Peripheral circulatory factors limit rate of increase in muscle O₂ uptake at onset of heavy exercise

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Central and peripheral circulatory limitations have an impact on the ability of humans to adapt to the metabolic demands of increased exercise intensities. Slower adaptation of O₂ uptake (V\(\text{O}_2\)) at the onset of very heavy exercise might reflect the interaction of central and peripheral circulatory limitations to O₂ transport and utilization (8, 15). Gerbino et al. (8) showed that V\(\text{O}_2\) increased more rapidly in response to a second bout of exercise, and MacDonald et al. (15) demonstrated even faster adaptation when arterial O₂ content was increased. These studies suggest that better adaptation is a consequence of improved O₂ transport in the second of two high-intensity exercise bouts.

Proposed mechanisms for the improved O₂ transport include elevated blood flow and greater O₂ extraction. Both could be consequences of alterations in local acid-base status, which might promote increased vascular conductance and a right shift of the O₂-hemoglobin dissociation curve, but direct confirmation is not available.

Our laboratory developed a forearm exercise experiment model that allows continuous monitoring of arterial blood flow in conjunction with measurement of arterial venous O₂ content difference for determination of muscle V\(\text{O}_2\) (11). In the present study, we investigated the mechanisms that enable skeletal muscle V\(\text{O}_2\) to adapt more rapidly to the second of two high-intensity exercise bouts. We hypothesized that venous blood would demonstrate an altered acid-base environment after the first bout of exercise. This alteration would then promote greater blood flow through more rapid vasodilation, as well as greater O₂ extraction, in the second bout of exercise, thus allowing muscle V\(\text{O}_2\) to increase more rapidly at the onset of exercise. The metabolic consequence of this rapid adaptation of oxidative metabolism would be reduction in venous blood lactate and less change in blood pH.

METHODS

Six healthy men (age 33 ± 5 yr, height 181 ± 2 cm, weight 74 ± 3 kg, mean ± SE) volunteered to participate in this study. They were not specifically trained, and all gave written consent on a form that was approved by the Office of Research Ethics at the University of Waterloo.

Exercise protocol. Subjects performed all exercises on a handgrip ergometer, in which the resistance was varied by adjusting the magnitude of load that was raised and lowered by repeated contractions with the right hand. The subjects were in a supine posture with the arm extended at heart level. The exercise protocol for all tests involved raising the weight for 0.5 s, lowering the weight for 0.5 s, and resting for 2 s before the next contraction, for a total cycle of 3 s. Initially, each subject performed a ramp handgrip exercise protocol, and the information from this test was used to determine the workloads to achieve 75% of peak load for the subsequent testing. In the ramp procedure, the resistance was continually increased by adding water at a flow rate of 1
l/min into a bucket that was raised and lowered. On a separate day, subjects reported to the laboratory in a rested state, at least 2 h after eating. They assumed a supine position, and a 21-gauge catheter (Angiocath, Becton-Dickinson, Sandy, UT) was inserted retrograde at the antecubital fossa in a deep forearm vein in the right arm. Another catheter was inserted in a dorsal hand vein on the left hand. This hand was warmed to obtain arterialized venous blood samples (7). The test session consisted of measurement of resting data for 5 min, which was followed, sequentially, by 5 min of exercise, 5 min of rest, 5 min of exercise, and an additional 5-min rest period.

