MUSCLE OXYGENATION DOES NOT AFFECT THE PRIOR EXERCISE EFFECT

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Andrés Hernández

Certificate of Approval:

_____	_____
David D. Pascoe Professor Kinesiology	L. Bruce Gladden, Chair Professor Kinesiology
_____	_____
Holly R. Ellis Associate Professor Chemistry and Biochemistry	Raymond P. Henry Professor Biological Sciences

_____

George T. Flowers
Dean
Graduate School

MUSCLE OXYGENATION DOES NOT AFFECT THE PRIOR EXERCISE EFFECT

Andrés Hernández

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MUSCLE OXYGENATION DOES NOT AFFECT THE PRIOR EXERCISE EFFECT

Andrés Hernández

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Signature of Author

_____
Date of Graduation

VITA

Andrés Hernández, proud son of Robert Paul Hernández and Mary Louise Hernández, was born on October 24, 1980 in Fresno, CA. He grew up in the nearby city of Clovis, CA. The interest in life science that Andrés has began at an early age when his father would teach him various scientific terminology as it pertained to living in the country. Andrés' interest in life science was further stimulated in high school and in college. He graduated from Floyd B. Buchanan High School where he took courses in biology and zoology. After high school, Andrés attended California State University, Fresno. He had always exercised since an early age, and in college, he began to ponder how muscles work. Accordingly, Andrés pursued the field of Exercise Physiology in which he earned a B.S. in 2003 and a M.A. 2005. Though beneficial, his time at California State University, Fresno left him frustrated as he had more questions than answers. This lead Andrés to pursue a Ph.D. in Exercise Physiology focused specifically on skeletal muscle physiology in Dr. L. Bruce Gladden's Laboratory at Auburn University. Though he once again had more questions than answers, his time under Dr. Gladden provided him with the means to pursue and elucidate physiological answers. Andrés' pursuit to learn about skeletal muscle physiology will never end. Accordingly, he will be joining Dr. Håkan Westerblad's Laboratory at the Karolinska Institutet in Stockholm, Sweden as a postdoctoral researcher.

DISSERTATION ABSTRACT

MUSCLE OXYGENATION DOES NOT AFFECT THE PRIOR EXERCISE EFFECT

Andrés Hernández

Doctor of Philosophy, August 10, 2009
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The controlling factors responsible for the 'turn-on' of oxidative phosphorylation at the onset of exercise (VO_2 on-kinetics) are controversial. Current hypotheses center on delayed O_2 delivery, the build-up of respiratory stimuli, and a combination of the two. Recently, speeded VO_2 on-kinetics after priming exercise have further fueled the debate over controlling factors. However, investigations into the mechanistic controllers of the prior exercise effect in exercising humans are limited by experimental techniques. It was the purpose of this study to examine the prior exercise effect in the highly-oxidative canine gastrocnemius muscle complex (gastrocnemius plus superficial digital flexor; GS) contracting *in situ*. With arterial [O_2] maintained constant, a step change in metabolic rate was elicited by stimulating canine GS muscles (n=5) via their sciatic nerves (6-8 V, 0.2 ms duration, 50 Hz, 200 ms train) at a rate of 2 contractions / 3 s for two, 2-min bouts

separated by 2 min of recovery. VO_2 on-kinetics were determined during both of these bouts for four experimental conditions: spontaneous adjustment of self perfused blood flow (spontaneous); maximized O_2 availability (elevated flow) in which blood flow was maintained at the end-contractile level throughout recovery and the second bout of contractions; maximized metabolic respiratory stimuli (resting flow) in which blood flow was rapidly returned to the pre-contractile level during recovery and the on-kinetics were the same between contractile bouts; and maximization of both metabolic respiratory stimuli and O_2 availability (additive) which was identical to the resting flow condition with the exception that blood flow was increased rapidly at the onset of the second bout. Near infrared spectroscopy (NIRS) was used to monitor muscle oxygenation ([O_2Hb] and [HHb]). Despite significant alterations in [O_2Hb] prior to the second contractile bout, tau remained unaltered (means: 11.8 vs. 10.6 s) for each condition. Time delay (mean: 6.2 s) and correspondingly mean response time (mean: 18.0 s) were significantly ($p<0.05$) speeded during bout 2 (mean: 1.9 and 12.5 s, respectively) and the amplitude of the VO_2 slow component was significantly reduced in all conditions after priming contractions (means: 11.0 vs. 28.2 $ml O_2 \bullet kg^{-1} \bullet min^{-1}$). These data indicate that altered O_2 delivery and muscle oxygenation as assessed by NIRS do not play a role in the prior exercise effect in highly-oxidative skeletal muscle. Thus, the prior exercise effect likely has its origin in elevations in metabolic respiratory stimuli prior to the second contractile bout (evidenced by an elevated bout 2 baseline VO_2 for all conditions). These data also provide evidence that reductions in the slow component amplitude after priming contractions do not require altered motor unit recruitment as has been suggested for human exercise.

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I. INTRODUCTION

At the onset of a square wave exercise transition, oxygen uptake (VO_2) increases more slowly than the energy requirement (66), rising exponentially and reaching a steady state in ≈2-3 min if the metabolic requirement is below the lactate (La^-) threshold (143). At the steady state, ATP demand is matched by oxidative phosphorylation. The delay in achievement of the steady-state VO_2 at the onset of exercise has been termed "VO_2 on-kinetics". Slowed VO_2 on-kinetics have been measured in individuals with type II diabetes (118), peripheral vascular disease (9, 12), aging (3), heart failure (2, 129), heart transplant (29, 51), heart and lung transplant (44), chronic respiratory diseases (64, 109), HIV infection (26), mitochondrial myopathies and McArdle's disease (52). Understanding the mechanisms involved in VO_2 on-kinetics might lead to potential therapeutic and pharmacological treatments that could increase the duration and quality of life in these individuals.

The mechanisms that control oxidative phosphorylation at the onset of exercise are yet to be fully elucidated. Two major hypotheses have been postulated: 1) there is a delay in the adjustment of blood flow and thereby O_2 delivery to active muscle that delays the turn-on of oxidative phosphorylation, and/or 2) VO_2 on-kinetics require the accumulation of metabolic signals that stimulate oxidative phosphorylation to drive the VO_2 response.

Decrements in the fraction of inspired PO_2 (76, 97, 98, 108), cardiac output (72, 75, 80), and perfusion pressure to the exercising muscles (73, 74, 79, 100) slow VO_2 on-kinetics. However, during conditions in which the blood flow response is unaltered, the blood flow response to exercising muscles is more than adequate (146); and at least as fast (54) or faster (33) than the VO_2 response. The most direct evidence against the O_2 delivery hypothesis comes from investigations that measured VO_2 directly across canine contracting muscle with elevated O_2 delivery (45, 46). VO_2 on-kinetics at the onset of a submaximal metabolic rate were not speeded in these experiments (45, 46). These data support the metabolic activation hypothesis for oxidative metabolism at the onset of exercise.

The metabolic activation hypothesis posits that a delay in the increase of metabolic signals that control respiration (e.g., [ADP], [P_i], [NADH]) is responsible for the lag in VO_2 at the onset of exercise. Metabolic pathways that buffer an increase in these metabolites may slow VO_2 on-kinetics. For example, inhibition of creatine kinase (CK) in skeletal muscle speeds VO_2 on-kinetics (50). The specific sites of metabolic activation are difficult to investigate. This is further convoluted by interaction of metabolic respiratory stimuli with O_2 delivery (136).

An interesting model that has been used to study the controlling mechanisms of VO_2 on-kinetics is prior exercise. In experiments of this type, two or more bouts of exercise are performed and VO_2 on-kinetics are compared. The first bout is considered a "priming" bout. These investigations have established that the priming exercise bout must be performed above the lactate threshold (supra-LT) (e.g., (124)) to alter the second

bout. Controversy exists as to which VO_2 on-kinetics parameters are affected by prior exercise (e.g., (124) and (135)). Similarly, the mechanism(s) by which priming exercise speeds VO_2 on-kinetics of a second bout are not yet well established, but current evidence indicates increased metabolic activation (e.g., (68)), enhanced O_2 availability (e.g., (34)), and/or altered motor unit recruitment (e.g., (21)) as possible causes. Understanding the mechanisms responsible for speeded kinetics after prior exercise can lead to elucidation of the mechanisms that control oxidative phosphorylation.

To date, the effect of priming exercise has not been investigated using a model that allows direct measurement of VO_2 across an exercising muscle and modulation of muscle blood flow. The isolated canine gastrocnemius muscle complex (gastrocnemius plus superficial digital flexor; GS) is ideal for this study as both can be done. These characteristics are advantageous for elucidating the roles of metabolic activation and O_2 delivery in the prior exercise effect. Additionally, as all motor units are stimulated synchronously, the role of altered motor unit recruitment as a main player in the prior exercise effect can be examined.

II. REVIEW OF LITERATURE

The ability of skeletal muscle to cope with a rapid and dramatic change in metabolic rate is key for the survival of animals and early humans, athletic performance, and exercise capacity. At the onset of a square wave exercise transition, VO_2 increases more slowly than the power output (66), rising exponentially and reaching a steady state in ≈2-3 min if the workrate is below the lactate threshold (143). At the steady state, ATP demand is matched by oxidative phosphorylation. The time delay in VO_2 at the onset of exercise has been termed "VO_2 on-kinetics".

During the delay in VO_2 adjustment, the difference between the ATP demand set forth by the workrate and the ATP supply provided by oxidative phosphorylation (O_2 deficit) is made up by anaerobic energy sources. These sources are stored ATP, phosphocreatine (PCr), the reaction catalyzed by adenylate kinase (ADP + ADP <---> ATP + AMP), and glycolysis/glycogenolysis to lactate. For simplicity, glycolysis and glycogenolysis will be referred to collectively as glycolysis. These processes result in substrate level phosphorylation and cellular disturbances that can cause fatigue. Thus, a faster adjustment of VO_2 to the ATP demand required by the workrate may prolong fatigue, increasing exercise capacity (43). This is of particular interest in clinical application as slowed VO_2 on-kinetics are seen in type II diabetes (118), peripheral vascular disease (9, 12), aging (3), heart failure (2, 129), heart transplant recipients (29, 51), heart and lung recipients (44), chronic respiratory diseases (64, 109), HIV infection

(26), and mitochondrial myopathies and McArdle's disease (52). Understanding the mechanisms involved in VO_2 on-kinetics can lead to potential therapeutic and pharmacological treatments that can increase the duration and quality of life in these individuals.

Oxidative phosphorylation can be summarized by the following reaction (136):

$$\mathbf{NADH + H^+ + 3ADP + 3Pi + 1/2O_2 ---> 3ATP + NAD^+ + H_2O}$$

From this equation, it is apparent that NADH, O_2, H^+, ADP, and Pi must be available for oxidative phosphorylation to occur. NADH provides reducing equivalents to the mitochondria needed to produce an electrochemical gradient that subsequently provides the energy needed to phosphorylate ADP to ATP. NADH is produced in glycolysis (reaction catalyzed by glyceraldehydes 3-phosphate dehydrogenase), in the complex of reactions linking glycolysis to aerobic metabolism (catalyzed by the pyruvate dehydrogenase complex (PDC)), in the tricarboxylic acid cycle (TCA) (reactions catalyzed by isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase, and malate dehydrogenase), and during the oxidation of free fatty acids into acetyl-CoA (beta-oxidation). It should be noted that $FADH_2$ also provides reducing equivalents to the mitochondria, and is produced by the reaction catalyzed by succinate dehydrogenase in the TCA cycle, in shuttling of reducing equivalents from NADH in the cytosol to the mitochondria in skeletal muscle using the glycerol phosphate shuttle, and during beta-oxidation.

ADP and Pi are produced from the hydrolysis of ATP (ATP <---> ADP + Pi). ATP hydrolysis is catalyzed by ATPases, which provide the energy for three important

components needed for skeletal muscle contraction: 1) the power stroke after myosin attaches to actin (myosin ATPase); 2) pumping of calcium ions from the cytosol into the sarcoplasmic reticulum (Ca^{2+} ATPase); and 3) providing for the increased energy demand of the Na^{+}-K^{+} ATPase pump (Na^{+}-K^{+} ATPase) to maintain excitability. The primary source for the increase in [Pi] seen during exercise in skeletal muscle is the phosphagen system. This system couples the energy from splitting phosphocreatine (PCr <---> Pi + Cr) via creatine kinase (CK) that results in rephosphorylation of ADP to ATP.

As indicated in the reaction above, O_2 must be present for oxidative phosphorylation to occur. Oxygen is the final electron acceptor at the end of the electron transport chain (ETC); a reaction catalyzed by cytochrome c oxidase. An increased metabolic demand requires an increase in O_2 delivery to match the increased rate of oxygen consumption by mitochondria. This is met via increases in blood flow to the active muscles and subsequent diffusion of oxygen from red blood cells (RBCs), across the capillary wall, and into the myocyte (125). Oxygen release from hemoglobin (Hb) in RBCs is aided during exercise by increases in temperature, PCO_2, and $[H^{+}]$.

PULMONARY VS. MUSCLE VO_2 KINETICS

At the onset of exercise, VO_2 at the muscle increases in a monoexponential fashion, displaying first order kinetics (141). Although biphasic responses and time delays (TD) have been measured, these may be due to methodological limitations such as transit delays (43). VO_2 on-kinetics measured at the mouth (pulmonary VO_2 on-kinetics) during exercise below the lactate threshold are composed of two phases: 1) delay or

cardiodynamic phase; and 2) primary phase (141). Exercise above the lactate threshold elicits a third phase referred to as a slow component (141). In this case, VO_2 continues to drift upwards after the monoexponential response. During the cardiodynamic phase, VO_2 increases rapidly (144). This rapid increase in VO_2 occurs as a result of an increased pulmonary blood flow and does not represent the VO_2 being taken up by the exercising muscles. The length of the cardiodynamic phase is primarily determined by the transit delay for the venous blood from the exercising muscles to reach the lungs (144). The primary phase matches closely to the VO_2 at the muscle, and is the phase used to investigate pulmonary VO_2 on-kinetics. The time from the onset of phase 2 until ≈63% of the achievement of the steady-state VO_2 is used to compare VO_2 responses at the onset of exercise. This is commonly referred to as the primary time constant (τ). Figure 1 depicts the VO_2 variables discussed in this paragraph. Although pulmonary VO_2 on-kinetics are within ≈90% of the response seen at the muscle (141), they are complicated by several factors (see next paragraph). Oxygen uptake across the exercising muscles has been investigated in humans, but these techniques are invasive, and are complicated by similar factors as pulmonary VO_2 measures (see next paragraph).

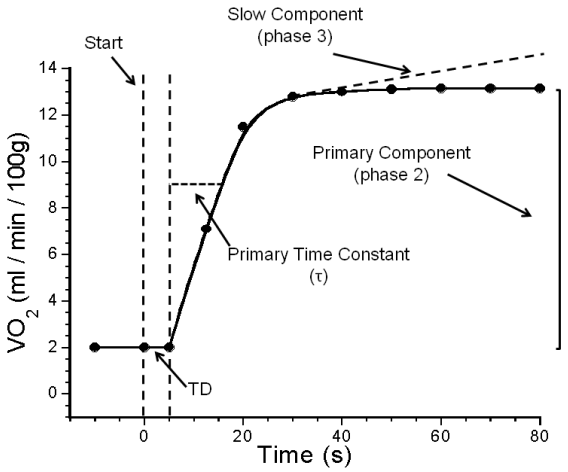

Figure 1. Idealized from Grassi et al. (49). Onset of contractions (Start); Time delay before a rise in VO_2 (TD).

Multiple skeletal muscles are often used to move a limb in the fashion required for exercise. Pulmonary VO_2 measurements are indicative of all of the muscles interacting, as well as those not used for the particular activity. Accordingly, relative blood flow distribution to working and non-working skeletal muscles can impact pulmonary VO_2 and VO_2 measured across the muscles (126). Although the use of blood sampling techniques to measure VO_2 on-kinetics may be more precise, they are complicated by the motor unit recruitment pattern during exercise (137). With these measures, the relative contribution of each muscle or muscle fiber to the VO_2 response still remains unclear. The fact that these studies cannot differentiate the VO_2 for each muscle or muscle fiber is further complicated by skeletal muscle fibers with different energetic (8, 18, 31, 67, 95) and metabolic (114) properties.

Measurements of VO_2 on-kinetics using an isolated muscle model are without many of the above limitations. Blood flow can be isolated to and from the muscle, allowing for determination of VO_2 at the muscle without 'dilution' from other sources.

Questions about which muscles and/or motor units are active are alleviated by stimulation of only the muscle being examined and the ability to evoke tetanic contractions. Also, the use of a muscle with a homogeneous muscle fiber metabolic profile prevents the influence of differences in muscle fiber energetic and metabolic properties within the same muscle. Although not without limitations, the use of isolated muscle models for the study of VO_2 on-kinetics provides an excellent model to elucidate the mechanisms that dictate the VO_2 on-response.

HYPOTHESES FOR THE LAG IN VO_2 AT EXERCISE ONSET

A lack of availability of any of the necessary substrates for oxidative metabolism can slow the increase in VO_2 at exercise onset. Exercise requires an increased need for oxidative phosphorylation and causes a decrease in intracellular PO_2 (107, 119). Accordingly, a lag in the rate of increase in any substrates required for oxidative phosphorylation can potentially slow the turn-on of oxidative metabolism. This means that the regulation of the kinetics of any of these substrates may have an effect on VO_2 on-kinetics. However, the exact role of these regulatory factors remains to be fully elucidated. Two major hypotheses have been put forward to explain the lag in VO_2 at exercise onset: 1) there is a delay in the adjustment of blood flow and thereby O_2 delivery to active muscle that delays the turn-on of oxidative phosphorylation, and 2) VO_2 on-kinetics require the accumulation of metabolic signals that stimulate oxidative phosphorylation to drive the VO_2 response.

Oxygen delivery hypothesis. Observations that endurance training increases capillarity of the trained skeletal muscles (25, 58, 59, 65, 116), speeds femoral artery blood velocity kinetics at the onset of cycling exercise (128), and speeds VO_2 on-kinetics (28, 30, 90) have provided circumstantial evidence that a delay in blood flow adjustment is responsible for the lag in oxygen uptake at the onset of exercise. Additionally, prior, supra lactate threshold exercise has been found to speed VO_2 on-kinetics (41), possibly by speeding the blood flow response at exercise onset. Data that show a limiting role of O_2 delivery on VO_2 on-kinetics have come from investigations that have modified the fraction of inspired PO_2 (76, 97, 98, 108), used transitions from one exercise intensity to another (35, 77, 78), decreased cardiac output via beta blockade (72, 75, 80), or altered perfusion pressure to the exercising muscles (73, 74, 79, 100).

Linnarsson et al. (97) measured oxygen uptake and calculated the O_2 deficit in subjects cycling submaximally and maximally during hypoxia, normoxia, and hyperoxia in the upright position. The O_2 deficit was significantly greater during the submaximal-hypoxia condition than control. Although VO_2 on-kinetics were not measured, the greater O_2 deficit may have been indicative of slower VO_2 on-kinetics during hypoxia. Following a similar trend, the O_2 deficit was significantly smaller during hyperoxia than during the normoxic condition during submaximal exercise. There were no significant differences between the three conditions during maximal exercise (fatigue in 3-4 min). Murphy et al. (108) investigated the effect of hypoxia vs. normoxia on VO_2 on-kinetics and the cardiac output response during step-transition exercise (25 to 105 W) using an upright cycle ergometer. Hypoxia resulted in a significantly longer lag time of VO_2 than

normoxia. Interestingly, the cardiac output response was not significantly different between conditions. These results indicate that O_2 delivery was reduced via reduced arterial O_2 saturation in hypoxia without an accompanying increase in cardiac output at the onset of exercise. Similar results were found by Hughson and Kowalchuk (76) during submaximal step changes in workrate during hypoxia. VO_2 on-kinetics were significantly slowed in hypoxia vs. normoxia and hyperoxia, indicating a role for O_2 delivery in VO_2 on-kinetics during upright cycling exercise.

To gain insight into the role of O_2 delivery on VO_2 on-kinetics during submaximal exercise below and above the ventilatory threshold, Macdonald et al. (98) had subjects exercise on an upright cycle ergometer during hyperoxia and normoxia. At exercise intensities below the ventilatory threshold, hyperoxia did not have an effect on VO_2 on-kinetics. However, hyperoxia significantly speeded VO_2 on-kinetics during exercise above the ventilatory threshold. These results differ from the data of Linnarsson et al. (97) that indicated a smaller O_2 deficit with hyperoxia vs. normoxia during submaximal, upright cycling exercise. It is important to point out that VO_2 on-kinetics were not measured in the study by Linnarsson et al. (97). Although ventilatory threshold was not measured in their study, it is very likely that the exercise intensity used (≈50% VO_2peak) was below the ventilatory threshold of their subjects. Differences in the exact exercise intensities and inspired PO_2, among other factors, may have contributed to the discrepancies.

Taken together, the data from Murphy et al. (108), Hughson and Kowalchuk (76), and Macdonald et al. (98) indicate that O_2 delivery as affected by the inspired PO_2 can

influence VO_2 on-kinetics. Indirect evidence was provided by Linnarsson et al. (97). These studies are consistent in the finding of a slowed response during hypoxia vs. normoxia during upright cycling exercise, but differ in the role of inspired PO_2 above normoxic values at the onset of exercise below the ventilatory threshold. It thus seems reasonable to conclude that, during upright cycling exercise in healthy individuals; O_2 delivery as affected by inspired PO_2 only affects VO_2 on-kinetics during sub-ventilatory threshold exercise at values below those indicative of normoxia.

Exercise transitions from one intensity to another have also been used to provide evidence in support of the O_2 delivery hypothesis. Hughson and Morrissey (77) investigated VO_2 on-kinetics during four exercise transitions: 1) rest to 80% ventilatory threshold; 2) rest to 40% ventilatory threshold; 3) 40-80% ventilatory threshold; 4) and 40-120% ventilatory threshold. VO_2 on-kinetics were slower in the transition from prior exercise than from rest. Similarly, di Prampero et al. (35) found a greater O_2 deficit in the transition from lower to heavier loads than that incurred for the same intensity change when the transition was from rest. In a follow-up to their 1982 study (77) using a similar protocol, Hughson and Morrissey (78) found that VO_2 on-kinetics were significantly slower during work-work transitions than rest-work transitions. Accordingly, heart rate kinetics were also significantly slower during the work-work transitions than the rest-work transitions. Hughson and Morrissey (78) and Hughson et al. (81) argue that this result indicates a slower adaptation of blood flow to the new workrate in a work-work transition, thus slowing oxygen delivery and VO_2 on-kinetics.

Beta-blockade has been used to reduce O_2 delivery to the exercising muscles at the onset of exercise. This causes a decrease in heart rate and stroke volume, thus decreasing cardiac output. Hughson and Smyth (80) induced beta-blockade in subjects via an oral dose of metroprolol or propranolol in subjects prior to commencing exercise at ≈80% of the ventilatory threshold. VO_2 on-kinetics were significantly slower after beta-blockade in comparison to control. Cardiac output was significantly lower after beta-blockade than without beta-blockade. These data indicate that VO_2 on-kinetics were slowed due to a decrease in O_2 delivery evoked by decreasing cardiac output. In a similar study, Hughson (72) induced beta-blockade in subjects with 100 mg metoprolol prior to a square wave increase in exercise intensity from loadless pedaling to 100 W. VO_2 on-kinetics were significantly slowed in the beta-blockade condition in comparison to control. Cardiac output was lower in the beta-blockade condition in comparison to control as evidenced by lower heart rates in the decreased cardiac output condition. Validating the assumption of decreased O_2 delivery after beta-blockade by Hughson and Smyth (80) and Hughson (72); Hughson and Kowalchuk (75) found that the cardiac output response at the onset of submaximal exercise was indeed slowed in subjects with beta-blockade. These data indicate that a decreased oxygen delivery to working muscles via decreased cardiac output kinetics induced by beta-blockade slow VO_2 on-kinetics, indicating an important role for oxygen delivery on the delay of oxygen uptake at the onset of exercise.

To examine the role of perfusion pressure to the exercising muscles, and thus delivery of blood to working muscles on VO_2 on-kinetics, Hughson et al. (73) had

subjects cycle in the upright and supine positions. In addition, subjects cycled in the supine position with lower body negative pressure. VO_2 on-kinetics were significantly slowed in the supine vs. upright position. This result indicated the effect of reduced perfusion pressure, and thus oxygen delivery to the working muscles. Further, exercise in the supine position with lower body negative pressure resulted in significantly faster VO_2 on-kinetics vs. the supine position without lower body negative pressure. The VO_2 on-kinetics were not significantly different from the upright cycling position, indicating a similar blood flow and oxygen delivery response between the two conditions. To gain further information related to this phenomenon, MacDonald et al. (100) investigated the blood flow response to knee extension exercise in the upright and supine positions. Leg blood flow was not different at rest between the two conditions. Exercise in the supine position resulted in significantly slower VO_2 on-kinetics than exercise in the upright position, confirming results of cycling exercise in these two positions (73). This result was accompanied by slower femoral artery blood flow kinetics in the supine vs. upright position. These investigations (73, 100) indicate that a reduction in perfusion pressure and blood flow, and thus O_2 delivery, may be responsible for the delayed VO_2 on-kinetics seen in supine vs. upright cycling exercise. It is likely that the use of lower body negative pressure in the study by Hughson et al. (73) increased the rate of blood flow adjustment to a rate similar to that observed during upright exercise.

Similar results have been found by Hughson and Imman (74) and Hughson et al. (79) using arm exercise. Hughson and Imman (74) occluded blood flow to the leg muscles prior to the onset of supine arm-crank exercise. VO_2 on-kinetics were

significantly faster with blood flow to the legs occluded in comparison to control, indicating that this condition provided for increased O_2 delivery to the exercising muscles in the arms, perhaps to values similar to upright arm-crank ergometer exercise. Hughson et al. (79) had subjects perform forearm exercise with the arm either above or below heart level to decrease or increase perfusion pressure, respectively. Oxygen uptake was determined across the forearm. The response of blood flow to and O_2 uptake by the forearm muscles was significantly slower with the exercising arm above vs. below heart level. The results of these studies using arm exercise (74, 79) are in agreement with those using cycle ergometer exercise (73, 100), and indicate that a decrease in perfusion pressure, resulting in a slowed O_2 delivery to the working muscles, slows VO_2 on-kinetics.

