON and IB at all the time points (Fig.3.11 A). FVC was calculated from FBF and MAP. FVC was lower through the exercise in PL compared to both CON and IB (Fig. 3.11 B).

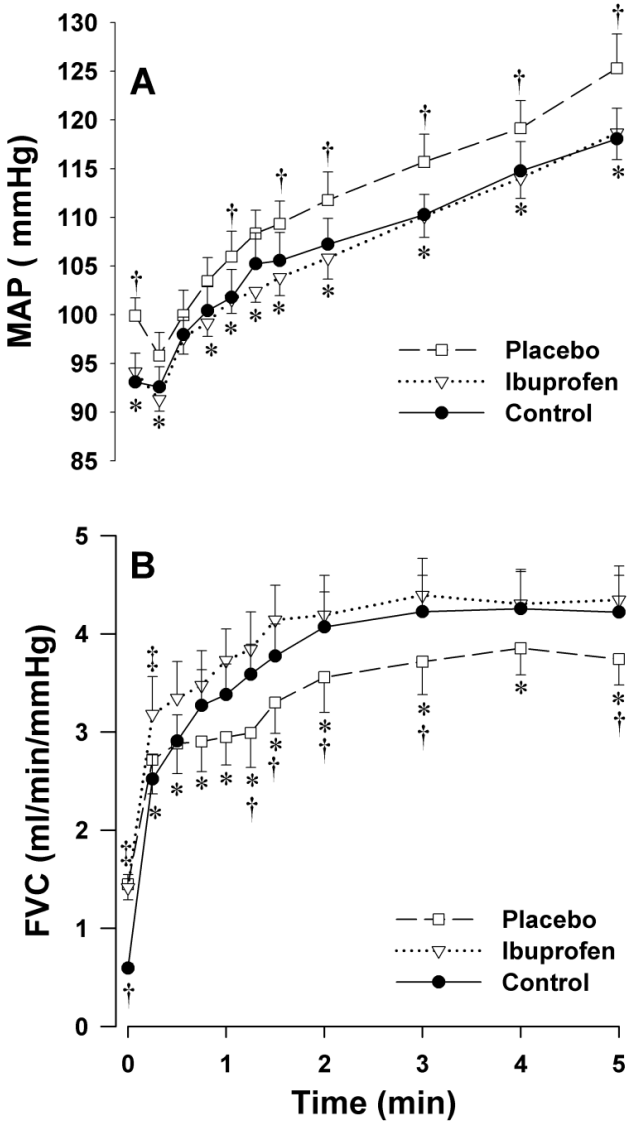

Figure 3.11: MAP (A) and FVC (B) responses during exercise in placebo and ibuprofen trials compared to control.

Prior occlusion without ibuprofen resulted in higher MAP and lower FVC throughout subsequent heavy exercise (Placebo) compared to Control and Ibuprofen. Data points are the average responses of 8 subjects. (Means ± SE) * P <0.01, † P < 0.05 (Placebo vs. Control), ‡ P < 0.05 (Ibuprofen vs. Control.

3.4.3 Group C

There were no differences between the baseline SBF across the three testing days (Protocol A: 0.21± 0.11, Ibuprofen: 0.26 ± 0.11, Placebo: 0.27 ± 0.16 AU, $P > 0.05$). Relative SBF was slightly greater in placebo compared to ibuprofen in both reactive hyperemia and during the exercise; this was in contrast to the total blood flow response during exercise seen with groups B. Prior occlusion elevated SBF in placebo with peak values of 6.5 fold vs. 5.5 in ibuprofen ($P = 0.22$), that decreased in placebo to approximately 3.5 fold vs. 2.5 fold in ibuprofen ($P = 0.04$) at the onset of subsequent exercise (Fig. 3.12). With the onset of exercise, SBF increased slightly to higher level in placebo (5 folds) compared to control, Heavy A and Ibuprofen (4, 3.5 and 3.5 folds, respectively; P > 0.05 for all, Fig. 3.12).

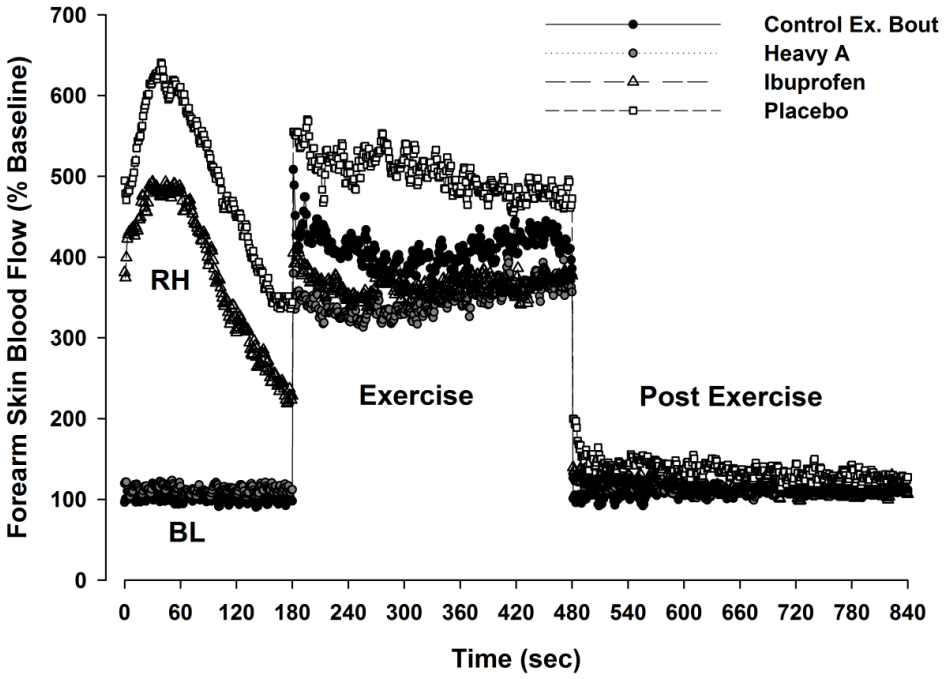

Figure 3.12: SBF responses during reactive hyperemia (RH), exercise and recovery in placebo and ibuprofen, Heavy A trials compared to control.

3.5 Discussion

Our results provided a unique observation that the $\dot{V}O_{2mus}$ response at the onset of heavy forearm exercise was depressed when exercise began 3 minutes after 15 minutes of forearm circulatory occlusion even though FBF was elevated at the start of exercise. The slower $\dot{V}O_{2mus}$ kinetics response in Heavy C was due to no improvement in FBF above the control condition once exercise commenced and smaller (a-v)DO_2. These differences were even more pronounced when contrasted with protocols that included prior exercise with or without additional circulatory occlusion, where FBF and $\dot{V}O_{2mus}$ kinetics were faster than control. Thus, our hypothesis that FBF and $\dot{V}O_{2mus}$ kinetics would be enhanced in a heavy bout of forearm hand-grip exercise that followed heavy exercise and/or occlusion was only partially supported. All priming conditions achieved elevated FBF immediately before the start of the subsequent heavy exercise bouts, but FBF and $\dot{V}O_{2mus}$ kinetics were enhanced only when the prior conditions included exercise. Our results contrast with other research that measured pulmonary $\dot{V}O_2$ during exercise that followed circulatory occlusion (Paganelli *et al.*, 1989; Walsh *et al.*, 2002). Overall though, our results demonstrated a strong linear relationship between the delivery of O_2 as reflected by the kinetics of FBF and the rate of increase in oxidative metabolism reflected by the kinetics of $\dot{V}O_{2mus}$ at the onset of heavy forearm exercise.

3.5.1 Methodological considerations

The primary objective of the current research was complement previous research that employed prior exercise by utilizing occlusion as a means to elevate

muscle blood flow prior to the onset of exercise. We selected 15 minutes prior circulatory occlusion with 3 minutes recovery period to achieve a relatively stable, elevated FBF similar to that shown with studies of prior exercise (Fukuba *et al.*, 2007; MacDonald *et al.*, 2001b). Shorter periods of occlusion caused the hyperemia to decline more rapidly toward baseline (Carlsson *et al.*, 1987). Previous research that examined muscle oxygenation and/or PCr concentrations during various combinations of occlusion and exercise provides information on the probable metabolic state of the muscle at the onset of exercise in our occlusion only protocol. During occlusion of the resting forearm muscle, O_2 stores were depleted within 5-6 minutes (Blei *et al.*, 1993; Boushel *et al.*, 1998) then increased above the resting state within 1 minute of reactive hyperemia, and returned to baseline level 3 minutes after cuff release (Hampson & Piantadosi, 1988). Muscle phosphate to PCr ratio was only slightly changed after 10 minutes occlusion and recovered to baseline within 2 minutes (Boushel *et al.*, 1998). PCr concentration was depleted less than 15% by 15 minutes of occlusion and, based on the measured time constant, would be expected to be within 5% of baseline with 3 minutes of recovery (Blei *et al.*, 1993). The rather modest changes in PCr after occlusion contrast for example with the 46% reduction with 3 minutes of repeated hand-grip contractions with intact circulation (Mole *et al.*, 1985). From these data we expect muscle O_2 stores to be no lower than baseline and PCr stores, as an indication of enzyme activity, within 5% of baseline. These conditions would allow us to achieve our objective of a high flow state with minimal metabolic disturbance at the onset of the heavy forearm exercise in protocol Heavy C.

The model of prior 5 minutes heavy exercise with 6 minutes recovery (Heavy A) is similar to that used in previous studies of prior exercise and was expected to

influence both blood flow and metabolic state (Gurd *et al.*, 2006; MacDonald *et al.*, 2001b; Rossiter *et al.*, 2001).

In Heavy B protocol, the priming heavy exercise bout was followed immediately by 2 minutes of circulatory occlusion to determine if the occlusion period modified the response to prior heavy exercise. The protocol of Heavy D incorporated the prior moderate exercise within the period of circulatory occlusion. It has been demonstrated that rapid depletion of PCr occurred when exercise was performed within occlusion and that recovery did not occur until circulation was restored (Blei *et al.*, 1993). Performing 1 minute of moderate exercise during the 5 minutes occlusion would be expected to elevate FBF through the 3 minutes after release of occlusion while inducing a relatively mild change in the metabolic environment that would have partially recovered by the start of the heavy exercise.

The kinetic response characteristics in the current study were quantified by calculation of the rise time (τ) from the transient responses of FBF and $\dot{V}O_{2mus}$. This calculation was based on the property of a linear first order system in which the time constant can be determined from the rate of increase of a variable toward the plateau value (Barstow & Mole, 1991). When calculated in this way the rise time is equivalent to the mean response time reported in other studies (Hughson *et al.*, 1996; MacDonald *et al.*, 1997) and includes the possibility of the appearance of a "slow component" with heavier exercise. A precise kinetics with multi-component exponential modeling is not possible in studies such as this without a marked increase in blood sampling frequency. Therefore, the rise time provides an estimate of the overall kinetics response and permits comparisons between FBF and $\dot{V}O_{2mus}$.

Brachial artery blood velocity and diameter were measured with the occlusion cuff placed distal to the elbow to avoid the potential influence of ischemia (Ramsey *et al.*, 1996) or myogenic dilation (Folkow, 1949) on arterial diameter. Proximal cuff placement has been shown to induce greater brachial diameter dilation than distal occlusion (Agewall *et al.*, 2001; Betik *et al.*, 2004; Guthikonda *et al.*, 2007). Further, distal occlusion provides a more accurate assessment of endothelial function compared to proximal occlusion (Guthikonda *et al.*, 2007; Peretz *et al.*, 2007).

3.5.2 Prior heavy exercise

The finding of faster FBF and $\dot{V}O_{2mus}$ kinetics in Heavy A than Con condition (group A) confirms previous research with both hand-grip (MacDonald *et al.*, 2001b) and knee-extension (Fukuba *et al.*, 2007; Hughson *et al.*, 2003; Krustrup *et al.*, 2001) exercise. However, some studies of knee-extension exercise have shown faster pulmonary $\dot{V}O_2$ kinetics without a change in leg blood flow kinetics (Endo *et al.*, 2005; Fukuba *et al.*, 2004; Koga *et al.*, 2005). A combination of several interacting mechanisms was likely responsible for the observed higher FBF and $\dot{V}O_{2mus}$ in Heavy A and Heavy B protocols (Figs. 3.3 and 3.5). Ferreira *et al.* (2005a) have shown a tight coupling between the kinetics of capillary blood flow estimated from near-infrared spectroscopy (NIRS) and $\dot{V}O_{2mus}$ during both moderate and heavy cycling exercise.

Improved O_2 delivery might have been achieved by accumulation of vasoactive metabolites from previous warm-up that enhances vasodilation increasing blood flow to exercising muscles and possibly promoting a right shift of the HbO_2 dissociation curve (Gerbino *et al.*, 1996; MacDonald *et al.*, 1997).

Prior warm-up exercise might reduce or abolish regional heterogeneities in muscle microvascular perfusion optimizing the match between O_2 delivery to O_2 utilization (DeLorey *et al.*, 2007; Fukuba *et al.*, 2002). Greater O_2 delivery can improves the oxidative phosphorylation contribution to energy production, while reducing the O_2 deficit and cellular homeostasis disturbance. The proposed mechanism involves an increase in the intracellular PO_2 (Hughson *et al.*, 2001; Tschakovsky & Hughson, 1999) and a subsequent reduction in PCr degradation and substrate level phosphorylation (Rossiter *et al.*, 2001).

The (a-v)DO_2 was significantly smaller at the start of exercise and at the first 15 s sample point in Heavy A and Heavy B than the Con condition, while there were no differences in (a-v)DO_2 after this time point. These results are in contrast to the findings of higher O_2 extraction during the second heavy bout of hand-grip (MacDonald *et al.*, 2001b) or leg extension (Krustrup *et al.*, 2001) exercise. The discrepancy may be due to the differences in subject characteristics. The present study examined highly trained varsity athletes who likely have higher O_2 extraction fraction during exercise (Kalliokoski *et al.*, 2001) that might not be further enhanced by a prior warm-up. Further, it has been reported that enhanced PDHa activity by prior heavy exercise can affect O_2 extraction during moderate (Gurd *et al.*, 2006) but not heavy (Bangsbo *et al.*, 2002) exercise. Thus, the faster $\dot{V}O_{2mus}$ kinetics in the prior heavy exercise conditions was attributable to the faster increase in FBF that facilitated greater O_2 delivery to the exercising forearm muscles. The patterns of FBF, (a-v)DO_2 and $\dot{V}O_{2mus}$ responses were similar in Heavy A and Heavy B bouts, with the small exception that the $\dot{V}O_{2mus}$ was significantly elevated above Con from 3-5 minutes only in Heavy A.

3.5.3 Prior ischemia

Previous research that employed circulatory occlusion prior to the start of exercise suggested that pulmonary $\dot{V}O_2$ kinetics were faster after the occlusion (Paganelli *et al.*, 1989; Walsh *et al.*, 2002). These results contrast with our findings of slower $\dot{V}O_{2mus}$ kinetics due to slower increase in FBF and smaller (a-v)DO_2 in the occlusion only condition (Heavy C), but could be explained by major differences in methodology. In the study by Walsh *et al.* (2002) the circulatory occlusion was released within 5 s prior to the onset of subsequent exercise and this condition resulted in a faster increase in $\dot{V}O_2$ than the Con condition. However, the authors were unable to evaluate the contribution of $\dot{V}O_2$ for the restoration of O_2 stores in blood and tissues compared to $\dot{V}O_2$ for oxidative phosphorylation. When both arms were occluded for 5-10 minutes prior to heavy arm-cranking exercise in the study by Paganelli *et al.* (1989) there was a small decrease (from 53 to 45 s) in the half time of $\dot{V}O_2$ on-response kinetics. Our observation that 15 minutes circulatory occlusion followed by 3 minutes recovery period significantly altered FBF and metabolic responses to subsequent heavy exercise (Heavy C) was unexpected and contrasted with our hypothesis that prior occlusion would facilitate a more rapid increase in FBF, O_2 delivery and $\dot{V}O_{2mus}$ at the onset of heavy forearm exercise.

The mechanisms responsible for the ischemic effects on muscle blood flow and metabolic responses are unknown. It appears that some factor associated with prolonged ischemia impaired FBF response at the onset of subsequent exercise. Previously, Naylor *et al.* (Naylor *et al.*, 1999) noted a 35% reduction in peak reactive

hyperemia after 5 minutes circulatory occlusion that included heavy exercise in a control (placebo) condition compared to similar occlusion with the PGs synthesis inhibitors ibuprofen and indomethacin. These authors speculated that the smaller reactive hyperemia in the placebo condition might have been due to the release of vasoconstrictor PGs or activation of platelet aggregation during the ischemic period. Interestingly, Shoemaker *et al.* (1996b) reported that PGs play no role in regulating FBF during forearm exercise, thus suggesting a unique ischemic effect on FBF control during a subsequent exercise.

To verify this surprising finding of slower $\dot{V}O_{2mus}$ during the transition phase in Heavy C, and in an attempt to determine a possible mechanism for reduced FBF, group B completed 2 trials of protocol C after ingesting ibuprofen or placebo. The responses of slower $\dot{V}O_{2mus}$ and reduced (a-v)DO_2 observed in Heavy C with group A were mirrored in the placebo trial in group B, whereas ibuprofen was able to restore $\dot{V}O_{2mus}$ to the control level primarily as a consequence of improved FBF with no difference in (a-v)DO_2 between placebo and ibuprofen, suggesting that altered O_2 extraction might be independent of a prostaglandin mechanism (Fig.3.10). In addition during the ibuprofen trial, the MAP response was identical to control while it was elevated with placebo in group B (Fig. 3.11) as seen in protocol C with group A (Fig. 3.9). These results suggested that 15 minutes of ischemia may activate a prostaglandin-mediated mechanism that had both local and systemic effects such as the observed release of thromboxane A_2 following 10 minutes of forearm circulatory occlusion (Mathieson *et al.*, 1983) causing some contraction of the vascular smooth muscle which was then exaggerated at the

onset of the subsequent heavy exercise 3 minutes after the release of occlusion, reducing FBF.

The selective perfusion of active muscle fibers is dependent upon several factors that constrict blood vessels to non-active regions and dilate vessels to active regions(Rowell, 1993). As reactive hyperaemia following ischemia includes blood flow to both the skin and muscle, it was important to consider how these two components might contribute to the total flow and O_2 extraction that we measured with and without inhibition of PGs in another group of subjects (group C). The oral administration of aspirin, COX (PGs) inhibitor, has markedly reduced SBF during reactive hyperemia (Binggeli *et al.*, 2003), suggesting that short period of occlusion might result in redistributions of blood to the periphery ~~al~~ and by consequence reducing FBF in subsequent exercise bout. However, local infusion of ketorolac, similar non-specific COX inhibitor, has shown increase (Medow *et al.*, 2007) or no changes (Lorenzo & Minson, 2007; McCord *et al.*, 2006) in SBF during reactive hyperemia. Likewise the study of Binggeli *et al.* (2003), SBF slightly reduced during reactive hyperemia and the subsequent heavy exercise after the oral administration of high dose of ibuprofen compared to placebo (Fig. 3.12). Yet, FBF was higher throughout the reactive hyperemia and subsequent exercise with ibuprofen suggesting that ischemia might have disparate effects on different tissue perfusion.

Venous blood was collected from the deep antecubital vein with the catheter oriented in a retrograde fashion (Mottram, 1955). Previous anatomical studies combined with an investigation in which increased SBF was induced by remote body heating suggested that the SBF would have minimal impact on O_2 saturation in the deep vein (Roddie *et al.*, 1956).The fact that there are differences in SBF between the ibuprofen and placebo conditions (group C, Fig. 3.12), while there are

similar (a-v)DO_2 patterns (group B, Fig. 3.4) provide further evidence that SBF is not affecting the (a-v)DO_2 calculation in the current study. However, care should be applied in interpretation of reduced (a-v)DO_2 during reactive hyperemia and exercise after occlusion.

The period of reactive hyperemia is associated with increased vascular conductance with dilation of the resistance vessels in skeletal muscle. O_2 stores are rapidly replaced during the hyperemic period as indicated by NIRS (Boushel *et al.*, 1998; Hampson & Piantadosi, 1988) as well as the marked reduction in the (a-v)DO_2 prior to the onset of exercise in the current study. What was unexpected in the current study was the continued reduction in (a-v)DO_2 over the first 90 s of hand-grip exercise in Heavy C in group A(Fig. 3.4)and group B (Fig. 3.10). It is not known if this was a consequence of blood flow being diverted away from the active muscle fibers, possibly to the skin, or if there was an alteration in the metabolic pathways that delayed the adaptation of oxidative phosphorylation. There was no difference in the end exercise [La^-] as a consequence of the delayed FBF and $\dot{V}O_{2mus}$ response so these data do not contribute to understanding potential metabolic differences. Future research is required to establish the mechanism for the altered (a-v)DO_2 after 15 minutes circulatory occlusion.

In the Heavy D protocol, where prior 5 minutes circulatory occlusion combined with 1 minute of moderate exercise, we cannot provide information on the internal metabolic state of the muscle when the subsequent heavy exercise bout started 3 minutes after release of circulatory occlusion. However, FBF was elevated to approximately the same value as in Heavy A and Heavy B protocols and the (a-v)DO_2 was similarly reduced below the CON condition at the start of exercise

as the other exercise protocols. As well as, in Heavy D FBF and $\dot{V}O_{2mus}$ were accelerated to same extent as in Heavy A and Heavy B protocols. The decrease in the rise time of $\dot{V}O_{2mus}$ in Heavy D is in agreement with Paganelli *et al.*(1989) who reported 50% reduction in the half response time of pulmonary $\dot{V}O_2$ kinetics during the heavy arm-cranking exercise bout after prior 3 minutes of circulatory occlusion combined with moderate arm-cranking exercise.

3.6 Limitations

Forearm $\dot{V}O_{2mus}$ was estimated from the Fick equation as the product of FBF and (a-v)DO_2. Venous blood was sampled from a deep forearm vein; however we were not able to measure arterial oxygen content to directly determine (a-v)DO_2. We assumed constant arterial O_2 content during steady state forearm exercise, as shown in both moderate (MacDonald *et al.*, 2000) and intense knee-extension exercise (Bangsbo *et al.*, 2000). We used a retrograde venous catheter to maximize the collection of blood from the confluence of venous drainage from the deep veins of the forearm extensor muscles; however, there is a possibility of contamination from other vascular beds (Corcondilas *et al.*, 1964). Our results show a similar pattern of change in the (a-v)DO_2 as seen in previous studies with directly measured arterial and venous O_2 content (Bangsbo *et al.*, 2002; Bangsbo *et al.*, 2000; Grassi *et al.*, 1996), or from venous blood samples with the assumption of constant arterial O_2 saturation (Hughson *et al.*, 1996; van Beekvelt *et al.*, 2001).

A limitation in all studies examining O_2 delivery and O_2 utilization is that there are no precise measurements of microvascular perfusion within the complex geometry of human muscle recruitment during voluntary exercise and the influence of the local environment on the HbO_2 dissociation curve can only be

speculated. Even with direct measures of muscle blood flow it is not feasible to determine whether perfusion and O_2 extraction are matched within the metabolically active regions of the muscles (Grassi *et al.*, 1996; Hughson *et al.*, 1996; MacDonald *et al.*, 2001b). The selective perfusion of active muscle fibers is dependent upon several factors that constrict blood vessels to non-active regions and dilate vessels to active regions (Rowell, 1993). Within the active muscle, microvascular perfusion related to local vasodilation and muscle O_2 consumption appears to follow complex dynamics (Iversen & Nicolaysen, 1989) as supported by recent data from multiple sites with near infrared spectroscopy (Koga *et al.*, 2007). However, during high intensity exercise, as was employed in the present study, there is relatively homogeneous recruitment of muscle fibers (Krustrup *et al.*, 2008), thus venous blood sampling is likely representative of O_2 utilization in the working muscles.

3.7 Conclusion

We demonstrated differences between prior active warm-up with "heavy exercise" and "occlusion plus exercise" compared to passive warm-up with "occlusion" only on the dynamic adaptation of $\dot{V}O_{2mus}$ at the onset of heavy forearm exercise. Our findings exposed differences in the mechanisms regulating $\dot{V}O_{2mus}$ with the active warm-ups resulting in a higher FBF but no changes in O_2 extraction to yield the faster increase in $\dot{V}O_{2mus}$ at the onset of subsequent heavy forearm exercise bouts. In contrast, prior occlusion slightly retarded the increase in FBF and significantly reduced O_2 extraction thus delaying $\dot{V}O_{2mus}$ kinetics. Over all there was a strong correlation between the rates of increase in FBF and oxidative metabolism suggesting that the acceleration of $\dot{V}O_{2mus}$ kinetics at the onset of heavy forearm exercise is linked to O_2 delivery in trained young men. Prolonged ischemia invoked a prostaglandin-mediated mechanism that affected FBF and O_2 extraction delaying the adaptation of oxidative metabolism during subsequent heavy exercise; but, the reduction in FBF was reversed by inhibition of prostaglandin synthesis with ibuprofen.

Chapter 4

Priming exercise induced attenuation of the $\dot{V}O_2$ slow component during heavy exercise and increased oxygen cost during moderate exercise are associated with changes in muscle EMG activity.

