Role of Resistance and Exchange Vessels in Local Microvascular Control of Skeletal Muscle Oxygenation in the Dog

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SUMMARY The effects of reduction in perfusion pressure, arterial hypoxia, muscle contraction, and adrenergic stimulation on the hindlimb muscle circulation were studied. Under normal conditions (venous Po2 ≥ 40 mm Hg), oxygen delivery to the muscle was maintained mainly by large increases in the capillary exchange capacity and the oxygen extraction ratio in accord with tissue demand following the application of the above stresses. The participation of the resistance vessels under these conditions was minimal. The prevailing venous oxygen tension then was reduced by several means and the response of vascular resistance and capillary exchange capacity to the same stresses was reexamined. At the lower prevailing venous Po2, the sensitivity of the resistance vessels to metabolic and hemodynamic disturbances was greatly increased. Consequently, blood flow autoregulation, functional hyperemia, and hypoxic hyperemia were more intense when venous oxygen tension was low. In contrast, the contribution of exchange capacity was diminished, probably owing to the fact that most of the capillaries already are open at low venous Po2. These data suggest that the focus of local microvascular control of muscle oxygenation shifts from the normally more sensitive precapillary sphincters to the proximal flow-controlling arterioles as the prevailing venous oxygen tension falls. Yet, although the relative contribution of the resistance and exchange vessels to intrinsic regulation of tissue oxygenation is related to the prevailing venous oxygen tension, the two compensatory mechanisms operating in concert maintain tissue Po2 above the critical level over a wide range of stresses.

THE DELIVERY of oxygen to skeletal muscle cells is critically dependent on the state of the local blood circulation. Because of this intimate relationship between parenchymal oxidative metabolism and the microcirculation, the concept of intrinsic metabolic regulation of microvascular tone has received much attention and support. In essence, the metabolic theory of local microvascular regulation proposes that the microvascular segments which determine blood flow and capillary exchange capacity are modulated by changes in tissue Po2, either directly or through the release of vasoactive metabolites. For example, a reduction in tissue oxygenation elicits increases both in blood flow and in exchange capacity, and consequently tissue oxygenation is returned toward the normal level. On the other hand, an elevation of tissue Po2 induces reductions of blood flow and capillary exchange capacity, thereby lowering tissue Po2 toward its normal value. Thus, coupling of microvascular tone to muscle metabolism provides a local feedback mechanism for maintaining optimal tissue oxygenation in the face of a variety of stresses. With this simple concept in mind, we investigated the relative contribution of changes in vascular resistance and capillary exchange capacity to local regulation of skeletal muscle oxygenation under a variety of conditions.

Methods

Thirty-six mongrel dogs weighing 16–22 kg were anesthetized with sodium pentobarbital (30 mg/kg, iv) and heparinized with sodium heparin (1,000 IU/kg). To ensure the absence of neurogenic influences during our study of local regulatory phenomena, most of the dogs were rendered areflexic by a technique described in detail elsewhere. Basically, the central nervous system was destroyed by injection of alcohol into the spinal cord and by decapitation. Respiration was maintained artificially and mean systemic arterial pressure was stabilized at 100 mm Hg by slow intravenous infusion of a 1:1 epinephrine-norepinephrine mixture (0.1–0.7 μg/kg per min). Rectal temperature was monitored and maintained at 38°C by infrared heat lamps. A reservoir containing the dog’s own blood was suspended from the ceiling and connected to a catheter in the right carotid artery. The height of the reservoir could be adjusted to change arterial pressure to any desired level.

The iliac artery and vein of the left hindlimb were isolated through an incision in the lower abdomen. A short (10 cm) loop of polyethylene tubing fitted with an extracorporeal flow probe (inside diameter = 5 mm) was inserted into the iliac artery. Blood flow was monitored with a Biotronix (BL-310) electromagnetic flowmeter. Iliac perfusion pressure was measured with a Statham P23AC transducer connected to the arterial loop via a T-connector. Another polyethylene loop was inserted into the iliac vein. Iliac vein pressure was measured with a Statham P23BC transducer connected to the venous loop. The arteriovenous oxygen difference across the hindlimb continuously was monitored by an Oxford arteriovenous oxygen difference analyzer. Arterial blood was withdrawn continuously from the femoral artery of the contralateral limb. Venous blood was withdrawn from the venous loop. Oxygen delivery to the muscle cells or O2 uptake was calculated by multiplying hindlimb blood flow by the arteriovenous oxygen difference. Arterial and venous Po2 also were determined during the steady state.