**Data collection.** Forearm blood flow (FBF) was measured by Doppler ultrasound, as described previously (6, 11, 20). This is a highly reproducible method that has a coefficient of variation of 3-4% for estimates of diameter and 13-20% for measurements of velocity and blood flow (20). Most variations in velocity and flow appear to arise from spontaneous fluctuations in the normal blood flow, as seen with beat-by-beat heart rate or blood pressure (2), and are not a function of error in the method (6, 20). Brachial artery diameter was estimated by echo Doppler ultrasound, with a 7.5-MHz linear array probe (model SSH-140A, Toshiba, Tochigi-Ken, Japan). Mean blood velocity (MBV) was determined with a 4-MHz pulsed Doppler ultrasound probe (model 500V, Multigon Industries, Mt. Vernon, NY). Brachial artery diameter was obtained twice at rest, at minutes 1 and 5 of each exercise bout, and during recovery. The spectrum of the pulsed Doppler was processed on-line with a mean velocity processor (17). Calibration signals in the Doppler shift frequency range were generated from the Doppler signal processor. The blood flow velocity of our system was calibrated against blood pumped through tubing (20). MBV was obtained through integration of the area under the curve for each heartbeat and by averaging the response over 3-s intervals, so that a contraction and relaxation phase were included during exercise. FBF was estimated beat by beat by multiplying the average MBV by the cross-sectional area \( \pi(D/2)^2 \), where diameter \( (D) \) was updated at the times indicated in Table 1. MBV and heart rate signals were collected on a computer-based system at 100 Hz.

**Blood samples.** Venous blood samples were obtained twice during rest, at 30 s, 2 min, and 5 min of exercise, and at 30 s and 2 min of recovery. Arterialized venous blood samples were obtained twice during rest and during the fifth minute of both exercise and recovery.

Approximately 1 ml of blood was collected in heparinized syringes that were immediately capped, gently agitated, and then stored in an ice bath. Within 1 h of collection, all whole blood samples were analyzed at 37°C for \( \text{PO}_2 \), \( \text{PCO}_2 \), hematocrit, plasma concentrations of lactate ([La\(^-\)]) and potassium ([K\(^+\)]), and plasma pH by using selective electrodes in a blood-gas electrolyte analyzer (NovaStat Profile Plus 9, Waltham, MA). The analyzer was calibrated at regular intervals. Hemoglobin concentration was calculated from the measured hematocrit by assuming normal mean corpuscular hemoglobin of 33% of the total cell volume. \( \text{O}_2 \) saturation and content were obtained from the output of the analysis system after application of standard equations.

**Blood data analysis.** Arteriovenous \( \text{O}_2 \) content difference was calculated from the difference in assumed arterial \( \text{O}_2 \) content and actual forearm venous \( \text{O}_2 \) content. To estimate arterial \( \text{O}_2 \) content, we assumed that it was constant for each subject, using 97% saturation for the calculation (arterial \( \text{O}_2 \) content = \( 1.34[\text{hemoglobin}] \times \text{O}_2 \) saturation/100). Assumption of a constant arterial \( \text{O}_2 \) content is reasonable in this study because we normally observe 97% saturation of arterial \( \text{O}_2 \) by noninvasive oximetry. Furthermore, forearm handgrip exercise represents a relatively small cardiovascular challenge that does not compromise arterial \( \text{O}_2 \) oxygenation in the lungs.

A 10-s average of FBF, centered on the time over which the blood samples were drawn, was used to match blood flow and \( \text{O}_2 \) extraction for calculation of forearm muscle \( \dot{\text{V}} \text{O}_2 \). Arterial \( \text{O}_2 \) content was estimated from the arterialized venous samples. Arterialized hand vein samples provided a reasonable estimation of arterial lactate concentrations because the \( \text{O}_2 \) saturation in the arterialized samples of a previous study was calculated as 93.7 ± 0.5 (SE) % \((n = 6)\) (7).

**Data analysis.** Effects of previous arm exercise and time on the values for FBF, arteriovenous \( \text{O}_2 \) content difference, muscle \( \text{VO}_2 \), pH, [La\(^-\)], [K\(^+\)], and \( \text{PCO}_2 \) were analyzed by using a two-way repeated-measures ANOVA. This first level of analysis revealed significant interactions of exercise bout time for all variables. Therefore, all subsequent analyses are reported from one-way ANOVA comparisons at each time point, thus testing for differences between the first and second exercise bouts. The level of significance was set at \( P < 0.05 \). Any differences were further analyzed with Student-Newman-Keuls post hoc test. All data are presented as means ± SE.

### RESULTS

The peak workload achieved in the ramp exercise protocol was 17.1 ± 0.9 kg. The workload used during the step test protocol was 12.8 ± 0.7 kg, which represented ~75% of the peak workload.

