CONTROL OF MUSCLE BLOOD FLOW DURING DYNAMIC EXERCISE:

MUSCLE CONTRACTION / BLOOD FLOW INTERACTIONS

by

BARBARA JUNE LUTJEMEIER

B.S., Kansas State University, 1999
M.S., Kansas State University, 2001

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Anatomy and Physiology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2006

Abstract

The interaction between dynamic muscle contractions and the associated muscle blood flow is very intriguing leading to questions regarding the net effect of these contractions on oxygen delivery and utilization by the working muscle. Study 1 examined the impact of contractions on muscle blood flow at the level of the femoral artery. We demonstrated that muscle contractions had either a facilitory, neutral, or net impedance effect during upright knee extension exercise as intensity increased from very light to ~70% peak work rate.

This led to the question of what impact a change in contraction frequency might have on the coupling of blood flow to metabolic rate during cycling exercise. The blood flow/$\dot{V}O_2$ relationship has been shown to be linear and robust at both the central (i.e., cardiac output/pulmonary $\dot{V}O_2$) and peripheral (leg blood flow/leg $\dot{V}O_2$) levels. However, an increase in contraction frequency has been reported to either decrease, have no effect, or increase the blood flow response during exercise. Study 2 determined if the steady state coupling between muscle blood flow and metabolic rate (centrally and/or peripherally) would be altered by varying contraction frequency. Our results indicate that both central and peripheral blood flow/$\dot{V}O_2$ relationships are robust and remain tightly coupled regardless of changes in contraction frequency.

Study 3 examined muscle microvascular hemoglobin concentration and oxygenation within the contraction/relaxation cycle to determine if microvascular RBC volume was preserved and if oxygen extraction occurred during contractions. We concluded that microvascular RBC volume was preserved during muscle contractions (i.e., RBCs remained in the capillaries), which could facilitate continued oxygen delivery. Further, there was a cyclic pattern of deoxygenation/oxygenation that corresponded with the contraction/relaxation phases of the contraction cycle, with deoxyhemoglobin

increasing significantly during the contractile phase. These data suggest that oxygen extraction continues to occur during muscle contractions.

Significant insight has been gained on the impact of muscle contractions on oxygen delivery to and exchange in active skeletal muscle. This series of studies forms a base of knowledge that furthers our understanding of the mechanisms which govern the control of skeletal muscle blood flow and its coupling to muscle metabolic rate.

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Acknowledgements

I would like to thank everyone at Kansas State University who has assisted me in my goal of earning a PhD and to all of the faculty and staff in the Departments of Anatomy & Physiology and Kinesiology who helped along the way. I would especially like to thank my major professor, Dr. Thomas Barstow, who has guided my path towards becoming a research scientist. I would also like to thank my committee members, Dr. Craig Harms, Dr. Mark Haub, Dr. Timothy Musch and Dr. David Poole, for their support during my program of study. A special thank you goes to Dana Townsend and Leonardo Ferreira, who have shared this experience with me and have given me unconditional support. I would also like to acknowledge the many wonderful people I have had the opportunity to work with: Dr. Akira Miura, Dr. Barry Scheuermann, Dr. Shunsaku Koga, Christine Ferguson, Pat Marteney and Tyler Barker. My research would have been impossible to complete without the tremendous effort by all of the subjects, students and volunteers who gave of their time so generously. To David Freeze and Allison Harper who helped with the endless data analysis, I am forever in your debt.

My greatest gratitude and appreciation go to my family, without their support, encouragement and belief in me, I could never have achieved this tremendous goal. My wonderful husband, Joe, has been by my side and has been a constant throughout this endeavor. Jessica and Jacob have been phenomenal, no one could ask for more supportive and understanding children. To all of my family and friends, this has been quite the journey, thank you for being there for me along the way.

Dedication

To Jessica and Jacob, you have been my inspiration.

To Joe, thank you...

CHAPTER 1 - INTRODUCTION

The regulation of blood flow to skeletal muscle has been studied for more than 100 years, yet there is much uncertainty regarding the mechanisms which regulate skeletal muscle blood flow (oxygen delivery). It is essential for our understanding of the mechanisms which underlie cardiovascular adjustments during dynamic exercise to examine the peripheral blood flow response to exercise in a way that characterizes the true nature of skeletal muscle blood flow. Historically, muscle blood flow during dynamic exercise has been determined over several seconds yielding an average value. Conceiving of the muscle blood flow response during exercise as a mean or steady-state value may not only obfuscate important physiological information but also limit our understanding of cardiovascular control mechanisms. This is especially true since rhythmic muscle contractions give rise to oscillations in muscle blood flow (2; 17). These oscillations in flow were first observed by Barcroft & Dornhorst in 1949 (2) using plethysmography. Wallow and Wesche in 1988 (17), were the first to apply Doppler technology to non-invasively follow blood flow continuously during dynamic exercise in humans. This study confirmed the oscillatory nature of muscle blood flow during exercise but did not systematically characterize the oscillations in flow. It is currently unclear what is the net effect of muscle contractions (impedance, enhancement, or neutral) on oxygen delivery to and utilization by the working muscle.

In the collection of studies described herein, we sought to characterize the net effect of rhythmic muscle contraction on oxygen delivery to and utilization by the working muscle. The study in Chapter 2 was developed to examine the impact of muscle contractions on blood flow at the level of the femoral artery utilizing Doppler ultrasound. The purpose of the study was to ascertain if muscle contractions had a facilitory, neutral or net impedance effect on muscle blood flow during dynamic knee extension exercise as work intensity increased. We chose dynamic knee extension exercise so as to be able to isolate the working muscles being examined (the quadriceps) along with the associated blood flow.

Given the impact of muscle contractions on blood flow with increasing work rate, the question arose: How would increases in contraction frequency impact blood flow and oxygen utilization? This led to the conceptualization of Study 2 (Chapter 3) where we examined if the steady state relationship between muscle blood flow and metabolic rate at two differing contraction frequencies (60 and 100 rpm) on a cycle ergometer would be different. The blood flow to oxygen uptake relationship has been shown to be linear and robust at both the central (cardiac output vs. pulmonary $\dot{V}O_2$) (5; 14) and peripheral (leg blood flow / leg $\dot{V}O_2$) (1; 10; 13) levels with increase work rate. However, an increase in contraction frequency has been reported to either decrease (3; 11; 12), have no effect (6; 11; 16), or increase (15) the blood flow response during exercise, potentially altering this relationship. We hypothesized that contraction frequency would not alter either the central or peripheral blood flow / Vo_2 relationship during discontinuous incremental upright cycle exercise (i.e., blood flow would remain tightly coupled to metabolic rate independent of contraction frequency).

Armed with the knowledge that dynamic muscle contractions can have a profound impact on muscle blood flow, we wanted to ascertain what impact rhythmic muscle contractions would have on capillary blood flow and gas exchange. Gray et al. (7) had demonstrated that maximal tetanic contractions did not cause microvascular collapse and Poole et al. (12) had observed red blood cells (RBCs) in myocardial capillaries during hypersystole. If RBCs remained trapped in capillaries during muscle contraction, this would predict the possibility of oxygen flux to the myocyte during contraction. To date, technical limitations have precluded assessment of this. In addition, Chung et al. (4) showed fluctuations in putative controllers of mitochondrial respiration during a muscle contraction cycle using ^{31}P-NMR, suggesting the possibility that oxidative phosphorylation might fluctuate as well. Thus, with this knowledge and our past observations of the oscillatory behavior in muscle blood flow during muscle contractions, we designed Study 3 described in Chapter 4 to elucidate if oxygen extraction could be occurring during muscle contractions. Utilizing NIRS we were able to examine (in real time) the muscle microvascular hemoglobin concentration ([Hb]) and oxygenation within the contraction / relaxation cycle to determine if microvascular RBC volume was preserved and if oxygen extraction continued during the actual contractile phase.

Collectively, these studies provide novel insights crucial to understanding the control of muscle blood flow during dynamic exercise and the interactions between muscle contractions and oxygen utilization. It is vitally important that control of muscle blood flow (oxygen delivery) and oxygen utilization by the working muscles are characterized in healthy individuals, so that mechanistic insights can be gained when examining disease states.

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CHAPTER 2 - MUSCLE CONTRACTION-BLOOD FLOW INTERACTIONS DURING UPRIGHT KNEE EXTENSION EXERCISE IN HUMANS

ABSTRACT

To test for evidence of a muscle pump effect during steady state upright submaximal knee extension exercise, seven male subjects performed seven discontinuous, incremental exercise stages (3 min/stage) at 40 contractions/min, at work rates ranging to 60-75% peak aerobic work rate. Cardiac-cycle-averaged muscle blood flow (MBF) responses and contraction-averaged blood flow responses were calculated from continuous Doppler sonography of the femoral artery. Net contribution of the muscle pump was estimated by the difference between mean exercise blood flow (MBF_M) and early recovery blood flow (MBF_R). MBF_M rose in proportion with increases in power output with no significant difference between the two methods of calculating MBF. For stages 1 and 5, MBF_M was greater than MBF_R; for all others, MBF_M was similar to MBF_R. For the lighter work rates (stages 1-4), there was no significant difference between exercise and early recovery mean arterial pressure (MAP). During stages 5 - 7 MAP was significantly higher during exercise and fell significantly early in recovery. From these results we conclude that: a) at the lightest work rate, the muscle pump had a net positive effect on mean exercise blood flow, b) during steady state moderate exercise (stages 2-4) the net effect of rhythmic muscle contraction was neutral (i.e., the impedance due to muscle contraction was exactly offset by the potential enhancement during relaxation), and c) at the three higher work rates tested (stages 5-7), any enhancement to flow during relaxation was insufficient to fully compensate for the contraction-induced impedance to muscle perfusion. This necessitated a higher MAP to achieve the mean exercise blood flow.

INTRODUCTION

We (13) and others have shown that blood flow to rhythmically contracting skeletal muscle can become limited or occluded during the contraction period due to an augmented intramuscular pressure (25), with the majority of blood flow occurring during the relaxation period between contractions (3,10,15,37,38). On the venous side, these repeated cycles of muscle contraction-relaxation may assist venous return and contribute to muscle blood flow via the muscle pump (16,28). However, the net contribution of the muscle pump to muscle blood flow during dynamic exercise is controversial (9,12,17). On the one hand, there is good evidence for a muscle pump effect in humans during upright cycle exercise at very light work rates (31) and in rats and dogs freely running on a treadmill (26,27). In contrast, Hamann et al. (12) did not find any evidence of a muscle pump effect under artificially, maximally adenosine-vasodilated conditions in which the onset of locomotion failed to further elevate blood flow. While these authors concluded that the muscle pump did not contribute to maximum skeletal muscle blood flow seen during exercise, their data does not rule out a role for the muscle pump under less-than-maximally dilated conditions.

In the present study we sought to clarify the interaction of muscle contraction and blood flow by examining the cardiovascular responses in naturally recruited muscles during voluntary upright knee extension exercise in the dependent limbs of humans. This was accomplished by comparing net blood flow during steady-state exercise with the blood flow observed during the first few cardiac cycles in recovery (after sufficient time for prior effects of muscle contraction, i.e. venous refilling and loss of pressure gradient, to be accounted for) across a series of dynamic work rates which ranged from light to heavy. We assumed that these first few cardiac cycles in recovery would reflect the level of vasodilation and vascular conductance during exercise, but without the influence of muscle contractions (9,12). Assuming also that mean arterial blood pressure (MAP) remained constant across the exercise-early recovery transition, we hypothesized that a) if blood flow early in recovery was less than the mean flow during exercise, this would imply a positive net influence of the mechanical effects of muscle contraction-relaxation to facilitate mean exercise blood flow above that achieved by vascular conductance and arterial pressure alone, b) if the early recovery flow was greater than the mean exercise blood flow, this would suggest that the net effect of the muscle contraction-relaxation cycle was

impedance to flow as seen by Hamann et al. (12) and Dobson and Gladden (9), or finally c) if early recovery flow was no different that the mean exercise level, this would suggest no net effect of the muscle contraction-relaxation cycle on exercise blood flow under these conditions. In addition, to gain further insight into the consequences of the muscle contraction/relaxation cycle on blood flow, we differentiated blood flow into that associated with muscle contraction (MBF_C , usually retrograde), and net flow during the subsequent relaxation (MBF_{NR}). To ascertain if the MBF_{NR} represented enhanced flow, we compared these values with the blood flow observed during the first few cardiac cycles in recovery (MBF_R).

METHODS

Subjects

Seven healthy male volunteers participated in the study. The physical characteristics of the subjects were (mean ± standard deviation): age 30 ± 15 yrs, height177.8 ± 5.0 cm, and weight 77.6 ± 12.7 kg. They represented a variety of activity backgrounds. Subjects were informed of all possible risks and discomforts associated with the experiment protocol prior to providing written consent. This study was approved by the Human Subjects Committee at Kansas State University where all exercise tests were conducted.

Experimental Design

For this experiment, subjects performed discontinuous, incremental one-leg knee extension exercise on a specially built leg ergometer (13). In contrast to our previous work (13) exercise was performed with the subject in the upright seated position, with the thigh parallel to, and the lower leg perpendicular to the ground. A strap was then placed around the ankle of the subject and attached to a pneumatic cylinder by means of a cable-pulley system. Work was accomplished by compressing the air in the cylinder as the lower leg was extended. Upon relaxation, expansion of the gas in the cylinder brought the leg back to the starting position, i.e., knee flexion was passive during the down stroke. This mode of contraction-relaxation is similar to most Krogh-ergometer based knee extension exercise, but is contrary to that used by Shoemaker et al. (32) who utilized both concentric and eccentric contractions, followed by relaxation, as their contraction cycle (which could have limited the effect of the muscle pump in

their study). For comparison of blood flow responses to our previous work (13), the distance traveled by the lower limb during knee extension was limited to a fixed linear displacement (d) of the piston of the pneumatic cylinder of 10.9 cm, which represented a range of motion about the knee of ~20°. The amount of work performed (per stroke) was calculated as $[P_{INT} + (P_F + P_{INT})/2] \cdot d$, where P_{INT} is the initial pressure in the cylinder and P_F is the final pressure at the end of the work stroke. This in turn was divided by the duration of the contraction cycle (~0.16 sec.) to yield power (W). Work rate was varied by adjusting the pressure in the cylinder. Actual work rates were 0.07, 1.39, 2.72, 4.05, 5.11, 7.49, and 9.80 watts. These represented exercise intensities ranging from 4-5% of maximum voluntary contraction at the lowest intensity to 30-40% at the highest intensity (see below), or up to 60-75% of the peak work rates as previously determined for this mode of exercise (13) . The contraction frequency was set at 40 kicks per minute. A metronome was used to assist the subjects in maintaining the appropriate kicking frequency. Ventilation was not measured so entrainment of breathing, with its potential effect on venous return, could not be assessed. However, from pilot work, this mode of exercise engenders such a small rise in metabolic rate (<700 ml/min) as to not appreciably affect the breathing rate. Each increment protocol consisted of 1 minute rest, 3 minutes of exercise, and one minute of recovery. Total time between stages was typically 10-15 minutes. Before the study, each subject was familiarized with the testing procedures and the exercise protocol by performing at least 3 to 4 practice sessions.

Measurements

Instantaneous blood velocity in the right femoral artery was continuously determined using a Doppler ultrasound velocimetry system (Model 500-V, Multigon Industries, NY) operating in pulsed mode. The pulsed-wave Doppler transducer, with an operating frequency of 4 MHz and a fixed isonation angle of 45°, was placed flat on the skin 2-3 cm below the inguinal ligament, above and parallel to the common femoral artery. This position was selected to minimize turbulent flow arising from the bifurcation of the common femoral artery into the superficial and profundus branches. The gate was set at full width to ensure complete femoral artery insonation. The frequency spectrum of Doppler audio signals was converted to an instantaneous mean blood velocity using a quadrature audio demodulator that was calibrated according to the specifications of the manufacturer (Hokanson Co., WA). Blood pressure was

continuously monitored noninvasively at the radial artery (Colin Model 7000, Colin, TX). ECG was obtained using a modified Lead I. Software developed in our laboratory (13) was used to calculate femoral artery blood velocity averaged over one cardiac cycle between the R-R interval. The instantaneous cardiovascular (blood velocity, blood pressure and ECG) and ergometer data (displacement, pressure) were digitized and stored for off-line analysis.

To test the reproducibility of the protocol and data analysis, a separate study was performed on one subject. On four consecutive days with approximately the same conditions each day, the subject performed a protocol similar to the primary study but utilizing only two work rates (2.3 and 7.0 W). All measurements and data analysis were the same as for the primary study.

On a separate day, the femoral artery cross sectional area (CSA) was determined using a duplex Doppler computed sonography system (Acuson, Model 128XP, CA) in two-dimension echo mode. The vessel diameter was determined at rest in the upright position from a cross-sectional view of the artery at the level used to measure blood velocity. From the images stored on VHS tape, ten to fifteen measurements were randomly made where the proximal and distal vessel walls were most accurately visualized. The mean of these measurements was then used to calculate an average CSA which in turn was used to convert velocity to flow.

To determine if the diameters of the resting femoral artery were representative of exercise values, and to validate the assumption of constant femoral artery diameters in the exercise-to-recovery transition, five different subjects performed a similar exercise protocol in which the femoral artery cross sectional area (CSA) was recorded continuously during the protocol using duplex Doppler computed sonography (Acuson, Model 128XP, CA). Work rates were set at 30% and 60% of the maximum work rate achieved during a previous incremental exercise test. Ten measurements were taken for each subject and work rate during both the systolic and diastolic phases at rest, exercise, and for the first 4-5 cardiac cycles in recovery (14).

In order to compare the generated muscle force with our previous work (13) and with other modes of exercise, maximum voluntary contraction (MVC) for each subject was determined in the same position that the knee extension was performed (upright with knee bent at 90°). Each subject performed three maximum isometric efforts for 5 seconds each, with 2 min rest in between, against a fixed cable connected to a force transducer.

Data Analysis

From the cardiac-cycle-averaged blood flow responses, three characteristics, two during exercise and one during early recovery, were determined as follows. Mean exercise blood flow (MBF_M) was defined as the average blood flow over the last 30 seconds of exercise. The peak oscillations in blood flow during exercise (MBF_{PO}) were calculated as the mean of the 10 highest values of blood flow during exercise. The early recovery muscle blood flow (MBF_R) was determined from the average of the first 4 cardiac cycles in recovery, after allowing for the equivalent time of a complete contraction-relaxation cycle for the last contraction of the exercise bout (see Fig. 1). This was done so as to allow for any residual effect of the last muscle contraction and muscle pump effect to dissipate.

From the contraction-averaged blood flow responses, the net relaxation blood flow between contractions (MBF_{NR}) and the blood flow during the contraction phases (MBF_C) were calculated along with the mean exercise blood flow during the steady state period.

For each of the time points of observation of the blood flow response (the means, peak oscillations, early recovery, net relaxation, and contraction), corresponding values of MAP were also determined. Because an Ohmic conductance does not exist across a pump (18), vascular conductance was only calculated for early recovery, as VC = muscle blood flow / MAP.

Statistics

Group summary data are presented as mean ± SD. For each of the cardiac-cycle-derived primary variables (blood flow and mean arterial pressure), data was initially analyzed using a two way ANOVA with 2 repeated measures to examine main effects of power output (stages 1-7) and data point (mean, peak oscillation, and early recovery) using NCSS 2000. Similarly, for the contraction-cycle based blood flows, data was initially analyzed using a two way ANOVA with 2 repeated measures to examine main effects of power output (stages 1-7) and data point (net muscle blood flow during relaxation and cardiac-cycle derived early recovery muscle blood flow) A two way ANOVA with two repeated measures (power output, time) was used to test the femoral artery diameters for rest, exercise and early recovery. Post-hoc testing of significant results was performed using a Fisher's LSD Multiple-Comparison test. In all cases, significance was declared when $P<0.05$.