Because we wanted to focus our attention on muscle, we...
skinned the hindlimb and occluded the paw with a metal clamp. To ensure that the majority of the blood supply to the limb muscles came from the iliac artery, the deep circumflex and internal iliac arteries were ligated. With this procedure, venous outflow was reduced to 1 ml/min or less during iliac artery occlusion, indicating absence of significant collateral circulation.

A fluid-filled plethysmograph was fitted on the hindlimb and connected to a reservoir suspended from a Grass FT 10C transducer for the purpose of monitoring changes in limb volume. An inflatable compression cuff wrapped around the lower abdomen was utilized to produce an increase in iliac vein pressure of 10 mm Hg. The slope of the weight increase was calculated every 30 seconds and plotted on semilog paper. Extrapolation of the slow component of weight gain to zero time provided an approximation of the initial rate of transcapillary filtration. Assuming 80% transmission of venous pressure to the capillary level, the capillary filtration coefficient was obtained by dividing the initial rate of transcapillary filtration by 8 mm Hg. The assumption of a constant percentage transmission of pressure to the capillaries is subject to error. During arterial hypotension, muscle contraction, and arterial hypoxia, the precapillary to postcapillary resistance ratio is altered and therefore the degree of transmission of venous pressure to the capillaries may change. Consequently, the capillary filtration may be underestimated by as much as 20%. This source of error must be kept in mind when quantifying changes in capillary exchange capacity.

The experimental procedure consisted of monitoring the response of the limb vasculature to (1) reduction in perfusion pressure, (2) muscle contraction, (3) arterial hypoxia, and (4) adrenergic stimulation. Perfusion pressure was reduced by lowering the arterial reservoir to 50 mm Hg. Muscle stimulation was accomplished by direct electrical stimulation of the thigh muscles. Arterial hypoxia was induced by respiring the dog with 15, 10, 8, and 6% mixtures of oxygen in nitrogen. Adrenergic stimulation was increased above the basal level by accelerating the rate of catecholamine infusion.

Iliac artery pressure, iliac vein pressure, iliac blood flow, arteriovenous oxygen difference, and hindlimb volume were monitored continuously on a Grass model 7 polygraph.

Results

Basal State of the Muscle Circulation

In many of the early experiments, values for vascular and metabolic variables were obtained prior to destruction of the spinal cord and decapitation. In general, these data suggested a normal flow rate of 7-10 ml/min per 100 g, a resting oxygen uptake of 0.3-0.5 ml/min per 100 g, and a normal venous PO₂ of 40 mm Hg or more. Because the reactions of the muscle circulation to various stresses were observed to depend on the initial venous PO₂, the catecholamine infusion required to support the areflexic preparation was maintained at a rate (0.1-0.7 µg/min per kg) compatible with an initial venous oxygen tension of 40 mm Hg or higher. With these physiological rates of catecholamine infusion, the vascular and metabolic variables of skeletal muscle in 36 dogs were: resting blood flow, 9.7 ± 2.0 ml/min per 100 g (mean ± SD); basal oxygen consumption, 0.44 ± 0.08 ml/min per 100 g; normal capillary filtration coefficient, 0.012 ± 0.003 ml/min/mm Hg-100 g; and a resting venous PO₂ of 44.2 ± 5.6 mm Hg.

