DISCREPANCY BETWEEN LEG AND CAPILLARY BLOOD FLOW KINETICS DURING KNEE EXTENSION EXERCISE

by

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B.S., Kansas State University, 2011

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Kinesiology
College of Human Ecology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2013

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Abstract

Previously in our laboratory, capillary blood flow (Q_{CAP}) kinetics were found to be significantly slower than femoral artery (Q_{FA}) kinetics following the onset of knee extension exercise. If the increase in Q_{CAP} does not follow a similar time course to Q_{FA}, blood must be flowing into the leg but not to the working muscle. One possible explanation for this discrepancy is that blood flow also increases to the nonworking lower leg muscles. **Purpose**: To determine if cuffing below the knee alters the kinetics of Q_{FA} and Q_{CAP} during knee extension exercise, and provide insight into the potential mechanisms controlling the rapid increase in Q_{FA}. **Methods:** Subjects performed a ramp max test to determine the work rate at which gas exchange threshold (GET) occurred. At least four constant work rate trials in each condition were conducted at work rates eliciting ~80% GET. Trials were performed with and without below knee occlusion. Pulmonary gas exchange, near-infrared spectroscopy, Q_{FA} and mean arterial pressure (MAP) measurements were taken. Muscle oxygen uptake (VO_2m) and deoxy[hemoglobin + myoglobin] were used to estimate Q_{CAP}. Conductance (C) was calculated (Q_{FA}/MAP) and the percent change from baseline at 60s into exercise was calculated to indicate a time course of change. **Results:** There was no significant difference between the uncuffed and cuffed conditions ($P>0.05$). The mean response times (MRT) of Q_{FA} were 18.7 ± 14.2s (uncuffed) and 24.6 ± 14.9s (cuffed). Q_{CAP} MRTs were 51.8 ± 23.4s (uncuffed) and 56.7 ± 23.2s (cuffed), which were not significantly different from the time constants (τ) of VO_2m (39.7 ± 23.2s (uncuffed) and 46.3 ± 24.1s (cuffed)). However, the MRT of Q_{FA} was significantly faster ($P<0.05$) than the MRT of Q_{CAP} and τVO_2m. τVO_2m and MRT Q_{CAP} were significantly correlated. The Q_{FA} and C percent increase from baseline at 60s were significantly different from MAP but not from each other. **Conclusion:** Cuffing below the knee did not significantly change the kinetics of Q_{FA}, Q_{CAP} or

VO_2m. Estimated Q_{CAP} kinetics tracked VO_2m following exercise onset, while changes in Q_{FA} appeared to be primarily driven by an increase in C, not an increase in MAP.

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Acknowledgements

I would first like to thank my lab colleagues and subjects without whom this research would not have happened. I would specifically like to thank Dr. Carl Ade and Ryan Broxterman for being my in the lab mentors and teaching me so much about all aspects of research.

Next, I would like to thank my major professor, Dr. Thomas Barstow, for allowing me to come into your lab as an undergraduate and stick around to pursue my master's degree. Your passion for research and extensive knowledge have inspired me to become a better scientist.

I would also like to thank my thesis committee, Dr. David Poole, Dr. Brett Wong, and Dr. Tim Musch. Your teaching, insight and criticism have been very valuable to my research specifically and to my education in general.

Finally, I would like to thank my family. Without your love and support, I never would have made it this far. Thank you for pushing me, helping me, listening to me complain and loving me. You have instilled in me the importance and love of learning that I will carry with me for the rest of my life.

Dedication

This thesis is dedicated to my family and friends. You have shaped me into the person I am today and I am so thankful for the impact you have had on my life.

Chapter 1 - Introduction

Following the onset of exercise, blood flow (Q) increases through the conduit artery and the working muscle microvasculature in order to supply the O_2 needed for muscle metabolism. Previous research has shown faster kinetics of femoral artery blood flow (Q_{FA}) than contracting muscle capillary blood flow ($Q_{CAP;}$ Harper et al., 2006), suggesting that there is blood flowing through the femoral artery that is not reaching the capillaries of the working muscle. This discrepancy suggests that there could be differences in the mechanisms associated with the increase in blood flow in these two locations. With regard to the potential mechanisms for the increase in blood flow in the conduit artery, MAP changes more slowly than total peripheral resistance (TPR), suggesting that a decrease in resistance (increase in conductance) is driving the increase in conduit artery flow during cycling exercise (Faisal et al., 2010). More specifically, the initial, rapid increase in Q at exercise onset is thought to occur through the effects of the muscle pump and rapid vasodilation (Tschakovsky and Sheriff, 2004) but the exact contribution of each of these mechanisms is unknown. In contrast, the kinetics of increase in Q_{CAP} have been shown to be tightly coupled to the kinetics of the increase in muscle metabolism (Ferreira et al., 2005b). This suggests that O_2 demand drives the increase in microvascular flow.

Q_{CAP} Estimation Technique

The kinetics of capillary blood flow can be estimated by rearranging the Fick equation as follows (Ferreira et al., 2005b):

$$Qcap\ (t) = \frac{VO2m\ (t)}{(CaO2 - CvO2)(t)} \propto \frac{VO2p\ (Phase\ 2)(t)}{deoxy[Hb + Mb](t)}$$

where Q_{CAP} is capillary blood flow, VO_2m is muscle oxygen uptake, (CaO_2-CvO_2) is the arterio-venous O_2 difference, VO_2p is pulmonary oxygen uptake and deoxy[Hb+Mb] is the deoxygenated hemoglobin plus myoglobin signal derived from near infrared spectroscopy (NIRS). This technique, first proposed by Ferreria et al. in 2005b, uses phase II VO_2p as a proxy measurement for VO_2m and deoxy[Hb+Mb] as a proxy measurement for the (CaO_2-CvO_2), making it possible to estimate Q_{CAP} kinetics noninvasively. Q_{CAP} kinetics were found to be tightly coupled to VO_2m kinetics and significantly slower than deoxy[Hb+Mb] kinetics during cycling exercise (Ferreira et al., 2005b).

The Q_{CAP} estimation technique has also been used during knee extension exercise to compare the changes in microvascular blood flow with those in the conduit artery. Harper et al. (2006) reported similar kinetic changes as Ferreria et al. (2005b) where Q_{CAP} was significantly slower than deoxy[Hb+Mb]. Additionally, the change in Q_{FA} was found to be significantly faster than the change in Q_{CAP}. This difference was unexpected as the authors had hypothesized that during knee extension exercise, blood flowing through the femoral artery would be primarily going to the working muscle capillaries. From this finding arose the question, if blood flowing into the lower limb is not going to the working muscle capillaries, where is it going? Further, what mechanisms controlled the increase in flow in both the femoral artery and the capillaries of the working muscles during knee extension exercise, and were they different from those reported by Faisal et al. (2010) for cycling exercise?

The purposes of the present study were twofold, a) to characterize the relative contribution of changes in driving pressure versus conductance to changes in Q_{FA} following the onset of knee extension exercise, and b) determine the mechanism(s) which produced the discrepancy between femoral artery blood flow kinetics and capillary blood flow kinetics seen by

Harper et al. (2006). We utilized a protocol similar to that of Harper et al. (2006) but added measurements of blood pressure and cuffing below the knee so as to eliminate the contribution of the nonworking muscles and vasculature of the lower leg. We hypothesized that during knee extension exercise, 1) occluding the leg below the knee would a) slow the kinetics of adjustment of Q_{FA}, and b) speed the kinetics of adjustment of Q_{CAP}, thus reducing the difference in the kinetics of Q_{FA} and $Q_{CAP,}$ and 2) the initial, rapid change in Q_{FA} would be caused primarily by an increase in conductance rather than an increase in pressure.

Chapter 2 - Review of Literature

Theory of Measurements

Near Infrared Spectroscopy

Near Infrared Spectroscopy (NIRS) is now widely used as an estimation of muscle tissue oxygenation status. The NIRS technique shines near infrared light into the tissue and detects the intensity of light returning through a known distance. Once the light enters the tissue, it will either be scattered or absorbed by a chromophore. Scattering occurs when the light contacts something, such as a cell wall, resulting in a change in the index of refraction. The main chromophores present in human tissues are hemoglobin (Hb), myoglobin (Mb) and water. Light wavelengths below 650nm do not penetrate into tissue, while wavelengths above 900nm will be almost completely absorbed by water, resulting in no detectable light returning to the sensor. Due to this, the ISS Oxiplex uses measurement wavelengths of 690nm and 830nm while assuming a constant water concentration. The presence or absence of oxygen bound to Hb and Mb alters the absorption spectra of each chromophore, thus allowing for the determination of the relative oxygenation of Hb and Mb.

While a distinction can be made between oxygenated and deoxygenated Hb, no distinction can be made between Hb and Mb contribution, as the absorption spectra for Hb and Mb are almost identical (Kreutzer and Jue, 1991). There is controversy in the literature as to how much Mb contributes to the overall [Hb+Mb] signal, with some studies suggesting a contribution of <10% (Mancini et al., 1994; Seiyama et al., 1988) and others suggesting a significant contribution (Molé et al., 1999; Richardson et al., 2001; Tran et al., 1999). Recent quantitative research has suggested that Mb can contribute >50% of the total [Hb+Mb] NIRS signal (Davis and Barstow, 2013). However, several studies have shown that Hb and Mb desaturate with a

similar time course during exercise, meaning that the NIRS deoxygenated signal can still provide an accurate representation of the O_2 extraction kinetics (Gayeski and Honig, 1988; Koga et al., 2012; Richardson et al., 1995).

The OxiplexTS system, used in the current study, can accurately determine the [Hb+Mb] concentration in tissue by measuring the absorption and scattering coefficients by way of frequency-domain multi-distance spectroscopy. With this technique, the light beam is modulated at a frequency of 110MHz and the intensity of the light is determined by changes in the average DC component, the alternating AC component and the phase shift between the light entering the tissue and the light leaving the tissue. The absorption and scattering coefficients can then be determined using the changes in these components as a function of the set distance between the light source and light detector.