4.1 Overview

The precise mechanisms for the development of the $\dot{V}O_2$ slow component during heavy exercise and the increase in O_2 cost during moderate exercise that follows heavy warm-up remain uncertain. We tested the hypothesis that changes in muscle activity are related to changes in slow component amplitude during heavy exercise and elevated steady state $\dot{V}O_2$ during moderate exercise following a heavy warm-up. Eight male endurance athletes performed two repetitions of two cycling protocols involving 6-min bouts of heavy and moderate intensity. $\dot{V}O_2$ was measured breath-by-breath and muscle activity was assessed by surface EMG. During heavy exercise, prior moderate and heavy exercise had a graded effect, attenuating the slow component amplitude by 19% and 40%, (prior moderate: 455 ± 52; prior heavy: 341 ± 54 vs. no warm-up: 564 ± 71 ml/min; $P < 0.01$ for both). Similarly, prior warm-up modified EMG activity between the 2nd and 6th min of exercise, shifting the increase in integrated EMG (iEMG) during control, to a smaller increase after a moderate bout and a decrease after heavy exercise. Principle components analysis showed a significant positive correlation between the slow component amplitude and the changes in iEMG of the knee extensor muscles (r = 0.45, $P = 0.03$). During moderate exercise, mean power frequency (MPF) was augmented by one or two prior heavy bouts, but no changes in iEMG were observed. The attenuation of slow component amplitude by moderate and heavy warm-up and the elevated moderate exercise steady state $\dot{V}O_2$ following a heavy warm-up appear to be related to some changes in surface EMG activity and this may be an indication of altered muscle fibre recruitment induced by the priming exercise.

4.2 Introduction

During constant-load heavy exercise above the ventilatory threshold (VT), pulmonary $\dot{V}O_2$ continues to rise above the fundamental exponential kinetic response with a superimposed delayed origin (≈ 90-150 s) termed the "slow component" (Whipp, 1994; Whipp & Wasserman, 1972). A large amount of the $\dot{V}O_2$ slow component can be attributed to the exercising muscles (Poole *et al.*, 1991; Rossiter *et al.*, 2002b), but the specifics of muscle activation patterns are still unclear. Several converging lines of evidence point to a progressive recruitment of muscle fibres potentially the less efficient type II muscle fibres as the principal mechanism by which O_2 consumption increases during the $\dot{V}O_2$ slow component. First, the amplitude of the $\dot{V}O_2$ slow component has been shown to be more pronounced in subjects with a high proportion of type II muscle fibres (Barstow *et al.*, 1996; Garland *et al.*, 2006; Pringle *et al.*, 2003). Also, Krustrup *et al.* (2004) have shown a marked depletion in the quadriceps muscle (mixed fibre type) PCr and glycogen from 3 to 6 minutes during heavy cycling exercise. Furthermore, investigations employing magnetic resonance imaging (MRI) have shown that changes in muscle recruitment are characteristic of the slow component (Endo *et al.*, 2007; Saunders *et al.*, 2000). However, studies using surface EMG are more varied and controversial (Bailey *et al.*, 2009b; Burnley *et al.*, 2002b; Cannon *et al.*, 2007; Garland *et al.*, 2006; Osborne & Schneider, 2006; Perrey *et al.*, 2001; Sabapathy *et al.*, 2005; Saunders *et al.*, 2000; Scheuermann *et al.*, 2001; Shinohara & Moritani, 1992).

Almost universally in previous research which applied a prior heavy warm-up, the amplitude of the $\dot{V}O_2$ slow component in the second heavy exercise bout was shown to be attenuated (Bailey *et al.*, 2009b; Burnley *et al.*, 2002b; Koppo & Bouckaert, 2000; Tordi *et al.*, 2003). However, the associated changes in EMG activity during the $\dot{V}O_2$ slow component are inconsistent (Burnley *et al.*, 2002b; Perrey *et al.*, 2003b; Scheuermann *et al.*, 2001; Tordi *et al.*, 2003). Burnley *et al.* (2002b) showed a reduction in integrated EMG (iEMG) across several muscles in conjunction with a smaller $\dot{V}O_2$ slow component in the second heavy bout.

Similarly, when Bailey *et al.* (2009b) investigated EMG and its relation to the $\dot{V}O_2$ slow component following different heavy warm-ups, they observed attenuation in the $\dot{V}O_2$ slow component amplitude and iEMG in the vastus lateralus (VL) muscle. Conversely, Scheuermann *et al.* (2001) observed a reduction in the slow component but no change in iEMG or mean power frequency (MPF) in VL muscle. Prior moderate exercise has also been shown to reduce the $\dot{V}O_2$ slow component amplitude during subsequent heavy exercise to a smaller extent than prior heavy exercise (Koppo & Bouckaert, 2000). However, there are no data available regarding the EMG responses in a heavy exercise bout that follows a single moderate warm-up bout. Therefore, investigating the $\dot{V}O_2$ slow component following moderate and heavy priming exercise in concert with EMG may provide useful information on the underlying mechanisms.

While muscle recruitment patterns have been thoroughly investigated as a potential mechanism for the $\dot{V}O_2$ slow component during heavy exercise, the role of muscle activity in higher O_2 cost during moderate exercise after priming heavy

exercise remains undefined. Gonzales and Scheuermann (2008) reported that elevated O_2 cost in moderate exercise after a heavy warm-up is not the result of additional recruitment of motor units or an appreciable recruitment of type II muscle fibres. In the current study, we employed repeated cycling protocols involving both moderate and heavy exercise transitions while measuring breath-by-breath $\dot{V}O_2$ concurrent with EMG activity in multiple leg muscles to examine the relation between the changes in muscle activity (as assessed by iEMG and MPF) and 1) the magnitude of the $\dot{V}O_2$ slow component during heavy exercise following both heavy and moderate warm-up; 2) the elevated O_2 cost in the moderate exercise that follows one and two prior heavy exercise bouts.

4.3 Methods

4.3.1 Subjects

Eight recreationally active ($\dot{V}O_{2peak}$ > 55 ml/kg/min) men (age: 23.2 ± 3.0 years, height: 176.0 ± 6.9 cm, weight: 70.3 ± 5.1 kg, $\dot{V}O_{2peak}$: 4190 ± 342 ml/min; mean ± SD) recruited from those who completed the first study (Chapter 2), participated in this study following informed consent. This study was approved by the Office of Research Ethics at the University of Waterloo.

4.3.2 Experimental design

Participants visited the laboratory on 4 occasions (non-consecutive days), to perform 2 sessions of each experimental protocol (Fig.4.1). $\dot{V}O_{2peak}$ and VT were determined from the incremental cycling test described previously in chapter 2. The experimental protocols involved a combination of three 6-min cycling bouts at moderate (M; 80% VT: 138 ± 25 W) and heavy (H; 85% $\dot{V}O_{2peak}$: 257 ± 24 W) intensities, interspersed with 6-min bouts at 20 W. Within each protocol, the three 6-min exercise bouts were labeled according to intensity, bout number and protocol letter, as follows: Protocol A = moderate ($M1_A$), heavy ($H2_A$), and moderate ($M3_A$); Protocol B = heavy ($H1_B$), heavy ($H2_B$), and moderate ($M3_B$); Protocols started immediately after 2 min of unloaded (0 W) cycling. $\dot{V}O_2$ and EMG from the lower limb musculature were measured continuously throughout the rides. The same procedures for the cycle ergometer set up and testing sessions were applied as mentioned in Chapter 2.

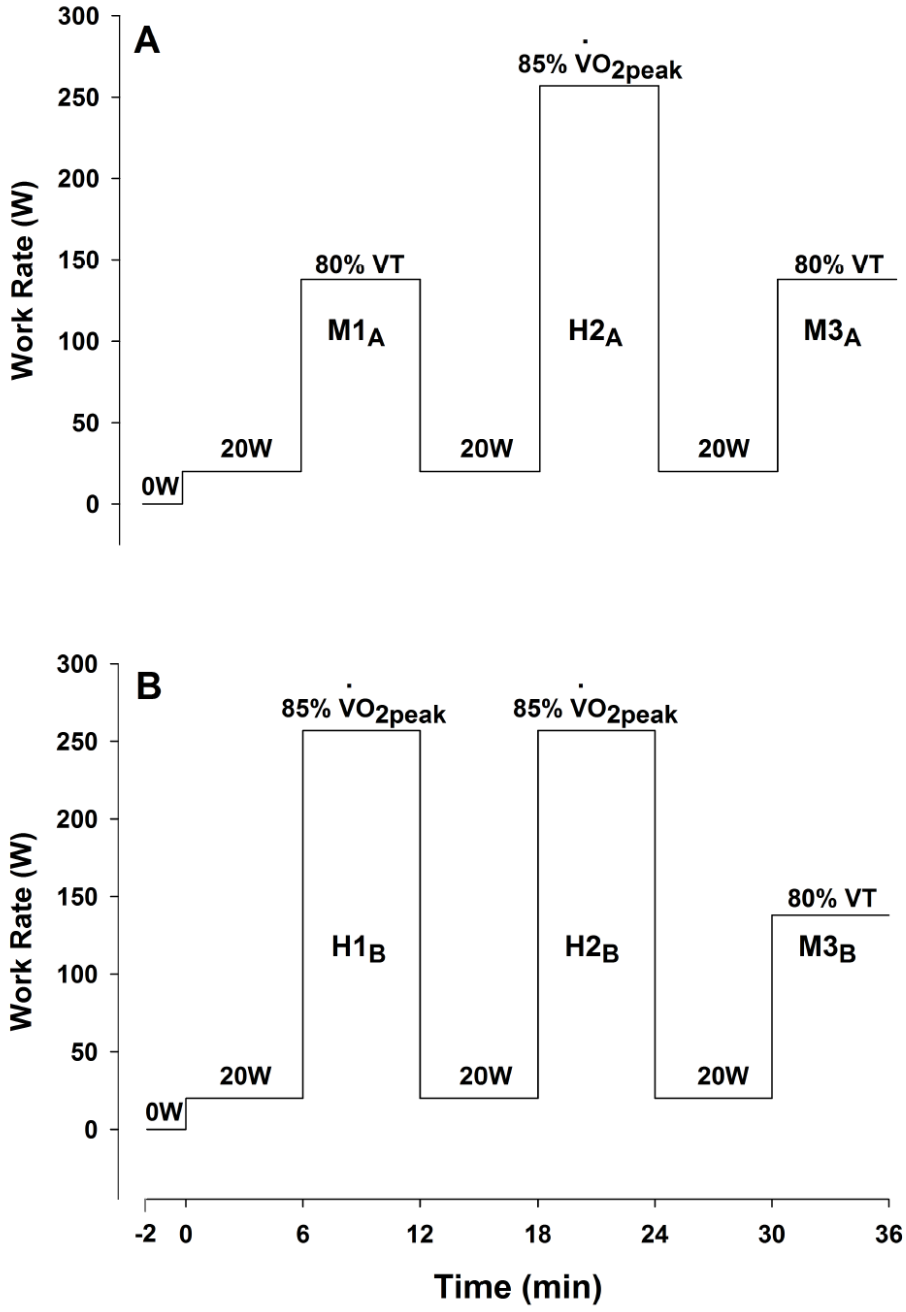

Figure 4.1: Two different cycling protocols.

Two different testing protocols employed to examine the relation between the changes in $\dot{V}O_2$ slow component amplitude during heavy exercise, increase O_2 cost during moderate that follow heavy warm-up and changes in EMG activity.

4.3.3 Breath-by-breath oxygen uptake

The set up of the breath-by-breath system and $\dot{V}O_2$ data collection were exactly the same as described in chapter 2. Non-interpolated, breath-by-breath $\dot{V}O_2$ data were blocked into 10-s windows and averaged together to yield a single data set for each subject in each protocol (Fig.4.2). The $\dot{V}O_2$ slow component amplitude was assessed by calculating the differences (Δ) in $\dot{V}O_2$ between the 2nd and 6th min [$\dot{V}O_{2(6-2)}$]. The 2nd min was calculated from 90 s to 150 s and was selected for the start of this analysis as curve-fitting analyses in chapter 2 have shown small τ_2 in these subjects, as well previous work has shown that the slow component started in the 2nd min of heavy exercise (Cannon *et al.*, 2007). In addition, the kinetics parameters of the $\dot{V}O_2$ slow component during heavy exercise bouts and the $Gain_{1+2}$ in both heavy and moderate exercise bouts (Fig. 4.3) for the 8 subjects were calculated from the rides they performed in the first study. $Gain_{1+2}$ was calculated as follows ($\Delta\dot{V}O_2/\Delta WR$), where $\Delta\dot{V}O_2$ is equal to the summation of phase I and phase II amplitudes (A_1+A_2)and ΔWR is equal to the difference between the work rate applied during exercise and 20 W baseline.

4.3.4 Electromyographic activity

EMG was obtained from the vastus lateralis (VL), vastus medialis (VM), rectus femoris (RF) and biceps femoris (BF) muscles. Prior to each session, EMG was recorded during 2 min of unloaded cycling (0 W). EMG was measured on the left leg using surface disposable pre-gelled EMG Ag-AgCl electrodes (Blue Sensor, Medicotest, Inc., Ølstykke, Denmark) with an inter-electrode distance of 2 cm. Skin was shaved and cleaned with alcohol to minimize skin impedance, before

electrodes were fixed to the skin in a bipolar configuration along the mid-line of the proximal and distal tendons. A reference electrode was placed over the anterior iliac crest. Electrode wires were taped to the skin to reduce movement artifacts. Electrode placement was marked with permanent ink to ensure consistent positioning during subsequent sessions. Raw EMG was amplified (Custom built amplifier, Waterloo, ON, Canada; bandwidth = 20 – 500 Hz, common mode rejection rate > 90 db, input impedance = 2 MΩ) and collected at 1000 Hz using a 16-bit A/D card with a ± 5 V range. To identify baseline muscle activity, the rectified signal was passed through a dual second-order Butterworth digital filter with a 5 Hz cut off frequency. Muscular contraction was considered to begin when the rectified signal exceeded the mean of the baseline EMG plus three standard deviations, and end when the signal fell below that level. Integrated EMG (iEMG) was calculated as the area under the rectified signal for each burst, blocked into 10-s windows throughout each protocol. Mean power frequency (MPF) was calculated using fast Fourier transformation analysis on the raw signal (95% of the total power was considered). Due to inter-subject variability and day to day variation in EMG signal, iEMG and MPF were normalized to the baseline activity measured during the first 2 min of unloaded cycling prior to each protocol. Differences in iEMG activity between the 2nd (90-150 s) and 6th (300-360 s) min (Δ iEMG $_{(6-2)}$) were determined for comparison with $\Delta \dot{V}O_{2(6-2)}$.

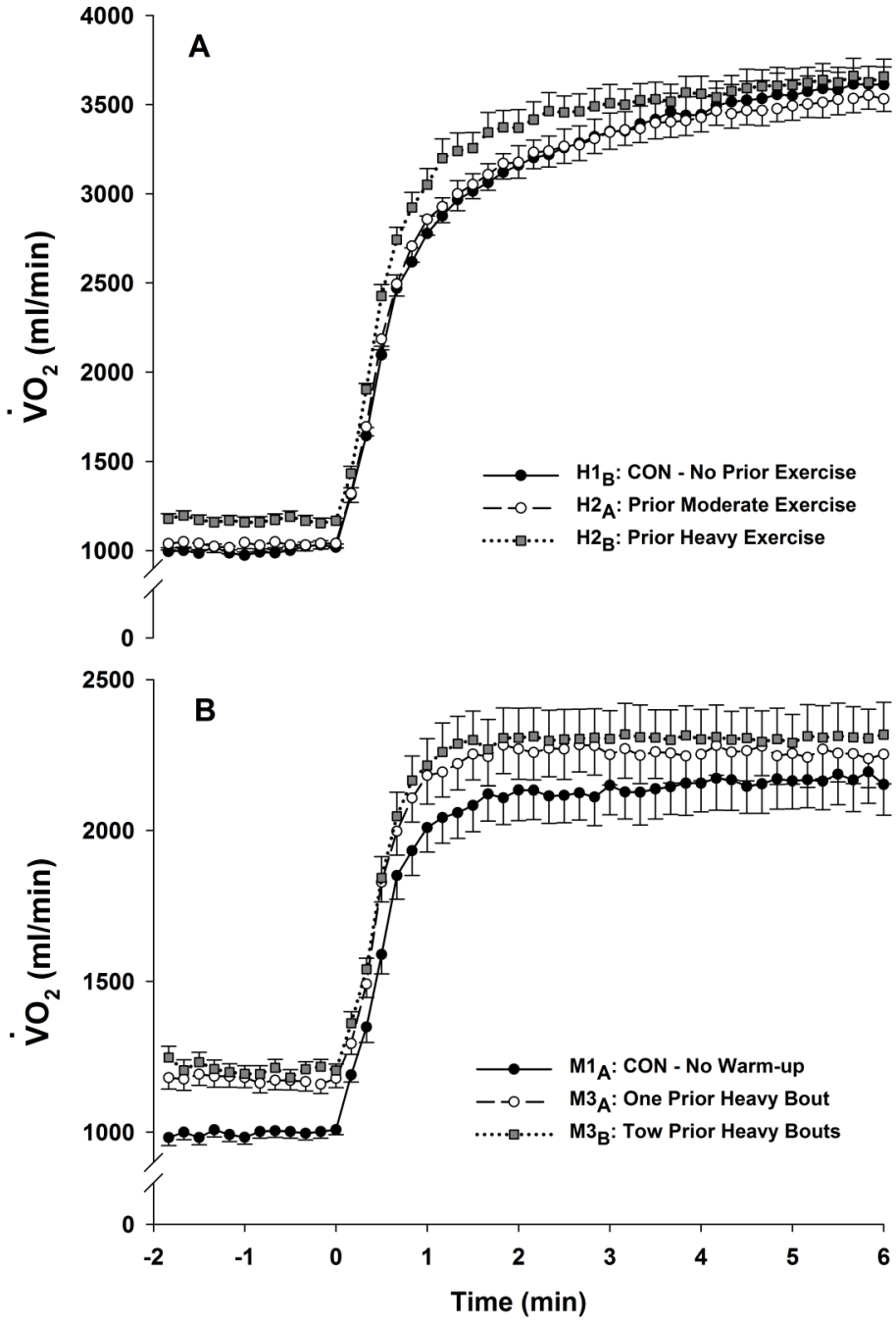

Figure 4.2: $\dot{V}O_2$ time series throughout heavy (A, top) and moderate (B, bottom) exercise bouts.

Data are the average of 10-s windows (± SE) from all 8 participants. The participants repeated each protocol on 2 occasions.

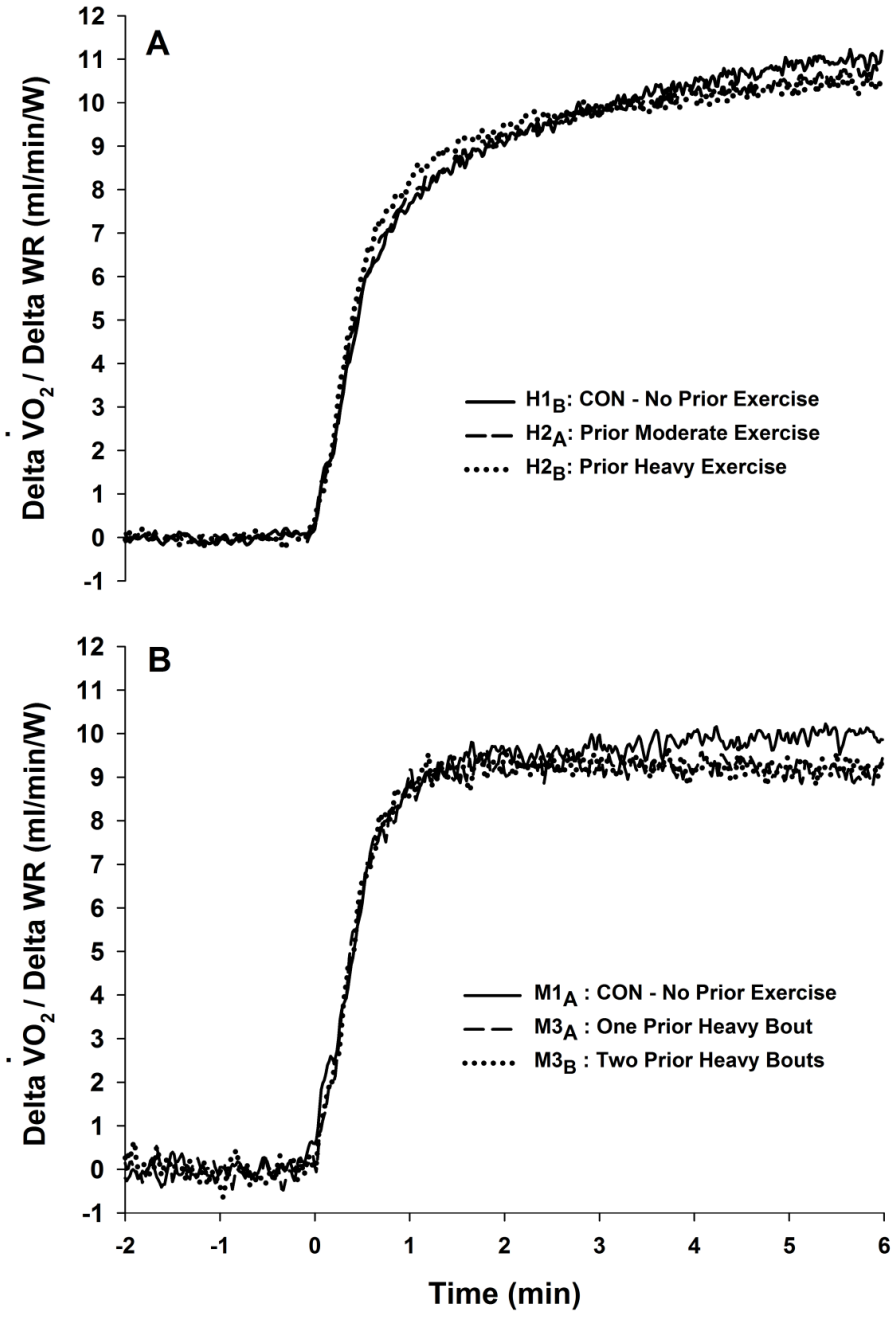

Figure 4.3: Aerobic gain time series throughout heavy (A) and moderate (B) exercise.

Data were averaged across all 8 participants with at least 4 repetitions per condition. $\Delta \dot{V}O_2$ is equal to the amplitude of phase I and phase II (A_1+A_2).

4.3.5 Blood sampling

Blood samples were drawn from the antecubital vein during the two rides of each protocol to measure lactate [La^-] concentrations by fluorometric assay (Lowry & Passonneau, 1972). Blood was drawn at rest, and between 5.5 and 6 minutes of each of the three work rates: 20 W, moderate, and heavy.

4.3.6 Statistical analysis

The effects of prior exercise on $\dot{V}O_2$ kinetics during subsequent moderate and heavy exercise were analyzed using one-way repeated measures ANOVA. The effects of prior exercise on one minute average values of iEMG and MPF during moderate and heavy bouts were analyzed using two-way repeated measures ANOVA. When significant effects were observed, the Tukey post hoc test was used for comparisons. T Test was used to examine the differences in MPF between the first 10 s and end exercise, and the differences in iEMG between the 2^{nd} and 6^{th} min of exercise in each heavy exercise condition. Principal components analysis (PCA) (Coste *et al.*, 2005) and regression analysis, which accounts for the correlation within subjects due to repetitions, were used to examine the relation between Δ iEMG $_{(6-2)}$ and $\dot{V}O_2$ slow component. All data were expressed as mean ± SD. The data were analyzed using Statistical Analysis Software (SAS) package 9.2 (SAS Institute, Cary, NC).

4.4 Results

4.4.1 Oxygen uptake kinetics

The fitting parameters of the $\dot{V}O_2$ kinetics during heavy and moderate exercise were modified by the warm-up bouts (Tables 4.1 and 4.2). The time constant (τ_2) during heavy exercise was significantly faster after either prior moderate ($H2_A$, 23.1 ± 2.2 s) or heavy ($H2_B$, 22.7 ± 2.3 s) warm-up compared to control ($H1_B$ 27.0 ± 1.9 s)**.** Similarly, τ_2 values were significantly lower in a moderate exercise bout that followed either one ($M3_A$) or two ($M3_B$) heavy bouts than during control ($M1_A$) (Table 4.2). Neither moderate nor heavy priming exercise affected the amplitude of $\dot{V}O_2$ during phase I (A_1) or phase II (A_2) in the subsequent bouts of moderate or heavy exercise (Tables 4.1 and 4.2). Prior moderate or heavy cycling exercises had a graded effect in attenuating the slow component amplitude (A_3) of subsequent heavy exercise ($H2_A$: 541 ± 103; $H2_B$: 385 ± 63 vs. $H1_B$: 610 ± 114 ml/min, $P < 0.01$ for both). This was also reflected by $\Delta\dot{V}O_{2(6-2)}$. The $\Delta\dot{V}O_{2(6-2)}$ during heavy exercise was 19 % lower following a moderate intensity warm up, and 40 % lower following a heavy intensity warm-up, than observed during the control bout ($H2_A$: 455 ± 52; $H2_B$: 341 ± 54 vs. $H1_B$: 564 ± 71 ml/min; $P < 0.01$ for both). There was greater attenuation with increasing intensity of the warm-up, such that the amplitude of slow component in $H2_B$ was significantly lower than in $H2_A$ for both the kinetics analyses and $\Delta\dot{V}O_{2(6-2)}$ ($P < 0.05$ for both). Prior moderate and heavy exercise reduced the time to initiate the slow component (TD3) during subsequent heavy exercise to a similar extent compared to control ($H2_A$: 81.4 ± 13.1; $H2_B$: 73.3 ± 10.6 vs. $H1_B$: 102.7 ± 10.4 s; $P < 0.01$ for both). Prior warm-up reduced the $Gain_{1+2}$ in subsequent moderate exercise, but not heavy cycling

compared to their respective controls (Moderate, $M3_A$: 9.2 ± 0.4; $M3_B$: 9.4 ± 0.5 vs. $M1_A$: 9.9 ± 0.4 ml/min/W; $P < 0.01$ for both; Heavy, $H2_A$: 8.8 ± 0.3; $H2_B$: 9.1 ± 0.5 vs. $H1_B$: 8.9 ± 0.6 ml/min/W; $P > 0.05$) (Fig. 4.3).