Autoregulation of Blood Flow

Autoregulation of blood flow has been defined as the "intrinsic tendency of an organ to maintain constant blood flow despite changes in arterial perfusion pressure." The typical response of the resting muscle circulation to a reduction of arterial pressure is illustrated in panel A of Figure 1. After a 50% reduction in perfusion pressure (Pₐ), the blood flow (F) through skeletal muscle was reduced by the same magnitude, indicating an absence of significant blood flow autoregulation. Yet, in spite of the reduced flow, oxygen delivery to the muscle cells (V₀₂) was maintained near the control level and oxygen extraction increased 2-fold. Associated with this greater O₂ extraction ratio was a 2-fold increase in the capillary filtration coefficient (Kᵣ), suggesting a doubling of the number of open capillaries.

In contrast to this behavior of the muscle vasculature under basal conditions, panel B of Figure 1 shows the effects of a perfusion pressure reduction of the same magnitude in the same preparation after the venous oxygen tension was lowered by steady state arterial hypoxia. Again, oxygen uptake was well maintained when perfusion pressure was decreased. However, at this lower prevailing venous O₂ level, flow autoregulation was evident and consequently contributed to the regulation of transcapillary oxygen flux. With this increase in the degree of autoregulation, the contribution of increased oxygen extraction was diminished. Associated with this lesser increase in oxygen extraction ratio was a smaller increase in effective capillary density as compared to the experiment in panel A. In a similar manner, the degree of flow autoregulation was increased when the prevailing venous PO₂ was lowered by muscle stimulation (Figure 2, panel B) or by increasing the rate of catechol-
amined infusion (Figure 3, panel B). The lowered venous oxygen tension during catecholamine infusion occurred secondary to a reduction in blood flow at a constant oxygen uptake. The microvascular and metabolic responses to a 50% reduction in perfusion pressure at different prevailing venous Po2 levels are summarized in Figure 4. Note that even if the capillary filtration coefficient at low oxygen levels is underestimated by 20% there is still a 3-fold difference between Kf values at high and low venous oxygen levels.

HYPOXEMIC HYPEREMIA

An alternative means of reducing oxygen availability at the capillary level is to lower the oxygen concentration of arterial blood. The effect of arterial hypoxemia on the muscle circulation is shown in Figure 5. Under resting conditions, a 6% oxygen-94% nitrogen atmosphere induced a 2.5-fold increase in limb blood flow (panel A). In addition, the calculated oxygen extraction fraction (arteriovenous O2 difference/arterial O2) increased and effective capillary density rose to nearly 2.5 times control. In contrast, after venous oxygen tension was lowered to 24 mm Hg by catecholamine infusion, blood flow increased more than 4-fold in the same preparation when the identical hypoxic stimulus was applied (Fig. 5, panel B). At this lower initial venous Po2, the oxygen extraction ratio and capillary filtration coefficient changes were much less than those

![Figure 2](image2.png)

**FIGURE 2** Typical response of muscle circulation to a perfusion pressure reduction under normal conditions (A) and during muscle contractions (B). Abbreviations as in Figure 1.

![Figure 3](image3.png)

**FIGURE 3** Typical response of muscle circulation to a perfusion pressure reduction under normal conditions (A) and during catecholamine infusion (B). Abbreviations as in Figure 1.

![Figure 4](image4.png)

**FIGURE 4** Modulating effect of initial venous Po2 on microvascular and metabolic responses to a 50% reduction of perfusion pressure. Initial venous oxygen tension lowered by mild arterial hypoxia (squares, n = 6), by muscle stimulation (triangles, n = 7), or by catecholamine infusion (circles, n = 8). Vertical bars indicate SEM.

![Figure 5](image5.png)

**FIGURE 5** Typical response of the muscle circulation to arterial hypoxemia under normal conditions (A) and during catecholamine infusion (B). Abbreviations as in Figure 1.
observed at the normal venous oxygen tension. Yet, whether the initial venous $P_{O_2}$ was 42 mm Hg (panel A) or 24 mm Hg (panel B), local regulation of transcapillary oxygen flux was nearly perfect. A similar pattern was observed when the initial venous oxygenation was lowered by mild muscle stimulation. Figure 6 summarizes the vascular and metabolic responses to graded arterial hypoxia under control conditions (solid line) and under low venous $P_{O_2}$ conditions (dotted line) produced by increased rate of catecholamine infusion (squares) or mild muscle stimulation (triangles).

