Regulation of human skeletal muscle perfusion and its heterogeneity during exercise in moderate hypoxia

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1Turku PET, Departments of 2Clinical Physiology and Nuclear Medicine, 3Anesthesiology and Intensive Care, 4Medicine, and 5Radiology, Turku University Hospital, University of Turku, Turku, Finland; 6Unit for Sports and Exercise Medicine, Institute of Clinical Medicine, University of Helsinki, Helsinki, Finland; and 7Centre for Healthy Aging, Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark

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Heinonen IH, Kemppainen J, Kaskinoro K, Peltonen JE, Borra R, Lindroos M, Oikonen V, Nuutila P, Knuutila J, Boushel R, Kalliokoski KK. Regulation of human skeletal muscle perfusion and its heterogeneity during exercise in moderate hypoxia. Am J Physiol Regul Integr Comp Physiol 299: R72–R79, 2010. First published April 28, 2010; doi:10.1152/ajpregu.00056.2010.—Although many effects of both acute and chronic hypoxia on the circulation are well characterized, the distribution and regulation of blood flow (BF) heterogeneity in skeletal muscle during systemic hypoxia is not well understood in humans. We measured muscle BF within the thigh muscles of nine healthy young men using positron emission tomography during one-leg dynamic knee extension exercise in normoxia and moderate physiological systemic hypoxia (14% O2 corresponding to ~3,400 m of altitude) without and with local adenosine receptor inhibition with femoral artery infusion of aminophylline. Systemic hypoxia reduced oxygen extraction of the limb but increased muscle BF, and this flow increment was confined solely to the exercising quadriceps femoris muscle. Exercising muscle BF heterogeneity was reduced from rest (P = 0.055) but was not affected by hypoxia. Adenosine receptor inhibition had no effect on capillary BF during exercise in either normoxia or hypoxia. Finally, one-leg exercise increased muscle BF heterogeneity both in the resting posterior hamstring part of the exercising leg and in the resting contralateral leg, whereas mean BF was unchanged. In conclusion, the results show that increased BF during one-leg exercise in moderate hypoxia is confined only to the contracting muscles, and the working muscle hyperemia appears not to be directly mediated by adenosine. Increased flow heterogeneity in noncontracting muscles likely reflects sympathetic nervous constraints to curtail BF increments in areas other than working skeletal muscles, but this effect is not potentiated in moderate systemic hypoxia during small muscle mass exercise.

positron emission tomography; exercise; hypoxia

IN 1919, AUGUST KROGH reported that, in addition to the importance of capillary density for tissue oxygenation, it is not sufficient to simply supply an adequate amount of oxygen to the organ as a whole, but oxygen has to be distributed within the organ precisely where it is needed (27, 28). Thus it must be appreciated that not only bulk blood flow but especially its appropriate distribution between and within working skeletal muscles is of importance for precise matching of oxygen supply and metabolism (11), especially during exercise. In this regard, we recently reported that blood flow heterogeneity decreases with increasing exercise intensity within contracting quadriceps femoris (QF) muscle (21). It is, however, not known how blood flow heterogeneity within active and nonactive thigh muscles changes when metabolism is challenged by reduced oxygen content of the blood, as is the case during exercise in systemic hypoxia.

Although many effects of both acute and chronic hypoxia on the circulation are well characterized, relatively little is known about how muscle blood flow is regulated in acute hypoxia in humans. In the working limb, mean blood flow is usually enhanced with systemic hypoxia (10, 26), yet vasomotor activity is preserved or enhanced (6, 43). In nonworking muscles, it is generally considered that already during normoxic exercise increasing sympathetic nervous activation prevents increases in muscle blood flow, and, thus, cardiac output is redirected effectively to active skeletal muscles. Sympathetic restraint of muscle blood flow is augmented during hypoxic exercise (43), but this pattern has been studied only sparsely in humans. Only a few previous human studies have addressed the effect of increased sympathetic activation in acute and chronic hypoxia on whole limb blood flow (6, 30), but flow distribution and its regulation specifically within muscle has never been determined.