Indeed, the studies outlined above indicate an important role for oxygen delivery on VO_2 on-kinetics. Hypoxia leads to an increased oxygen deficit (97) and slowed VO_2 on-kinetics (76, 108). Hyperoxia has been shown to decrease the oxygen deficit during submaximal exercise (97) and speed VO_2 on-kinetics at exercise intensities above the ventilatory threshold (98). Delayed VO_2 on-kinetics when proceeding from a prior exercise intensity to a higher exercise intensity (35, 77) are accompanied by a delayed heart rate response (78). Additionally, a reduction of oxygen delivery via reducing cardiac output at the onset of exercise (72, 75, 80) and perfusion pressure (73, 74, 79, 100) have slowed VO_2 on-kinetics. A critical evaluation of the literature manifests an important trend in these investigations. Oxygen delivery only impacted VO_2 on-kinetics when O_2 delivery was decreased. These data show that when oxygen delivery is

decreased, VO_2 on-kinetics are slowed. However, the opposite is not necessarily true in that the delay in turn-on of oxidative metabolism at the onset of exercise is due to an oxygen limitation when oxygen delivery is not decreased. Likewise, an increase in the capacity to deliver blood to skeletal muscles in trained individuals (25, 58, 59, 65, 116, 128), as well as speeded VO_2 on-kinetics (28, 30, 90), does not show a cause and effect relationship between O_2 delivery and the rate of VO_2 adjustment.

Evidence from De Cort et al. (33) shows that the blood flow response to the exercising muscles during upright cycling (unloaded cycling to 70-80 W) was faster than the VO_2 response. Williamson et al. (146) found that reduced skeletal muscle blood flow via lower body positive pressure did not alter VO_2 on-kinetics during upright cycling exercise at intensities below or slightly above the lactate threshold. Thus, upright cycling exercise elicits a blood flow response in healthy subjects that is more than sufficient to not limit the rate of VO_2 on-kinetics. Additional evidence comes from Grassi et al. (54) who found that bulk blood flow delivery to the exercising muscles during upright cycle ergometer exercise followed a similar time course to that of VO_2. Elaborate data obtained by Grassi et al. (53) provide even further insight. Grassi et al. (53) determined the oxygenation of the vastus lateralis muscle during the onset of upright cycling below and above the ventilatory threshold. The balance between O_2 delivery and O_2 uptake was assessed via the change in signal of deoxygenated Hb + myoglobin (Mb) using near-infrared spectroscopy (NIRS). At the onset of exercise, there was a delay in the increase of the deoxygenated Hb + Mb signal that was significantly different from 0 for both exercise intensities. If O_2 delivery had been inadequate, the deoxygenated signal would

have increased immediately upon the start of exercise. These results indicate that ample O_2 delivery was available under both conditions, providing powerful evidence against the O_2 delivery theory.

Studies on the role of O_2 delivery to exercising muscles using humans have provided insight into whether or not VO_2 on-kinetics are limited by O_2 delivery. However, technical limitations prevent determination of VO_2 at the specific muscle(s) exercising as well as the blood flow response to the muscle(s). With animal models, it is possible to isolate the blood flow to and from the muscle which allows for determination of VO_2 and blood flow kinetics at the muscle under investigation. Using the isolated canine gastrocnemius muscle complex (gatrocnemius plus superficial digital flexor; GS), Grassi et al. (45) found no difference in VO_2 on-kinetics when blood flow was increased to the steady-state level prior to initiating contractions. Similar results were found when blood flow was increased in addition to peripheral O_2 diffusion via administration of a drug that causes a rightward shift in the oxyhemoglobin dissociation curve (46). Studies examining microvascular oxygen pressure kinetics have indicated a delay in the decrease in microvascular PO_2 at the onset of contractions in certain rat muscles (14, 15, 17, 40, 104), indicating ample blood flow and thus oxygen delivery at the onset of contractions. Using single skeletal muscle fibers, Hogan (68) found that there was a delay in the fall of intracellular PO_2 at the onset of contractions, indicating delayed metabolic activation. Interestingly, a recent study found an immediate decrease in microvascular PO_2 in muscles with a low oxidative capacity (104), indicating that oxygen delivery to lowly-oxidative muscles may be a factor in VO_2 on-kinetics. VO_2 on-kinetics have not been

measured in lowly-oxidative muscle. The data from human and animal studies, and most data from microvascular PO_2 kinetics, indicate that sufficient oxygen delivery is present at the onset of contractions under "normal" conditions. This supports the metabolic activation hypothesis for oxidative metabolism at the onset of exercise.

Metabolic activation hypothesis. Considerable debate exists as to the controllers of respiration (4, 106, 147). Possible factors include [ADP] (or [ADP]/[ATP]), interaction of [ADP], [Pi], [NAD], [NADH] and reduced cytochrome c, creatine phosphate shuttling, and thermodynamic control models. It is beyond the scope of this review to review respiratory control. The important point in terms of VO_2 on-kinetics is that any of these could influence the rate of adaptation of oxygen consumption to the steady-state level. These regulators provide the basis for the metabolic activation hypothesis of VO_2 on-kinetics. This hypothesis posits that a delay in the increase of metabolic signals that control respiration is responsible for the lag in VO_2 at the onset of exercise. It is the availability of the regulators of respiration that dictate the rate of metabolic activation. Understanding the effects of these regulators on skeletal muscle VO_2 on-kinetics is complicated by potential interactions of the metabolic signals (4).

Based on the ADP hypothesis for respiratory control, a delayed decrease in energy charge (increase in [ADP] and/or [Pi]) could be a regulator of the turn-on of oxidative phosphorylation at the onset of exercise. ADP concentration is buffered by degradation of PCr via CK. Roman et al. (120) calculated a faster rise in [ADP] that was accompanied by a slower breakdown of PCr in MM CK knockout mice. Thus, CK may serve as part of the delay in metabolic activation in the turn-on of oxidative

phosphorylation by buffering the rise in [ADP]. Meyer (105) found that [PCr] decreased to a steady-state level after the onset of contractions, and that increases in stimulation frequency led to an even further reduced steady-state level. Barstow et al. (10, 11) and McCreary et al. (103) found that PCr kinetics at the onset of contractions were similar to VO_2 on-kinetics. With the use of techniques designed by Whipp et al. (142), Rossiter et al. (121, 123) showed that PCr kinetics at the onset of contractions match very similarly to the primary component of oxygen uptake kinetics during moderate leg kick exercise. When the kinetics were examined at the onset of high intensity leg kick exercise (123), the fundamental phase for a single bout preceded by only rest was not significantly different for the PCr and oxygen uptake kinetics. Interestingly, the time constants for PCr degradation and the fundamental component of VO_2 were not significantly different between metabolic rates (121, 123), confirming previous measurements using an *in situ* preparation (105). According to these data, PCr kinetics play a significant role in metabolic activation at the onset of exercise.

Based on the data above, a faster decrease in [PCr] via inhibition and/or knockout of CK could increase [ADP], and speed VO_2 on-kinetics. Kindig et al. (84) found that intracellular PO_2 declined more rapidly in contracting single skeletal muscle fibers with CK inhibition, indicating a faster turn-on of oxidative phosphorylation. Harrison et al (63) found an increase in response time for VO_2 in isolated rabbit hearts with acute CK inhibition. Using isolated hearts from mice with knockouts of cytoplasmic (M) CK and the mitochondrial isoform gene, Gustafson and Van Beek (57) reported speeded VO_2 on-kinetics. These data indicate that it is very likely that CK plays a role in VO_2 on-kinetics,

possibly by buffering the increase in [ADP]. However, extrapolation to skeletal muscle cannot be done emphatically as the study by Kindig et al. (84) did not measure VO_2, cardiac muscle differs from skeletal muscle in its regulation of metabolism (4), and CK knockout mice demonstrate compensatory adaptations (84, 132, 138).

Another pathway that is activated rapidly at the onset of exercise and may delay the increase in [ADP] and [NADH] is glycolysis. The flux through the glycolytic pathway increases with workrate (131). Increases in signals to turn-on oxidative phosphorylation directly (ADP and Pi) and indirectly via an increase in enzymatic stimulators (i.e., Ca^{2+}) that result in an increase in stimulators of respiration, also turn on glycolysis (131). This is an interesting cycle in that the increase in stimulators needed to increase respiratory rate may actually delay the rate of increase in respiration by stimulating glycolysis and buffering [ADP], and potentially [NADH]. Inhibition of glycolysis has been shown to speed the response time of VO_2 in isolated rabbit hearts (62). However, this has not been investigated in skeletal muscle.

The rate of production and/or use of reducing equivalents (NADH, $FADH_2$) could impact VO_2 on-kinetics through the potential roles of NADH and $FADH_2$ in the regulation of respiration. Thus, enzymes that control the production and/or shuttling of these reducing equivalents to the mitochondria could limit the turn-on of oxidative phosphorylation. The main producer of reducing equivalents, the TCA cycle, is dependent upon substrate entry into the cycle. One of these substrates is acetyl-CoA. The pyruvate dehydrogenase complex (PDC) provided an interesting potential controller for oxidative metabolism because of its role in the production of acetyl-CoA and

reducing equivalents (NADH). Specifically, PDH is the enzyme in the PDC that catalyzes a rate-limiting reaction and results in the direct production of acetyl-CoA.

The PDC is activated by stimuli indicative of a greater metabolic demand (i.e., increased [Ca^{2+}], decreased [ATP]/[ADP] and [NAD^{+}]/[NADH]) (130), and its activity is increased during exercise (130). Infusion of dichloroacetate (DCA) to activate the PDC prior to the onset of exercise in humans has resulted in a smaller calculated O_2 deficit (110, 130, 134). However, VO_2 on-kinetics were not assessed in these studies. When VO_2 on-kinetics have been measured after DCA infusion in humans (6, 82, 93, 122) and in the isolated canine GS (47), no speeding was observed. Thus, the calculated differences in O_2 deficit between the two conditions (110, 130, 134) was likely due to other effects of DCA at the onset of exercise, possibly by lessening the rate of fatigue (47). These data indicate that the supply of acetyl groups and reducing equivalents through the PDC does not limit VO_2 on-kinetics.

Though activation of the PDC does not appear to limit the rate of VO_2 adjustment, it does not necessarily mean that accumulation of NADH is unimportant. For example, an increased flux through the TCA cycle would cause an increase in the production of reducing equivalents to power the ETC. This can be accomplished by other means besides PDC activation. The rate-limting reactions of the TCA cycle catalyzed by isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase, and citrate synthase all provide possible sites of investigation. Interestingly, the maximal flux rate through this cycle appears to be very close to the maximal flux rate through extracted citrate synthase (4), and it remains possible that citrate synthase activity may modulate VO_2 on-kinetics.

However, Bangsbo et al. (5) found that accumulation of TCA cycle intermediates is not essential for oxidative phosphorylation at the onset of exercise in humans.

Ca^{2+} activates many mitochondrial enzymes, including those responsible for the production of reducing equivalents. A delay in Ca^{2+} uptake by mitochondria may prevent a necessary increase of reducing equivalents. However, Hak et al. (60) found that inhibition of mitochondrial Ca^{2+} uptake by administration of ruthenium red slowed VO_2 on-kinetics in isolated rabbit hearts only after a strong blockade. Kindig et al. (85) suggested that metabolic signals (i.e., ADP) play a more major role than $[Ca^{2+}]$ in the rate of VO_2 adjustment at the onset of contractions in single skeletal muscle fibers. In this investigation (85), intracellular PO_2 kinetics were unaltered after inhibition of contractile activation with 2,3-butanedione monoxime. Ca^{2+} kinetics as they pertain to VO_2 on-kinetics in skeletal muscle mitochondria in intact, contracting skeletal muscles are yet to be investigated.

As investigation of the metabolic activation hypothesis gains momentum in the laboratory, important regulators of the needed metabolic stimuli for an increase in respiratory rate may become elucidated. Research in this area should focus on non-equilibrium reactions as these are the rate-limiters for their respective pathways. Activation of these limiting enzymes prior to the onset of exercise should cause an increase in their respective products. It is the evaluation of VO_2 on-kinetics in these conditions; in skeletal muscle, that will provide needed information for this hypothesis.

Interaction between O_2 delivery and metabolic activation. Although there are two main hypotheses for the controller(s) of VO_2 on-kinetics, they may not be mutually exclusive from one another. Oxygen delivery and metabolic regulators of oxidative metabolism interact to achieve the steady-state level of VO_2. Specifically, the mitochondrial PO_2, stimulators of respiration (i.e., [ADP] (or [ATP]/[ADP]), [Pi] (or [ATP]/[ADP] * [Pi], [NADH] (or [NADH]/[NAD^+]), and reduced cytochrome c availability interact to determine the rate of oxidative metabolism (4, 136, 147). An understanding of these interactions requires a more detailed reaction scheme for oxidative metabolism than depicted previously. The first two sites of oxidative phosphorylation are near-equilibrium under physiological conditions and can be summarized as follows (147):

$$\mathbf{NADH_i + 2c^{3+} + 2ADP_e + 2Pi_e <---> NAD^+_i + 2c^{2+} + 2ATP_e + H^+}$$

The subscripts i and e indicate intracellular and extracellular concentrations, respectively. The abbreviations c^{3+} and c^{2+} represent the oxidized and reduced forms of cytochrome c, respectively. The third site of oxidative phosphorylation is irreversible and can be summarized as follows (147):

$$\mathbf{2c^{2+} + 1/2O_2 + ADP_e + Pi_e + 2H^+ ---> 2c^{3+} + H_2O + ATP_e}$$

This site is considered the rate limiting step in the overall reaction, and involves the enzyme cytochrome c oxidase.

Mitochondrial PO_2 is determined by the interactions of the driving pressure for O_2 from capillary to cell and local diffusion capacity (139) with the rate of O_2 consumption by the mitochondria. The capillary PO_2 is determined by muscle blood flow and dissociation of O_2 from Hb (125). An ample increase in oxygen delivery by a rapid blood

flow adjustment is important to provide the driving gradient for O_2 diffusion. If O_2 consumption by mitochondria increases without an increase in blood flow, the diffusion gradient will be greatly diminished, possibly compromising the rate of O_2 consumption. Thus, O_2 delivery is important for an adequate mitochondrial PO_2, and subsequent oxidation of reduced cytochrome c. Mitochondrial PO_2 cannot be measured in intact, exercising skeletal muscles. Instead, intracellular PO_2 is often used as in indicator of O_2 availability. Intracellular PO_2 decreases during exercise (107, 119), indicating the likelihood of a decrease in mitochondrial PO_2. Using the equation for the third site of oxidative phosphorylation, the question becomes: How does respiration increase and remain elevated with a decreased mitochondrial PO_2?

Increases in the amount of reduced cytochrome c have been seen in cell suspensions with a decreasing PO_2 (148), possibly as a compensatory mechanism. This increase would allow for the achievement of a given rate of respiration with a decreasing PO_2 (equation for third site of oxidative phosphorylation). An increased flux through the first two sites of oxidative phosphorylation can increase the amount of reduced cytochrome c available. Increases in the concentrations of the metabolic stimulators NADHi, ADPe, or Pie would increase this flux due to the near-equilibrium properties of these reactions (equation for first two sites of oxidative phosphorylation). Data from Hogan et al. (69, 70), using the isolated canine GS contracting *in situ* show that greater increases were elicited in proposed controllers of respiration in conditions with decreased O_2 availability in comparison to control. Greater metabolic disturbances (71), likely due to an increase in respiratory regulators, has also been observed during hypoxia. It is

unlikely, however, that solely [ADP] or [NADH] control the turn-on of mitochondrial respiration as fixing the concentration of one of these stimulators makes respiration highly dependent on the other (148). This implies that the concentrations of these regulators with respect to each other are important.

Oxygen availability as determined by blood flow kinetics is not independent of the need for metabolic stimuli for a given rate of VO_2, nor vice versa. This idea raises interesting questions, particularly: Does the mitochondrial PO_2 ultimately dictate the amount of increase needed in metabolic regulators of oxidative phosphorylation for a given respiratory rate? Or, does the rapidity of the increase in metabolic regulators of oxidative phosphorylation ultimately determine the mitochondrial PO_2 needed for a given respiratory rate? Via this idea, O_2 delivery and metabolic stimuli regulate mitochondrial PO_2, and interact to determine the rate of VO_2. How quickly these factors interact at the onset of an increased energy demand determines the rate of VO_2 on-kinetics.

PRIMING EXERCISE

An interesting model that has been used to study the controlling mechanisms of VO_2 on-kinetics is prior exercise. In these experiments, two bouts of exercise are performed. The first is considered a "priming" bout. After a recovery period, a second exercise bout is undertaken and the VO_2 on-kinetics are compared to the first bout. Early investigations into the effects of prior exercise were hindered by the lack of sophisticated fitting techniques crucial for quantification of the VO_2 on-response. One of the first experiments to show potential effects of priming exercise on VO_2 on-kinetics was

conducted by Buono and Roby (20). In this experiment, subjects performed two 5-min bouts of cycle ergometry at VO_2peak, separated by 25 min of active recovery. During the second exercise bout, the VO_2 response was elevated in comparison to the first bout for the first 2 min of exercise suggesting speeded kinetics. In contrast to the results from Buono and Roby (20), Martin et al. (101) and De Bruyn-Prevost (32) showed no effect of priming exercise on VO_2 response at the onset of exercise. Martin et al. (101) measured the VO_2 response in two trained runners with and without a prior warm-up bout of moderate intensity. The rate constant for the change in VO_2 for the first minute of exercise was unchanged by prior exercise. De Bruyn-Prevost (32) found no effect of various warm-up durations and intensities on the response to a sub-maximal exercise bout. These seemingly conflicting results led to an intriguing paper by Gerbino et al. (41).

Prior exercise above the lactate threshold (supra-LT). In 1996, Gerbino et al. (41) conducted a pivotal priming exercise study. They (41) sought to examine if VO_2 on-kinetics for high-intensity exercise could be speeded by prior exercise at an intensity below the lactate threshold (sub-LT) and/or exercise at an intensity above the lactate threshold (supra-LT). Subjects performed unloaded pedaling for 3-min followed by a 6-min of constant-load exercise. This 6-min bout was succeeded by 6 min of unloaded pedaling as a recovery regimen. After the recovery period, another 6-min bout of constant-load exercise was performed. The 6-min constant-load exercise bouts were arranged so that every combination of sub- and supra-LT exercise could be examined (e.g., sub-sub, supra-supra, sub-supra, supra-sub). Gerbino et al. (41) found that a supra-

LT intensity exercise bout speeded the VO_2 on-kinetics of a second supra-LT intensity exercise bout. Prior, moderate intensity exercise had no effect. Another key finding by Gerbino et al. (41) was that a prior bout of supra-LT exercise reduced the VO_2 slow component amplitude during a successive supra-LT bout. This was the first experiment that showed a speeding of VO_2 on-kinetics after prior exercise.

MacDonald et al. (98) examined the effect of 10 min of exercise at an intensity above the ventilator threshold (supra-VT) exercise bout on a second, identical exercise bout after 6 min of recovery via unloaded pedaling. Subjects were tested under both normoxic and hyperoxic conditions, such that the following exercise bout combinations were conducted: Normoxic-normoxic, hyperoxic-hyperoxic, normoxic-hyperoxic, hyperoxic-normoxic. The VO_2 on-kinetics of the second supra-VT exercise bout were speeded by prior supra-VT exercise. Interestingly, hyperoxia with prior supra-VT exercise speeded the VO_2 on-kinetics of the second bout to a greater extent than hyperoxia or prior exercise alone. MacDonald et al. (98) also found that the amplitude of the VO_2 slow component in the second supra-VT bout was reduced after previous supra-VT bout. The results obtained by MacDonald et al. (98) confirmed those of Gerbino et al. (41).

MacDonald et al. (99) investigated the role of prior forearm exercise on a second bout of forearm exercise. In this experiment, VO_2 was measured across the exercising limb. In comparison to the first bout, muscle VO_2 for the second bout was elevated at 30 s. The absolute VO_2 achieved after 5 min of exercise was the same between bouts.

Unfortunately, due to limitations that prevented rapid sampling, VO_2 kinetics could not be determined, and the fundamental component could not be quantitatively examined.

The investigations by Gerbino et al. (41), MacDonald et al. (98), and Bohnert et al. (19) indicated speeded VO_2 on-kinetics after a prior bout of high-intensity exercise, and that the amplitude of the VO_2 slow component was reduced in the second bout. However, due to technical limitations of these investigations, how the fundamental component of VO_2 on-kinetics is impacted by prior exercise was not examined directly. In light of this, Burnley et al. (24) examined the effect of prior exercise on the fundamental component of the VO_2 response during a second bout of exercise. Subjects performed a 6-min priming bout of exercise followed by 6-min of active recovery. The active recovery period was followed by a 6-min exercise bout. The intensity for each bout was either sub- or supra-LT such that the following conditions were used: supra-supra, sub-sub, supra-sub, sub-supra. No effect was seen when the second bout was sub-LT. The mean response time of the second supra-LT bout was speeded by prior supra-LT exercise. However, the amplitude of neither the fundamental component nor the τ was significantly altered (Figure 2). The amplitude of the slow component was significantly reduced by prior supra-LT exercise (Figure 2). According to these results, the fundamental component of VO_2 on-kinetics is not altered by prior exercise.

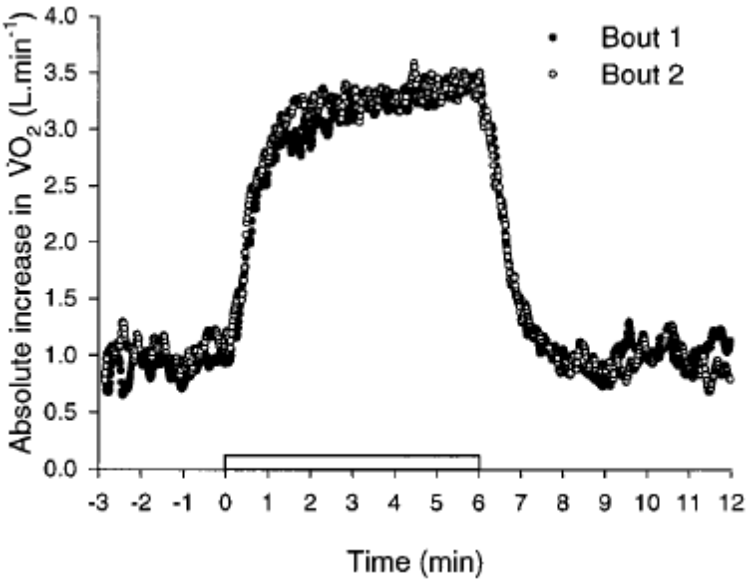

Figure 2. Pulmonary VO_2 response to a first (bout 1) and second (bout 2) supra-LT exercise bout. From Burnley et al. (24).

Koppo and Bouckaert (88) found similar results as Burnley et al. (24). Subjects cycled at 90% VO_2 peak for 3 min, followed by a 6-min recovery period. The recovery period consisted of 3 min rest and 3 min of unloaded pedaling. After the recovery period, a second bout of exercise was performed that was identical to the first bout. In agreement with the findings of Burnley et al. (24), the fundamental component was not altered during the second exercise bout and a reduction in the amplitude of the slow component was found. Also, Koppo and Bouckaert (88) found that the time constant for the slow component was significantly reduced for the second exercise bout.

A prior bout of supra-VT cycle ergometry exercise was found to alter a second identical bout by Bearden and Moffatt (13). In this study, subjects cycled for 10 min followed by a 10 min recovery period. The amplitude of the fundamental component of the second bout was significantly greater than the first. The time delay and tau were not

significantly different between bouts. Also, the amplitude of the slow component was significantly lower for the second bout.

Scheurmann et al. (127) found that prior sub-LT exercise did not alter the VO_2 on-kinetics during a successive bout of exercise above the LT. This includes no change in amplitude of the fundamental or slow component, tau of the fundamental component, and the gain of the fundamental component. However, when the supra-LT bout was preceded by an identical supra-LT bout, the slow component amplitude was decreased in the second bout. The time constant, amplitude, and gain of the fundamental component were not different.

To examine not only the effect of prior supra-LT exercise on a successive bout but also the effect of recovery duration and total work performed in the second bout, Burnley et al. (22) modified their previous protocol (24). In addition to two 6-min supra-LT exercise bouts separated by 6 min of recovery, two additional conditions were added: one in which the recovery time was extended to 12 min; and one in which the intensity of the first bout was sub-LT but the total work accomplished was the same as the 6-min supra-LT bouts. When the prior exercise was performed above LT, the absolute VO_2 achieved during the fundamental phase was greater during the second bout and the amplitude of the slow component was decreased. These results were repeated by Burnley et al. (21) in 2002 with a recovery period of 12 min between 6-min supra-LT bouts. The sub-LT bout did not elicit any changes in the VO_2 on-kinetics for the successive supra-LT bout (22). The lack of a change in the time constant of the fundamental component and a

decrement in the slow component amplitude is consistent with what has been reported previously (24, 88).

Burnley et al. (23) examined the effect of the duration of a prior supra-LT bout on a subsequent 6-min bout of supra-LT exercise. Specifically, a 30 s bout of maximal sprint cycling was used for the first bout, and the results were compared to those obtained from a second bout after a 6-min bout of supra-LT exercise. Both bout 1 protocols significantly increased the absolute VO_2 amplitude and reduced the slow component amplitude. The amplitudes of the fundamental component and slow component were not different between the 30 s sprint exercise and 6-min supra-LT bout conditions. These results again indicate that the intensity of the first bout is of upmost importance.

Perrey et al. (113) investigated the effect of cycling at 80% VO_2peak for 10 min on a subsequent identical cycling period. The exercise bouts were separated by a 10 min recovery period at 35% VO_2peak. The overall VO_2 response was faster during the second bout. Specifically, the amplitude of the fundamental component was greater than that observed in the first bout. This was accompanied by a significant decrease in the amplitude of the slow component. The time constant of the fundamental component was not different between the exercise bouts.

To examine the role of muscle fatigue and acidosis on VO_2 on-kinetics, Tordi et al. (135) had subjects perform a supra-LT exercise bout, three Wingate tests, and then a second supra-LT bout that was identical to the first. Interestingly, the amplitudes of the fundamental and slow component were not affected by prior exercise. The time delay of the fundamental component was not affected either. The time constant of the

fundamental component, however, was significantly faster after the prior exercise bouts in comparison to the first bout of supra-LT exercise. This was the first investigation to measure a speeded primary time constant after prior exercise.

Sahlin et al. (124) examined the effect of prior supra-VO_2peak exercise on the VO_2 on-kinetics during a bout of cycling at 75% VO_2peak. Baseline VO_2 was significantly greater after prior exercise in comparison to the first bout at 75% VO_2peak without prior exercise. Time delay, mean response time, and the tau of the primary component were not significantly different between submaximal exercise bouts. The primary component VO_2 asymptote and amplitude were significantly greater after supra-VO_2peak exercise.