At the onset of the first bout of exercise, FBF immediately began a rapid adaptation (Fig. 1), which is characteristic of the response for both forearm and leg.

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<th>Table 1. Resting, exercise, and recovery responses of brachial artery diameter and MBV in first and second exercise bouts</th>
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<td><strong>MBV, cm/s</strong></td>
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Values are means ± SE; \( n = 6 \) subjects. Diameter, brachial artery diameter; MBV, brachial artery mean blood velocity. *Significantly different from 1st exercise bout, \( P < 0.05 \).
exercise (16, 21). The pattern of adaptation altered in the second bout, resulting in a faster adjustment to steady state. This response was mainly due to increases in MBV, with relatively small changes in diameter over the course of exercise or recovery, in either the first or second bout (Table 1). In the second bout of high-intensity exercise, FBF was significantly elevated before the start of exercise (111 ± 23 vs. 36 ± 6 ml/min, \(P < 0.05\)) and at 30 s of exercise (234 ± 18 vs. 187 ± 4 ml/min, \(P < 0.05\)) compared with the first bout of exercise (Fig. 2). With cessation of muscle contractions, there was a marked elevation in FBF after both the first and second bouts of exercise (Fig. 1). Postexercise hyperemia was prolonged after both exercise bouts, with FBF still markedly elevated above the preexercise resting value at the end of the 5-min recovery period.

Resting muscle \(\dot{V}O_2\) was not significantly different before the first or second exercise bouts (Fig. 2). This was achieved by the combination of lower FBF and greater arteriovenous \(O_2\) content difference in the true resting state before the start of the first exercise bout. Before the second exercise bout, FBF was elevated, whereas the arteriovenous \(O_2\) content difference was significantly reduced (4.6 ± 0.9 vs. 7.2 ± 0.7 ml \(O_2/dl\), \(P < 0.05\)) and higher by 30 s of exercise (11.2 ± 0.7 vs. 10.8 ± 0.7 ml \(O_2/dl\), \(P < 0.05\)). At 30 s of exercise, muscle \(\dot{V}O_2\) was significantly greater in the second compared with the first exercise bout (Fig. 2). The elevated muscle \(\dot{V}O_2\) was caused by significantly greater FBF and arteriovenous \(O_2\) content difference in the 30-s sample in the second exercise bout. The arteriovenous \(O_2\) content difference was significantly lower in the second exercise bout compared with the first at the 5-min sampling point. This did not result in a difference in muscle \(\dot{V}O_2\) at this time point, as the FBF was slightly, but not significantly, elevated in the second test.

Arterialized venous pH, [La\(^-\)], and [K\(^+\)] did not change significantly across the entire duration of the exercise protocol.

The elevated venous [La\(^-\)] and reduced venous pH in the second exercise bout, before the start of exercise and at 30 s, are consistent with the expected metabolic acidosis in the exercising forearm after an initial bout of high-intensity exercise (Figs. 3 and 4). Also, after the first bout of exercise, there was an elevation in venous blood PCO\(_2\). Before the start of the second bout, PCO\(_2\) had declined to the point that it was not significantly elevated over the initial resting values. As exercise progressed, the relative acidosis was greater in the first exercise bout compared with the second. This was observed as significantly lower venous [La\(^-\)] and PCO\(_2\), which contributed to the significantly greater pH at the end of the second bout of exercise. The differences in acid-base were maintained throughout recovery, showing the greater acidosis after the first exercise bout.

Before the onset of the second exercise bout, venous plasma [K\(^+\)] was reduced compared with the first bout. Although there was a marked increase in venous [K\(^+\)] with exercise, there were no differences in [K\(^+\)] between exercise bouts. At 30 s after the second exercise bout, venous plasma [K\(^+\)] was elevated compared with after the first bout (Fig. 3).