4.4.2 Lactate

The baseline [La^-] leading into heavy exercise was elevated following a prior heavy bout compared to that following a prior moderate bout or no warm-up exercise ($H2_B$: 4.8 ± 0.7 vs. $H2_A$ = 1.1 ± 0.2; $H1_B$: 0.8 ± 0.2 mmol/l, $P < 0.001$ for both). At the end of the heavy exercise bouts, [La^-] remained significantly lower in $H1_B$ (4.2 ± 1.2 mmol/l) than $H2_B$ (6.3 ± 1. 2 mmol/l, $P < 0.01$). Also, it tended to stay lower in $H2_A$ (4.9 ± 1.0 mmol/l, $P = 0.06$) compared to $H2_B$. Immediately prior to moderate exercise, the baseline [La^-] was higher for bouts following a heavy intensity warm-up than the bout with no warm up ($M3_A$: 4.7 ± 1.0; $M3_B$: 5.4 ± 1.3 vs. $M1_A$: 0. 9 ± 0. 2 mmol/l, $P < 0.01$ for both). At the end of the moderate exercise bouts that followed a heavy warm-up, [La^-] was lower than baseline ($M3_A$: 3.3 ± 0.9 mmol/l; $M3_B$: 3.7 ± 1.3 mmol/l, $P < 0.01$ for both).

Table 4.1: Fitting parameters during heavy exercise bouts

Parameters	Heavy Bouts		
	$H1_B$	**$H2_A$**	**$H2_B$**
A_0, ml/min	987 ± 50	1021 ± 76	1155 ± 48 *‡
A_1, ml/min	447 ± 148	463 ± 107	424 ± 103
A_2, ml/min	1661 ± 201	1610 ± 166	1733 ± 245
A_1+A_2, ml/min	2108 ± 273	2074 ± 219	2157 ± 275
A_3, ml/min	610 ± 114	541 ± 103	385 ± 63
τ_2, s	27.0 ± 1.9	23.1 ± 2.2 *	22.7 ± 2.3 *
τ_3, s	154.3 ± 36.3	144.6 ± 35.8	141.4 ± 27.2
TD_2, s	11.5 ± 1.9	11.7 ± 1. 5	10.2 ± 1.0 †§
TD_3, s	102.7 ± 10.4	81.4 ± 13.1 *	73.3 ± 10.6 *
$Gain_{1+2}$, ml/min/W	8.9 ± 0.6	8.8 ± 0.3	9.1 ± 0.5
End Exercise, ml/min	3600 ± 277	3540 ± 230	3635 ± 256

Mean ± SD, n = 8

* $P < 0.01$ $H1_B$ vs. $H2_A$-$H2_B$; † $P < 0.05$ $H1_B$ vs. $H2_A$-$H2_B$;

‡ $P < 0.01$ $H2_A$ vs. $H2_B$; § $P < 0.05$ $H2_A$ vs. $H2_B$

Amplitude parameters A_0, A_1, A_2 and A_3 for the baseline, phase I, phase II and phase III (slow component) respectively; time constants (τ_2, τ_3) and time delays (TD_2, TD_3) for the corresponding phases; end bout is the average $\dot{V}O_2$ in the last minute of each exercise bout. The heavy bouts for protocols A and B are shown in Fig. 4.1A and 4.1B respectively.

Table 4.2: Fitting parameters during moderate exercise bouts

Parameters	Moderate Bouts		
	$M1_A$	**$M3_A$**	**$M3_B$**
A_0, ml/min	990 ± 48	1159 ± 73 *	1196 ± 66 *
A_1, ml/min	304 ± 130	255 ± 79	279 ± 87
A_2, ml/min	861 ± 141	837 ± 176	834 ± 191
A_1+A_2, ml/min	1165 ± 254	1092 ± 228	1112 ± 259
τ_2, s	23.7 ± 4.4	19.3 ± 3.4 †	17.0 ± 2.9 *
TD_2, s	13.2 ± 1.2	12.9 ± 1.9	14.3 ± 1.7
$Gain_{1+2}$, ml/min/W	9.9 ± 0.4	9.2 ± 0.4 †	9.4 ± 0.5 †
End Exercise, ml/min	2179 ± 278	2259 ± 276	2307 ± 303

Mean ± SD, n = 8

* $P < 0.01$ $M1_A$ vs. $M3_A$ - $M3_B$; † $P < 0.05$ $M1_A$ vs. $M3_A$ - $M3_B$;

‡ $P < 0.01$ $M3_A$ vs. $M3_B$; § $P < 0.05$ $M3_A$ vs. $M3_B$

Amplitude parameters A_0, A_1 and A_2 for the baseline, phase 1 and phase 2 respectively; time constant (τ_2) and time delay (TD_2) for the primary phase; end bout is the average $\dot{V}O_2$ in the last minute of each exercise bout. The moderate bouts for protocols A and B are shown in Fig. 4.1A and 4.1B respectively.

4.4.3 EMG

The baseline EMG was not different between days and protocols in all muscles (iEMG Protocol A vs. Protocol B, VL: 12.3 ± 2.6 vs. 12.6 ± 2.7, VM: 14.4 ± 6.6 vs. 13.9 ± 6.5, RF: 10.8 ± 1.6 vs. 11.6 ± 2.4 and BF: 24.8 ± 17.6 vs. 27.2 ± 22.2 mV, $P > 0.05$ for all; MPF Protocol A vs. Protocol B, VL: 56.0 ± 3.8 vs. 55.1 ± 2.7, VM: 54.4 ± 6.0 vs. 54.7 ± 3.8, RF: 57.1 ± 2.5 vs. 58.6 ± 4.2 and BF: 64.5 ± 7.9 vs. 63.7 ± 10.1 Hz, $P > 0.05$ for all). There were also no differences in iEMG or MPF between the 20 W baselines that preceded each moderate and heavy condition (Table 4.3), or the end exercise iEMG within the moderate and heavy conditions (Table 4.4). During heavy exercise, MPF (Fig. 4.4) was significantly lower in all the extensor (agonist) muscles at the end of exercise compared to the first 10 s ($P < 0.05$ for all comparisons, Tables 4.5 – 4.7). However, MPF in the flexor (antagonist) BF muscle was slightly but not significantly higher at the end of exercise [$H1_B$: 101.4 ± 8.6 vs. 106.5 ± 11.7, $H2_A$: 101.2 ± 6.7 vs. 105.6 ± 7.4 and $H2_B$: 107.7 ± 9.1 vs. 108.4 ± 9.4 % of baseline MPF; $P > 0.05$ for all comparisons, Fig. 4.5]. Prior heavy exercise significantly elevated MPF throughout the subsequent heavy bout ($H2_B$) compared to the control heavy bout ($H1_B$) in all the extensor VL, VM and RF muscles (over the 6 minutes of exercise P values were less than 0.05, Tables 4.5 – 4.7), however, no effect was observed in the antagonist BF muscle. Prior moderate exercise resulted in a significantly higher MPF in VL and VM muscles throughout the first minutes in subsequent heavy bout ($H2_A$) compared to control ($P < 0.05$, Tables 4.5 and 4.6), but no effects have shown in RF and BF muscles.

Table 4.3: Baseline iEMG and MPF during 20W cycling prior to heavy and moderate bouts

Variables	Heavy Bouts			Moderate Bouts		
	$H1_B$	$H2_A$	$H2_B$	$M1_A$	$M3_A$	$M3_B$
VL – iEMG	126.5 ± 22.7	133.5 ± 19.9	134.2 ± 37.3	132.2 ± 18.8	133.5 ± 19.9	136.1 ± 42.8
VM - iEMG	124.9 ± 20.2	129.7 ± 42.9	128.3 ± 30.5	127.2 ± 33.6	128.1 ± 26.1	131.5 ± 24.5
RF - iEMG	103.6 ± 26.1	116.2 ± 24.7	112.3 ± 22.4	113.2 ± 18.9	116.4 ± 24.7	113.3 ± 26.9
BF - iEMG	122.0 ± 24.6	109.5 ± 25.4	127.8 ± 35.2	117.4 ± 14.9	119.6 ± 24.1	115.9 ± 22.3
VL - MPF	97.3 ± 2.3	98.7 ± 3.5	98.7 ± 5.4	98.0 ± 3.9	98.0 ± 7.1	102.9 ± 8.4
VM - MPF	96.7 ± 8.6	94.7 ± 11.3	96.9 ± 5.4	96.6 ± 9.3	95.9 ± 10.8	102.8 ± 9.9
RF - MPF	96.4 ± 7.9	100.9 ± 7.4	97.7 ± 8.1	100.1 ± 5.6	101.3 ± 6.4	101.8 ± 10.0
BF - MPF	103.1 ± 12.8	101.1 ± 18.4	110.4 ± 13.8	105.9 ± 9.4	108.1 ± 14.9	112.2 ± 14.7

Mean ± SD, n = 8

iEMG and MPF values are shown as % of unloaded cycling

Table 4.4: End exercise iEMG during heavy and moderate bouts

Variables	Heavy Bouts			Moderate Bouts		
	$H1_B$	$H2_A$	$H2_B$	$M1_A$	$M3_A$	$M3_B$
VL – iEMG	617.2 ± 29.5	591.0 ± 22.7	585.5 ± 28.0	339.7 ± 9.6	348.6 ± 8.7	305.0 ± 10.3
VM - iEMG	598.7 ± 18.1	560.3 ± 21.0	572.7 ± 24.1	315.5 ± 11.6	301.6 ± 8.2	330.7 ± 11.7
RF - iEMG	419.6 ± 21.6	392.8 ± 18.5	392.3 ± 19.3	230.7 ± 10.0	238.5 ± 9.6	234.9 ± 11.5
BF - iEMG	311.8 ± 12.3	259.1 ± 10.7	293.3 ± 14.1	193.0 ± 19.9	166.1 ± 17.0	208.0 ± 16.0

Mean ± SD, n = 8

iEMG values are shown as % of unloaded cycling

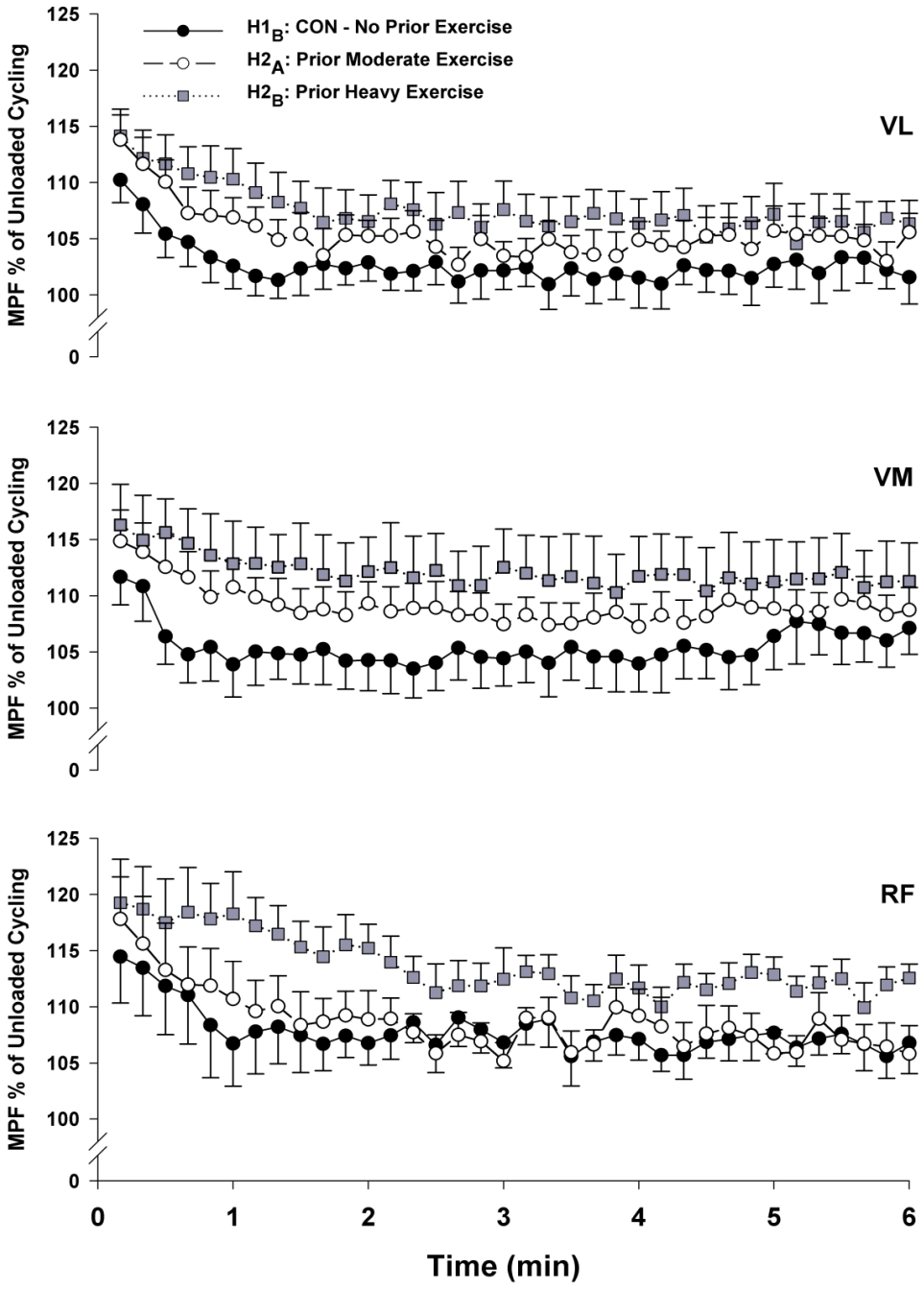

Figure 4.4: MPF time series throughout heavy cycling bouts.

Muscle activity is shown from vastus lateralis (VL), vastus medialis (VM) and rectus femoris (RF) muscles. MPF is reported as a percentage of unloaded cycling (0 W). Data are the average (± SE) of all 8 participants with 2 repetitions per condition.

Table 4.5: MPF in VL muscle during heavy and moderate exercise bouts

Variables	Heavy Bouts			Moderate Bouts		
	$H1_B$	**$H2_A$**	**$H2_B$**	**$M1_A$**	**$M3_A$**	**$M3_B$**
MPF – 10 s	110.2 ± 5.7 ‡	113.8 ± 6.3 ‡	114.2 ± 6.7 ‡	106.9 ± 6.6	109.9 ± 6.2	110.9 ± 7.1 *
MPF - Min 1	105.7 ± 5.8	109.5 ± 5.6 *	111.5 ± 6.5 *	104.5 ± 4.3	110.6 ± 6.7 *	112.4 ± 6.5 *
MPF - Min 2	102.2 ± 5.0	105.1 ± 4.7 *	107.4 ± 6.9 *	103.8 ± 3.6	109.4 ± 5.1 *	112.6 ± 6.7 *†
MPF - Min 3	102.1 ± 5.1	104.4 ± 4.5	107.1 ± 6.5 *	103.6 ± 4.1	109.2 ± 5.8 *	112.5 ± 5.5 *†
MPF - Min 4	101.8 ± 5.9	104.0 ± 4.4	106.7 ± 6.1 *	104.2 ± 4.3	109.3 ± 6.7 *	113.3 ± 5.8 *†
MPF - Min 5	102.0 ± 5.5	104.8 ± 5.1	106.3 ± 6.5 *	104.7 ± 4.1	109.0 ± 6.5 *	113.6 ± 6.3 *†
MPF - Min 6	102.6 ± 6.4	104.9 ± 5.0	106.1 ± 6.1 *	105.3 ± 4.1	109.0 ± 6.9 *	114.1 ± 6.1 *†

Mean ± SD, n = 8

* $P < 0.05$ $H1_B$ vs. $H2_A$ and $H2_B$; $M1_A$ vs. $M3_A$ and $M3_B$

† $P < 0.05$ $M3_A$ vs. $M3_B$

‡ $P < 0.05$ 10 s vs. Min 6 in each heavy and moderate exercise condition

MPF values are shown as % of unloaded cycling

Table 4.6: MPF in VM muscle during heavy and moderate exercise bouts

Variables	Heavy Bouts			Moderate Bouts		
	$H1_B$	$H2_A$	$H2_B$	$M1_A$	$M3_A$	$M3_B$
MPF – 10 s	111.7 ± 7.0 ‡	114.9 ± 7.9 ‡	116.3 ± 10.2 ‡	109.3 ± 7.1	111.0 ± 11.7	118.0 ± 10.6 *†
MPF - Min 1	107.2 ± 7.5	112.3 ± 7.0 *	114.5 ± 9.4 *	107.1 ± 8.2	110.2 ± 10.6	117.5 ± 10.6 *†
MPF - Min 2	104.7 ± 7.4	109.0 ± 5.5 *	112.3 ± 9.2 *†	104.1 ± 9.2	109.6 ± 9.3 *	115.6 ± 10.5 *†
MPF - Min 3	104.3 ± 7.3	108.4 ± 5.6 *	111.7 ± 9.4 *†	103.6 ± 7.3	108.8 ± 8.5 *	116.0 ± 9.8 *†
MPF - Min 4	104.6 ± 7.8	107.8 ± 5.0	111.3 ± 10.2 *†	103.3 ± 7.1	109.5 ± 10.3 *	115.4 ± 9.5 *†
MPF - Min 5	105.2 ± 7.9	108.6 ± 5.5	111.4 ± 10.3 *	104.0 ± 7.4	110.2 ± 9.8 *	115.2 ± 10.2 *†
MPF - Min 6	106.9 ± 7.6	108.9 ± 5.7	111.5 ± 9.7 *	104.0 ± 7.5	109.8 ± 9.9 *	116.2 ± 9.7 *†

Mean ± SD, n = 8

* $P < 0.05$ $H1_B$ vs. $H2_A$ and $H2_B$; $M1_A$ vs. $M3_A$ and $M3_B$

† $P < 0.05$ $H2_A$ vs. $H2_B$; $M3_A$ vs. $M3_B$

‡ $P < 0.05$ 10 s vs. Min 6 in each heavy and moderate exercise condition

MPF values are shown as % of unloaded cycling

Table 4.7: MPF in RF muscle during heavy and moderate exercise bouts

Variables	Heavy Bouts			Moderate Bouts		
	$H1_B$	$H2_A$	$H2_B$	$M1_A$	$M3_A$	$M3_B$
MPF – 10 s	116.0 ± 11.4 ‡	118.1 ± 10.5 ‡	120.7 ± 11.3 ‡	104.1 ± 7.2	110.8 ± 8.0 *	115.6 ± 9.1 *†
MPF - Min 1	111.0 ± 11.6	113.5 ± 10.1	118.4 ± 10.2 *†	103.9 ± 7.1	108.8 ± 7.0 *	114.4 ± 7.4 *†
MPF - Min 2	107.4 ± 7.4	109.1 ± 6.7	115.7 ± 6.5 *†	103.4 ± 6.4	109.5 ± 7.4 *	112.1 ± 8.8 *
MPF - Min 3	107.8 ± 5.6	107.0 ± 4.4	112.3 ± 6.2 *†	102.4 ± 6.2	108.5 ± 8.7 *	112.4 ± 8.0 *
MPF - Min 4	107.4 ± 5.1	108.3 ± 4.2	111.8 ± 4.2 *	101.9 ± 5.8	108.7 ± 8.6 *	111.6 ± 7.8 *
MPF - Min 5	106.8 ± 4.5	107.3 ± 5.8	111.9 ± 4.2 *†	101.6 ± 5.6	109.7 ± 9.2 *	111.4 ± 8.1 *
MPF - Min 6	106.7 ± 5.1	106.8 ± 5.2	111.8 ± 4.1 *†	101.2 ± 6.0	108.6 ± 8.4 *	111.6 ± 8.7 *

Mean ± SD, n = 8

* $P < 0.05$ $H1_B$ vs. $H2_A$ and $H2_B$; $M1_A$ vs. $M3_A$ and $M3_B$

† $P < 0.05$ $H2_A$ vs. $H2_B$; $M3_A$ vs. $M3_B$

‡ $P < 0.05$ 10 s vs. Min 6 in each heavy and moderate exercise condition

MPF values are shown as % of unloaded cycling

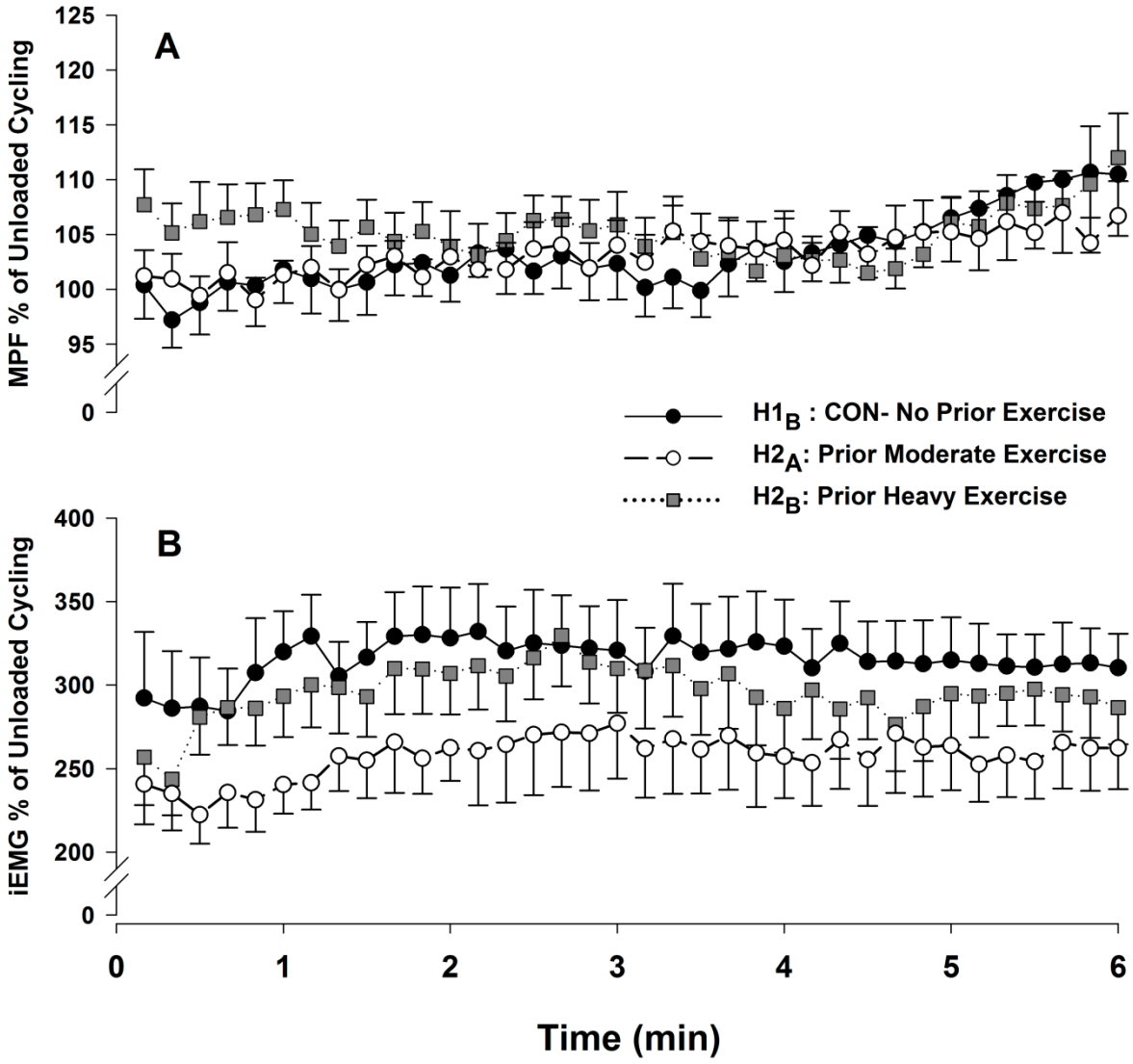

Figure 4.5: MPF (A) and integrated EMG (B) activity in BF muscle.

Time series for MPF and iEMG are reported as a percentage of unloaded cycling (0 W). Data are the average (± SE) of all 8 participants with 2 repetitions per condition.