Finger Photoplethysmography

Blood pressure (BP) can be monitored continuously using noninvasive finger photoplethysmography. For this technique, the unstretched volume (where the transmural pressure is zero) of the finger arteries must be determined via a physiological calibration introduced by Wesseling and colleagues (1995). The finger cuff pressure is maintained constant for one or more heart beats and changes in the finger arterial plethysmogram are observed and subsequently used to determine an unstreched arterial volume. To monitor changes in pressure, the volume clamp method, first introduced by Peñáz in 1967, is used, which clamps the artery at the unstreched volume and then monitors changes in finger arterial volume continuously with a photoplethysmograph built into the finger cuff. The finger arterial blood volume is kept constant by using the signal from the photo plethysmograph to rapidly adapt the air pressure in the cuff. As the artery is clamped at its unstreched volume, the pressure in the finger cuff is equal to the

finger arterial pressure at all times. The finger BP waveform is then transformed into a brachial BP waveform using a transfer function and correction factor for the brachial-finger pressure gradient. BP measured via finger photoplethysmography has been validated against auscultatory BP measures and direct intra-arterial BP measures (Eeftinck Schattenkerk et al., 2009; Imholz et al., 1993). In both resting and exercising states, finger photoplethysmography has been shown to provide accurate BP measurements (Eeftinck Schattenkerk et al., 2009; Idema et al., 1989; Imholz et al., 1993).

Doppler Ultrasound

Doppler ultrasound has been used to noninvasively, instantaneously measure blood velocity (BV) during exercise. This technique uses the Doppler effect, a phenomenon that occurs when there is relative motion between a source and receiver of sound. If a sound wave is reflected by a moving object, the wave will return at a different frequency than it was transmitted. This is called a Doppler shift and is influenced by the speed and direction at which the reflecting object is moving. When measuring BV in a conduit artery, the red blood cells (RBC) moving through the artery reflect the emitted sound waves causing them to be returned to the receiver at a different frequency than they were transmitted. If the RBCs are moving away from the sound source, the sound waves will be returned to the receiver at a slower frequency than they were emitted. Conversely, when the RBCs are moving toward the sound source, the sound waves will be returned at a faster frequency. This permits distinction between antegrade and retrograde flow in the conduit artery. In order to detect differences in movement direction, the Doppler angle, the angle between the light beam and the RBC flow, must be known. If the light beam is perpendicular to the RBC flow, no relative motion of the RBCs can be detected. When the system is used in pulse wave mode, the researcher can set the depth from which

motion is being detected to narrow the field of view to just the artery being measured. Using the Doppler shift and Doppler angle, the blood velocity is computed by the ultrasound system and reported beat-by-beat.

In order to calculate blood flow (Q), cross-sectional area (CSA) of the artery must also be measured. It has previously been shown during knee extension exercise that the diameter of the conduit artery does not significantly change (Harper et al., 2006; Lutjemeier et al., 2005; Radegran, 1997). This allows the CSA to be determined with subjects at rest prior to exercise when the vessel walls can be accurately detected. The vessel diameter is measured using two-dimensional sonography and CSA is calculated using the equation $CSA = \pi r^2$, where r is the radius of the vessel.

Control of Blood Flow

Blood flow is determined by the equation $Q=\Delta P/R$, where Q is blood flow, P is pressure and R is resistance. Thus, the changes in Q following the onset of exercise can either be caused by an increase in the pressure gradient across the muscle, a decrease in resistance (increase in conductance) or a combination of the two. There is evidence to suggest that an increase in the pressure gradient occurs with the onset of exercise (DiCarlo and Bishop, 1992). One mechanism thought to increase the pressure gradient during exercise is the muscle pump (Folkow et al., 1970; Pollack and Wood, 1949). Of the total energy necessary to perfuse skeletal muscle during upright exercise, it has been proposed that as much as 30% is supplied by the muscle pump (Stegall, 1966). One way the muscle pump improves muscle perfusion is by reducing the venous hydrostatic pressure. Many studies, using various human and animal models, have arrived at the conclusion that muscle contraction can decrease venous hydrostatic pressure sufficiently to improve muscle Q and perfusion (Folkow et al., 1970; Leyk et al., 1994; Pollack and Wood,

1949; Radegran and Saltin, 1998; Sheriff and Van Bibber, 1998; Shiotani et al., 2002; Tschakovsky et al., 1996). It is important to note that the muscle pump appears to only be effective in the presence of a hydrostatic column and at moderate work rates, while at higher work rates, muscle contraction may actually impede blood flow (Lutjemeier et al., 2005).

Another, less supported, mechanism of the muscle pump is its ability to propel or "suck" blood from the arteries into the veins. This idea goes beyond reducing the venous hydrostatic pressure and indicates that muscle contraction can generate a negative pressure in the veins. This negative pressure causes an increase in flow upon relaxation as the blood follows the pressure gradient from the arteries into the veins. This mechanism is largely supported by indirect evidence as no one has been able to actually measure a negative pressure within the veins. Almen and Nylander (1962) indirectly demonstrated this mechanism by infusing contrast medium into superficial cutaneous veins during calf contractions and observing that the contrast medium was rapidly propelled into the deep veins upon muscle relaxation. While they were only observing flow from superficial to deep veins, they presumed that the rapid transport of blood seen from superficial to deep vein would also happen from artery to vein. Also supporting this idea are arterial inflow tracings which show a brief period of elevated arterial inflow immediately following relaxation during forearm contractions (Saunders and Tschakovsky, 2004).

A correlation between contraction frequency and Q has been shown, providing additional evidence in support of a muscle pump. The initial rise in Q witnessed in dogs at the onset of treadmill exercise is in proportion to contraction frequency when autonomic nerve activation is blocked to prevent any effects from sympathetic vasoconstriction (Sheriff and Hakeman, 2001). Using treadmill running with rats, it was shown that changes in muscle Q correspond directly to

changes in treadmill speed and therefore contraction frequency, suggesting a direct mechanical link between Q and contraction frequency (Sheriff and Hakeman, 2001).

While changes in Q appear to be correlated with contraction frequency, contraction intensity can also influence the muscle pump and therefore Q. The muscle pump does not appear to have the same effect at all exercise intensities (Lutjemeier et al., 2005). At low work rates, Q during exercise was greater than immediately in recovery suggestive of an enhancement in flow as a result of the muscle pump. At intermediate work rates, Q was the same during recovery as it was during exercise suggesting no net effect of the muscle pump. Finally, at high work rates, Q remained the same from exercise into recovery, but a drop in recovery mean arterial pressure (MAP) suggested a muscle-contraction-induced impedance to flow during exercise. This was likely the result of the increased flow during relaxation not being adequate to make up for the flow impedance caused by muscle contraction. Therefore, MAP had to increase in order to maintain overall flow in the muscle.

Despite substantial evidence indicating an increase in the pressure gradient across the muscle contributes to increasing Q, an increase in conductance must also be considered. This increase in conductance can be caused by rapid vasodilation in the contracting muscle. Research on rapid vasodilation has been conducted either in isolated muscle preparations or in the intact muscles. Interestingly, these two preparations appear to lead to different results. In isolated muscle preparations, a delay of at least 5-10s has been seen before the onset of vasodilation (Cohen et al., 2000; Marshall, 1984). For example, after a single twitch contraction, it took ~10s for terminal arteriole vasodilation to begin and ~20s for primary arteriole dilation to begin (Marshall, 1984). This data suggests that the vasodilation is not quick enough to explain the initial change in conduit artery Q, at least during single twitch contractions. More recent isolated

muscle preparation data, however, show a shorter delay prior to the onset of vasodilation (Behnke et al., 2010; Kindig et al., 2002; VanTeeffelen and Segal, 2006; Wunsch et al., 2000). A time delay of only 3-4s has been consistently shown in isolated rat arterioles (Behnke et al., 2010; Wunsch et al., 2000). These time delays are more consistent with those seen in intact muscle preparations. When intact muscle preparations are used, Q appears to increase in proportion to muscle contraction intensity when using a single contraction model (Anrep and von Saalfeld, 1935; Corcondilas et al., 1964; Tschakovsky et al., 2004). Tschakovsky (1996) found Q remained elevated longer in response to a single forearm contraction than it did in response to a single forearm cuff inflation/deflation (simulation of muscle pump). This indicates that rapid vasodilation is contributing to the initial increase in Q in addition to any increase caused by the muscle pump. It is likely that Q increases at the onset of exercise through some interplay of these two systems.

After the initial, rapid rise in Q following the onset of exercise, further increases in Q may be needed to facilitate the matching of oxygen delivery to the metabolic demand of the working muscle. This appears to occur through vasodilatory mechanisms (Berg et al., 1997). There are many potential vasodilators that contribute to exercise-induced hyperemia including potassium ions, adenosine, carbon dioxide, inorganic phosphate, lactate and hydrogen ions (Hughson, 2003). One recently studied vasodilatory compound is adenosine triphosphate (ATP). RBCs release ATP into the vascular lumen in response to metabolic and mechanical stimuli such as reduced PO_2 and pH, mechanical deformation of RBCs and increased shear stress. The ATP binds to endothelial P_2Y purinergic receptors and causes the release of endothelial nitric oxide, prostaglandins and endothelium derived hyperpolarizing factor, all of which induce vasodilation (González Alonso, 2012). It has been difficult to isolate the contribution of each vasodilatory

compound as there is redundancy in the system, such that removing or blocking one vasodilator results in compensation by the other mechanisms so no change in blood flow occurs (Hughson, 2003). These vasodilators are released near the active motor units in the working muscle causing local vasodilation. In addition, cell-to-cell communication allows the dilatory signal to be propagated up the vascular tree so that flow from the feed arteries can be directed to the active muscle fibers (Berg et al., 1997; Segal and Kurjiaka, 1995; Song and Tyml, 1993).

If the vasodilation caused by the compounds described above exceeds the heart's ability to pump an adequate amount of blood to maintain MAP, the arterial baroreflex will be activated and cause sympathetic vasoconstriction, which can limit the increase of muscle Q (Remensnyder et al., 1962). If skeletal muscle vasodilates enough to cause a drop in MAP, sympathetic nerves will fire causing the release of norepinephrine. Norepinephrine will activate the α-adrenoreceptors which will produce vasoconstriction. This vasoconstriction will override the vasodilatory signals and protect MAP (Remensnyder et al., 1962).

Capillary Blood Flow

Direct measurement of muscle capillary blood flow (Q_{CAP}) cannot be performed in humans because it is difficult and invasive. Therefore, Ferreira and colleagues (2005b) proposed a way of estimating Q_{CAP} using a rearrangement of the Fick equation and noninvasive measures. The Fick equation states that $Q_{CAP}=VO_2m/(a\text{-}v)O_2$, where VO_2m is muscle oxygen uptake and $(a\text{-}v)O_2$ is the arteriovenous O_2 difference. To determine Q_{CAP} noninvasively, proxy measurements are needed for VO_2m and $(a\text{-}v)O_2$. The primary (phase 2) component of pulmonary O_2 uptake (VO_2p) is used to estimate VO_2m and deoxy[Hb+Mb] is used in place of $(a\text{-}v)O_2$ (Ferreira et al., 2005b).

