Resistance and Venous Oxygen Dynamics during Sinusoidal Exercise of Dog Skeletal Muscle

By David E. Mohrman and Harvey V. Sparks

ABSTRACT

We observed the response of vascular resistance and venous oxygen (O₂) saturation to sinusoidally modulated continuous exercise of the isolated dog calf. Stimulus frequency was varied sinusoidally between 0.5 Hz and 1 Hz with modulation frequencies ranging from 0.005 Hz to 0.05 Hz. Venous O₂ responses were corrected for dispersion due to vascular and catheter transit. As modulation frequency was increased, the amplitude of the response of both resistance and venous O₂ to changing stimulation frequency decreased. The dynamics of the resistance response depended on flow rate. At high constant flow (56 ± 8 ml/100 g min⁻¹) the resistance response followed higher modulation frequencies without loss of amplitude (Bode corner frequency = 0.016 ± 0.003 Hz) than it did at low constant flow (25 ± 7 ml/100 g min⁻¹) (Bode corner frequency = 0.0049 ± 0.0006 Hz). The dynamics of the venous O₂ saturation response were not significantly altered by flow. In the low constant-flow range, vascular resistance and venous O₂ saturation had similar dynamics in response to sinusoidal modulation of exercise rate. This finding indicates that oxidative metabolism may be linked to the resistance changes at low flow. At high constant flow, however, the vascular resistance response followed higher modulation frequencies without loss of amplitude than did venous O₂ saturation in three of four dogs. Given certain assumptions, it is doubtful that changes in oxidative metabolism are linked to changes in vascular resistance at high constant flow. Vascular resistance, under the conditions of this experiment, appeared to be controlled by at least two mechanisms, the slower of which may be related to oxidative metabolism.

KEY WORDS  exercise hyperemia  metabolic vasodilator  blood flow frequency response  constant-flow perfusion

Increased skeletal muscle blood flow during steady-state exercise is linearly related to increased muscle oxygen (O₂) consumption (1). However, a direct functional link between increased skeletal muscle O₂ consumption and decreased vascular smooth muscle tone has not been demonstrated. O₂ apparently is required for the return of vascular tone during reactive hyperemia (2), but the evidence that low tissue oxygen tension (Po₂) is causally involved in exercise hyperemia is less convincing (3). Indeed, many factors associated with muscular exercise but not directly linked to oxidative metabolism may participate in the production of exercise hyperemia. Among these factors are elevated interstitial potassium (4), osmolarity (5), adenosine triphosphate (6), and inorganic phosphate (7). The concept that exercise hyperemia may result from a combination of many influences which decrease vascular smooth muscle tone has received strong support from the demonstration by Skinner and Costin (8) that low Po₂, elevated interstitial potassium, and elevated osmolarity act additively to decrease muscle vascular resistance.

The relative influence of various local factors on exercise hyperemia may possibly be defined by comparing the time course of changes in these factors with the time course of changes in vascular resistance during exercise transients. We have previously reported (9) that, during high constant-flow perfusion, the resistance response to a brief tetanus of skeletal muscle appears to be too rapid to be controlled by factors related to tissue oxidative metabolism. However, the vascular response to a brief tetanus during lower constant-flow perfusion contains a slow phase that could be related to oxidative metabolism. Therefore, different mechanisms may influence the local control of vascular
resistance under different experimental conditions. The present study attempted to determine whether (1) the rapid vascular control mechanism responsible for the resistance response to a brief tetanus is operative during continuous exercise, (2) the dynamics of exercise hyperemia are dependent on blood flow rate during continuous twitch exercise, and (3) the dynamics of venous O₂ saturation are compatible with the possibility that an event related to oxidative metabolism is involved in the control of exercise hyperemia.

**Methods**

**PREPARATION**

Male mongrel dogs weighing 20–25 kg were anesthetized with sodium pentobarbital (35 mg/kg, iv). They were heparinized (500 units/kg plus 100 units/kg hour⁻¹) just prior to artificial perfusion of the calf muscle preparation.