There were no significant differences in iEMG activity at any time point between the three heavy exercise conditions in all muscles. There were also no significant differences in iEMG activity between the 2nd and 6th min of exercise within the three exercise conditions in all extensor muscles (Fig. 4.6) and flexor muscle (Fig.4.5). The Δ iEMG$_{(6\text{-}2)}$ in the control heavy bout ($H1_B$) were positive in the extensor muscles during [VL (5.1 %), VM (4.9 %) and RF (5.2 %)]. This response was altered by warm-up exercise, such that in $H2_A$ Δ iEMG$_{(6\text{-}2)}$ were less than control in VL (3.1 %) and VM (3.8 %), and was negative in RF (-2.1 %). There was an even more dramatic shift in $H2_B$, where the Δ iEMG$_{(6\text{-}2)}$ were negative in all three muscles [VL (-1.5 %), VM (-2.2 %) and RF (-6.3 %); Fig. 4.6]. In the flexor (antagonist) BF muscle, iEMG was slightly decreased in $H1_B$ (-3.5 %), unchanged in $H2_A$ (1.0 %) and further decreased in $H2_B$ (-13.6 %) (Fig. 4.5). Including all the muscles in PCA retained 53% of the variation of the $\dot{V}O_2$ slow component. However, excluding the flexor BF muscle (eigenvalue < 1) from PCA showed that Δ iEMG$_{(6\text{-}2)}$ in the extensor VL, VM and RF muscles explained 74% of the variation in $\dot{V}O_2$ slow component. Therefore, the three extensor muscles were used in PCA to retain a single factor for the correlation. There were no significant correlation between $\Delta \dot{V}O_{2(6-2)}$ and Δ iEMG$_{(6\text{-}2)}$ in all three heavy bout conditions, while when the data from all subjects and conditions were clustered, there was a moderate but significant correlation between $\Delta \dot{V}O_{2(6-2)}$ and Δ iEMG$_{(6\text{-}2)}$ in VL, VM and RF muscles ($r = 0.45$, $P = 0.029$).

In all moderate bouts, there were no changes in MPF throughout the 6 min of exercise. Yet, prior one or two heavy bouts significantly elevated MPF in the extensor VL, VM and RF muscles throughout the 6 min of subsequent moderate

exercise in a graded manner (Fig. 4.7, Tables 4.5 – 4.7). Relative to the control bout, average MPF throughout the exercise bout was elevated during $M3_A$ in VL (4.9 %), VM (5.1 %) and RF (6.5 %) muscles, and during $M3_B$ in VL (6.8 %), VM (13.0 %) and RF (10.8 %) muscles. MPF was significantly higher in VL and VM muscles through the 6 min of exercise after two heavy bouts ($M3_B$) compared to after one heavy bout ($M3_A$) ($P < 0.05$; Fig. 4.7). There were no changes in iEMG throughout the 6 min of moderate exercise. Also, there were no significant differences in iEMG activity at any time point between the three exercise conditions in all extensor muscles (Fig. 4.8).

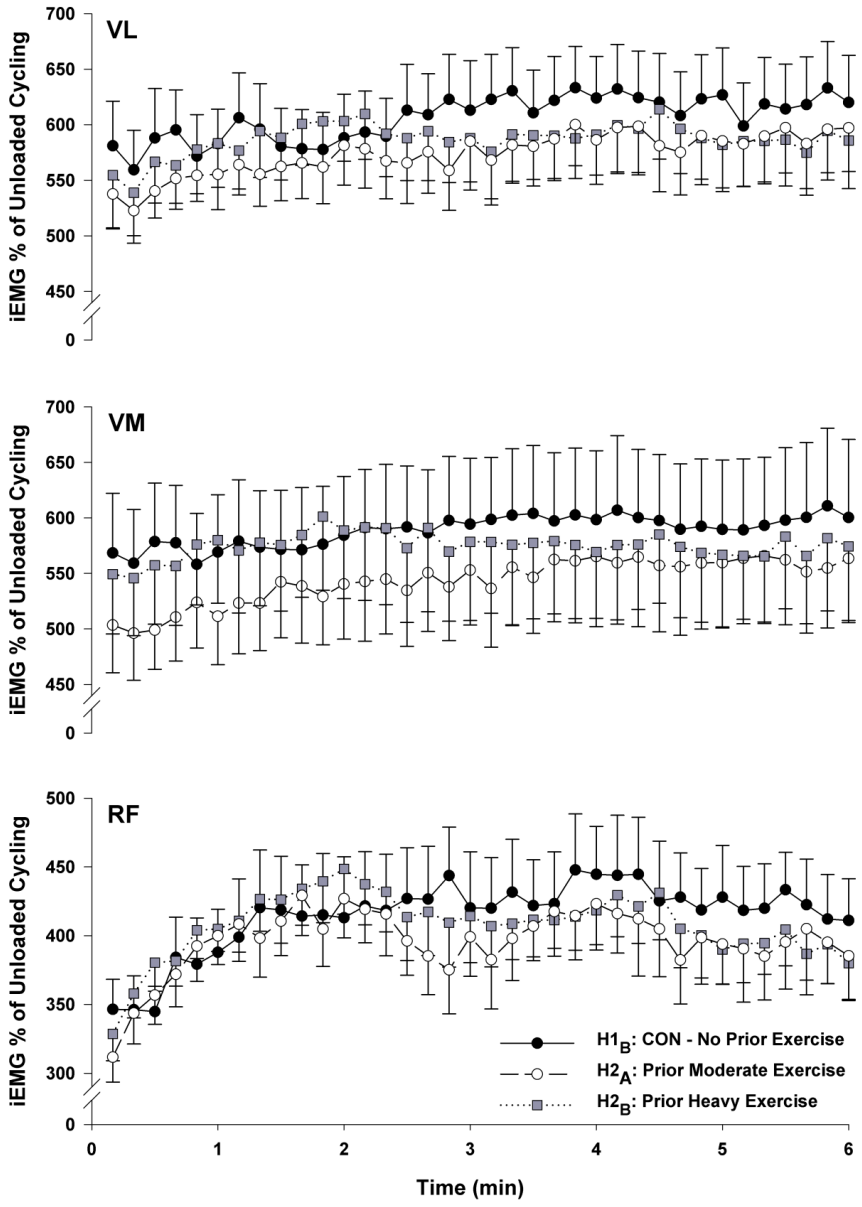

Figure 4.6: Integrated EMG time series throughout heavy cycling bouts.

Muscle activity is shown from vastus lateralis (VL), vastus medialis (VM) and rectus femoris (RF) muscles. MPF is reported as a percentage of unloaded cycling (0 W). Data are the average (± SE) of all 8 participants with 2 repetitions per condition.

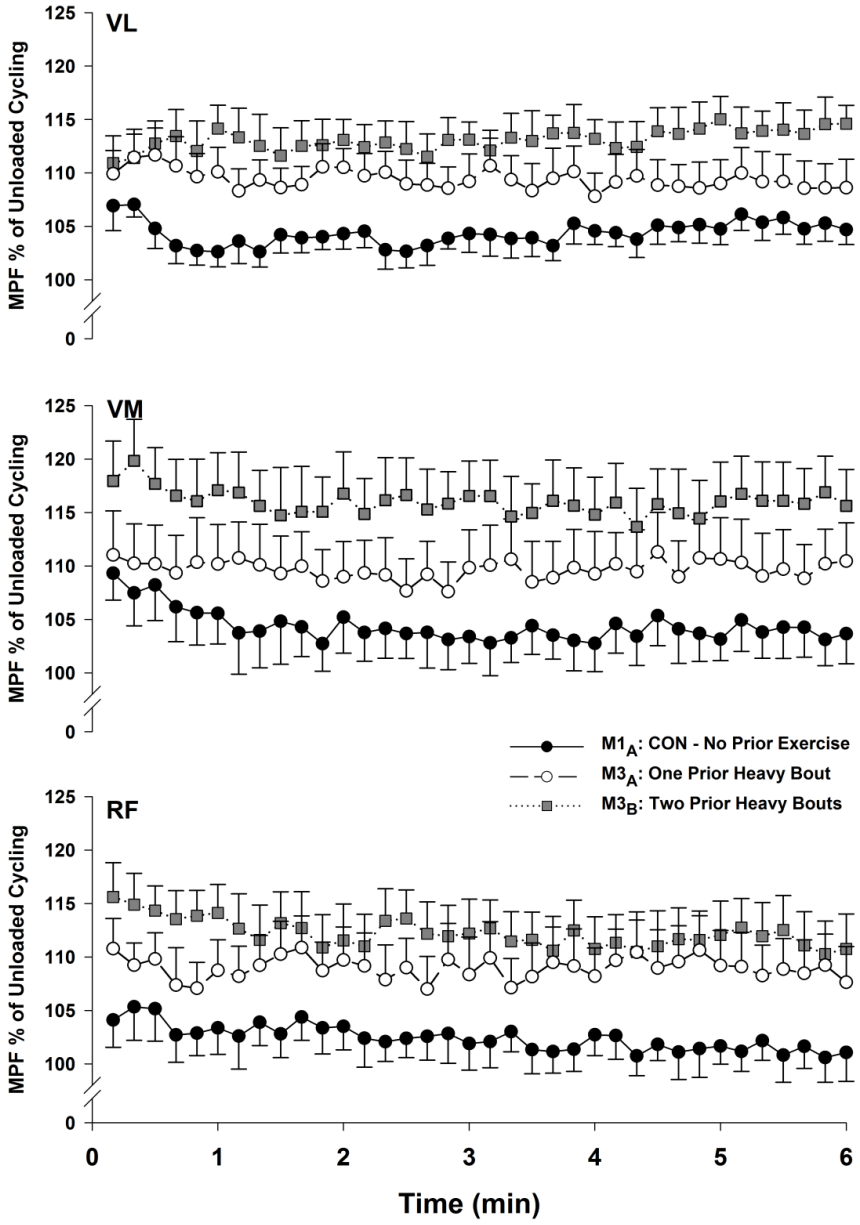

Figure 4.7: MPF time series through moderate cycling bouts.

Muscle activity is shown from vastus lateralis (VL), vastus medialis (VM) and rectus femoris (RF) muscles. MPF is reported as a percentage of unloaded cycling (0 W). Data are the average (± SE) of all 8 participants with 2 repetitions per condition.

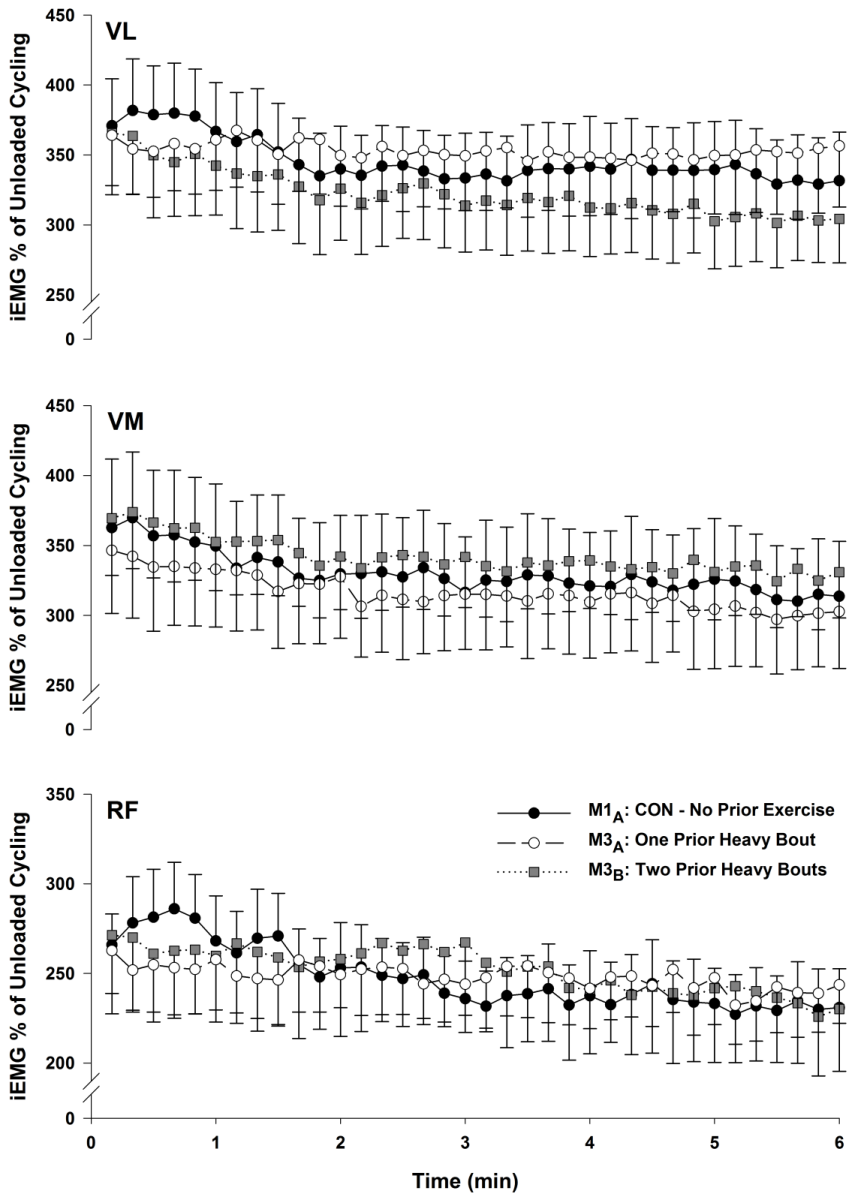

Figure 4.8: Integrated EMG time series through moderate cycling bouts. Muscle activity is shown from vastus lateralis (VL), vastus medialis (VM) and rectus femoris (RF) muscles. MPF is reported as a percentage of unloaded cycling (0 W). Data are the average (± SE) of all 8 participants with 2 repetitions per condition.

4.5 Discussion

The results of this study clearly demonstrate the capacity of prior heavy and moderate exercise to diminish the amplitude of the $\dot{V}O_2$ slow component in addition to the important findings that: 1) the attenuation in slow component amplitude appears to be related to some changes in muscle activity, as measured by surface EMG; and 2) the higher O_2 cost of moderate exercise is associated with higher MPF which may suggest changes in fibre types recruitment during moderate exercise after a heavy warm-up.

4.5.1 Methodological considerations

Two minutes of unloaded cycling was employed prior to each protocol to serve as baseline condition in order to compare all changes in EMG activity within and between the testing protocols on different days. This allowed comparison of the different 20W baselines, as well as between the moderate and heavy bouts. The normalization of EMG data to the unloaded baseline condition did not impact the analyses as the baseline EMG was identical between days and protocols. As well as, no differences were shown in EMG activity between the 20 W baselines that precede each moderate and heavy condition (Table 4.3).

The principal components analysis (PCA) (Coste et al., 2005) was used to examine the relation between changes in iEMG activity and $\dot{V}O_2$ slow component. The central idea of using PCA is to aggregate the EMG activity measured in all muscles into one factor. PCA with VARIMAX rotation was used on the 4 muscles and eigenvalues greater than one were used to decide the number of muscles that show the most variation to be included in the analysis (Jolliffe, 2002). This method of analysis enabled the inclusion of all the extensor muscles (VL, VM and RF) to

discern how they may contribute in concert to the development of $\dot{V}O_2$ slow component. However, the PCA was not able to detect the significant relationships between the changes in iEMG and slow component amplitude in the individual heavy exercise conditions. This might have been due to small number of participants, but could also reflect the inability of iEMG to relate to the mechanism involved for the increased $\dot{V}O_2$ slow component.

Finally, the $\dot{V}O_2$ slow component was calculated as the increase in amplitude between the 2nd and 6th min of the exercise bout. While some previous work has used the 3rd-6th min (Whipp & Wasserman, 1972; Whipp & Wasserman, 1986), the slow component was shown to generally begin to manifest within the last 30 s of the second minute in our fitting analysis (Chapter 2), which has been shown in previous work (Cannon *et al.*, 2007).

4.5.2 Potential sources of the $\dot{V}O_2$ slow component

Consistent with previous work (Gerbino *et al.*, 1996; MacDonald *et al.*, 1997; Whipp, 1994) we observed the development of a $\dot{V}O_2$ slow component during heavy exercise that was not present during moderate exercise. Several hypotheses, including peripheral and central factors, have been proposed to explain the excess of $\dot{V}O_2$ during the slow component. Direct measurements of (a-v)DO_2 have shown that the exercising legs are responsible for approximately 86% of the $\dot{V}O_2$ slow component (Poole *et al.*, 1991); therefore, the pulmonary $\dot{V}O_2$ slow component reflects to a large extent the changes in O_2 cost of the exercising muscles. However, increased cardiorespiratory work may still play a role (Aaron *et al.*, 1992; Carra *et al.*, 2003; Gaesser & Poole, 1996). While the primary anatomical source of

increased $\dot{V}O_2$ during the slow component appears to be the skeletal muscle, the precise mechanisms which account for the majority of excess $\dot{V}O_2$ remain unclear.

The prevailing hypothesis, originally posited by Shinohara and Moritani (1992), asserts that as Type I fibres fatigue, the recruitment of Type II muscle fibres increases in order to maintain appropriate force production. Since then, many supportive (Barstow *et al.*, 1996; Burnley *et al.*, 2002b; Krustrup *et al.*, 2004; Pringle *et al.*, 2003; Sahlin *et al.*, 2005) and contradictory (Cannon *et al.*, 2007; Garland *et al.*, 2006; Perrey *et al.*, 2003b; Scheuermann *et al.*, 2001) findings have been reported regarding the role of additional muscle fibre recruitment in the development the slow component.

In support of the progressive recruitment hypothesis, Sahlin *et al.* (1997) showed marked increase in the degradation of PCr and rise of energy requirement by the end of submaximal cycling exercise at 75% $\dot{V}O_2$max. Furthermore, at the whole body level, both proportion of Type II fibres (Barstow *et al.*, 1996) and plasma [NH_3^+], a marker of Type II fibre activation (Sabapathy *et al.*, 2005), were correlated with slow component amplitudes. Additional support for the progressive recruitment hypothesis comes from studies examining half-relaxation time (T2) using MRI. Saunders *et al.* (2000) observed an increase in the O_2 cost per unit of active muscle during high intensity, but not moderate intensity exercise, suggesting the recruitment of less efficient Type II muscle fibres. Furthermore, Endo *et al.* (2007) showed that in 10 muscles of the thigh, there is an increasing difference in the activation of muscles between the 3rd and 6th minute of exercise as intensity progressed from moderate, to heavy, to severe, and that this correlated significantly with $\dot{V}O_2$ slow component amplitude.

It has been suggested previously that agonist-antagonist co-activation might increase as exercise duration increases, resulting in reduced efficiency, increased internal work rate and elevated O_2 cost (Kellis, 1998). However, Borrani *et al.* (2003) showed that slow component is not a result of a change in external or internal mechanical work with fatigue and, in the current study, there was no evidence of increased co-activation as there was a decrease in the iEMG activity of the antagonistic BF muscle (Fig. 4.5). In trained subjects, co-activation may actually decrease with the progress of exercise (Hautier *et al.*, 2000). These subjects may have learned to use their agonist and antagonist muscles more efficiently to lower the O_2 demand and attenuate the $\dot{V}O_2$ slow component amplitude during the second exercise bout.

Another possible mechanism suggested for the development of the $\dot{V}O_2$ slow component is the accumulation of [La^-] with exercise progression. A high correlation between the increase in blood [La^-] and the excess of $\dot{V}O_2$ slow component has been observed (Roston *et al.*, 1987); however, similar to other studies (Koppo & Bouckaert, 2000; Poole *et al.*, 1994; Sahlin *et al.*, 2005) our data quite convincingly show that lactic acidosis could not be a factor, since blood [La^-] levels were significantly elevated throughout the second heavy bout ($H2_B$), despite the fact that the slow component amplitude was significantly reduced (Table 4.1). Sahlin *et al.* (2005) showed no changes in muscle or blood [La^-] from 3 to 10 min of heavy exercise where the slow component was manifested. Moreover, there were no correlations observed between changes in muscle and blood [La^-] and the amplitude of the $\dot{V}O_2$ slow component (Duffield *et al.*, 2007).

4.5.3 Effects of warm-up exercise on the $\dot{V}O_2$ slow component and EMG

Using a prior exercise model described in Chapter 2, there were graded reductions in the amplitude of the slow component by 22% and 49% when the heavy exercise bout was preceded by moderate and heavy exercise, respectively. It was hypothesized that changes in muscle recruitment might explain the graded attenuation in slow component amplitude following moderate and heavy exercise; therefore, in this study the same protocols were repeated in concert with surface EMG measurements. In this study, prior moderate and heavy exercise resulted in 19% and 40% reductions in the slow component amplitude [$\Delta \dot{V}O_{2(6-2)}$] during subsequent heavy exercise bouts, respectively.

As noted in some previous studies (Burnley *et al.*, 2002b; Garland *et al.*, 2006; Scheuermann *et al.*, 2001), cycling exercise involves activation of a large group of muscles within the thigh, making it necessary to examine EMG in more than one muscle; therefore, EMG activity was measured in multiple thigh muscles. It was observed that the moderate Δ iEMG $_{(6-2)}$ across the three knee extensor muscles in the control heavy bout ($H1_B$) was attenuated in a graded fashion during the $H2_A$ and $H2_B$ bouts following moderate and heavy warm-ups, respectively (Fig. 4.6). Principal components analysis was also employed in order to discern if alterations in recruitment within this muscle group as a whole may account for the development of the slow component. Although there were no significant differences in iEMG between the 2nd and 6th min of exercise, prior warm-up exercise slightly attenuated the overall activation increase (Δ iEMG $_{(6-2)}$), and this attenuation was moderately correlated with slow component amplitudes. This correlation between slow component amplitude and principal components analysis of Δ iEMG $_{(6-2)}$ from

all three thigh muscles provides some evidence that lower muscle activation in the second half of the bout may be associated with attenuation of the slow component. On the other hand, it cannot be overlooked that no significant change was noted in iEMG between the 2nd and 6th min suggesting that other factors, such as redistribution of the blood flow to the periphery and mismatching between O_2 supply and demand (Tordi *et al.*, 2003), reduced efficiency (Sahlin *et al.*, 2005) or elevated temperature (Krustrup *et al.*, 2001), likely play a role to the development of slow component in addition to changes in muscle fibre recruitment. Furthermore, the iEMG activities during the 20W baselines prior to each heavy and moderate bouts (Table 4.3) as well as, at the end of exercise during moderate exercise (Table 4.4) were the same between conditions, when $\dot{V}O_2$ was significantly higher after prior heavy warm-up (Table 4.1 and 4.2), and slightly elevated at the end of moderate exercise that followed the heavy warm-ups (Table 4.2) indicating that changes in muscle recruitment are insufficient to explain all differences in $\dot{V}O_2$ during exercise.

In this study, the attenuation of iEMG during a second heavy exercise bout following short periods of recovery from moderate and heavy warm-up is consistent with another study that examined EMG activity (Bailey *et al.*, 2009b). However, the additional observation of a high correlation between Δ iEMG $_{(6\text{-}2)}$ and the slow component may be explained by differences in analysis methods. Fitting analysis (Table 4.1) and the calculation of $\dot{V}O_{2(6-2)}$ clearly showed that the $\dot{V}O_2$ slow component amplitude was truly diminished without changes in the primary phase amplitude. In contrast, other studies have observed larger primary phase amplitudes associated with an increase in fibre recruitment as suggested by

increased iEMG, early in exercise (Burnley *et al.*, 2002b; Sahlin *et al.*, 2005). In this study, there was also a reduction in the slow component amplitude even after a short bout of moderate exercise, similar to that shown after a longer period of moderate exercise (Koppo & Bouckaert, 2000; Koppo & Bouckaert, 2002).

While iEMG data provide some evidence of changes in muscle fibre recruitment, the MPF data could not discern the types of muscle fibres being recruited. Additionally, there was a decreasing trend in MPF for all extensor muscles within heavy bouts (Fig. 4.4). This trend and the lack of correlation between MPF and the slow component is in contrast to a temporal relationship observed between the beginning of the slow component and increases in MPF in trained runners (Borrani *et al.*, 2001). The graded increases between each condition are suggestive of increasing fibre recruitment throughout the exercise bout from start to finish. It was interesting that in heavy exercise MPF tended to decrease early in exercise and then leveled off in contrast to moderate exercise where no changes occurred throughout the exercise. The early decrease in MPF observed during the heavy bouts may be due to a greater force needed to overcome the inertia of the cycle ergometer at the onset. Indeed, MPF toward the end of the control bouts was similar between moderate and heavy exercise.

The graded reductions in the $\dot{V}O_2$ slow component were observed to occur in concert with accelerated primary phase kinetics. It was shown in Chapter 2 that the acceleration of primary phase $\dot{V}O_2$ kinetics during heavy exercise was related, in part, to improved O_2 delivery, since $\dot{Q}$ kinetics were similarly accelerated by both moderate and heavy warm-ups. If bulk O_2 delivery and matching of O_2 supply to demand in the microvasculature is improved early in exercise, this may have

important implications for any putative fibre type recruitment shift, since the energetic state of the cell is determined by the interplay of oxygen partial pressure, enzyme/substrate levels, and ADP concentrations (Hughson, 2009; Hughson *et al.*, 2001; Tschakovsky & Hughson, 1999). Reduced metabolic fatigue early in exercise might generate a lower demand for additional fibre recruitment, and therefore reduce the $\dot{V}O_2$ slow component. Burnley *et al.* (2002b) suggested that greater recruitment of Type II muscle fibres early in exercise might reduce the force demand and metabolic strain per single fibre; however, this response was not observed in this study or recent studies from the same group (Bailey *et al.*, 2009b; DiMenna *et al.*, 2008). Additionally, Layec *et al.* (2009) showed that during second heavy exercise bout, muscle oxygenation, as measured by NIRS, was higher and muscle [ADP], measured by ^{31}P-MRS, was lower while ATP production remained similar to control. These authors raised the possibility that this could enhance the phosphorylation potential of ATP compared to control conditions. The improved metabolic conditions may limit the reduction in contractile efficiency (Rossiter *et al.*, 2001; Zoladz *et al.*, 2008), thereby reducing the O_2 cost of force production and resulting in a smaller slow component.