VO_2p as a proxy measure for VO_2m

The first question to consider with the proxy measures is whether VO_2p can serve as a reliable estimate of VO_2m. Much of the research in this area has used computer simulations to determine the relationship between VO_2m and VO_2p as the direct measurement of VO_2m is difficult and invasive. Barstow and Molé (1987) used a computer simulation to model blood flow in the exercising legs, O_2 consumption of the leg muscles, and blood flow in other regions of the body such as the splanchnic and renal regions and resting smooth muscle. This model was used to estimate the influence of each region on VO_2p during leg exercise. The model showed that VO_2p increases in two phases after the onset of exercise. The first phase (phase 1) reflects the time it takes venous blood to travel to the lungs from the working muscle; during this phase any increase in VO_2 does not represent the metabolic activity in the working muscle but rather an increase in cardiac output. However, the second phase (phase 2) at the lung is very closely coupled to VO_2m (Barstow and Molé, 1987).

Further computer simulations have examined phase 1 and phase 2 separately. VO_2p was measured and compared to simulated VO_2m during the onset of moderate, heavy and very heavy exercise. At the onset of exercise at all intensities, phase 1 VO_2p was found to differ from VO_2m (Lai et al., 2006). A simulation study looking specifically at phase 2 supported the findings of Barstow and Molé (1987) and used a much wider variety of manipulations. This study also used simulations to model the working leg muscles as one compartment and the rest of the body as another, with the ability to vary parameters such as the time constants for blood flow and muscle O_2 uptake. Throughout various manipulations, it was found that phase 2 VO_2p was primarily determined by, and therefore closely correlated with, VO_2m (Barstow et al., 1990). However, while computer simulations can be very useful and informative, they cannot fully replace human in vivo studies.

To determine whether the results of the computer simulations were valid, invasive studies have been conducted on human subjects. Grassi and colleagues (1996) used a constant-infusion thermodilution technique to rapidly measure Q in the leg during the transition to exercise while measuring (a-v)O_2 using arterial and femoral venous blood draws. This permitted the determination of leg VO_2, which was assumed to be a close representation of VO_2m as the majority of the VO_2 in the leg is consumed by the working muscles. This human study demonstrated similar results to the computer simulations performed by Barstow et al. (1987, 1990) where VO_2m was demonstrated to closely match VO_2p during phase 2. It was also shown, as in the previously mentioned computer simulation, that phase 1 VO_2p did not reflect VO_2m (Barstow and Molé, 1987; Grassi et al., 1996). Krustrup et al. (2009) measured VO_2p and VO_2m simultaneously using the same technique as Grassi et al. (1996) during knee extension exercise and recovery. Their findings during exercise agreed with those of Grassi et al. (1996), as there was a strong correlation between VO_2p and VO_2m. These authors were able to determine a more exact time course of change and found that within the first 20s of exercise, contracting muscles begin contributing to VO_2p. The relationship between VO_2p and VO_2m was also measured during recovery and it was found that VO_2p and VO_2m are not as strongly correlated. Therefore VO_2p should not be used as an estimate of VO_2m during recovery (Krustrup et al., 2009).

Rossiter et al. (1999) took a different approach to measuring the relationship between VO_2p and VO_2m by using phosphocreatine concentration [PCr] as a surrogate of VO_2m. [PCr] and VO_2p were measured simultaneously using a whole-body nuclear magnetic resonance spectrometer. Despite the different technique, the study concluded that phase 2 VO_2p serves as a good representation of [PCr] and therefore VO_2m (Rossiter et al., 1999). Human studies and

computer simulations alike thus agree that during phase 2, VO_2p and VO_2m are closely matched and therefore using phase 2 VO_2p as an estimate of VO_2m is a valid practice.

Using deoxy[Hb+Mb] as a proxy measure of (a-v)O_2

The second proxy measurement that needs to be considered is the use of deoxy[Hb+Mb] in place of (a-v)O_2. Deoxy[Hb+Mb] is measured using NIRS, which assesses tissue oxygenation changes in small blood vessels, capillaries and intracellularly. NIRS can be used to assess the relationship between oxygen delivery and oxygen consumption in the muscle being measured (DeLorey et al., 2003; Ferreira et al., 2005b; Grassi et al., 2003; Harper et al., 2006). A change in deoxy[Hb+Mb] has been interpreted as a change in oxygen extraction, which enables the use of deoxy[Hb+Mb] as an estimate of (a-v)O_2 (DeLorey et al., 2003).

Despite ample evidence supporting the use of NIRS in monitoring muscle oxygenation status, a few studies have raised concerns over the validity of this measurement. MacDonald and colleagues (1999) had subjects perform double-leg knee extension exercise while measuring leg blood flow, arterial and venous blood gases and tissue saturation using NIRS. For the first minute of exercise, both signals changed similarly. Following the first minute, however, the NIRS-derived O_2 saturation began to increase while the directly measured venous O_2 saturation continued to decrease or plateau. The authors concluded that because of this discrepancy, NIRS was not a reliable estimate of venous blood oxygenation. In an additional study conducted by Hicks and colleagues (1999), isometric contractions at 10 and 30% maximal voluntary contraction (MVC) were performed, while venous O_2 saturation and NIRS measurements were taken. At 10% MVC, venous O_2 saturation decreased while no change was seen in the NIRS oxygenation signal. At 30% MVC, both signals decreased initially, but the NIRS signal started to increase after 30s of exercise similar to the response seen by MacDonald (1999). It was also

concluded in this study that NIRS measures are not reliable to use as estimates of venous O_2 saturation.

The discrepancies seen in the previous studies can potentially be explained by differences in methodology. First, the venous blood being analyzed was not exclusively from the same muscle tissue that was being sampled by the NIRS probe, but rather came from the entire limb, which included working and nonworking muscles, so the actual contribution of the working muscle to the sample is unknown. Second, the NIRS probe is measuring venous, capillary and arterial Hb+Mb saturation so it is logical that the NIRS derived oxygen saturation be higher than that of the venous blood alone. If a blood sample could be taken from the artery, capillary and vein, the volume or hematocrit-weighted average oxygen saturation of all three might more closely represent the NIRS oxygen saturation signal. Third, up to 50% of the NIRS signal may be coming from Mb instead of Hb so the relative oxygen saturation of the Mb being measured may also account for the differences seen between the NIRS oxygen saturation signal and the measured venous O_2 saturation (Davis and Barstow, 2013). A slower, progressive increase in blood flow and therefore arterial and capillary oxygen saturation following the first minute of exercise could explain the increase in the NIRS oxygen saturation signal seen by MacDonald (1999) and Hicks (1999). Due to the fact that NIRS is measuring the artery, capillary and vein, one limitation with the use of deoxy[Hb+Mb] as a proxy measurement for $(a\text{-}v)O_2$, is that the precise contribution from each compartment to the total signal cannot be determined.

When using NIRS, it is essential to remember that only a small muscle volume is actually being measured and therefore probe placement is critical to assure that the working muscle is being measured properly (Grassi et al., 2003). It has been shown that during leg extension exercise, the vastus lateralis, vastus medialis and rectus femoris muscles show a similar

deoxy[Hb+Mb] response, thus implying that oxygen delivery and extraction are similar within the microvasculature of the quadriceps muscles (duManoir et al., 2010). However, Koga and colleagues (2011) challenged the notion that muscle deoxygenation was homogenous throughout the quadriceps muscles. They used a custom built multi-site NIRS system to show that the magnitude and temporal profile of the deoxy[Hb+Mb] signal was variable between measurement sites. These findings suggest that the quadriceps muscles are not homogenous in their matching of O_2 delivery to O_2 utilization. The limitation with this study, however, is the multi-site NIRS system had fixed light emitters and receivers and therefore could not be adjusted for each subject to ensure that light was being transmitted through the same region of each muscle across subjects. This could have led to erroneous signals if light was being transmitted through connective tissue or tendons. One final consideration with NIRS is that the overall amplitude of change can vary widely between subjects based on adipose tissue thickness (ATT) (McCully and Hamaoka, 2000).

Using the Q_{CAP} estimation technique during exercise

VO_2p as an estimate of VO_2m and deoxy[Hb+Mb] as an estimate of $(a\text{-}v)O_2$ have been shown to be acceptable proxy measurements (Barstow and Molé, 1987; Barstow et al., 1990; Boushel et al., 2001; Grassi et al., 1996; Lai et al., 2006; Mancini et al., 1994; Rossiter et al., 1999), so theoretically, the use of the Fick equation to estimate capillary blood flow should be possible. The first study to use this Q_{CAP} estimation technique compared the kinetics of the estimated Q_{CAP} to the kinetics of estimated VO_2m (Ferreira et al., 2005b). The authors predicted that the Q_{CAP} kinetics would be faster than the VO_2m kinetics. Subjects performed both moderate and heavy intensity cycling exercise and for both intensities, Q_{CAP} was found to be closely coupled with VO_2m kinetics. Q_{CAP} also exhibited biphasic kinetics demonstrating distinct phase

1 and phase 2 responses similar to those seen in VO_2p and femoral artery Q. Q_{CAP} was also estimated during recovery and demonstrated biphasic kinetics, similar to exercise onset, but with slower kinetics than VO_2m (Ferreira et al., 2005a). This was an expected outcome considering Krustrup et al. (2009) found that VO_2m and VO_2p are not as closely matched during recovery.

Harper and colleagues (2006) estimated Q_{CAP} and compared it with conduit artery blood flow (Q_{FA}) as well as VO_2m kinetics to determine whether conduit artery blood flow could be used to estimate Q_{CAP} during knee extension exercise. They hypothesized that Q_{CAP} and Q_{FA} kinetics would be faster than VO_2m kinetics, but that Q_{CAP} and Q_{FA} kinetics would be similar. As expected, Q_{FA} kinetics were faster than VO_2m kinetics, but surprisingly, Q_{CAP} kinetics were significantly slower than both Q_{FA} and VO_2m kinetics. There are many possible explanations why this occurred including the use of knee extension exercise instead of cycling, where Q_{CAP} and VO_2m were shown to have similar mean response times (MRT) (Ferriera et al., 2005b). Harper et al. (2006) also measured deoxy[Hb+Mb] of the rectus femoris while Ferriera et al. (2005b) measured the vastus lateralis. Additionally, the cycling transition was measured from unloaded to loaded exercise (Ferriera et al., 2005b) while the knee extension transition was measured from rest to loaded exercise (Harper et al., 2006). Finally, neither study accounted for blood flowing to the nonworking muscles or skin.