RESULTS

Figure 1 shows an expanded view of the raw analog data for one subject spanning the transition from exercise to rest recovery. Figure 2 shows the blood flow and MAP responses, averaged for each cardiac cycle, for another subject performing one 3 min stage protocol, while Figure 3 shows the same exercise bout with blood flow averaged during each contraction and relaxation. Note the reduction, but not elimination, of oscillations when blood flow is averaged over each contraction and relaxation (Fig. 3) compared to cardiac-cycle averages (Fig. 2). Figure 4 shows the group mean responses for MBF and MAP for the net flow during relaxation (MBF_{NR}) and for the first four cardiac cycles in recovery. The highest blood flow values during recovery occurred during these four cardiac cycles in all 49 individual trials and there was no significant difference among them. Thus, the average of these four cardiac cycles was used for the early recovery value.

Blood flow (Cardiac cycle-based):

There was no significant difference between the exercise mean blood flow values calculated from the cardiac cycle-based data and the contraction-based data for any stage. For the cardiac cycle-based data, MBF_M, MBF_{PO} and MBF_R all increased significantly as functions of power output (Fig. 5A). Post-hoc testing showed that MBF_{PO} was significantly greater than MBF_M for stages 2 through 7; i.e., blood flow calculated over each cardiac cycle demonstrated significant oscillations about the mean value for all but the lightest work rate. MBF_R was similar to MBF_M except for stages 1 and 5. For no stage was the peak blood flow in recovery (MBF_R) greater than the peak exercise oscillations (MBF_{PO}) (Fig. 5A).

Blood flow (Contraction based):

Muscle contraction was associated with retrograde blood flow in the femoral artery (Fig. 1), even at the lightest work rate. The volume of blood per contraction progressively and significantly increased over the first 2 stages, but did not change further from stages 3 to 7 (Fig. 7). This impedance to flow was balanced by enhancement to flow such that MBF_{NR} was significantly higher than MBF_R for stages 1-6 (Fig 5B). At the highest work rate, stage 7, MBF_{NR} was not significantly different than MBF_R.

Mean Arterial Pressure (MAP):

Overall, MAP tended to increase with power output, especially at the higher work rates (Fig. 6). Post-hoc testing revealed that MAP_M and MAP_{PO} values were similar for each individual power output, i.e. the oscillations in cardiac-cycle derived muscle blood flow were not associated with oscillations in blood pressure. MAP did not fall significantly in early recovery compared to the exercise mean for the first four stages. However, at the higher work rates (stages 5-7), MAP_R was significantly lower than MAP_M (Fig. 6).

Reproducibility of blood flow responses:

Reproducibility of the blood flow responses in one subject who repeated two work rates (corresponding approximately to stages 3 and 6) on four consecutive days is shown in Table 1. Coefficients of variation were $\leq$10%, and with the exception of MBF_R were lower at the higher work rate.

Femoral artery diameter changes with exercise:

For the different group of subjects, the average femoral artery diameter at rest was 9.2 ± 1.1 mm during systole and 8.8 ± 1.1 mm for diastole. During exercise, diameter was 8.9 ± 1.3 mm during systole and 8.6 ± 1.3 mm for diastole, and was not significantly different either between work rates or compared to the resting values. Finally, there were no significant differences in diameters between the exercise values and the first few cardiac cycles in recovery.

DISCUSSION

To our knowledge, this is the first study to quantify the net contribution of the muscle pump during steady state exercise across a wide range of submaximal work rate intensities (up to ~60-75% peak work rate, based on our previous work (13)) during upright knee extension exercise. The primary finding is that at the lightest work rate, rhythmic muscle contraction enhanced mean exercise blood flow, compared to blood flow early in recovery. Above this work rate, there was no systematic enhancement of mean muscle blood flow during steady state exercise. In fact, at the higher work rates there was evidence that the net effect of muscle contraction/relaxation was impedance to flow.

During dynamic exercise, muscle blood flow is determined by skeletal muscle vascular conductance, the perfusion pressure gradient, and the efficacy of the muscle pump (6,18,23). With regard to the last, there is ample evidence from a variety of studies that muscle contraction transiently increases venous outflow from the muscle/limb (9,29). The fundamental controversy lies in whether the overall effect of muscle contraction (pump) is to enhance the net (average) muscle blood flow, decrease the net flow, or have no discernable effect. There is evidence that the muscle pump can actively assist muscle perfusion (26,29) and accounts for much of the immediate rise in vascular conductance at the onset of exercise, before metabolic vasodilation (19,27,28). Tschakovsky et al. (35) demonstrated with rhythmic cuff inflation/deflation that muscle blood flow at rest can be enhanced by a mechanical muscle pump effect. Further, Shiotani et al (31) found that in humans, cycling in the upright position under near unloaded conditions (5W) was associated with twice the femoral arterial blood flow, presumably via the venous muscle pump, compared to cycling at the same light work rate in the supine position. However, this difference disappeared as the work rate was ramped up to 45W over 60 sec (31). Our results are similar to those of Shiotani et al (31) in that mean exercise blood flow was significantly greater than early recovery for the lightest work rate, but this difference disappeared as the work rates became greater (with the isolated exception of stage 5).

In contrast, a recent study by Hamann et al.(12) found no evidence for a muscle pump enhancement of peak muscle blood flow. In their preparation, conscious dogs standing on a treadmill were maximally adenosine vasodilated. The onset of locomotion failed to further elevate blood flow. Dobson and Gladden (9) also found no evidence of the muscle pump enhancing peak muscle blood flow during electrical stimulation of isolated dog gastrocnemius muscle. However, the unphysiological recruitment patterns of electrically activated muscle in that study may not have engaged the muscle pump effectively (16). Our current data, gathered under conditions of natural muscle and vascular recruitment during moderate steady-state exercise, extend these observations. As such, our results suggest that any net enhancement to muscle blood flow by the muscle pump occurred only at very light work rates, but that for moderate and higher work rates, there was generally no net effect of the muscle pump to augment net muscle blood flow during steady state submaximal, upright knee extension exercise.

To assess any potential effect of the muscle pump to augment net exercise blood flow, we compared mean muscle blood flow during steady state exercise to early recovery blood flow,

after allowing time for any effects from the exercising period (i.e., venous refilling and loss of pressure gradient) to elapse. Since blood flow during the first four cardiac cycles during early recovery were not significantly different from each other, this suggested that changes in vascular tone (conductance) had not yet occurred. Generally, mean exercise blood flow was similar to recovery blood flow (except for Stages 1 and 5). There was no significant change in MAP from exercise to early recovery for stages 1-4, suggesting that for stages 2-4, the muscle pump did not augment blood flow above that achievable by blood pressure and vascular conductance alone (Fig. 8). From Stages 5 to 7 MAP was progressively higher during exercise than during recovery. Since MBF_{NR} again was generally similar to MBF_R , this suggested that the net effect of the muscle contraction/relaxation cycle was progressive impedance to flow, which necessitated a higher MAP in order to achieve the mean exercise flow under these conditions (Fig. 8).

To gain further insight into the hemodynamic effects of the muscle contraction/relaxation cycle and extend the observations of Hamann et al. (12), we also partitioned the Doppler-derived blood flow into contraction (usually retrograde, discussed below) and relaxation flows. At the lighter work rates (Stages 1-4), the average relaxation blood flow was significantly greater than early recovery blood flow, indicating that blood flow during relaxation was enhanced above that attained by pressure and conductance alone (i.e., a muscle pump effect). For Stages 5 and 6, relaxation blood flow was also higher than early recovery, but was associated with higher MAP during exercise; in this case the enhancement to flow during relaxation could not be attributed solely to a muscle pump effect, but was also due at least in part to elevated pressure during exercise. At the highest work rate (Stage 7), MBF_{NR} was similar to MBF_R , even though MAP was higher during exercise. This suggested that any enhancement to flow during relaxation was insufficient to fully compensate for the contraction-induced impedance to muscle perfusion at the highest work rate.

A critical assumption in the present study was that vascular conductance (VC_R) and blood flow (MBF_R) during the first few cardiac cycles in recovery from dynamic exercise reflected the state of the peripheral circulation during the exercise period, but in the absence of any mechanical influence of muscle contraction itself (analogous to a sub-maximally vasodilated animal model)(9,12). To our knowledge, little work has been reported to date regarding the timing of changes in vascular conductance immediately upon cessation of exercise. Since vascular conductance cannot be measured across a pump such as rhythmically contracting

muscle (18), we could not directly determine vascular conductance during exercise so as to validate this assumption. However, evidence regarding the onset of vasodilation after application of a dilatory stimulus indirectly supports our assumption. For example, Wunsch et al. (39) found that potassium chloride, adenosine, acetylcholine, and sodium nitroprusside applied directly to isolated rat arterioles each exhibited a delay of ~5 s before vasodilation occurred. Similarly Sheriff and Zidon (30) found ~5 s delay in the onset of vasodilation following grade changes during treadmill running in rats. Further, once vasodilation had been initiated, the overall response took several seconds to reach a peak (30,34,39), and relevant to our assumption, several more seconds to decay following even a single contraction (35). In contrast, smooth muscle hyperpolarization n (11) and/or contraction-induced mechanical distortion of the arterial resistance vessels (34) have recently been suggested as putative mechanisms for rapid (within 2 s) vasodilation following exercise onset. It remains uncertain if removal of either of these two stimuli/mechanisms upon cessation of exercise is rapid enough to invalidate our assumption. Thus, based on current data, our assumption that early recovery blood flow reflects the state of the peripheral circulation during the exercise period, without mechanical interference from muscle contraction, appears to be valid. A similar conclusion was reached by Shiotani et al (31) for femoral artery blood flow in the immediate recovery from upright cycle exercise. (N.B. If this assumption is wrong, i.e., if the first few cardiac cycles in recovery do reflect a reduced vascular conductance relative to that of exercise, this would imply that the method used here (the difference between exercise relaxation and early recovery blood flow) would overestimate the contribution of the muscle pump to the exercise blood flow).

Contraction-induced oscillations in muscle blood flow have been recognized for over 50 years (3). In each investigation in which muscle blood flow has been reported on a contraction-by-contraction basis (2,4,20,22,23,32,33,37) impedance of blood flow during contractions, due to increased intramuscular pressure (25), has been noted. In the current study, we were able to observe significant flow impedance, and even retrograde flow in the femoral artery, at surprisingly low muscle tensions (4-5% MVC, Fig. 7), similar to that reported by Robergs et al. (24) for forearm exercise (6% MVC), but half of the lowest work intensity (10% MVC) for knee extension exercise observed by Walloe and Wesche (37). We have previously observed that retrograde blood flow during incremental continuous exercise in the supine position reached a constant, maximum value of about 3-4 ml/contraction at very light muscle forces and power

outputs (13), similar to that observed in the present study (~4 ml/contraction). Kagaya & Ogita (15) also reported no significant change in blood flow during the contraction phase of rhythmic handgrip exercise over a range of muscle forces (10% and 30% MVC) similar to that employed here. This pattern of response (relatively constant minimum blood flow during contraction at higher power outputs) is similar to those reported for brachial artery blood flow during wrist flexion/extension exercise (24).

It is interesting to note that the mode of exercise performed here (knee extension exercise over a limited (20°) range of motion) might be considered less likely than normal locomotion to demonstrate an effect of the muscle pump (16). For comparison, running typically is associated with a range of motion of ~37° around the knee (from 165° at contact to 128° at maximum flexion) (7,8), whereas cycling may elicit a range of knee movement of ~74°, depending on relative seat height (21). Also, the more common Krogh-style knee extension ergometer is associated with a range of motion of up to 80° (1) Thus, while not isometric, our mode of muscle contraction represents a smaller range of motion than would be encountered in other forms of exercise, both stationary and locomotory. Even so, we were able to detect wide oscillations about the mean blood flow that represented the impedance and subsequent enhancement to flow (muscle pump) caused by our mode of muscle contractions.

Due to conflicting data in the literature regarding vessel diameter changes during dynamic exercise (5,15,20,22,36), we assessed the diameter of the femoral artery during the rest-to-exercise and the exercise-to-recovery transitions, in a different group of subjects. We found no change in femoral artery diameter from rest to exercise to early recovery for two work rates comparable to those performed in the present study, validating our use of diameters determined at rest as representative of exercise values (similar to the conclusions of Radegran (22), Shiotani et al. (31)and DeLorey et al. (5).

In summary, we have quantified the net contribution of the muscle pump during steady state upright knee extension exercise by comparing mean blood flow during exercise to early recovery blood flow across a wide range of submaximal work rates in humans. Further, by comparing blood flow during the relaxation phase between contractions to the early recovery flow, assuming vascular conductance remained unchanged from the exercise level, we were able to quantify the potential enhancement effect of the muscle contraction/relaxation cycle to muscle blood flow. For the lightest work rate, mean exercise blood flow was greater than during

recovery, suggesting a muscle-pump-induced enhancement to flow. For the next intermediate work rates (stages 2-4), the net effect of the muscle pump was neutral, i.e., it neither enhanced nor impeded net blood flow. At the heavier work rates the increased impedance to flow with increased contraction force was not fully compensated for by any enhanced flow during relaxation. This required an increase in MAP during the exercising phase so as to maintain mean muscle blood flow at a level equivalent to that set by vasodilation.

ACKNOWLEDGMENTS

I would like to thank Akira Miura, Barry W. Scheuermann, Shunsaku Koga, Dana K. Townsend and Thomas J. Barstow for their collaboration on this manuscript. I would also like to thank Allison Harper for assistance with data processing. This work was supported in part by American Heart Association Grant-in-Aid #0151183Z to TJB.

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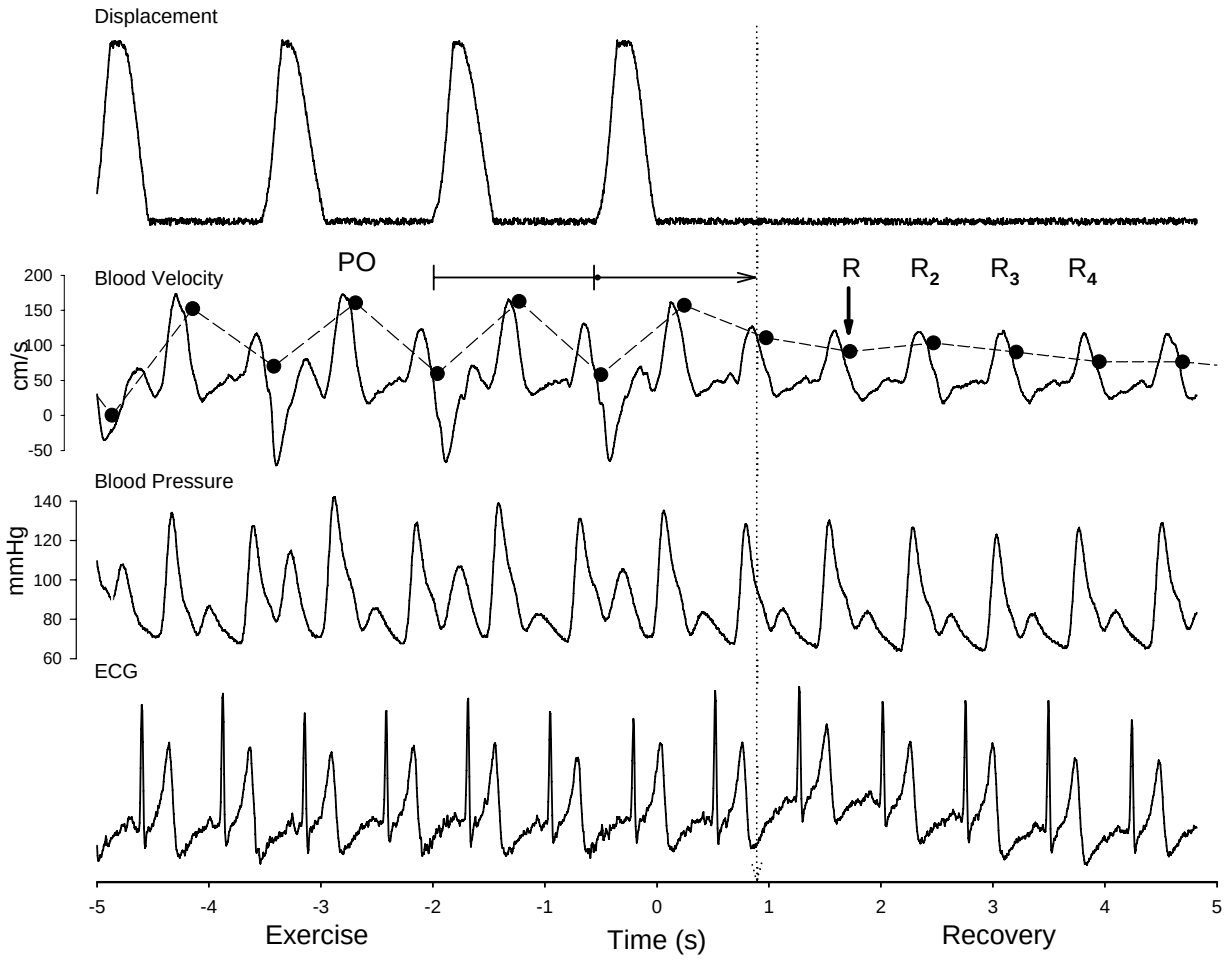
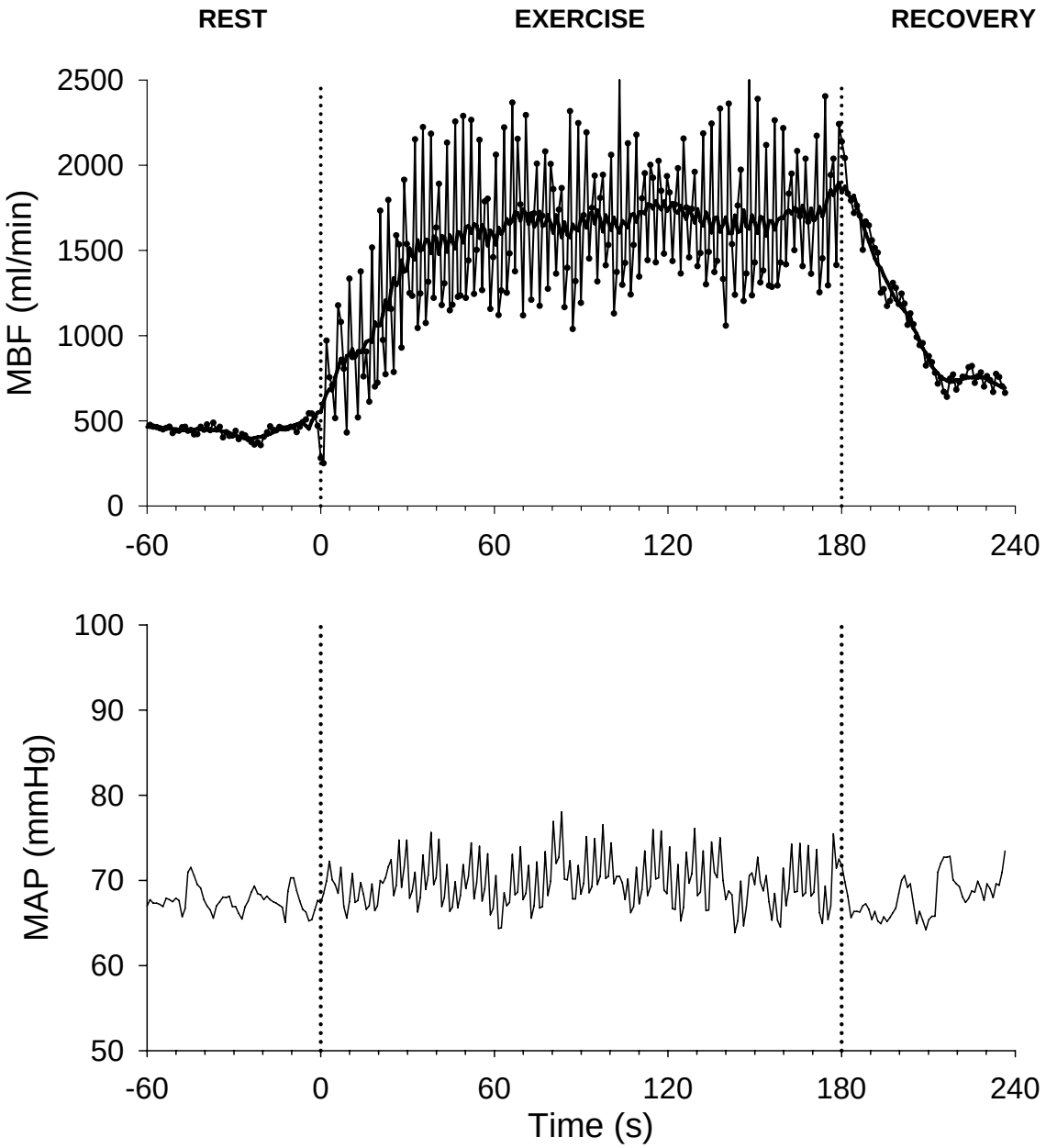
FIGURES

Figure 2-1 Raw analog signals showing responses at the Exercise: Recovery transition.