4.5.4 Impact of prior exercise on moderate exercise oxygen consumption

As expected, during moderate exercise there was no evidence of the $\dot{V}O_2$ slow component and no changes in MPF (Fig. 4.8) and iEMG (Fig. 4.9) throughout the exercise bouts with and without prior heavy warm-up. There was; however, a graded and constant increase in MPF after one ($M3_A$) and two ($M3_B$) heavy warm-ups bouts compared to control (Fig. 4.8). While Gonzales and Scheuermann (2008) observed an elevation in median power frequency during a moderate bout after

heavy warm-up exercise, they saw no associations with changes in $\dot{V}O_2$ or $\dot{V}O_2$ gain. In contrast in the current study, a consistent increase in MPF was observed in all muscles studied. Subjects with a higher proportion of Type II fibres tend to show lower gain during moderate exercise transitions (Pringle *et al.*, 2003). The higher absolute $\dot{V}O_2$ during moderate exercise after heavy warm-up (Table 4.2) could be explained by fatigue resulting in higher O_2 cost per ATP generated (Rossiter *et al.*, 2001) or the recruitment of additional muscle fibres which have a lower phosphate to O_2 ratio (Crow & Kushmerick, 1982).

There may be several possible reasons for observing a larger gradation in MPF compared to the heavy exercise bouts. First, due to the size principle of motor unit recruitment (Henneman *et al.*, 1965) it would be expected that fewer Type II fibres would be employed during control moderate exercise, so any increase in muscle fibre recruitment after heavy exercise might then have a more dramatic effect on overall MPF. Secondly, the warm-ups involved for heavy bouts were one moderate or one heavy bout; whereas the moderate bouts were preceded by one moderate plus one heavy bout, or two heavy bouts possibly resulting in elevated muscle temperature (Krustrup *et al.*, 2001) that could have increased conduction velocity (Bigland-Ritchie & Woods, 1984).

4.6 Limitations

While surface EMG is a well-established method for measuring electrical activation of muscle fibres and a tight relationship between iEMG and O_2 consumption has been observed (Bigland-Ritchie & Woods, 1974), there are several considerations that should be made when employing its various derivatives to make statements about changes in muscle activity and relating them to the slow

component. First, as with breath-by-breath $\dot{V}O_2$ measurements, surface EMG measures contain significant variability, which needs to be reduced in order to discern any real physiological changes (Farina & Mesin, 2005). The use of repeated measures on both $\dot{V}O_2$ and EMG increases the confidence that our results might represent true changes in muscle recruitment and that the relation to ~~VO2~~ $\dot{V}O_2$ is accurate. Still, the resolution of EMG does not necessarily allow the ability to discern small changes in motor unit recruitment. Electrode placement between days may have been different and therefore may have affected EMG signals, however the baseline EMG did not vary between days and protocols verify that care was taken in precise electrode placement. From a theoretical standpoint, an increase in MPF measured at the surface should correspond to an increase in conduction velocity within the muscle (Stulen & DeLuca, 1981), indicating the recruitment of larger, faster muscle fibres (Kupa *et al.*, 1995). However, any increase in conduction velocity related to increased recruitment of fast Type II fibres may be overwhelmed by the MPF depressing effects of higher extracellular $[K^+]$, leading to no change in overall MPF measured at the surface (Fortune & Lowery, 2009). Additionally, intramuscular temperature increases greatly during exercise and changes in muscle temperature could certainly affect MPF by increasing conduction velocity (Gamet *et al.*, 1993). Therefore, it is unclear how much of the gradation in MPF responses were due to actual changes in recruitment and to what degree changes in body temperature influenced the frequency, since both variables might alter MPF similarly.

4.7 Conclusion

It was observed that the $\dot{V}O_2$ slow component amplitude and increase in iEMG during heavy cycling exercise were attenuated in a graded manner following moderate and heavy warm-up. There was a moderate correlation between the attenuation in the $\dot{V}O_2$ slow component and the simultaneous changes of iEMG across the extensor muscles of the thigh when all the data from all subjects across all exercise conditions were clustered. Pooling of results from principal components analysis across conditions might not be appropriate so further work is required to explore the hypothesis that changes in progressive fibre recruitment during heavy exercise could affect the amplitude of the $\dot{V}O_2$ slow component. Furthermore, the increased O_2 cost of moderate exercise after a heavy warm-up was associated with a higher MPF of contractions in the thigh extensor muscles. However, the effect of temperature on increased MPF during moderate exercise following prior heavy exercise should be taken in consideration. Despite some limitations, measurement of leg muscle EMG may provide some evidence for increased muscle fibre recruitment during heavy and moderate exercise that follow prior heavy warm-up.

Chapter 5
General discussion and future considerations

5.1 General discussion

Oxygen uptake kinetics reflect the dynamic balance between O_2 transport and utilization mechanisms affecting muscle energetics during work rate transitions. The rate of increase in oxidative phosphorylation at the onset of exercise has been debated to be limited by the adaptations of O_2 transport and O_2 utilization mechanisms (Grassi, 2001; Hughson, 2009; Poole *et al.*, 2008; Tschakovsky & Hughson, 1999; Whipp *et al.*, 2005). Interventions that can accelerate $\dot{V}O_2$ kinetics will increase the proportional contribution of oxidative metabolism to the total energy demands and could lead to enhanced exercise performance. Prior heavy exercise is an experimental paradigm that accelerates $\dot{V}O_2$ kinetics at the onset of subsequent exercise, potentially through several mechanisms that affect both O_2 transport (Faisal *et al.*, 2010; Gerbino *et al.*, 1996; MacDonald *et al.*, 1997) and O_2 utilization (Bangsbo *et al.*, 2000; Grassi *et al.*, 1996; Gurd *et al.*, 2006). Alternatively, prior circulatory occlusion is an intervention that elevates muscle blood flow prior to the onset of subsequent exercise (Carlsson *et al.*, 1987) and has been shown to accelerate pulmonary $\dot{V}O_2$ kinetics (Paganelli *et al.*, 1989; Walsh *et al.*, 2002).

The main observation of this thesis is that simultaneous measurements of O_2 transport and O_2 utilization can provide considerable insight into the control mechanisms regulating $\dot{V}O_2$ kinetics at the onset of exercise. In Chapter 2 the time course of the increase in $\dot{Q}$ and O_2 uptake during the transition to heavy and moderate cycling exercise was characterized. Prior moderate and heavy exercise

was applied to examine whether these interventions would result in more rapid $\dot{V}O_2$ and $\dot{Q}$ responses in subsequent moderate and heavy exercise. An interesting finding from this study was that the temporal responses of $\dot{Q}$ and alveolar O_2 uptake seemed to parallel each other at the onset of heavy exercise, and to a lesser extent during moderate exercise. This finding is in agreement with a tight coupling between the kinetics of capillary blood flow measured by near-infrared spectroscopy (NIRS) and $\dot{V}O_{2mus}$ during both moderate and heavy cycling exercise (Ferreira *et al.*, 2005a).

Contrary to the majority of previous cycling studies (i.e. Burnley *et al.*, 2000; Gerbino *et al.*, 1996; Jones *et al.*, 2006; Koppo *et al.*, 2003), in this study there was actual acceleration in $\dot{V}O_2$ kinetics during the second heavy bout as shown by a smaller τ_2. For the first time, prior moderate exercise was shown to accelerate the $\dot{V}O_2$ kinetics and reduce τ_2 in subsequent heavy exercise in endurance athletes. Faster $\dot{V}O_2$ kinetics following prior moderate and heavy exercise were coupled with similar acceleration in $\dot{Q}$ kinetics. A faster increase in $\dot{Q}$ at the onset of heavy exercise can impact the regulation of O_2 uptake. Greater O_2 delivery can improve the oxidative phosphorylation contribution to energy production, while reducing the O_2 deficit and cellular homeostasis disturbance (MacDonald *et al.*, 1997). The proposed mechanism involves an increase in PaO_2 as a function of increased O_2 delivery (Hughson, 2009; Tschakovsky & Hughson, 1999) and a subsequent reduction in PCr degradation and substrate level phosphorylation (Rossiter *et al.*, 2001). The acceleration of $\dot{V}O_2$ kinetics at the onset of moderate exercise that followed a heavy warm-up was associated with a small increase in $\dot{Q}$ and greater

(a-v)DO_2 differences. These results do not rule out the potential role of prior exercise to stimulate the O_2 utilization machinery and regulate $\dot{V}O_2$ kinetics during moderate exercise. Gurd *et al.* (2006) showed that prior heavy exercise accelerated the VO_2 kinetics during a subsequent moderate bout (τ_2 reduced from 24 to 19 s) due to faster activation of pyruvate dehydrogenase.

Numerous non-invasive techniques such as foreign gas rebreathing (Bell *et al.*, 2003; Hunt *et al.*, 1997; Johnson *et al.*, 2000), Doppler echocardiography (Ihlen *et al.*, 1987) and vascular impedance cardiography (Perrey *et al.*, 2003a; Yoshida & Whipp, 1994), have been applied to measure $\dot{Q}$ during exercise. However, these techniques were limited to steady state exercise. The finger cuff Modelflow technique (Wesseling *et al.*, 1993) offered a continuous beat-to-beat estimate of SV and $\dot{Q}$ during transitions to moderate and heavy cycling exercise with a high time resolution thereby providing valuable information on the acute time course of changes in $\dot{Q}$. ModelFlow $\dot{Q}$ estimation was validated during steady-state exercise using C_2H_2 rebreathing (Faisal *et al.*, 2009). One of the most interesting findings in this study was the observation of a rapid SV overshoot at the onset of moderate and heavy exercise. The SV overshoot reflects the sudden increase in venous return mediated primarily by the action of the muscle pump during upright cycling exercise (Sheriff *et al.*, 1993; Tschakovsky *et al.*, 1996).

The observation of accelerated $\dot{V}O_2$ and $\dot{Q}$ during heavy cycling exercise following prior moderate and heavy exercise shown in the first study (Chapter 2) led to further investigations into the impact of elevated muscle blood flow prior to the onset of heavy exercise on blood flow and $\dot{V}O_{2mus}$ kinetics. In Chapter 3 the

dynamic responses of FBF, measured directly by Doppler ultrasound, and $\dot{V}O_{2mus}$ during the transition to heavy hand-grip exercise were investigated following prior heavy exercise and circulatory occlusion to examine whether these interventions would result in a similar rapid blood flow response and acceleration in $\dot{V}O_{2mus}$ during subsequent heavy exercise. In agreement with previous studies that employed a prior heavy exercise paradigm (Fukuba *et al.*, 2007; Hughson *et al.*, 2003; MacDonald *et al.*, 2001b), it was observed that prior heavy exercise resulted in a faster increase in muscle blood flow and $\dot{V}O_{2mus}$ at the onset of a subsequent heavy exercise bout (Faisal *et al.*, 2010). This finding supported the original hypothesis that increased oxidative metabolism at the onset of exercise is linked to increased O_2 delivery to the working muscles. It has been shown that the degree of tissue oxygenation may play a role in modulating the levels of other regulators of oxidative phosphorylation such that altered tissue oxygenation by reduced PaO_2 will result in a greater change in the regulators of cellular respiration (e.g. phosphocreatine, ADP) to achieve a given $\dot{V}O_{2mus}$ (Haseler *et al.*, 1998; Hogan *et al.*, 1992). There was a direct linear relationship between the PCr degradation and the magnitude of the O_2 deficit during submaximal exercise while breathing gases with varying inspired PaO_2 (Linnarsson *et al.*, 1974). Additionally, Hughson (2005; 2009) suggested theoretical models that illustrate the impact of dynamic changes in the PaO_2 on enzyme activity, metabolic substrates and how the oxidative metabolism at the onset of exercise is modulated by a dynamic interaction between O_2 delivery and utilization mechanisms.

Chapter 3 also presented results from an alternative model designed to enhance muscle blood flow and O_2 delivery at the onset of exercise based on prior

circulatory occlusion. Although prior occlusion elevated blood flow prior to exercise onset, blood flow and O_2 extraction were depressed in the early phase of exercise, resulting in slower $\dot{V}O_{2mus}$ dynamics with the onset of heavy exercise (Faisal *et al.*, 2010). These results contrast with previous studies that reported faster pulmonary $\dot{V}O_2$ kinetics following circulatory occlusion (Paganelli *et al.*, 1989; Walsh *et al.*, 2002). Based on previous observations that post-occlusion reactive hyperaemia was significantly elevated by indomethacin and ibuprofen, inhibitors of cycloxygenase (Naylor *et al.*, 1999), it was speculated that vasoconstrictor PGs might have contributed to the suppression of muscle blood flow during exercise following ischemia. Ingestion of a high dose of ibuprofen prior to the circulatory occlusion protocol was able to restore FBF and $\dot{V}O_{2mus}$ to control levels, although (a-v)DO_2 remained suppressed relative to the control condition. These results suggest that blood flow was influenced by a vasoconstrictor prostaglandin mechanism, and perhaps prior occlusion had an independent effect on muscle metabolism.

Chapter 4 described a study designed to investigate whether changes in muscle activity as assessed by EMG are related to changes in $\dot{V}O_2$ slow component amplitude and increased O_2 cost during moderate exercise after heavy warm-up. Although there was no change in iEMG activity during each of the heavy and moderate exercise conditions, there was a significant increase in O_2 consumption between the 2nd and 6th min of heavy exercise and throughout moderate exercise after prior heavy warm-up. This observation may suggest that additional factors other than the progressive recruitment of muscle fibres may be involved in the development of slow component during heavy exercise and elevate steady state

$\dot{V}O_2$ during moderate exercise after the heavy warm-up. Interestingly, applying the principal component analysis to aggregate the EMG activity in all muscles into one factor showed a significant correlation between Δ iEMG $_{(6\text{-}2)}$ activity in the knee extensor muscles and $\dot{V}O_2$ slow component amplitude. Additionally, the increase of O_2 cost during moderate exercise following the heavy warm-up was associated with increase in MPF. These later observations provide some evidence for the progressive recruitment of type II muscle fibres during the slow component and throughout the moderate exercise following the heavy warm-up.

This thesis focused on the study of O_2 transport and O_2 utilization mechanisms in highly fit young men; therefore, the data might not reflect the general population, but rather a particular subset of the population with well-adapted cardiovascular and metabolic responses. Training status might be an important factor that has rarely been addressed in the studies employing experimental models designed to improve O_2 availability (i.e. prior exercise or prior ischemia) in examining blood flow and $\dot{V}O_2$ kinetics. There is some evidence that subjects of higher fitness (Knight *et al.*, 1993) are more sensitive to O_2 delivery limitations than subjects of lower fitness (Cardus *et al.*, 1998)to improve their maximal oxygen uptake. Although, untrained healthy individuals with slower $\dot{V}O_2$ kinetics have shown greater acceleration of τ_2 during moderate exercise after prior heavy warm-up than those who have fast $\dot{V}O_2$ kinetics (Gurd *et al.*, 2005). Elite athletes might be more susceptible to limitations in oxygen delivery at the onset of heavy exercise. Endurance athletes tend to show fast $\dot{V}O_2$ kinetics (Berger *et al.*, 2006c) and a high O_2 extraction fraction during exercise (Kalliokoski *et al.*, 2001)

that may slightly be enhanced by prior warm-up. Tordi *et al.* (2003) reported a faster primary component of $\dot{V}O_2$ kinetics (τ_2 reduced from ~29 to ~22 s) during subsequent heavy cycling exercise following prior multiple-sprint exercise in well trained athletes because O_2 delivery was also enhanced. Similarly, endurance athletes in the current study have shown an acceleration in $\dot{V}O_2$ and $\dot{Q}$ kinetics (τ_2 reduced from ~27 to ~22 s). Although individuals show large variation in matching blood flow to $\dot{V}O_{2mus}$ at the onset of exercise, endurance athletes tend to have a better matching between muscle blood flow and $\dot{V}O_{2mus}$ than untrained men (Kalliokoski *et al.*, 2005). More recently, Hernandez *et al.* (2010) showed that a prior bout of contractions speeds blood flow and $\dot{V}O_{2mus}$ onset kinetics during a subsequent bout in highly oxidative skeletal muscle. Additionally, Bailey *et al.* (2010) have shown that 4 weeks of inspiratory muscle training were able to reduce the respiratory muscles fatigue, increased blood flow to the exercising muscle and improved $\dot{V}O_2$ kinetics following the onset of high intensity exercise suggesting that enhanced O_2 delivery enabled the increase of oxidative phosphorylation during the transition to heavy exercise. Therefore, it could be inferred that O_2 delivery will play a greater role to accelerate $\dot{V}O_2$ kinetics in trained humans who have a well adapted respiratory system.

5.2 Conclusion

In summary, the results of the studies presented in this thesis ~~clearly~~ provided evidence that enhanced O_2 supply to the exercising muscles following prior exercise enabled a faster increase in the rate of oxidative phosphorylation during the transition to heavy exercise in trained humans. On the other hand, prior circulatory occlusion depressed $\dot{V}O_{2mus}$ kinetics by retarding the increase in muscle blood flow and reducing O_2 extraction. The surface electromyography provided partial evidence for the role of muscle fibre recruitment to the development of slow component and increased O_2 cost during moderate exercise following a heavy warm-up.

Warm-up intervention is a regular routine to enhance the performance during subsequent training sessions or during competition. Although trained subjects would be expected to show small acceleration in $\dot{V}O_2$ kinetics following prior warm-up, this increase may provide a boost to the performance during an Olympic medal or world record attempt. Therefore, the investigation of different warm-up interventions that could accelerate the rate of oxidative phosphorylation is very essential for sport performance.

5.3 Future considerations

The studies enclosed in this thesis addressed mechanisms regulating $\dot{V}O_2$ kinetics at the onset of exercise. Several interventions were applied to manipulate the rate of increase in O_2 delivery prior to exercise. The measurements of O_2 transport were primarily non-invasive in nature. The following recommendations address specific methodological issues in this thesis.

In Chapter 2, estimates of $\dot{Q}$ were obtained during cycling exercise by the beat-to-beat method of finger arterial blood pressure by the Modelflow algorithm. The derived kinetics of $\dot{Q}$ were used as a surrogate marker for leg blood flow kinetics during cycling exercise. Increased $\dot{Q}$ after heavy warm-up indicated a partial redistribution of blood flow to serve thermoregulatory demands, and therefore one cannot be sure of the flow to the working muscle. However, previous studies (Hughson *et al.*, 2003; Krustrup *et al.*, 2001; MacDonald *et al.*, 2001b) as well as the current research in Chapter 3 have directly measured an increase in blood flow to the muscle following a heavy warm-up, which supports the notion that at least some of the increase in $\dot{Q}$ observed in the present study is directed to the working muscle. Additionally, ModelFlow $\dot{Q}$ was validated during "steady state" phases of exercise; it was not possible to calibrate Modelflow $\dot{Q}$ during dynamic transitions at the onset of exercise. The validation of Modelflow $\dot{Q}$ during exercise transition will be of great advantage in the exercise modalities where direct measurements of blood flow is not possible; though we know of no method by which this could be performed.

In Chapter 3 of this study, muscle blood flow and O_2 extraction were depressed following prolonged circulatory occlusion. Oral administration of a prostaglandin synthase inhibitor was able to restore muscle blood flow at the onset of exercise that followed prolonged circulatory occlusion to the control level (Faisal *et al.*, 2010), and others have demonstrated marked reductions in SBF during reactive hyperemia (Binggeli *et al.*, 2003). These observations suggest that ischemia might have disparate effects on perfusion in different tissues. It will be interesting to examine whether circulatory occlusion, with the potential release of the vasoconstrictor thromboxane A_2 (Mathieson *et al.*, 1983) has a systemic effect on tissue perfusion by measuring muscle and SBF during exercise when ischemia followed by reperfusion has been applied to a distant, non-exercising limb.

Repeated episodes of ischemia followed by reperfusion (ischemic preconditioning) represent an endogenous protective mechanism that delays cell injury. Recent observations have shown that remote ischemic preconditioning improved endothelial function (Kharbanda *et al.*, 2002), decreased myocardial infarction effect (Botker *et al.*, 2010) as well as, increased maximal O_2 consumption and maximal power output during subsequent incremental cycling test (de Groot *et al.*, 2010). It would be interesting to examine muscle blood flow and $\dot{V}O_{2mus}$ kinetics responses following short periods of intermittent occlusion rather than prolonged period of ischemia.

Furthermore, changes in the metabolic environment and PaO_2 during and after intermittent and prolonged ischemia as well as during subsequent exercise also deserve future attention. Magnetic resonance spectroscopy is a powerful, non-invasive method that could offer a great potential to investigate human

biochemistry. This technique would help to assess PaO_2 and provide a clear picture about the time course of PCr degradation during exercise transition following prolonged ischemia. Duplicating the prolonged ischemia study using magnetic resonance spectroscopy would provide more precise information about the metabolic environment and the mechanisms that could contribute to the depressed O_2 extraction shown in the current study.

Appendix A

Oxygen uptake and blood pressure regulation at the onset of exercise: Interaction of circadian rhythm and priming exercise

This appendix is the basis for the manuscript accepted (September 28, 2010) for publication in the *American Journal of Physiology - Heart and Circulation.* Yet it is not available online as an Article in Press

A.1 Overview

Circadian rhythm has an influence on several physiological functions that contribute to athletic performance. We tested the hypothesis that circadian rhythm would affect BP responses but not $\dot{V}O_2$ kinetics during the transitions to moderate and heavy cycling exercises. Nine male athletes ($\dot{V}O_{2peak}$: 60.5 ± 3.2 ml/kg/min) performed multiple rides of two different cycling protocols, involving 6-minute bouts at moderate and heavy intensities, in the morning (7 am) and evening (5 pm). Breath-by-breath $\dot{V}O_2$ and beat-by-beat BP estimated by finger cuff plethysmography were measured simultaneously throughout the protocols. Circadian rhythm did not affect $\dot{V}O_2$ onset kinetics during either moderate (M) or heavy (H) exercise (τ_2 M: morning 22.5 ± 4.6 s vs. evening 22.2 ± 4.6 s; τ_2 H: morning 26.0 ± 2.7 s vs. evening 26.2 ± 2.6 s; $P > 0.05$). Priming exercise induced the same robust acceleration in $\dot{V}O_2$ kinetics during subsequent moderate and heavy exercise in the morning and evening. A novel finding was an overshoot in the estimate of BP from finger cuff plethysmography in the first minutes of each moderate and heavy exercise bout. After the initial overshoot, BP declined in association with increasing SBF between the 3rd and 6th minute of the exercise bout. Priming exercise showed a greater effect in modulating the BP responses in the evening. These findings suggest that circadian rhythm interacts with priming exercise to attenuate BP responses during exercise with a greater influence in the evening due to increased SBF.

A.2 Introduction

Circadian rhythm has shown potent affects on a wide range of cardiovascular functions (Guo & Stein, 2003). However, only two studies have examined the effect of circadian rhythm on $\dot{V}O_2$ and BP during exercise. Circadian rhythm displays small effects on resting $\dot{V}O_2$; however, this influence weakens and disappears with increasing exercise intensity (Reilly & Brooks, 1990). The previous studies that examined the effect of circadian rhythm on $\dot{V}O_2$ kinetics have offered diametrically opposing results. Carter *et al.* (2002) reported no diurnal variation of $\dot{V}O_2$ kinetics during either moderate or heavy exercise, while Brisswalter *et al.* (2007) showed a robust acceleration of $\dot{V}O_2$ kinetics and a higher steady-state $\dot{V}O_2$ during moderate exercise in the evening. It is unclear, then, whether or not $\dot{V}O_2$ kinetics are affected by circadian rhythm. It has been shown in Chapter 2 that prior moderate and heavy exercise can accelerate $\dot{V}O_2$ kinetics in endurance athlete, but it is unknown if these responses would be affected by the time of day.

The circadian variation in BP has been known for over 100 years (Hill & Lond, 1898), with the lowest BP during sleeping, a morning "surge" around the waking hours and the highest BP at midday (Kario *et al.*, 2003; Millar-Craig *et al.*, 1978; Verdecchia *et al.*, 1990). Recent studies have shown a greater effect of circadian rhythm on post exercise BP responses in the afternoon (Jones *et al.*, 2008a; Jones *et al.*, 2008b). However, no previous study has investigated the effect of circadian rhythm on BP during exercise transitions. Altered SBF may alter the control of arterial BP, particularly after warm-up when the thermoregulatory mechanisms are engaged. Exercise induces a specific heat load that results in

smaller temperature increases and larger cutaneous vasodilatory responses in the afternoon (Aldemir *et al.*, 2000; Waterhouse *et al.*, 2007) suggesting a potential for greater BP reduction in the afternoon. This attenuation of BP response may sustain through subsequent daily activity and exercise (MacDonald *et al.*, 2001a).

$\dot{V}O_2$ onset kinetics and beat-by-beat BP in association with SBF were examined at two different times of day (morning and evening) and during different protocols involving transitions from light exercise to moderate and heavy exercise. It has been hypothesized that circadian rhythm would attenuate the BP responses during moderate and heavy exercise in the evening due to greater thermoregulatory effects but would not affect $\dot{V}O_2$ kinetics during either moderate or heavy exercise. Additionally, prior moderate and heavy exercise would have different effects on BP responses during subsequent exercise in the morning compared to the evening, but would have the same effects on $\dot{V}O_2$ kinetics during subsequent exercise regardless of time of day.