Another method of lower body exercise that has been used to investigate the effects of prior exercise is knee extension exercise. Endo et al. (37) had subjects perform this type of exercise above LT for 6 min followed by a 4-min bout of recovery knee extension exercise. The second exercise bout was the same as the first. Neither the amplitude of the fundamental component nor the time delay was altered after knee extension exercise. The calculated tau using a monoexponential was significantly faster for the second bout in comparison to the first. The amplitude of the slow component was also reduced for the second bout of exercise.

Gurd et al. (56) examined VO_2 on-kinetics in subjects with a relatively fast versus slow primary time constant. Subjects pedaled on a cycle ergometer at 80% LT for 6 min followed by 6 min above LT and then 6 min at 80% LT. Each transition was preceded by 6 min of pedaling at 20 W. Both groups of subjects displayed significantly faster time

constants for the second bout at 80% LT. This is the first study to show that VO_2 on-kinetics can be speeded during exercise below the LT after prior exercise.

Paterson et al. (112) examined VO_2 on-kinetics of a second bout of single leg knee extension exercise. Subjects performed single leg knee extension exercise at an intensity of 80% VO_2peak for 6 min followed by 6 min of unloaded exercise. After recovery, they once again exercised at 80% VO_2peak for 6 min. The baseline VO_2, phase 1 and 2 amplitudes, and phase 3 amplitude were not significantly different between bouts. Calculated time delay was also not different between bouts. The time constant for the second bout of single leg knee extension exercise was significantly faster than that measured in the first bout. Interestingly, the end exercise VO_2 was significantly greater for the second bout of exercise.

Using a similar cycle ergometer protocol as used previously (56), Gurd et al. (55) once again examined the effect of prior exercise above LT on the VO_2 on-kinetics of a successive bout below LT. In this experiment, the baseline VO_2 was significantly elevated prior to the second bout. This likely led to the measured decrease in amplitude for the VO_2 response at the onset of exercise in the second bout compared to the first bout below LT. Similarly to their previous findings (56), Gurd et al. (55) found that the time constant for the second bout below LT was significantly faster than the first. In agreement with Paterson et al. (112), the end exercise VO_2 was significantly greater for the second bout, although this was moderate intensity cycle ergometry exercise in comparison to single leg knee extension exercise.

DeLorey et al. (34) examined VO_2 on-kinetics during alternating knee extension exercise. Exercise was performed for 8 min at a supra-LT intensity. Following a recovery period, another bout of exercise was performed that was of identical intensity as the first. The baseline VO_2 prior to the second bout was significantly greater than in the first bout and the amplitude was not significantly different. The time constant for the VO_2 response at the onset of exercise was significantly faster for the second exercise bout.

Sub-LT exercise prior to supra-LT exercise. Although most data support the necessity of a supra-LT exercise bout to speed VO_2 on-kinetics in a subsequent bout (see above), data do exist in which the subsequent bout was speeded by a prior exercise bout of sub-LT intensity. Koppo and Bouckaert (89) investigated the role of the amount of work performed in the first bout of exercise on the VO_2 on-kinetics of the second bout. Two protocols were used: the first consisted of two, 6 min cycling bouts at 90% VO_2peak separated by 6 min of recovery. The second protocol consisted of a cycling bout at 50% VO_2peak for a duration in which the work performed was tantamount to the 6 min, 90% VO_2peak bout during the first protocol. The second bout was the same as the second bout of the first protocol. The VO_2 slow component during the second bout was significantly reduced during both protocols. These data indicate that prior moderate intensity exercise can influence a bout of supra-LT exercise when the total work performed is the same for the first bout. Koppo and Bouckaert provided additional support for these findings in 2002 (87) when a 12-min moderate bout of exercise preceded a 6-min supra-LT bout. Interestingly, a 6-min moderate exercise bout also

reduced the VO_2 slow component (though to a lesser extent) during a supra-LT exercise bout (87).

Sub-LT exercise prior to sub-LT exercise. Campbell-O'Sullivan et al. (27) provided support for the work of Koppo and Bouckaert (87, 89) in their finding that a prior bout of moderate intensity exercise can affect the VO_2 on-kinetics of a second bout of exercise. However, Campbell-O'Sullivan et al. (27) investigated the effect of prior moderate intensity exercise (10 min cycling at 55% VO_2peak) on a subsequent bout of moderate intensity exercise (10 min cycling at 75% VO_2peak). Mean response time and the O_2 deficit were significantly reduced when moderate intensity exercise preceded 10 min cycling at 75% VO_2peak. Unfortunately, Campbell-O'Sullivan et al. (27) did not characterize the VO_2 kinetic response with complex modeling techniques. Thus, it is unclear which parameter(s) (tau, time delay, primary amplitude, slow component amplitude) were altered in the speeding of the overall kinetics.

Priming exercise with different muscle groups. As proposed by Gerbino et al. (41), the prior exercise effect may lie in enhanced blood flow to the contracting muscles due to an acid-base dependent vasodilatory response. Accordingly, an adjustment in acid-base status via a mechanism other than contraction of the muscles used in the exercise bout could cause a similar speeding effect. Examining the VO_2 on-kinetics response after priming exercise with a different musculature (e.g., arm exercise prior to cycling exercise) allows for examination of Gerbino et al.'s (41) postulation.

The protocols used by Gerbino et al. (98) and MacDonald et al. (41) involved prior exercise of the same modality as the second exercise bout (upright cycle

ergometry). Bohnert et al. (19) sought to investigate the effect of a priming bout of exercise with a different muscle mass than the second exercise bout. Subjects performed a 6-min supra-LT exercise bout on a cycle ergometer which was preceded by either an identical bout on the cycle ergometer, or a 6-min bout of supra-LT arm-crank ergometry. The VO_2 slow-component was reduced for the second bout of cycle ergometry exercise when it was preceded by either leg cycle ergometry or arm-crank ergometry exercise. The effect of prior leg cycling was greater than the effect of prior arm exercise. These data are interesting in that they indicate a potential mechanism by which prior exercise may influence VO_2 on-kinetics via a mechanism not directly related to the contracting muscle in the second bout.

Fukuba et al. (38) examined the effect of prior supra-LT exercise with the arms or legs on a subsequent bout of supra-LT leg cycle ergometry. When the data were fit with a double exponential, the time delay was significantly less and gain of the primary component VO_2 was significantly greater with prior exercise. Interestingly, these values for prior arm exercise or leg exercise were not significantly different from each other. The double exponential fit was used as the VO_2 residuals are biased by the slow component (111). When the data were compared with a monoexponential fit, mean response time after prior leg exercise was shorter. This was due to a significantly shorter primary time constant. The monoexponential fit was used to obtain the 'effective' tau (41) for the fundamental component.

Koppo et al. (91) examined the effect of prior supra-LT arm exercise on a subsequent bout of cycling at 90% VO_2peak. The exercise bouts were 6 min in duration

and were separated by 6 min of recovery. In comparison to a second bout that was preceded by a cycling bout at 90% VO_2peak, the time constant for the fundamental component was not different. The amplitude of the slow component, however, was significantly reduced with prior exercise. This amplitude was lower for exercise preceded by leg cycling exercise in comparison to arm exercise. Whereas prior supra-LT arm exercise did have an effect on the successive cycle bout, Koppo et al. (91) suggested that their data indicate that the primary factor causing changes in VO_2 on-kinetics are located within the muscle itself.

PROPOSED MECHANISMS FOR THE PRIOR EXERCISE EFFECT

The sections above that discussed the effect of prior exercise on the VO_2 on-kinetics of a second bout of exercise provided evidence for the prior exercise effect. The mechanism(s) by which prior exercise speeds VO_2 on-kinetics was not discussed. The purpose of this section is to discuss the proposed mechanisms for the prior exercise effect. Currently, four proposed mechanisms exist to explain the prior exercise effect: 1) increased muscle temperature; 2) altered motor unit recruitment; 3) increased metabolic activation; and 4) increased O_2 delivery and availability. Understanding the mechanisms responsible for speeded kinetics after prior exercise can lead to elucidation of the mechanisms that control oxygen uptake, particularly at the onset of exercise.

Increased muscle temperature. The temperature of the exercising muscle(s) increases during contractions (42). If the duration and intensity of the prior bout are sufficiently great and the duration of the recovery period is sufficiently short, the

temperature of the exercising muscle(s) will be elevated above rest prior to the second bout. Koga et al. (86) investigated the role of temperature on VO_2 on-kinetics by passively heating the exercising musculature of subjects by ≈3 deg C. Both a sub-LT exercise bout and a supra-LT bout were examined (on separate occasions). The primary time constant and primary amplitude were not significantly different with or without passive heating for both conditions. The slow component amplitude for the supra-LT bout was significantly less after passive heating in comparison to control.

In 2002, Koppo et al. (92) measured the temperature of the vastus lateralis during exercise via an indwelling thermistor. Muscle temperature was measured during two consecutive bouts of supra-LT cycling exercise. The temperature was higher at the start of the second bout than at the start of the first. To isolate the role of temperature, Koppo et al. (92) passively heated the exercising muscles on a separate day until the same temperature was reached as the start of the previous second bout. Subjects then performed an exercise bout identical to the previous day but starting at the same muscle temperature as the second bout without any other prior exercise effects. The VO_2 slow component was reduced only after prior supra-LT exercise. Passively heating the exercising muscles prior to a supra-LT bout did not alter the VO_2 slow component. Similar results were obtained by Burnley et al. (23). Burnley et al. (23) passively heated the legs of subjects in a hot water bath prior to a 6 min supra-LT bout of exercise. The water bath elevated muscle temperature by ≈2.6 deg C. Passive heating of the legs did not alter the VO_2 response to supra-LT exercise. Thus, muscle temperature does not appear to play a major role in the prior exercise effect.

Altered motor unit recruitment. Reduction and/or elimination of the VO_2 slow component (e.g., (124)) when exercise is preceded by a supra-LT bout suggests that the factors involved in the slow component are altered by prior supra-LT exercise. It has been suggested that a cause of the VO_2 slow component lies in the recruitment of additional motor units as the exercise bout progresses (115, 140). If recruitment of additional motor units is in fact a cause of the VO_2 slow component, a reduction in its amplitude after prior supra-LT exercise suggests that motor unit recruitment has been altered. Further, the primary component amplitude has been shown to be sensitive to muscle fiber composition (e.g., (117)). Thus, alterations in either the primary or slow component amplitude by prior exercise could be due to alterations in motor unit recruitment.

Scheuermann et al. (127) provided evidence that alterations in the VO_2 slow component after prior exercise are not accompanied by changes in EMG activity of exercising muscle. Despite a reduction in the slow component amplitude during a second supra-LT bout of exercise, EMG activity was not significantly different between bouts. Tordi et al. (135) found that the tau for a second bout of supra-LT exercise separated by fatiguing sprint exercise was faster. However, the amplitudes of the primary and slow components were not significantly different between bouts. This was accompanied by a non-significant difference in EMG activity of the exercising muscles. It is important to point out, however, that there was a trend for greater EMG activity during the primary phase for the second bout of exercise in comparison to the first in the investigations by

Scheuermann et al. (127) and Tordi et al. (135). Contrary results were obtained by Burnley et al. (21).

Burnley et al. (21) measured activity of three leg muscle during cycling via EMG. Subjects cycled at a supra-LT intensity for two, 6 min bouts separated by 12 min of rest. Time delay and tau of the primary component were not significantly different between bouts. The amplitude of the primary component VO_2 was significantly greater for the second exercise bout, and the slow component amplitude was less. Total EMG activity during the primary phase was significantly greater for the second bout of exercise. When the EMG activity was expressed relative to the primary VO_2, no significant difference was found. The results from Burnley et al. (21) indicate that the greater amplitude of the primary component is due to a greater recruitment of motor units at the onset of the second bout of exercise. Thus, motor unit recruitment appeared to be altered by prior supra-LT exercise.

Recently, DiMenna et al. (36) provided evidence for a motor unit recruitment effect on the primary time constant. Subjects cycled for two, 6 min exercise bouts at an intensity above the LT. Each 6-min exercise bout was separated by 6 min of recovery. To examine the role of motor unit recruitment, 35 and 115 rpm were used. When the first exercise bout was performed at 115 rpm, the primary component tau was significantly reduced during the second bout. The priming bout at 115 rpm likely recruited more type II muscle fibers than the priming bout at 35 rpm. Thus, the authors speculated that the second bout was speeded after priming at 115 rpm due to specific effects on these muscle fibers. No EMG data, however, were included.

Increased metabolic activation. Another proposed method by which prior exercise speeds VO_2 on-kinetics is by alterations in metabolites within the contracting muscle fibers. Since the metabolic activation hypothesis was covered in detail previously, this section will provide an overview specific to the prior exercise effect. Campbell-O'Sullivan et al. (27) found that a 10 min cycling bout at 55% VO_2peak resulted in an elevated amount of acetyl groups prior to the second bout of exercise. This amount was greater than without prior exercise. The increased acetyl group availability indicated that the PDC may have been more greatly activated at the onset of the second bout in comparison to the first. In addition, the amount of skeletal muscle PCr degradation and lactate accumulation was less after prior exercise. These results indicate that there was a decreased reliance on substrate level phosphorylation with prior exercise. This was further supported by a lower O_2 deficit during the second bout and a faster mean response time in comparison to exercise without a prior bout.

Gurd et al. (55) provided evidence that PDC activity and thus acetyl group availability may be part of the delayed metabolic activation at the onset of exercise. Prior supra-LT exercise resulted in a speeding of the VO_2 on-kinetics during a subsequent sub-LT exercise bout (55). The primary time constant was significantly speeded after prior exercise and was accompanied by a significantly greater [acetyl-CoA] at the start of and 30 s into the second bout in comparison to the first. Activity of a rate limiting enzyme in the PDC, PDHa, was significantly greater prior to the start of the second bout and 6 min into exercise in comparison to the first exercise bout. Interestingly, PDHa activity was not significantly different between exercise bouts 30 s into exercise. In contrast to the

results of Campbell-O'Sullivan et al. (27), [PCr] was significantly less at the end of the second exercise bout in comparison to the first. However, Gurd et al. (55) indicate that the slower rate of PCr degradation at the onset of the second exercise bout indicates increased mitochondrial oxidation during the transition phase.

Although increased acetyl group availability and PDC activation have been suggested to be sites of delayed metabolic activation (e.g., (27, 55, 134)), direct activation of the PDC has not resulted in speeded VO_2 on-kinetics in humans (6, 82, 93, 122) or the isolated canine GS (47). Further, despite an elevated amount of acetyl groups prior to and throughout a second exercise bout, Sahlin et al. (124) found no change in the primary time constant or mean response time. Despite the stockpiling of acetyl groups found by Campbell-O'Sullivan et al. (27) and Gurd et al. (55) prior to the start of a second bout of exercise and a speeding of VO_2 on-kinetics, this does not necessarily mean that the speeded kinetics were caused by increased acetyl group availability. Exercise results in multiple changes within the metabolic state of contracting skeletal muscle fibers. In fact, increased acetyl group availability was accompanied by increased PCr degradation during the second bout of exercise in two investigations (55, 124). This result is yet to be examined further, but indicates a lack of change in oxidative phosphorylation between exercise bouts.

Intriguing evidence for an intracellular origin for the effect of prior exercise was provided by Hogan (68) and Behnke et al. (16). Using single amphibian skeletal muscle fibers, Hogan (68) found that a 3 min contractile bout led to a shorter time delay and t1/2 during a second 3 min contractile bout. As O_2 delivery to these fibers is not reliant on

adjustments in blood flow, the speeding of the VO_2 on-kinetics during the second contractile bout suggests increased metabolic activation at the onset of the second bout. With use of the rat spinotrapezius muscle contracting *in situ*, Behnke et al. (16) provided additional evidence for an intracellular effect of prior contractions in 2002. A priming contractile bout resulted in a shorter time delay and time to 63% of the final response for microvascular PO_2 during a second bout. Similar to the study by Hogan (68), O_2 delivery at the start of both contractile bouts was the same. Together, both of these investigations indicate an intracellular origin for the effect of priming exercise.

Increased O_2 delivery and availability. The original proposal to explain the prior exercise effect was that alterations in acid-base status caused by a prior exercise bout could alter VO_2 on-kinetics during a subsequent bout by increasing O_2 delivery (41, 133). This postulation is considered plausible due to feedback control of muscle blood flow (136) and the role of skeletal muscle in overriding sympathetic-induced vasoconstriction (functional symphatholysis) (61). Increased O_2 delivery to contracting skeletal muscle after priming exercise has been indirectly supported by the finding of increased heart rate (13, 21, 56, 135) and estimated cardiac output (135) after a supra-LT priming exercise bout. Investigations that have measured blood flow to the working muscles have shown conflicting results. Bangsbo et al. (7) found thigh blood flow to be elevated prior to the start of a second bout of exercise in comparison to the first. Flow remained elevated for ≈3 min of knee extensor exercise. Using a similar exercise protocol, Krustrup et al. (94) also found that leg blood flow was elevated during a second and third bout of exercise in comparison to the first. MacDonald et al. (99) measured forearm blood flow during two

bouts of exercise. Forearm blood flow was higher at the onset of the second bout of exercise compared to the first and remained elevated for the first 30 s of exercise. Recently, however, evidence for an elevation in blood flow at the onset of a second bout of exercise has not been supported (34, 37, 39, 112).

In 2004, Fukuba et al. (39) measured a speeding of the mean response time after supra-LT exercise. Although leg blood flow was significantly elevated prior to the start of the second exercise bout, blood flow kinetics were not altered in comparison to the first bout. Similarly, Endo et al. (37) and Paterson et al. (112) measured an elevated blood flow baseline prior to a second bout of exercise in comparison to the first but the kinetics were not altered nor was blood flow elevated above the first bout. Using a cycle ergometer exercise protocol, DeLorey et al. (34) found no difference in the baseline leg blood flow or blood flow kinetics between two supra-LT exercise bouts. These recent data (34, 37, 39, 112) indicate that bulk O_2 delivery to the exercising muscles is not a requirement for speeded VO_2 on-kinetics. However, O_2 delivery may still be an essential component to the speeding of VO_2 on-kinetics after a priming bout of exercise. In this case, O_2 availability may be enhanced without enhancing O_2 delivery to the exercising muscles. Near Infrared Spectroscopy has been used to investigate O_2 availability at the site of use (21, 34, 38, 56).

NIRS continuously measures changes in the concentrations of total Hb (tHb), O_2Hb, and HHb and can be used to examine the relationship between O_2 delivery and uptake at the onset of exercise. Burnley et al. (21) measured an elevated [O_2Hb] and [tHb] prior to a second bout of exercise. [O_2Hb] and [tHb] remained elevated throughout

the second exercise bout. Fukuba et al. (38) measured an elevated [tHb] at the onset of a second bout of exercise in comparison to a first bout. Gurd et al. (56) observed elevated [tHb] and [O_2Hb] at the beginning of a second bout of exercise in comparison to a first bout. The calculated time delay for the increase in [HHb] was significantly shorter after priming exercise. Blood flow and muscle oxygenation kinetics at the onset of exercise with and without a priming bout were provided by DeLorey et al. (34). Baseline muscle blood flow and exercise blood flow kinetics were not different between exercise bouts. However, [tHb] and [O_2Hb] were significantly greater at baseline for the second bout of exercise. This trend continued throughout the exercise period. Interestingly, the amplitude of [HHb] kinetics during the second exercise bout was significantly greater than the first bout suggesting an increased O_2 extraction.

Summary of the prior exercise effect. Priming exercise performed with different muscles has a minimal effect on VO_2 on-kinetics (19, 38, 91). This indicates that the prior exercise effect is located within the exercising muscle(s). Further, VO_2 on-kinetics are not altered or altered to a minor extent when the priming exercise bout is of sub-LT intensity (21, 22, 27, 32, 87, 89, 101). It is well established that VO_2 on-kinetics are speeded after a priming bout of supra-LT exercise (13, 19, 22-24, 34, 37, 38, 55, 56, 88, 91, 124, 135), although conflict exists as to which VO_2 kinetics parameters are affected. Based on this speeding, four mechanisms have been proposed: 1) increased muscle temperature; 2) altered motor unit recruitment; 3) increased metabolic activation; and 4) increased O_2 delivery and availability. Understanding the mechanisms responsible for

speeded kinetics after prior exercise can lead to elucidation of the mechanisms that control oxidative phosphorylation, particularly at the onset of exercise.

To date, the available evidence for the prior exercise effect indicates increased metabolic activation and/or increased O_2 delivery and availability as the major causes. Altered motor unit recruitment appears to also play a role. As discussed previously, the cause may not be one factor but rather a complimentary effect between multiple factors. Though the human data are interesting, technical limitations have prevented elucidation of the prior exercise effect mechanisms. Blood flow manipulation using human subjects is extremely limited. Finally, the VO_2 of the contracting muscle cannot be directly assessed in humans. This is particularly a disadvantage as the prior exercise effect likely lies in the muscle itself.

VO_2 ON-KINETICS AT THE MUSCLE LEVEL

To gain insight into VO_2 on-kinetics at the muscle, a series of experiments was designed to investigate both the oxygen delivery hypothesis (45, 46, 48) and metabolic activation hypothesis (47, 49) in the highly oxidative (102) canine GS *in situ*. Grassi et al. (45) investigated the effect of an increased rate of blood flow (O_2 delivery) to the exercising muscle vs. spontaneous blood flow on VO_2 on-kinetics. A metabolic rate of ≈60-70% VO_2peak was elicited via isometric tetanic contractions of the isolated GS (1 contraction per 2 seconds), for a 3 min period. For the control condition, blood flow was spontaneous and self controlled (Figure 3). The fast flow delivery condition consisted of

increasing blood flow to the muscle ≈15-30 s prior to the start of contractions to the rate observed at steady state during the control period (Figure 3).

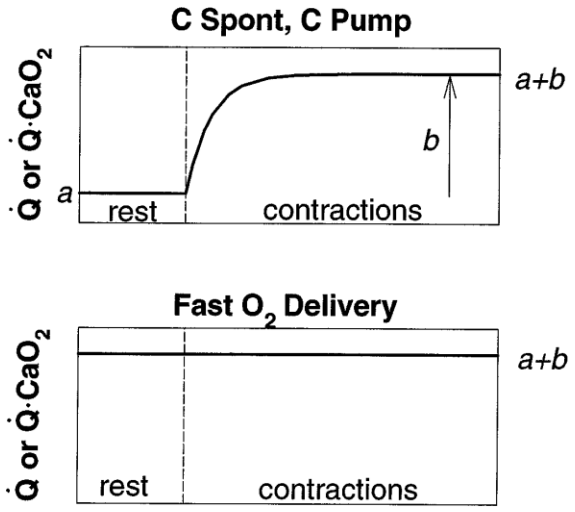

Figure 3. Depiction of experimental conditions (45). Top: spontaneous adjustment of blood flow/O_2 delivery. Bottom: elevated flow condition removing any delay in blood flow/O_2 delivery adjustment at the onset of contractions.

Arterial and venous blood samples were taken at rest, every 5-7 s during the first 75 s of contractions, and every 30-45 s after the initial 75 s of contractions until the end of the 3-min contraction period. These samples were analyzed for PO_2, PCO_2, pH, [Hb], O_2 saturation, O_2 content, and [La^-]. Blood flow and O_2 delivery were higher at the onset of contractions in the fast-flow condition. VO_2 was not significantly different between conditions at rest or upon achievement of a steady state. Despite the increased O_2 delivery in the fast-flow condition, VO_2 on-kinetics were not significantly affected. According to these results, during the onset of contractions to 60-70% VO_2 peak, the lag in oxygen uptake is not due to a delayed convective O_2 delivery to the muscle. This finding provides evidence that muscle oxidative metabolism is slow to respond even without a convective O_2 delivery limitation.

In a follow up study to the fast-flow experiments (45), Grassi et al. (46) investigated the role of peripheral O_2 diffusion on VO_2 on-kinetics. Specifically, this study was conducted to investigate whether the driving pressure gradient for O_2 to the contracting muscle limits the rate at which oxygen uptake rises at the onset of contractions. Peripheral O_2 diffusion was increased with hyperoxia, and hyperoxia plus a rightward shift of the oxyhemoglobin dissociation curve. Isometric tetanic contractions were induced in the same fashion as previously described (45), evoking approximately 60-70% VO_2peak. The experimental design consisted of three conditions: 1) a control condition in which the dogs were ventilated with ambient air with blood flow increased to the steady state level ≈15-30 s prior to the start of contractions; 2) identical to the control condition except that the dogs inspired 100% O_2; and 3) identical to 2) except that the dogs were given RSR-13 at a dose of 100 mg•kg^{-1} 15 min prior to the start of contractions. RSR-13 allosterically inhibits the binding of O_2 to Hb, thus causing a rightward shift of the oxyhemoglobin dissociation curve (1). Arterial and venous blood samples were taken at rest, every 5-7 s during the first 75 s of contractions, and every 30-45 s after the initial 75 s of contractions until the end of the 3-min contraction period. These samples were analyzed for PO_2, PCO_2, pH, [Hb], O_2 saturation, O_2 content, and [La^-].

Resting and steady state arterial and venous PO_2 was significantly greater ($p<0.05$) in the hyperoxia and hyperoxia plus RSR-13 conditions in comparison to control. A rightward shift of the oxyhemoglobin dissociation curve in the hyperoxia plus RSR-13 condition was evidenced by a significant increase in P50 vs. control. The

calculated rate of O_2 delivery to the muscle was significantly greater in hyperoxia plus hyperoxia with RSR-13 than the control condition. The O_2 diffusive gradient from capillary to myocyte was increased ≈2 fold with hyperoxia and ≈4 fold with hyperoxia plus RSR-13. Despite the increased O_2 delivery and pressure gradient for O_2 diffusion into the muscle, VO_2 on-kinetics were not speeded. These results (46), along with the results of increasing convective O_2 delivery to the muscle (45), indicate that a slow turn-on of oxidative metabolism at the onset of contractions to a metabolic rate of ≈60-70% VO_2peak in a highly oxidative muscle occurs without an O_2 limitation.

In addition to direct experiments on VO_2 on-kinetics in the isolated canine GS, a study on VO_2 on-kinetics using data from the previous experiments has been conducted *in silico* (96). Lai et al. (96) investigated factors controlling VO_2 at the muscle at contraction onset using experimental data (45, 46) and mechanistic modeling. The results suggest that oxygen consumption by the mitochondria at contraction onset is faster than oxygen uptake by the muscle, and thus results obtained by venous sampling could indicate slower kinetics. Also, it is suggested that the time delay between muscle O_2 uptake and mitochondrial O_2 consumption is increased with hyperoxia due to an increase in muscle O_2 stores, preventing detection of potentially speeded on-kinetics as measured via the arteriovenous oxygen difference. This model suggests that VO_2 on-kinetics may indeed have been speeded with increased O_2 delivery to the working muscle (45, 46), but technical limitations of measurement may have prevented detection.