Calf muscles were isolated in a manner similar to that reported by Kjellmer (4). All structures in the popliteal region except the femoral artery and vein and the femur were transected. The calf was skinned, and the paw was removed. The femoral vein (carrying all venous return from the calf) was cannulated just proximal to the popliteal junction; the effluent returned to a reservoir connected to the contralateral femoral vein. The popliteal junction; the effluent returned to a reservoir connected to the contralateral femoral vein. The calf was skinned, and the paw was removed. The femoral vein (carrying all venous return from the calf) was cannulated just proximal to the popliteal junction; the effluent returned to a reservoir connected to the contralateral femoral vein. The popliteal junction; the effluent returned to a reservoir connected to the contralateral femoral vein. The preparation was pump perfused throughout. During each run, flow was held constant at low values (25.2 ± 7.3 ml/100 g min⁻¹) or high values (56.0 ± 8.4 ml/100 g min⁻¹). Muscles were excised and weighed at the conclusion of each experiment. Although we did not systematically compare the experimental muscle's weight with that of the contralateral muscle, whenever the comparison was made, the weight of the experimental muscle was approximately 30% greater than that of the contralateral muscle. This observation indicates significant edema formation over the course of the day. Since we alternated beginning with the high and the low modulation frequencies, we doubt that any systematic change observed would be due to edema formation.

**DATA ANALYSIS AND PREPARATION**

The response of vascular resistance and venous O₂ saturation to the sinusoidal variations in exercise rate used in this study may be described as periodic but not necessarily sinusoidal. By Fourier series analysis (11), these periodic responses can be expressed as the algebraic sum of a fundamental sinusoid at the modulation frequency and as higher harmonic sinusoids at multiples of the modulation frequency. We analyzed only the fundamental sinusoidal components of the vascular resistance and the venous O₂ saturation responses. These fundamental components were obtained by cross-correlating (12) the tape-recorded resistance and the venous O₂ saturation signals with the simultaneously recorded stimulus modulation signal using a Hewlett-Packard 2115A computer. For each stimulus modulation frequency, the amplitude of the fundamental sinusoidal components of the responses was determined. Therefore, all processed data described the fundamental component of the responses of vascular resistance and venous O₂ saturation to sinusoidal modulation of exercise rate. These data are
Consequently, only the gain portion of the frequency saturation dynamics were viewed as the dynamics of saturation was constant, as was flow, end-capillary $O_2$ of the oximeter itself. The measured frequency the net transport of $O_2$ across the capillary mem-
ition gives the transfer function of the vascular transit process. The vasculare and venous $O_2$ responses to a brief tetanus of this preparation can be compared with the responses to continuous sinusoidal exercise because, if the system is linear and time invariant, the information needed to make the Bode diagrams in Figures 2 and 3 is contained in the response to an impulse of exercise (brief tetanus). This information can be obtained by taking the Fourier transform of the impulse reported earlier (9).

**Results**

Segments of strip chart records from various portions of a sinusoidal exercise experiment are shown in Figure 1. This particular experiment required 6 hours during which the preparation was continuously exercised on the average at 0.75 twitches/sec. Gastrocnemius tension development did not diminish over this period. In this experiment, as in others, mean perfusion pressure increased throughout the 6-hour experimental pe-

<table>
<thead>
<tr>
<th>Freq</th>
<th>Gain</th>
<th>Phase</th>
</tr>
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<tbody>
<tr>
<td>0.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
<td>0</td>
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Table 1 shows the average values of mean perfusion pressure and mean venous $O_2$ observed during sinusoidal variation of exercise rate between 0.5 and 1.0 twitch/sec for the low- and high-flow experiments. In addition, the maximum peak-to-

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smaller. There was no statistically significant difference in either the mean value or the amplitude of fluctuations of O$_2$ consumption between high- and normal-flow experiments.