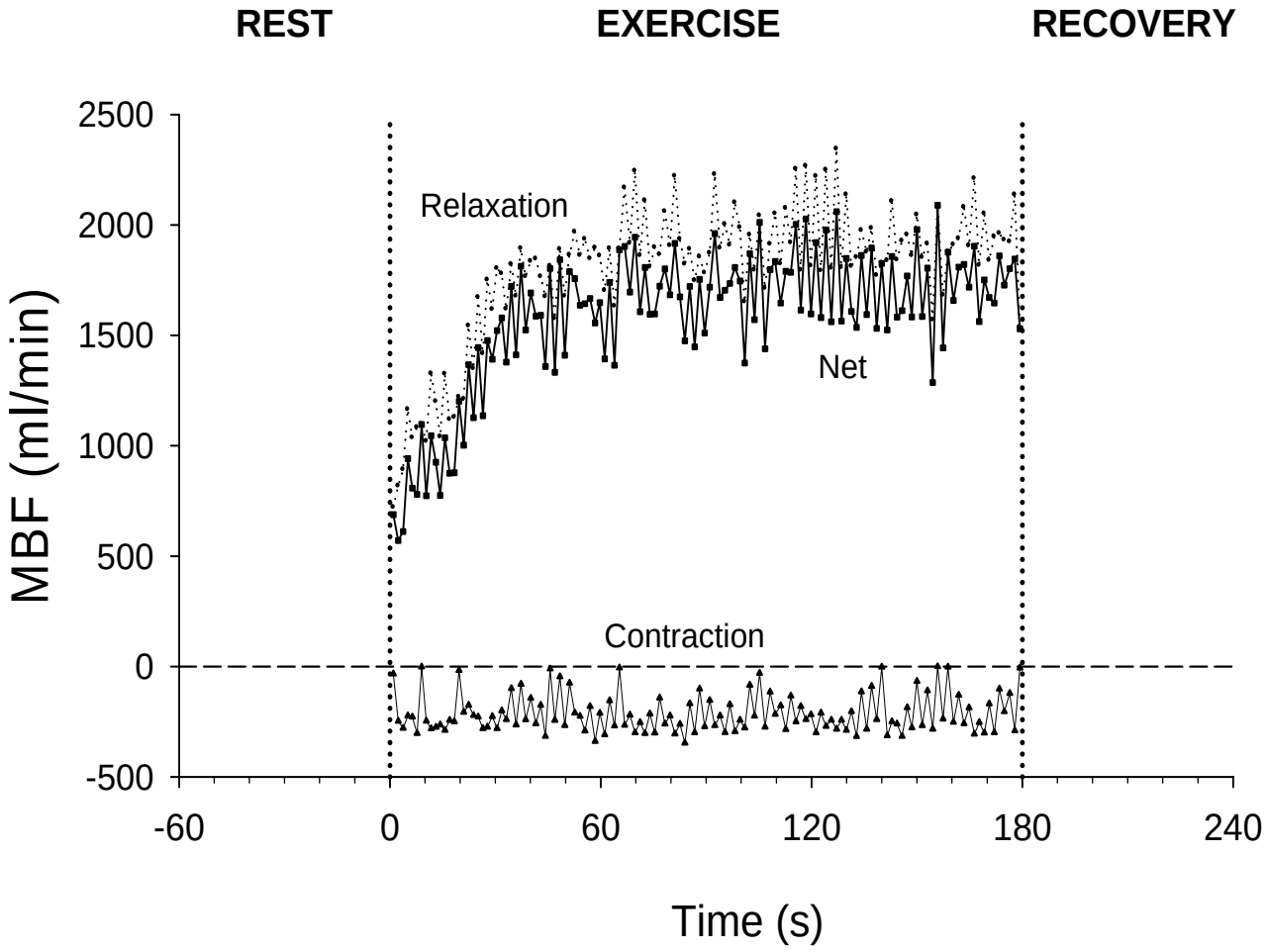
Solid circles in Blood Velocity tracing represent average blood flow during corresponding cardiac cycle. Dotted vertical line shows equivalent time for contraction:relaxation cycle for last kick (i.e., the time of onset of the next contraction, if existed). PO represents peak exercise oscillations. R represents first cardiac cycle in recovery fully after the time for the last relaxation period (i.e., after the dotted line). R2, R3, and R4 represent the next three consecutive cardiac cycles in recovery. Note oscillatory behavior of blood velocity/flow curves due to intermittent effect of muscle contraction to impede flow, followed by augmented flow during the relaxation phase, relative to early recovery blood flow. Also note secondary, intermittent smaller peak in blood pressure associated with muscle contraction and retrograde flow in the femoral artery.

Figure 2-2 Blood flow response and corresponding mean arterial pressure during knee extension exercise at 10 W for the same subject.



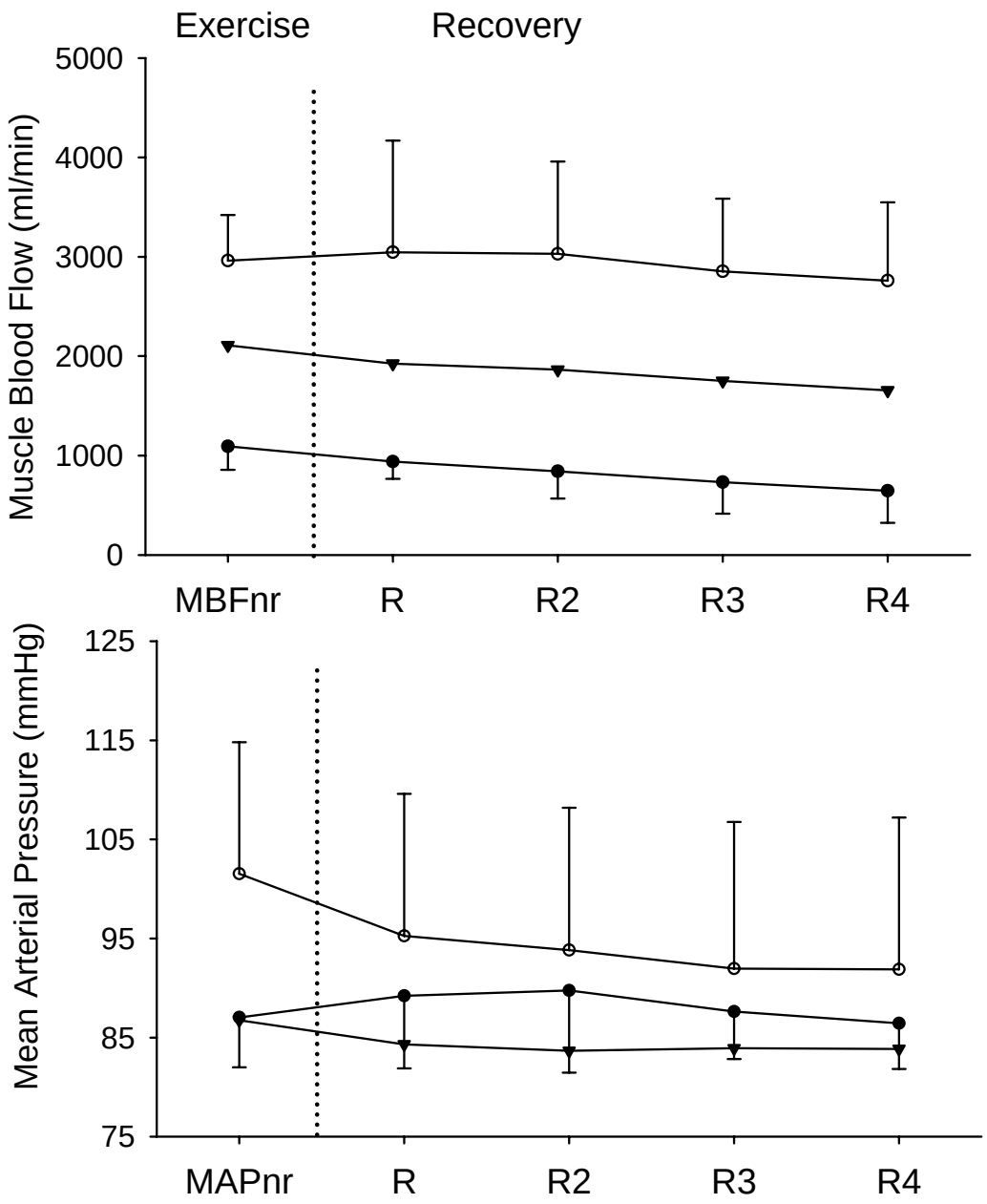
***Upper** panel* Blood flow response during knee extension exercise at 10 W in one subject showing cardiac cycle-by-cycle values (dotted line) along with typical mean response (solid line, calculated using 11 point rolling average). *Lower panel* Corresponding mean arterial pressure (MAP) for the same subject.

Figure 2-3 Blood flow responses during each contraction/relaxation cycle for the same exercise bout as in Fig. 2.



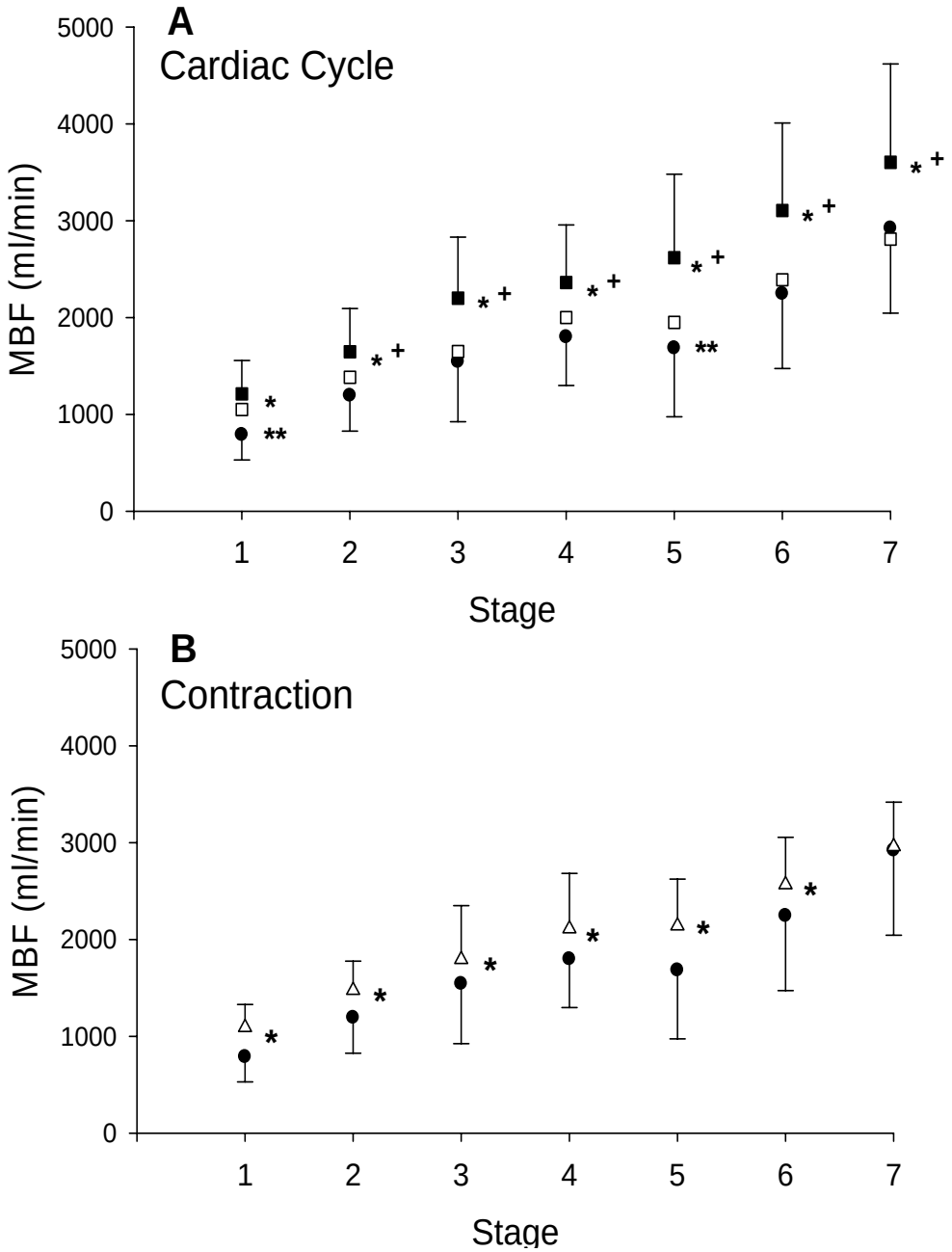
Contraction=retrograde blood flow during muscle contraction, Relaxation=blood flow during relaxation periods between contractions (MBF_{nr}), Net=net blood flow for entire contraction-relaxation cycle.

Figure 2-4 Group mean responses for average muscle blood flow and mean arterial pressure.



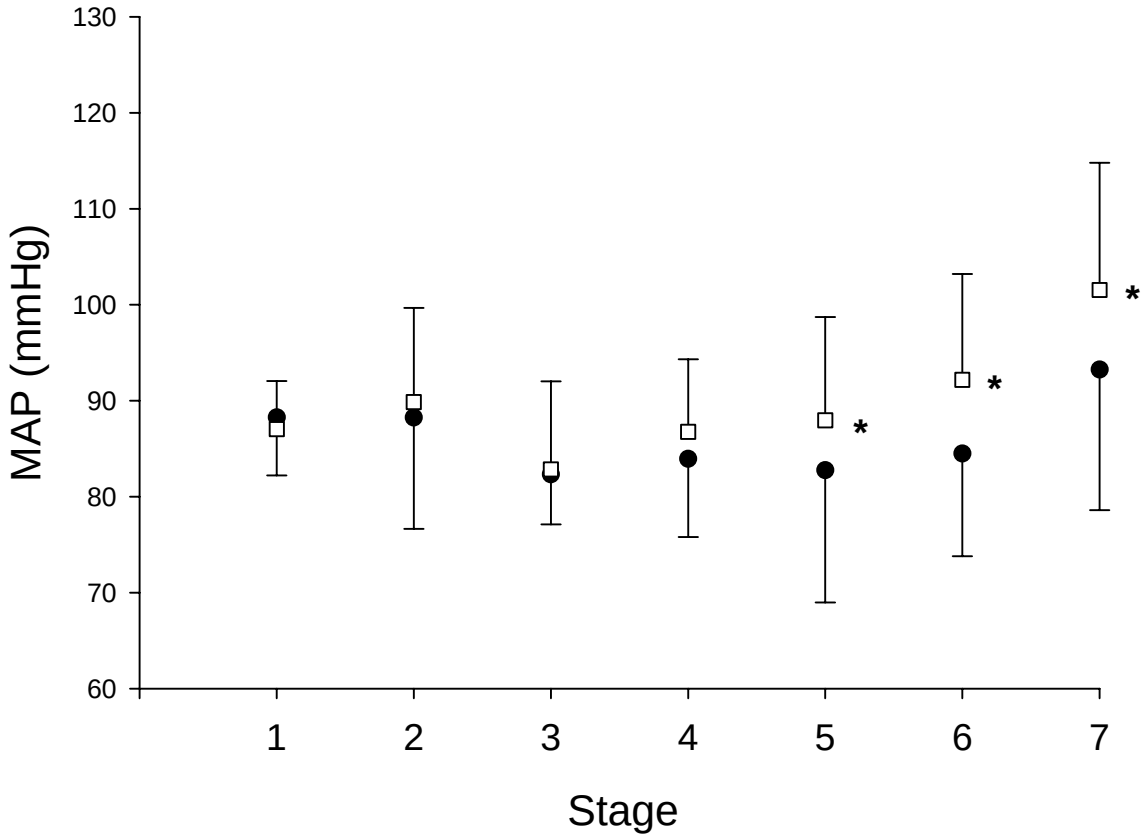
Group mean responses for average muscle blood flow (*upper panel*) and mean arterial pressure (*lower panel*) during relaxations between contractions (MBF_{NR} and MAP_{NR}), first cardiac cycle in recovery (R), and the three following cardiac cycles (R2-R4). For clarity only the first (●), fourth (▾), and seventh (○) stages are shown.

Figure 2-5 Muscle blood flow for the seven exercise stages.



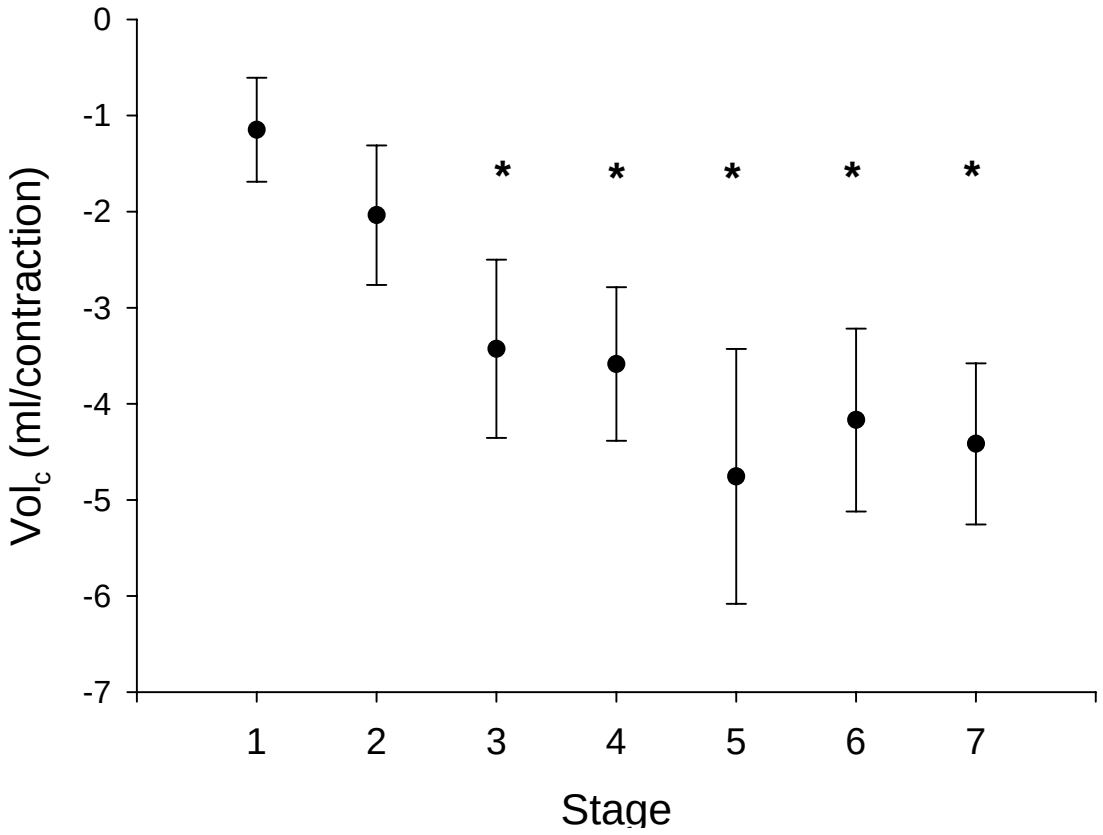
Muscle blood flow (MBF) (mean +/- SD), for the seven exercise stages. *Upper panel* (□) exercise mean (MBF_M), (■) peak exercise oscillation (MBF_{PO}), (●) early recovery hyperemia (MBF_R) for the cardiac cycle based data. Error bars omitted from MBF_M for clarity. MBF_M, MBF_R, and MBF_{PO} significantly increased with power output ($P<0.05$). * MBF_{PO} significantly greater than MBF_R. + MBF_{PO} sign.greater than MBF_M ($P<0.05$). ** MBF_M sign. greater than MBF_R ($P<0.05$). *Lower panel* (▵) net flow during relaxations between contractions (MBF_{NR}) and (●) early recovery hyperemia (MBF_R) as in panel A. * MBF_{NR} significantly higher than MBF_R.