Based on human data that indicate faster VO_2 on-kinetics during exercise of high intensity with increased O_2 delivery (e.g., (98)), Grassi et al. (48) sought to investigate

the effect of increasing O_2 delivery on the rate of VO_2 on-kinetics to a work rate that elicits VO_2peak. Isomteric contractions were elicited at a rate of 1/s for 4 min. The experimental conditions were the same as (45) with the exception of the achieved metabolic rate (VO_2peak). Increasing blood flow and thus O_2 delivery to the muscle prior to contractions caused a significant speeding of VO_2 on-kinetics in comparison to control. These data indicate that O_2 delivery to the muscle is a factor in determining the rate of oxygen uptake at the onset of contractions to a metabolic rate of VO_2peak.

The delay in oxygen uptake at the onset of contractions to ≈60-70% VO_2peak is not part of the lag in O_2 uptake at the onset of exercise in highly-oxidative skeletal muscle (45, 46). These results indicated a slow turn-on of oxidative metabolism that was due to delayed metabolic activation. Slow activation of the PDC may limit acetyl group availability, thereby slowing the flux through the TCA cycle and subsequently, the turn-on of oxidative metabolism. To investigate this part of the metabolic activation hypothesis, Grassi et al. (47) investigated VO_2 on-kinetics in the isolated canine GS with prior activation of PDH with 300 mg•kg^{-1} dichloroacetate (DCA). The contraction protocol has been previously described (48). The experimental design consisted of two conditions: 1) a control condition in which 45 ml of saline was infused over a period of ≈45 min prior to the start of contractions; and 2) ≈45 min infusion of DCA (300 mg•kg $^{-1}$) in 45 ml saline. Muscle biopsies were taken from the experimental muscle at rest and with 15 s of contractions remaining. These samples were analyzed for ATP, PCr, Cr, and La^- concentrations. PDC activity was assessed by measuring free carnitine and acetyl-carnitine concentrations.

Free carnitine and La^- concentrations at rest were lower, and [acetylcarnitine] was greater after administration of DCA, indicating activation of PDH with DCA prior to the onset of contractions. [Acetylcarnitine] was also greater at the end of contractions in the condition with DCA versus control. pH was significantly greater in the DCA condition versus control at steady-state VO_2. Interestingly, less fatigue was encountered with DCA in comparison to control. Despite the activation of PDH with DCA, VO_2 on-kinetics were not speeded in comparison to control. These results indicate that the PDC, and thus acetyl group availability, is not responsible for the delayed metabolic activation at the onset of contractions.

Another potential metabolic site of interest is cytochrome c oxidase. Nitric oxide is a vasodilator that can bind to the O_2 binding site of cytochrome c oxidase in the electron transport chain, competitively inhibiting the enzyme. The enzyme nitric oxide synthase plays a critical role in nitric oxide production, and inhibition of this enzyme has been shown to speed pulmonary VO_2 on-kinetics in humans (83, 145). Grassi et al. (49) investigated the effect of nitric oxide synthase inhibition on oxygen uptake kinetics at the muscle in the isolated canine GS. The isometric tetanic contraction protocol was the same as previously described (48). During the control condition, a physiological saline solution was infused for 3-min prior to contractions. Inhibition of nitric oxide synthase with 20 $mg \bullet kg^{-1}$ N^{ω}-nitro-L-arginine methyl ester (L-NAME) in 10 ml of physiological saline for three min prior to the start of contractions comprised the treatment condition. Muscle biopsies were obtained in the manner as (47) and analyzed for [ATP], free [ADP], [PCr], [Cr], and $[La^-]$.

L-NAME infusion reduced vascular effects of nitric oxide as evidenced by an ≈50% lower decline in blood pressure after administration of 0.3 μg•kg wet muscle weight $(ww)^{-1}$ acetylcholine in comparison to control. The arteriovenous O_2 difference, resting VO_2, and O_2 extraction percentages were significantly greater at rest after L-NAME infusion in comparison to control. These values were not different upon achievement of steady state VO_2. Significantly less fatigue was seen at min 1, 2, 3, and 4 after L-NAME infusion versus control. No differences in muscle metabolites were seen between conditions. The time to achieve 50% of the steady state VO_2 was not different between conditions. Interestingly, the time to achieve 63% of the steady state VO_2 (mean response time) was slightly but significantly longer in the L-NAME condition in comparison to control. Grassi et al. (49) proposed that the longer mean response time after L-NAME administration was due to a longer time delay, likely caused by the higher VO_2 at rest in comparison to the control condition. The VO_2 kinetics after the time delay, however, showed a similar increase between conditions. Grassi et al. (49) concluded that inhibition of cytochrome c oxidase by nitric oxide does not limit VO_2 on-kinetics at the onset of contractions.

Information from experiments by Grassi et al. (45, 46) using the highly oxidative canine GS contracting *in situ* has provided evidence for the presence of delayed metabolic activation at the onset of contractions to a metabolic rate equal to ≈60-70% VO_2peak. This delayed metabolic activation does not appear to be due to a delay in available acetyl-groups (47) or inhibition of cytochrome c oxidase via nitric oxide (49). The prior exercise effect has not been investigated using the isolated canine GS. This

preparation is ideal for this study as it provides an excellent means by which VO_2 can be measured directly across the contracting muscle, and blood flow can be controlled via pump perfusion. These characteristics are particularly advantageous for elucidating blood flow and metabolic roles. Additionally, as all motor units are stimulated synchronously, the role of altered motor unit recruitment as a main player in the prior exercise effect can be examined.

PURPOSE

It was the purpose of this investigation to: 1) determine the effect of priming contractions on VO_2 on-kinetics in highly-oxidative skeletal muscle; and 2) determine whether O_2 delivery and/or delayed metabolic activation play a role in any measured speeding of VO_2 on-kinetics.

III. Journal Manuscript

MUSCLE OXYGENATION DOES NOT AFFECT THE PRIOR EXERCISE EFFECT

ABSTRACT

It was the purpose of this study to examine the prior exercise effect in highly-oxidative skeletal muscle. A step change in metabolic rate was elicited by stimulating canine gastrocnemius-superficialis muscles (n=5) via their sciatic nerves (6-8 V, 0.2 ms duration, 50 Hz, 200 ms train) at a rate of 2/3 s for two, 2-min bouts separated by 2 min of recovery. VO_2 on-kinetics were determined during four conditions: spontaneous adjustment of self perfused blood flow (spontaneous); maximized O_2 availability (elevated flow); maximized metabolic respiratory stimuli (resting flow); and maximization of both respiratory stimuli and O_2 availability (additive). Near infrared spectroscopy (NIRS) was used to monitor muscle oxygenation. Despite significant alterations in [O_2Hb] prior to the second contractile bout, tau remained unaltered (means: 11.8 vs. 10.6 s) for each condition. Time delay (mean: 6.2 s) and mean response time (mean: 18.0 s) were significantly ($p<0.05$) speeded during bout 2 (mean: 1.9 and 12.5 s, respectively) and the amplitude of the VO_2 slow component was significantly reduced in all conditions after priming contractions (means: 11.0 vs. 28.2 $mlO_2 \bullet kg^{-1} \bullet min^{-1}$). These data indicate that altered O_2 delivery and muscle oxygenation as assessed by NIRS do not play a role in the prior exercise effect in highly-oxidative skeletal muscle.

INTRODUCTION

At the onset of a square wave exercise transition, oxygen uptake (VO_2) increases more slowly than the energy requirement (38), rising exponentially and reaching a steady state in ≈2-3 min if the metabolic requirement is below the lactate (La^-) threshold (78). At the steady state, ATP demand is matched by oxidative phosphorylation. The field of study investigating the controlling factors of O_2 uptake at the onset of exercise is termed "VO_2 on-kinetics". Slowed VO_2 on-kinetics have been measured in individuals with type II diabetes (66), peripheral vascular disease (4, 5), aging (2), heart failure (1, 70), heart transplant (13, 32), heart and lung transplant (26), chronic respiratory diseases (37, 62), HIV infection (12), mitochondrial myopathies and McArdle's disease (33).

At the onset of exercise, VO_2 at the muscle increases in a monoexponential fashion after a short time delay (TD), displaying first order kinetics (141). VO_2 on-kinetics measured at the mouth (pulmonary VO_2 on-kinetics) during exercise performed below the lactate threshold are composed of two components: 1) delay or cardiodynamic component; and 2) primary component (141). Exercise at intensities above the lactate threshold elicit a third component referred to as a slow component (141). In this case, VO_2 continues to drift upwards after the monoexponential response. VO_2 on-kinetics measured across a contracting muscle display primary and slow components after a short TD. The time from the onset of the primary component until ≈63% of the achievement of the steady-state VO_2 (primary time constant; τ) is used to compare VO_2 responses at the onset of exercise. Further, TD and τ are often summated to express mean response time (MRT). Figure 1 depicts VO_2 variables of interest for quantification of the response.

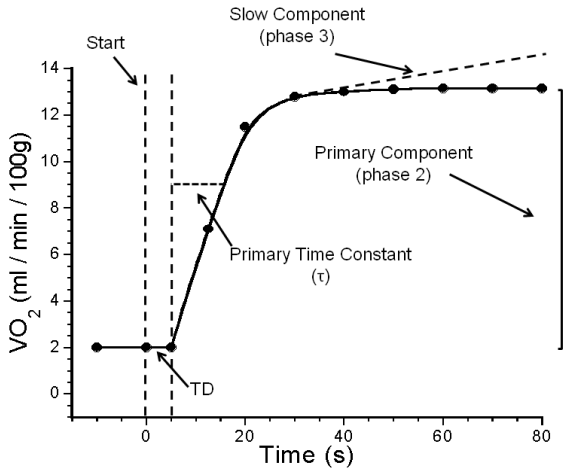

Figure 1. Idealized from Grassi et al. (49). Onset of contractions (Start); Time delay before a rise in VO_2 (TD).

The mechanisms that control oxidative phosphorylation at the onset of exercise remain to be fully elucidated. Two major hypotheses have been postulated: 1) there is a delay in the adjustment of blood flow and thereby O_2 delivery to active muscle that delays the turn-on of oxidative phosphorylation, and/or 2) there is a delay in the accumulation of metabolic signals that stimulate oxidative phosphorylation to drive the VO_2 response.

Decrements in the fraction of inspired O_2 (45, 54, 55, 61), cardiac output (41, 44, 47), and perfusion pressure to the exercising muscles (42, 43, 46, 57) slow VO_2 on-kinetics. However, in normal conditions, the blood flow response to exercising muscles is more than adequate (80); and at least as fast (34) or faster (14, 22) than the VO_2 response for upright exercise. The most direct evidence against the O_2 delivery hypothesis, at least in highly-oxidative skeletal muscle, comes from investigations that measured VO_2 directly across the contracting muscle with elevated O_2 delivery (27, 28).

VO_2 on-kinetics at the onset of a submaximal metabolic rate were not speeded in these experiments (27, 28). Thus, increases in necessary metabolic stimuli of respiration within the contracting muscle fibers themselves appear to be responsible for the lag in VO_2 at the onset of submaximal exercise.

The metabolic activation hypothesis posits that a delay in the increase of metabolic signals that control respiration (e.g., [ADP], [P_i], [NADH]) is responsible for the lag in VO_2 at the onset of exercise. Metabolic pathways that buffer an increase in these metabolites may slow VO_2 on-kinetics. For example, inhibition of creatine kinase (CK) in skeletal muscle (31) speeds VO_2 on-kinetics. The specific metabolic sites of importance are difficult to investigate and are convoluted by interaction of metabolic respiratory stimuli with O_2 delivery (40, 74).

An interesting model that has been used to study the controlling mechanisms of VO_2 on-kinetics is prior exercise. In these experiments, two or more bouts of exercise are performed and VO_2 on-kinetics are compared. The first bout is considered a "priming" bout. These investigations have established that the priming exercise bout must be performed above the lactate threshold (supra-LT) (e.g., (68)) to alter the second bout. Controversy exists as to which VO_2 on-kinetics parameters are affected by prior exercise (e.g., (68) and (73)). Similarly, the mechanism(s) by which priming exercise speeds VO_2 on-kinetics of a second bout are not yet well established, but current evidence indicates increased metabolic activation (e.g., (39)), enhanced O_2 availability (e.g., (15)), and/or altered motor unit recruitment (e.g., (8)) as possible causes.

Understanding the mechanisms responsible for speeded kinetics after prior exercise might lead to elucidation of the mechanisms that control oxidative phosphorylation *in vivo*.

To date, the effect of priming exercise has not been investigated using a model that allows direct measurement of VO_2 across an exercising muscle and modulation of muscle blood flow. The isolated canine gastrocnemius muscle complex (gastrocnemius plus superficial digital flexor; GS) is ideal for this study as both can be done. These characteristics are advantageous for clarifying the roles of metabolic activation and O_2 delivery in the prior exercise effect. Additionally, as all motor units are stimulated synchronously in this model, the role of altered motor unit recruitment as a main player in the prior exercise effect can be examined. Therefore, it was the purpose of this investigation to: 1) determine if a bout of priming contractions alters VO_2 on-kinetics in highly-oxidative skeletal muscle; and 2) determine whether O_2 delivery and/or metabolic activation play a role in any measured speeding of VO_2 on-kinetics.

METHODS AND PROCEDURES

***Animals*.** Five, adult, mongrel hounds (canis familiaris) of either sex were used. All procedures performed were approved by the Auburn University Institutional Care and Use Committee (PRN 2007-1185). Dogs had access to food and water *ad libitum*.

***Animal Preparation*.** In all cases, animals were anesthetized and intubated. Briefly, dogs were anesthetized with an intravenous injection of sodium pentobarbital (30 $mg \bullet kg^{-1}$) with maintenance doses given as required to maintain a deep, surgical plane of

anesthesia. Upon anesthetization, animals were intubated with an endotracheal tube. A heating pad was placed under the animal and adjusted as needed to maintain the rectal temperature near 37° C. Prior to the start of the experimental protocols, animals were mechanically ventilated (tidal volume: ≈20 ml•kg^{-1}; breath frequency: ≈15 breaths•min^{-1}) for the duration of the experiment.

***Surgical Preparation*.** In these experiments, the left GS muscle group was surgically isolated as previously described (72). Briefly, a medial incision was made through the skin of the left hindlimb from midthigh to the ankle. All muscle which overlaid the GS (sartorius, gracilis, semitendinosus, and semimembranosus) were cut with a cauterizing blade at their insertions and laid back. Venous outflow from the GS was isolated by ligating all veins draining into the popliteal vein except the GS veins. The popliteal vein was cannulated, and its venous blood flow was returned to the animal via a reservoir attached to a cannula in the left jugular vein. Arterial circulation to the GS was isolated by ligation of all vessels from the popliteal artery that did not enter the GS. The right carotid artery was also cannulated and a blood pressure transducer (model RP-1500, Narco Biosystems) inserted for measurement of systemic and perfusion (pump controlled) pressure. This was facilitated by a T-connector in the tubing. In the case of pump perfusion, blood from the carotid artery was passed through tubing to a peristaltic pump (Gilson Miniplus 3, Gilson Incorporated, Middleton, WI) and through another cannula into the contralateral, isolated popliteal artery supplying the GS.

A portion of the calcaneus, with the two tendons from the GS attached, was cut away at the heel and clamped around a metal rod for connection to an isometric

myograph via a load cell (Interface SM-250) and a universal joint coupler. The universal joint coupler allowed the muscle to consistently pull in a direct line with the load cell and thus prevented the development of significant torque. The other end of the muscle remained attached to its origin. Both the femur and the tibia were fixed to the base of the myograph by bone nails. A turnbuckle strut was placed parallel to the muscle between the tibial bone and the arm of the myograph to minimize flexing of the myograph.

The sciatic nerve was exposed and isolated near the GS. The distal stump of the nerve, ≈1.5-3.0 cm in length, was pulled through a small epoxy electrode containing two wire loops for stimulation. Exposed tissues were covered with saline-soaked gauze and a thin plastic sheet to minimize drying and cooling. After each experiment, the GS was removed from the animal, cleared of surface connective tissue and weighed. Weight was used to normalize several variables to muscle mass (e.g., VO_2). All dogs were euthanized at the end of the experiment with an overdose of sodium pentobarbital and potassium chloride.

At the start of each experiment, the GS was set at optimal length (L_o) by progressively lengthening it until a peak in developed tension was obtained (stimulated at a rate of 0.2 Hz). Once L_o was obtained, five minutes of rest were allowed before studies began. Optimal length was reset prior to each bout of contractions.

***Experimental Design*.** Each dog (n=5) was used for four experimental protocols (Figure 1). In all cases, the GS was stimulated (Grass S48 stimulator, West Warwick, RI, USA) to contract tetanically (8 V, 50 Hz, .2 ms; train duration 200 ms) for 2 min at a rate of 2 contractions per 3 s. This contraction protocol elicits a metabolic rate of ≈70-80%

VO_2 peak. After 2 min, contractions were stopped and a 2 min recovery period began. A recovery period of 2 min was chosen due to the rapid recovery of this highly-oxidative muscle (64). Upon completion of the 2-min recovery period, contractions were once again elicited for 2 min. Protocol 1 (Spontaneous) was designed to study VO_2 on-kinetics during spontaneous adjustment of self-perfused blood flow with and without a prior bout of contractions.

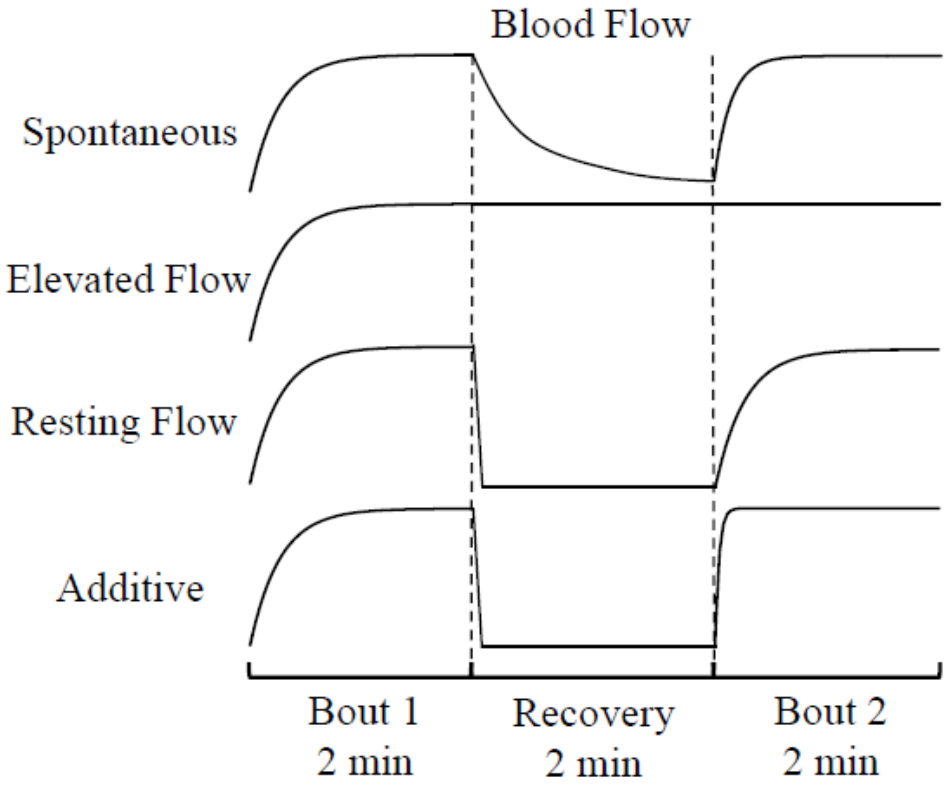

Figure 2. Graphic representation of the four experimental protocols.

For protocols 2-4, blood flow from the right carotid artery was directed to the left popliteal artery via tubing. This tubing was passed through a peristaltic pump (Minipuls 3, Gilson Incorporated, Middleton, WI) to allow for control of blood flow to the contracting GS. Computer software (706 developer's kit, Gilson) and a program developed in-house were used to control the pump via an interface box (RS-232 to RS-

485 converter; 508 box, Gilson). Baseline and steady-state blood flow and the primary time constant (τ) for the first bout of protocols 2-4 were set to the values measured during the first bout of protocol 1. All differences in protocols 2-4 occurred upon cessation of the first contractile bout. These experimental protocols were randomized.

Protocol 2 (Elevated Flow) – This protocol was designed to maximize O_2 delivery to the contracting muscle during recovery. Upon completion of the first 2-min contractile bout, blood flow was maintained at the steady-state contraction level for the duration of the 2-min recovery period and through the second 2-min contractile bout. Maintenance of blood flow at the steady-state contraction level will increase O_2 delivery and decrease metabolite concentrations (e.g., ADP). Accordingly, the purpose of this protocol was to maximize O_2 delivery to the muscle during recovery and at the onset of the second bout of contractions while minimizing metabolic signals of respiration prior to and during the second contractile bout.

Protocol 3 (Resting Flow) – Upon completion of the first 2-min contractile bout, blood flow was rapidly decreased to the resting flow measured prior to bout 1 of protocol 1 for the 2-min recovery period. At the start of the second contractile bout, the same blood flow kinetics as the first bout were implemented. The purpose of this protocol was to maximize metabolic stimuli of respiration by minimizing O_2 delivery and availability during the recovery period.

Protocol 4 (Additive) – This protocol was designed to examine the possibility of an additive effect between O_2 delivery and metabolic stimuli of respiration. At the end of the first contractile bout, blood flow was rapidly returned to the resting blood flow as in

Protocol 3. However, the τ for blood flow turn-on at the onset of the second bout of contractions was set to 2 s, to yield an extremely rapid adjustment of convective O_2 delivery.

***Measurements*.** Outputs from the pressure transducer and load cell (first through strain gauge couplers), ultrasonic flowmeter (T206, Transonic Systems, Ithaca, NY; first through a transducer coupler), and indwelling inline oximeter probe (Transonic Systems Incorporated, Ithaca, NY) connected to an oximeter (Oximetrix 3, Abbott Laboratories, North Chicago, IL) were fed into a computerized data acquisition system (Oxymon MkIII, Artinis Medical Systems BV). All signals were sampled at a rate of 125 Hz. The load cell reaches 90% of full response within 1 ms while the flowmeter was set to its maximal pulsatile cutoff frequency of 100 Hz. The load cell was calibrated with known weights prior to each experiment. The flowmeter was manually calibrated with a graduated cylinder and clock during and after each experiment. The Oximetrix 3 sampled percent hemoglobin saturation (SO_2, %) at a rate of 244 samples per second, averaged the samples each second, and then gave an output of a 5-s rolling average each second. This output has a 90% response time of 5 s. The time response of this output was further decreased via mathematical deconvolution based on the Oximetrix 3's response to square-wave changes induced by rapidly moving the probe between two tubes of blood containing different SO_2 values.

Samples of arterial blood entering the muscle and of venous blood from the popliteal vein were drawn anaerobically into 3 ml plastic syringes. Since the arterial values varied only slightly throughout each experiment, arterial samples were taken

before and after each experimental protocol. Venous blood samples were collected from the catheter draining the muscle at rest and at the end of the second contraction bout for each condition. These samples were used to calibrate the Oximetrix 3 signal. Blood samples were capped and immediately stored in ice water until analyzed (within 30 min of collection). Both arterial and venous blood samples were analyzed at 37° C for PO_2, PCO_2, and pH by a blood gas, pH analyzer (GEM Premier 3000, Instrumentation Laboratory Company, Lexington, MA), and for hemoglobin concentration ([Hb]) and SO_2 with a CO-oximeter (682 CO-Oximeter, Instrumentation Laboratory Company, Lexington, MA) set for dog blood. These instruments were calibrated before and during each set of sample measurements.

VO_2 of the GS was calculated by Fick's principle as $VO_2 = Q \bullet C(a\text{-}v)O_2$, where Q is the blood flow and C(a-v) is the difference in O_2 concentration between the arterial and venous blood. Samples for blood flow and venous SO_2 (following deconvolution) were averaged over each contraction cycle (≈1.5 s) to obtain contraction-by-contraction VO_2. Blood samples were also taken prior to each experimental protocol to ensure physiological arterial values. Sodium bicarbonate and O_2 were administered and ventilation adjusted as required to maintain appropriate pH, PCO_2, PO_2, and SO_2. Normal saline (0.9%) was infused at a rate of 0.03 $ml \bullet kg^{-1} \bullet min^{-1}$ for most of the experiments.

Analysis of VO_2 and blood flow on-kinetics. VO_2 and blood flow on-kinetics data were fit by the monoexponential function (29):

$$\mathbf{y(t) = y_{Bas} + A(1 - e^{-(t - TD)/\tau})}$$

in order for trials to be compared mathematically. The abbreviations are as follows (29): y_{Bas} is the baseline value, A is the amplitude between y_{Bas} and the steady-state value during contractions, TD is the time delay (time before any observed change), and τ is the time constant (time to achieve ≈63% of steady-state) for the function. To facilitate comparison with results obtained by previous investigations using this model (27, 28, 30), mean response time (MRT) was calculated via summation of TD and τ. In order to attain the highest confidence interval for τ values, only the primary component of each VO_2 and blood flow response was fit (67, 77). The fitting window was progressively expanded and curve fits compared. The end of the primary component (and thus start of a slow-component) was determined as the data point before which the confidence interval for τ, Chi^2/degrees of freedom ratio, and residuals became progressively worse. This also allowed for determination of primary and slow-component amplitudes. VO_2 on-kinetics variables (baseline, tau, primary amplitude, slow component amplitude, time delay, and mean response time) for all four conditions were compared.

NIRS. Muscle oxygenation was analyzed with a continuous wave near-infrared spectroscopy (NIRS) system (Oxymon MkIII, Artinis Medical Systems, BV). Briefly, two fiber-optic bundles communicate between the data acquisition system and the muscle. At the end of one cable, NIR light is emitted from an optode in two wavelengths (784 and 860 nm); at the end of the other cable, NIR light is absorbed through an optode

and transmitted back to the data acquisition center. Since deoxyhemoglobin (HHb) and oxyhemoglobin (O_2Hb) absorb NIR light maximally at different wavelengths, it is possible to distinguish between the relative oxygenation of these chromophores. Total [Hb] ([tHb]) is calculated from the sum of [O_2Hb] and [HHb]. Currently, controversy exists over how much myoglobin (Mb) contributes to the NIRS signal (e.g., (6)). However, it is beyond the scope of this paper to discuss the controversy. In current experiments, the optodes were fixed in place on the GS by a Velcro strap. Opaque black plastic was placed over the optodes to block external light. Signals are relative (μM) and were biased to zero prior to each contraction period. Signals were averaged over each contraction cycle.