A.3 Methods

Subjects

Nine men who regularly participated in endurance training activities (age: 22.3 ± 2.7 years, height: 178.6 ± 7.8 cm, weight: 71.9 ± 6.5 kg, $\dot{V}O_{2peak}$: 4325 ± 516 ml/min; mean ± SD), same subjects participated in the study in Chapter 2, gave consent to participate in this study following full description of the protocols. To minimize the between-subject variability in aerobic fitness, an inclusion criterion of $\dot{V}O_{2peak}$ > 55 ml/kg/min was used during recruitment. This study was approved by the Office of Research Ethics at the University of Waterloo.

Experimental design

All subjects first performed an incremental exercise test to volitional exhaustion on an electrically-braked cycle ergometer (Excalibur, Lode, Groningen, Netherlands) to determine their $\dot{V}O_{2peak}$ and ventilatory threshold (VT) as described in Chapter 2. All subjects performed multiple rides of two different cycling protocols either in the early morning (7am) or early evening (5pm). Both protocols involved 6-minute bouts at moderate (M; 80% VT) and heavy (H; 85% $\dot{V}O_{2peak}$) intensities (M; 146 ± 33 W and H; 266 ± 35 W) interspersed with 6-minute bouts at 20 W. Moderate and heavy work rates were calculated from the incremental test and assigned names identifying the intensity, the bout number within the protocol, and the protocol itself as follows: Protocol A = moderate ($M1_A$) followed by heavy ($H2_A$) followed by moderate ($M3_A$); Protocol B = heavy ($H1_B$) followed by heavy ($H2_B$) followed by moderate ($M3_B$). The protocols were shown previously in Chapter 2 (Fig. 2.1).

Pedaling frequency was maintained at 80 rpm throughout each protocol and the same cycle ergometer and individual set up (handle bar and seat position) was used throughout the study. Subjects were asked to report to the laboratory in a rested, hydrated state and abstain from consuming caffeine for 12 h and alcohol for 24 h prior to test sessions. All rides were performed at least 24 h after the participants' last exercise regimen and 36 h following their last study ride. The tests were completed in a quiet, air-conditioned laboratory at a temperature of ~23°C.

Breath-by-breath oxygen uptake

Pulmonary gas exchange was measured continuously in at least 4 rides of each protocol (morning and evening) on a breath-by-breath basis (First Breath, Waterloo, ON) by measuring inspired and expired concentrations of O_2, CO_2, and N_2 via mass spectrometry (Innovision, Amis 2000, Odense, Denmark). Gas volumes and concentrations were measured as described in Chapter 2.

Blood pressure and cardiac output

Arterial BP was measured continuously in all protocols using finger arterial pressure pulse wave analysis (Finometer, Finapres Medical System, Arnhem, Netherlands), which also estimated $\dot{Q}$ beat-by-beat. In order to minimize the effect of arm and hand movement on BP and $\dot{Q}$ signals, a sling apparatus supported the left arm slightly below heart level while subjects cycled. An appropriately sized cuff was wrapped around the distal end of the third or fourth digit to measure finger artery pressure, and a glycerin column and pressure transducer were used to correct for hydrostatic pressure differences between the level of the hand and the heart. The Finometer used a transfer function to estimate the brachial pressure waveform from finger pressure waveform. During setup, a return-to-flow

calibration, using an automated arm-cuff, was used to further validate the transformed pressure waveform (Bos *et al.*, 1996). To maintain signal validity throughout the rides, an automatic physiologic calibration ("physiocal") was run periodically after ≤ 70 beats to calibrate the finger artery size at which finger cuff air pressure equals finger arterial blood pressure. The BP, $\dot{Q}$ and electrocardiogram (ECG) (Pilot 9200, Colin Medical Instruments, San Antonio, TX) signals were sampled at 1 kHz (PowerLab, AD Instruments, Colorado Springs, CO). The Finometer's cardiac output estimations were validated against an acetylene rebreathing technique as described in Chapter 2.

Skin blood flow

In one ride of each protocol (morning and evening), relative forearm SBF was estimated continuously by a laser Doppler probe (MoorLAB, Moor Instruments Ltd, Devon, UK) placed over the wrist extensors, 5cm distal to the lateral epicondyle.

Data analysis

Oxygen uptake kinetics

The analyses of $\dot{V}O_2$ kinetics were exactly the same as described in chapter 2. The moderate and heavy bouts were curve fitted by two- (phases I and II) and three-component (phases I, II and III) exponential models respectively.

Blood pressure and cardiac output

The $\dot{Q}$ signal was shifted -1s and 1 beat backward in order to compensate for the Finometer's internal digital signal processing delay. The BP, $\dot{Q}$, and HR signals were linearly interpolated at 1-s intervals, and the rides were time aligned and averaged together to yield a single data set for each subject in each protocol at each time of day.

Total peripheral resistance and forearm skin blood flow

Total Peripheral Resistance (TPR) throughout the exercise tests was calculated as $\dot{Q}$/MAP. The SBF signal was normalized to the average of resting SBF, measured over the 5 min immediately prior to the start of each exercise test.

Statistical analysis

A two-way ANOVA with repeated measures was used to determine significant differences in $\dot{V}O_2$ kinetics and BP responses during all moderate and heavy bouts. When significant effects were observed, the Tukey post hoc test was used for comparisons. All data are expressed as means ± SD and a probability of $P < 0.05$ was accepted as statistically significant. The data were analyzed using Statistical Analysis Software package 9.1 (SAS Institute, Cary, NC).

A.4 Results

Oxygen uptake kinetics

The fitting parameters for $\dot{V}O_2$ kinetics in the heavy control ($H1_B$) and moderate control ($M1_A$) bouts were identical in the morning and evening sessions (Fig. A.1, Tables A.1 and A.2), revealing no effect of circadian rhythm on $\dot{V}O_2$ kinetics in young trained men. Moreover, priming exercise modified the $\dot{V}O_2$ kinetics in subsequent heavy and moderate bouts at 7am and 5pm to similar extents. During a heavy exercise bout that followed either prior moderate ($H2_A$, 22.5 ± 4.0 s) or heavy ($H2_B$, 21.8 ± 2.8 s) warm-up, $\dot{V}O_2$ kinetics were accelerated compared to control ($H1_B$ 26.0 ± 2.7 s) in morning sessions with similar results during the evening sessions, as shown by the significantly smaller τ_2 values (Fig. A.2, Table A.1). Similarly, in a moderate exercise bout that followed either one ($M3_A$) or two ($M3_B$) heavy bouts, τ_2 values were significantly lower than control ($M1_A$) in both morning and evening sessions (Fig. A.3, Table A2).

Blood pressure

During all heavy and moderate cycling exercise bouts (morning and evening), there was an overshoot of the peak BP response through the second minute of exercise with lower values by the end (6th minute) of exercise (Figs. A.4 and A.5, Tables A.3 – A.8). The BP response tended to be lower throughout the last three minutes in both control moderate and heavy exercise bouts in the evening. Generally, there were no differences between the morning and evening sessions in the baseline, peak, or end exercise BP responses during the control bouts ($H1_B$ and $M1_A$), the heavy bout that followed prior heavy exercise ($H2_B$) or the moderate bout that followed two heavy bouts ($M3_B$) (Tables A.3 – A.8). However, there were differences

between the morning and evening sessions in BP responses during the heavy bout that followed a moderate warm-up ($H2_A$) where end exercise BP was lower during the evening compared to the morning ($P < 0.05$ for SBP, MAP and DBP, Fig. A.4, Tables A.3 – A.5), with greater decline in the SBP from the overshoot peak to end exercise during the evening compared to the morning ($P < 0.05$, Table A.3). In moderate exercise that followed one heavy bout ($M3_A$), BP was more like exercise that followed two heavy bouts ($M3_B$) in the evening (Fig. A.5). Peak MAP and DBP were significantly lower during the evening ($P < 0.05$ for both, Tables A.7 and A.8). At the end of the $M3_A$ exercise bout, SBP, MAP and DBP were all significantly lower in the evening compared to morning ($P < 0.05$, Tables A.6 – A.8). The decline in the SBP from the overshoot peak to end exercise in the control ($M1_A$) was more evident during the evening compared to the morning ($P < 0.05$, Table A.6). Prior heavy warm-up had a greater effect on attenuating the initial rise in BP responses during subsequent heavy ($H2_B$) and moderate ($M3_A$ and $M3_B$) cycling in the morning (Tables A.3 – A.8). Prior moderate warm-up showed a similar effect as prior heavy exercise in attenuating the BP during subsequent heavy bouts in the evening. Likewise, prior one heavy bout showed the similar effect as prior two heavy bouts in attenuating the BP response during subsequent moderate bouts in the evening.

Cardiac output

In both morning and evening sessions, baseline values for $\dot{Q}_{MF}$ were significantly elevated in a heavy bout that followed prior heavy exercise ($H2_B$) compared to both control ($H1_B$) and a heavy bout that was preceded by moderate exercise ($H2_A$) ($P < 0.05$, Fig. A.6 and A.7, Table A.9). The moderate exercise bouts that followed one prior heavy bout ($M3_A$) and two prior heavy bouts ($M3_B$) had

higher baseline $\dot{Q}_{MF}$ than the no warm-up condition ($M1_A$) ($P < 0.05$, Fig. A.8 and A.9, Table A.10). By the end of exercise, there were no differences between the three moderate bouts in the morning, but $M3_B$ had a higher $\dot{Q}_{MF}$ than $M1_A$ in the evening ($P < 0.05$, Table A.10).

Total peripheral resistance

Baseline values for TPR were significantly lower in a heavy bout that followed prior heavy exercise ($H2_B$) compared to both control ($H1_B$) and a heavy bout that was preceded by moderate exercise ($H2_A$) ($P < 0.05$, Figs. A.6 and A.7, Table A.9). TPR remained lower in $H2_B$ compared to $H1_B$ until the end of exercise in both the morning and evening (Figs. A.6 and A.7); however, TPR was lower in $H2_B$ compared to $H2_A$ only in the morning. End exercise TPR was significantly lower in $H2_A$ in the evening compared to the morning ($P < 0.05$, Table A.9). Prior heavy warm-up (one and two heavy bouts) lowered baseline TPR in $M3_A$ and $M3_B$, respectively, compared to the moderate control ($M1_A$) ($P < 0.05$, Figs. A.8 and A.9, Table A.10). TPR remained significantly lower in $M3_A$ and $M3_B$ compared to $M1_A$ until the end of exercise ($P < 0.05$, Table A.10). End exercise TPR was significantly lower in $M3_A$ in the evening compared to the morning ($P < 0.05$, Table A.10).

Skin blood flow

Resting SBF (immediately prior to the start of exercise protocols) was not significantly different in the evening compared to the morning (0.33 ± 0.18 vs. 0.27 ± 0.14 arbitrary units, $P = 0.43$). During the heavy exercise bouts, baseline SBF was significantly elevated in a heavy bout that followed prior heavy exercise ($H2_B$) compared to both control ($H1_B$) and a heavy bout that was preceded by moderate exercise ($H2_A$) ($P < 0.05$, Figs. A.6 and A.7, Table A.9). By the end of exercise there

were no differences between $H2_A$ and $H2_B$ and both were significantly higher than $H1_B$; the end exercise values for $H2_A$ and $H2_B$ were significantly higher in the evening compared to morning ($P < 0.05$, Table A.9). One and two prior heavy bouts resulted in a higher baseline SBF in $M3_A$ and $M3_B$ than in the no warm-up condition ($M1_A$) ($P < 0.05$, Figs. A.8 and A.9, Table A.10). The SBF responses remained significantly higher in $M3_A$ and $M3_B$ throughout the bout and until the end of exercise compared to $M1_A$ ($P < 0.05$, Table A.10). End exercise SBF was significantly higher in $M3_A$ in the evening compared to the morning ($P < 0.05$, Table A.10).

Table A.1: Morning and evening $\dot{V}O_2$ fitting parameters during heavy cycling bouts

Parameters	Morning			Evening		
	$H1_B$	**$H2_A$**	**$H2_B$**	**$H1_B$**	**$H2_A$**	**$H2_B$**
A_0, ml/min	1016 ± 54	1019 ± 77	1192 ± 55 *†	1014 ± 44	1038 ± 62	1172 ± 46 *†
A_1, ml/min	482 ± 132	454 ± 160	382 ± 155	403 ± 112	456 ± 142	374 ± 169
A_2, ml/min	1703 ± 240	1709 ± 216	1821 ± 209 *†	1748 ± 246	1687 ± 213	1808 ± 165 *†
A_1+A_2, ml/min	2186 ± 341	2163 ± 327	2203 ± 333	2151 ± 327	2143 ± 332	2157 ± 306
A_0+A_1+A_2, ml/min	3203 ± 395	3182 ± 403	3395 ± 388 *†	3165 ± 371	3181± 395	3270 ± 335 *†
A_3, ml/min	651 ± 118	563 ± 124 *	404 ± 69 *†	621 ± 115	533 ± 133 †	405 ± 111 *†
τ_2, s	26.0 ± 2.7	22.5 ± 4.0 *	21.8 ± 2.8 *	26.2 ± 2.6	22.0 ± 2.6 *	21.6 ± 4. 1 *
τ_3, s	153.0 ± 16.5	136.6 ± 20.0	128.4 ± 25.2 *	144.5 ± 19.6	137.9 ± 23.6	131. 2 ± 25.8
TD_2, s	11.4 ± 1. 9	11.3 ± 1.9	9.7 ± 1.9 *†	10.3 ± 2.5	11.4 ± 1.5 *	9.5 ± 2.0 *†
TD_3, s	96.7 ± 12.7	83.7 ± 14.8 *	74.6 ± 12.4 *	95.7 ± 14.3	79.6 ± 10.7 *	71.7 ± 16.1 *
Gain, ml/min/W	8.9 ± 0.6	8. 8 ± 0.5	9.0 ± 0.4	8.7 ± 0.5	8.7 ± 0.3	8.8 ± 0.8
End Bout, ml/min	3718 ± 409	3653 ± 390	3748 ± 385	3667 ± 375	3628 ± 356	3704 ± 362

Mean ± SD, n = 9; * $P < 0.05$ $H1_B$ vs. $H2_A$, $H2_B$; † $P < 0.05$ $H2_A$ vs. $H2_B$

Table A.2: Morning and evening $\dot{V}O_2$ fitting parameters during moderate cycling bouts

Parameters	Morning			Evening		
	$M1_A$	**$M3_A$**	**$M3_B$**	**$M1_A$**	**$M3_A$**	**$M3_B$**
A_0, ml/min	981 ± 55	1157 ± 76 *	1210 ± 80 *	1012 ± 54	1181 ± 66 *	1197 ± 53 *
A_1, ml/min	317 ± 137	273 ± 92	308 ± 137	322 ± 148	244 ± 98	269 ± 102
A_2, ml/min	924 ± 253	926 ± 297	884 ± 242	888 ± 261	925 ± 290	908 ± 263
A_1+A_2, ml/min	1240 ± 364	1199 ± 351	1191 ± 365	1210 ± 340	1168 ± 343	1178 ± 354
τ_2, s	22.5 ± 4.6	17. 8 ± 3.6 *	16.8 ± 3.6 *	22.2 ± 4.6	18.0 ± 3.2 *	17.1 ± 3.0 *
TD_2, s	14.5 ± 3. 0	13.5 ± 1.3	14.4 ± 2.0	14.1 ± 2.0	13.1 ± 1.8	13.7 ± 1.5
Gain, ml/min/W	9.8 ± 0.5	9.5 ± 0.7	9.4 ± 0.6	9.6 ± 0.3	9.2 ± 0.5	9.3 ± 0.6
End Bout, ml/min	2226 ± 354	2352 ± 356 *	2407 ± 373 *	2232 ± 375	2343 ± 351 *	2373 ± 352 *

Mean ± SD, n = 9; * $P < 0.05$ $M1_A$ vs. $M3_A$, $M3_B$

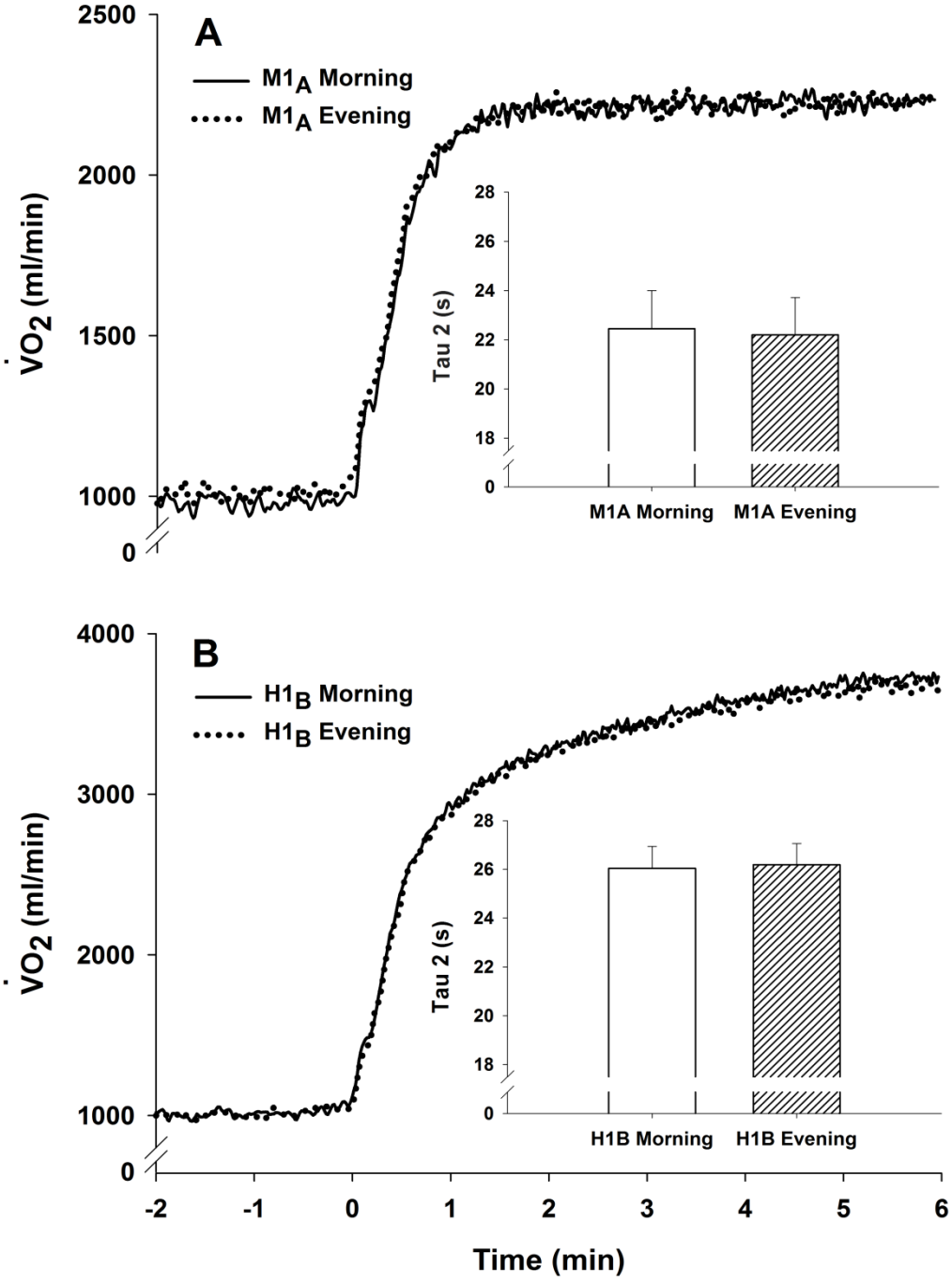

Figure A.1: $\dot{V}O_2$ time series data for the morning and evening responses in moderate (top, A) and heavy (bottom, B) exercise control conditions.

Data are the average of all 9 participants with ≥ 4 repetitions per each exercise condition. *Inset*: Tau_2 values (mean ± SE).

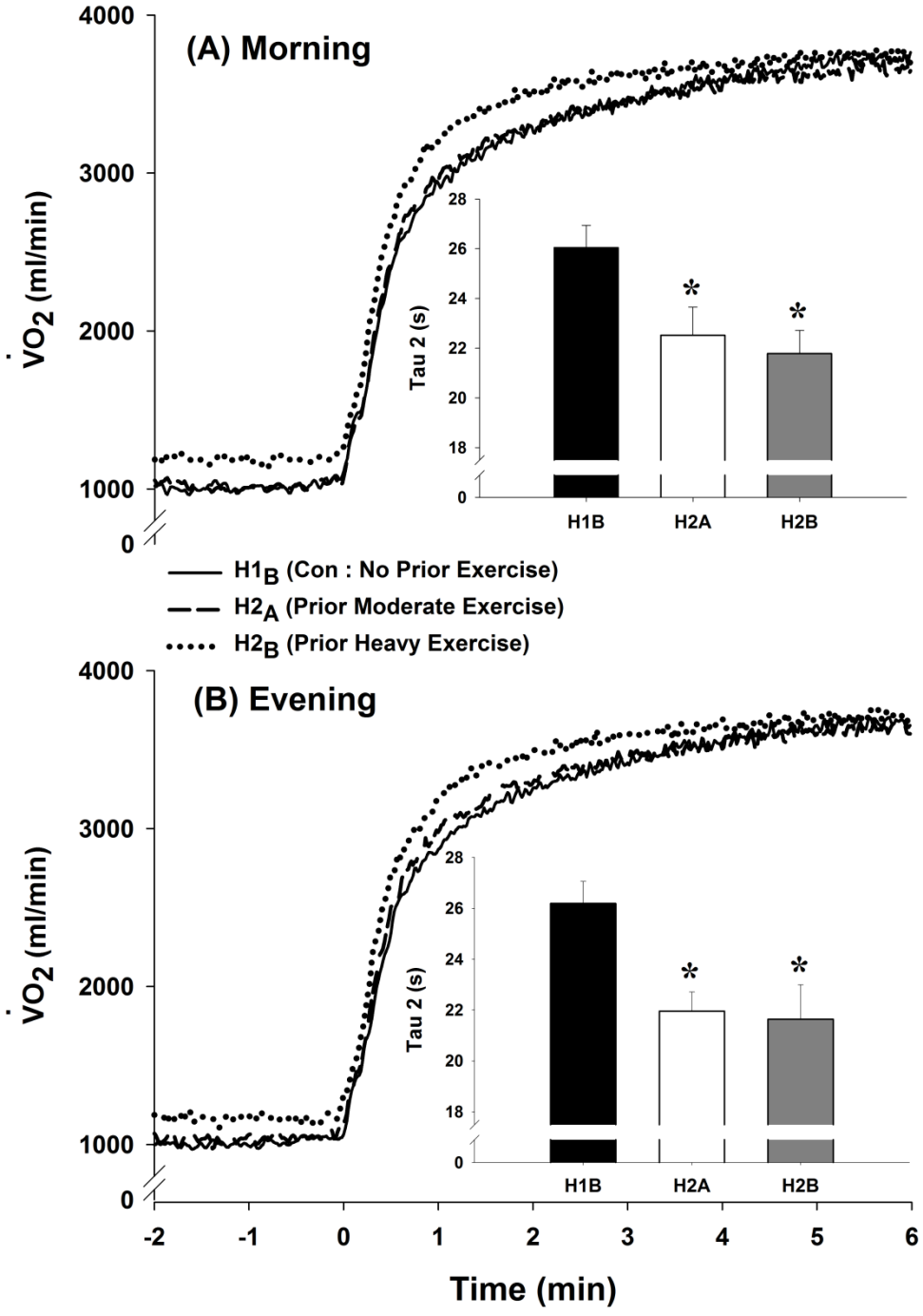

Figure A.2: $\dot{V}O_2$ time series data for the three heavy exercise bouts in the morning (top, A) and evening (bottom, B).

Data are the average of all 9 participants with ≥ 4 repetitions per each exercise condition. *Inset*: Tau_2 values (mean ± SE); * $P < 0.05$ compared to $H1_B$ (control).