A recent study by Murias and colleagues (2012) has called into question the validity of the Q_{CAP} estimation technique. They found the kinetic profile of Q_{CAP} to be dependent on the deoxy[Hb+Mb] baseline-to-amplitude ratio with those subjects whose deoxy[Hb+Mb] amplitude was greater than the baseline deoxy[Hb+Mb] actually showing a decrease in the estimated Q_{CAP} response. However, this response was seen during the second bout of moderate intensity exercise (Mod 2) which directly followed a bout of heavy exercise. Due to this experimental set-up, the

baseline values in Mod 2 started out elevated above the baseline values from the first bout of moderate intensity exercise (Mod 1). Ferriera et al. (2005b) and Harper et al. (2006) only did single transitions from rest to exercise and never claimed that the Q_{CAP} calculation technique would work for repeated bouts of exercise. If just considering the Mod 1 data from Murias et al. (2012), it actually shows a very similar response to those seen by Ferriera et al. (2005b) and Harper et al. (2006) and provides further support for the use of the Q_{CAP} calculation. It is important to note, however, that the Q_{CAP} calculation technique can only provide an estimation of the kinetic change of Q_{CAP}, not an absolute volume change in capillary flow.

The Q_{CAP} estimation technique proposed by Ferreira and colleagues (2005b) is thought to be an accurate representation of actual Q_{CAP} kinetics during a single exercise transition. Unfortunately, direct measurement of Q_{CAP} in humans is not possible so there is no way to determine, for sure, whether the estimation technique is an accurate representation of the physiology. Phase 2 VO_2p and VO_2m kinetics are closely matched and therefore it is possible to use phase 2 VO_2p as an estimate of VO_2m (Barstow and Molé, 1987; Barstow et al., 1990; Grassi et al., 1996; Lai et al., 2006; Rossiter et al., 1999). Deoxy[Hb+Mb] is thought to be representative of the matching between O_2 delivery and O_2 extraction (DeLorey et al., 2003; Grassi et al., 2003) and therefore can be used as a proxy measurement for (a-v)O_2 although the contribution from arterial and venous blood cannot be determined. Successful application of this technique has shown that Q_{CAP} kinetics are biphasic and closely coupled with VO_2m during exercise onset but not closely coupled with VO_2m during recovery (Ferreira et al., 2005a, Ferreira et al., 2005b). When estimated Q_{CAP} kinetics were compared with measured Q_{FA} kinetics, however, Q_{CAP} kinetics were found to be significantly slower than Q_{FA} kinetics (Harper

et al., 2006). Further investigation is needed to explain the mechanisms related to the discrepancy between Q_{CAP} and Q_{FA} kinetics.

Chapter 3 - Methods

Subjects

Six men age (mean ± SD) 25 ± 1.8 years; height 176 ± 7.5 cm; weight 83.8 ± 17.9 kg participated in the present study. All subjects were free of known cardiovascular, respiratory and metabolic disease, as determined by medical questionnaire, and were nonsmokers. The exercise protocol, benefits and risks of the study were explained and informed consent was obtained prior to participation. All procedures were approved by the Institutional Review Board for Research Involving Human Subjects at Kansas State University and conformed to the *Declaration of Helsinki.*

Exercise Protocol

All exercise bouts consisted of two-leg knee extension exercise using a dolphin-style kick as described previously by Harper et. al (2006). During the first visit, subjects performed an incremental test to exhaustion. Following 2 minutes of resting baseline, the test began at a work rate of 5 watts and a rate of 40 contractions per minute. The work rate was increased every 10 seconds so as to produce a total increase of 5W/min. Subjects continued until volitional fatigue or until they were unable to maintain the target contraction rate.

During visits 2-5, subjects performed square-wave transitions at a work rate which elicited ~80% of each individual's lactate threshold (see below). These tests consisted of 3 minutes of unloaded exercise followed by 6 minutes of constant-load exercise. Three to four transitions were performed during each testing session with at least 10 minutes of rest between transitions. Each transition was randomly selected to be uncuffed or cuffed. For the cuffed trials, pneumatic cuffs (DE Hokanson Inc, Bellevue, Washington) were rapidly inflated immediately

distal to the knee joint on each leg to ~300mmHg, an adequate pressure to occlude blood flow to the lower leg and foot, as confirmed by Doppler ultrasound of the tibial artery. The cuffs were inflated two minutes prior to the start of exercise and remained inflated for the duration of exercise plus the first two minutes of recovery, at which time they were rapidly deflated. At least four transitions were performed in each condition for each subject in order to increase the signal-to-noise ratio so as to enhance the precision of curve fitting and analysis.

Measurements

All exercise was performed on a double leg knee extension ergometer, constructed by researchers at Kansas State University. Muscle oxygenation status as deoxyhemoglobin + myoglobin (deoxy[Hb+Mb]) was measured using near infrared spectroscopy (NIRS; OxiplexTS, ISS, Champaign, IL, USA). The NIRS probe consisted of 8 light sources, four emitting at 690nm and four emitting at 830nm, and a light source detector. The light source detector separation distances were 2.0, 2.5, 3.0 and 3.5 cm. The device was calibrated before each testing session according to manufacturer guidelines and all data were stored at 25 Hz. The probe was placed on the belly of the rectus femoris and secured using a velcro strap and marked to ensure consistent placement during subsequent testing sessions.

An open-circuit breath-by-breath metabolic system (Ultima CardiO_2, Medical Graphics, St. Paul, MN, USA) was used to measure pulmonary oxygen consumption (VO_2), carbon dioxide production (VCO_2) and minute ventilation (V_E). The system was flow calibrated using a 3 liter syringe and the O_2 and CO_2 sensors were calibrated with concentrations of known gases before each testing session. The highest 15 second averages obtained during the incremental test for VO_2, VCO_2 and V_E were considered peak responses. The VO_2 corresponding to the gas exchange threshold (GET) was determined using the V-slope method first outlined by Beaver et.

al (1986) and confirmed using ventilatory equivalents, end tidal CO_2 and O_2 and the respiratory exchange ratio.

Blood velocity in the left femoral artery was measured using Doppler ultrasound (Vivid 3; GE Medical Systems, Milwaukee, WI, USA) with a linear array transducer probe operating at an imaging frequency of 6.7 MHz. All velocity measurements were made proximal to the bifurcation with an angle of insonation $<60^{o}$ and the system operating at a Doppler frequency of 4.0 MHz in pulsed wave mode. Femoral artery diameter in this location (D_{FA}) was measured at rest. From the velocity and diameter measurements, femoral artery blood flow ($\dot{Q}_{FA}$) was calculated using the following equation:

$$\dot{Q}_{FA} = V_{FA} \cdot \pi \cdot (D_{FA}/2)^2$$

where V_{FA} is mean femoral artery blood velocity (cm/s) averaged over 5s windows. These 5s windows occurred every 30s during unloaded exercise, continuously for the first 90s of loaded exercise and then every 30s for the duration of exercise and two minutes of recovery.

Beat-by-beat mean arterial pressure (MAP) was measured continuously via finger photoplethysmography (NexFinHD, BMEYE, Amsterdam, The Netherlands). The finger cuff was placed on the third finger of each subject's left hand. Subjects were instructed to keep this hand relaxed throughout the entire experiment as contracting the hand can influence measurements (Imholz et al., 1993). Heart rate (HR) was monitored via 3-lead electrocardiogram.

Data Analysis

VO_2 breath-by-breath data were converted to second-by-second values and time aligned to the onset of exercise. Deoxy[Hb+Mb] and Q_{FA} were also time aligned to the onset of exercise and all variables were ensemble-averaged across trials for each subject. This produced a single

data set for each variable in each condition. The averaged VO_2 was filtered using a low pass frequency filter set to remove 90% of the high frequencies (SigmaPlot 10.0 Systat Software; Point Richmond, CA, USA) as previously used by Harper et. al (2006) and Ferreira et. al (2005b). Kinetic analysis for VO_2 and Q_{FA} was conducted using nonlinear regression with a least squares technique (SigmaPlot 10.0 Systat Software; Point Richmond, CA,USA). VO_2 responses were fit as follows:

$$\dot{V}O_2(t) = Baseline + \mathrm{H}(t - TD1) * a1\left(1 - e^{-(t-TD1)\tau 1}\right) + \mathrm{H}(t - TD2) * a2(1 - e^{-(t-TD2)\tau 2})$$

$$\text{where } \mathrm{H}(t) = \begin{cases} 0 & t < 0 \\ 1 & t \geq 0 \end{cases}$$

1 and 2 refer to phase 1 and phase 2 respectively and "a" represents the amplitude, "TD" the time delay and "τ" the time constant of each exponential response. The amplitude of the phase 1 response at TD_2 was truncated as $a1' = a1(1 - e^{-(TD2)\tau 1})$. The overall amplitude of the phase 2 response was calculated as $a2' = a1' + a2$. Q_{FA} responses were fit in a similar manner, using the above equations.

Capillary blood flow (Q_{CAP}) was estimated by rearranging the Fick equation (Harper et. al (2006); Ferreira et. al (2005b)) as shown below:

$$Qcap\ (t) = \frac{VO2m\ (t)}{(CaO2 - CvO2)(t)} \propto \frac{VO2p\ (Phase\ 2)(t)}{Deoxy[Hb + Mb](t)}$$

where VO_2p is the pulmonary oxygen uptake. The resulting Q_{CAP} responses were then fit similarly to VO_2 and Q_{FA} using the above equation.

Finally the mean response times of VO_{2m}, Q_{FA} and Q_{CAP} were calculated using the following equation:

$$MRT = \left({a_1'}/{a_2'} \right)(TD1 + \tau 1) + \left({a_2}/{a_2'} \right)(TD2 + \tau 2)$$

MAP was converted to second-by-second values and then averaged over 5s at the same distinct time points as the Q_{FA} data points. MAP was then time aligned with Q_{FA} and conductance (C) was calculated as Q_{FA} divided by MAP at each time point. Not all signals showed a distinctly exponential response so to estimate of the time course of change for each variable, the percent change from baseline occurring by 60s was calculated.

Statistical Analysis

Significant differences between the means of MRT Q_{CAP}, MRT Q_{FA} and τ VO_2m in the uncuffed versus cuffed trials were initially determined using a two-way repeated measures analysis of variance (ANOVA). Since no significant differences were seen between the uncuffed and cuffed conditions, all trials were combined to increase statistical power and the kinetic parameters compared using a one-way ANOVA. These ANOVAs were followed, when appropriate, by a Student-Newman-Keuls *post hoc* test for pair-wise comparisons. Pearson product-moment correlation analysis was used to determine correlations between MRT of Q_{CAP}, Q_{FA} and τ VO_2m. ANOVA was used to determine differences among the average % change occurring by 60s in Q_{FA}, MAP and C in the uncuffed and cuffed conditions. This ANOVA was also followed, when appropriate, by a Student-Newman-Keuls *post hoc* test for pair-wise comparisons. All statistical tests were performed using SigmaPlot 10.0 Software (Systat Software; Point Richmond, CA,USA). For all statistical analyses, significance was declared when $p<0.05$.