**FUNCTIONAL HYPEREMIA**

Another facet of local control of the muscle circulation is evinced by the elevated blood flow or functional hyperemia induced by increased metabolic demand during muscle stimulation. A typical response of the normal muscle circulation to stimulation at 1.6 impulses/sec is shown in panel A of Figure 7. At a normal venous $P_{O_2}$ of 42 mm Hg, muscle stimulation produced a 3-fold increase in blood flow and a 3-fold increase in oxygen extraction. In contrast, when the venous oxygen tension in the same preparation was lowered to 32 mm Hg by a 2-fold increase in the catecholamine infusion rate (panel B), stimulation at the same frequency induced a 6-fold increase in blood flow and only a 40% increase in the oxygen extraction ratio. Thus, although the increase in oxygen uptake was nearly identical in the two experiments, the mechanism utilized to accelerate oxygen flux varied according to the venous $P_{O_2}$ before muscle stimulation. The effect of graded muscle stimulation on the blood flow, capillary filtration coefficient, and venous tension, and oxygen extraction are summarized in Figure 8. Note that similar results were obtained whether the venous oxygen level prior to stimulation was lowered by catecholamine infusion (triangles) or by mild arterial hypoxia (squares).

**AUTOREGULATORY ESCAPE OF EXCHANGE CAPACITY AND VASCULAR RESISTANCE FROM ADRENERGIC STIMULATION**

As discussed in the previous sections, we frequently utilized adrenergic stimulation to increase vascular tone in the muscle circulation and thereby achieve lower blood flow and venous oxygen levels. Although blood flow decreased during adrenergic stimulation, effective capillary density consistently increased, suggesting an unexpected relaxation of the vascular structures involved in regulation of exchange capacity. This phenomenon, known as "autoregulatory escape from adrenergic stimuli," is evident in Figures 3 and 5. The initial values of the filtration coefficient are higher during catecholamine infusion at high rates (panel B of Figs. 3 and 5) than during the slower infusion required to maintain normal arterial pressure in the areflexic preparation (panel A of Figures 3 and 5). To investigate the effect of initial oxygen tension on vascular escape, adrenergic stimulation was studied under control conditions, during arterial hypoxemia, and during muscle contraction (Table 1). At normal venous oxygenation, a 3-fold increase in catecholamine infusion rate caused a reduction of blood flow to nearly half of normal. However, oxygen uptake remained near the normal level and the oxygen extraction ratio increased. In addition, the capillary filtration coefficient increased above the control level. During arterial hypoxia or muscle stimulation, the capillary filtration coefficient and oxygen extraction increase to high values, as demonstrated in previous sections of this paper. Superimposing the same adrenergic stimulus on hypoxemia and muscle contractions results in an initial increase in vascular resistance; however,
within 2–3 minutes blood flow returns toward the preinfusion level. These findings suggest that the resistance vessels escape from the constrictor action of catecholamines when tissue oxygenation is lower than normal.

EXPERIMENTS IN INTACT, ANESTHETIZED DOGS

In three dogs flow autoregulation, functional hyperemia, and adrenergic stimulation were studied prior to destruction of the nervous system. Blood flow was monitored with a noncannulating flow probe fitted around the external iliac artery. The probe was connected to a Carolina square wave flowmeter. Perfusion pressure was decreased by graded compression of the abdominal aorta just below the renal arteries. Adrenergic stimulation was achieved by electrical stimulation of the sympathetic plexus coursing the length of the iliac artery. The results obtained in these experiments were similar both qualitatively and quantitatively to those obtained in the areflexic preparation. Unfortunately, we were unable to study arterial hypoxia in the reflexic dog, because of the effect of low arterial Po2 on chemoreceptors and the cardiovascular centers in the central nervous system. Recently, we completed a more extensive study of flow autoregulation in the anesthetized, intact dog.14 In eight dogs, the degree of flow autoregulation was similar to that observed in the present study. These findings in the intact, anesthetized dog suggest that the results of the present study are not artifacts of an unphysiological preparation. On the contrary, a large body of experimental evidence has demonstrated the extreme usefulness of the areflexic dog preparation in studying a multitude of cardiovascular functions without interference from neural control loops.8–15–20