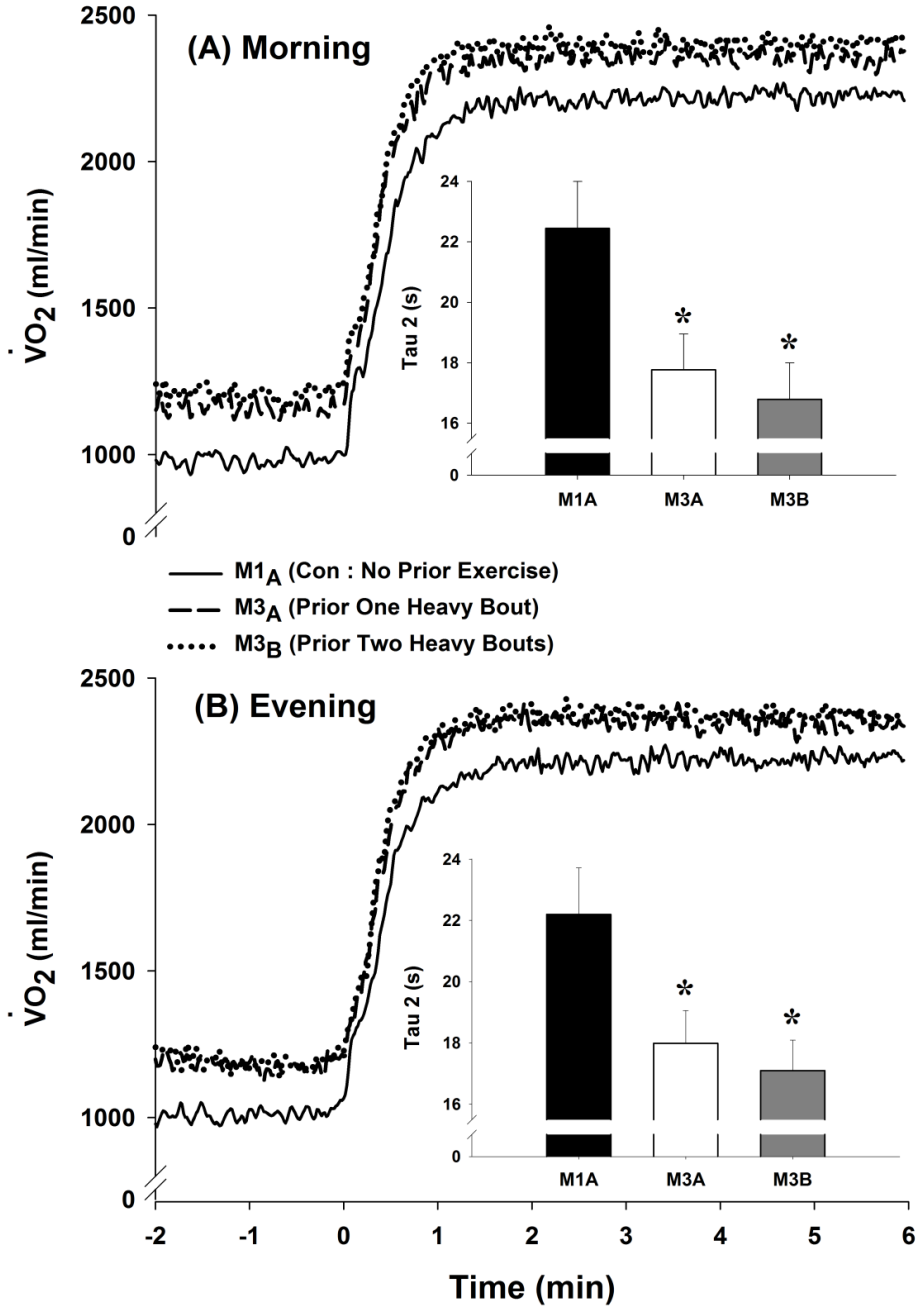

Figure A.3: $\dot{V}O_2$ time series data for the three moderate exercise bouts in the morning (top, A) and evening (bottom, B).

Data are the average of all 9 participants with ≥ 4 repetitions per each exercise condition. *Inset*: Tau_2 values (mean ± SE); * $P < 0.05$ compared to control ($M1_A$)

Table A.3: Morning and evening SBP responses during heavy cycling bouts

Parameters	Morning			Evening		
	$H1_B$	**$H2_A$**	**$H2_B$**	**$H1_B$**	**$H2_A$**	**$H2_B$**
Baseline SBP, mmHg	147.3 ± 9.8	146.1 ± 14.9	142.7 ± 10.3	152.4 ± 13.3	148.2 ± 14.4	150.8 ± 13.5
Peak SBP, mmHg	219.7 ± 15.5	220.6 ± 14.8	209.7 ± 14.1*†	219.7 ± 12.3	218.7 ± 13.4	211.0 ± 9.8 *†
End Bout SBP, mmHg	199.6 ± 11.7	196.8 ± 17.2	183.8 ± 8.7 *†	197.5 ± 13.2	185.5 ± 16.8 *‡	184.4 ± 12.6 *
Peak - Baseline SBP, mmHg	72.4 ± 9.6	74.5 ± 11.0	67.0 ± 14.3 †	67.3 ± 9.7	70.5 ± 7.8	60.2 ± 9.3 *†‡
Peak - End SBP, mmHg	20.1 ± 7.8	23.8 ± 9.7	25.9 ± 8.2	22.2 ± 6.2	33.2 ± 10.2 *‡	26.6 ± 8.9 †

Mean ± SD, n = 9;

* $P < 0.05$ $H1_B$ vs. $H2_A$, $H2_B$; † $P < 0.05$ $H2_A$ vs. $H2_B$; ‡ $P < 0.05$ (Morning vs. Evening in the same bout)

Table A.4: Morning and evening MAP responses during heavy cycling bouts

Parameters	Morning			Evening		
	$H1_B$	**$H2_A$**	**$H2_B$**	**$H1_B$**	**$H2_A$**	**$H2_B$**
Baseline MAP, mmHg	103.8 ± 5.6	105.3 ± 10.1	103.3 ± 6.4	105.1 ± 9.6	103.5 ± 8.2	104.3 ± 9.4
Peak MAP, mmHg	145.8 ± 12.9	145.6 ± 13.4	140.0 ± 10.8	145.1 ± 8.4	141.7 ± 10.5	139.0 ± 7.5
End Bout MAP, mmHg	134.9 ± 8.0	134.1 ± 11.2	127.3 ± 7.3 *†	132.2 ± 8.0	126.7 ± 9.5 *‡	125.8 ± 7.2 *
Peak - Baseline MAP, mmHg	42.0 ± 9.2	40.3 ± 10.8	36.7 ± 9.3	40.0 ± 8.9	38.2 ± 8.1	34.7 ± 5.6
Peak - End MAP, mmHg	10.9 ± 5.9	11.5 ± 2.9	12.7 ± 4.7	12.9 ± 2.6	15.0 ± 5.9	13.2 ± 4.5

Mean ± SD, n = 9;

* $P < 0.05$ $H1_B$ vs. $H2_A$, $H2_B$; † $P < 0.05$ $H2_A$ vs. $H2_B$; ‡ $P < 0.05$ (Morning vs. Evening in the same bout)

Table A.5: Morning and evening DBP responses during heavy cycling bouts

Parameters	Morning			Evening		
	$H1_B$	**$H2_A$**	**$H2_B$**	**$H1_B$**	**$H2_A$**	**$H2_B$**
Baseline DBP, mmHg	77.4 ± 3.0	80.2 ± 7.4	78.6 ± 3.8	77.9 ± 7.0	77.4 ± 4.9	78.1 ± 6.9
Peak DBP, mmHg	107.1 ± 10.5	106.5 ± 10.7	100.9 ± 9.0 *†	104.8 ± 6.2	103.0 ± 7.3	99.4 ± 5.5 *
End Bout DBP, mmHg	96.5 ± 5.7	96.1 ± 9.4	90.0 ± 5.8 *†	93.6 ± 5.7	89.5 ± 7.3 ‡	87.4 ± 5.3 *
Peak - Baseline DBP, mmHg	29.7 ± 8.7	26.3 ± 7.8 *	22.3 ± 8.14 *†	26.9 ± 6.5	25.6 ± 6.7	21.3 ± 5.0 *†
Peak - End DBP, mmHg	10.6 ± 5.4	10.4 ± 2.4	10.9 ± 4.6	11.2 ± 1.8	13.5 ± 4.9	12.0 ± 3.9

Mean ± SD, n = 9;

* $P < 0.05$ $H1_B$ vs. $H2_A$, $H2_B$; † $P < 0.05$ $H2_A$ vs. $H2_B$; ‡ $P < 0.05$ (Morning vs. Evening in the same bout)

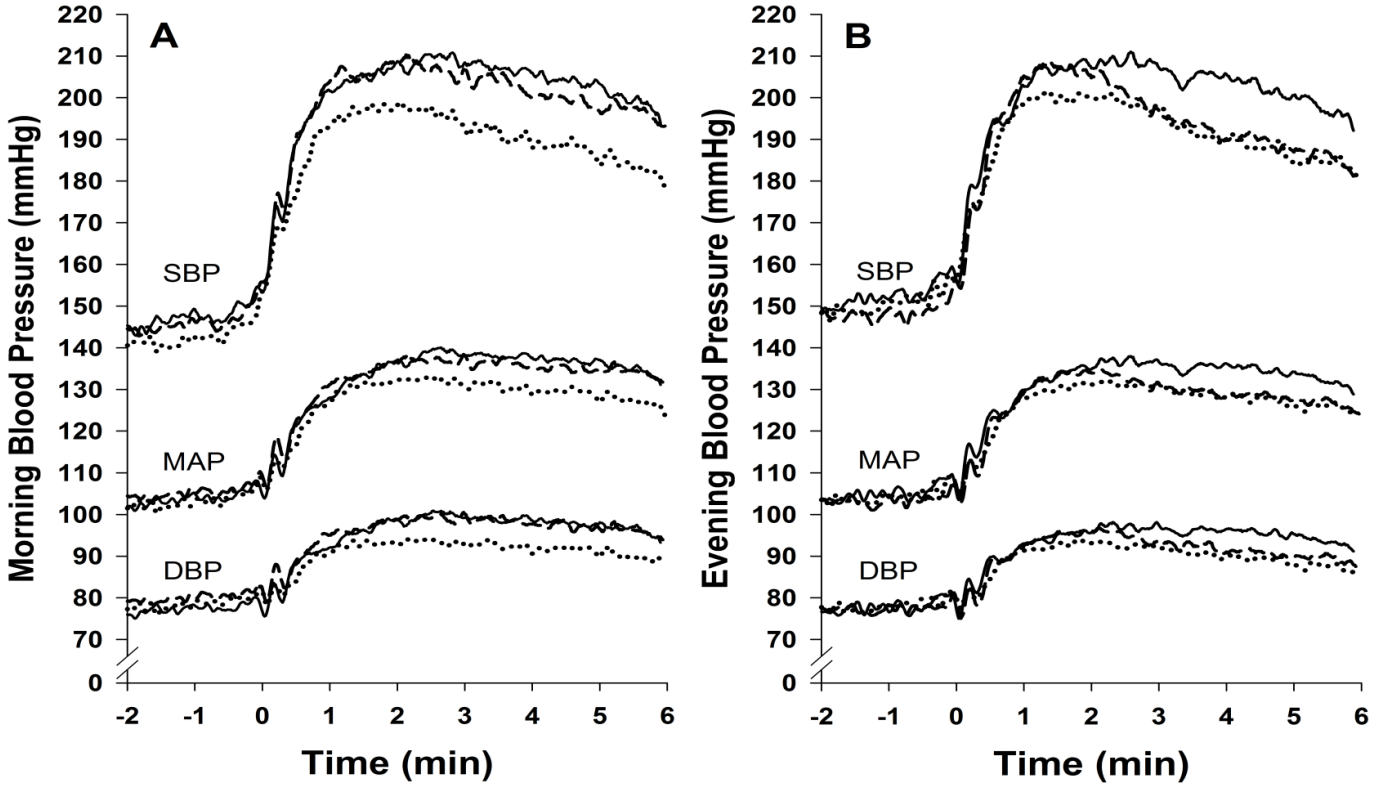

Figure A.4: SBP, MAP and DBP time series responses during the three heavy exercise conditions in the morning (A) and evening (B).

Data lines are the 5 s moving average for all 9 participants with ≥ 4 repetitions per each exercise condition. **Solid line**: $H1_B$, heavy control - no prior warm-up; **dashed line**: $H2_A$, prior moderate warm-up; **dotted line**: $H2_B$, prior heavy warm-up.

Table A.6: Morning and evening SBP responses during moderate cycling

Parameters	Morning			Evening		
	$M1_A$	**$M3_A$**	**$M3_B$**	**$M1_A$**	**$M3_A$**	**$M3_B$**
Baseline SBP, mmHg	141.9 ± 13.5	140.8 ± 13.0	132.4 ± 8.8 *†	142.9 ± 13.6	136.5 ± 11.2	135.9 ± 11.9 *
Peak SBP, mmHg	186.4 ± 23.7	172.4 ± 22.9 *	156.8 ± 16.2 *†	189.5 ± 16.6	166.2 ± 17.2 *	162.3 ± 19.0 *
End Bout SBP, mmHg	166.7 ± 21.4	152.9 ± 19.8 *	140.8 ± 14.0 *†	162.4 ± 17.8	144.6 ± 15.3 *‡	142.9 ± 15.4 *
Peak - Baseline SBP, mmHg	44.5 ± 16.2	31.6 ± 12.9 *	24.4 ± 11.2 *†	46.6 ± 14.7	29.7 ± 10.6 *	26.4 ± 12.5 *
Peak - End SBP, mmHg	19.7 ± 9.0	19.5 ± 8.4	16.0 ± 6.1	27.1 ± 11.0 ‡	21.6 ± 5.7 *	19.4 ± 5.3 *

Mean ± SD, n = 9;

* $P < 0.05$ $M1_A$ vs. $M3_A$, $M3_B$; † $P < 0.05$ $M3_A$ vs. $M3_B$; ‡ $P < 0.05$ (Morning vs. Evening in the same bout)

Table A.7: Morning and evening MAP responses during moderate cycling

Parameters	Morning			Evening		
	$M1_A$	**$M3_A$**	**$M3_B$**	**$M1_A$**	**$M3_A$**	**$M3_B$**
Baseline MAP, mmHg	101.7 ± 8.8	103.5 ± 9.8	98.6 ± 7.6 †	99.7 ± 6.9	99.1 ± 7.6	98.6 ± 9.1
Peak MAP, mmHg	124.5 ± 15.9	119.5 ± 15.6	111.6 ± 11.1 *†	123.7 ± 10.6	114.0 ± 10.5 *‡	112.1 ± 12.8 *
End Bout MAP, mmHg	114.5 ± 14.6	109.0 ± 15.0 *	101.9 ± 10.0 *†	110.0 ± 10.6	102.4 ± 10.9 *‡	101.0 ± 11.2 *
Peak - Baseline MAP, mmHg	22.8 ± 10.9	16.0 ± 7.2 *	13.0 ± 6.9 *	24.0 ± 9.3	14.9 ± 5.5 *	13.5 ± 5.7 *
Peak - End MAP, mmHg	10.0 ± 2.8	10.5 ± 4.2	9.7 ± 3.7	13.7 ± 5.1 ‡	11.6 ± 3.0	11.1 ± 3.2

Mean ± SD, n = 9;

* $P < 0.05$ $M1_A$ vs. $M3_A$, $M3_B$; † $P < 0.05$ $M3_A$ vs. $M3_B$; ‡ $P < 0.05$ (Morning vs. Evening in the same bout)

Table A.8: Morning and evening DBP responses during moderate cycling

Parameters	Morning			Evening		
	$M1_A$	**$M3_A$**	**$M3_B$**	**$M1_A$**	**$M3_A$**	**$M3_B$**
Baseline DBP, mmHg	77.0 ± 6.0	80.1 ± 7.4	76.9 ± 5.6	74.6 ± 3.7	75.6 ± 4.9	75.9 ± 6.4
Peak DBP, mmHg	93.5 ± 11.3	92.3 ± 11.6	87.3 ± 9.0 *†	90.7 ± 6.8	87.3 ± 8.4 ‡	85.7 ± 8.8 *
End Bout DBP, mmHg	85.2 ± 10.7	82.2 ± 12.4	76.5 ± 7.6 *†	80.7 ± 7.3 ‡	76.3 ± 8.1 *‡	74.9 ± 8.6 *
Peak - Baseline DBP, mmHg	16.5 ± 8.0	12.2 ± 5.8 *	10.4 ± 6.1 *	16.1 ± 6.7	11.7 ± 4.8 *	9.8 ± 4.1 *
Peak - End DBP, mmHg	8.3 ± 1.9	10.1 ± 4.3	10.8 ± 3.8	10.0 ± 3.6	11.0 ± 3.2	10.8 ± 4.0

Mean ± SD, n = 9;

* $P < 0.05$ $M1_A$ vs. $M3_A$, $M3_B$; † $P < 0.05$ $M3_A$ vs. $M3_B$; ‡ $P < 0.05$ (Morning vs. Evening in the same bout)

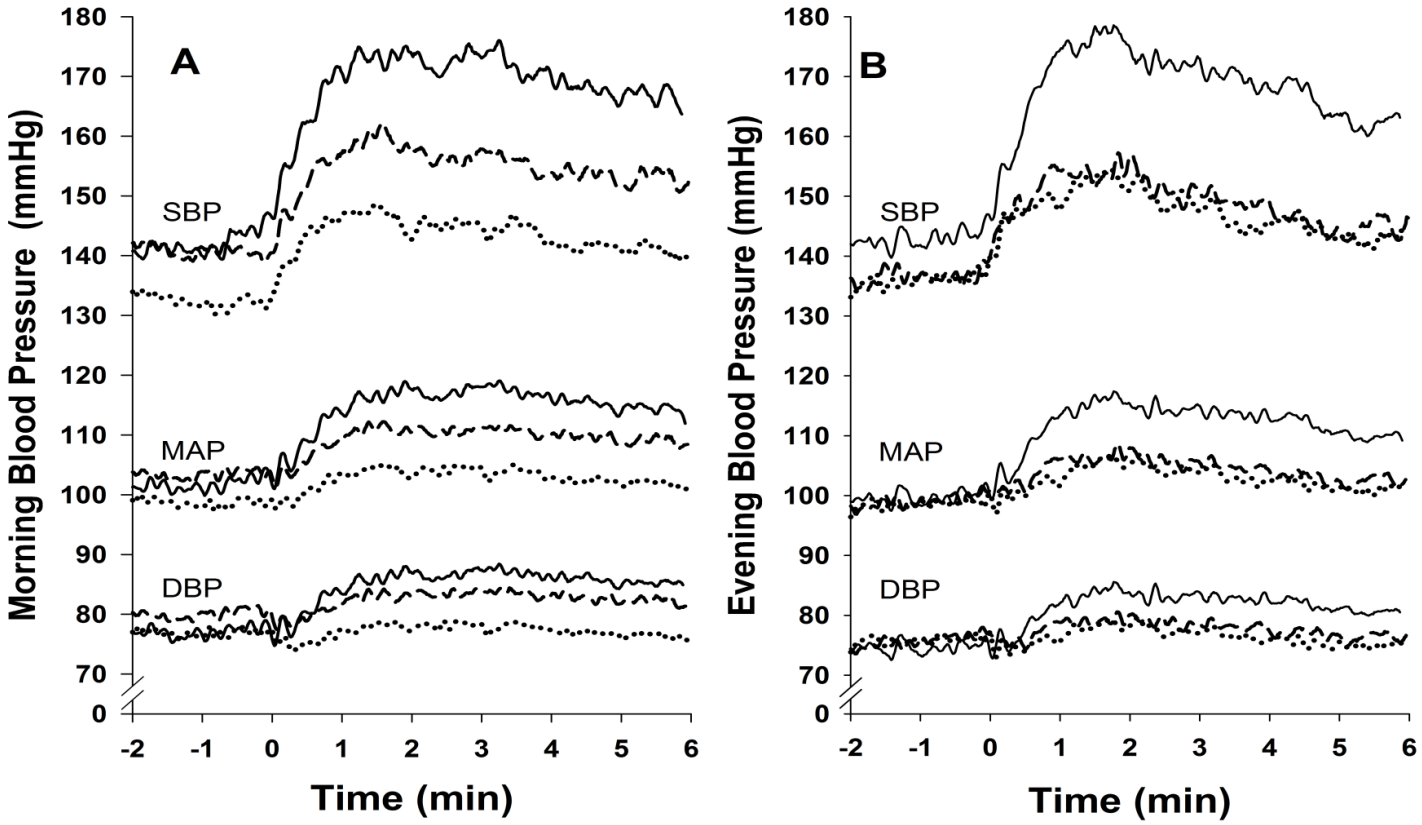

Figure A.5: SBP, MAP and DBP time series responses during the three moderate exercise conditions in the morning (A) and evening (B).

Data lines are the 5 s moving average for all 9 participants with ≥ 4 repetitions per each exercise condition. **Solid line**: $M1_A$, moderate control - no prior warm-up; **dashed line**: $M3_A$, one prior heavy bout; **dotted line**: $M3_B$, two prior heavy bouts.

Table A.9: Morning and evening $\dot{Q}_{MF}$, TPR and SBF responses during heavy cycling bouts

Parameters	Morning			Evening		
	$H1_B$	**$H2_A$**	**$H2_B$**	**$H1_B$**	**$H2_A$**	**$H2_B$**
Baseline Q, l/min	10.2 ± 1.0	10.4 ± 0.6	11.8 ± 1.2 *†	10.4 ± 0.9	10.7 ± 0.8	12.6 ± 0.6 *†
End Bout Q, l/min	22.3 ± 1.8	22.5 ± 1.8	23.9 ± 1.8 *†	23.4 ± 1.7	23.1 ± 0.9	24.6 ± 1.6 *†
Baseline TPR, mmHg/l/min	10.4 ± 1.0	10.3 ± 0.9	9.0 ± 0.8 *†	10.0 ± 1.5	9.8 ± 0.5	8.4 ± 0.9 *†
End Bout TPR, mmHg/l/min	6.1 ± 0.6	6.0 ± 0.8	5.4 ± 0.6 *†	5.7 ± 0.6	5.5 ± 0.5 ‡	5.2 ± 0.5 *
Baseline SBF, % Baseline	169 ± 26	170 ± 38	269 ± 101 *†	149 ± 42	209 ± 41	341 ± 134 *†
End Bout SBF, % Baseline	352 ± 135	555 ± 144 *	655 ± 198 *	463 ± 150	815 ± 319 *‡	937 ± 215 *‡

Mean ± SD, n = 9 for $\dot{Q}_{MF}$ and TPR, n = 8 for SBF;

* $P < 0.05$ $H1_B$ vs. $H2_A$, $H2_B$; † $P < 0.05$ $H2_A$ vs. $H2_B$; ‡ $P < 0.05$ (Morning vs. Evening in the same bout)

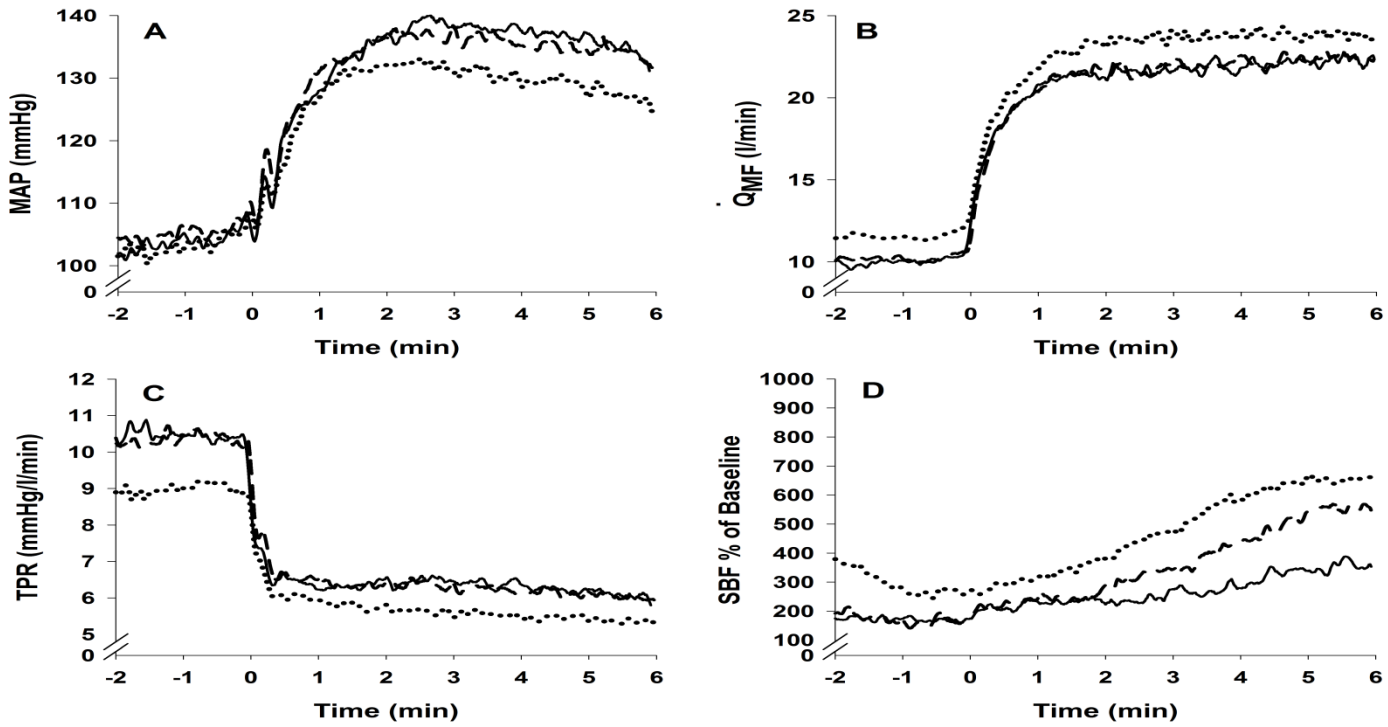

Figure A.6: MAP (A), $\dot{Q}_{MF}$ (B), TPR (C), and SBF (D) time series responses during the three heavy exercise conditions in the morning.

MAP, $\dot{Q}_{MF}$ and TPR data are the 5 sec moving average for all 9 participants with ≥ 4 repetitions per each exercise condition. SBF data are the 5 sec moving average from one ride for 8 participants. **Solid line**: $H1_B$, heavy control - no prior warm-up; **dashed line**: $H2_A$, prior moderate warm-up; **dotted line**: $H2_B$, prior heavy warm-up.