Chapter 4 - Results

Table 1 shows subject characteristics and group mean responses during the incremental peak exercise test. The average peak WR was 49.2 ± 6.2 watts eliciting a peak relative VO_2 of 18.9 ± 4.1 ml/kg $_{body\ weight}$/min. Average GET was 900 ± 70.7 ml/min which occurred at 59.7 ± 10.8% peak VO_2. Table 2 shows group mean responses during the constant work rate tests. The average WR for the constant exercise tests was 10.8 ± 1.5 watts which elicited VO_2 responses that were 88.3 ± 11.3 and 81.8 ± 11.5% GET in the uncuffed and cuffed conditions, respectively.

Figure 1 illustrates estimated VO_2m, measured deoxy[Hb+Mb] and calculated Q_{CAP} for a representative subject. Figure 2 shows a comparison between the kinetic responses of Q_{CAP} and Q_{FA} in a different subject demonstrating that the increase in Q_{CAP} occurred more slowly than the change in Q_{FA}.

Table 3 shows the kinetic parameters of VO_2, Q_{CAP} and Q_{FA} while Figure 3 provides a graphical comparison of the overall kinetics. Since there is no TD for VO_2m, τ was used in place of the MRT. The MRTs for Q_{CAP} were 51.8 ± 23.4, 56.7 ± 23.2s (uncuffed, cuffed), for Q_{FA} were 18.7 ± 14.2, 24.6 ± 14.9s (uncuffed, cuffed), while τ VO_2m were 39.7 ± 23.2, 46.3 ± 24.1s (uncuffed, cuffed). There were no significant differences between the uncuffed and cuffed conditions for any variables ($P>0.05$) so uncuffed and cuffed data for the overall kinetics were combined to increase statistical confidence. The MRT of Q_{CAP} and τ VO_2m were both significantly slower than the MRT of Q_{FA} ($P<0.05$) but were not statistically different from each other.

Figure 4 shows the relationships between the kinetic responses of VO_2m, Q_{CAP} and Q_{FA} for both the uncuffed and cuffed conditions. The correlation between VO_2m and Q_{CAP} was

significant in the uncuffed and cuffed conditions (r^2 =0.95, r^2 = 0.70 respectively). None of the other correlations between parameters were found to be significant.

Figure 5 illustrates Q_{FA}, MAP and calculated conductance (C) changes from unloaded to loaded exercise in one subject. Table 4 shows the average percent change from baseline occurring by 60s in each variable. Q_{FA} and C were significantly different from MAP but not from each other suggesting that the change in Q_{FA} was primarily driven by the change in C. No differences were seen between the uncuffed and cuffed conditions in any of these variables.

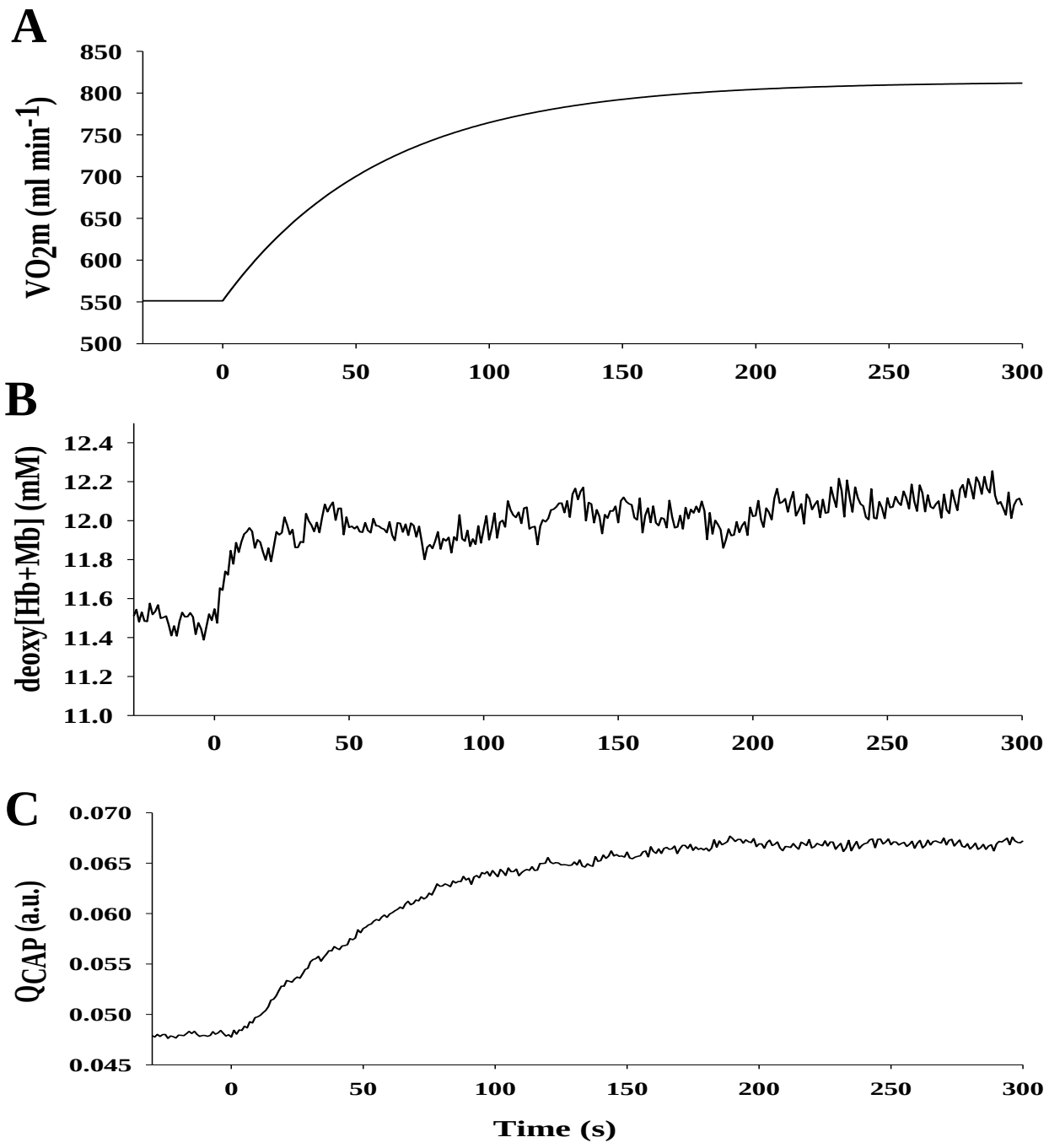

Figure 1. Representative data from one subject during unloaded to loaded exercise transition.

A: VO_2m (ml min-1) estimated from kinetic parameters of VO_2p. B: deoxyhemoglobin + myoglobin concentration (deoxy[Hb+Mb], μM). C: estimated Q_{CAP} profile calculated by dividing VO_2m by deoxy[Hb+Mb]. The amplitude of Q_{CAP} is displayed in arbitrary units (a.u.) (Ferriera, 2005; Harper, 2006).

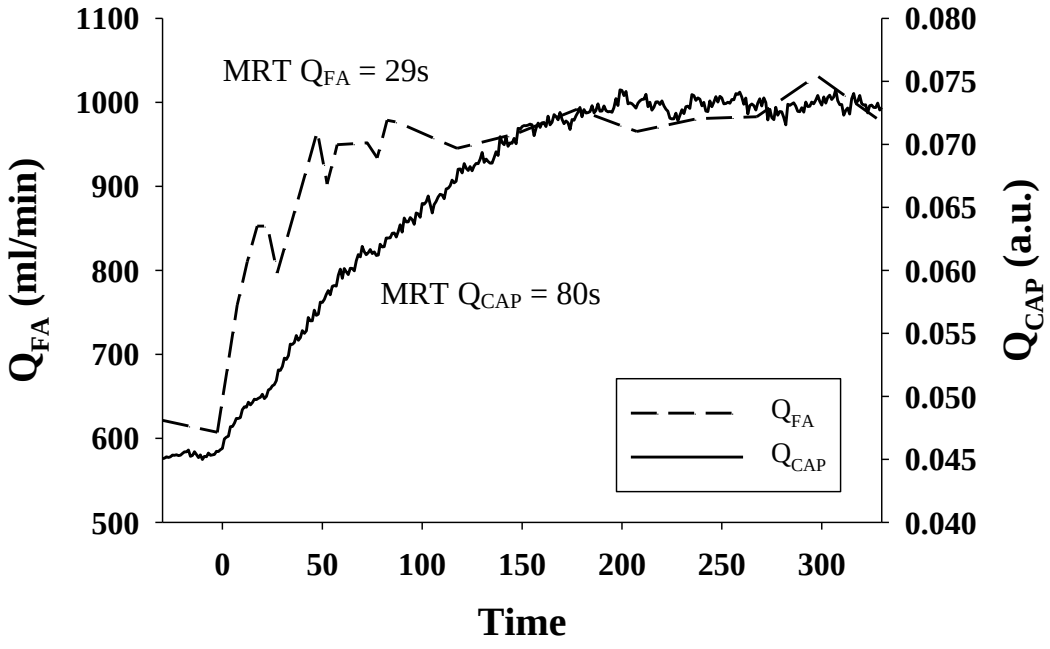

Figure 2. Q_{FA} and Q_{CAP} kinetic responses in a representative subject.

Mean response times (MRT) are shown.

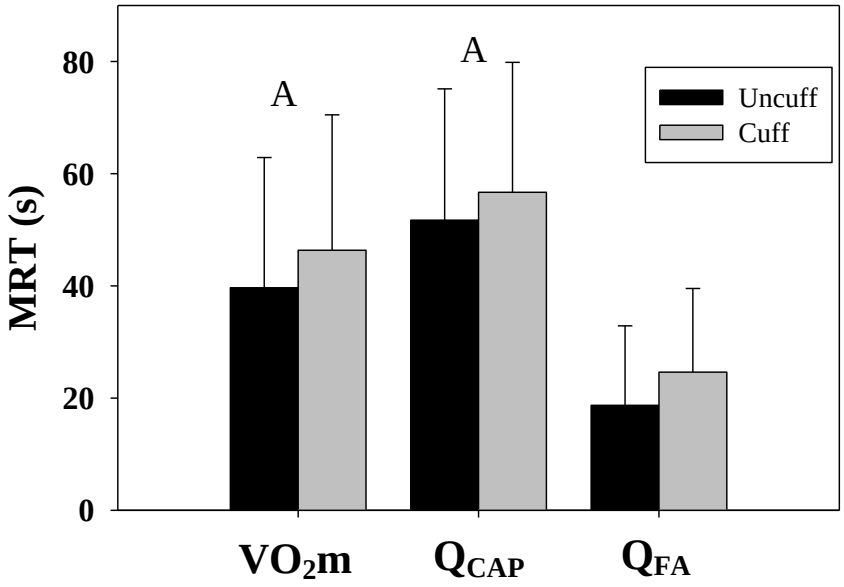

Figure 3. Average MRT of VO_2m, Q_{CAP} and Q_{FA}.