**FREQUENCY RESPONSE AT HIGH CONSTANT FLOW**

The gain portions of the fundamental components of the vascular resistance and the end-capillary O$_2$ responses obtained during high constant flow (57.5 ± 8.3 ml/100 g min$^{-1}$) are shown in Figure 2A. Three of the four O$_2$ responses lost amplitude at a lower stimulus modulation frequency than did vascular resistance in that preparation. Thus in these three dogs, sinusoidal changes in O$_2$ became relatively smaller than the changes in resistance at higher modulation frequencies. In one case the O$_2$ changes were decreased no more than the resistance changes with increasing modulation frequency. When the individual O$_2$ gain responses were fitted by the response of a first-order system, the average corner frequency (frequency at which gain equals $-3$db) was 0.0093 ± 0.0044 Hz. Fits of a first-order system to the resistance response gave a corner frequency of 0.0155 ± 0.0032 Hz. These corner frequencies were not different statistically ($P = 0.29$).

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>Flow (ml/100 g min$^{-1}$)</th>
<th>Perfusion pressure (mm Hg)</th>
<th>O$_2$ consumption (ml/100 g min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Peak-to-peak</td>
<td>Mean Peak-to-peak</td>
<td>Mean Peak-to-peak</td>
</tr>
<tr>
<td>Low flow</td>
<td>27.2 ± 7.3</td>
<td>100 ± 22</td>
<td>3.4 ± 1.1</td>
</tr>
<tr>
<td>High flow</td>
<td>57.5 ± 8.3*</td>
<td>161 ± 22*</td>
<td>4.4 ± 2.9</td>
</tr>
</tbody>
</table>

$n =$ number of dogs. All values are means ± so.

*Difference between high and low flow is statistically significant at the 0.05 level.
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Gain portion of a Bode plot of the fundamental component of perfusion pressure and end-capillary O₂ response to sinusoidal modulation of exercise rate during high (A) and low (B) constant flow. Each type of line indicates data from an individual dog.

FIGURE 2

Gain portion of a Bode plot of the fundamental component of perfusion pressure and end-capillary O₂ response to sinusoidal modulation of exercise rate during high (A) and low (B) constant flow. Each type of line indicates data from an individual dog.

FREQUENCY RESPONSES AT LOW CONSTANT FLOW

Figure 2B shows the mean frequency responses of the fundamental components of vascular resistance and end-capillary O₂ obtained during lower constant flow (27.2 ± 8.4 ml/100 g min⁻¹). The O₂ gain curves at this lower flow were very similar to those obtained at the higher flow rate (Fig. 2A). The amplitude of the vascular resistance responses, however, began to fall off at much lower modulation frequencies during low constant flow, so that at low flow the vascular resistance and the end-capillary O₂ frequency response curves were nearly superimposed. The corner frequencies obtained by fitting first-order dynamics to individual resistance and O₂ responses were, respectively, 0.0049 ± 0.0006 and 0.0104 ± 0.0053 Hz; there was no statistical difference (P = 0.29).

COMPARISON WITH DYNAMICS OF RESPONSE TO BRIEF TETANUS

In Figure 3 the dynamics of the sinusoidal response of vascular resistance (A) and end-capillary O₂ (B) at high and low flow are compared with each other and with the dynamics predicted from the response of these variables to 1 second of tetanus. In each case, the open circles represent the frequency-response characteristics predicted by taking the Fourier transform of the time courses of the transient responses of vascular resistance and end-capillary O₂ which were previously reported (9). The frequency response of O₂ to sinusoidal variations in twitch rate (Fig. 3B) was similar at both high- and low-flow rates. In addition, end-capillary O₂ appeared to have approximately the frequency response expected from the O₂ response to a brief tetanus. The rate of gain decrease was approximately 6 db/octave at modulation frequencies above 0.01 Hz. The corner frequencies obtained by fitting first-order dynamics to individual responses were 0.0104 ± 0.0053 for low flow and 0.0093 ± 0.0044 Hz for high flow and were not different statistically (P = 0.88).