Discussion

LOCAL MICROVASCULAR CONTROL OF MUSCLE OXYGENATION

Recent measurements of cell Po2 in skeletal muscle yielded values of 5–10 mm Hg.21–24 Because muscle oxygen utilization is limited by oxygen availability below oxygen tensions of 1 mm Hg, several investigators have suggested that the muscle cell normally is on the verge of hypoxia.22–25 Yet, a number of studies,26–29 including our present one, indicate that capillary Po2 can fall by as much as 30 mm Hg before cell Po2 reaches the critical level and oxygen uptake is compromised. This maintenance of transcapillary oxygen flux in accord with the demands of the muscle under a variety of stresses suggests the existence of local microvascular mechanisms involved in stabilization of cell Po2 above the critical level.

According to current concepts of microvascular structure and function,18,28 the loci of local vascular control of tissue oxygenation are arterioles and precapillary sphincters. Local regulation of blood flow occurs at the arterioles, the major resistance segment of the muscle microvasculature.29 In essence, intrinsic metabolic regulation of blood flow serves to minimize changes in capillary oxygen tension during hemodynamic and metabolic stresses.28,30 Many studies28–34 have demonstrated that capillary exchange ca-

### Table 1 Effect of Norepinephrine Infusion on Muscle Circulation at Normal and Low Venous Po2

<table>
<thead>
<tr>
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<th>Control (N = 18)</th>
<th>Hypoxia (N = 9)</th>
<th>Contraction (N = 9)</th>
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<tr>
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<td>P</td>
<td>I</td>
<td>P</td>
</tr>
<tr>
<td><strong>Venous Po2 (mm Hg)</strong></td>
<td></td>
<td></td>
<td>43.8 ± 5.2</td>
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<tr>
<td><strong>Blood flow (ml/min per 100 g)</strong></td>
<td>9.8 ± 2.2</td>
<td>4.8 ± 1.0*</td>
<td>11.3 ± 2.9</td>
</tr>
<tr>
<td><strong>A-V O2 (vol %)</strong></td>
<td>4.7 ± 0.7</td>
<td>9.3 ± 1.1*</td>
<td>3.5 ± .8</td>
</tr>
<tr>
<td><strong>O2 consumption (ml/min per 100 g)</strong></td>
<td>0.45 ± 0.08</td>
<td>0.44 ± 0.07</td>
<td>0.39 ± 0.08</td>
</tr>
<tr>
<td><strong>Capillary filtration coefficient (ml/min/mm Hg per 100 g)</strong></td>
<td>0.012 ± 0.004</td>
<td>0.023 ± 0.006*</td>
<td>0.026 ± 0.007</td>
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* Significant differences from preinfusion values, P < 0.01.
capillary exchange capacity have been demonstrated in a large number of studies, very few data are available concerning the relative contributions of the arterioles and precapillary sphincters to prevention of muscle cell hypoxia under normal and stress conditions. In our study, muscle preparations characterized by normal blood flow, normal venous Po2, and resting oxygen consumption exhibited only small degrees of local flow control in response to changes in perfusion pressure, arterial oxygen concentration, and oxygen demand. However, effective capillary density increased markedly in these preparations under the same stress conditions. Hence, the experimental results suggest that in normal resting muscle the metabolic sensitivity of the vascular elements exerting control over exchange capacity is greater than that of the resistance vessels. The relative insensitivity of the arterioles at normal and high venous Po2 is not due to lack of adequate resting vascular tone, because dilation of the muscle vasculature during perfusion with oxygen-free blood or with cyanide allowed a 4- to 5-fold increase in blood flow. Furthermore, as discussed below, high degrees of blood flow regulation were observed under conditions in which vascular tone actually was lower than normal.