***Statistical Analyses*.** Data for blood flow tau, baseline, primary amplitude, and slow component amplitude for the spontaneous bouts were compared via a 1-way repeated measures ANOVA. All VO_2 on-kinetics, NIRS, baseline and end-exercise data were compared between conditions and bouts using a 2-way ANOVA with repeated measures on both factors (Bout and Condition). In cases where significant differences were found in the absence of significant interaction, a 1-way repeated measures ANOVA was used to examine the significance. When a significant interaction was found, simple main effects were determined. Duncan's post hoc test was used to elucidate the site of significance in the absence of interaction and also when a significant simple main effect was found. Level of significance for all statistical procedures was set to $p < 0.05$. Significance values of $p > 0.05$ but < 0.10 were taken as a tendency or trend for significance.

RESULTS

Baseline values for bout 1 and 2. Although some statistically significant differences were noted for PaO_2 and $PaCO_2$, they were physiologically minor. Baseline PaO_2 ranged from 82 to 149 and 84.5 to 145 Torr for bouts 1 and 2, respectively. $PaCO_2$ at baseline ranged from 29 to 35 Torr for bout 1 and 29.5 to 35 Torr for bout 2. CaO_2 (20.4±2.4 ml•dl^{-1}), [tHb] (15.1±1.8 g•dl^{-1}), and arterial pH (7.39±0.02) did not differ at baseline between bouts.

Baseline values for blood flow (Q), O_2 delivery (Q • CaO_2), and VO_2 are presented in Table 1. Baseline values for these variables did not differ between conditions prior to bout 1, indicating a physiologically similar state. Prior to the start of the second bout, VO_2 was significantly greater for each condition in comparison to the first bout (Table 1). In addition, VO_2 for the elevated flow and resting flow conditions was significantly greater than the spontaneous and additive conditions for bout 2. Of specific interest to the experimental protocol, Q and Q • CaO_2 were significantly greater prior to the second bout of the elevated flow condition. Blood flow and Q • CaO_2 did not differ between the spontaneous, resting, or additive conditions prior to bout 2. Thus, modulation of blood flow and O_2 delivery as outlined in the *Experimental Design* section was successful.

Table 1. Baseline blood flow, O_2 delivery, and O_2 utilization.

Q ($ml \bullet kg^{-1} \bullet min^{-1}$)

	Spontaneous	Elevated Flow	Resting Flow	Additive
Bout 1	136.1±51.6	211.2±50.9	204.5±39.5	221.2±54.0
Bout 2	233.0±63.7*	1202.6±140.9*†‡	221.4±51.0	216.2±52.8

Q • CaO_2 ($mlO_2 \bullet kg^{-1} \bullet min^{-1}$)

	Spontaneous	Elevated Flow	Resting Flow	Additive
Bout 1	28.8±13.0	42.9±14.5	42.9±10.8	45.0±15.1
Bout 2	48.0±14.6	245.8±57.4*†‡	46.2±13.6	43.9±14.4

VO_2 ($ml \bullet kg^{-1} \bullet min^{-1}$)

	Spontaneous	Elevated Flow	Resting Flow	Additive
Bout 1	3.3±0.9	4.8±0.7	3.8±1.2	3.9±1.2
Bout 2*	11.3±2.2	22.0±3.8†	20.7±6.3†	15.1±5.2

Data are presented as means±SD. Q: blood flow; Q • CaO_2: O_2 delivery. * indicates significance ($p<0.05$) from bout 1. † indicates significance ($p<0.05$) from the spontaneous and additive conditions. ‡ indicates significance ($p<0.05$) from the resting flow condition. Per kg indicates per kg of wet muscle weight.

Bout 1 and 2 end-exercise values for each condition. Mean end-exercise CaO_2 (20.4±2.4 $ml \bullet dl^{-1}$) did not differ between conditions or bouts. Values for Q, Q • CaO_2, and VO_2 are presented in Table 2. Blood flow and Q • CaO_2 were significantly greater for the elevated flow, resting flow, and additive conditions in comparison to the spontaneous condition. No differences existed between bouts 1 and 2. End-exercise VO_2 did not differ among conditions or between bouts.

Table 2. End-exercise blood flow, O_2 delivery, and O_2 utilization.

Q ($ml \cdot kg^{-1} \cdot min^{-1}$)

	Spontaneous	Elevated Flow*	Resting Flow*	Additive*
Bout 1	1085.0±115.8	1230.5±138.4	1245.9±137.8	1232.8±138.0
Bout 2	1107.5±135.8	1208.8±134.3	1229.4±134.0	1228.4±132.4

$Q \bullet CaO_2$ ($mlO_2 \cdot kg^{-1} \cdot min^{-1}$)

	Spontaneous	Elevated Flow*	Resting Flow*	Additive*
Bout 1	226.1±43.6	257.1±53.2	260.5±55.3	257.8±55.1
Bout 2	229.4±49.2	251.1±51.8	255.5±53.8	255.3±54.1

VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)

	Spontaneous	Elevated Flow	Resting Flow	Additive
Bout 1	187.5±30.1	177.3±28.8	182.4±27.7	172.1±29.3
Bout 2	188.8±31.4	180.5±29.5	184.4±30.4	182.6±32.9

Data are presented as means±SD. Q: blood flow; $Q \bullet CaO_2$: O_2 delivery. * indicates significance ($p<0.05$) from the spontaneous condition. Per kg indicates per kg of wet muscle weight.

Blood flow kinetics. Mean blood flow kinetics for bouts 1 and 2 of the spontaneous condition are depicted in Figure 2. Data pertaining to blood flow kinetics for bouts 1 and 2 of the spontaneous condition are presented in Table 3. Baseline, primary amplitude, and slow component amplitude were not significantly different between bouts. Mean response time for the spontaneous condition, however, was significantly shorter for the second bout of contractions.

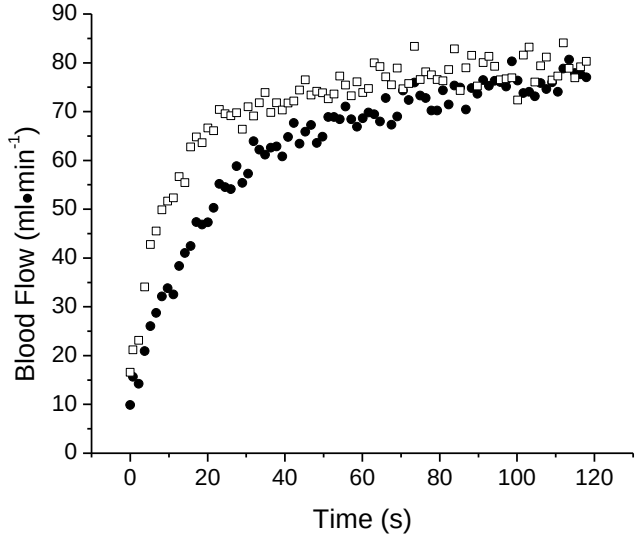

Figure 3. Mean blood flow kinetics for bout 1 (closed circles) and 2 (open squares) during the spontaneous condition.

Table 3. Blood flow kinetics for the spontaneous condition.

	Baseline ($ml \cdot kg^{-1} \cdot min^{-1}$)	PA ($ml \cdot kg^{-1} \cdot min^{-1}$)	MRT (s)	SCA ($ml \cdot kg^{-1} \cdot min^{-1}$)
Bout 1	9.9±4.6	63.2±14.0	19.7±4.0	4.5±3.3
Bout 2	16.6±4.7	57.7±15	9.9±1.8*	4.8±2.0

Data are presented as means±SD. * indicates significance ($p<0.05$) from bout 1. PA: primary amplitude; MRT: mean response time; SCA: slow component amplitude. Per kg indicates per kg of wet muscle weight.

Blood flow kinetics as assessed via MRT were similar for all conditions for bout 1 (Table 4). Mean response time was also similar between bouts for the resting flow condition. Blood flow kinetics for bout 2 of the additive condition were faster than both bouts 1 and 2 of the spontaneous condition (Table 4). Thus, the planned blood flow kinetics protocol as outlined in the *Experimental Design* section was successful.

Table 4. Blood flow mean response time (s) for bouts 1 and 2 of each condition.

	Spontaneous	Elevated Flow	Resting Flow	Additive
Bout 1	19.7±4.0	20.0±5.4	20.1±5.7	20.1±4.7
Bout 2	9.9±1.8	0±0	20.0±4.6	4.1±0.4

Data are presented as means±SD.

VO_2 on-kinetics. Figure 3 (A-D) depicts the mean contraction-by-contraction VO_2 data obtained for bouts 1 and 2 of each condition. Data pertaining to tau, TD, and MRT are presented in Table 5. Tau values were not different among the different conditions for bout 1 or bout 2, nor for bout 2 vs. bout 1 for any of the conditions. However, TD was significantly shorter for bout 2 of each condition (Table 5). Additionally, the TD for bout 2 of the additive condition was significantly shorter than TD in the other conditions. Mean response time was significantly shorter for bout 2 of each condition (Table 5).

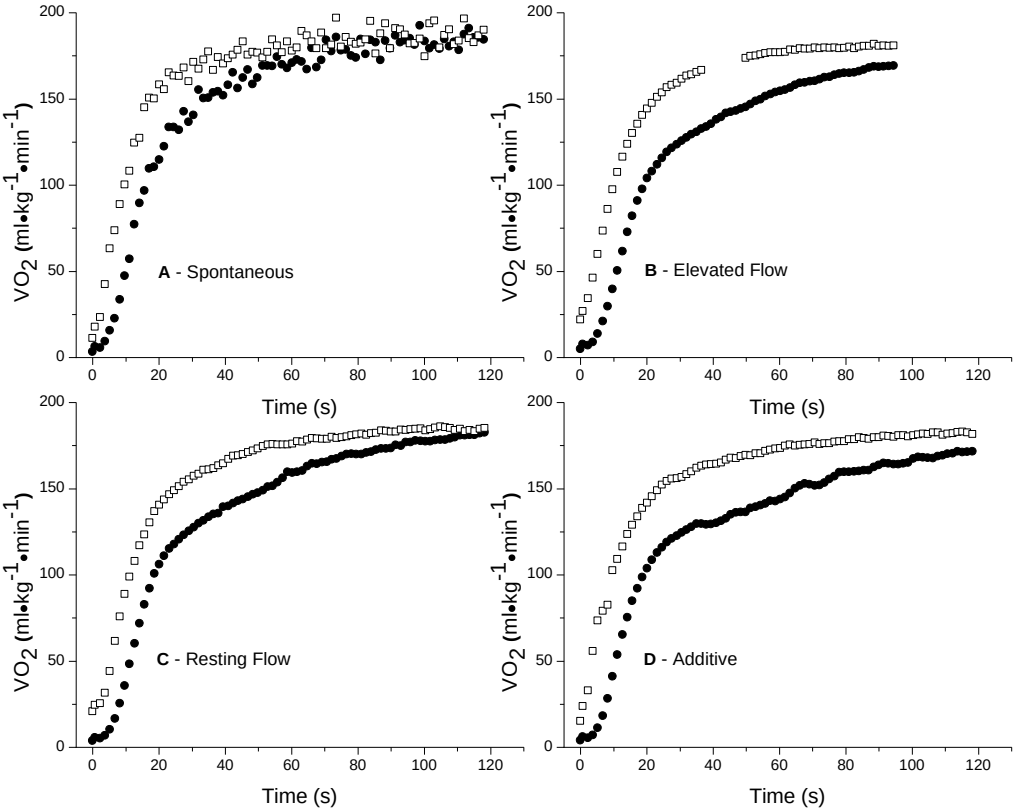

Figure 4. Mean contraction-by-contraction VO_2 data for bout 1 (closed circle) and 2 (open square) of the spontaneous (A), elevated flow (B), resting flow (C), and additive (D) conditions. Data were removed from ≈40-50 s in bout 2 of the elevated flow condition due to non-physiological artifact from the indwelling oximeter in one animal. The artifact was determined as such on the basis of normal force, blood flow, blood pressure, and muscle oxygenation during this time. Data were clipped prior to 120 s in the elevated flow condition for the same reason.

Table 5. VO_2 on-kinetics data.

tau (s)

	Spontaneous	Elevated Flow	Resting Flow	Additive
Bout 1	12.5±4.5	13.2±2.7	11.1±1.6	10.2±1.4
Bout 2	9.8±3.6	10.7±1.4	10.4±2.0	11.6±2.8

TD (s)

	Spontaneous	Elevated Flow	Resting Flow	Additive
Bout 1	5.6±1.2	5.9±0.2	6.9±0.6	6.4±0.6
Bout 2*	1.9±1.7	2.2±0.7	3.4±0.4	0.02±0.04†

MRT (s)

	Spontaneous	Elevated Flow	Resting Flow	Additive
Bout 1	18.1±4.0	19.2±2.8	18.1±2.0	16.6±1.2
Bout 2*	11.7±3.0	12.9±1.7	13.8±2.1	11.6±2.8

Data are presented as means±SD. TD: time delay; MRT: mean response time. * indicates significance ($p<0.05$) from bout 1. † indicates significance ($p<0.05$) from other three conditions.

The amplitude and asymptote of the primary VO_2 component and amplitude of the VO_2 slow component are presented in Table 6. Initial 2-way ANOVA analysis revealed that the primary amplitude was greater in the spontaneous condition than in the elevated flow, resting flow, and additive conditions. A follow up 1-way ANOVA suggested that this difference was due to bout 1. The 2-way ANOVA also indicated that the primary amplitude of bout 1 was different from bout 2. Follow-up ANOVAs suggested that this significant main effect was likely due to a significantly lower bout 1 in the elevated flow and additive conditions.

Table 6. VO_2 amplitudes and asymptotes.

Primary Amplitude ($mlO_2 \cdot kg^{-1} \cdot min^{-1}$)

	Spontaneous	Elevated Flow	Resting Flow	Additive
Bout 1	165.6±25.2	144.5±25.3†	142.4±14.7†	133.9±25.8†
Bout 2*	168.3±30.7	151.5±27.9	147.9±29.1	155.2±29.6

Primary Asymptote ($mlO_2 \cdot kg^{-1} \cdot min^{-1}$)

	Spontaneous	Elevated Flow	Resting Flow	Additive
Bout 1	168.9±24.7	149.4±25.4†	146.2±14.0†	137.9±26.4†
Bout 2*	179.5±29.5	173.5±26.9	168.6±30.6	169.9±32.8

Slow Component Amplitude ($mlO_2 \cdot kg^{-1} \cdot min^{-1}$)

	Spontaneous	Elevated Flow	Resting Flow	Additive
Bout 1	17.4±15.7	26.3±4.2	35.4±16.6†	33.6±11.0†
Bout 2*	8.6±5.0	7.2±9.2	15.8±12.4	12.5±15.2

Data are presented as means±SD. * indicates significance ($p<0.05$) from bout 1. † indicates significance ($p<0.05$) from the spontaneous condition. Per kg indicates per kg of wet muscle weight.

A significant main effect for condition and bout was found for the asymptote of the primary component (Table 6). Follow up ANOVAs suggested that bout 1 of the spontaneous condition was significantly greater than bout 1 of the other three conditions. A follow-up 1-way ANOVA also suggested that the primary asymptotes for bout 2 of the elevated flow and additive conditions were greater than their respective bout 1 values. Though not significantly different, bout 1 and 2 of the resting flow condition tended towards significance ($p=0.067$).

The amplitude of the VO_2 slow component for each condition and bout is presented in Table 6. The amplitudes of all bout 2 curves were significantly lower than the bout 1 amplitudes. Follow up ANOVAs suggested that all of the bout 2 slow component amplitudes were less than the bout 1 amplitudes with the exception of the spontaneous condition. The absence of a significant difference for the spontaneous

condition arose due to a lack of reduction in the amplitude of one animal. Also of note is that the slow component amplitudes of bout 1 for the resting flow and additive conditions were significantly greater than that of the spontaneous condition.

NIRS. Figure 4 depicts the mean NIRS responses for bouts 1 and 2 of each condition. Data pertaining to muscle oxygenation are presented in Tables 7 (baseline) and 8 (end-exercise). Baseline [O_2Hb] was not significantly different between bouts for the spontaneous or additive conditions. However, [O_2Hb] was significantly greater for bout 2 in comparison to bout 1 for the elevated flow condition. Bout 2 [O_2Hb] for the elevated flow condition was also significantly greater than [O_2Hb] for bout 2 of the other three conditions. Thus, the elevated flow condition maximized O_2 availability. [O_2Hb] was significantly lower prior to bout 2 in comparison to bout 1 for the resting flow condition. Further, bout 2 [O_2Hb] for the resting flow condition was significantly lower than bout 2 for the other three conditions. Accordingly, the resting flow condition minimized O_2 availability at the start of bout 2.

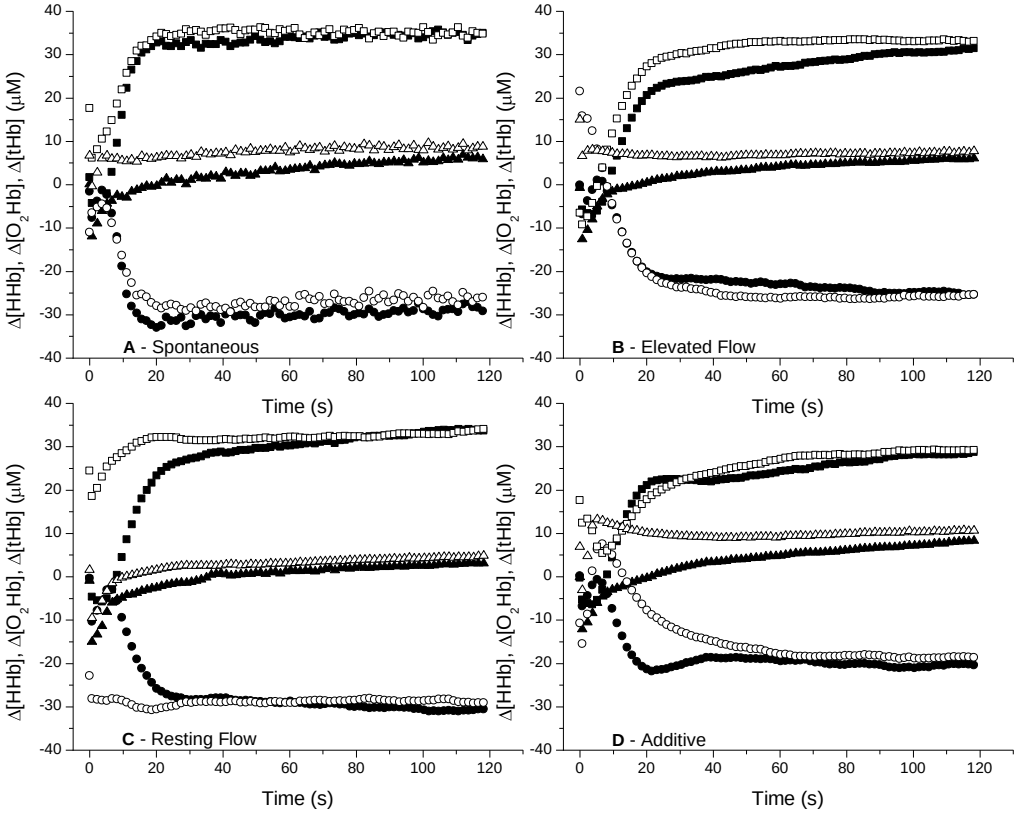

Figure 5. Mean NIRS data for bouts 1 and 2 of the spontaneous (A), elevated flow (B), resting flow (C), and additive (D) condition. Closed circle (bout 1 [O_2Hb]), closed square (bout 1 [HHb]), closed triangle (bout 1 [tHb]); Open circle (bout 2 [O_2Hb]), open square (bout 2 [HHb]), open triangle (bout 2 [tHb]).

Table 7. Baseline [O_2Hb] and [HHb] as assessed by NIRS.

[O_2Hb] (μM)

	Spontaneous	Elevated Flow	Resting Flow	Additive
Bout 1	-1.6±2.8	-0.1±1.0	-0.4±0.5	0.2±1.6
Bout 2	-11.0±6.1	21.5±9.6*†	-22.8±8.3*‡	-10.7±15.0

[HHb] (μM)

	Spontaneous	Elevated Flow	Resting Flow	Additive
Bout 1	1.7±0.4	-0.6±0.4	-0.6±2.1	-0.3±1.3
Bout 2	17.7±1.8*	-6.5±3.3†	24.4±3.6*	17.6±13.8*

Data are presented as means±SD. * indicates significance ($p<0.05$) from bout1. † indicates significantly ($p<0.05$) greater than other three conditions. ‡ indicates significantly ($p<0.05$) less than other three conditions. Negative values indicate a decrease in concentration after optodes were biased.

Table 8. End-exercise [O_2Hb] and [HHb] as assessed by NIRS.

[O_2Hb] (μM)

	Spontaneous	Elevated Flow	Resting Flow	Additive
Bout 1	-32.4±3.7	-25.3±5.8	-30.6±7.0	-20.4±11.8
Bout 2	-30.4±7.5	-25.4±8.3	-29.1±7.4	-18.6±11.8

[HHb] (μM)

	Spontaneous	Elevated Flow	Resting Flow	Additive
Bout 1	38.2±8.2	31.7±4.0	33.7±3.4	28.7±13.8
Bout 2	38.4±10.0	33.1±6.5	34.0±4.5	20.6±22.2

Data are presented as means±SD. Negative values indicate a decrease in concentration after optodes were biased.

[HHb] at baseline of the second bout was significantly greater in comparison to bout 1 for the spontaneous, resting flow, and additive conditions, respectively. Baseline [HHb] prior to bout 2 of the elevated flow condition was significantly lower than in the other conditions, but not significantly different from bout 1. [O_2Hb] and [HHb] at the end of each contractile bout were not significantly different between bouts or among conditions (Table 8).

DISCUSSION

The purpose of this investigation was to: 1) determine the effect of a priming bout of contractions on VO_2 on-kinetics in highly-oxidative skeletal muscle; and 2) determine whether O_2 delivery and/or metabolic activation play a significant role in any measured alterations in VO_2 on-kinetics. The main findings of this investigation were that: 1) prior contractions speeded TD for all conditions; and this speeding resulted in a shorter MRT; 2) prior contractions did not speed the primary τ in any condition; 3) prior contractions reduced the slow component amplitude of all conditions; and 4) muscle oxygenation status did not alter the VO_2 on-kinetics response (tau, TD, and MRT). In other words, regardless of the intervention employed during recovery and throughout the second contractile bout (i.e., elevated flow, resting flow, rapid flow adjustment), TD (and therefore MRT) was shorter and the slow component was reduced in the second bout.

TD. One of the key findings of this investigation was that TD was decreased for all four conditions after priming exercise. This differs from most of the human pulmonary VO_2 data in which a speeding of TD (or a faster MRT due to a shortened TD) was absent (9-11, 17, 18, 21, 22, 35, 52, 63, 68, 73). At least part of the TD is due to the transit time for venous blood from the exercising musculature to reach the site of measurement (3), and correction for this time yields faster MRT values (TD + tau) (3, 24, 75). Accordingly, the duration of the TD will vary with the rate of blood flow (19, 34). A lack of alteration in TD after priming exercise in humans coincides with non-speeded blood flow kinetics (18, 22, 63), and thus unaltered transit times. It is therefore possible that at least part of the reduction in TD for the second bout of the spontaneous condition

in the current study was due to faster blood flow kinetics in the second bout (MRT of 9.9±1.8 vs. 19.7±4.0 s). Further, this may at least partially explain the shortened TD when blood flow was maintained at the steady-state value during recovery and throughout the second contractile bout in the elevated flow condition. However, using indocyanine green to correct for transit time, Bangsbo et al. (3) found that a TD still existed, albeit shorter than previously suggested (34). This suggested that at least part of the TD exists in the muscle itself.

In support of an intramuscular origin to the TD, the resting flow condition of the present experiment yielded a significantly shorter TD for bout 2 (3.4±0.4 vs. 6.9±0.6 s). In this condition, the reduced TD could not have been due to a faster transit time as the blood flow at baseline (Table 1) and blood flow kinetics (Table 4) were not different for bout 2 vs. bout 1. Though no muscle biopsy samples were taken, the elevated VO_2 at baseline of the second contractile bout accompanied by similar O_2 delivery as in the first bout indicates that metabolic stimuli of respiration (e.g., ADP) were likely elevated. The reduction in TD for the second bout of the resting flow condition agrees with findings by other researchers using animal models (7, 39, 49). For example, Kindig et al. (49) calculated an ≈2 s TD at the onset of a single bout of contractions in isolated myocytes. The existence of a delay without a blood flow transit time supports an intracellular origin. Also using isolated myocytes, Hogan (39) found that the TD for the fall in intracellular PO_2 at contraction onset was significantly speeded by a prior contractile bout. Similarly, Behnke et al. (7) observed a shorter TD for the decrease in microvascular PO_2 at the

onset of a second contractile bout in the spinotrapezius muscle of rats, implying a shorter TD in VO_2.

Of particular interest is that the additive condition of the present investigation yielded a TD for the second bout of contractions (0.02±0.04 s) that was significantly shorter than all other conditions. The rapid blood flow kinetics surely shortened the transit time. However, if this incredibly short TD (essentially zero) was solely due to a shortened transit time, then it would not have been faster than the second bout of the elevated flow condition. An elevated VO_2 in comparison to the first contractile bout with similar O_2 delivery implies elevated concentrations of respiratory stimuli. As oxidative metabolism would already be 'activated' prior to the second bout, this 'activation' in combination with the rapid increase in convective O_2 delivery at the onset of the second bout could have led to the shortest TD (81, 82).

Primary τ. Reports on the effect of priming exercise on the primary τ in humans are inconsistent (e.g., (9, 11, 15, 68, 73)). Burnley et al. (11) found no effect of prior high-intensity leg cycling on the primary τ of a second, moderate bout. These results were duplicated by the same research group (8-11) and others (e.g., (68)) for supra-LT exercise in both bouts. In contrast, Tordi et al. (73) measured a speeding of tau during a second bout of high-intensity cycling. Recently, DeLorey et al. (15) reported a speeding of the primary τ for moderate intensity exercise preceded by high-intensity exercise.