In contrast, the frequency response of vascular resistance to sinusoidal variation of exercise rate was markedly flow dependent (Fig. 3A). At low constant flow, the resistance response lost amplitude at lower modulation frequencies than it did at high constant flow. The corner frequencies obtained by fitting first-order dynamics to individual curves were 0.0049 ± 0.0006 Hz at low flow and 0.0155 ± 0.0032 Hz at high flow. These values were different statistically (P = 0.008). The final rate of gain loss with increasing modulation frequency was nearly 6 db/octave at high flow but nearer to 9 db/octave at low flow. The frequency response expected from the vascular response to brief tetanus was approximately that observed during constant high-flow perfusion.

Discussion

USE OF FREQUENCY ANALYSIS

The experimental protocol and the data analysis employed in this study were patterned after the frequency-response techniques developed in linear control theory. The predictive power of these techniques depends on their use for the analysis of
linear, time-invariant systems, and therefore great caution must be used in drawing conclusions from the application of such techniques to systems whose basic characteristics are unknown (16). It is very likely that the processes between skeletal muscle activation and both decreased vascular resistance and increased blood-to-tissue O₂ transfer are not linear. The nonlinearity of the vascular resistance response to a brief tetanus is evidenced by the lack of algebraic summation of the vascular response to two closely spaced periods of brief tetanus (9). In the case of capillary O₂ transport, the shape of both the myoglobin and the hemoglobin O₂ dissociation curves confer nonlinearity on blood-to-tissue O₂ delivery. Despite these reservations, the response to sinusoidal variations in exercise rate may be a useful method to characterize the dynamics of the mechanisms controlling vascular resistance and end-capillary O₂ consumption during ongoing exercise. Extrapolation to the dynamic behavior of exercise hyperemia in other situations must be tested, but some confidence in the predictive power of the frequency-analysis approach can be derived from the excellent correlation of results from the impulse and the sinusoidal experiments.

END-CAPILLARY O₂ SATURATION

The similarity of the frequency response of end-capillary O₂ at low and high constant flow (Fig. 3B) suggested that delivery per se did not affect the dynamics of end-capillary O₂ under the conditions of our experiments. Moreover, since the Fourier transform of the O₂ response to brief tetanus very nearly superimposed on the observed frequency response of O₂ at high and low flow (Fig. 3B), it appeared that end-capillary O₂ was controlled by mechanisms having essentially the same dynamics in these three situations. The 0.01-Hz corner frequency and the 6-db/octave loss of gain with increasing frequency of exercise modulation indicated that end-capillary O₂ behaved, in the modulation frequency range investigated, as a first-order system with a rate constant of approximately 0.06/sec.

VASCULAR RESISTANCE

The frequency response of vascular resistance to sinusoidal modulation of twitch rate was flow dependent (Fig. 3A). Vascular control was faster at high-flow rates than it was at low-flow rates, because resistance followed higher modulation frequencies with less gain loss at high-flow rates than it did at low-flow rates.
Flow rate could alter the dynamics of the vascular response by altering the rate at which vasodilator metabolites are removed from tissue by flow washout. However, the speed of the vascular responses made it unlikely that flow removal of dilators explained the flow dependence of the vascular response in this study. The fastest conceivable washout rate constant of a highly diffusible, flow-limited substance was 0.0006/sec at the high-flow rate (57.5 ml/100 g min⁻¹). Thus, even at the high-flow rate, a system of vascular control that depended on flow removal of dilator could not follow exercise modulations at greater than approximately 0.0015 Hz (corner frequency). O₂ is an exception because of the high solubility of O₂ in blood which allows delivery that would otherwise require much higher flow rates. Since resistance followed considerably higher modulation frequencies without appreciable gain loss at both flow rates used in this study, dilator washout cannot explain the different dynamics at high and low flow nor can washout be a major route for dilator removal in either case (9). Dornhorst and Whelan (17) also concluded that removal of the exercise vasodilator(s) is not solely flow dependent. Therefore, local degradation and cell uptake remain as the most likely important routes of removal of vasodilator from the interstitial space. Certain experimental situations, e.g., periods of exercise without flow (18), produce resistance alterations which last for 30 minutes or more after cessation of exercise. The slow time course of vascular recovery in these extreme circumstances might well be compatible with a vasodilator washout mechanism.