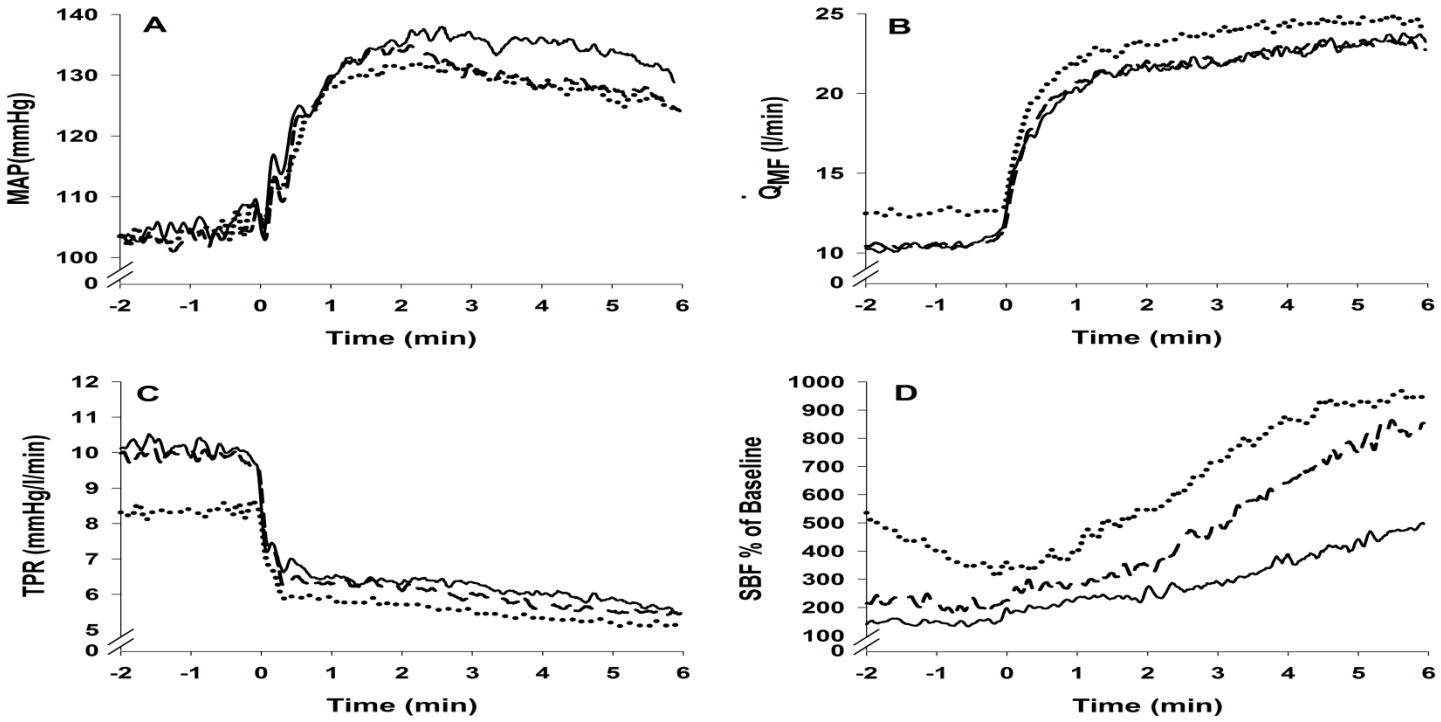

Figure A.7: MAP (A), $\dot{Q}_{MF}$ (B), TPR (C), and SBF (D) time series responses during the three heavy exercise conditions in the evening.

MAP, $\dot{Q}_{MF}$ and TPR data are the 5 sec moving average for all 9 participants with ≥ 4 repetitions per each exercise condition. SBF data are the 5 sec moving average from one ride for 8 participants. **Solid line**: $H1_B$, heavy control - no prior warm-up; **dashed line**: $H2_A$, prior moderate warm-up; **dotted line**: $H2_B$, prior heavy warm-up.

Table A.10: Morning and evening $\dot{Q}_{MF}$, TPR and SBF responses during moderate cycling bouts

Parameters	Morning			Evening		
	$M1_A$	**$M3_A$**	**$M3_B$**	**$M1_A$**	**$M3_A$**	**$M3_B$**
Baseline Q, l/min	9.74 ± 0.91	10.78 ± 0.63 *	10.70 ± 0.93 *	9.65 ± 0.90	11.02 ± 0.60 *	11.54 ± 0.40 *‡
End Bout Q, l/min	15.80 ± 1.07	16.24 ± 1.20	16.46 ± 2.00	16.16 ± 1.39	16.36 ± 1.37	17.10 ± 1.40 *
Baseline TPR, mmHg/l/min	10.64 ± 1.11	9.68 ± 0.84 *	9.31 ± 0.87 *	10.59 ± 0.87	9.08 ± 0.50 *	8.59 ± 0.59 *
End Bout TPR, mmHg/l/min	7.24 ± 0.69	6.73 ± 0.86 *	6.27 ± 0.91 *†	6.81 ± 0.40	6.27 ± 0.41*‡	5.93 ± 0.62 *
Baseline SBF, % Baseline	152 ± 29	369 ± 125 *	473 ± 135 *	183 ± 47	477 ± 125 *	580 ± 143 *
End Bout SBF, % Baseline	234 ± 47	581 ± 160 *	633 ± 190 *	274 ± 94	785 ± 224 *‡	753 ± 229 *

Mean ± SD, n = 9;

* $P < 0.05$ $H1_B$ vs. $H2_A$, $H2_B$; † $P < 0.05$ $H2_A$ vs. $H2_B$; ‡ $P < 0.05$ (Morning vs. Evening in the same bout)

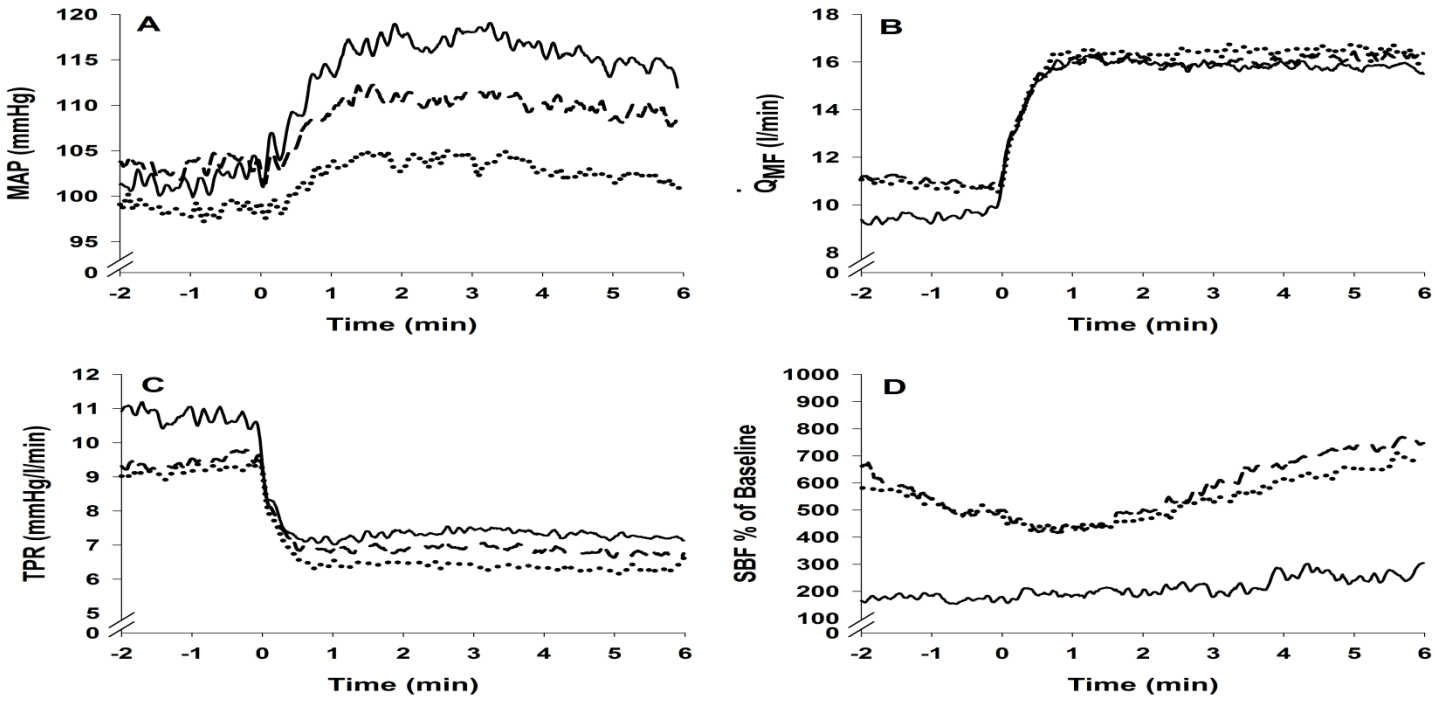

Figure A.8: MAP (A), $\dot{Q}_{MF}$ (B), TPR (C), and SBF (D) time series responses during the three moderate exercise conditions in the morning.

MAP, $\dot{Q}_{MF}$ and TPR data are the 5 sec moving average for all 9 participants with ≥ 4 repetitions per each exercise condition. SBF data are the 5 sec moving average from one ride for 8 participants. **Solid line**: $M1_A$, moderate control - no prior warm-up; **dashed line**: $M3_A$, one prior heavy bout; **dotted line**: $M3_B$, two prior heavy bouts.

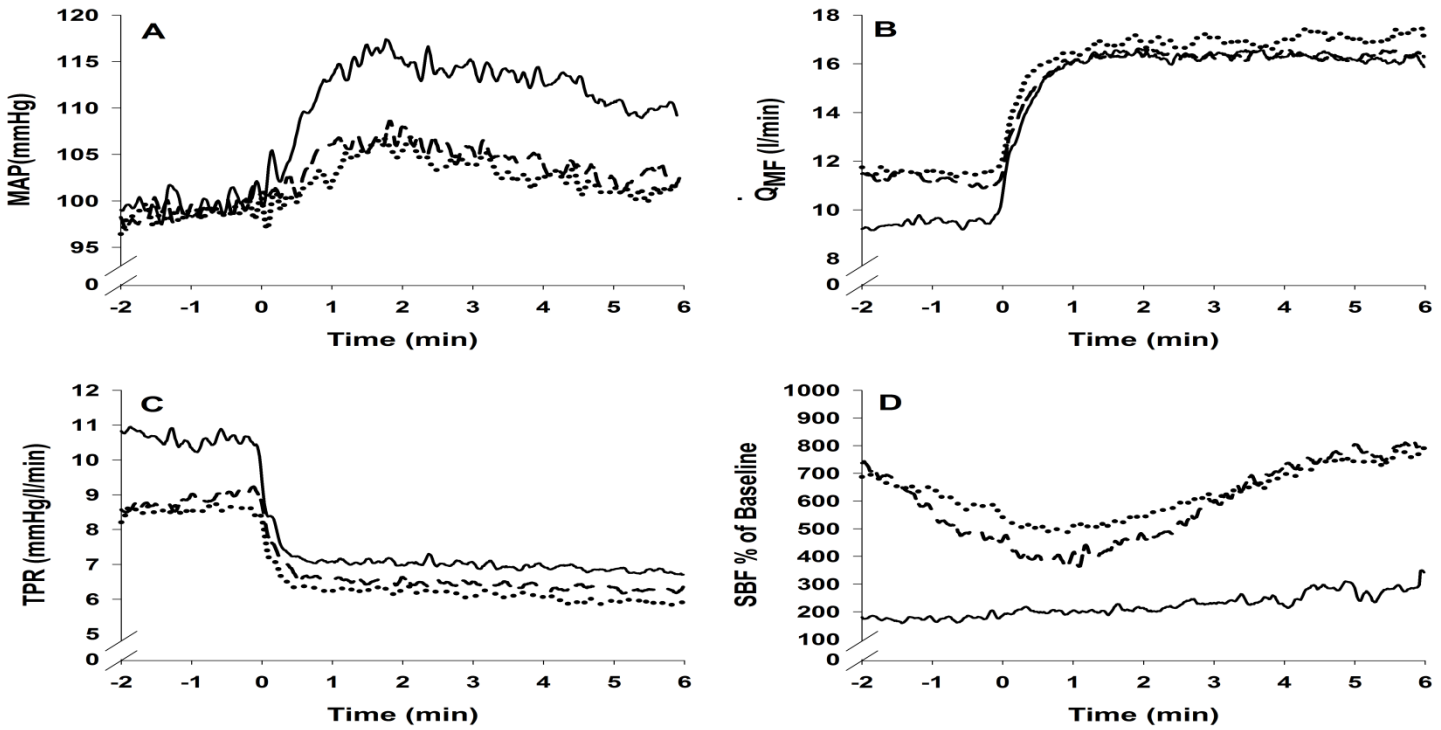

Figure A.9: MAP (A), $\dot{Q}_{MF}$ (B), TPR (C), and SBF (D) time series responses during the three moderate exercise conditions in the evening.

MAP, $\dot{Q}_{MF}$ and TPR data are the 5 sec moving average for all 9 participants with ≥ 4 repetitions per each exercise condition. SBF data are the 5 sec moving average from one ride for 8 participants. **Solid line**: $M1_A$, moderate control - no prior warm-up; **dashed line**: $M3_A$, one prior heavy bout; **dotted line**: $M3_B$, two prior heavy bouts.

A.5 Discussion

The current research has confirmed our previous findings of significant speeding of $\dot{V}O_2$ kinetics during both moderate and heavy exercise following priming exercise (Chapter 2) and has shown that these responses were identical in the morning and evening. Thus, our results concerning possible circadian influence on O_2 transport-utilization mechanisms contrast with those reported by Brisswalter *et al.*(2007) but supported our hypothesis and were in agreement with the only other study of circadian effects on $\dot{V}O_2$ kinetics (Carter *et al.*, 2002). In addition, this is the first study to report several key findings regarding the BP response to exercise. First, there was a consistent overshoot in BP during the first two minutes of both moderate and heavy exercise, then BP declined in association with increasing SBF between the 3rd and 6th minute of exercise. This overshoot response seems to be controlled by the feed forward central command mechanism (Gallagher *et al.*, 2006; Rowell & O'Leary, 1990) independent of circadian rhythm. Second, the BP response was shown to be lower throughout the last three minutes in both control moderate and heavy exercise bouts in the evening, suggesting an influence of time of day on BP response during exercise after the initial BP resetting. Third, BP responses during both moderate and heavy exercise were significantly affected by priming exercise, and the effects of prior moderate and heavy exercise differed between the morning and evening. One or two bouts of prior heavy exercise caused graded reductions in BP during subsequent moderate exercise in the morning while the effects were similar in the evening. During heavy exercise, prior moderate exercise had no effect on BP response in the morning

while it reduced BP to a similar degree as prior heavy exercise in the evening. Fourth, the BP overshoot magnitude and the subsequent BP reduction were modulated to a greater extent by priming exercise in the evening due to a larger increase in SBF.

Circadian rhythm and $\dot{V}O_2$ ***kinetics***

Despite considerable research into $\dot{V}O_2$ kinetics, very little has been done to investigate the effect of circadian rhythm on breath-by-breath $\dot{V}O_2$ during exercise transitions. We observed no differences in any parameter of $\dot{V}O_2$ kinetics during the control moderate ($M1_A$) and heavy ($H1_B$) cycling exercise bouts between the morning and afternoon sessions (Fig. A1, Tables A.1 and A.2). These findings agree with the only previous study that examined circadian effects on $\dot{V}O_2$ kinetics during treadmill running at moderate and heavy intensities (Carter *et al.*, 2002). Additionally, priming exercise exhibited the same robust effects on $\dot{V}O_2$ kinetics during subsequent moderate and heavy exercise bouts irrespective of time of day.

Both moderate and heavy warm-ups accelerated $\dot{V}O_2$ kinetics in subsequent heavy exercise (Fig. A.2 and Table A.1), and one or two prior heavy bouts accelerated the $\dot{V}O_2$ kinetics in subsequent moderate exercise (Fig. A.3 and Table A.2). The effects of prior exercise on $\dot{V}O_2$ kinetics have been discussed extensively in Chapter 2. Conversely, our results strongly contrast with those of Brisswalter *et al.* (2007) who reported a 50% improvement in $\dot{V}O_2$ kinetics, a 15% reduction in

the $\dot{V}O_2$ amplitude, and a 3% increase in net efficiency during 80%VT cycling exercise in the afternoon. While Brisswalter *et al.* (2007) used a simple mono-exponential model fitted to data collected using a COSMED device, even these methodological differences seem inadequate to explain the magnitude changes observed and the disparity with our results and those of Carter *et al.* (2002).

In addition to the $\dot{V}O_2$ kinetics results, there were no differences in $\dot{V}O_2$ during either the initial 20W periods, steady state moderate exercise, or at the end of exercise during heavy bouts at different times of day. While resting $\dot{V}O_2$ has been observed to follow the circadian rhythm of core temperature, with resting values achieving their peak in the early evening (Reilly & Brooks, 1990), the rhythm, if detected, becomes weaker during light exercise and disappears during moderate and heavy exercise (Deschenes *et al.*, 1998; Reilly & Brooks, 1990; Reilly & Garrett, 1998). Therefore our results are consistent with previous literature in showing no circadian effect on $\dot{V}O_2$ during exercise.

Circadian rhythm and the blood pressure response to exercise

In contrast with finding no circadian effect on $\dot{V}O_2$, we observed novel circadian effects on the regulation of BP during exercise that add to the established circadian pattern on resting BP (Millar-Craig *et al.*, 1978), as well as the recent observations of differences in the post-exercise hypotension (PEH) (Jones *et al.*, 2008a; Jones *et al.*, 2008b). BP displays a consistent circadian rhythm with the highest pressures observed at midday, a reduction of 10-20% through the

afternoon and during sleep, and a morning "surge" in the hours before and following waking (Kario *et al.*, 2003; Millar-Craig *et al.*, 1978; Verdecchia *et al.*, 1990). Additionally, the response of BP after exercise is altered by the time of day, with greater PEH achieved in the afternoon than in the morning (Jones *et al.*, 2008a; Jones *et al.*, 2008b; Jones *et al.*, 2009). However, no research has focused on the circadian effects on BP during exercise transitions. Using beat-by-beat measurement of BP by finger plethysmography, we observed a lower BP response throughout the last three minutes in both control moderate ($M1_A$) and heavy ($H1_B$) exercise bouts in the evening. Elevated $\dot{Q}$ and SBF during exercise in the evening, consistent with reports of altered thermoregulation (Aldemir *et al.*, 2000; Jones *et al.*, 2008a; Jones *et al.*, 2008b; Waterhouse *et al.*, 2007), could have contributed to the lower arterial BP during exercise in the evening.

Notably, we observed a distinct overshoot of BP within the first minutes of both moderate and heavy exercise, which was followed by a slow decline between the 3rd and 6th minute of each exercise bout. The BP overshoot was consistent in all moderate and heavy bouts, morning and evening, regardless of whether or not warm-up exercise was performed. This rapid overshoot in BP has been observed previously in humans via intra-arterial catheterization during supine kicking exercise in humans (MacDonald *et al.*, 1998). Additionally, sphymomanometry BP measurements during cycling show the largest response early in exercise, then a slow decline up to 15 min of exercise (MacDonald *et al.*, 1999; Nakas-Icindic *et al.*, 2004). The overshoot measured here by finger cuff plethysmography in both

moderate and heavy cycling exercise is consistent with these previous observations. Peak BP values reported in the tables are slightly greater than the response pattern in the figures due to small differences between individuals in the time to peak response.

Effect of prior exercise on blood pressure during subsequent exercise

The BP responses following priming exercise at different times of day could be influenced by large increases in relative SBF, reductions in TPR, and in some cases, increases in $\dot{Q}$. Prior heavy but not moderate exercise caused sustained elevations in $\dot{Q}$ prior to and throughout the subsequent heavy exercise bout ($H2_B$) in both the morning and evening. TPR was reduced only after prior heavy exercise and SBF was elevated to a greater extent in the evening after heavy and moderate exercise (Fig.A.7). One or two prior heavy bouts elevated $\dot{Q}$ prior to subsequent moderate bouts in the both morning and evening, but $\dot{Q}$ was only elevated at the end of the moderate bout ($M3_B$) after two prior heavy bouts in the evening. Simultaneously, TPR was reduced and SBF was increased following one or two bouts of prior heavy exercise (Figs. A.8 and A.9). SBF increases to a greater extent during exercise in the evening compared to morning, these observations may be closely linked to differences in thermoregulatory processes at different times of day. The "body clock", located in the suprachiasmatic nucleus of the hypothalamus, induces circadian rhythmicity of core temperature which is generated via heat gain and heat loss modes in the morning and evening, respectively (Aldemir *et al.*, 2000; Waterhouse *et al.*, 2007; Waterhouse *et al.*, 2004). Exercise-induced heat loads

may raise core temperature more and faster in the morning (Aldemir *et al.*, 2000) in association with smaller increases in SBF (Aldemir *et al.*, 2000; Waterhouse *et al.*, 2007). These results align with our observation of attenuated SBF responses to exercise in the morning compared to the evening. Thus, the greater effect of priming exercise in modulating/lowering BP responses during subsequent exercise in the evening appears to be linked to the enhanced thermoregulatory response in the evenings compared to the morning. Athletes generally have better responses to thermal stress with larger SBF increases for a given change in temperature (Fritzsche & Coyle, 2000; Johnson, 1998; Lenasi & Strucl, 2004), which may imply that the robustness of response we observed is a result of the subjects' fitness. While the observed attenuation of BP during subsequent exercise following warm-up seems to be a unique physiological response, it is prudent to compare this phenomenon to similar studies involving PEH. Importantly, our results are consistent with those of MacDonald *et al.* (2001a) who observed that PEH, measured intra-arterially, is maintained in subsequent rest, exercise, and simulated activities of daily living. Our results also bear striking similarity to a series of previous studies examining the response of BP after exercise at different points in the circadian cycle (Jones *et al.*, 2008a; Jones *et al.*, 2008b; Jones *et al.*, 2009). These studies showed that after a continuous exercise bout, normotensive subjects only experienced PEH in the afternoon. Our observations expand upon these previous results and show that in trained subjects, BP is lowered during subsequent exercise, but requires a greater exercise stimulus in the morning to achieve the same reductions as in the evening. Interestingly, intermittent exercise

has been shown to be more effective than continuous exercise in altering post exercise BP response. Jones *et al.*(2009) have observed greater PEH immediately following an intermittent exercise protocol similar to our protocols, involving three 10 min exercise periods interspersed with 10 min of rest. Furthermore, the use of intermittent exercise was able to induce reductions in BP in the morning, where continuous exercise could not. These observations provide evidence that the recovery period in between bouts of exercise may play an important role in reducing BP during subsequent exercise bouts. Additional study is necessary to determine whether or not the recovery period, or simply exercise intensity, play a critical role in lowering the BP responses during subsequent exercise.

Implications to blood pressure control during exercise

At the initiation of exercise the arterial baroreflex response curve is reset to a higher BP, though the maximal gain remains essentially unchanged (Bevegard & Shepherd, 1966; Dicarlo & Bishop, 1992; Melcher & Donald, 1981; Ogoh *et al.*, 2003; Walgenbach & Donald, 1983). The immediate resetting of arterial baroreceptors at the exercise onset is likely achieved through the feed forward central command mechanism (Dicarlo & Bishop, 1992; Gallagher *et al.*, 2006; Ludbrook & Graham, 1985; Rowell & O'Leary, 1990). We observed the same peak BP during the overshoot in the control moderate ($M1_A$) and heavy ($H1_B$) bouts, irrespective of time of day, consistent with an immediate resetting by central command that is dependent on motor neural output (Gallagher *et al.*, 2001; Potts *et al.*, 1993; Rowell & O'Leary, 1990) independent of circadian rhythm. However, the large increases in SBF and the short exercise time to the onset of cutaneous

vasodilation in trained subjects (Fritzsche & Coyle, 2000) might influence the dynamic resetting of the arterial baroreceptors, potentially through the cardiopulmonary baroreflex (Ogoh *et al.*, 2006) leading to an adjustment in the BP set-point to lower values after just a few minutes of exercise.

Priming exercise, which generates heat stress, and the time of day may interact to alter the regulation of SBF and arterial BP during exercise. Passive heat stress shifted the carotid-vascular baroreflex response curves downward to the prevailing cardiovascular conditions with a reduction in maximal gain (Crandall, 2000). The impact of the priming exercise might also be through residual effects that have been observed as reductions in baroreflex sympathetic outflow (Hara & Floras, 1992), vascular responsiveness (Halliwill *et al.*, 1996) and arterial baroreflex operating point (Chandler *et al.*, 1998) during the period of PEH.

The current research demonstrating a reduction in exercise BP with time and after prior exercise was conducted with trained, physically fit subjects. The results could have important implications for exercise prescription and for the post-exercise hypotension (Piepoli *et al.*, 1994; Quinn, 2000) in the general population and for individuals at risk for cardiovascular disease. It is important, however, to replicate these studies in these other groups to determine if the BP responses to exercise are reduced by prior priming or “warm-up” exercise.

A.6 Conclusion

We observed that circadian rhythm does not impact $\dot{V}O_2$ kinetics, end exercise $\dot{V}O_2$ in moderate or heavy exercise, or the acceleration of $\dot{V}O_2$ kinetics by prior warm-up during moderate and heavy exercise in trained humans. However, we did find that BP overshoots early in both moderate and heavy exercise and then steadily falls in association with increases in SBF, a phenomenon that was influenced by both prior exercise and time of day. The effects of prior moderate and heavy exercise were different between the morning and evening. In the evening, moderate exercise and a single prior heavy warm-up had greater attenuating influences on the BP response of subsequent heavy and moderate exercise, respectively.

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