In the classic experiment by Gerbino et al. (23), it was suggested that the speeding of pulmonary VO_2 kinetics after prior supra-LT exercise was due to an acidosis-linked vasodilation that resulted in greater and more rapid muscle perfusion (23, 56, 73). In

support of this postulation, MacDonald et al. (56) found that alterations in PCO_2, pH, and $[La^-]$ after prior exercise resulted in elevated blood flow prior to the start and for the first 30 s of a second exercise bout. Tordi et al. (73) implemented prior fatiguing sprint exercise to induce acidosis (60, 71) and assess VO_2 on-kinetics. The primary τ was speeded during the second bout in comparison to the first. However, a recent investigation by Sahlin et al. (68) found that the primary τ was not speeded despite an $\approx$16 fold greater blood $[La^-]$ prior to the second bout and a greater acidosis throughout this bout in comparison to the first. Although blood or muscle $[La^-]$ was not measured in the present investigation, a recent report (29) found only a modest increase in muscle $[La^-]$ after 4 min of contractions in the contracting canine GS using the same stimulation pattern as the present set of experiments. The rapid recovery of this highly-oxidative muscle (64) suggests that $[La^-]$ and pH would only be minimally/moderately altered at the onset of the second contractile bout.

A distinct advantage to our investigation is that we were able to measure and control blood flow to the contracting GS. Thus, we were able to directly investigate the role of blood flow. During the spontaneous condition, blood flow MRT was significantly shorter for the second contractile bout in comparison to the first (9.9±1.8 vs. 19.7±4.0 s). Despite this more rapid adjustment of blood flow delivery, the VO_2 primary τ was not significantly altered (Table 5). This differs from the work of MacDonald et al. (56) who observed an elevated blood flow and VO_2 for the first 30 s of a second exercise bout in comparison to a first. No sophisticated modeling techniques were used, however, and thus it is unclear whether the primary VO_2 τ was speeded or rather the primary amplitude

was increased. It has been previously reported that a speeding of the primary τ after priming exercise can occur independent of an increase in blood flow during the second contractile bout (15, 18, 22, 63). However, our condition in which metabolic signals were maximized (resting flow) also failed to show a speeding of tau after a bout of priming contractions. Thus, the role of bulk O_2 delivery in the prior exercise effect is unclear.

If prior contractions speed the primary τ of a second exercise bout via increased O_2 delivery (56), then the elevated flow condition used in the present experiments certainly would have shown an effect. However, no speeding of the primary τ occurred despite maximization of O_2 delivery to the muscle (Table 5). This is in agreement with previous observations which have reported that maximizing O_2 delivery prior to contractions does not speed VO_2 on-kinetics in highly-oxidative skeletal muscle contracting at a submaximal metabolic rate (27, 28). It is important to note however, that the lack of significance for bout 2 tau in comparison to bout 1 in the spontaneous and elevated flow conditions occurred due to one animal displaying a slightly slower tau during the second bout. Further investigation into this discrepancy is currently planned. Interestingly, tau was not speeded (nor did it display a trend for speeding) during the second bout of the additive condition in which the blood flow MRT was ≈5 fold shorter than in the first bout. Though the results of the present investigation support most of the literature (8-11, 21, 51, 52, 68) in finding that a prior bout of high-intensity exercise/contractions does not alter tau for the second bout, results from the spontaneous and elevated flow conditions indicate the potential for a speeding with more rapid O_2

delivery. Thus, it cannot be definitively stated whether or not O_2 delivery *per se* at the onset of contractions mediates faster primary VO_2 kinetics after priming exercise.

Slow component amplitude. Another interesting finding of this investigation was that the VO_2 slow component amplitude was reduced after a prior bout of contractions. The slow component amplitude comprised ≈10-20% of the total VO_2 response for the first bout of contractions and was reduced to ≈5-10% during the second bout. This result is in agreement with much of the data from human studies (8-11, 52, 68, 69) yet differs from the main proposed mechanism (65, 76). Classically, the VO_2 slow-component has been suggested to occur as a consequence of recruitment of additional motor units as the exercise bout progresses (65, 76). For example, Burnley et al. (8) found that the primary amplitude was increased and slow component amplitude reduced jointly with greater motor unit recruitment at the onset of a second exercise bout. Scheuermann et al. (69) and Tordi et al. (73) found a trend for greater motor unit recruitment during a second bout of exercise. Accordingly, data from exercising humans suggest that altered motor unit recruitment after prior exercise may reduce the amplitude of the slow component.

A key difference in our model is that all motor units are recruited synchronously, and thus progressive motor unit recruitment during a contractile bout is absent. Recently, a "slow component-like response" in the canine GS was reported by Zoladz et al. (83) when VO_2 was corrected for peak force and force-time integral. Slow component responses without correction are not commonly reported for muscle contracting *in situ* (25, 29). This is the first experiment to show a consistent VO_2 slow component for each animal at a submaximal metabolic rate. The reason for this is unclear, but likely lies in

the new measurement techniques being utilized to obtain contraction-by-contraction VO_2. The large increase in the number of data samples obtained during the key transitional period (≈30 samples (current investigation) vs. ≈5 samples (27)) provides a more confident means by which the primary VO_2 response can be fit (77) and separated from the slow component. The appearance of a VO_2 slow component in our model suggests that progressive motor unit recruitment is not a requirement for this phenomenon. Additionally, the results from our investigation suggest that altered motor unit recruitment is not required for reductions in the slow-component amplitude to occur.

The VO_2 slow component in humans is often determined as the rise in VO_2 after the initial three min of exercise (e.g., (68)). However, recent investigations that have fit the different responses to each phase indicate that the slow component can begin as early as ≈1-2 min into exercise (11, 17, 79). Thus, the time of onset of the VO_2 slow component in the current investigation (≈45 s) indicates that the mechanisms responsible for a slow component in our model may be similar to that in humans. Intriguingly, breathing of a hyperoxic gas mixture attenuated the VO_2 slow component in human subjects cycling at supra-LT intensities (55, 79). Hyperoxic gas breathing has also been found to eliminate the slow component of PCr hydrolysis (36). Wilkerson et al. (79) recently suggested that the VO_2 slow component may be linked to O_2 supply to the contracting musculature. This could explain the reduction in the slow component amplitude for the second contractile bout of the spontaneous, elevated flow, and additive conditions as O_2 delivery was more rapid in the second bout in comparison to the first in all three of these conditions. However, increased O_2 delivery cannot explain the reduced

slow component amplitude for the second contractile bout of the resting flow condition (35.4±16.6 vs. 15.8±12.4 $mlO_2 \cdot kg\ ww^{-1} \cdot min^{-1}$). Further, Grassi et al. (30) found that elevations in convective O_2 delivery did not reduce the slow component amplitude in the canine GS contracting at peak VO_2. However, that study (30) did not have the benefit of measuring contraction-by-contraction VO_2.

The result of an attenuated VO_2 slow component in the second contractile bout in the present investigation (Figure 3) is strikingly similar to observations by Sahlin et al. (68) in exercising humans (Figure 5). They (68) suggested that the slow component 'merged' into the primary component; possibly due to factors that reduced the efficiency of contraction within the muscle. The results obtained in the present investigation and by Sahlin et al. (68) suggest that the second contractile/exercise bout likely began with stimuli of the VO_2 slow component already present. Thus, the asymptote of the primary component was increased and the amplitude of the slow component was considerably reduced in the second bout.

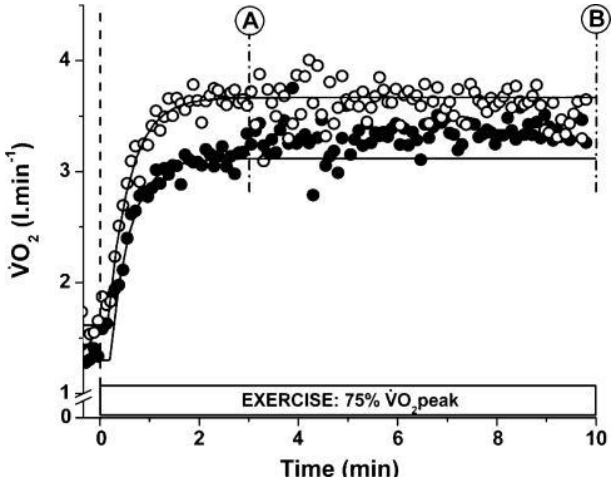

Figure 6. Data from a representative subject from Sahlin et al. (68). Closed circles: bout 1. Open circles: bout 2. Slow component amplitude was measured as the rise in VO_2 between points A and B.

***Muscle oxygenation*.** Recently, it has been suggested that enhanced O_2 availability in the microvasculature prior to the second exercise bout is responsible for the speeded primary τ (15, 17, 35). Using NIRS, Gurd et al. (35) observed an elevated [O_2Hb] at the onset of a second sub-LT bout that was preceded by supra-LT exercise. [O_2Hb] remained elevated throughout the second exercise bout in comparison to the first. These results were repeated by DeLorey et al. (15) who also measured no difference in bulk blood flow or O_2 delivery between exercise bouts. An elevation in [O_2Hb] prior to and throughout a second exercise bout was also reported for supra-LT exercise at extremely slow (35 rpm) and rapid (115 rpm) pedal rates (17). In contrast to previous reports (15, 17, 35), Burnley et al. (8) did not observe a speeding of the primary τ after priming exercise despite elevations in [O_2Hb]. The results from our spontaneous condition suggest that microvascular O_2 availability as assessed by [O_2Hb] is not a requirement for the speeding of the primary τ. As stated in the *Results* section, four out of five animals displayed a shorter tau for the second spontaneous bout. However, during this condition, [O_2Hb] was not statistically different prior to the start of the second bout in comparison to the first (Table 7). In fact, these values were numerically lower than the first bout. Also different from prior studies in humans (8, 15, 17, 35) is the fact that the end-exercise values for [O_2Hb] were not different between contractile bouts (Table 8).

We successfully maximized O_2 availability during recovery and throughout the second contractile bout with our elevated flow condition (≈200 fold increase in [O_2Hb]; Table 7). However, this did not result in a shorter primary tau. Further, [O_2Hb] did not remain elevated in comparison to the first bout (Figure 4, Panel B), and the end-

contractile values were not different between contractile bouts (Table 8). Of striking interest is that during the condition in which we minimized O_2 availability during recovery and throughout a second bout of contractions (resting flow), tau was not significantly altered in either direction (i.e., speeded or slowed) and [O_2Hb] remained lower than the first bout for only the first ≈25 s of contractions (Figure 4, Panel C). On the basis of our results, O_2 availability as assessed by [O_2Hb] does not play an important role in determining the prior exercise effect, at least in highly-oxidative skeletal muscle. It thus seems premature to conclude that enhanced local O_2 delivery is the cause for speeded VO_2 on-kinetics (15).

In the present investigation, no differences were observed between bouts or conditions for [HHb] at the end of contractions (Table 8). This indicates an equal O_2 balance between O_2 delivery and O_2 consumption for all conditions at the end of contractions, and matches the end-exercise blood flow and VO_2 data. Fitting the bout 1 mean [HHb] data (Figure 4) with a monoexponential function suggests that a slow component was present in each condition. This is consistent with observations in muscle of exercising humans (16, 17). Fitting the bout 2 mean [HHb] data suggests that this slow component was reduced after a priming bout of contractions in all conditions (Figure 4). As [HHb] can be altered via changes in either blood flow or muscle O_2 extraction, it is unclear whether the reduced [HHb] slow component after prior contractions was caused by a reduced VO_2 slow component or vice versa (17, 48, 58). Manifestation of the relationship between [HHb] kinetics and the VO_2 slow component is further complicated by the fact that all conditions appeared to have a reduction in [HHb]

slow component (Figure 4). Thus, bulk O_2 delivery did not appear to affect the [HHb] slow component.

[HHb] kinetics can be used to provide information on the time course of O_2 utilization when combined with measurements of VO_2 (16). A delay in change in the [HHb] signal indicates that O_2 extraction is matched by O_2 delivery. Fitting of the mean [HHb] curves in Figure 4 suggests that the TD may be shorter for the second bout of the elevated flow condition in comparison to the first. As the delay in O_2 delivery at the onset of the second bout was absent in the elevated flow condition, this indicates a more rapid O_2 extraction by the contracting skeletal muscle. However, the VO_2 kinetics were not speeded. This investigation into [HHb] kinetics also indicates that the resting flow condition had a shorter TD and tau for the second bout of contractions. This could be explained by an increased O_2 extraction during the transition period in comparison to the first bout as blood flow kinetics were identical for both bouts. It would also appear to indicate faster VO_2 on-kinetics for both TD and tau; however only TD was significantly shorter for the second bout in comparison to the first. Further investigation is needed to clarify these kinetics.

A reduction in TD for [HHb] has been reported in some (20, 35, 58), but not all (17) investigations in humans after priming exercise. In all of these investigations, the TD for the VO_2 response at the onset of exercise was not speeded (17, 20, 35, 58). Interestingly, the tau for [HHb] kinetics at the onset of the second bout of exercise was not altered in investigations that did (15, 17, 35) or did not (20, 58) find a speeding of the primary τ for VO_2 after priming exercise. This could be explained by increased blood

flow at the onset of the second contractile bout and thus maintenance of [HHb] kinetics with increased O_2 extraction. However, as discussed previously, blood flow kinetics during a second bout are not always speeded (15, 18, 22, 63).

Interpretation of NIRS data can be convoluted by several factors. A rightward shift of the oxyhemoglobin dissociation curve during muscle contraction can cause an increase in [HHb] that is not indicative of increased O_2 extraction over O_2 delivery. Further, as stated by DiMenna et al. (17), "…NIRS data only reflect changes within the superficial area of muscle under interrogation and as such may not be representative of the entire muscle mass." This, along with heterogeneous muscle oxygenation dynamics within a given muscle and between muscles (50) indicates that mechanistic inferences about data acquired from NIRS and VO_2 on-kinetics should be made with caution. In this context our experimental model presents several distinct advantages. First, a greater percentage of the contracting muscle mass is interrogated in comparison to human studies. Second, the canine GS has a rather homogeneous metabolic profile (59). And third, motor unit recruitment heterogeneities are eliminated by the use of maximal tetanic contractions. It would be interesting to further investigate the NIRS response at the onset of contractions by comparison with CvO_2 kinetics. Computational modeling techniques have recently been employed to examine this relationship (53) and further investigation is clearly needed. Though the determinants of [HHb] kinetics in our model warrant further investigation, the fact that VO_2 on-kinetics were altered to the same extent regardless of muscle oxygenation status indicates that muscle oxygenation as assessed by NIRS does not play a significant role in determining VO_2 on-kinetics.

Conclusions. A prior bout of contractions speeds VO_2 on-kinetics in highly-oxidative skeletal muscle by reductions in TD and the amplitude of the VO_2 slow component. The shorter TD leads to a faster MRT. In addition, maximizing or reducing O_2 availability in the microvasculature by changing blood flow (as confirmed by $[O_2Hb]$ via NIRS) does not predictably alter the prior exercise effect in isolated, highly-oxidative skeletal muscle. These results suggest an intracellular origin to the prior exercise effect. Elevations in the baseline VO_2 prior to the second contractile bout indicate that metabolic stimuli of respiration were likely increased in comparison to conditions before the first bout. These stimuli were likely the cause of the prior exercise effects observed in this highly-oxidative skeletal muscle. Apparently, these intracellular factors are not consistently linked to oxygenation status. The results from this investigation also suggest that progressive motor unit recruitment is not a requirement for the manifestation of a VO_2 slow component. Likewise, altered motor unit recruitment at the onset of a second bout is not a prerequisite for reductions in the VO_2 slow component amplitude after a priming contractile bout. As human exercise requires a combination of different muscles with heterogeneous muscle fiber compositions, further examinations into the prior exercise effect should be undertaken with lowly-oxidative skeletal muscle.

REFERENCES

1. **Arena R, Humphrey R, and Peberdy MA**. Measurement of oxygen consumption on-kinetics during exercise: implications for patients with heart failure. *J Card Fail* 7: 302-310, 2001.

2. **Babcock MA, Paterson DH, Cunningham DA, and Dickinson JR**. Exercise on-transient gas exchange kinetics are slowed as a function of age. *Medicine and science in sports and exercise* 26: 440-446, 1994.

3. **Bangsbo J, Krustrup P, Gonzalez-Alonso J, Boushel R, and Saltin B**. Muscle oxygen kinetics at onset of intense dynamic exercise in humans. *American journal of physiology* 279: R899-906, 2000.

4. **Barker GA, Green S, Green AA, and Walker PJ**. Walking performance, oxygen uptake kinetics and resting muscle pyruvate dehydrogenase complex activity in peripheral arterial disease. *Clin Sci (Lond)* 106: 241-249, 2004.

5. **Bauer TA, Regensteiner JG, Brass EP, and Hiatt WR**. Oxygen uptake kinetics during exercise are slowed in patients with peripheral arterial disease. *J Appl Physiol* 87: 809-816, 1999.

6. **Behnke BJ, Kindig CA, Musch TI, Koga S, and Poole DC**. Dynamics of microvascular oxygen pressure across the rest-exercise transition in rat skeletal muscle. *Respiration physiology* 126: 53-63, 2001.

7. **Behnke BJ, Kindig CA, Musch TI, Sexton WL, and Poole DC**. Effects of prior contractions on muscle microvascular oxygen pressure at onset of subsequent contractions. *The Journal of physiology* 539: 927-934, 2002.

8. **Burnley M, Doust JH, Ball D, and Jones AM**. Effects of prior heavy exercise on VO(2) kinetics during heavy exercise are related to changes in muscle activity. *J Appl Physiol* 93: 167-174, 2002.

9. **Burnley M, Doust JH, Carter H, and Jones AM**. Effects of prior exercise and recovery duration on oxygen uptake kinetics during heavy exercise in humans. *Experimental physiology* 86: 417-425, 2001.

10. **Burnley M, Doust JH, and Jones AM**. Effects of prior heavy exercise, prior sprint exercise and passive warming on oxygen uptake kinetics during heavy exercise in humans. *European journal of applied physiology* 87: 424-432, 2002.

11. **Burnley M, Jones AM, Carter H, and Doust JH**. Effects of prior heavy exercise on phase II pulmonary oxygen uptake kinetics during heavy exercise. *J Appl Physiol* 89: 1387-1396, 2000.

12. **Cade WT, Fantry LE, Nabar SR, Shaw DK, and Keyser RE**. Impaired oxygen on-kinetics in persons with human immunodeficiency virus are not due to highly active antiretroviral therapy. *Arch Phys Med Rehabil* 84: 1831-1838, 2003.

13. **Cerretelli P, Grassi B, Colombini A, Caru B, and Marconi C**. Gas exchange and metabolic transients in heart transplant recipients. *Respiration physiology* 74: 355-371, 1988.

14. **De Cort SC, Innes JA, Barstow TJ, and Guz A**. Cardiac output, oxygen consumption and arteriovenous oxygen difference following a sudden rise in exercise level in humans. *The Journal of physiology* 441: 501-512, 1991.

15. **DeLorey DS, Kowalchuk JM, Heenan AP, Dumanoir GR, and Paterson DH**. Prior exercise speeds pulmonary O2 uptake kinetics by increases in both local muscle O2 availability and O2 utilization. *J Appl Physiol* 103: 771-778, 2007.

16. **DeLorey DS, Kowalchuk JM, and Paterson DH**. Relationship between pulmonary O2 uptake kinetics and muscle deoxygenation during moderate-intensity exercise. *J Appl Physiol* 95: 113-120, 2003.

17. **DiMenna FJ, Wilkerson DP, Burnley M, Bailey SJ, and Jones AM**. Influence of priming exercise on pulmonary O2 uptake kinetics during transitions to high-intensity exercise at extreme pedal rates. *J Appl Physiol* 106: 432-442, 2009.

18. **Endo M, Okada Y, Rossiter HB, Ooue A, Miura A, Koga S, and Fukuba Y**. Kinetics of pulmonary VO2 and femoral artery blood flow and their relationship during repeated bouts of heavy exercise. *European journal of applied physiology* 95: 418-430, 2005.

19. **Essfeld D, Hoffmann U, and Stegemann J**. A model for studying the distortion of muscle oxygen uptake patterns by circulation parameters. *European journal of applied physiology and occupational physiology* 62: 83-90, 1991.

20. **Ferreira LF, Lutjemeier BJ, Townsend DK, and Barstow TJ**. Dynamics of skeletal muscle oxygenation during sequential bouts of moderate exercise. *Experimental physiology* 90: 393-401, 2005.

21. **Fukuba Y, Hayashi N, Koga S, and Yoshida T**. VO(2) kinetics in heavy exercise is not altered by prior exercise with a different muscle group. *J Appl Physiol* 92: 2467-2474, 2002.

22. **Fukuba Y, Ohe Y, Miura A, Kitano A, Endo M, Sato H, Miyachi M, Koga S, and Fukuda O**. Dissociation between the time courses of femoral artery blood flow and pulmonary VO2 during repeated bouts of heavy knee extension exercise in humans. *Experimental physiology* 89: 243-253, 2004.

23. **Gerbino A, Ward SA, and Whipp BJ**. Effects of prior exercise on pulmonary gas-exchange kinetics during high-intensity exercise in humans. *J Appl Physiol* 80: 99-107, 1996.

24. **Gladden LB, Hogan MC, Kelley KM, Dobson JL, and Grassi B**. Metabolic response time of canine skeletal muscle. *Medicine and science in sports and exercise* 34: S79, 2002.

25. **Grassi B**. Skeletal muscle VO2 on-kinetics: set by O2 delivery or by O2 utilization? New insights into an old issue. *Medicine and science in sports and exercise* 32: 108-116, 2000.

26. **Grassi B, Ferretti G, Xi L, Rieu M, Meyer M, Marconi C, and Cerretelli P**. Ventilatory response to exercise after heart and lung denervation in humans. *Respiration physiology* 92: 289-304, 1993.

27. **Grassi B, Gladden LB, Samaja M, Stary CM, and Hogan MC**. Faster adjustment of O2 delivery does not affect V(O2) on-kinetics in isolated in situ canine muscle. *J Appl Physiol* 85: 1394-1403, 1998.

28. **Grassi B, Gladden LB, Stary CM, Wagner PD, and Hogan MC**. Peripheral O2 diffusion does not affect V(O2)on-kinetics in isolated insitu canine muscle. *J Appl Physiol* 85: 1404-1412, 1998.

29. **Grassi B, Hogan MC, Greenhaff PL, Hamann JJ, Kelley KM, Aschenbach WG, Constantin-Teodosiu D, and Gladden LB**. Oxygen uptake on-kinetics in dog gastrocnemius in situ following activation of pyruvate dehydrogenase by dichloroacetate. *The Journal of physiology* 538: 195-207, 2002.

30. **Grassi B, Hogan MC, Kelley KM, Aschenbach WG, Hamann JJ, Evans RK, Patillo RE, and Gladden LB**. Role of convective O(2) delivery in determining VO(2) on-kinetics in canine muscle contracting at peak VO(2). *J Appl Physiol* 89: 1293-1301, 2000.

31. **Grassi B, Hogan MC, Rossiter HB, Howlett RA, Harris JE, Goodwin ML, Dobson JL, and Gladden LB**. Effects of acute creatine kinase inhibition on skeletal muscle O2 uptake kinetics. *Medicine and science in sports and exercise* 38: S519-S520, 2006.

32. **Grassi B, Marconi C, Meyer M, Rieu M, and Cerretelli P**. Gas exchange and cardiovascular kinetics with different exercise protocols in heart transplant recipients. *J Appl Physiol* 82: 1952-1962, 1997.

33. **Grassi B, Morandi L, Pogliaghi S, Rampichini S, Marconi C, and Cerretelli P**. Functional evaluation of patients with metabolic myopathies during exercise. *Medicine and science in sports and exercise* 34: S78, 2002.

34. **Grassi B, Poole DC, Richardson RS, Knight DR, Erickson BK, and Wagner PD**. Muscle O2 uptake kinetics in humans: implications for metabolic control. *J Appl Physiol* 80: 988-998, 1996.

35. **Gurd BJ, Scheuermann BW, Paterson DH, and Kowalchuk JM**. Prior heavy-intensity exercise speeds VO2 kinetics during moderate-intensity exercise in young adults. *J Appl Physiol* 98: 1371-1378, 2005.

36. **Haseler LJ, Kindig CA, Richardson RS, and Hogan MC**. The role of oxygen in determining phosphocreatine onset kinetics in exercising humans. *The Journal of physiology* 558: 985-992, 2004.

37. **Hebestreit H, Hebestreit A, Trusen A, and Hughson RL**. Oxygen uptake kinetics are slowed in cystic fibrosis. *Medicine and science in sports and exercise* 37: 10-17, 2005.

38. **Hill AV, and Lupton H**. Muscular exercise, lactic acid, and the supply and utilization of oxygen. *Quarterly Journal of Medicine* 16: 135-171, 1923.

39. **Hogan MC**. Fall in intracellular PO(2) at the onset of contractions in Xenopus single skeletal muscle fibers. *J Appl Physiol* 90: 1871-1876, 2001.

40. **Hogan MC, Arthur PG, Bebout DE, Hochachka PW, and Wagner PD**. Role of O2 in regulating tissue respiration in dog muscle working in situ. *J Appl Physiol* 73: 728-736, 1992.

41. **Hughson RL**. Alterations in the oxygen deficit-oxygen debt relationships with beta-adrenergic receptor blockade in man. *The Journal of physiology* 349: 375-387, 1984.

42. **Hughson RL, Cochrane JE, and Butler GC**. Faster O2 uptake kinetics at onset of supine exercise with than without lower body negative pressure. *J Appl Physiol* 75: 1962-1967, 1993.

43. **Hughson RL, and Imman MD**. Faster kinetics of VO2 during arm exercise with circulatory occlusion of the legs. *International journal of sports medicine* 7: 22-25, 1986.

44. **Hughson RL, and Kowalchuk JM**. Beta-blockade and oxygen delivery to muscle during exercise. *Can J Physiol Pharmacol* 69: 285-289, 1991.

45. **Hughson RL, and Kowalchuk JM**. Kinetics of oxygen uptake for submaximal exercise in hyperoxia, normoxia, and hypoxia. *Canadian journal of applied physiology = Revue canadienne de physiologie appliquee* 20: 198-210, 1995.

46. **Hughson RL, Shoemaker JK, Tschakovsky ME, and Kowalchuk JM**. Dependence of muscle VO2 on blood flow dynamics at onset of forearm exercise. *J Appl Physiol* 81: 1619-1626, 1996.

47. **Hughson RL, and Smyth GA**. Slower Adaptation of VO2 to steady state of submaximal exercise with beta-blockade. *European journal of applied physiology and occupational physiology* 52: 107-110, 1983.

48. **Jones AM, Fulford J, and Wilkerson DP**. Influence of prior exercise on muscle [phosphorylcreatine] and deoxygenation kinetics during high-intensity exercise in men. *Experimental physiology* 93: 468-478, 2008.