Figure 2-6 Mean arterial pressure for the seven exercise stages.



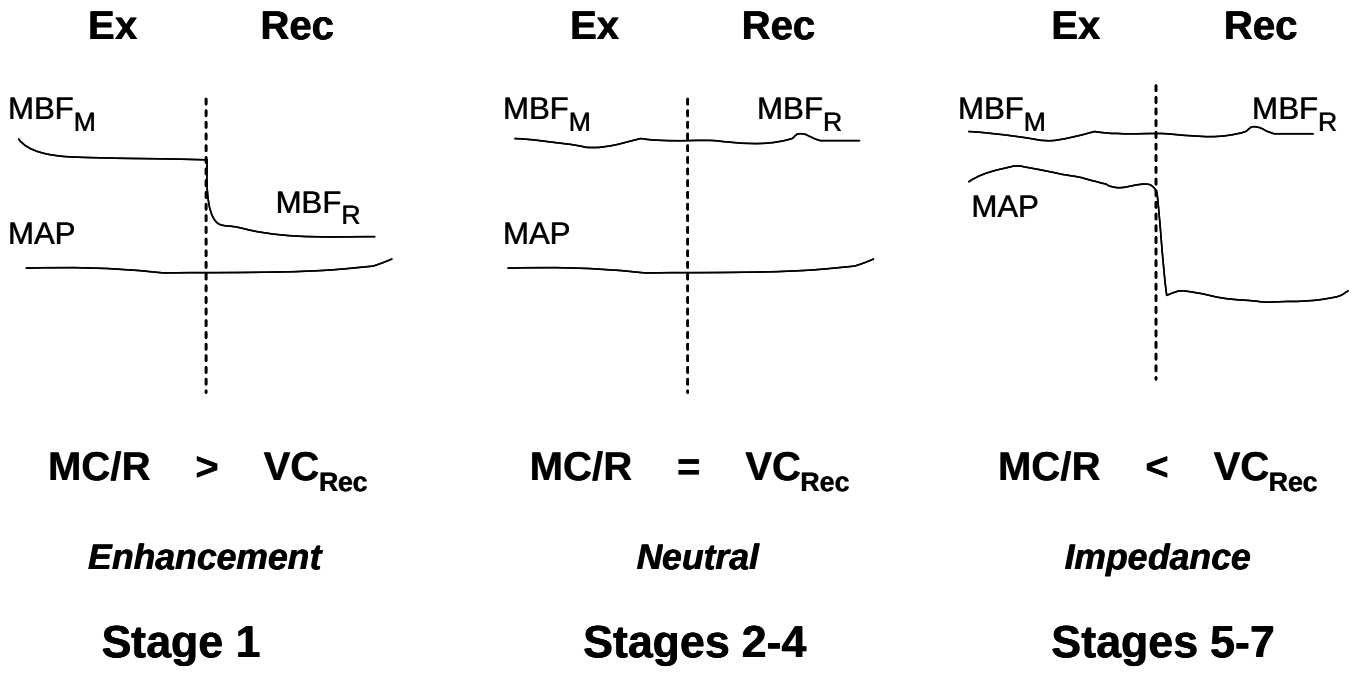
Mean arterial pressure (MAP) (mean +/- SD) for the seven exercise stages. (□) exercise mean (MAP_M), (●) peak recovery hyperemia (MAP_R). * MAP_R sign. different from MAP_M ($P<0.05$).

Figure 2-7 Volume of blood sent retrograde in the femoral artery during knee extension contraction (MBV_C) for each stage.



*Retrograde volume was significantly greater for stages 3-7 than for stages 1-2 ($P<0.05$), but there was no significant difference among stages 3-7.

Figure 2-8 **Schematic summarizing results for current study.**



Ex: exercise; Rec: recovery; MBF_M: mean exercise blood flow; MBF_R: muscle blood flow early in recovery; MAP: mean arterial pressure; MC/R: muscle contraction/relaxation cycle (muscle pump); VC_{Rec}: vascular conductance in recovery. For *Stage 1*, mean blood flow fell from exercise to early recovery (i.e., $MBF_M > MBF_R$). Since MAP remained constant, results suggest increased flow during exercise was due to muscle pump enhancement to flow over that achieved by MAP and VC_{Rec} alone. For *Stages 2-4*, $MBF_M = MBF_R$, which with no change in MAP suggests neutral effect of muscle pump. For *Stages 5-7*, $MBF_M = MBF_R$, but MBF_M was achieved with a progressively higher MAP during exercise than required during recovery. This indicated a net impedance effect of the muscle contraction/relaxation cycle at the higher work rates.

TABLE

Table 2.1 Representing the reproducibility of the blood flow responses.

Work Rate		Mean	Std Dev	Co Var (%)
MBF_{PO}	2.3 w	2512	195.14	7.8
	7.0 w	3669	129.01	3.5
MBF_{M}	2.3 w	1816	149.26	8.2
	7.0 w	2473	52.60	2.1
MBF_{R}	2.3 w	2293	126.93	5.5
	7.0 w	3153	320.84	10.2

CHAPTER 3 - THE EFFECT OF CONTRACTION FREQUENCY ON THE CENTRAL ANDPERIPHERAL BLOOD FLOW / VO_2 RELATIONSHIP

ABSTRACT

The blood flow / $\dot{V}O_2$ relationship has been shown to be linear and robust at both the central (i.e., cardiac output / pulmonary $\dot{V}O_2$) and peripheral (leg blood flow / leg $\dot{V}O_2$) levels. However, an increase in contraction frequency has been reported to either decrease, have no effect, or increase the blood flow response during exercise, potentially altering this relationship. **PURPOSE:** To determine if the steady state coupling between muscle blood flow and metabolic rate (centrally and peripherally) would be altered by varying contraction frequency (pedal rate) during upright cycle exercise. **METHODS:** Six healthy subjects performed discontinuous ramp protocols (4.5 min. stages) on a cycle ergometer at either 60 or 100 cpm with 90 minutes of rest between protocols. Pulmonary gas exchange was measured breath-by-breath. Cardiac output, measured as pulmonary blood flow ($\dot{Q}_C$), was determined by the single breath acetylene absorption technique. Leg blood flow (LBF) was measured by the constant-infusion thermodilution technique and leg $\dot{V}O_2$ was calculated as the product of leg blood flow and arteriovenous O_2 content difference across the leg. **RESULTS:** Central findings: both $\dot{Q}_C$ and V O_2 increased linearly with power output. Results from paired t-test showed no significant difference (p=0.39) between the slopes of $\dot{Q}_C$ vs. $\dot{V}O_2$ at 60cpm (4.95 ± 0.91) compared to 100cpm (5.23 ± 0.37). Peripheral findings: both LBF and leg $\dot{V}O_2$ increased linearly with power output. There was no significant difference (p=0.37) between the slopes of LBF vs. leg $\dot{V}O_2$ at 60cpm (5.79 ± 0.40) compared to 100cpm (5.50 ± 0.39). **CONCLUSIONS:** These results indicate that the central and peripheral blood flow / $\dot{V}O_2$ relationships are robust and remain tightly coupled regardless of changes in contraction frequency.

INTRODUCTION

The blood flow to oxygen uptake relationship has been shown to be linear and robust at both the central (cardiac output vs. pulmonary $\dot{V}O_2$) (6; 28) and peripheral (leg blood flow / leg $\dot{V}O_2$) (1; 16; 26) levels with increase workrate. However, an increase in contraction frequency has been reported to either decrease (3; 14; 15), have no effect (8; 24; 31), or increase (30) the blood flow response during exercise, potentially altering this relationship.

Historically, it has been reported that muscle blood flow increases linearly with increases in work rate (1; 16; 26). However, changes in contraction frequency have been shown to impact this relationship with several studies reporting a plateau or decrease in muscle blood flow with increases in contraction frequency (3; 14; 15) while others have observed an enhancement to muscle blood flow with increases in contraction frequency (i.e., muscle pump effect) (30). However, it has also been shown that contraction frequency does not limit blood flow when contractions are performed at a constant duty cycle (8; 24; 31). It has also been reported that for moderate pedal rates (i.e., 60-70 rpm), the cardiac output / oxygen uptake relationship is linear and reproducible (6), although cardiac output has been shown to increase at a fixed work rate of 200 watts with increased contraction frequencies (70 to 100 cpm) due to lower intramuscular pressures developed during the contraction period at 100 cpm (12). Hence, increases in contraction frequency may potentially alter the coupling between the peripheral and/or central blood flow and oxygen uptake.

Thus, our purpose was to ascertain if the steady state coupling between blood flow and metabolic rate (both centrally and peripherally) would be altered by varying contraction frequency (as pedal rate) during upright cycle exercise. Further, we wanted to observe whether or not muscle blood flow would become limited at the higher contraction frequencies since increased impedance to muscle blood flow has been suggested during high contraction frequencies due to the shorter amount of time spent in the relaxation phase {662, 661, 671, 460}}. Specifically, we hypothesized that contraction frequency would not alter either the central or peripheral blood flow / $\dot{V}O_2$ relationship, i.e., blood flow would remain tightly coupled to $\dot{V}O_2$.

METHODS

Subjects

Experimental procedures and all possible risks and discomforts associated with the experiment protocol were explained to each subject prior to their providing written consent. This study was approved by the Institutional Review Board for Research Involving Human Subjects at Kansas State University, where all exercise tests were conducted.

Preliminary Tests

After being fully familiarized with the protocol and equipment, each subject performed two maximal incremental exercise tests, one at 60 cpm, and the other at 100cpm, on separate days on an electronically-braked cycle ergometer (Corival 400, Lode, The Netherlands). The incremental exercise test involved 4 min of baseline cycling (20 W) at the desired cadence (60 or 100 rpm) followed by a progressive (ramp) increase in exercise intensity (25-35 $W \cdot min^{-1}$) until volitional fatigue or until pedal cadence fell below 55 or 95 rpm respectively, for 5-10 seconds despite strong verbal encouragement. The incremental work rate, seat height and handlebar position on the cycle ergometer were recorded in the first test and reproduced on the second test. Pulmonary gas exchange ($\dot{V}O_2$ and $\dot{V}CO_2$) and minute expired ventilation ($\dot{V}E$) was measured breath-by-breath (CardiO_2, Medical Graphics Corporation, St. Paul, MN). Before each exercise test the volume signal was calibrated by pumping a 3-liter syringe at flow rates spanning the range expected during the exercise studies, while the O_2 and CO_2 analyzers were calibrated with gases of known concentration. Heart rate was recorded from the electrocardiogram using a modified lead I configuration and stored in the breath-by-breath file. Peak oxygen uptake ($\dot{V}O_{2peak}$) was defined as the highest $\dot{V}O_2$ achieved during the test averaged over a 15s interval. On the basis of these tests, work rates corresponding to 10, 20, 30, 40, 50, 60, 70, and 80% of $\dot{V}O_{2peak}$ were identified for each cpm condition.

Experimental Protocol

After completion of the catheterization the subjects returned to the exercise physiology laboratory for testing. Figure 1 shows a schematic of the discontinuous incremental protocol (upper panel) and the timing of blood draws (BD), thermodilution (TD), and cardiac output ($\dot{Q}_C$) measurements (lower panel). Each subject completed two exercise bouts (one at 60 and the other at 100 cpm). Each bout began with two minutes of pedaling at 30 W and then for 4.5 minutes sequentially at those WRs calculated to elicit ~10-80% $\dot{V}O_{2\ max}$ on the basis of the incremental tests. Each work rate stage was separated by two minutes of pedaling at 30 W. Two blood draws were performed during each stage (2 and 3 min.) followed immediately by infusion of the iced saline. One $\dot{Q}_C$ measurement was taken during the last ~15 seconds of each stage. The order of the two exercise bouts was randomly assigned and counterbalanced across the subjects with 90 minutes of rest between exercise bouts.

Peripheral Measurements

Subject preparation and leg blood flow measurements. Leg blood flow was determined by a constant-infusion thermodilution technique (for detail, see Refs (25) and (26)) derived from the method originally described by Anderson and Saltin (1). Briefly, within 1 week of the preliminary tests, subjects reported to the medical clinic after consuming a light meal with no caffeinated beverages. A catheter (DSA 4OOL, Cook, Bloomington, IN) was placed in the femoral vein ~2 cm below the inguinal ligament and advanced distally ~10 cm. This catheter, utilized for the injection of sterile cold normal saline and for blood gas sampling, is open ended with 10 pinhole side ports in the distal 2.5 cm oriented in all directions. This allows thin streams of saline to be ejected at all orientations into the vein, facilitating mixing across the vein lumen. The second catheter contained the teflon coated thermocouple (Physitemp IT-18, Physitemp Instruments, Clifton, NJ) and was advanced from approximately the same location proximally ~10 cm into the same femoral vein. It was not possible to ensure that the thermocouple tip was positioned distal to the site of entry of the saphenous flow. However, during severe-intensity exercise, the contribution of saphenous vein flow should be small. During exercise, iced saline was infused through the Cook catheter at flow rates sufficient to decrease blood temperature at

the thermocouple sensor by - 1° C. Injectate flow was continued for ~15 s until femoral vein temperature had stabilized. Rate of saline injection was determined by weight change in the reservoir bag. The calculation of blood flow was performed using thermal balance principles as described by Andersen and Saltin (1).

Measurement of leg $\dot{V}O_2$. The venous blood-gas samples were collected anaerobically in heparinized syringes, placed on ice, and analyzed within ~ 1 hour. PO_2, PCO_2, and pH were measured by a calibrated blood gas analyzer (CIBA-Corning 238 pH/Blood Gas Analyzer, Ciba Corning Diagnostic Corp., Medfield, Massachusetts). Hemoglobin concentration ([Hb]) and O_2 saturation (SO_2) were measured by the OSM3 Hemoximeter (Radiometer Medical, Copenhagen, Denmark). All blood gas measurements were corrected to the femoral vein blood temperature obtained immediately before blood sampling. Arterial blood O_2 content was calculated as (1.39 × total hemoglobin concentration × 98 % O_2 saturation) + (0.003 × blood PO_2 of 90). Venous blood O_2 content was calculated as (1.39 × total hemoglobin concentration × % O_2 saturation) + (0.003 × blood PO_2). Leg arteriovenous O_2 content difference [(a-v)O_2] and leg blood flow (2 legs) were then multiplied to give leg $\dot{V}O_2$ (l/min) according to the Fick principle.

Central Measurements

Measurement of cardiac output. Cardiac output was measured by the single-breath constant exhalation acetylene uptake technique (Sensormedics 229, Sensormedic Corp., Loma Linda CA, USA) using a test gas of acetylene (C_2H_2) (0.3%), carbon monoxide (CO) (0.3%), methane (0.3%), oxygen (O_2) (21%), with the balance of nitrogen (N_2). This method is based upon absorption of acetylene relative to an inert gas (methane) during constant expiration. At ~ 4'15" mark of each stage, the subjects were instructed to fully exhale, and then maximally inhale the C_2H_2 gas mixture to total lung volume. The subjects then performed a ~1 s breath-hold followed by a slow 3 second exhalation at a constant rate (1 L/sec). The non-invasive cardiac output was calculated from regression equations of acetylene and methane concentration curves.

The day before the trials, each subject performed multiple practice breathing maneuvers at various work rates to familiarize themselves with the procedure and to ensure proper

technique. The day of the testing, the subjects were reminded of the procedure and practiced before the tests began.

Measurements of pulmonary $\dot{V}O_2$, heart rate and stroke volume. Pulmonary gas exchange ($\dot{V}O_2$ and $\dot{V}CO_2$) and minute expired ventilation ($\dot{V}E$) were measured breath-by-breath with the same metabolic cart (Sensormedics 229, Sensormedic Corp., Loma Linda CA, USA. Heart rate (HR) was recorded from the electrocardiogram using a lead IV configuration and stored in the breath-by-breath file. Stroke volume (SV) was calculated from the $\dot{Q}_C$ and HR data ($SV = \dot{Q}_C / HR$).

Statistical Analysis

To test for linear behavior of the central and peripheral measurements to work rate and the blood flow / $\dot{V}O_2$ relationship, linear regression analysis was performed. To test for significant differences between two means, a two-tailed Student's paired t-test was performed. A repeated measures analysis of variance was performed to compare more than two means, with the Tukey-Kramer post hoc test was used for pair wise comparisons (NCSS 2000, Statistical Software, Kaysville, UT). Statistical significance was accepted when $P<0.05$. Values are reported as mean ± SD, unless otherwise specified.

RESULTS

Six male subjects participated in the study. The physical characteristics of the subjects were (mean ± standard deviation): age 23 ± 2 yrs, height 186 ± 10 cm, and weight 77 ± 10 kg. The peak pulmonary $\dot{V}O_2$ during the preliminary continuous ramp tests was 3.92 ± 0.31 L/min at 60 cpm and 3.92 ± 0.22 L/min at 100 cpm (NS, $P>0.05$). The peak work rates were 335 ± 22W for 60 cpm and 318 ± 46 W for 100 cpm (NS, $P>0.05$).

Central Responses.

The responses of $\dot{Q}_C$, pulmonary $\dot{V}O_2$, HR, and SV for 60 and 100 rpm are shown for one subject in Fig. 2. $\dot{Q}_C$, HR and $\dot{V}O_2$ increased linearly with power output. For the same external work rate, contraction frequency elicited different $\dot{V}O_2$ and $\dot{Q}_C$ responses at 100 cpm compared

to 60 cpm ($P<0.05$) (Fig. 2, Table 1). There was no significant difference between the slopes of the HR vs. WR at 100 cpm compared to 60 cpm ($P>0.05$) (Fig. 2, Table 1). Stroke volume was not systematically different at 100 compared to 60 cpm (Fig. 2). The $\dot{Q}_C$ / $\dot{V}O_2$ relationship for the same subject whose data is shown in Fig. 2 is graphed in Fig. 3. There was no significant difference between the slopes of $\dot{Q}_C$ vs. $\dot{V}O_2$ at 100 cpm compared to 60 cpm ($P>0.05$) (Table 2). The slope of heart rate-to-$\dot{V}O_2$ at 60 cpm had a tendency to be significantly different from that at 100 cpm ($p = 0.06$). However, the peak heart rates were not significantly different at 60 vs. 100 cpm ($P>0.05$) (60 = 172 ± 15 bpm; 100 = 175 ± 14 bpm).

Peripheral Responses.

The responses of LBF and $\dot{V}O_2$ for 60 and 100 rpm are shown for one subject in Fig. 4. Both LBF and leg $\dot{V}O_2$ increased linearly with power output (Fig. 4). In contrast to what was observed centrally, there was no significant difference between the slopes of LBF vs. WR or leg $\dot{V}O_2$ vs. WR at 100 cpm compared to 60 cpm ($P>0.05$) (Fig. 4, Table 1). The LBF / leg $\dot{V}O_2$ relationship for the same subject whose data is shown in Fig. 4 is graphed in Fig. 5. There was no significant difference between the slopes of LBF vs. leg $\dot{V}O_2$ at 100 cpm compared to 60 cpm ($P>0.05$) (Table 2).

DISCUSSION

The findings from the present study, consistent with our hypothesis, indicate that both the central and peripheral blood flow / $\dot{V}O_2$ relationships are robust in exercising humans and remain tightly coupled regardless of changes in contraction frequency. Consistent with this, these data indicate that muscle blood flow does not become limited at higher contraction frequencies for this mode of exercise. Thus, the increased metabolic demands of the working muscle are met primarily by an increase in oxygen delivery (i.e., blood flow) (8), not oxygen extraction (22).