Metabolic vasodilation is considered an important signaling mechanism controlling muscle blood flow in exercising muscle in both normoxia and hypoxia. Endogenous adenosine can be one of the signals mediating metabolic vasodilation in skeletal muscle. The adenosine hypothesis proposes that adenosine functions as a distress signal to match oxygen supply to cellular metabolism in hypoxic and/or ischemic tissue (3). Evidence to support that increased blood flow in the exercising limb under moderate hypoxia is indeed the result of enhanced metabolic signals is inferred, since sympatholysis does not contribute to the enhanced blood flow in hypoxic forearm exercise (47). A major role of adenosine for enhancing limb blood flow during hypoxic forearm exercise was not substantiated by a recent study, which found no difference between flow in normoxia and hypoxia with adenosine receptor blockade (8). To the best of our knowledge, the contribution of adenosine to the muscle nutritive blood flow response during exercise with decreased arterial oxygen supply in hypoxia has never been studied in humans.

Taken together, there were several purposes in the present study. First, we aimed to elucidate the patterns of muscle blood flow redistribution in active and nonactive muscles during dynamic one-leg knee extension exercise during physiological


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systemic hypoxia corresponding to \( \sim 3,400 \) meters of altitude (inspired \( O_2 \) 14%). Second, we wanted to study whether blood flow heterogeneity changes within the active and inactive muscles during exercise in hypoxia. Third, we studied whether adenosine is involved in the regulation of muscle capillary blood flow in hypoxic exercise by applying similar antagonism procedures as in several recent studies (8, 29, 32). We hypothesized that, in hypoxia, 1) mean blood flow would increase along with a decrease in flow heterogeneity in contracting muscles; 2) the opposite response would occur in inactive muscle; and 3) if adenosine indeed plays a role in matching oxygen supply to metabolic demands, capillary blood flow in exercising muscle would be reduced and flow heterogeneity increased during adenosine receptor blockade and the reduced blood flow would be compensated for by increased oxygen extraction to assure similar muscle oxygen consumption and work performance.

METHODS

Subjects. Nine healthy fit men (25 ± 5 yr, 184 ± 6 cm, 76 ± 9 kg) volunteered to participate in the study. The purpose, nature, and potential risks of the study were explained to the subjects before they gave their written informed consent to participate. The subjects were requested to abstain from caffeine-containing beverages for at least 24 h before the experiments and to avoid strenuous exercise within 48 h before the study. The subjects were not taking any regular medication. The study was performed at least 3 h after the subjects had eaten a light breakfast. The study was performed according to the Declaration of Helsinki and was approved by the Ethical Committee of the Hospital District of South-Western Finland and the National Agency for Medicines.

Study design. Skeletal muscle blood flow in the femoral region (Fig. 1) was measured using positron emission tomography (PET) with \([^{15}O]H_2O\), as described below. Muscle blood flow was measured first under normal resting conditions and then during systemic hypoxia (14% inspired \( O_2 \) in \( N_2 \); equivalent to altitude of \( \sim 3,400 \) m). After these measurements at rest, blood flow was measured during one-leg dynamic exercise (Fig. 1) in a counterbalanced setting without and with locally administered adenosine receptor antagonism by aminophylline with the subject breathing either normal room air or hypoxic gas. Additionally, radial artery and femoral vein blood samples for blood gas parameters were drawn for analysis in each occasion mentioned above.

We recently reported muscle blood flow values at rest and during normoxic exercise (20). A portion of the data gathered from that study in the same subjects is included in the present paper. In this study, we present mostly new data on the effect of hypoxia and adenosine receptor blockade by aminophylline on muscle blood flow during exercise.