49. **Kindig CA, Kelley KM, Howlett RA, Stary CM, and Hogan MC**. Assessment of O2 uptake dynamics in isolated single skeletal myocytes. *J Appl Physiol* 94: 353-357, 2003.

50. **Koga S, Poole DC, Ferreira LF, Whipp BJ, Kondo N, Saitoh T, Ohmae E, and Barstow TJ**. Spatial heterogeneity of quadriceps muscle deoxygenation kinetics during cycle exercise. *J Appl Physiol* 103: 2049-2056, 2007.

51. **Koppo K, and Bouckaert J**. The effect of prior high-intensity cycling exercise on the VO2 kinetics during high-intensity cycling exercise is situated at the additional slow component. *International journal of sports medicine* 22: 21-26, 2001.

52. **Koppo K, Jones AM, and Bouckaert J**. Effect of prior heavy arm and leg exercise on VO2 kinetics during heavy leg exercise. *European journal of applied physiology* 88: 593-600, 2003.

53. **Lai N, Saidel GM, Grassi B, Gladden LB, and Cabrera ME**. Model of oxygen transport and metabolism predicts effect of hyperoxia on canine muscle oxygen uptake dynamics. *J Appl Physiol* 103: 1366-1378, 2007.

54. **Linnarsson D, Karlsson J, Fagraeus L, and Saltin B**. Muscle metabolites and oxygen deficit with exercise in hypoxia and hyperoxia. *J Appl Physiol* 36: 399-402, 1974.

55. **Macdonald M, Pedersen PK, and Hughson RL**. Acceleration of VO2 kinetics in heavy submaximal exercise by hyperoxia and prior high-intensity exercise. *J Appl Physiol* 83: 1318-1325, 1997.

56. **MacDonald MJ, Naylor HL, Tschakovsky ME, and Hughson RL**. Peripheral circulatory factors limit rate of increase in muscle O(2) uptake at onset of heavy exercise. *J Appl Physiol* 90: 83-89, 2001.

57. **MacDonald MJ, Shoemaker JK, Tschakovsky ME, and Hughson RL**. Alveolar oxygen uptake and femoral artery blood flow dynamics in upright and supine leg exercise in humans. *J Appl Physiol* 85: 1622-1628, 1998.

58. **Marles A, Perrey S, Legrand R, Blondel N, Delangles A, Betbeder D, Mucci P, and Prieur F**. Effect of prior heavy exercise on muscle deoxygenation kinetics at the onset of subsequent heavy exercise. *European journal of applied physiology* 99: 677-684, 2007.

59. **Maxwell LC, Barclay JK, Mohrman DE, and Faulkner JA**. Physiological characteristics of skeletal muscles of dogs and cats. *The American journal of physiology* 233: C14-18, 1977.

60. **McCartney N, Spriet LL, Heigenhauser GJ, Kowalchuk JM, Sutton JR, and Jones NL**. Muscle power and metabolism in maximal intermittent exercise. *J Appl Physiol* 60: 1164-1169, 1986.

61. **Murphy PC, Cuervo LA, and Hughson RL**. A study of cardiorespiratory dynamics with step and ramp exercise tests in normoxia and hypoxia. *Cardiovasc Res* 23: 825-832, 1989.

62. **Nery LE, Wasserman K, Andrews JD, Huntsman DJ, Hansen JE, and Whipp BJ**. Ventilatory and gas exchange kinetics during exercise in chronic airways obstruction. *J Appl Physiol* 53: 1594-1602, 1982.

63. **Paterson ND, Kowalchuk JM, and Paterson DH**. Effects of prior heavy-intensity exercise during single-leg knee extension on VO2 kinetics and limb blood flow. *J Appl Physiol* 99: 1462-1470, 2005.

64. **Piiper J, and Spiller P**. Repayment of O2 debt and resynthesis of high-energy phosphates in gastrocnemius muscle of the dog. *J Appl Physiol* 28: 657-662, 1970.

65. **Poole DC, Barstow TJ, Gaesser GA, Willis WT, and Whipp BJ**. VO2 slow component: physiological and functional significance. *Medicine and science in sports and exercise* 26: 1354-1358, 1994.

66. **Regensteiner JG, Bauer TA, Reusch JE, Brandenburg SL, Sippel JM, Vogelsong AM, Smith S, Wolfel EE, Eckel RH, and Hiatt WR**. Abnormal oxygen uptake kinetic responses in women with type II diabetes mellitus. *J Appl Physiol* 85: 310-317, 1998.

67. **Rossiter HB, Ward SA, Doyle VL, Howe FA, Griffiths JR, and Whipp BJ**. Inferences from pulmonary O2 uptake with respect to intramuscular [phosphocreatine] kinetics during moderate exercise in humans. *The Journal of physiology* 518 (Pt 3): 921-932, 1999.

68. **Sahlin K, Sorensen JB, Gladden LB, Rossiter HB, and Pedersen PK**. Prior heavy exercise eliminates VO2 slow component and reduces efficiency during submaximal exercise in humans. *The Journal of physiology* 564: 765-773, 2005.

69. **Scheuermann BW, Hoelting BD, Noble ML, and Barstow TJ**. The slow component of O(2) uptake is not accompanied by changes in muscle EMG during repeated bouts of heavy exercise in humans. *The Journal of physiology* 531: 245-256, 2001.

70. **Sietsema KE, Ben-Dov I, Zhang YY, Sullivan C, and Wasserman K**. Dynamics of oxygen uptake for submaximal exercise and recovery in patients with chronic heart failure. *Chest* 105: 1693-1700, 1994.

71. **Spriet LL, Lindinger MI, McKelvie RS, Heigenhauser GJ, and Jones NL**. Muscle glycogenolysis and H+ concentration during maximal intermittent cycling. *J Appl Physiol* 66: 8-13, 1989.

72. **Stainsby WN, and Welch HG**. Lactate metabolism of contracting dog skeletal muscle in situ. *The American journal of physiology* 211: 177-183, 1966.

73. **Tordi N, Perrey S, Harvey A, and Hughson RL**. Oxygen uptake kinetics during two bouts of heavy cycling separated by fatiguing sprint exercise in humans. *J Appl Physiol* 94: 533-541, 2003.

74. **Tschakovsky ME, and Hughson RL**. Interaction of factors determining oxygen uptake at the onset of exercise. *J Appl Physiol* 86: 1101-1113, 1999.

75. **Van Beek JH, and Westerhof N**. Response time of cardiac mitochondrial oxygen consumption to heart rate steps. *The American journal of physiology* 260: H613-625, 1991.

76. **Whipp BJ**. The slow component of O2 uptake kinetics during heavy exercise. *Medicine and science in sports and exercise* 26: 1319-1326, 1994.

77. **Whipp BJ, and Rossiter HB**. The kinetics of oxygen uptake: physiological inferences from parameters. In: *Oxygen Uptake Kinetics in Sport, Exercise and Medicine*, edited by Jones AM, and Poole DC. Abingdon: Routledge, 2005, p. 62-94.

78. **Whipp BJ, and Ward SA**. Physiological determinants of pulmonary gas exchange kinetics during exercise. *Medicine and science in sports and exercise* 22: 62-71, 1990.

79. **Wilkerson DP, Berger NJ, and Jones AM**. Influence of hyperoxia on pulmonary O2 uptake kinetics following the onset of exercise in humans. *Respiratory physiology & neurobiology* 153: 92-106, 2006.

80. **Williamson JW, Raven PB, and Whipp BJ**. Unaltered oxygen uptake kinetics at exercise onset with lower-body positive pressure in humans. *Experimental physiology* 81: 695-705, 1996.

81. **Wilson DF**. Factors affecting the rate and energetics of mitochondrial oxidative phosphorylation. *Medicine and science in sports and exercise* 26: 37-43, 1994.

82. **Wilson DF, and Rumsey WL**. Factors modulating the oxygen dependence of mitochondrial oxidative phosphorylation. *Adv Exp Med Biol* 222: 121-131, 1988.

83. **Zoladz JA, Gladden LB, Hogan MC, Nieckarz Z, and Grassi B**. Progressive recruitment of muscle fibers is not necessary for the slow component of VO2 kinetics. *J Appl Physiol* 105: 575-580, 2008.

CUMULATIVE REFERENCES

1. **Ameredes BT, Brechue WF, and Stainsby WN**. Mechanical and metabolic determination of VO2 and fatigue during repetitive isometric contractions in situ. *J Appl Physiol* 84: 1909-1916, 1998.

2. **Arena R, Humphrey R, and Peberdy MA**. Measurement of oxygen consumption on-kinetics during exercise: implications for patients with heart failure. *J Card Fail* 7: 302-310, 2001.

3. **Babcock MA, Paterson DH, Cunningham DA, and Dickinson JR**. Exercise on-transient gas exchange kinetics are slowed as a function of age. *Medicine and science in sports and exercise* 26: 440-446, 1994.

4. **Balaban RS**. Regulation of oxidative phosphorylation in the mammalian cell. *Am J Physiol* 258: C377-389, 1990.

5. **Bangsbo J, Gibala MJ, Howarth KR, and Krustrup P**. Tricarboxylic acid cycle intermediates accumulate at the onset of intense exercise in man but are not essential for the increase in muscle oxygen uptake. *Pflugers Arch* 452: 737-743, 2006.

6. **Bangsbo J, Gibala MJ, Krustrup P, Gonzalez-Alonso J, and Saltin B**. Enhanced pyruvate dehydrogenase activity does not affect muscle O2 uptake at onset of intense exercise in humans. *American journal of physiology* 282: R273-280, 2002.

7. **Bangsbo J, Krustrup P, Gonzalez-Alonso J, and Saltin B**. ATP production and efficiency of human skeletal muscle during intense exercise: effect of previous exercise. *Am J Physiol Endocrinol Metab* 280: E956-964, 2001.

8. **Barclay CJ, Constable JK, and Gibbs CL**. Energetics of fast- and slow-twitch muscles of the mouse. *The Journal of physiology* 472: 61-80, 1993.

9. **Barker GA, Green S, Green AA, and Walker PJ**. Walking performance, oxygen uptake kinetics and resting muscle pyruvate dehydrogenase complex activity in peripheral arterial disease. *Clin Sci (Lond)* 106: 241-249, 2004.

10. **Barstow TJ, Buchthal S, Zanconato S, and Cooper DM**. Muscle energetics and pulmonary oxygen uptake kinetics during moderate exercise. *J Appl Physiol* 77: 1742-1749, 1994.

11. **Barstow TJ, Buchthal SD, Zanconato S, and Cooper DM**. Changes in potential controllers of human skeletal muscle respiration during incremental calf exercise. *J Appl Physiol* 77: 2169-2176, 1994.

12. **Bauer TA, Regensteiner JG, Brass EP, and Hiatt WR**. Oxygen uptake kinetics during exercise are slowed in patients with peripheral arterial disease. *J Appl Physiol* 87: 809-816, 1999.

13. **Bearden SE, and Moffatt RJ**. VO2 and heart rate kinetics in cycling: transitions from an elevated baseline. *J Appl Physiol* 90: 2081-2087, 2001.

14. **Behnke BJ, Barstow TJ, Kindig CA, McDonough P, Musch TI, and Poole DC**. Dynamics of oxygen uptake following exercise onset in rat skeletal muscle. *Respir Physiol Neurobiol* 133: 229-239, 2002.

15. **Behnke BJ, Kindig CA, Musch TI, Koga S, and Poole DC**. Dynamics of microvascular oxygen pressure across the rest-exercise transition in rat skeletal muscle. *Respir Physiol* 126: 53-63, 2001.

16. **Behnke BJ, Kindig CA, Musch TI, Sexton WL, and Poole DC**. Effects of prior contractions on muscle microvascular oxygen pressure at onset of subsequent contractions. *The Journal of physiology* 539: 927-934, 2002.

17. **Behnke BJ, McDonough P, Padilla DJ, Musch TI, and Poole DC**. Oxygen exchange profile in rat muscles of contrasting fibre types. *The Journal of physiology* 549: 597-605, 2003.

18. **Bockman EL**. Blood flow and oxygen consumption in active soleus and gracilis muscles in cats. *Am J Physiol* 244: H546-551, 1983.

19. **Bohnert B, Ward SA, and Whipp BJ**. Effects of prior arm exercise on pulmonary gas exchange kinetics during high-intensity leg exercise in humans. *Experimental physiology* 83: 557-570, 1998.

20. **Buono MJ, and Roby FB**. Acid-base, metabolic, and ventilatory responses to repeated bouts of exercise. *J Appl Physiol* 53: 436-439, 1982.

21. **Burnley M, Doust JH, Ball D, and Jones AM**. Effects of prior heavy exercise on VO(2) kinetics during heavy exercise are related to changes in muscle activity. *J Appl Physiol* 93: 167-174, 2002.

22. **Burnley M, Doust JH, Carter H, and Jones AM**. Effects of prior exercise and recovery duration on oxygen uptake kinetics during heavy exercise in humans. *Experimental physiology* 86: 417-425, 2001.

23. **Burnley M, Doust JH, and Jones AM**. Effects of prior heavy exercise, prior sprint exercise and passive warming on oxygen uptake kinetics during heavy exercise in humans. *European journal of applied physiology* 87: 424-432, 2002.

24. **Burnley M, Jones AM, Carter H, and Doust JH**. Effects of prior heavy exercise on phase II pulmonary oxygen uptake kinetics during heavy exercise. *J Appl Physiol* 89: 1387-1396, 2000.

25. **Cabric M, and James NT**. Morphometric analyses on the muscles of exercise trained and untrained dogs. *Am J Anat* 166: 359-368, 1983.

26. **Cade WT, Fantry LE, Nabar SR, Shaw DK, and Keyser RE**. Impaired oxygen on-kinetics in persons with human immunodeficiency virus are not due to highly active antiretroviral therapy. *Arch Phys Med Rehabil* 84: 1831-1838, 2003.

27. **Campbell-O'Sullivan SP, Constantin-Teodosiu D, Peirce N, and Greenhaff PL**. Low intensity exercise in humans accelerates mitochondrial ATP production and pulmonary oxygen kinetics during subsequent more intense exercise. *The Journal of physiology* 538: 931-939, 2002.

28. **Caputo F, Mello MT, and Denadai BS**. Oxygen uptake kinetics and time to exhaustion in cycling and running: a comparison between trained and untrained subjects. *Arch Physiol Biochem* 111: 461-466, 2003.

29. **Cerretelli P, Grassi B, Colombini A, Caru B, and Marconi C**. Gas exchange and metabolic transients in heart transplant recipients. *Respir Physiol* 74: 355-371, 1988.

30. **Cerretelli P, Pendergast D, Paganelli WC, and Rennie DW**. Effects of specific muscle training on VO2 on-response and early blood lactate. *J Appl Physiol* 47: 761-769, 1979.

31. **Crow MT, and Kushmerick MJ**. Chemical energetics of slow- and fast-twitch muscles of the mouse. *J Gen Physiol* 79: 147-166, 1982.

32. **De Bruyn-Prevost P**. The effects of various warming up intensities and durations upon some physiological variables during an exercise corresponding to the WC170. *European journal of applied physiology and occupational physiology* 43: 93-100, 1980.

33. **De Cort SC, Innes JA, Barstow TJ, and Guz A**. Cardiac output, oxygen consumption and arteriovenous oxygen difference following a sudden rise in exercise level in humans. *The Journal of physiology* 441: 501-512, 1991.

34. **DeLorey DS, Kowalchuk JM, Heenan AP, Dumanoir GR, and Paterson DH**. Prior exercise speeds pulmonary O2 uptake kinetics by increases in both local muscle O2 availability and O2 utilization. *J Appl Physiol* 103: 771-778, 2007.

35. **di Prampero PE, Mahler PB, Giezendanner D, and Cerretelli P**. Effects of priming exercise on VO2 kinetics and O2 deficit at the onset of stepping and cycling. *J Appl Physiol* 66: 2023-2031, 1989.

36. **DiMenna FJ, Wilkerson DP, Burnley M, Bailey SJ, and Jones AM**. Influence of priming exercise on pulmonary O2 uptake kinetics during transitions to high-intensity exercise at extreme pedal rates. *J Appl Physiol* 106: 432-442, 2009.

37. **Endo M, Okada Y, Rossiter HB, Ooue A, Miura A, Koga S, and Fukuba Y**. Kinetics of pulmonary VO2 and femoral artery blood flow and their relationship during

repeated bouts of heavy exercise. *European journal of applied physiology* 95: 418-430, 2005.

38. **Fukuba Y, Hayashi N, Koga S, and Yoshida T**. VO(2) kinetics in heavy exercise is not altered by prior exercise with a different muscle group. *J Appl Physiol* 92: 2467-2474, 2002.

39. **Fukuba Y, Ohe Y, Miura A, Kitano A, Endo M, Sato H, Miyachi M, Koga S, and Fukuda O**. Dissociation between the time courses of femoral artery blood flow and pulmonary VO2 during repeated bouts of heavy knee extension exercise in humans. *Experimental physiology* 89: 243-253, 2004.

40. **Geer CM, Behnke BJ, McDonough P, and Poole DC**. Dynamics of microvascular oxygen pressure in the rat diaphragm. *J Appl Physiol* 93: 227-232, 2002.

41. **Gerbino A, Ward SA, and Whipp BJ**. Effects of prior exercise on pulmonary gas-exchange kinetics during high-intensity exercise in humans. *J Appl Physiol* 80: 99-107, 1996.

42. **Gonzalez-Alonso J, Quistorff B, Krustrup P, Bangsbo J, and Saltin B**. Heat production in human skeletal muscle at the onset of intense dynamic exercise. *The Journal of physiology* 524 Pt 2: 603-615, 2000.

43. **Grassi B**. Skeletal muscle VO2 on-kinetics: set by O2 delivery or by O2 utilization? New insights into an old issue. *Medicine and science in sports and exercise* 32: 108-116, 2000.

44. **Grassi B, Ferretti G, Xi L, Rieu M, Meyer M, Marconi C, and Cerretelli P**. Ventilatory response to exercise after heart and lung denervation in humans. *Respir Physiol* 92: 289-304, 1993.

45. **Grassi B, Gladden LB, Samaja M, Stary CM, and Hogan MC**. Faster adjustment of O2 delivery does not affect V(O2) on-kinetics in isolated in situ canine muscle. *J Appl Physiol* 85: 1394-1403, 1998.

46. **Grassi B, Gladden LB, Stary CM, Wagner PD, and Hogan MC**. Peripheral O2 diffusion does not affect V(O2)on-kinetics in isolated insitu canine muscle. *J Appl Physiol* 85: 1404-1412, 1998.

47. **Grassi B, Hogan MC, Greenhaff PL, Hamann JJ, Kelley KM, Aschenbach WG, Constantin-Teodosiu D, and Gladden LB**. Oxygen uptake on-kinetics in dog gastrocnemius in situ following activation of pyruvate dehydrogenase by dichloroacetate. *The Journal of physiology* 538: 195-207, 2002.

48. **Grassi B, Hogan MC, Kelley KM, Aschenbach WG, Hamann JJ, Evans RK, Patillo RE, and Gladden LB**. Role of convective O(2) delivery in determining VO(2) on-kinetics in canine muscle contracting at peak VO(2). *J Appl Physiol* 89: 1293-1301, 2000.

49. **Grassi B, Hogan MC, Kelley KM, Howlett RA, and Gladden LB**. Effects of nitric oxide synthase inhibition by L-NAME on oxygen uptake kinetics in isolated canine muscle in situ. *The Journal of physiology* 568: 1021-1033, 2005.

50. **Grassi B, Hogan MC, Rossiter HB, Howlett RA, Harris JE, Goodwin ML, Dobson JL, and Gladden LB**. Effects of acute creatine kinase inhibition on skeletal

muscle O2 uptake kinetics. *Medicine and science in sports and exercise* 38: S519-S520, 2006.

51. **Grassi B, Marconi C, Meyer M, Rieu M, and Cerretelli P**. Gas exchange and cardiovascular kinetics with different exercise protocols in heart transplant recipients. *J Appl Physiol* 82: 1952-1962, 1997.

52. **Grassi B, Morandi L, Pogliaghi S, Rampichini S, Marconi C, and Cerretelli P**. Functional evaluation of patients with metabolic myopathies during exercise. *Medicine and science in sports and exercise* 34: S78, 2002.

53. **Grassi B, Pogliaghi S, Rampichini S, Quaresima V, Ferrari M, Marconi C, and Cerretelli P**. Muscle oxygenation and pulmonary gas exchange kinetics during cycling exercise on-transitions in humans. *J Appl Physiol* 95: 149-158, 2003.

54. **Grassi B, Poole DC, Richardson RS, Knight DR, Erickson BK, and Wagner PD**. Muscle O2 uptake kinetics in humans: implications for metabolic control. *J Appl Physiol* 80: 988-998, 1996.

55. **Gurd BJ, Peters SJ, Heigenhauser GJ, LeBlanc PJ, Doherty TJ, Paterson DH, and Kowalchuk JM**. Prior heavy exercise elevates pyruvate dehydrogenase activity and speeds O2 uptake kinetics during subsequent moderate-intensity exercise in healthy young adults. *The Journal of physiology* 577: 985-996, 2006.

56. **Gurd BJ, Scheuermann BW, Paterson DH, and Kowalchuk JM**. Prior heavy-intensity exercise speeds VO2 kinetics during moderate-intensity exercise in young adults. *J Appl Physiol* 98: 1371-1378, 2005.

57. **Gustafson LA, and Van Beek JH**. Activation time of myocardial oxidative phosphorylation in creatine kinase and adenylate kinase knockout mice. *Am J Physiol Heart Circ Physiol* 282: H2259-2264, 2002.

58. **Gute D, Fraga C, Laughlin MH, and Amann JF**. Regional changes in capillary supply in skeletal muscle of high-intensity endurance-trained rats. *J Appl Physiol* 81: 619-626, 1996.

59. **Gute D, Laughlin MH, and Amann JF**. Regional changes in capillary supply in skeletal muscle of interval-sprint and low-intensity, endurance-trained rats. *Microcirculation* 1: 183-193, 1994.

60. **Hak JB, Van Beek JH, Eijgelshoven MH, and Westerhof N**. Mitochondrial dehydrogenase activity affects adaptation of cardiac oxygen consumption to demand. *Am J Physiol* 264: H448-453, 1993.

61. **Hansen J, Sander M, and Thomas GD**. Metabolic modulation of sympathetic vasoconstriction in exercising skeletal muscle. *Acta physiologica Scandinavica* 168: 489-503, 2000.

62. **Harrison GJ, van Wijhe MH, de Groot B, Dijk FJ, Gustafson LA, and van Beek JH**. Glycolytic buffering affects cardiac bioenergetic signaling and contractile reserve similar to creatine kinase. *Am J Physiol Heart Circ Physiol* 285: H883-890, 2003.

63. **Harrison GJ, van Wijhe MH, de Groot B, Dijk FJ, and van Beek JH**. CK inhibition accelerates transcytosolic energy signaling during rapid workload steps in isolated rabbit hearts. *Am J Physiol* 276: H134-140, 1999.

64. **Hebestreit H, Hebestreit A, Trusen A, and Hughson RL**. Oxygen uptake kinetics are slowed in cystic fibrosis. *Medicine and science in sports and exercise* 37: 10-17, 2005.

65. **Hermansen L, and Wachtlova M**. Capillary density of skeletal muscle in well-trained and untrained men. *J Appl Physiol* 30: 860-863, 1971.

66. **Hill AV, and Lupton H**. Muscular exercise, lactic acid, and the supply and utilization of oxygen. *Quarterly Journal of Medicine* 16: 135-171, 1923.

67. **Hochachka PW, Bianconcini MS, Parkhouse WS, and Dobson GP**. On the role of actomyosin ATPases in regulation of ATP turnover rates during intense exercise. *Proc Natl Acad Sci U S A* 88: 5764-5768, 1991.

68. **Hogan MC**. Fall in intracellular PO(2) at the onset of contractions in Xenopus single skeletal muscle fibers. *J Appl Physiol* 90: 1871-1876, 2001.

69. **Hogan MC, Arthur PG, Bebout DE, Hochachka PW, and Wagner PD**. Role of O2 in regulating tissue respiration in dog muscle working in situ. *J Appl Physiol* 73: 728-736, 1992.

70. **Hogan MC, Nioka S, Brechue WF, and Chance B**. A 31P-NMR study of tissue respiration in working dog muscle during reduced O2 delivery conditions. *J Appl Physiol* 73: 1662-1670, 1992.

71. **Hogan MC, and Welch HG**. Effect of altered arterial O2 tensions on muscle metabolism in dog skeletal muscle during fatiguing work. *Am J Physiol* 251: C216-222, 1986.

72. **Hughson RL**. Alterations in the oxygen deficit-oxygen debt relationships with beta-adrenergic receptor blockade in man. *The Journal of physiology* 349: 375-387, 1984.

73. **Hughson RL, Cochrane JE, and Butler GC**. Faster O2 uptake kinetics at onset of supine exercise with than without lower body negative pressure. *J Appl Physiol* 75: 1962-1967, 1993.

74. **Hughson RL, and Imman MD**. Faster kinetics of VO2 during arm exercise with circulatory occlusion of the legs. *International journal of sports medicine* 7: 22-25, 1986.

75. **Hughson RL, and Kowalchuk JM**. Beta-blockade and oxygen delivery to muscle during exercise. *Can J Physiol Pharmacol* 69: 285-289, 1991.

76. **Hughson RL, and Kowalchuk JM**. Kinetics of oxygen uptake for submaximal exercise in hyperoxia, normoxia, and hypoxia. *Canadian journal of applied physiology = Revue canadienne de physiologie appliquee* 20: 198-210, 1995.

77. **Hughson RL, and Morrissey M**. Delayed kinetics of respiratory gas exchange in the transition from prior exercise. *J Appl Physiol* 52: 921-929, 1982.

78. **Hughson RL, and Morrissey MA**. Delayed kinetics of VO2 in the transition from prior exercise. Evidence for O2 transport limitation of VO2 kinetics: a review. *International journal of sports medicine* 4: 31-39, 1983.

79. **Hughson RL, Shoemaker JK, Tschakovsky ME, and Kowalchuk JM**. Dependence of muscle VO2 on blood flow dynamics at onset of forearm exercise. *J Appl Physiol* 81: 1619-1626, 1996.

80. **Hughson RL, and Smyth GA**. Slower Adaptation of VO2 to steady state of submaximal exercise with beta-blockade. *European journal of applied physiology and occupational physiology* 52: 107-110, 1983.

81. **Hughson RL, Tschakovsky ME, and Houston ME**. Regulation of oxygen consumption at the onset of exercise. *Exerc Sport Sci Rev* 29: 129-133, 2001.

82. **Jones AM, Koppo K, Wilkerson DP, Wilmshurst S, and Campbell IT**. Dichloroacetate does not speed phase-II pulmonary VO2 kinetics following the onset of heavy intensity cycle exercise. *Pflugers Arch* 447: 867-874, 2004.