Contracting skeletal muscle has an increased demand for oxygen that is met by an increase in blood flow (oxygen delivery) and oxygen extraction (1). The blood flow ($\dot{Q}$) to oxygen uptake ($\dot{V}O_2$) response during steady-state exercise has been shown to be linear for whole-body [cardiac output *vs.* pulmonary $\dot{V}O_2$ (6; 28)] and exercising limbs (1; 16; 26) with

similar slopes ($\Delta\dot{Q}/\Delta\dot{V}O_2$= 5-6) for both. However, discrepancies in the literature (2; 3; 10; 14; 30), suggest that this fundamental relationship may be altered when the frequency of muscle contractions is increased. The crucial point from the present study is that the potential alterations in blood flow with changes in contraction frequency need to be defined in the context of metabolic rate. Thus, "enhanced" blood flow would be defined as an increase in flow that is greater than that which would be predicted by an increase in metabolic rate from the above relationship. Likewise, an "impedance" to blood flow would be evidenced by a plateau or decrease in flow such that the leg oxygen extraction would have to increase (a-vO_2 difference widen) in order for the metabolic needs of the working muscles to be met (8; 22). Both of these scenarios would result in a change in the blood flow / $\dot{V}O_2$ relationship. Our data shows that neither is the case; i.e., there is neither an increase in blood flow above that predicted by the increase in metabolic rate, nor a plateau or decrease in blood flow, with increases in contraction frequency. Thus, as stated earlier, the increased metabolic demands of the working muscle are met primarily by an increase in oxygen delivery (i.e., blood flow).

Naamani et al., (23) examined the mechanical effects of muscle contractions to determine if the increase in muscle blood flow was independent of metabolic factors. They found that the mechanical effect of muscle contraction alone produced only a modest increase in muscle blood flow. However, they utilized an instrumented vasculature preparation in an anesthetized, mechanically ventilated dog model that may have negated the mechanical effects of upright, naturally perfused, locomotory exercise. As stated by Laughlin & Schrage (17), there are two possible explanations for Naamani et al. (23) findings. First, spontaneous contractions may have produced the muscle pump effect but artificial stimulation of muscle contraction did not. Second, the muscle pump effect may be abolished by instrumentation of the veins associated with *in vitro/in situ* preparations. Therefore, there may have been alterations in the venous vascular mechanics with cannulation such that the interactions of muscle contraction with blood flow were also impacted.

Increased impedance to muscle blood flow has been suggested during high contraction frequencies due to the shorter amount of time spent in the relaxation phase (2; 3; 10; 14). It is important to keep in mind that the upper limit of pedaling rate that even trained subjects can

maintain for prolonged periods of exercise is ~120 cpm (2 Hz) (34). Thus, it has been shown that when exercise is performed at a constant duty cycle (~1/3 for this study), elevations in contraction frequency do not impede muscle blood flow during cycling exercise (8) or during knee extension exercise (24; 31). In contrast, in many animal studies, the contraction frequency shown to have an impedance affect on muscle blood flow is above that attainable by humans (> 2 Hz) (2; 3; 10). Further, if duty cycle is allowed to lengthen in concert with the increase in contraction frequency, impedance to blood flow can also occur at lower frequencies (60-80 cpm) during supine knee extension exercise (14). Thus, while it has been shown that very high contraction frequencies and/or changes in duty cycle can have an impedance affect on muscle blood flow, an increase in contraction frequency alone at a constant duty cycle does not appear to impede flow in exercising humans.

Enhancement of muscle blood flow with increases in contraction frequency has also been reported. Sheriff (30), observed an enhancement in muscle blood flow with increases in contraction frequency in running rats, which they attributed to a greater muscle pump effect. However, since oxygen uptake was not measured (30), it is unclear if the increased flow was simply due to increase metabolic rate ($\dot{V}O_2$) or due to a true "enhancement" effect. The close coupling of blood flow to metabolic rate found in the present study revealed no enhancement to flow above that which was predicted by an increase in metabolic rate, irrespective of an increase in contraction frequency.

Part of the discrepancy in assessing the effect of contraction frequency on the blood flow response also relates to the use of external power (EP) vs. total power [TP = (EP) + internal power (IP)] (24; 31; 32). IP is generated by the muscles to overcome inertial and gravitational forces related to the movement of the lower limbs at different contraction frequencies (7; 31). On the one hand, Ferguson et al. (7) reported leg blood flow to be higher when the same TP was performed at 100 cpm compared with 60 cpm. However, when the leg blood flow response is related to the total work rate, it has been demonstrated that blood flow is matched directly and linearly to the work performed irrespective of the contraction frequency (24; 31). Assuming a close similarity in metabolic efficiency for IP as for EP (31), these data support the very close coupling between the metabolic activity (as $\dot{V}O_2$) and the leg blood flow response to exercise.

These discrepancies may have been due exclusively to the differences in the models for IP calculation (energetic (31) vs. biomechanical (7)), but they could also have arisen from differences in experimental setups (knee angular displacement of ~45° in the upright position for Sjogaard et al. (31) vs. 80 ° in the supine for Ferguson et al. (7)). Further, the supine position could influence the magnitude of the blood flow response by altering venous return, stroke volume, and cardiac output (13; 18-20). Nonetheless, with direct measurements of oxygen uptake at both the central and peripheral levels in the present study, we were able to substantiate and extend the findings of Osada and Radegran (24) and Sjogaard et al. (31) of a close matching of blood flow to metabolic rate irrespective of contraction frequency.

We have shown that the central and peripheral blood flow / $\dot{V}O_2$relationships are robust and remain tightly coupled regardless of changes in contraction frequency. What regulates this relationship (i.e., why is the slope set at $\dot{Q}$ /$\dot{V}O_2$= 5-6) is not currently known. What is known is that this is a very reproducible phenomenon. The present study adds to the accumulating data that demonstrates that this relationship is quite robust regardless of exercise training (21; 28), aging (21), anemia (27; 29), hypoxia (27), hyperoxia (11) or fiber type (9). The underlying mechanisms, and even the regulated variable(s) related to this response must await further study.

Methodological Considerations:

For this study it was important for the subjects to approach a steady state at each power output as well as to remain in the linear portion of the $\dot{V}O_2$/ work rate relationship so as to be able to examine the affect of contraction frequency on the blood flow to oxygen uptake relationship. Thus, we intentionally avoided power outputs greater than 80% of the subjects' peak work rates due to the reported plateauing of leg $\dot{V}O_2$ associated with a plateauing of leg blood flow (16; 22).

We chose a discontinuous exercise protocol in order to allow the subjects to partially recover from the previous exercise bout in the expectation that they would be able to reach higher work levels and sustain them sufficiently long enough to make the described measurements near a steady state. One single breath acetylene uptake maneuver was performed near the end of each stage to measure cardiac output. A short break (i.e. cycling at 30 W for 2

min.) was introduced before the next increase in work intensity to allow for the acetylene to "washout" so as to minimize any impact on the next cardiac output measurement.

The single breath constant exhalation acetylene uptake technique has reportedly high correlations with both the direct Fick method and thermodilution at rest (5; 33). It has also been proven to be a reliable technique for use during exercise up to 200 W (4). However, the single breath maneuver requires a constant, slow exhalation rate which makes the procedure difficult to perform at higher exercise intensities (4). We minimized this potential complication by having the subjects practice the technique prior to test day at the highest work rate they were to perform (80% max) until they were able to competently accomplish the maneuver.

The constant-infusion thermodilution technique has been proven to be a reliable measure of leg blood flow (1; 25); however, it is not without possible sources of error. These have previously been reviewed (25) and include: 1.) *Fluctuations in blood flow over the measurement period.* It is important that the measurements are taken during or near the steady state of exercise. In the present study, care was taken to avoid the period of rapid blood flow changes when work rate was increased. Further, to insure that the measurements were reproducible, two thermodilutions were taken during each exercise stage; no significant difference was found between the two. 2.) *Recirculation.* As previously reported (25), it is extremely unlikely that the typical temperature decrease of ~1 °C seen in the femoral vein blood during cold saline infusion would reduce recirculated blood temperature. 3.) *Effective mixing of cold saline and blood.* The catheter used for the saline infusion has multiple pinhole side ports to enable effective mixing. As reported previously (25), streaming in the femoral vein is highly unlikely. 4.) *Loss of cold saline between sites of injection and measurement*. Though care was taken, it is possible that during the exercise tests the catheter could have shifted, allowing one or more of the proximal pinholes to pull out of the vessel, thus allowing saline to leak into the interstitial space. Thus, more saline would have to be introduced in order to have the ~1 °C temperature drop in venous blood, which would overestimate flow. The linearity of the blood flow to work rate and $\dot{V}O_2$ data in the present study suggest that this was not a significant problem. 5.) *Adjusting for cooling of the perivenous tissue.* The femoral venous temperature does not always immediately return to the pre-infusion level after cold saline infusion. Instead,

after the infusion is stopped, femoral vein temperature increases rapidly and sometimes plateaus slightly below that seen immediately before the cold saline infusion. This indicates a cold "loss" into the perivenous tissue and adjustments were made by averaging pre- and post-infusion baselines for the blood flow calculation.

Conclusions:

We have shown that for cycle exercise, across a large range of power outputs, the blood flow / $\dot{V}O_2$ response, both centrally and peripherally, remained robust despite changes in contraction frequency. Thus, muscle blood flow is matched to the metabolic needs of the working muscle, increasing linearly with work rate with neither an impedance to flow nor any enhancement above that which would be predicted by the increase in metabolic rate. It is important to establish the conditions in which this relationship remains coupled so that mechanistic conclusions can be drawn in circumstances in which this relationship is altered (i.e., disease states).

ACKNOWLEDGMENTS

I would like to thank Craig A. Harms, Leonardo F. Ferreira, Dana Townsend, Allison J. Harper, Christine S. Ferguson and Thomas J. Barstow their collaboration on this manuscript. I would also like to thank David Freeze for assistance with data processing and Drs. David Pauls and Lance Saville for their assistance with catheterizations. This work was supported in part by American Heart Association, Grant-in-Aid # 0151183Z to T.J. Barstow. L.F. Ferreira was supported by a Fellowship from the Ministry of Education/CAPES - Brazil.

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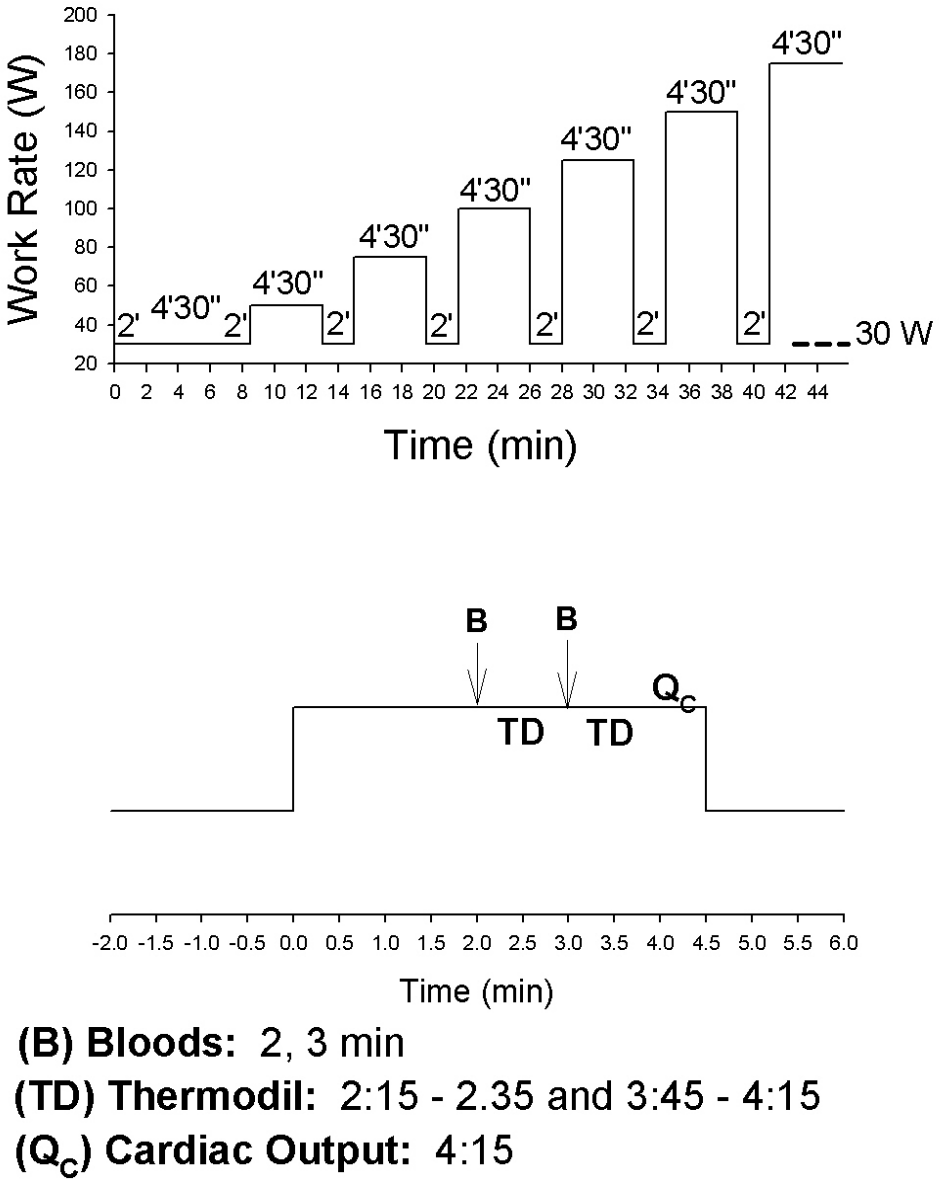
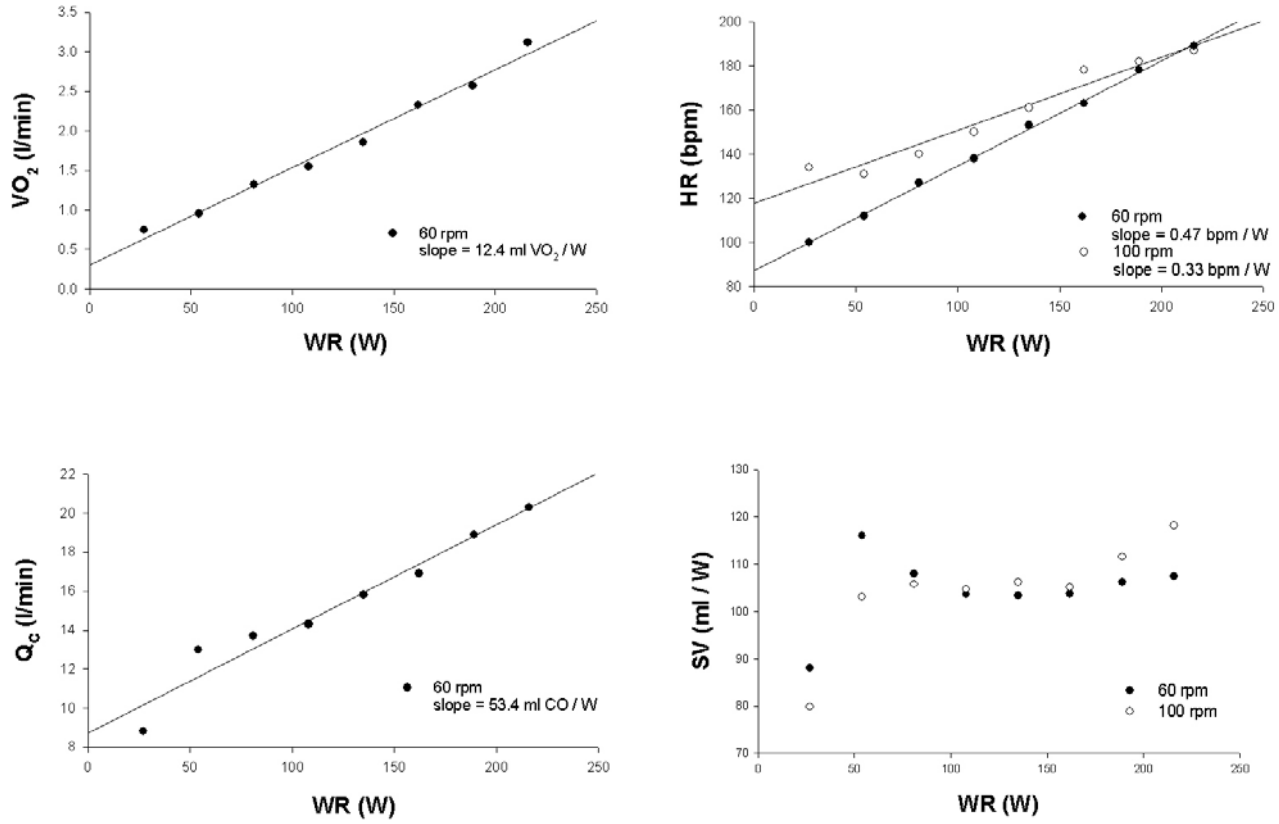
FIGURES

Figure 3-1 **Schematics of the exercise protocol.**



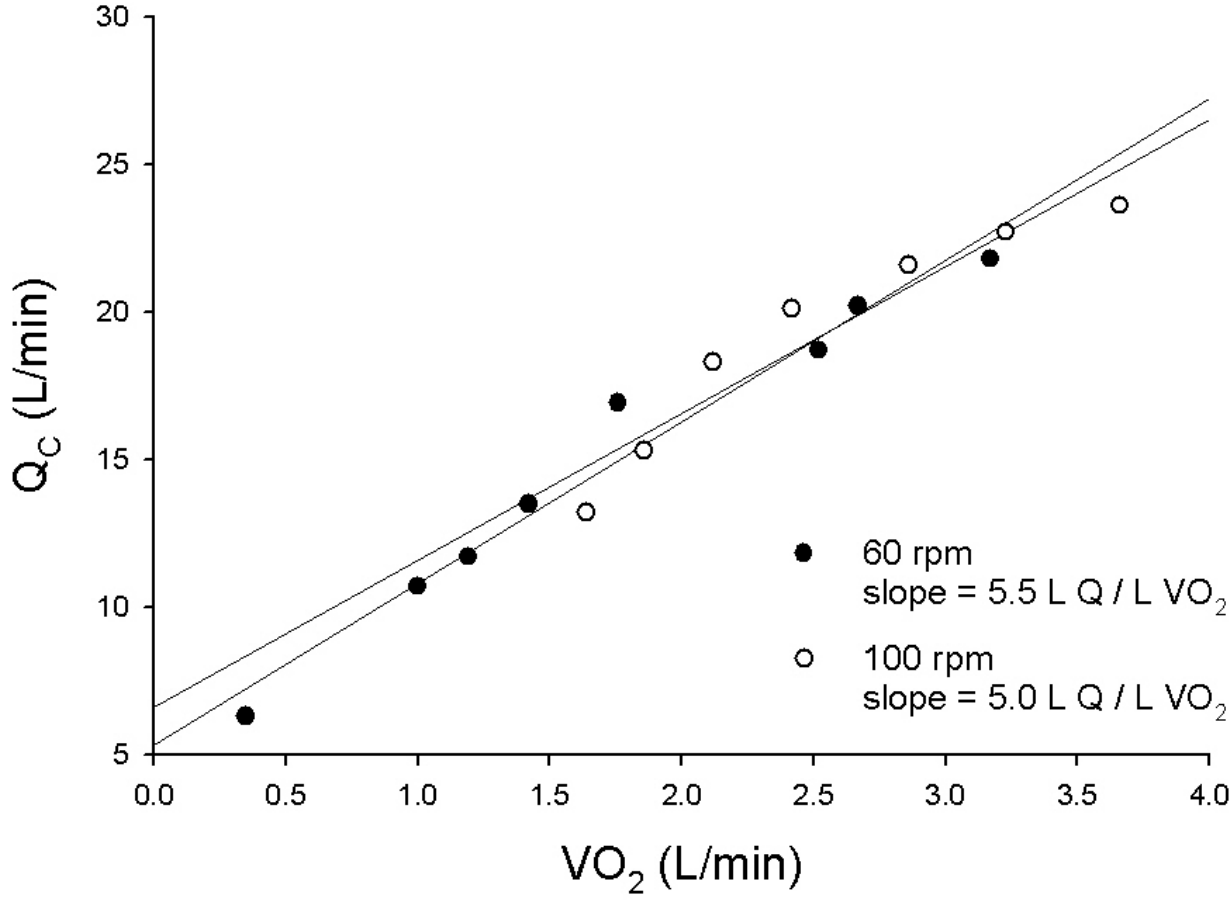
Top panel shows the discontinuous incremental protocol. Note that baseline and the two minutes between stages are all at 30 W. The first stage was performed at 10% of the subject's maximum work load but never lower than 30 W. The lower panel shows the timing of blood draws (B), thermodilution (TD), and cardiac output ($\dot{Q}_C$) measurements within each stage.