Other procedures before and after PET measurements. Before the PET experiments, the antecubital vein was cannulated for tracer administration. For blood sampling, a radial artery cannula was placed under local anesthesia in the contralateral arm. Additionally, cannulas were placed under local anesthesia in the femoral artery and vein for local drug infusion (aminophylline) and blood sampling, respectively.

Subjects were then moved to the PET scanner with the femoral region under local anesthesia in the contralateral arm. Additionally, cannulas for blood gas parameters were drawn for analysis in each occasion mentioned above.

Other procedures before and after PET measurements. Before the PET experiments, the antecubital vein was cannulated for tracer administration. For blood sampling, a radial artery cannula was placed under local anesthesia in the contralateral arm. Additionally, cannulas were placed under local anesthesia in the femoral artery and vein for local drug infusion (aminophylline) and blood sampling, respectively.

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Fig. 1. Positron emission tomography imaging. A: the subject was positioned in the gantry so that the middle thigh was in the middle of the imaging area (12 cm). B and C: one-legged dynamic exercise was performed in the range of almost 90 degrees. Dynamic muscular work for the quadriceps femoris (QF) muscle consisted of a lifting phase and subsequent braking phase back to the starting position. D: regions of interest for blood flow analysis were drawn to represent whole thigh muscle, QF muscle, and the posterior muscle of the thigh. At rest, thigh muscle blood flow was fairly uniform (D), but, during exercise, the increment of blood flow was solely concentrated on exercising QF muscle (E). Color scaling for the respective blood flow values in images are provided on left. Thus the more green, yellow, or red is seen, the higher is the local blood flow. In general, increased heterogeneity in resting contralateral leg [left leg (L)] during one-leg exercise is readily seen in E. R, right leg.
intra-arterial aminophylline infusion at rest and at baseline hypoxia, as well as during exercise in NORMO and HYPO without and with aminophylline infusion, and scanning consisted of the following frames: 6 × 5, 12 × 10, and 7 × 30 s at rest and 6 × 5 and 12 × 10 s during exercise. During systemic hypoxia, breathing 14% oxygen gas began 5 min before imaging. During exercise, scanning commenced 3 min after exercise onset to obtain a metabolic steady-state situation and continued until the end of the exercise bout, which every time lasted 2.5 half-minutes, thus, altogether 5.5 min. Arterial blood radioactivity was also sampled continuously with a detector during imaging for blood flow quantification. Exercise consisted of dynamic one-leg exercise at 40 rpm with individually chosen workloads (4.3 ± 2.1 kg) with a knee angle range of motion of ~70–80 degrees (Fig. 1B).

Table 1. Heart rates, blood pressures, leg vascular resistance and conductance, and blood oxygen in radial artery and femoral vein at rest and at baseline hypoxia, as well as during exercise in NORMO and HYPO without and with intra-arterial aminophylline infusion