83. **Jones AM, Wilkerson DP, Koppo K, Wilmshurst S, and Campbell IT**. Inhibition of nitric oxide synthase by L-NAME speeds phase II pulmonary .VO2 kinetics in the transition to moderate-intensity exercise in man. *The Journal of physiology* 552: 265-272, 2003.

84. **Kindig CA, Howlett RA, Stary CM, Walsh B, and Hogan MC**. Effects of acute creatine kinase inhibition on metabolism and tension development in isolated single myocytes. *J Appl Physiol* 98: 541-549, 2005.

85. **Kindig CA, Stary CM, and Hogan MC**. Effect of dissociating cytosolic calcium and metabolic rate on intracellular PO2 kinetics in single frog myocytes. *The Journal of physiology* 562: 527-534, 2005.

86. **Koga S, Shiojiri T, Kondo N, and Barstow TJ**. Effect of increased muscle temperature on oxygen uptake kinetics during exercise. *J Appl Physiol* 83: 1333-1338, 1997.

87. **Koppo K, and Bouckaert J**. The decrease in VO(2) slow component induced by prior exercise does not affect the time to exhaustion. *International journal of sports medicine* 23: 262-267, 2002.

88. **Koppo K, and Bouckaert J**. The effect of prior high-intensity cycling exercise on the VO2 kinetics during high-intensity cycling exercise is situated at the additional slow component. *International journal of sports medicine* 22: 21-26, 2001.

89. **Koppo K, and Bouckaert J**. In humans the oxygen uptake slow component is reduced by prior exercise of high as well as low intensity. *European journal of applied physiology* 83: 559-565, 2000.

90. **Koppo K, Bouckaert J, and Jones AM**. Effects of training status and exercise intensity on phase II VO2 kinetics. *Medicine and science in sports and exercise* 36: 225-232, 2004.

91. **Koppo K, Jones AM, and Bouckaert J**. Effect of prior heavy arm and leg exercise on VO2 kinetics during heavy leg exercise. *European journal of applied physiology* 88: 593-600, 2003.

92. **Koppo K, Jones AM, Vanden Bossche L, and Bouckaert J**. Effect of prior exercise on VO(2) slow component is not related to muscle temperature. *Medicine and science in sports and exercise* 34: 1600-1604, 2002.

93. **Koppo K, Wilkerson DP, Bouckaert J, Wilmshurst S, Campbell IT, and Jones AM**. Influence of DCA on pulmonary (.-)V(O2) kinetics during moderate-intensity cycle exercise. *Medicine and science in sports and exercise* 36: 1159-1164, 2004.

94. **Krustrup P, Gonzalez-Alonso J, Quistorff B, and Bangsbo J**. Muscle heat production and anaerobic energy turnover during repeated intense dynamic exercise in humans. *The Journal of physiology* 536: 947-956, 2001.

95. **Kushmerick MJ**. Energetics studies of muscles of different types. *Basic Res Cardiol* 82 Suppl 2: 17-30, 1987.

96. **Lai N, Saidel GM, Grassi B, Gladden LB, and Cabrera ME**. Model of oxygen transport and metabolism predicts effect of hyperoxia on canine muscle oxygen uptake dynamics. *J Appl Physiol* 103: 1366-1378, 2007.

97. **Linnarsson D, Karlsson J, Fagraeus L, and Saltin B**. Muscle metabolites and oxygen deficit with exercise in hypoxia and hyperoxia. *J Appl Physiol* 36: 399-402, 1974.

98. **Macdonald M, Pedersen PK, and Hughson RL**. Acceleration of VO2 kinetics in heavy submaximal exercise by hyperoxia and prior high-intensity exercise. *J Appl Physiol* 83: 1318-1325, 1997.

99. **MacDonald MJ, Naylor HL, Tschakovsky ME, and Hughson RL**. Peripheral circulatory factors limit rate of increase in muscle O(2) uptake at onset of heavy exercise. *J Appl Physiol* 90: 83-89, 2001.

100. **MacDonald MJ, Shoemaker JK, Tschakovsky ME, and Hughson RL**. Alveolar oxygen uptake and femoral artery blood flow dynamics in upright and supine leg exercise in humans. *J Appl Physiol* 85: 1622-1628, 1998.

101. **Martin BJ, Robinson S, Wiegman DL, and Aulick LH**. Effect of warm-up on metabolic responses to strenuous exercise. *Medicine and science in sports* 7: 146-149, 1975.

102. **Maxwell LC, Barclay JK, Mohrman DE, and Faulkner JA**. Physiological characteristics of skeletal muscles of dogs and cats. *Am J Physiol* 233: C14-18, 1977.

103. **McCreary CR, Chilibeck PD, Marsh GD, Paterson DH, Cunningham DA, and Thompson RT**. Kinetics of pulmonary oxygen uptake and muscle phosphates during moderate-intensity calf exercise. *J Appl Physiol* 81: 1331-1338, 1996.

104. **McDonough P, Behnke BJ, Padilla DJ, Musch TI, and Poole DC**. Control of microvascular oxygen pressures in rat muscles comprised of different fibre types. *The Journal of physiology* 563: 903-913, 2005.

105. **Meyer RA**. A linear model of muscle respiration explains monoexponential phosphocreatine changes. *Am J Physiol* 254: C548-553, 1988.

106. **Meyer RA, and Foley JM**. Testing models of respiratory control in skeletal muscle. *Medicine and science in sports and exercise* 26: 52-57, 1994.

107. **Molé PA, Chung Y, Tran TK, Sailasuta N, Hurd R, and Jue T**. Myoglobin desaturation with exercise intensity in human gastrocnemius muscle. *Am J Physiol* 277: R173-180, 1999.

108. **Murphy PC, Cuervo LA, and Hughson RL**. A study of cardiorespiratory dynamics with step and ramp exercise tests in normoxia and hypoxia. *Cardiovasc Res* 23: 825-832, 1989.

109. **Nery LE, Wasserman K, Andrews JD, Huntsman DJ, Hansen JE, and Whipp BJ**. Ventilatory and gas exchange kinetics during exercise in chronic airways obstruction. *J Appl Physiol* 53: 1594-1602, 1982.

110. **Parolin ML, Spriet LL, Hultman E, Matsos MP, Hollidge-Horvat MG, Jones NL, and Heigenhauser GJ**. Effects of PDH activation by dichloroacetate in human skeletal muscle during exercise in hypoxia. *Am J Physiol Endocrinol Metab* 279: E752-761, 2000.

111. **Paterson DH, and Whipp BJ**. Asymmetries of oxygen uptake transients at the on- and offset of heavy exercise in humans. *The Journal of physiology* 443: 575-586, 1991.

112. **Paterson ND, Kowalchuk JM, and Paterson DH**. Effects of prior heavy-intensity exercise during single-leg knee extension on VO2 kinetics and limb blood flow. *J Appl Physiol* 99: 1462-1470, 2005.

113. **Perrey S, Scott J, Mourot L, and Rouillon JD**. Cardiovascular and oxygen uptake kinetics during sequential heavy cycling exercises. *Canadian journal of applied physiology = Revue canadienne de physiologie appliquee* 28: 283-298, 2003.

114. **Peter JB, Barnard RJ, Edgerton VR, Gillespie CA, and Stempel KE**. Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits. *Biochemistry* 11: 2627-2633, 1972.

115. **Poole DC, Barstow TJ, Gaesser GA, Willis WT, and Whipp BJ**. VO2 slow component: physiological and functional significance. *Medicine and science in sports and exercise* 26: 1354-1358, 1994.

116. **Poole DC, and Mathieu-Costello O**. Relationship between fiber capillarization and mitochondrial volume density in control and trained rat soleus and plantaris muscles. *Microcirculation* 3: 175-186, 1996.

117. **Pringle JS, Doust JH, Carter H, Tolfrey K, Campbell IT, Sakkas GK, and Jones AM**. Oxygen uptake kinetics during moderate, heavy and severe intensity "submaximal" exercise in humans: the influence of muscle fibre type and capillarisation. *European journal of applied physiology* 89: 289-300, 2003.

118. **Regensteiner JG, Bauer TA, Reusch JE, Brandenburg SL, Sippel JM, Vogelsong AM, Smith S, Wolfel EE, Eckel RH, and Hiatt WR**. Abnormal oxygen uptake kinetic responses in women with type II diabetes mellitus. *J Appl Physiol* 85: 310-317, 1998.

119. **Richardson RS, Newcomer SC, and Noyszewski EA**. Skeletal muscle intracellular PO(2) assessed by myoglobin desaturation: response to graded exercise. *J Appl Physiol* 91: 2679-2685, 2001.

120. **Roman BB, Meyer RA, and Wiseman RW**. Phosphocreatine kinetics at the onset of contractions in skeletal muscle of MM creatine kinase knockout mice. *Am J Physiol Cell Physiol* 283: C1776-1783, 2002.

121. **Rossiter HB, Ward SA, Doyle VL, Howe FA, Griffiths JR, and Whipp BJ**. Inferences from pulmonary O2 uptake with respect to intramuscular [phosphocreatine] kinetics during moderate exercise in humans. *The Journal of physiology* 518 (Pt 3): 921-932, 1999.

122. **Rossiter HB, Ward SA, Howe FA, Wood DM, Kowalchuk JM, Griffiths JR, and Whipp BJ**. Effects of dichloroacetate on VO2 and intramuscular 31P metabolite kinetics during high-intensity exercise in humans. *J Appl Physiol* 95: 1105-1115, 2003.

123. **Rossiter HB, Ward SA, Kowalchuk JM, Howe FA, Griffiths JR, and Whipp BJ**. Dynamic asymmetry of phosphocreatine concentration and O(2) uptake between the on- and off-transients of moderate- and high-intensity exercise in humans. *The Journal of physiology* 541: 991-1002, 2002.

124. **Sahlin K, Sorensen JB, Gladden LB, Rossiter HB, and Pedersen PK**. Prior heavy exercise eliminates VO2 slow component and reduces efficiency during submaximal exercise in humans. *The Journal of physiology* 564: 765-773, 2005.

125. **Saltin B**. Hemodynamic adaptations to exercise. *Am J Cardiol* 55: 42D-47D, 1985.

126. **Sarelius IH**. Cell and oxygen flow in arterioles controlling capillary perfusion. *Am J Physiol* 265: H1682-1687, 1993.

127. **Scheuermann BW, Hoelting BD, Noble ML, and Barstow TJ**. The slow component of O(2) uptake is not accompanied by changes in muscle EMG during repeated bouts of heavy exercise in humans. *The Journal of physiology* 531: 245-256, 2001.

128. **Shoemaker JK, Phillips SM, Green HJ, and Hughson RL**. Faster femoral artery blood velocity kinetics at the onset of exercise following short-term training. *Cardiovasc Res* 31: 278-286, 1996.

129. **Sietsema KE, Ben-Dov I, Zhang YY, Sullivan C, and Wasserman K**. Dynamics of oxygen uptake for submaximal exercise and recovery in patients with chronic heart failure. *Chest* 105: 1693-1700, 1994.

130. **Spriet LL, and Heigenhauser GJ**. Regulation of pyruvate dehydrogenase (PDH) activity in human skeletal muscle during exercise. *Exerc Sport Sci Rev* 30: 91-95, 2002.

131. **Spriet LL, Howlett RA, and Heigenhauser GJ**. An enzymatic approach to lactate production in human skeletal muscle during exercise. *Medicine and science in sports and exercise* 32: 756-763, 2000.

132. **Steeghs K, Benders A, Oerlemans F, de Haan A, Heerschap A, Ruitenbeek W, Jost C, van Deursen J, Perryman B, Pette D, Bruckwilder M, Koudijs J, Jap P, Veerkamp J, and Wieringa B**. Altered Ca2+ responses in muscles with combined mitochondrial and cytosolic creatine kinase deficiencies. *Cell* 89: 93-103, 1997.

133. **Stringer W, Wasserman K, Casaburi R, Porszasz J, Maehara K, and French W**. Lactic acidosis as a facilitator of oxyhemoglobin dissociation during exercise. *J Appl Physiol* 76: 1462-1467, 1994.

134. **Timmons JA, Gustafsson T, Sundberg CJ, Jansson E, and Greenhaff PL**. Muscle acetyl group availability is a major determinant of oxygen deficit in humans during submaximal exercise. *Am J Physiol* 274: E377-380, 1998.

135. **Tordi N, Perrey S, Harvey A, and Hughson RL**. Oxygen uptake kinetics during two bouts of heavy cycling separated by fatiguing sprint exercise in humans. *J Appl Physiol* 94: 533-541, 2003.

136. **Tschakovsky ME, and Hughson RL**. Interaction of factors determining oxygen uptake at the onset of exercise. *J Appl Physiol* 86: 1101-1113, 1999.

137. **van Bolhuis BM, Medendorp WP, and Gielen CC**. Motor unit firing behavior in human arm flexor muscles during sinusoidal isometric contractions and movements. *Exp Brain Res* 117: 120-130, 1997.

138. **van Deursen J, Heerschap A, Oerlemans F, Ruitenbeek W, Jap P, ter Laak H, and Wieringa B**. Skeletal muscles of mice deficient in muscle creatine kinase lack burst activity. *Cell* 74: 621-631, 1993.

139. **Wagner PD**. Central and peripheral aspects of oxygen transport and adaptations with exercise. *Sports Med* 11: 133-142, 1991.

140. **Whipp BJ**. The slow component of O2 uptake kinetics during heavy exercise. *Medicine and science in sports and exercise* 26: 1319-1326, 1994.

141. **Whipp BJ, Rossiter HB, and Ward SA**. Exertional oxygen uptake kinetics: a stamen of stamina? *Biochemical Society transactions* 30: 237-247, 2002.

142. **Whipp BJ, Rossiter HB, Ward SA, Avery D, Doyle VL, Howe FA, and Griffiths JR**. Simultaneous determination of muscle 31P and O2 uptake kinetics during whole body NMR spectroscopy. *J Appl Physiol* 86: 742-747, 1999.

143. **Whipp BJ, and Ward SA**. Physiological determinants of pulmonary gas exchange kinetics during exercise. *Medicine and science in sports and exercise* 22: 62-71, 1990.

144. **Whipp BJ, Ward SA, Lamarra N, Davis JA, and Wasserman K**. Parameters of ventilatory and gas exchange dynamics during exercise. *J Appl Physiol* 52: 1506-1513, 1982.

145. **Wilkerson DP, Campbell IT, and Jones AM**. Influence of nitric oxide synthase inhibition on pulmonary O2 uptake kinetics during supra-maximal exercise in humans. *The Journal of physiology* 561: 623-635, 2004.

146. **Williamson JW, Raven PB, and Whipp BJ**. Unaltered oxygen uptake kinetics at exercise onset with lower-body positive pressure in humans. *Experimental physiology* 81: 695-705, 1996.

147. **Wilson DF**. Factors affecting the rate and energetics of mitochondrial oxidative phosphorylation. *Medicine and science in sports and exercise* 26: 37-43, 1994.

148. **Wilson DF, and Rumsey WL**. Factors modulating the oxygen dependence of mitochondrial oxidative phosphorylation. *Adv Exp Med Biol* 222: 121-131, 1988.

APPENDIX A

Protocol for Dog Studies (Priming Contractions)

Weeks Before

1. Order dogs.
2. Order cartridge for blood gas machine.
3. Ensure ample reagents, solutions and cal dye are available for the CO-Oximeter.
4. Check that sutures, umbilical tape and string are in ample supply.
5. Inform other lab members of upcoming experiment days.
6. Run any pilot work that can be run without an animal to ensure equipment is working.
7. Obtained a keycard to kennel facilities so that we can be admitted before 7am.

Day Before

8. Benchtop paper on table and dog board.
9. Heating pad on dog board.
10. Strings cut for tying limbs of dog to board.
11. Surgical equipment clean and placed on tray.
12. Soldering guns cleaned, checked, and working.
13. Blood gas and CO-Ox machines turned on and working.
14. Syringes for blood samples labeled and arranged.
15. Set up Oxymon for appropriate measurements.
16. Be sure that ice is available.
17. Prepare suture if needed.
18. Check that sufficient pentobarbital has been diluted from 390 $mg \bullet ml^{-1}$ to 65 $mg \bullet ml^{-1}$.
19. Check that sufficient normal saline (0.9%) is available.
20. Soak flow probe in saline.
21. Check that sufficient saturated KCl solution is available for euthanasia.
22. Check the laptop with "flow program" is ready and working.

Day of

Getting Dog

23. Double-check the anesthesia toolbox.
    a. Catheters
    b. Syringes
    c. Needles
    d. StopcocksGauze
    e. Gloves
    f. Pentobarbital
    g. Endotracheal tubes
    h. Laryngoscope
    i. Flashlight
    j. Muzzle
    k. Leash
    l. Calculator
    m. Extension cord
    n. Clippers
    o. Keys to kennel
    p. Key/card to enter facility if before 7:30am
    q. Scale
    r. Lab coats
24. Take any frozen carcass with you and drop off at incinerator
25. Walk dog from kennel to examination room
26. Obtain weight, anesthetize animal:
    s. Anesthesia dosage:

$$\frac{30\text{ mg}}{(?)\text{ kg dog mass}} \times \frac{1\text{ ml sol'n}}{65\text{ mg sol'n}} = \text{ml's sol'n per kg dog mass}$$

27. Insert endotracheal tube.
28. Transport dog via cart to lab.

Setting up in lab

29. Place dog on table.
30. Shave hair from surgical areas while vacuuming.
31. Place heating pad under dog and maintain at 37°C.
32. Place rectal probe through anus into rectum and check temperature.
33. Tie limbs to table and begin Surgery (steps 35-37).
34. While surgery is being performed:
    a. Set up jugular reservoir with saline.

b. Prepare heparin syringe (3,000 Units per kg dog mass).
c. Check blood gas, pH, and CO-Ox machines (run cal dyes on CO-Ox).
d. Turn on all equipment (Flowmeter, indwelling oximeter, physiograph, data acquisition center, laptop for pump-control, pumps) and prepare accordingly.
e. Set up perfusion pump by pumping saline through all of the tubing until all bubbles are removed.
f. Put fresh blade in scalpel.

35. Surgically isolate the left-side GS.
36. Surgically isolate both jugular veins.
37. Inserter catheter into right-side jugular vein.
38. **Without delay**, begin infusion of saline into right-side jugular vein at a rate of 0.03 ml•kg^{-1}•min^{-1}.
39. Surgically isolate the right-side carotid artery.
40. Place 12" X 12" plastic sheet under isolated GS and into V-groove of board.
41. Drive bone nails.
42. Place connector on Achilles tendon.
43. Insert jugular reservoir catheter into jugular vein and suspend on ring stand.
44. **IMMEDIATELY add heparin to jugular reservoir**.
45. Insert catheter into popliteal vein and attach oxicath, flow probe, and tubing to jugular reservoir.
46. Thread sciatic nerve through stimulator cuff.
47. Run data acquisition calibration:
    a. Pressure – 0 and 100 mmHg pressure.
    b. Force – 0 and 198 N on load cell.
    c. Flow – 0 and 1,000 ml•min^{-1} on the flowmeter.
    d. Indwelling Oximeter is set to alternately output 0 and 100% saturation throughout the 30 sec of calibration.
48. Insert catheter into right-side carotid artery.
49. Connect blood pressure transducer to catheter in right-side carotid artery.
50. Set up muscle myograph and attach Achilles tendon connector to the load cell.
51. Set up Oxymon and attach optodes to the muscle with elastic.
52. Place dog on ventilator and check appropriate settings: tidal volume = 20 ml•kg^{-1}, 50% inspiration, 15-20 breaths•min^{-1}.
53. Set optimal length of muscle. Use tetanic stimuli (8.0 V, 0.2 ms duration, trains at 50 Hz for 200 ms duration).
54. Check blood gas, pH, Hb, O_2Sat values and adjust as necessary according to the following algorithm:
    a. <u>"Normal" arterial dog values in this prep and how to fix problems:</u>
        i. pH = ~7.38 – 7.4
            1. If pH is lower than 7.38 – 7.40, then:
                a. If PCO_2 is adequate or high, consider increasing ventilation (perhaps 5-10 breaths/min per 0.03 pH units?).

b. If PCO_2 is adequate or low, consider adding bicarbonate (use a 1.0 M bicarb solution and titrate ~10 mL per hour per 0.05 pH units).

2. If pH is higher than 7.38 – 7.4, then:
   a. If PCO_2 is lower than 28-35 Torr, consider decreasing ventilation (perhaps 5-10 breath/min per 0.03 pH units?).
   b. If PCO_2 is higher than usual, then slow ventilation slightly and add acid. This would be most unusual!

ii. PCO_2 = ~28-35 Torr

1. If pH is normal and PCO_2 is higher, then we probably do not need to worry too much about it. Ventilation could be speeded slightly.
2. If pH is normal and PCO_2 is lower, then we probably do not need to worry too much about it. Ventilation could be slowed slightly.

iii. O_2 Sat = 95-98%

1. If O_2Sat is below 94, consider giving titrating small amounts of 100% O_2 flow into ventilator until 95-98% is reached.

iv. **If PCO_2 is high and pH is very low, or anything else looks really goofy** – check to be sure that trach tube cuff is properly inflated.

55. Throughout, check blood gas, pH, Hb, and O_2Sat and adjust as needed.
56. Throughout, check palpebral and plantar reflexes throughout and add maintenance doses of pentobarbital as needed (usually in 1-2 ml dosages).
57. Sometime during the day be sure to copy dog tag and place with strip chart recording at end of day.

Experimental Protocols

Spontaneous

58. Take a blood flow cal and mark rate.
59. Take and arterial blood sample.
60. Take a venous blood sample.
61. Stimulate muscle (8.0 V, 0.2 ms duration, trains at 50 Hz for 200 ms duration) at a rate of 2 contractions / 3 seconds for 2 min.
62. Let muscle recover for 2 min.
63. During recovery, reset resting tension on GS to optimal (i.e., if the resting tension on the GS was 20 N prior to the first contractile bout, reset it to 20 N prior to the second bout).
64. After 2 min of recovery, stimulate the GS to contraction.
65. Take a venous blood sample 2 min into the second bout.
66. After 2 min of contractions, take a flow cal and mark the flow rate.

67. Cease contractions.
68. Take an arterial sample.

Set-Up for Next Experimental Protocols

69. Insert catheter from perfusion pump into right side carotid artery.
70. Allow blood to move through tubing and be sure bubbles are removed.
71. Insert catheter from perfusion pump into the left popliteal artery supplying the GS.
72. After inserting catheter, turn on perfusion pump and set blood flow to the rate measured at rest during the Spontaneous condition.
73. Determine the blood flow tau for the first contractile bout of the spontaneous condition.

The next three experimental protocols are to be randomized:

Elevated Flow

74. Set optimal length of GS.
75. Set-up pump and enter needed variables (resting and steady-state blood flows from spontaneous condition)
76. The tau for the first bout will be what was measured during the spontaneous condition.
77. Take a blood flow cal and mark rate.
78. Take and arterial blood sample.
79. Take a venous blood sample.
80. Start contractions at 2 tetanic contractions every 3 seconds (8.0 V, 0.2 ms duration, trains at 50 Hz for 200 ms duration) for 2 min
    AT THE SAME TIME that we start the blood flow response.
    a. **Gladden: Countdown and start contractions**
    b. **Colonel: Start flow program**
    c. **Hernández: Event Marker**
81. Stop contractions after 2 min.
82. Maintain flow at steady state throughout recovery period.
83. Reset optimal length of GS during recovery.
84. After 2 min of recovery, start contractions again.
85. Take a venous blood sample 2 min into the second bout.
86. After 2 min of contractions, take a flow cal and mark the flow rate.
87. Cease contractions.
88. Take an arterial sample.
89. Return blood flow to resting rate.
90. Allow muscle to recover for at least 35 min after end of contractions.

Resting Flow

91. Set optimal length of GS.
92. The resting and steady-state blood flows and tau for the first and second bouts will be what was measured during the first bout of the spontaneous condition.
93. Take and arterial blood sample.
94. Take a venous blood sample.
95. Start contractions at 2 tetanic contractions every 3 seconds (8.0 V, 0.2 ms duration, trains at 50 Hz for 200 ms duration) for 2 min
    AT THE SAME TIME that we start the blood flow response.
    a. **Gladden: Countdown and start contractions**
    b. **Colonel: Start flow program**
    c. **Hernández: Event Marker**
96. Stop contractions after 2 min.
97. Rapidly return blood flow to the resting rate and set-up pump variables (identical to bout 1)
98. Reset optimal length of GS during recovery.
99. After 2 min of recovery, start contractions again.
100. Take a venous blood sample 2 min into the second bout.
101. Cease contractions.
102. Take an arterial sample.
103. Return blood flow to resting rate.
104. Allow muscle to recover for at least 35 min after end of contractions.

Additive

101. Set optimal length of GS.
102. The resting and steady-state blood flows and tau for the first bout will be what was measured during the first bout of the spontaneous condition.
103. Take and arterial blood sample.
104. Take a venous blood sample.
105. Start contractions at 2 tetanic contractions every 3 seconds (8.0 V, 0.2 ms duration, trains at 50 Hz for 200 ms duration) for 2 min
    AT THE SAME TIME that we start the blood flow response.
    a. **Gladden: Countdown and start contractions**
    b. **Colonel: Start flow program**
    c. **Hernández: Event Marker**
106. Stop contractions after 2 min.
107. Rapidly return blood flow to the resting rate and set-up pump variables (2 second tau, resting and steady-state blood flows identical to bout 1)
108. Reset optimal length of GS during recovery.
109. After 2 min of recovery, start contractions again.
110. Take a venous blood sample 2 min into the second bout.
111. Take an arterial sample.
112. Cease contractions and blood flow.

113. Monitor the HHb NIRS signal until a max has been reached.
114. Restart blood flow at resting rate.

After experiments

115. Remove dog from ventilator.
116. Inject remainder of Pentobarbital into animal and then pour saturated KCl into jugular reservoir.
117. After heart and breathing appear to stop, perform a bilateral pneumothorax.
118. Remove muscle, trim non-muscle tissue, place onto weighed and labeled aluminum foil, and record weight as follows:
    a. Tare weight: (weight of aluminum foil)
    b. Total weight: (weight of foil + muscle)
    c. Net weight: (total weight – tare weight)
119. Place muscle wrapped in aluminum foil into drying oven at 80°C.
120. Record aluminum foil tare weight and muscle weight on recording paper (strip chart).
121. Clean up and place dog in freezer.
122. Turn off all gases used during experiment.
123. Turn off other pertinent equipment and shut down, etc.
124. Remove tubes from pumps (unclamp the pumps).