Figure 3-2 Oxygen uptake ($\dot{V}O_2$), cardiac output ($\dot{Q}_C$). heart rate (HR) and stroke volume (SV) responses at 60 and 100 cpm for one subject.



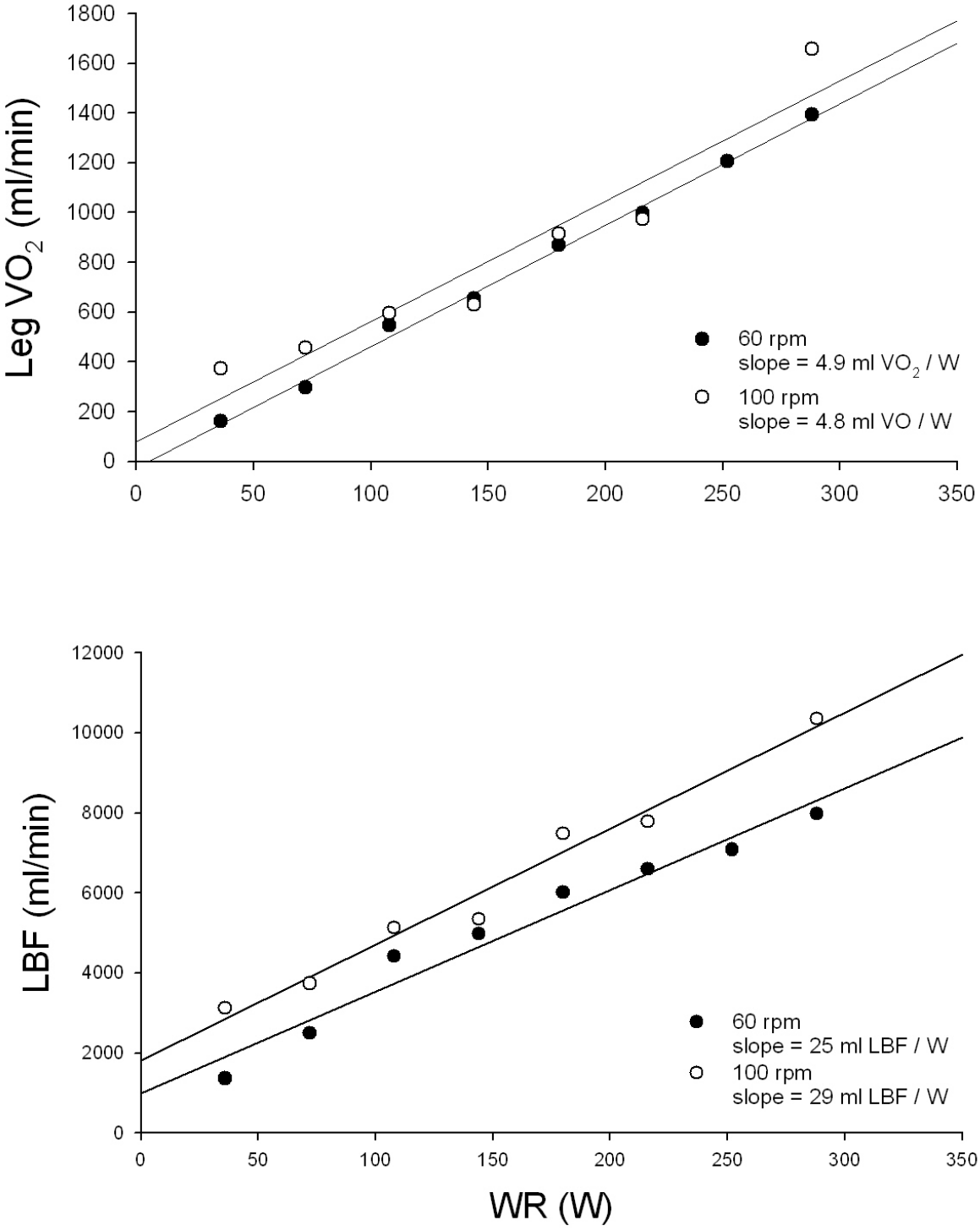
VO_2, $\dot{Q}_C$ and HR increased linearly with work rate.

Figure 3-3 The cardiac output ($\dot{Q}_C$) / oxygen uptake ($\dot{V}O_2$) relationship at 60 and 100 cpm for the same subject as in Fig. 2.



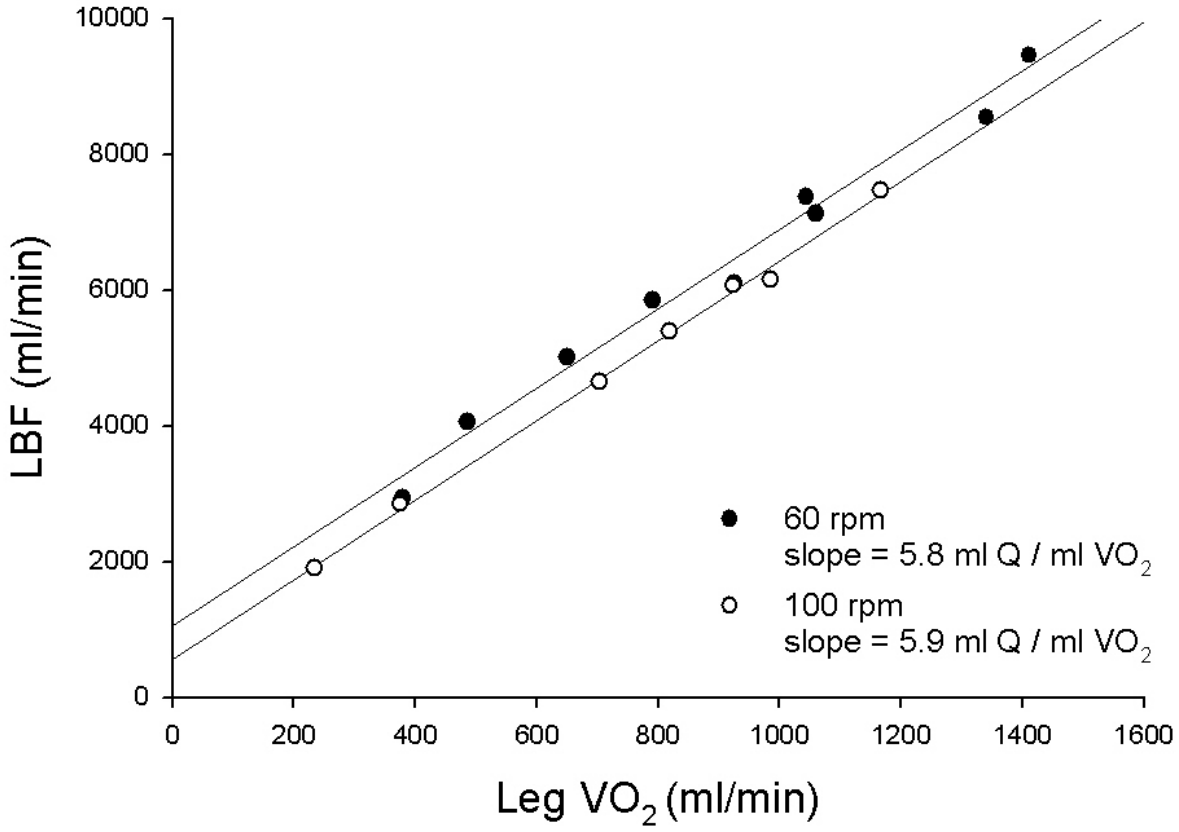
Both $\dot{Q}_C$ and VO2 increased linearly with power output.

Figure 3-4 Leg oxygen uptake ($\dot{V}O_2$) and leg blood flow (LBF) responses at 60 and 100 cpm for one subject.



$\dot{V}O_2$ and LBF increased linearly with work rate.

Figure 3-5 The leg blood flow (LBF) / leg oxygen uptake ($\dot{V}O_2$) relationship at 60 and 100 cpm for the same subject as in Fig. 4.



TABLES

Table 3.1 Slopes of central and peripheral hemodynamics / work rate relationships.

	CENTRAL			PERIPHERAL	
	Q_C (ml/w)	**VO_2** (ml/w)	**HR** (bpm/w)	**LBF (1 leg x 2)** (ml/w)	**VO_2** (ml/w)
60 cpm	55 ± 8.3	10.4 ± 1.1	0.36 ± 0.08	57.8 ± 13.0	10.0 ± 2.0
100 cpm	51 ± 5.8	8.7 ± 0.9	0.26 ± 0.04	50.4 ± 13.4	9.0 ± 2.8

Table 3.2 Slopes of central and peripheral blood flow / $\dot{V}O_2$ relationships.

	CENTRAL	PERIPHERAL
	Q_C vs. VO_2	**Leg BF vs. VO_2**
60 cpm	4.59 ± 0.9	5.79 ± 0.4
100 cpm	5.27 ± 0.4	5.50 ± 0.4

CHAPTER 4 - MUSCLE MICROVASCULAR HEMOGLOBIN CONCENTRATION AND OXYGENATION WITHIN THE CONTRACTION-RELAXATION CYCLE

ABSTRACT

Inability to directly measure microvascular oxygen distribution and extraction in striated muscle during a contraction/relaxation cycle limits our understanding of oxygen transport to and utilization by contracting muscle. We examined muscle microvascular hemoglobin concentration ([Hb]) and oxygenation within the contraction/relaxation cycle to determine if microvascular RBC volume would be preserved and if oxygen extraction continued during the actual contraction phase. Eight subjects performed dynamic knee extension exercise (40 contractions/min) at moderate (~30% of peak work rate) and heavy (~80% of peak) work rates. Total hemoglobin ([THb]) and deoxyhemoglobin ([HHb]) were measured in the rectus femoris using NIRS to determine if microvascular [Hb] would be preserved during the contraction, and to estimate microvascular oxygen extraction, respectively. Mean values during the relaxation (RP) and contractile phases and the peak values during the contractile phase for both moderate and heavy exercise were calculated. [THb] increased from rest to steady-state exercise (6.36 ± 5.08 μM moderate; 5.72 ± 4.46 μM heavy exercise, both $p<0.05$), but did not change significantly within the contraction/relaxation cycle (Fig.3). Muscle contractions were associated with a significant (1.29 ± 0.98 μM moderate; 2.16 ± 2.12 μM heavy exercise, $p<0.05$) increase in [HHb] relative to RP. We conclude that microvascular RBC volume is preserved during muscle contractions (i.e., RBCs were present in the capillaries) which, combined with the cyclical pattern of deoxygenation/oxygenation during the respective contraction/relaxation phases of the contraction cycle suggests the notion that oxygen extraction continues to occur during muscle contractions.

INTRODUCTION

Rhythmic muscle contractions cause oscillations of inflowing arterial (3,31,44) and outflowing venous (18,40) blood flow. However, technological constraints have precluded analysis of any oscillatory patterns in microvascular blood volume and their effects on blood-myocyte gas exchange within the contraction cycle in human muscle. Even with analysis of muscle blood flow and VO_2 kinetics, the tacit assumption has been that O_2 exchange proceeds unimpeded and uniformly across the contraction-relaxation cycle (18).

Notwithstanding the above, contraction-induced increase in intramuscular pressure has been considered to at least partially evacuate the muscle vascular bed (43) which would account for the retrograde arterial (24,31,44) and anterograde venous (1,43) blood "spurts." If the source of these blood "spurts" was the microcirculation, in addition to intervening large arteriolar and venular sites, the reduction in microvascular red blood cell (RBC) volume during contraction would reduce or abolish the potential for blood-myocyte O_2 delivery and relegate that delivery to the following relaxation phase. Such behavior would reduce the effective mean capillary RBC transit time and mandate a higher O_2 flux density from each RBC during relaxation. In addition, because intramyocyte phosphocreatine concentration falls and those of phosphagen-linked mitochondrial controllers (e.g., free ADP, inorganic phosphate) rise rapidly during the contractile phase of the contraction cycle (7), the O_2 supply would be decreasing at the very instant that O_2 demand were rising.

In marked contrast to the notion that the microvascular bed is emptied of RBCs during contraction, Gray et al. (20) demonstrated that maximal tetanic contractions did not cause microvascular collapse in the rat spinotrapezius muscle, while Poole et al. (35) observed RBCs in myocardial capillaries during barium-induced hypersystole. These latter findings suggest that during the muscle contractile phase RBCs would be present in the capillaries - albeit at a reduced flux - to facilitate continued myocyte O_2 delivery. Resolution of this issue in human muscle(s) during exercise is crucial for construction of O_2 transport models that have physiological validity (34).

Near infrared spectroscopy (NIRS) technology can track changes in muscle total hemoglobin concentration ([Hb]) as well as that of oxygenated/deoxygenated Hb + myoglobin (Mb) (11,17,29). Recent advances in NIRS technology now allow for very rapid sampling (19), providing the temporal resolution necessary to dynamically examine muscle microvascular hemoglobin concentration ([Hb]) and oxygenation dynamically within the contraction-relaxation cycle. We conducted the experiment described herein to test the following hypotheses: 1) Microvascular [Hb] would be preserved during contraction, and 2) There would be a cyclical pattern of deoxygenation/oxygenation that corresponded with the contraction/relaxation phases of the contraction cycle. The results obtained support these hypotheses and suggest that although convective RBC flux may be decreased during contraction, capillary RBC content and therefore the potential for O_2 diffusion is sustained, resulting in an increase in deoxyhemoglobin ([HHb]) during contractions.

METHODS

Subjects

Seven male and one female volunteers participated in the study. The physical characteristics of the subjects were (mean ± standard deviation): age 23 ± 2 yrs, height 175 ± 9 cm, and weight 71 ± 13 kg. Experimental procedures and all possible risks and discomforts associated with the experiment protocol were explained to each subject prior to their providing written consent. This study was approved by the Institutional Review Board for Research Involving Human Subjects at Kansas State University, where all exercise tests were conducted.

Experimental Design

Subjects performed upright one-leg knee extension exercise on a specially built leg ergometer. A strap was placed around the ankle of the subject and attached to a pneumatic cylinder by means of a cable-pulley system. The distance traveled by the lower limb during knee extension was limited to a range of ~20°, which produced a fixed linear displacement (d) of the piston of the pneumatic cylinder of 10.9 cm. Work was accomplished by compressing the air in the cylinder as the lower leg was extended. The amount of work performed (per stroke) was calculated as $[P_{INT} + (P_F + P_{INT})/2] \cdot (\pi r^2 \cdot d)$, where P_{INT} is the initial pressure in the cylinder and P_F is the final pressure at the end of the work stroke, and was divided by the duration of the contraction cycle to yield power (W). Two work bouts were completed: moderate (~30% of peak work rate), and heavy (~80% of peak work rate). At least 20 minutes separated the two work bouts. The contraction frequency was set at 40 contractions per minute. A metronome was used to assist the subjects in maintaining the appropriate contraction frequency. Each exercise bout consisted of 1 minute rest followed by 3 minutes of exercise.

Measurements

Skeletal muscle O_2 extraction, estimated by deoxy-hemoglobin concentration ([HHb]) was determined using a frequency-domain multi-distance (FDMD) near-infrared spectroscopy (NIRS) system (OxiplexTS, ISS, Champaign, IL) during the exercise tests. The principles and

algorithms utilized in the equipment were reviewed by Gratton et al. (19). This device consists of eight light-emitting diodes (LED) operating at wavelengths of 690 (four LEDs) and 830 nm (four LEDs), with one detector fiber bundle. Light source-detector separation distances were 2.0, 2.5, 3.0 and 3.5 cm. The NIRS data was stored at 31.25 Hz. The probe was placed longitudinally along the belly of the right rectus femoris which had been identified by palpation during isometric contraction of the muscle. To minimize motion artifacts and contamination of the signal by ambient light, the margins of the probe were bound to the thigh with a skin cement (Skin-Bond, Smith & Nephew, Largo, FL) after careful shaving and drying of the area, and secured with Velcro straps around the thigh. A black cloth was then secured over the probe to further shield the probe from ambient light. The NIRS probe was calibrated prior to each test according to the manufacturer's recommendations.

The [HHb] signal can be regarded as being essentially blood-volume insensitive during exercise (9,13,17). Thus, it was assumed to be a reliable estimator of changes in fractional oxygen extraction in the field of interrogation (10,12,13,17,14).

The FDMD NIRS provides a measurement of the absolute concentrations (expressed in μM) of deoxy- ([HHb]) and oxy- [HbO_2] hemoglobin and myoglobin. [HHb] and [HbO_2] were calculated incorporating the dynamic measurement of reduced scattering coefficients made throughout the exercise test. Measures of total hemoglobin concentration ([THb] = [HHb] + [HbO_2]), and tissue oxygen saturation ($StO_2 = [HbO_2]/[Hb]_{tot}$ (%)) were also calculated. The last 30 seconds of the exercise period (i.e. steady state) were used to calculate the mean values during the relaxation (RP) and contractile phases (CP), respectively and the peak values during the contractile phase for both moderate and heavy exercise. To determine the reproducibility of the NIRS signal two subjects performed the identical protocol on two separate days.

Statistical Analysis

To determine differences between two means, a two-tailed Student's paired t-test was performed. A repeated measures analysis of variance was performed to compare more than two means and the Tukey-Kramer post hoc test was used for pairwise comparisons (NCSS 2000—Statistical Software, Kaysville, UT). Statistical significance was accepted when $P<0.05$. Values are reported as mean ± SD, unless otherwise specified.

RESULTS

Power output for moderate exercise was the same for all subjects (4.1 W) and mean power output for heavy exercise was (9.8 ± 1.7 W). Figure 1 shows the raw data from five muscle contractions for one subject during steady state exercise. Importantly, we observed good reproducibility of the NIRS signal comparing two different days: (e.g., Fig. 2). There was a significant increase (i.e., rest to mean exercise) in exercise [THb] of 6.36 ± 5.08 μM for moderate and 5.72 ± 4.46 μM for heavy exercise and in [HHb] of 3.78 ± 3.02 μM for moderate and 5.12 ± 4.37 μM for heavy ($p<0.05$). In addition, StO_2 decreased significantly for moderate (3.53 ± 3.32 %) and for heavy exercise (5.93 ± 4.14 %) ($p<0.05$ for both). However, there was an insignificant increase in [HbO_2] of 2.28 ± 3.01 μM for moderate and 0.53 ± 1.85 μM for heavy exercise ($p>0.05$). Despite the increase from rest to steady-state exercise, [THb] did not change significantly within the contraction-relaxation cycle (Fig. 3) at both moderate and heavy exercise. However, muscle contractions were associated with a significant ($P<0.05$) increase in [HHb] (RP vs. Peak) for both moderate and heavy exercise (Fig. 4). Fig. 5 shows the individual changes in [HHb] from relaxation to contraction during moderate and heavy exercise. These represented ~ 40% of the total increases in [HHb] from rest to exercise (Fig. 6).

StO_2 decreased significantly ($P<0.05$) from rest to RP for both moderate and heavy exercise (Table 1). In addition, StO_2 was lower during contractions compared to RP ($P<0.05$). [HbO_2] did not change significantly during contractions nor was significantly different between work rates (Table 1).