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Hypoxia</th>
<th>Exercise Without Aminophylline</th>
<th>Exercise With Aminophylline</th>
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<tr>
<td></td>
<td>NORMO</td>
<td>HYPO</td>
<td>NORMO</td>
<td>HYPO</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>61 ± 10</td>
<td>69 ± 10</td>
<td>92 ± 12a</td>
<td>102 ± 10ab</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>98 ± 7</td>
<td>98 ± 12</td>
<td>108 ± 6a</td>
<td>112 ± 10b</td>
</tr>
<tr>
<td>BPs, mmHg</td>
<td>125 ± 9</td>
<td>137 ± 18</td>
<td>146 ± 7a</td>
<td>152 ± 11b</td>
</tr>
<tr>
<td>BPd, mmHg</td>
<td>74 ± 6</td>
<td>79 ± 10</td>
<td>90 ± 9ab</td>
<td>92 ± 12</td>
</tr>
<tr>
<td>Leg VR, mmHg/ml-100 (g^-1 min^-1)</td>
<td>39 ± 19</td>
<td>37 ± 22</td>
<td>3.2 ± 1.0</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>Leg VC, (ml-100 g^-1 min^-1)-mmHg^-1</td>
<td>0.03 ± 0.02</td>
<td>0.04 ± 0.02</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Oxygen saturation-A, %</td>
<td>98 ± 1</td>
<td>91 ± 5a</td>
<td>98 ± 1</td>
<td>88 ± 5</td>
</tr>
<tr>
<td>Oxygen content-A, ml/l</td>
<td>186 ± 9</td>
<td>186 ± 13c</td>
<td>205 ± 9</td>
<td>182 ± 11d</td>
</tr>
<tr>
<td>Oxygen saturation-V, %</td>
<td>71 ± 9</td>
<td>71 ± 10</td>
<td>43 ± 16</td>
<td>37 ± 13b</td>
</tr>
<tr>
<td>Oxygen content-V, ml/l</td>
<td>152 ± 23</td>
<td>141 ± 18a</td>
<td>88 ± 32</td>
<td>78 ± 26b</td>
</tr>
<tr>
<td>Oxygen extraction, ml/l</td>
<td>48 ± 16</td>
<td>45 ± 14</td>
<td>117 ± 33b</td>
<td>101 ± 29b</td>
</tr>
<tr>
<td>OEF, %</td>
<td>24 ± 9</td>
<td>24 ± 8</td>
<td>57 ± 16b</td>
<td>56 ± 16</td>
</tr>
<tr>
<td>Leg VO2, ml-100 g^-1 min^-1</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>4.1 ± 1.0</td>
<td>4.0 ± 1.3</td>
</tr>
</tbody>
</table>

The results are means ± SD. NORMO, normoxia; HYPO, hypoxia; HR, heart rate; MAP, mean arterial pressure; BPs, systolic blood pressure; BPd, diastolic blood pressure; VR, vascular resistance; VC, vascular conductance; oxygen saturation and content-A, arterial oxygen saturation and content; oxygen saturation and content-V, venous oxygen saturation and content; OEF, oxygen extraction fraction; VO2, oxygen consumption. *P < 0.01 and **P < 0.05 compared with rest. aP < 0.05, bP < 0.01, and cP < 0.001 compared with NORMO and resting baseline. dP < 0.01 compared with baseline rest and hypoxia.
not affect blood flow heterogeneity at rest (63 ± 16% in normoxia and 60 ± 13% in hypoxia, P = 0.72).

**Exercise measurements.** Blood pressure and heart rate increased from rest to exercise. Systolic, diastolic, and mean arterial blood pressures were all similar during exercise in normoxia, hypoxia, and under aminophylline infusion. Heart rate was increased during hypoxic compared with normoxic exercise (P = 0.02), and there was a tendency for aminophylline to increase heart rate in the hypoxia compared with normoxic exercise (P = 0.08) (Table 1 and Figs. 1 and 2).