**DISCUSSION**

This study provides confirmation that circulatory factors can limit the adaptation of muscle \(\dot{V}O_2\) at the onset of high-intensity exercise. Muscle \(\dot{V}O_2\) was measured during two, identical, high-intensity forearm exercise challenges in which the second challenge was 5 min after the first. We found that muscle \(\dot{V}O_2\) was significantly elevated during the first minute of the second exercise bout compared with the first bout. Consistent with our hypotheses, the residual effects of the first exercise bout on the acid-base status of the muscle were associated with an elevation in FBF and a greater \(O_2\) extraction during the second exercise bout. Despite the differences between the present model and whole body exercise, these data, obtained in the exercising forearm, suggest that adaptation of aerobic metabolism at the onset of whole body high-intensity exercise might also be limited by the availability of \(O_2\) in healthy subjects and in patients with heart disease (1, 13, 22).

\(\dot{V}O_2\) at the onset of exercise. The present experiments allowed us to explore the potential roles of bulk \(O_2\) supply, as determined by FBF and \(O_2\) extraction, in limiting \(\dot{V}O_2\) at the onset of heavy exercise. Both factors were proposed, in previous modeling (3) and experimental studies (8, 11, 15), as potential rate-limiting steps in the delivery of \(O_2\) for aerobic metabolism. The data from this experiment provide the first direct confirmation of the important role of both effects in mod-
ifying \( \text{O}_2 \) utilization during the rest-to-exercise transition for small muscle mass exercise. The forearm exercise model used in this study has a very different feature compared with repeated, high-intensity cycling exercise. Whereas cycling challenges both the central and peripheral circulatory capacities, the forearm model focuses on potential peripheral limitations. Thus any improvements in exercise performance that occur with whole body exercise might be further enhanced if cardiac output increases in conjunction with the altered peripheral factors identified in this study (15). The extent to which \( \text{O}_2 \) transport contributes to muscle \( \text{V}_\text{O}_2 \) at the onset of submaximal exercise is still being debated (9, 11).

**Limitations to the methodology.** Limitations to the methodology used in the present study include both instrumental constraints as well as theoretical limitations of the model. As previously shown in our laboratory (20), and confirmed by Rådegran (18), Doppler ultrasound is a highly reliable and accurate method for determining both MBV and conduit artery diameter at rest and during exercise. The estimation accuracy of FBF in the present study may have been limited by the lack of repeated data collections; however, FBF variability was similar to that previously observed using these methods (18, 20).

In the present study, venous blood was sampled from a single deep forearm vein. Possible limitations to this include the assumption that the blood sampled from this vein accurately reflected the metabolic rate of the muscles of interest and that the distribution of venous return from the forearm was not altered with changes in flow. The patterns of change seen in the arteriovenous \( \text{O}_2 \) content difference and venous \( \text{O}_2 \) content were consistent with previously published patterns (11).

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**Fig. 2.** Time course of changes in muscle oxygen uptake (\( \text{V}_\text{O}_2; \) top), FBF (middle), and arteriovenous \( \text{O}_2 \) content difference [\( \text{a-v}\text{O}_2; \) bottom] during the first (●) and second bouts (○) of high-intensity handgrip exercise. Exercise started at time 0. Values are means ± SE; \( n = 6. \) Note that the first ○ at time = 0 min for the second exercise bout is identical to the ● at time = 10 min in the first exercise bout. *Significant difference between first and second exercise bouts (\( P < 0.05 \)).
FBF. Blood flow adapts in the transition from rest to forearm or leg exercise in a two-phase pattern. The rapid increase in blood flow observed within the first 15–20 s of exercise is a consequence of the action of the muscle pump emptying veins and allowing a greater pressure gradient in combination with a limited vasodilation (19, 23). Subsequent increases in flow are primarily a consequence of further vasodilation in response to release of local vasoactive metabolites (such as \( \text{K}^+ \) and \( \text{H}^+ \), as shown in this study) and, possibly, endothelial factors (14). During this adaptive phase, blood flow distribution is coordinated to deliver more blood flow to metabolically active sites.