Data are mean ± SE. For VO_2m there is no time delay so τ is used as MRT. There was no significant difference between the uncuffed and cuffed conditions ($P>0.05$), so overall means were compared for each condition. ^{A} significantly slower than MRT Q_{FA} ($P<0.05$).

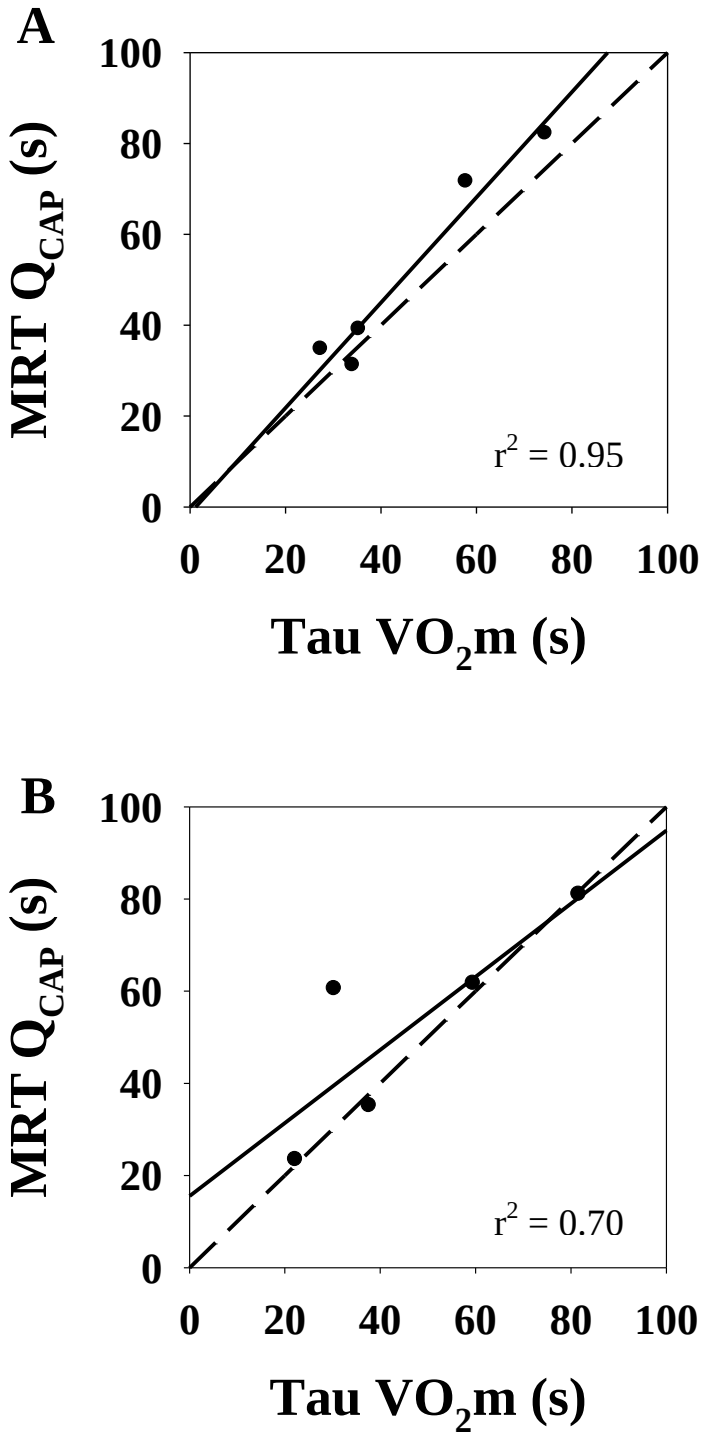

Figure 4. Correlations between the kinetics of VO_2m and Q_{CAP}.

Dashed lines show the line of identity for each plot. r^2 values are shown for each plot. A: uncuffed. B: cuffed. In both conditions, τ VO_2m and MRT Q_{CAP} were significantly correlated.

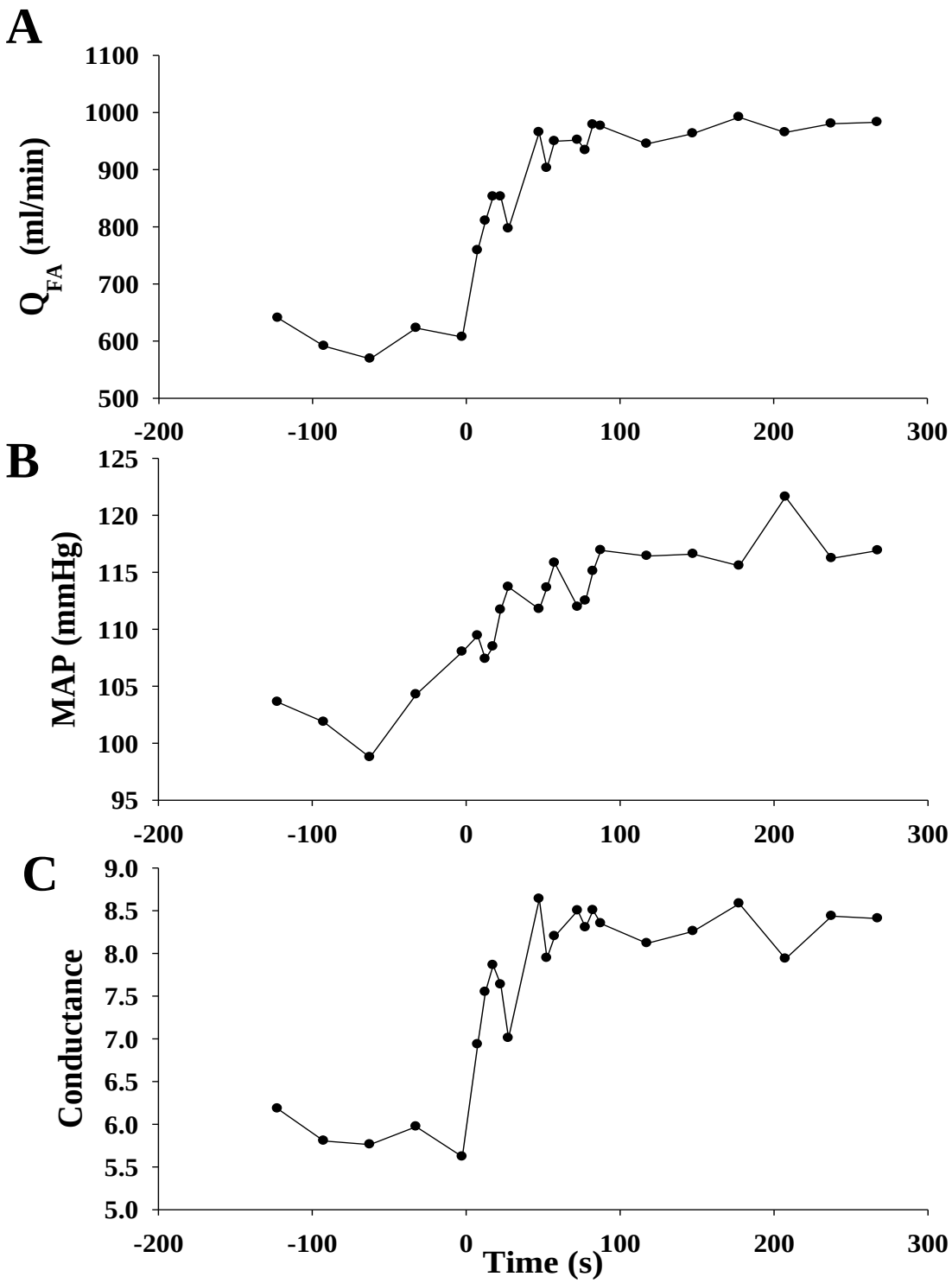

Figure 5. Representative data from one subject during unloaded to loaded exercise transition.

A: femoral artery blood flow (Q_{FA}, ml min^{-1}). B: mean arterial pressure (MAP, mmHg). C: estimated conductance calculated as Q_{FA}/MAP.

Table 1. Subject characteristics and peak exercise responses.

Age	25 ± 2
Height (m)	1.76 ± .07
Weight (kg)	83.8 ± 17.9
ATT (mm)	9.2 ± 2.2
D_{FA} Uncuff (mm)	7.9 ± 1.4
D_{FA} Cuff (mm)	8.0 ± 1.4
VO_2 peak (ml/min)	1543 ± 259
VO_2 peak (ml/kg/min)	18.9 ± 4.1
Peak WR (Watts)	49.2 ± 6.2
GET (ml/min)	900 ± 71
GET (% VO_2 peak)	59.7 ± 10.8

Values are mean ± SD. ATT is adipose tissue thickness underneath NIRS probe. D_{FA} is femoral artery diameter. Gas exchange threshold (GET) and peak exercise values were obtained during the initial incremental exercise test.

Table 2. Responses during constant work rate tests.

	Uncuff	Cuff
WR (watts)	10.8 ± 1.5	10.8 ± 1.5
VO_2 (ml/min)	794 ± 114	732 ± 88
VO_2, % GET	88.3 ± 11.3	81.8 ± 11.5

Values are mean ± SD.

Table 3. Kinetic parameters of pulmonary VO_2, Q_{CAP} and Q_{FA} for uncuffed and cuffed exercise.