Mean blood flow and oxygen consumption in working QF muscle was higher during exercise than at rest (P < 0.001). There was also a significant redistribution of blood flow in thigh muscles from rest to exercise (Fig. 1) and in absolute heterogeneity values over all thigh muscles (63 ± 16% at rest and 95 ± 14% during exercise, P < 0.001). Whole thigh blood flow heterogeneity was not different between the exercise interventions studied (P > 0.45, data not shown), whereas blood flow was higher in QF in hypoxic compared with normoxic exercise (P = 0.02), and aminophylline had no effect in either normoxia or hypoxia (P = 0.5) (Fig. 2A). Oxygen saturation (~88%) and oxygen content of arterial blood was significantly lower during one-leg exercise in hypoxia compared with normoxia (Table 1). Arterial-venous oxygen extraction was reduced under hypoxic exercise (P = 0.02), and aminophylline had no effect on extraction (P = 0.92). Muscle oxygen consumption was similar in all four exercise interventions (P > 0.15; Table 2). Blood flow heterogeneity in working QF muscle tended to be significantly reduced compared with rest (60 ± 19% normoxia at rest and 46 ± 6% normoxia during exercise; P = 0.055), but neither hypoxia nor aminophylline changed this heterogeneity during exercise (P > 0.61; Fig. 2B). There were no differences in vascular resistance or conductance between different exercise interventions (Table 1), but vascular resistance decreased and conductance increased from rest. Of importance, the hypoxia-induced increase in blood flow was confined only to working QF muscle, since blood flow did not change significantly in the posterior part of the thigh muscles from rest for any of the exercise conditions (P > 0.4; Fig. 3A). Posterior hamstring muscle blood flow during normoxic control exercise was comparable to flow at rest (Fig. 3A). Blood flow heterogeneity was, however, increased significantly from rest to exercise in posterior muscles of the thigh, but there were no changes in posterior muscle blood flow heterogeneity between the different exercise interventions (Fig. 3B). Vascular conductance or resistance did not change significantly in posterior muscles in any condition studied (Table 2). Mean blood flow and vascular conductance or resistance in whole thigh musculature of the resting contralateral leg were not changed from rest to one-leg normoxic control exercise (Table 2), but heterogeneity of blood flow was markedly increased (Table 2 and Fig. 1E). There were no changes between different exercise conditions in blood flow or its heterogeneity during exercise (Table 2).

**DISCUSSION**

We examined the skeletal muscle blood flow response directly within the thigh muscles of the exercising and resting contralateral limb in normoxia and systemic hypoxia (14% O2) and under nonspecific adenosine receptor inhibition. The main findings were that 1) limb oxygen extraction was reduced and

### Table 2. Vascular conductance, resistance as well as mean blood flow and flow heterogeneity in noncontracting muscles at resting baseline, at rest in hypoxia, and during exercise in different conditions studied

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Exercise Without Aminophylline</th>
<th>Exercise With Aminophylline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Hypoxia</td>
<td>NORMO</td>
</tr>
<tr>
<td>VR-Post (ml·100 g⁻¹·min⁻¹)·mmHg⁻¹</td>
<td>0.03 ± 0.02</td>
<td>0.04 ± 0.02</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>VR-Post, mmHg (ml·100 g⁻¹·min⁻¹)</td>
<td>40 ± 22</td>
<td>39 ± 23</td>
<td>31 ± 13</td>
</tr>
<tr>
<td>VC-CL (ml·100 g⁻¹·min⁻¹)·mmHg⁻¹</td>
<td>0.03 ± 0.01</td>
<td>0.04 ± 0.02</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>VC-CL, mmHg·(ml·100 g⁻¹·min⁻¹)</td>
<td>38 ± 19</td>
<td>39 ± 22</td>
<td>47 ± 39</td>
</tr>
<tr>
<td>Muscle BF-CL, ml·100 g⁻¹·min⁻¹</td>
<td>3.1 ± 1.9</td>
<td>3.4 ± 1.9</td>
<td>3.7 ± 2.6</td>
</tr>
<tr>
<td>Muscle BF heterogeneity-CL, %</td>
<td>56 ± 12</td>
<td>52 ± 10</td>
<td>113 ± 14*</td>
</tr>
</tbody>
</table>

The results are means ± SD. Post, posterior muscles; CL, (resting) contralateral leg; BF, blood flow. Blood flow and its heterogeneity are reported here only form resting contralateral whole thigh muscles, since data from posterior muscles from working leg are shown in Fig. 3. *P < 0.001 compared with resting baseline and hypoxia.
Oxygen content was larger in the arterial than venous circulation and remained the same. During exercise, however, the drop in blood flow to muscles, such as in skin (40, 45), the reduced arterial oxygen availability possibly because blood flow increases in tissues other than the forearm, net flow increases are often observed (45, 47), systemic hypoxia does not change resting leg blood flow (5, 6), and during exercise in four different study conditions. Although posterior muscle blood flow did not change significantly from rest to any of the exercise conditions (P > 0.3), flow heterogeneity in posterior muscles was increased from rest when exercise was performed by QF muscles. Similar but more pronouncedly enhanced flow heterogeneity was also observed in resting contralateral musculature (see the end of RESULTS for details). ***P < 0.001 compared with resting baseline and hypoxia at rest. The results are means ± SD.