If we assume linearity, the high-flow vascular response can be characterized as first order with a time constant of approximately 10 seconds or a halftime of 7 seconds in the frequency range investigated. This time constant was the same as the time constant for the monoeexponential return of resistance to control following a single brief tetanus (9). The similarity between the dynamics of the resistance response to brief tetanus and the frequency response of resistance at high flow was also indicated by the close approximation of the Fourier transform of the brief tetanus response to the high-flow frequency response of resistance (Fig. 3A). Therefore, vascular control mechanisms as rapid as those that produce the response to brief tetanus must also be operative during continuous exercise.

End-capillary O₂ saturation dynamics are probably a reasonable reflection of the dynamics of the Po₂ to which resistance values are exposed (9). If this statement is true, we can conclude that vascular wall Po₂ appears to have slower dynamics than the resistance response at high flow and is unlikely to be the mediator of this response. The resistance response at low flow appears to have dynamics similar to those of end-capillary O₂ and so could be controlled by a factor related to vascular wall Po₂.

Our estimate of the dynamics of end-capillary O₂ or blood-to-tissue transport may allow statements about the dynamics of cell oxidative metabolism if certain assumptions are correct. These assumptions are that (1) end-capillary Po₂ is, on the average, in equilibrium with some average muscle Po₂, and (2) myoglobin and dissolved O₂ do not supply any sizable portion of the total O₂ used in the portion of the cycle when twitch rate is increasing. Whether end-capillary O₂ is in equilibrium with some average muscle Po₂ depends on the diffusion barriers for O₂ and the rate of flow. The critical venous Po₂ for constant O₂ consumption is high according to Landis and Pappenheimer (19) and close to an equilibrium value according to Stainsby and Otis (20). Since O₂ is a lipid-soluble gas and several such substances appear to come into diffusion equilibrium in skeletal muscle capillaries (21), the diffusion barriers should be minimal for O₂. However, there is considerable evidence for heterogeneity of flow per volume in muscle on a macroscopic level (22) and Po₂ on a microscopic level (23); therefore, the best we could expect would be an averaged approximation of cell O₂ dynamics.

If myoglobin supplies part of the O₂ delivered across the mitochondrial boundary during increasing twitch rate and then takes up O₂ during decreasing twitch rate, true oxidative metabolism dynamics could be buffered so that the dynamics revealed by blood-tissue transport (end-capillary O₂) are slower than those of oxidative metabolism. If so, doubling flow from 37.2 ± 7.3 to 57.5 ± 8.3 ml/100 g min⁻¹ might be expected to raise cellular Po₂, increase saturation of myoglobin, and reduce the buffering effect because of the nonlinear Po₂-myoglobin association curve. The end-capillary dynamics for O₂ consumption are the same at the high- and low-flow rates (Fig. 3B), and this observation argues against a major effect of tissue O₂ storage in our observed dynamics.

At high flow, the resistance response followed considerably higher modulation frequencies than did the end-capillary O₂ in three of four dogs (Fig.
Thus, if our assumptions are correct, it seems unlikely that the vascular resistance changes are secondarily related to changes in venous \( O_2 \) at high flow. Piiper et al. (24) noted that, at the onset of continuous exercise, flow rose at a faster rate than did \( O_2 \) consumption. In that situation, however, it is possible to assume certain dose-response curves between tissue \( P_O_2 \) and vascular resistance which would make the rate of the response appear faster than the rate of change of the controller. It is not possible to explain the discrepancy between the high-flow resistance and the end-capillary \( O_2 \) frequency responses reported in this paper on that basis because we are presumably operating on the same portion of any such dose-response curves at all modulation frequencies. Any systematic shifts in \( O_2 \) consumption over the day cannot participate since the order in which the modulation frequencies were employed was varied from dog to dog.