In contrast to this normal pattern, the contribution of the local flow control mechanism becomes increasingly important as the prevailing venous oxygen tension is progressively lowered. This behavior was observed when the venous Po2 was decreased by adrenergic stimulation, by arterial hypoxia, or by muscle contractions. Thus, in our study, autoregulation of blood flow, functional hyperemia, and hypoxic hyperemia were all facilitated at low venous oxygen tensions. Previous studies in other laboratories support this viewpoint. Jones and Berne37 noted that muscle preparations showing spontaneously high venous oxygen levels autoregulated flow poorly, whereas preparations exhibiting high vascular tone and low venous Po2 demonstrated strong flow autoregulation. In addition, several investigators38, 39 have observed better autoregulation in exercising muscle than in resting tissue. To our knowledge, our present study is the first to demonstrate greater flow autoregulation during arterial hypoxia and during adrenergic stimulation. In any event, our results and those of others37, 39 suggest that the sensitivity of the resistance vessels to changes in tissue oxygenation is increased at low venous O2 tensions. Thus, when the prevailing venous Po2 is low, adequate delivery of oxygen to the muscle cells is maintained mainly by the ability of the flow control mechanism to prevent large decreases in capillary Po2. The relative unimportance of the precapillary sphincter mechanism under these conditions may be due to the fact that a large fraction of the capillaries are already open.

At present, we have no evidence to establish the mechanism involved in the shift of control from the precapillary sphincter to the arterioles as metabolic and hemodynamic stresses become more severe. One possibility is that the sphincters are more sensitive to changes in local oxygen tension or metabolite concentrations. Alternatively, the arterioles and sphincters may be equally sensitive to local oxygen tension or metabolite levels, but significant changes in the effective stimulus in the vicinity of the arteriolar smooth muscle may not occur until venous Po2 falls to low levels. In the absence of data at the microvascular level, these and other possibilities must remain speculative.

INTERACTION OF LOCAL AND ADRENERGIC CONTROLS OF MUSCLE CIRCULATION-AUTOREGULATORY ESCAPE FROM ADRENERGIC STIMULI

Although muscle cell Po2 is maintained within narrow limits despite a variety of local stresses, it also is a well established fact that nervous control of vascular resistance in skeletal muscle is of major importance for reflex regulation of systemic arterial pressure.40 Our results suggest that when neurogenic and local mechanisms are brought into opposition, local tissue oxygenation and systemic arterial pressure can be regulated simultaneously under physiological conditions. If systemic pressure falls suddenly, baroreceptor-induced vasoconstriction of the muscle vasculature assists in the return of pressure to normal. Yet, the diminished muscle blood flow associated with neurogenic vascular constriction decreases the flow of oxygen to the exchange vessels and, consequently, capillary oxygen tension decreases. In addition, effective capillary density is reduced during the early stages of sympathetic stimulation.13 In turn, reduction of capillary Po2 and exchange capacity precipitate a dramatic fall in tissue oxygenation. Apparently, vasodilator metabolites released at low tissue oxygen levels override the constrictor effect of norepinephrine on the metabolically sensitive sphincters. Consequently, the sphincters escape from neurogenic influences and effective capillary density rises above the control level. The increase in diffusion capacity compensates for the neurogenic reduction of blood flow and tissue oxygen delivery is maintained by a rise in oxygen extraction. Thus, the hierarchical arrangement of local and nervous control loops allows systemic arterial pressure and muscle oxygen delivery to be simultaneously regulated under normal conditions.

Although the resistance vessels do not escape from adrenergic stimulation under normal conditions, this is not the case when the prevailing venous oxygen tension is low (see Table 1). Perhaps this ability of the resistance vessels to escape the influences of norepinephrine at low venous Po2 represents another manifestation of the transfer of the locus of local control to the proximal arterioles at low levels of tissue oxygenation. Several studies from other laboratories support this concept. Remensnyder et al.46 demonstrated a "functional sympatholysis" in contracting hindlimbs sub-
jected to sympathetic stimulation. Lewis and Mellander observed less vasoconstriction during sympathetic stimulation in limbs perfused at low flow than in preparations perfused at normal flow rate. In the present study, we also observed decreased sensitivity of resistance vessels to catecholamines during arterial hypoxia. Low venous oxygen tension is a common denominator in the exercise, low flow, and arterial hypoxia experiments.

Acknowledgments

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