Muscle blood flow increased, which was confined only to the exercising muscles in moderate hypoxic exercise; 2) in the working muscles, blood flow heterogeneity tended to be reduced from rest (P = 0.055), which was not affected by hypoxia; 3) the hypoxia-induced increase in exercising muscle blood flow was not mediated via adenosine; and 4) increased blood flow heterogeneity but unchanged mean blood flow was observed in noncontracting muscles (posterior hamstring in working limb and resting contralateral leg), which likely reflects sympathetic nervous constraints to muscle vasculature to blunt blood flow increments elsewhere than in working skeletal muscles.

Muscle blood flow was not significantly affected by the moderate hypoxic exposure at rest in the present study. This is in line with recent studies showing that even more severe systemic hypoxia does not change resting leg blood flow (5, 36), which can be attributed to a profound increase in leg muscle sympathetic nervous activation (35, 38). In contrast, in the forearm, net flow increases are often observed (45, 47), possibly because blood flow increases in tissues other than muscle, such as in skin (40, 45). The reduced arterial oxygen content was compensated for by a concomitant reduction in venous oxygen content such that oxygen extraction rate remained the same. During exercise, however, the drop in blood oxygen content was larger in the arterial than venous circulation; thus, limb oxygen extraction was reduced in hypoxic exercise, and oxygen supply to working muscles was provided by increased blood flow. In this respect, our study adds novel information to this well-established hemodynamic hypoxic response (10, 26, 36) by showing for the first time in humans that hypoxia-induced blood flow enhancement is confined only to working muscle while blood flow in other muscles (posterior hamstring part, and also resting contralateral leg) remained similar to that in normoxic exercise. Because heart rate was higher in hypoxia, the absence of flow increase in posterior muscles suggests increased sympathetic nervous system activation (18, 22, 42), which likely restrained local muscle vasodilation. Furthermore, that blood flow heterogeneity was increased significantly from rest to exercise in posterior muscles of the thigh even without any change in mean blood flow supports the likelihood of increased sympathetic nervous activity. Hypoxia did not evoke a further increase in flow heterogeneity in posterior muscles during exercise compared with normoxia (39), which suggests that sympathetic activity was not markedly enhanced by this moderate level of hypoxia during small muscle mass knee extension exercise. These data support the conclusion that, rather than primarily reducing inactive muscle blood flow as we hypothesized, neural restraints on blood flow in hypoxia with a small muscle mass occur also in contracting muscles, as recent animal and human studies suggest (6, 43).

Muscle blood flow heterogeneity in exercising muscle and the effect of hypoxia. Changes in muscle blood flow heterogeneity from rest to exercise, and oxygen supply to working muscles was provided by increased blood flow. In this respect, our study adds novel information to this well-established hemodynamic hypoxic response (10, 26, 36) by showing for the first time in humans that hypoxia-induced blood flow enhancement is confined only to working muscle while blood flow in other muscles (posterior hamstring part, and also resting contralateral leg) remained similar to that in normoxic exercise. Because heart rate was higher in hypoxia, the absence of flow increase in posterior muscles suggests increased sympathetic nervous system activation (18, 22, 42), which likely restrained local muscle vasodilation. Furthermore, that blood flow heterogeneity was increased significantly from rest to exercise in posterior muscles of the thigh even without any change in mean blood flow supports the likelihood of increased sympathetic nervous activity. Hypoxia did not evoke a further increase in flow heterogeneity in posterior muscles during exercise compared with normoxia (39), which suggests that sympathetic activity was not markedly enhanced by this moderate level of hypoxia during small muscle mass knee extension exercise. These data support the conclusion that, rather than primarily reducing inactive muscle blood flow as we hypothesized, neural restraints on blood flow in hypoxia with a small muscle mass occur also in contracting muscles, as recent animal and human studies suggest (6, 43).