The vascular resistance frequency response at low constant flow was approximately that of the end-capillary \( O_2 \) response (Fig. 2B). Thus at low flow, changes in vascular resistance may be secondarily related to changes in tissue \( O_2 \). If this hypothesis is correct, the near superposition of the vascular and end-capillary \( O_2 \) frequency responses at low flow indicate that the processes linking resistance to tissue \( O_2 \) may be rapid compared with those controlling \( O_2 \) consumption itself; i.e., the rates of change of resistance could be limited almost entirely by the rates of change of \( O_2 \) consumption. Penaz et al. (25) have studied the vascular frequency response to sinusoidal modulation of sympathetic nerve stimulation of the femoral bed of rabbits. Their results indicate that the vascular response follows modulation of sympathetic stimulation well up to a frequency of approximately 0.05 Hz. Basar et al. (26) have shown that the renal vascular response to sinusoidal alterations in perfusion pressure reaches a maximum at 0.3 Hz. These observations indicate that vascular smooth muscle itself is capable of must faster changes in tension than either of the vascular resistance frequency responses in this paper indicate.

The vascular response to a brief tetanus at low constant flow (9) is biphasic with distinct rapid and slow components. The time course of the slow component appears to be nearly that of increased \( O_2 \) consumption following brief tetanus, but the magnitude of this slow component is small in relation to the entire vascular response following a single brief tetanus. However, the magnitude of this slow component increases much more than that of the rapid phase when two closely spaced tetani are performed (9). This phenomenon indicates that the mechanism(s) responsible for the second phase of the resistance response to brief tetanus might become increasingly more important and perhaps even dominate if exercise were continued at low flow. The similarity between the low-flow resistance and the end-capillary \( O_2 \) frequency responses reported in this paper support that possibility, i.e., the frequency response of resistance during low flow is close to that which is predicted from the slow phase of the vascular response to a brief tetanus observed at low flow.

The discrepancy between the resistance response at high and low constant flow raises two questions. (1) Do different mechanisms, with inherently different dynamics, dominate the control of vascular resistance during exercise at high and low flow? (2) Does a single mechanism, the dynamics of which are flow dependent, control vascular resistance? The biphasic response to a brief tetanus suggests the possibility that at least two distinct mechanisms are involved. If only one mechanism were acting, a simple slowing of the vascular response would have been expected rather than a biphasic response caused by the appearance of a second slow phase (9).

Vascular wall \( P_O_2 \) is an example of a potential mechanism for vascular control which might contribute differently to the control of vascular resistance at different flow rates. At high flow, average tissue \( O_2 \) levels must certainly be higher than they are during the performance of the same exercise at low flow. Fluctuations about the high average \( P_O_2 \) level at high flow might have little effect on vascular smooth muscle. Thus other mechanisms of vascular control might dominate at high flow. At low flow, the average tissue \( P_O_2 \) might be at a level where vascular smooth muscle is sensitive to \( O_2 \) and thus fluctuations in tissue \( P_O_2 \) might play an important role in controlling vascular resistance.

We agree with other investigators (8) who suggest that multiple mechanisms control the vascular response to skeletal muscle exercise. The study of the dynamics of exercise hyperemia may be a fruitful approach to identifying and quantifying the importance of these mechanisms. This approach has allowed characterizations of at least two mechanisms of local vascular control with inherently different dynamics. At least one of these mechanisms appears to be too rapid to be directly or secondarily related to tissue oxidative metabo-
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In addition, these data indicate that different mechanisms may dominate the control of vascular resistance under different flow conditions and exercise patterns.

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References

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