It is important to note in the present experiments that a relatively steady FBF was not achieved until ~2 min after the onset of exercise. These data are consistent with a negative feedback control mechanism. Even though FBF appeared to be relatively steady, it is obvious from the marked postexercise hyperemia that FBF was inadequate during exercise. During high-intensity exercise, FBF was unable to meet the demands because of periodic compression and occlusion of blood vessels with each contraction. Indeed, on stopping the last contraction, the high value of FBF (\( \sim 600 \text{ ml/min} \); Fig. 1) indicates how high flow was during the muscle relaxation phases in the latter part of exercise. Figure 5 shows the beat-by-beat values for MBV throughout the entire protocol. It is apparent in all tests that individual beat values achieved the same high velocity in the pause between contractions as in the period immediately after exercise. FBF recovered during the 5-min period between exercise bouts, but it did not return to resting baseline values. Thus, just before the start of the second exercise bout, FBF was approximately twice the value from before the first bout. The two-phase pattern of blood flow adaptation was also less obvious at the start of the second bout of exercise, possibly due to the elevation of resting flow and the increased vasodilation that may have existed in the exercising muscle vascular bed. The elevated FBF can be attributed, at least in part, to the vasodilatory effect of a significant reduction in pH, measured in venous blood, along with the significant elevation in

![Fig. 3. Venous blood lactate concentration ([La\(^-\); top] and venous potassium concentration ([K\(^+\); bottom] during rest, exercise (0–5 min), and recovery for the first (●) and second (○) exercise bouts. Values are means ± SE; n = 6. *Significant difference between first and second exercise bouts (P < 0.05).](http://jap.physiology.org/)

![Fig. 4. Venous blood pH (top) and PCO\(_2\) (bottom) during rest, exercise (0–5 min), and recovery for the first (●) and second (○) exercise bouts. Values are means ± SE. *Significant difference between first and second exercise bouts (P < 0.05).](http://jap.physiology.org/)
**Metabolic consequences of altered O₂ transport.** The more rapid increase in muscle \( \dot{V}O_2 \) that we found in the second exercise bout would mean that a greater proportion of the total ATP resynthesis occurred through oxidative metabolism. Consequently, utilization of phosphocreatine stores and anaerobic glycolysis should contribute less. We do not have information on the phosphocreatine stores in the present study. It might be anticipated that these stores were not completely recovered before the onset of the second exercise bout, but their final levels are unknown. Although we do not have intracellular measurements of \( [La^-] \), we do have information from venous blood that indicates production was probably decreased in the second compared with the first exercise bout. Previous investigations of the effects of warm-up exercise have reported either no effect on (8), or a reduction in, blood \( [La^-] \) compared with exercise without a warm-up (12). However, these studies did not measure \( [La^-] \) in blood as it left the exercising muscle, and many factors could have impacted systemic venous blood \( [La^-] \). Because the total muscle mass being exercised was quite small, our finding of no change in arterialized blood \( [La^-] \) was expected.

In the first 30 s of the second exercise bout, we observed a 30% increase in muscle \( \dot{V}O_2 \), which was a consequence of a 25.1% increase in FBF and a 3.7% increase in arteriovenous O₂ content difference. These relative contributions indicate that the major factor that influenced muscle \( \dot{V}O_2 \) was the increase in FBF. Indeed, the relatively small changes in the local acid-base environment had only a small contribution to
increased O$_2$ extraction. We used the mean values of venous blood pH and PCO$_2$ to estimate the 50% saturation of hemoglobin by O$_2$ (P$_{50}$). For exercise test 1 (pH = 7.38, PCO$_2$ = 49.5 Torr), the P$_{50}$ was 27.7 Torr, whereas, for test 2 (pH = 7.36, PCO$_2$ = 51.0 Torr), the P$_{50}$ was 28.3 Torr. The difference might be greater if the effect of temperature was included. The slightly but significantly greater O$_2$ extraction seen at the 30-s point of test 2 probably reflects the small shift of the O$_2$-hemoglobin dissociation curve as well as better distribution of hemoglobin by O$_2$ (P$_{50}$). For exercise

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