Muscle blood flow heterogeneity in exercising muscle and the effect of hypoxia. Changes in muscle blood flow heterogeneity from rest to exercise depend largely on exercise intensity. We previously showed that blood flow heterogeneity in working QF actually increases from rest to mild exercise intensity and starts to diminish when exercise intensity is increased to a moderate level (21). Blood flow heterogeneity in working QF in the present study tended to be reduced compared with the resting state (P = 0.055), which can be explained by relatively high exercise intensity. Hypoxia, however, did not change blood flow heterogeneity in exercising muscle, despite a 10% increase in mean blood flow.

Structural and functional properties likely play an important role in changes in blood flow heterogeneity in exercising muscle. At rest, the diameter of arterioles controlling blood flow in a group of capillaries changes rhythmically (vasomotion). During muscle contraction, capillary units are likely to be recruited (9, 23), and the time frequency of diminished diameter of arteriole is reduced (17), contributing to the often-noted decrease in voxel-by-voxel heterogeneity. Regarding heterogeneity in hypoxia, we hypothesized that, if more capillaries are recruited and/or the basal contraction frequency of smooth muscle cells is reduced in hypoxic exercise, muscle blood flow would be even more uniform. In contrast to our hypothesis, heterogeneity remained essentially the same during exercise in hypoxia. It is highly likely that the capillary recruitment was already maximal during this level of exercise, or hypoxia per se simply does not induce greater capillary recruitment within the contracting muscle. This pattern may be altered by more pronounced hypoxia and/or intensive exercise (4). However, although it is commonly known that the arterial blood oxygen saturation is tightly coupled to inspired O2 fraction, even with the combination of more severe hypoxic exposure (12% O2) and exercise intensity (maximal one-legged exercise), arterial oxygen saturation does not necessarily decrease lower than the
88% value we also observed in the present study (2). This is because lower inspired oxygen usually also elicits marked ventilatory compensation trying to maintain arterial $O_2$. Thus, during exercise with a small muscle mass, pulmonary gas exchange and muscle $O_2$ delivery are well preserved in hypoxia by virtue of the capacity to increase blood flow and $O_2$ delivery to a given recruited muscle mass (6). In addition, oxygen extraction fraction values (~57% in control exercise) in general suggest that the one-leg exercise indeed was of moderate to high intensity, since they are close to the well-established maximal value of 70% in comparable (exercise duration, etc.) one-leg exercise (1). Thus severity of hypoxia or exercise intensity is unlikely to explain similar blood flow heterogeneity during hypoxic and normoxic exercise in the one-leg knee extension model.

No evidence for adenosine mediation in hypoxia-induced muscle capillary blood flow increase. The importance of adenosine in blood flow control during exercise in normoxia and hypoxia was examined in the present study by femoral arterial infusion of the nonspecific adenosine receptor blocker aminophylline as previously applied in the studies of forearm blood flow in systemic hypoxia at rest (29) and in normoxic (32) and hypoxic (8) forearm exercise. These studies showed that aminophylline is effectively delivered from luminal to muscle interstitial spaces and that it not only inhibited the normal flow increase with contractions (32) but also effectively inhibited intraluminal adenosine acting on the endothelium, since resting hypoxic vasodilation was abolished (29). As mentioned, in the present study, blood flow was enhanced substantially from rest and was significantly higher in hypoxic exercise compared with normoxia. Aminophylline did not affect blood flow in exercising QF, suggesting no contribution of adenosine to capillary blood flow in normoxic or hypoxic exercise in the conditions studied. Additionally, adenosine receptor blockade did not change blood flow heterogeneity either during normoxic or hypoxic exercise. The fact that flow heterogeneity was not changed during hypoxia under adenosine receptor blockade is logical, since hypoxia per se did not affect exercising muscle blood flow heterogeneity.