DISCUSSION

Two principal new findings arise from the present study. First, microvascular RBC volume was preserved during muscle contractions. Thus, RBCs are present in the capillaries – albeit at a reduced flux - which could facilitate continued oxygen delivery to the myocyte. Second, there was a cyclical pattern of deoxygenation /oxygenation that corresponded with the contraction/relaxation phases of the contraction cycle, with [HHb] increasing significantly during the contractile phase. This suggests that oxygen extraction continues to occur during muscle contractions.

Contracting skeletal muscle has an increased demand for oxygen that is met by an increase in blood flow (oxygen delivery) and oxygen extraction (2). Because oxygen diffusion from capillaries to tissues is a time-dependent process, the time RBCs stay in capillaries can have a direct effect on oxygen extraction. RBC velocity will determine the RBC transit time in muscle capillaries. In the presence of fast RBC velocity there is an increased likelihood of RBCs with transit times that are short enough to compromise gas exchange (33,41). During exercise, capillary transit time shortens, but the mean transit time usually remains long enough to enable sufficient oxygen extraction (26,39). Takaishi et al. (42) examined blood volume and oxygenation (NIRS) in humans during cycle ergometry and observed an increase in bulk flow and oxygenation immediately following contraction. This corresponds with the findings of Kindig et al. (27) of an increase in RBC flux, velocity and capillary tube hematocrit immediately following contraction in their rat spinotrapezius preparation, suggesting that a bulk volume of blood had entered the vascular bed upon relaxation. As suggested by Lash and Bohlen (30), an increase in bulk flow between contractions would decrease the time available for oxygen extraction between the blood and tissue. Indeed, Takaishi et al. (42) reported the "lump" of muscle blood volume that passed under their NIRS probe immediately following contraction did so without becoming deoxygenated. In contrast, the suppression of flow during the contractile phase would reduce RBC velocity and likely prolong capillary transit time, which would facilitate further oxygen extraction during the contraction. Thus, our finding that microvascular

RBC volume was preserved during muscle contraction agrees with Gray et al. (20), who concluded that the manner in which the vascular bed is occluded keeps red cells in the capillaries, providing a source of oxygen that would help to offset the reduction in capillary RBC transit time observed during the relaxation phase when capillary RBC velocity increases. Consistent with this prediction, we were able to detect an increase in [HHb] during contraction, suggesting that oxygen extraction continues to occur during muscle contractions.

Based on the Fick principle, O_2 extraction [$C(a–v)O_2$] is equal to muscle oxygen uptake ($\dot{V}O_{2M}$) / muscle blood flow ($\dot{Q}_M$). Thus, the increase in O_2 extraction during contraction might be caused by an increase in $\dot{V}O_{2M}$ (flow being constant), a decrease in $\dot{Q}_M$ ($\dot{V}O_{2M}$ being constant) or a combination of both. Chung et al. (7) reported transient decreases in PCr and increases in P_i which corresponded closely to the time of force generation during contraction. They stated that such a large drop in PCr during contraction would require substantial energy restoration arising from either glycolytic or oxidative phosphorylation sources. In a later study (6), they concurred that oxidative phosphorylation supplied a significant fraction of the energy during steady state exercise over the dynamic, milliseconds-duration muscle contraction cycle. However, they assumed that O_2 in the vasculature and/or in the interstitial space would not contribute during the contractile phase but would only readily diffuse during the relaxation phase. For the first time, we were able to examine (in real time) microvascular gas exchange during the contractile phase and provide original data supporting that substantial Hb was present and continued O_2 extraction was occurring during contraction.

This raises a couple of intriguing possibilities: 1. The observed increase in deoxy Hb+Mb associated with contractions in the present study, combined with the observation of a rapid resynthesis of PCr prior to full relaxation (7), is consistent with an accelerated response of mitochondrial oxidative phosphorylation with consequent O_2 utilization during contraction. 2. Alternatively, the cyclical reduction of [PCr] during contractions (7)may be caused or at least exacerbated by the transiently reduced pressure of O_2 (23,25}.

As noted above, contracting skeletal muscle has an increased demand for oxygen that is met by an increase in blood flow (oxygen delivery) and oxygen extraction (2). Since 80-90% of capillaries are perfused under resting conditions (15,27), if both RBC velocity and capillary tube

hematocrit (number of RBCs per unit capillary length) increase during muscle contractions, this would produce a large increase in the RBC flux. Our data show a significant though proportionately modest increase in [THb] from rest to exercise; thus RBCs needed to provide additional oxygen for muscle metabolism are available, and further significant increases occur with elevated exercise intensity. Further, those RBCs are trapped in the microcirculation during a contraction allowing for oxygen extraction to continue which helps meet the metabolic needs of the working muscle.

Methodological Considerations:

The assumption that an increase in [HHb] provides an estimate of O_2 extraction involves several essential considerations (14,16,22). First is the inability of NIRS to separate light absorption by hemoglobin from that by myoglobin. We assumed that the relative contribution of the vascular compartments to the NIRS signal was similar and unchanging during exercise and across work rates. Myoglobin deoxygenation is relatively unchanged from 50–60% of the peak work rate up to the peak in work rate (37), while [HHb] continues to increase above 50% of the work rate peak, which is qualitatively similar to leg O_2 extraction (28,38). This suggests that change in the NIRS signal originates primarily from changes in hemoglobin. However, irrespective of the relative contribution of Mb or Hb to the HHb signal, this study suggests that Hb/Mb desaturation along the entire diffusional path from capillary to mitochondria transiently increases (i.e., PO_2 decreases) during muscle contraction. The second consideration is the validity of the assumption of the relative contribution of arterioles, capillaries and venules to the NIRS signal which has not been clarified; nor is it clear if these relative proportions remain constant across exercise intensities. However, as capillaries comprise the majority of the muscle microvascular volume (36) it appears reasonable to consider that the changes in [HHb] will primarily reflect capillary O_2 extraction. Another consideration is the small sampling volume of the NIRS probe, which determines the oxygenation state of only a small (relatively superficial) portion of the working muscle. Due to potential regional differences in fiber type distribution, motor unit recruitment, muscle blood flow and regional difference in oxygenation status it is unclear if the area sampled is representative of the entire muscle (4,32). The contribution of skin blood flow to NIRS measurements also needs to be considered. There is the potential for

confounding interpretation of the NIRS-derived signal during conditions where both skin and muscle blood flows are elevated concomitantly (e.g., high-intensity and/or prolonged exercise) (8,5) although it is currently unclear if changes in skin blood flow and volume would affect the [HHb] signal used here. This might explain the unexpected [HbO_2] findings from the present study, where [HbO_2] did not decrease consistently as would be expected during exercise. The [HHb] signal from NIRS however, is less sensitive to blood volume changes, and thus is considered an appropriate estimate of capillary O_2 extraction (14,21,29) and because of the more prominent influence of [HHb] , we see the concomitant drop in StO_2 during a contraction.

Conclusions:

Our current understanding of oxygen transport to and utilization by contracting muscle has been limited due to an inability to directly measure microvascular oxygen distribution and extraction in striated muscle during a contraction and thus it has not been possible to detect if oxygen extraction occurs during contractions. For the first time, microvascular gas exchange has been estimated (in real time) during the contractile phase of rhythmic knee extension exercise. In agreement with previous studies in animal muscles (20,35) microvascular RBC volume was preserved during contraction in human muscle. Further, the availability of RBCs in the microcirculation during contraction facilitated O_2 continued extraction (as evidenced by increased [HHb]), at a time when the metabolic demands of the working muscle were transiently increased.

ACKNOWLEDGMENTS

I would like to thank Leonardo F. Ferreira, David C. Poole, Dana Townsend and Thomas J. Barstow for their collaboration on this manuscript. This work was supported in part by American Heart Association, Grant-in-Aid # 0151183Z to T.J. Barstow. L.F. Ferreira was supported by a Fellowship from the Ministry of Education/CAPES - Brazil.

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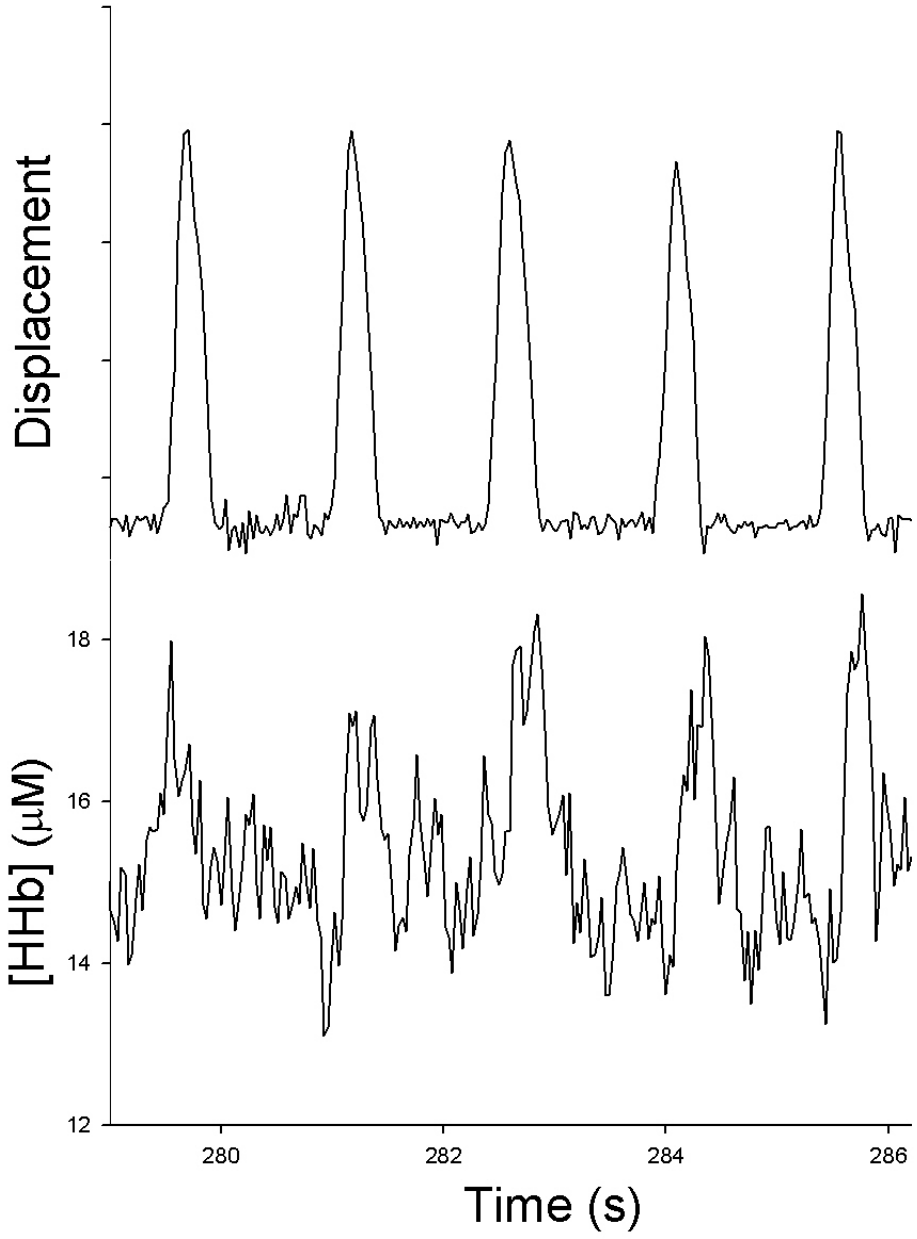
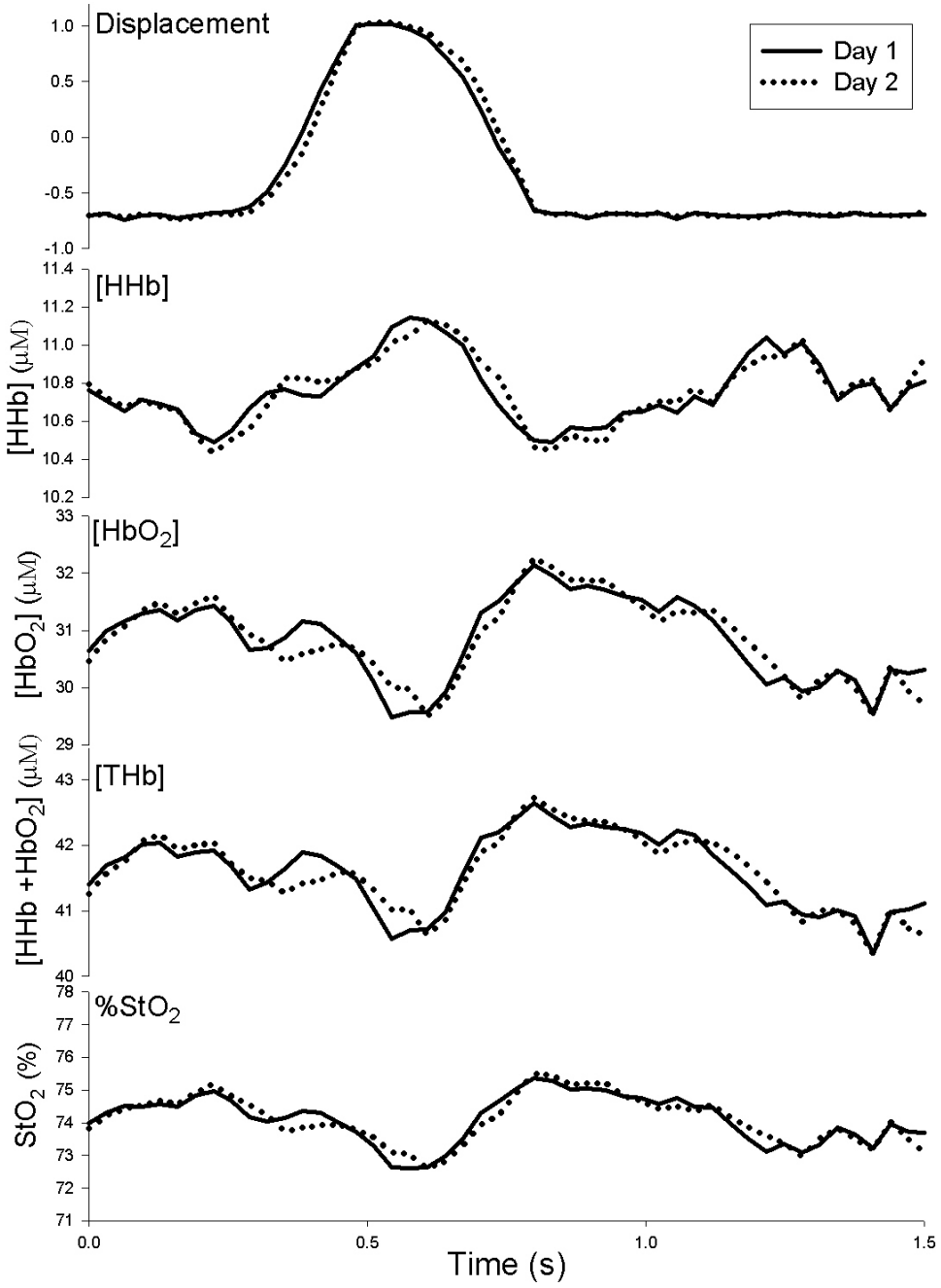
FIGURES

Figure 4-1 Raw signals of ergometer displacement and NIRS deoxy-hemoglobin concentration [HHb] for one subject during steady state knee extension.



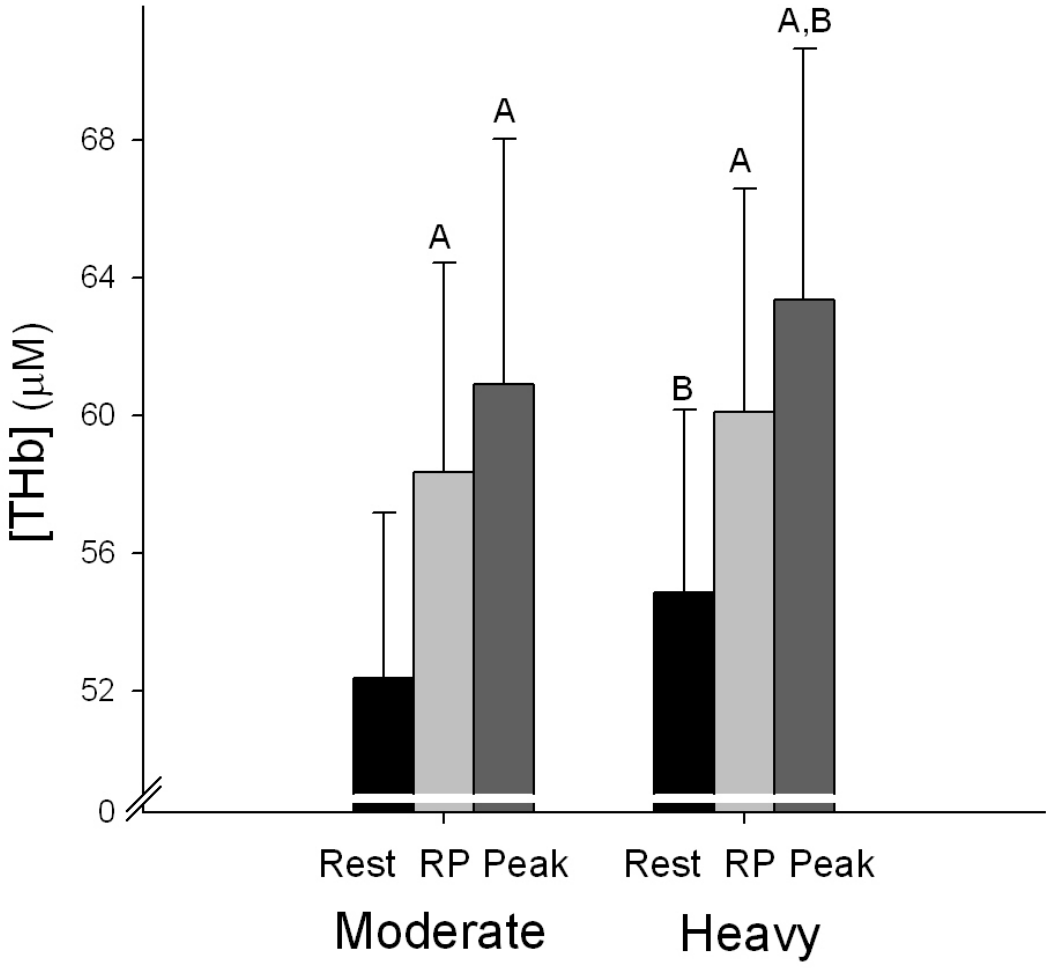
Note oscillatory behavior of [HHb] which corresponds with muscle contraction.

Figure 4-2 Reproducibility of the NIRS signals.



Five consecutive contractions were time aligned and ensemble-averaged. Solid lines are from Day 1 and dotted lines represent Day 2. Displacement shows the contractile phase of the exercising period. Deoxyhemoglobin concentration ([HHb]), oxyhemoglobin concentration ($[HbO_2]$), total hemoglobin concentration ([THb]), and percent tissue saturation of oxygen ($\%StO_2$) are represented.