Parameter	VO_2		Q_{CAP}		Q_{FA}	
	Uncuff	**Cuff**	**Uncuff**	**Cuff**	**Uncuff**	**Cuff**
Baseline	532 ± 66	479 ± 58	39.5 ± 18	32.4 ± 13	733 ± 520	690 ± 504
A_1[a]	137 ± 12.6	137 ± 45	5.44 ± 6.3	4.8 ± 1.7	388 ± 191	234 ± 43
τ_1 (s)	1.8 ± 2.5	1.8 ± 1.0	7.9 ± 9.5	19.0 ± 18.1	9.7 ± 18.8	10.6 ± 17.1
A_2[a]	127 ± 68	130 ± 45	12.8 ± 7.6	9.9 ± 9.5	184 ± 111	250 ± 336
TD_2 (s)	39.0 ± 26.6	40.7 ± 16.4	17.8 ± 17.4	36.0 ± 25.3	37.5 ± 23.2	21.8 ± 12.0
τ_2 (s)	39.7 ± 23.2	46.3 ± 24.1	46.3 ± 14.2	47.3 ± 15.0	12.5 ± 9.2	23.4 ± 11.8
MRT (s)	36.8 ± 16.1	41.3 ± 7.6	51.8 ± 23.4	56.7 ± 23.2	18.7 ± 14.2	24.6 ± 14.9

Values are mean ± SD. A, amplitude. τ, time constant. TD, time delay. 1, phase 1. 2, phase 2. MRT is the time to approximately 63% of the total response (see text for calculation). [a] Units for VO_2, ml min^{-1}; Q_{CAP}, arbitrary; Q_{FA}, ml min^{-1}.

Table 4. Average baseline, steady state and % change from baseline seen at 60s into exercise for Q_{FA}, MAP and C during uncuffed and cuffed exercise.

	QFA		MAP		C	
	Uncuff	**Cuff**	**Uncuff**	**Cuff**	**Uncuff**	**Cuff**
Baseline	742 ± 526	684 ± 500	97.6 ± 7.4	98.3 ± 8.0	7.9 ± 6.3	7.1 ± 5.4
Steady State	1215 ±832	1115 ± 772	102 ±11	110 ± 10	12.5 ± 10.2	10.2 ± 7.3
% Change at 60s	62 ± 23[A]	60 ± 18[A]	5 ± 4	6 ± 3	53 ± 30[A]	51 ± 21[A]

Values are mean ± SD. [A] indicates a significant difference from MAP.

Chapter 5 - Discussion

The purpose of this study was to better understand how limb and capillary blood flow are controlled following the onset of exercise, and more specifically to explain the discrepancy between femoral artery blood flow kinetics and capillary blood flow kinetics seen by Harper et al. (2006). The major findings were as follows: contrary to our first hypothesis, occluding the nonworking muscle in the lower limb did not change the MRTs of Q_{FA} and $Q_{CAP,}$ or the difference between the two. Additionally, the change in Q_{FA} following exercise onset was caused primarily by an increase in conductance, rather than an increase in pressure, as predicted by our second hypothesis.

Our current findings are in agreement with previous work by Harper et al. (2006) and Ferriera et al. (2005b). During knee extension exercise, Harper et al. (2006) found MRT Q_{FA} to be significantly faster than both τ VO_2m and MRT $Q_{CAP,}$ findings that were also seen in the current study. In contrast to Harper et al. (2006), but in agreement with Ferriera et al. (2005b), we found MRT Q_{CAP} and τ VO_2m to be similar and significantly correlated. One possible explanation for the difference between the present study and that of Harper et al. (2006) is that the current study used an unload-to-loaded exercise transition while the previous study used a rest-to-exercise transition.

Limitations/Assumptions

Two fundamental assumptions are made when estimating Q_{CAP}; the use of phase 2 VO_2p as an estimate of VO_2m and deoxy[Hb+Mb] in place of (a-v)O_2. VO_2m was estimated using the kinetics of phase 2 VO_2p. This relationship was first examined using computer modeling, which predicted that phase 2 VO_2p was primarily determined by, and therefore closely correlated with, VO_2m (Barstow and Molé, 1987; Barstow et al., 1990; Lai et al., 2006). These predictions were

verified using invasive determination of leg VO_2 from measurement of leg Q and (a-v)O_2 during both cycling (Grassi et al., 1996) and knee extension exercise (Krustrup et al., 2009). Finally, the similarity between phase 2 VO_2p and VO_2m was further supported from comparison of [PCr] as a surrogate of VO_2m with phase 2 VO_2p (Rossiter et al., 1999). These studies collectively validate the use of phase 2 VO_2p as an estimate of VO_2m.

The second assumption in the calculation of Q_{CAP} is that deoxy[Hb+Mb] can directly substitute for the (a-v)O_2. NIRS can be used to assess the relationship between O_2 delivery and O_2 consumption in the muscle being measured (Grassi et al., 2003; DeLorey et al., 2003; Ferriera et al., 2005b; Harper et al., 2006). A constant deoxy[Hb+Mb] during exercise implies that there is a close coupling, at the level of the muscle microvasculature, between increasing O_2 delivery and increasing O_2 consumption (DeLorey et al., 2003; Grassi et al., 2003). A change in deoxy[Hb+Mb], therefore, has been interpreted as a change in O_2 extraction, which enables the use of deoxy[Hb+Mb] as an estimate of (a-v)O_2 (DeLorey et al., 2003).

There are, however, some limitations with the use of deoxy [Hb+Mb]. First, the absorption spectra for Hb and Mb are almost identical so no distinction can be made between Hb and Mb contribution (Kreutzer and Jue, 1991). There is controversy in the literature as to how much Mb contributes to the overall [Hb+Mb] signal, with some studies suggesting a contribution of <10% (Mancini et al., 1994; Seiyama et al., 1988), while others suggest a much greater contribution (Tran et al., 1999; Richardson et al., 2001; Mole et al., 1999). Recent quantitative research has suggested that Mb can contribute >50% of the total [Hb+Mb] NIRS signal (Davis and Barstow, 2013). Despite not being able to determine the exact contribution of Hb and Mb, several studies have shown that Hb and Mb desaturate with a similar time course during exercise,

meaning that the NIRS deoxygenation signal can still provide an accurate representation of the kinetics of O_2 extraction (Gayeski and Honig, 1988; Koga et al., 2012; Richardson et al, 1995).

It has been shown that during knee extension exercise, the vastus lateralis, vastus medialis and rectus femoris muscles show a similar deoxy[Hb+Mb] response, thus implying that O_2 delivery and extraction are similar within the microvasculature of the quadriceps muscles (duManoir et al., 2010). However, Koga et al. (2011) challenged the notion that muscle deoxygenation was homogenous throughout the quadriceps muscles. Using a custom built multi-site NIRS system, they found that the magnitude and temporal profile of the deoxy[Hb+Mb] signal was variable between measurement sites, suggesting that the matching of O_2 delivery to O_2 utilization was not homogenous in the quadriceps muscles. One limitation with this study, however, is the multi-site NIRS system had a fixed arrangement of light emitters and receivers and therefore could not be adjusted for each subject to ensure that light was being transmitted through the same region of each muscle across subjects. This could explain some of the signal variability because measurements may have been made over non-muscle tissue or across the edges of two muscles. Another limitation in this study is a lack of EMG data so muscle recruitment patterns during the knee extension exercise are not known in this study. If one head of the quadriceps muscle had a higher percentage of recruited fibers, and was therefore producing more force, a greater change in the deoxy[Hb+Mb] would be expected in that location, indicating a greater O_2 extraction.

One final limitation with NIRS is the influence of ATT on signal strength. A dampened signal is produced by subjects with larger ATT causing a lower overall amplitude change (McCully and Hamaoka, 2000). Without correcting for ATT, there will be variability in the

overall amplitude of change in the NIRS signals among subjects that is due to differences in ATT.

These sources of variability in NIRS then carry over into the Q_{CAP} calculation when deoxy[Hb+Mb] is substituted for (a-v)O_2. The amplitude of deoxy[Hb+Mb] can vary widely because of the above mentioned limitations. Additionally, the NIRS signals arise from arterioles, capillaries and venules, and the precise contribution from each compartment to the total signal cannot be determined (Davis and Barstow, 2013). Due to these limitations with the deoxy[Hb+Mb] signal, the amplitude of Q_{CAP} is not quantitatively accurate. Therefore, only the temporal change (kinetics) of Q_{CAP} can be used with confidence and the amplitude is reported in arbitrary units.

A recent study by Murias and colleagues (2012) called into question the validity of the Q_{CAP} estimation technique. They found the kinetic profile of Q_{CAP} to be dependent on the deoxy[Hb+Mb] baseline-to-amplitude ratio, with those subjects whose deoxy[Hb+Mb] amplitude was greater than the baseline actually showing a decrease in estimated Q_{CAP} at exercise onset. However, this response was seen during moderate intensity exercise bouts which directly followed bouts of heavy exercise. Due to the protocol, there was residual hyperemia so that the baseline values started out elevated above those seen from the first control bout of moderate intensity exercise with no prior heavy exercise. In the current study (as well as the previous studies by Ferriera et al. (2005b) and Harper et al. (2006)), only single transitions from unloaded to loaded exercise were performed, similar to the first bout of moderate exercise performed by Murias et al. (2012). When only the first bout of moderate exercise was considered in the work of Murias et al. (2012), the response was very similar to that of Ferriera et al. (2005b), Harper et al. (2006) and the current study, and provides further support for the use of

the Q_{CAP} calculation. It is clear that the Q_{CAP} estimation technique may not be valid when baseline values are elevated above resting or unloaded values, as seen with prior heavy exercise, (Murias et al., 2012) but when performing single exercise transitions from a non-elevated baseline, it appears to produce reliable kinetic information (Ferreira et al., 2005b; Harper et al., 2006; Murias et al., 2012).

Effect of Cuffing on Temporal Profiles

In the current study, we were seeking to explain the difference between the increase in Q_{FA} and the increase in Q_{CAP} of the working muscles. One explanation of this difference, suggested by Harper et al. (2006), was that the blood might be going to nonworking muscles, specifically below the knee. In order to test this hypothesis, we occluded blood flow below the knee and compared the temporal profiles of Q_{FA} and Q_{CAP}. We found that preventing perfusion to the nonworking tissues and vasculature below the knee did not significantly change the temporal profiles of Q_{FA} or Q_{CAP}. This suggests that the difference between the MRT of Q_{FA} and MRT of Q_{CAP} is not due to blood flow to nonworking muscles and vasculature of the lower leg. One alternative potential vascular compartment through which blood flow might occur is the skin. However, this seems unlikely because with the onset of moderate exercise in normothermic conditions, cutaneous vascular conductance actually decreases (Johnson et al., 1986; Johnson, 1992). If any change in skin blood flow occurred during the current exercise protocol, it would have been later in the exercise bout (Johnson, 1992) and thus would not explain the discrepancy between Q_{FA} and Q_{CAP} during the first minute following the onset of exercise.