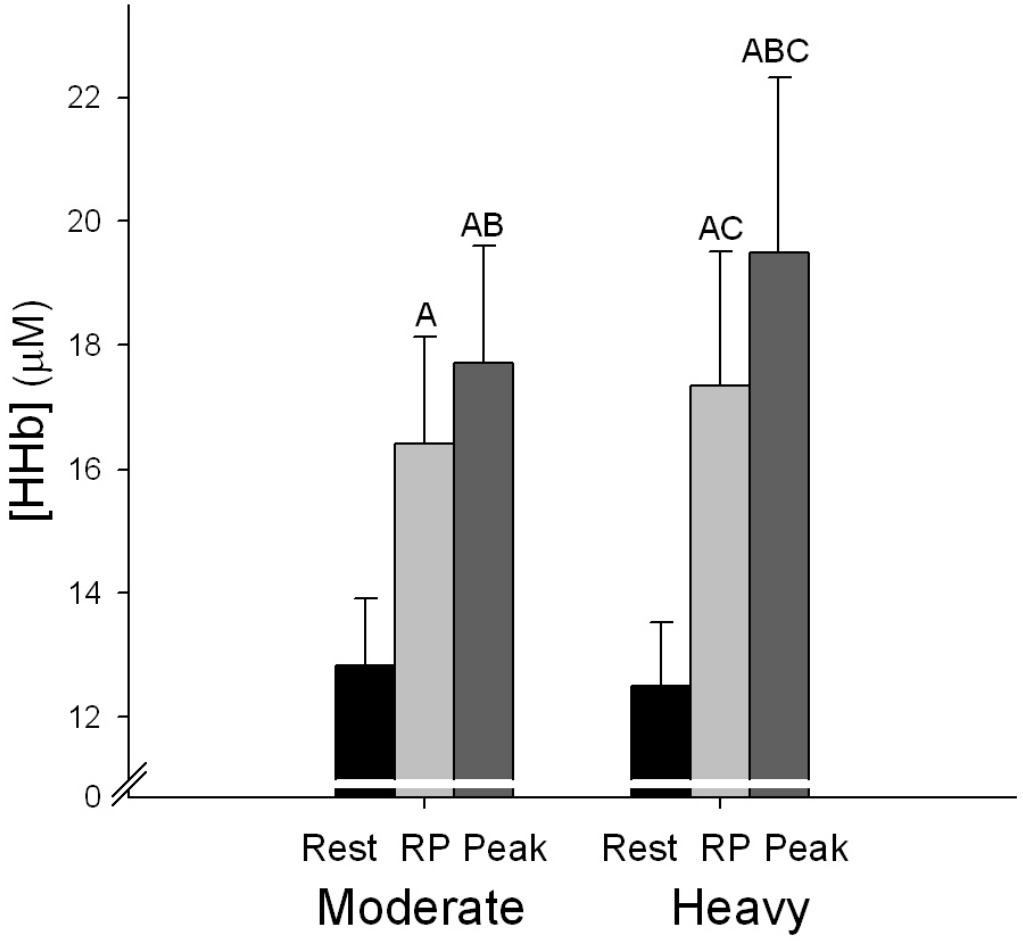
Figure 4-3 Total hemoglobin ([THb]) during rest, relaxation phase (RP) and peak values during the contractile phase for both moderate and heavy exercise.



[A] Significantly different from rest ($P < 0.05$).

[B] Significantly different from moderate exercise ($P < 0.05$).

Figure 4-4 Deoxyhemoglobin concentration ([HHb]) during rest, relaxation phase (RP) and peak values during the contractile phase for both moderate and heavy exercise.

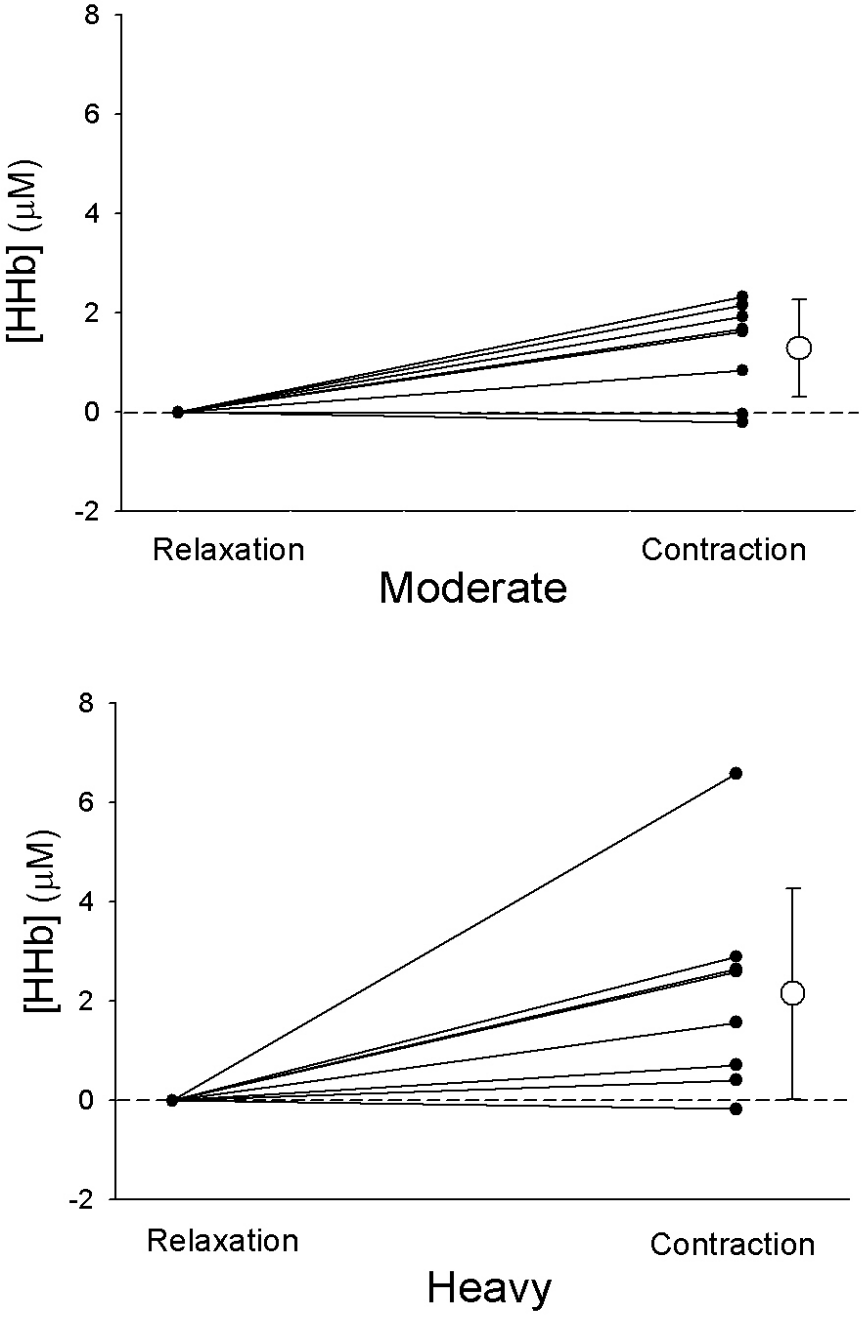


[A] Significantly different from rest ($P < 0.05$).

[B] Significantly different from relaxation phase (RP) ($P < 0.05$).

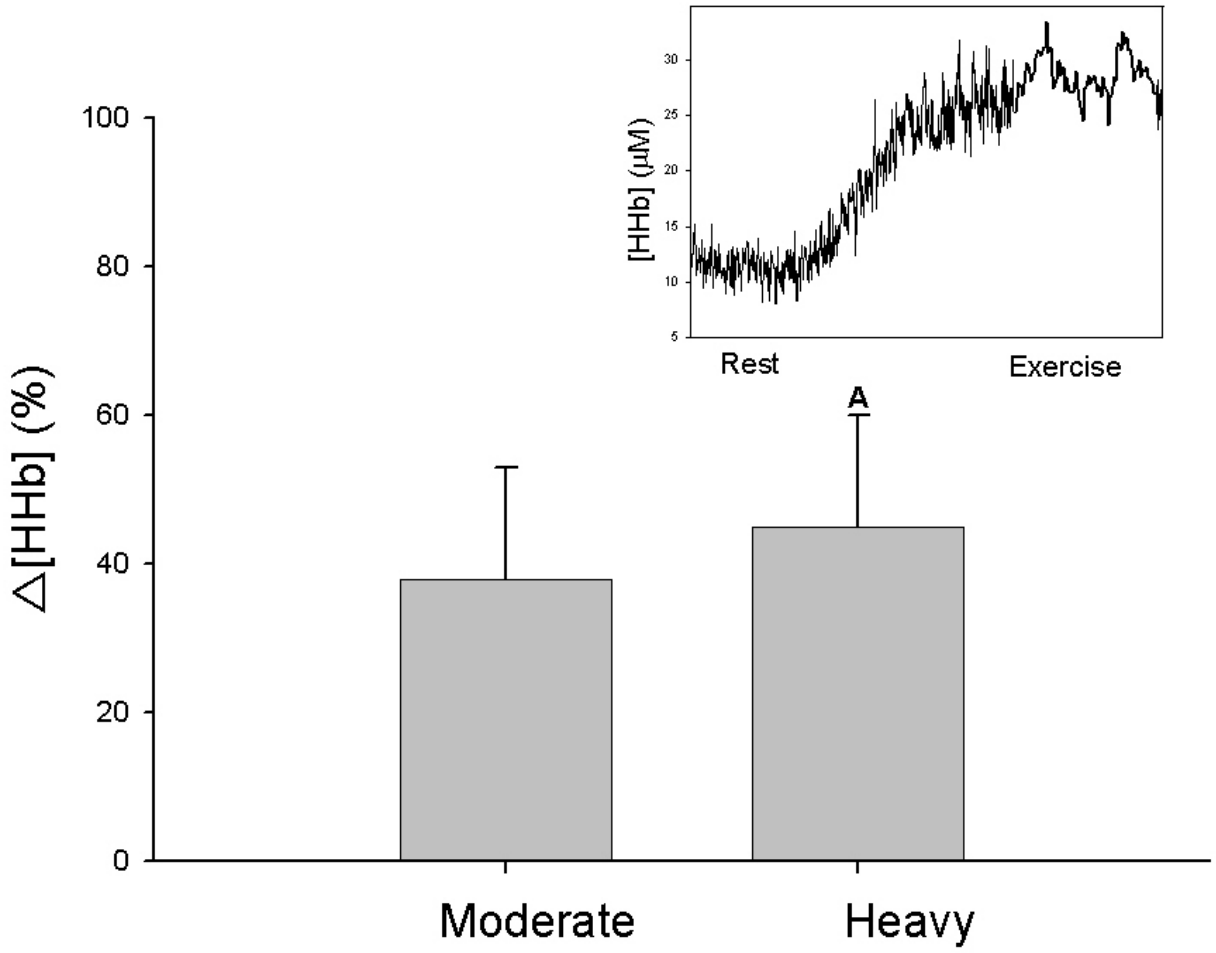
[C] Significantly different from moderate exercise ($P < 0.05$).

Figure 4-5 Absolute change in deoxyhemoglobin concentration [HHb].



Absolute change in deoxyhemoglobin concentration [HHb] from the relaxation phase to the peak during contractions for all eight subjects during moderate and heavy exercise.

Figure 4-6 Change in deoxyhemoglobin concentration ((Δ[HHb]) during contractions as a percentage of the total increase from rest to exercise for moderate and heavy exercise.



Inset shows raw [HHb] signal during one rest-to-exercise transition with two contractions expanded in time to facilitate visualization of the data.
[A] Significantly different from moderate exercise ($P < 0.05$).

TABLE

Table 4.1 Muscle oxygenation during moderate and heavy knee extension exercise.

	Moderate			Heavy		
	Rest	**Relaxation Phase**	**Peak**	**Rest**	**Relaxation Phase**	**Peak**
[HbO_2] (M)	39.5 ± 11.4	41.9 ± 12.7	43.2 ± 15.0	42.3 ± 12.4	42.8 ± 13.2	43.6 ± 13.7
StO_2 (%)	75 ± 3.9	72 ± 3.1 [a]	66 ± 3.1 [a,b]	77 ± 2.4	71 ± 4.3 [a]	70 ± 4.2 [a,b,c]

Values are mean ± standard deviation. [HbO_2], oxyhemoglobin concentration; StO_2, tissue oxygen saturation.

[a] Significantly different ($P < 0.05$) from rest;

[b] Significantly different ($P < 0.05$) from relaxation phase (RP);

[c] Significantly different ($P < 0.05$) from moderate exercise.

CHAPTER 5 - CONCLUSION

With the unique ability to observe the interactions between skeletal muscle blood flow and dynamic muscle contractions at the levels of the conduit artery, venous outflow and the capillary bed in the collection of studies reported herein, significant insight has been gained on the impact of muscle contractions on oxygen delivery to and exchange with active skeletal muscle.

In the first study (Chapter 2), we quantified the net contribution of the muscle pump to skeletal muscle blood flow during steady-state upright knee extension exercise. This was accomplished by comparing mean blood flow during exercise to early recovery blood flow across a wide range of submaximal work rates in humans. Furthermore, by comparing blood flow during the relaxation phase between contractions to the early recovery flow, assuming vascular conductance remained unchanged from the exercise level, we were able to quantify the potential enhancement effect of the muscle contraction-relaxation cycle to blood flow. We found that at the lightest work rate, rhythmic muscle contraction enhanced mean exercise blood flow, compared to blood flow early in recovery. Above this work rate, there was no systematic enhancement of mean muscle blood flow during steady state exercise. In fact, at the higher work rates there was evidence that the net effect of muscle contraction/relaxation was impedance to flow.

In Chapter 3, we found that across a large range of cycle exercise power outputs, the blood flow / $\dot{V}O_2$ response, both centrally (as cardiac output to pulmonary $\dot{V}O_2$) and peripherally (as leg blood flow to leg $\dot{V}O_2$), remained robust despite changes in contraction frequency. Thus, muscle blood flow is matched to the metabolic needs of the working muscle, increasing linearly with work rate with neither an impedance to flow nor any enhancement above that which would be predicted by the increase in metabolic rate.

Finally, our previous understanding of oxygen transport to and utilization by contracting muscle had been limited due to an inability to directly measure microvascular oxygen

distribution and extraction in striated muscle during a contraction; thus it had not been possible previously to detect if oxygen extraction occurred during contractions themselves. In chapter 4, for the first time, microvascular gas exchange has been described (in real time) during the contractile phase of rhythmic knee extension exercise. Microvascular red blood cell volume was shown to be preserved during contraction in human muscle, which facilitated continued O_2 extraction (as evidenced by increased [HHb]), at a time when the metabolic demands of the working muscle were transiently increased.

This series of studies constitutes a base of knowledge that furthers our understanding of the mechanisms which govern the control of skeletal muscle blood flow and its coupling to muscle metabolic rate.

Appendix A - Curriculum Vitae

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EDUCATION:

December 1999	Kansas State University	Kinesiology (B.S.)
May 2001	Kansas State University	Kinesiology (M.S.)
Fall 2006	Kansas State University	Anatomy and Physiology (PhD)

RESEARCH/WORK EXPERIENCE:

2001-2006; *Kansas State University*: Research Assistant, Exercise Physiology Laboratory.

2006 - *Kansas State University*: Post-doctorate Fellow, Department of Anatomy and Physiology

ACADEMIC AWARDS:

1999 Graduated with Honors; *Summa Cum Laude*.
Casablanca Award for Outstanding Performance in Human Body Course at KSU.

SOCIETY MEMBERSHIP:

American College of Sports Medicine
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HONORS:

2001 Doctoral Student Research Award, Central States chapter, American College of Sports Medicine.

RESEARCH PAPERS:

1. **Lutjemeier, B.J.**, A. Miura, B.W. Scheuermann, S. Koga, D. Townsend and T.J. Barstow. Muscle contraction-blood flow interactions during upright knee extension exercise in humans. *J. Appl. Physiol*. 98:1575-83, 2005.

2. Ferreira, L.F., D.K. Townsend, **B. J. Lutjemeier** and T.J. Barstow. Muscle capillary blood flow kinetics estimated from pulmonary O_2 uptake and near-infrared spectroscopy. *J. Appl. Physiol.*, 98:1820-1828, 2005.

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5. Ferreira, L.F., **B.J. Lutjemeier**, D.K. Townsend and T.J. Barstow. Effects of pedal frequency on estimated muscle microvascular O_2 extraction. *Eur. J. Appl. Physiol. 96:558-63, 2006.*

6. Harper A.J., L.F. Ferreira,, **B.J. Lutjemeier**, D.K. Townsend and T.J. Barstow. Femoral artery and estimated muscle capillary blood flow kinetics following the onset of exercise. *Exp. Physiol. 91(4):661-71, 2006.*

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1. Barstow, T.J., **B.J. Lutjemeier** and L.F. Ferreira. Kinetics of restoration of arteriolar tone after exercise. *J. Appl. Physiol*. Aug, 2005.

RESEARCH PAPERS, in review:

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1. **Lutjemeier, B.J.**, C.A. Harms, L.F. Ferreira, D.K. Townsend, A.J. Harper and T.J. Barstow. The effect of contraction frequency on the central and peripheral blood flow / Vo_2 relationship.

ABSTRACTS and PRESENTATIONS:

1. **Lutjemeier, B.J.**, A. Miura, B.W. Scheuermann, S. Koga and T.J. Barstow. Post exercise hyperemia does not overshoot peak oscillatory blood flow during exercise. Central States ACSM 2000.

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3. Miura, A., **B.J. Lutjemeier**, B.W. Scheuermann, S. Koga and T.J. Barstow. The effect of muscle tension and body position on leg blood flow during exercise. ACSM 2001.

4. **Lutjemeier, B.J.**, D. Townsend, B. Hoelting, A. Miura, B.W. Scheuermann and T.J. Barstow. Relationship between mean and oscillations in muscle blood flow during dynamic exercise. CSACSM 2001.

5. **Lutjemeier, B.J.**, D. Townsend, B. Hoelting, A. Miura, B.W. Scheuermann and T.J. Barstow. Relationship between mean and oscillations in muscle blood flow during dynamic exercise. ACSM 2002.

6. **Lutjemeier, B.J.**, D.K. Townsend, S. Koga, C.A. Harms and T.J. Barstow. Oscillations in femoral venous blood flow during dynamic exercise do not simply reflect muscle pump. CSACSM 2002.

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8. Townsend, D.K., **B.J. Lutjemeier**, A. Miura, B.W. Scheuermann and T.J. Barstow. Skeletal muscle vascular conductance during dynamic knee extension exercise. ACSM 2003.

9. **Lutjemeier B.J.**, D.J. Stevens, S. Warren, C.A. Harms, and T.J. Barstow. The impact of muscle contraction on blood flow delivery: examining oscillations in venous blood flow.

10. **Lutjemeier, B.J.**, D. Townsend, L. Ferreira and T.J. Barstow. Impact of muscle contraction on arterial blood flow and tissue gas exchange by NIRS. ACSM 2004.

11. Ferreira, L., **B.J. Lutjemeier**, D. Townsend, T.J. Barstow. NIRS-Derived estimate of muscle blood flow kinetics during moderate- and heavy-intensity cycling exercise. ACSM 2004.

12. Townsend, D., L. Ferreira, **B.J. Lutjemeier** and T.J. Barstow. The influence of adipose tissue thickness on near-infrared spectrometry during intra-contraction knee extension exercise. ACSM 2004.

13. Barstow, T.J., L. Ferreira, **B.J. Lutjemeier** and D. Townsend. Tissue oxygenation by NIRS as a function of pedal rate during incremental exercise. ACSM 2004.

14. **Lutjemeier, B.J.**, C.A. Harms, A.J. Harper, L. Ferreira, D.K. Townsend and T.J. Barstow. Pedal frequency does not alter the cardiac output : Vo2 relationship during cycling. ACSM 2005.

15. Townsend, D., M.D. Haub, **B.J. Lutjemeier**, L. Ferreira, A.J. Harper and T.J. Barstow. Dissociation of glucose homeostasis from insulin sensitivity in college-age subjects at risk for type 2 diabetes. ACSM 2005.

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17. Harper, A.J., L.F. Ferreira, **B.J. Lutjemeier**, D.K. Townsend and T.J. Barstow. Estimated kinetics of muscle capillary blood flow during recovery from exercise. ACSM 2005.

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PRESENTATIONS:

1. "Muscle contractions / blood flow interactions during dynamic exercise." Seminar series; Anatomy and Physiology Department, Kansas State University. Fall 2003.

2. "Evidence for a Muscle Pump? Facilitation of blood flow during steady state exercise in humans." Seminar series; Anatomy and Physiology Department, Kansas State University. Fall 2004.

3. "Impact of Contractions on Muscle Blood Flow and Microvascular Gas Exchange." Seminar series; Anatomy and Physiology Department, Kansas State University. Fall 2005.

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Figure B. 1. Stamps of Permission