Another vascular compartment that could be receiving blood at the onset of exercise is the nonworking hamstring muscles. However, without electromyographic information, we do not know with certainty that the hamstrings were not recruited during our study, although previous

work by Andersen et al. (1985) showed no recruitment of the hamstrings during knee extension exercise. More recently, Heinonen et al. (2010) used positron emission tomography (PET) to show that mean hamstring Q did not change following the onset of knee extension exercise, further supporting the idea that the hamstrings are not recruited during knee extension. NIRS measurements in concert with EMG on the hamstring muscles may provide some indication as to whether or not the hamstrings could be serving as a compartment for blood perfusing the femoral artery rather than the working quadriceps muscles.

Discrepancy Between Q_{FA} and Q_{CAP}

The difference in measurement location of the Q_{FA} and Q_{CAP} signals may help explain the discrepancy between the temporal profiles. Q_{FA} is the blood flowing to the entire lower limb. Since cuffing below the knee had no effect, it is reasonable to conclude that Q_{FA} reflects blood flow primarily to the working quadriceps muscles. In the current protocol, with the leg dependent to the heart, following the onset of exercise, much of the increase in Q_{FA} is caused by the muscle pump (Tschakovsky and Sheriff, 2004). This mechanism causes a nonspecific increase in Q that is not directed solely to working motor units, so there is likely initial overperfusion of inactive motor units (Koga, in preparation). With both cycling and knee extension, an initial drop in deoxy[Hb+Mb] has been shown following the onset of exercise supporting the idea that the muscle under the NIRS probe (whether actively recruited or not) is temporarily overperfused (DeLorey et al., 2007; Ferreira et al., 2005b; Koga et al., 2007).

Q_{FA} is presumably going to all four heads of the quadriceps but it does not appear to do so evenly. PET has shown that Q to the vastus intermedius (VI) is higher than Q to the other, more superficial, muscles of the quadriceps during low-to-moderate intensity knee extension exercise (Koga, in preparation). The VI is the deepest of the quadriceps muscles and is composed

of mostly Type I muscle fibers while the superficial muscles have a higher percentage of Type II fibers (Johnson et al., 1973). In addition to across muscle variability, fiber type distribution has been shown to be heterogeneous within a single muscle with a predominance of Type II fibers appearing superficially (Johnson et al., 1973; Lexell et al., 1983).

Q_{CAP} is calculated from a global VO_2 measurement but a very specific deoxy[Hb+Mb] measurement. VO_2m, as estimated by phase II VO_2p, is an average value coming from all the recruited motor units in the quadriceps. Deoxy[Hb+Mb], however, is coming from a very small, superficial sample of tissue. The NIRS probe we utilized is only capable of measuring up to a maximum depth of ~17mm (ie. 35mm/2; Chance et al., 1988). In the current study, the average ATT was ~9 mm, meaning that the NIRS signal was coming on average from 8 mm of rectus femoris (RF) tissue closest to the skin. Since the RF has a higher percentage of Type II fibers overall (Johnson et al., 1973) and Type II fibers tend to be more prevalent on the surface of the muscle (Johnson et al., 1973; Lexell et al., 1983), it is likely that most of the deoxy[Hb+Mb] reflects the oxygenation status of primarily Type II fibers. The implication of this is that the NIRS signals measured on the surface may not be representative of the relative oxygenation of fibers deeper in the RF or VI, where there is a higher percentage of Type I fibers.

Differences can exist between the response of Type I and Type II fibers following the onset of exercise. Numerous studies have reported higher Q in Type I fibers (Ferreira et al., 2006; Kindig and Poole, 1998; Laughlin and Armstrong, 1982). In addition to this difference in flow, there is also a kinetic difference between fiber types with Type I fibers showing a more rapid rate of increase in Q than Type II fibers during exercise (Behnke et al., 2003). Using phosphorescence quenching, Behnke et al. (2003), showed a slower decrease in microvascular

PO_2 in Type I fibers, suggesting a better matching of O_2 delivery to O_2 demand in Type I fibers as compared to Type II fibers.

Thus, Q_{CAP} is likely reflecting primarily superficial Type II fibers which receive less blood flow, have slower Q kinetics and thus do not match O_2 delivery to O_2 demand as well as Type I fibers. Simultaneously, Q_{FA} is primarily directed to deeper Type I fibers which receive more blood (Koga, in preparation), have faster Q kinetics and better matching of O_2 delivery to O_2 demand. These physiological differences between the muscle region where Q_{CAP} is measured and the region within the exercising muscles and limb where Q_{FA} is predominantly directed, could explain the temporal difference between Q_{CAP} and Q_{FA} during moderate knee extension exercise.

Implications of Similar MRT Q_{CAP} and τVO_2m

MRT Q_{CAP} and τ VO_2m were found to be similar in the present study, which is consistent with previous research (Ferreira et al., 2005b; Grassi et al., 1996; Koga et al., 2005). This suggests that the rate of increase in microvascular Q is closely matched to the rate of increase in metabolic demand in the working muscle. If MRT Q_{CAP} was significantly faster than τ VO_2m, it would suggest that muscle metabolism was not being limited by O_2 delivery. Alternatively, if MRT Q_{CAP} was significantly slower than τ VO_2m, it would suggest that O_2 delivery could possibly be limiting muscle metabolism. A matching of MRT Q_{CAP} and τ VO_2m indicates a matching of O_2 delivery and O_2 utilization. The regulatory processes that make this matching possible have not been specifically determined, but are likely influenced by several factors. The red blood cell (RBC) has been suggested as a mechanism for O_2 sensing as it releases ATP when exposed to a low O_2 environment such as working muscle (Dietrich et al., 2000). This observation was originally made in rats (Dietrich et al., 2000) but recently, ATP released by red

blood cells (RBCs) when O_2 is offloaded has been shown in humans to also be a local signaling substance that could act to increase or redirect blood flow at the microvascular level to the capillary networks of the contracting fibers (González Alonso, 2012).

MAP and Compliance Components of Q_{FA}

Changes in Q are determined by changes in pressure and/or conductance. We measured the time course of change of MAP, C and Q_{FA} in an attempt to ascertain whether MAP or C had a greater influence on the change of Q_{FA}. Unfortunately, physiological variability in the measurements precluded the use of an exponential fit to determine kinetics of the MAP and C signals and thus we used percent change from baseline occurring at 60s into exercise as an approximation of kinetics. We found the change in C occurred much faster and to a greater extent than that of MAP and was closely related to the change in Q_{FA}. These findings are similar to previous work showing that, following the onset of cycling exercise, the change in MAP occurs more slowly than the change in total peripheral resistance (TPR), the inverse of conductance (Faisal et al., 2010). Although Faisal et al. (2010) measured whole body resistance, not resistance in the exercising limb, their findings, combined with the findings of the current study, suggest that the rise in MAP is too slow to be causing the initial, rapid rise in Q_{FA}.

Using MAP as the indicator of changes in driving pressure could overlook one mechanism that may contribute to the initial rise in Q_{FA}, the muscle pump. The muscle pump may be causing a pressure change across the microvasculature that is not reflected in the overall measurement of MAP. Many studies, using various human and animal models, have concluded that muscle contraction can decrease venous hydrostatic pressure sufficiently to improve muscle Q and perfusion (Pollack and Wood, 1949; Folkow et al., 1970; Sheriff and Van Bibber, 1998; Tschakovsky et al., 1996; Leyk et al., 1994; Radegran and Saltin, 1998; Shiotani et al., 2002).

The initial rise in Q is proportional to contraction frequency in dogs at the onset of treadmill exercise when sympathetic vasoconstriction is prevented by autonomic nerve activation blockade (Sheriff and Hakeman, 2001). In rats, changes in muscle Q correspond directly to changes in treadmill speed and therefore contraction frequency (Sheriff and Hakerman, 2001). These studies show the potential importance of a muscle pump by suggesting a direct mechanical link between Q and contraction frequency. It is important to note that the muscle pump appears to only be effective in the presence of a hydrostatic column and at moderate work rates, since muscle contraction has been shown to impede blood flow at higher work rates (Lutjemeier et al., 2005). Thus, while overall MAP does not increase rapidly enough to explain the initial increase in Q_{FA}, there is evidence to suggest that the muscle pump could be playing a role in the initial increase in Q_{FA}.

The other potential mechanism responsible for the initial increase in Q_{FA} is rapid vasodilation, which increases conductance. When intact muscle preparations are used, Q increases in proportion to muscle contraction intensity when using a single contraction model to minimize the effect of the muscle pump (Anrep and von Saalfeld, 1935; Corcondilas et al., 1964; Tschakovsky et al., 2004). Also, when the venous hydrostatic column was eliminated by performing contractions above heart level, the immediate increase in Q was in proportion to the contraction intensity (Tschakovsky et al., 2004). These studies support the current findings by showing that vasodilation, or an increase in conductance, contributes to the initial, rapid increase in Q seen in this study.

Future Research

While the present study has ruled out the lower leg as a compartment for blood flowing through the femoral artery but not to the working capillary during exercise, the skin also needs to

be examined as a possible compartment. There are many issues with NIRS that await further inspection such as quantifying heterogeneity within the quadriceps muscles, evaluating if the intersite variability can be predicted based on muscle recruitment, determining the relative contribution of arterioles, capillaries and venules to the NIRS signal and developing techniques to quantify the relative contribution of Mb to the NIRS signal. It is also unknown how the temporal profiles of Q_{CAP}, Q_{FA} and VO_2m might change in older subjects or patient populations. Finally, it would be beneficial to compare the metabolic and cardiovascular responses between the double leg "dolphin" style kick used in the present study and the more traditional bi-pedal kick.

Conclusions

We have shown that occluding the circulation below the knee did not alter the kinetics of the responses of Q_{FA} and Q_{CAP}, and thus did not reduce or eliminate the difference between the kinetic profiles of Q_{FA} and $Q_{CAP.}$ This finding rules out the nonworking muscle below the knee as a possible compartment for the blood flowing through the femoral artery but not to the working capillary. A likely explanation for this discrepancy between the kinetic profiles of Q_{FA} and Q_{CAP} is that the tissue being measured to estimate Q_{CAP} is not representative of the deeper tissue that is primarily receiving Q_{FA}. Q_{CAP} is likely reflecting primarily superficial Type II fibers while Q_{FA} is mainly directed to deeper Type I fibers. In both the uncuffed and cuffed conditions, we saw a significant correlation between MRT Q_{CAP} and τVO_2m, suggestive of a matching between O_2 demand and O_2 delivery following the onset of exercise. Finally, the time course of change of Q_{FA} was significantly faster than that of MAP but similar to that of C, suggesting that the rapid increase in C is primarily driving the rapid increase in Q_{FA}.

Chapter 6 - References

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