Some animal studies suggest that adenosine could account for up to 40% of exercise hyperemia, but there are some disparate findings on the significance of adenosine that relate to species differences, measurement methods, the inhibitors used, and the exercise conditions, such as intensity (31). Also, some (33, 34), but not all (8, 21), human studies suggest that adenosine would play a role in exercise hyperemia. In the present study, we did not find any support for adenosine in the regulation of muscle capillary blood flow during exercise. Clearly, methodological factors account for these different outcomes. While Doppler ultrasound or thermodilution methods measure bulk limb blood flow, PET allows determination of nutritive capillary blood flow within the muscles. Thus it seems that capillary blood flow is not as easily disturbed by adenosine blockade as bulk blood flow. Supporting this, it is known from the coronary circulation that, when stenosis limits blood flow in large coronary arteries, there is a substantial compensatory downstream vasodilation (12). Because it is obvious that $Q_F$ is at least slightly overperfused during one-leg knee extension (20), it may well be that blockade affects resistance vessels, and overall conduit blood flow may be slightly reduced (33, 34). Simultaneously, there is a compensatory vasodilation in more distal resistance vessels close to the capillaries preserving more critical working muscle capillary nutritive blood flow. In this respect, it is noteworthy that blood flow in conduit arteries does not always represent blood flow in capillaries, especially in the onset of exercise (19), but possibly also in other conditions. This mainly unexplored issue warrants future experimentation. It is also highly likely that compensatory vasodilators, such as ATP derived from red blood cells, nitric oxide, and/or prostanooids or $\beta$-adrenergic vasodilation, appeared to preserve exercising muscle blood flow during inhibition (7, 15, 16, 45, 46).

Although the data in the present and our previous study (21) clearly suggest that adenosine is not obligatory for the increase in muscle capillary blood flow in exercising muscle, it does not, however, exclude the possibility that adenosine is one of the many tonic regulators of skeletal muscle blood flow as shown by Duncker and colleagues (13) in swine myocardium and Edlund & Sollevi (14) in the human heart. Normally adenosine emanates mainly from extracellular pathways, and intracellular adenosine formation and its subsequent release to muscle interstitial space to the vicinity of smooth muscle cells of resistance vessels is triggered only when muscle metabolic demands exceed oxygen delivery (12). Thus the physiological contribution of adenosine to muscle hyperemia seems not to be substantially activated in steady-state exercise with a small muscle mass in moderate systemic hypoxia but may require more severe ischemic conditions or higher exercise intensities (32) to be activated. There were no signs of this in the present study since, for instance, blood lactate remained essentially similar during all conditions (data not shown). Therefore, hyperemic mediation by adenosine needs to be further examined in maximal whole body exercise where a mismatch between metabolism and oxygen delivery can be assumed to exist. The conclusion that adenosine is not mandatory for the regulation of skeletal muscle nutritive capillary blood flow during exercise with a small muscle mass in normoxia or physiological systemic hypoxia is thus well in accordance with cardiac studies showing no indication for adenosine mediation in normal coronary exercise hyperemia in dogs, swine, or humans (12). Only when stenosis or severe ischemia affects cardiac perfusion does adenosine contribute importantly to coronary vasodilation (12). Other groups, having studied the contraction-induced skeletal muscle hyperemia and reviewed the evidence for adenosine mediation, conclude that, despite long-standing interest in adenosine as a mediator of metabolic vasodilator, there is strong evidence against its involvement in exercise hyperemia (44). Finally, although we (20) and others (32) have found substantial variation in blood flow responses with adenosine infusion, no subdivision into “responders and nonresponders” was observed in the present study regarding the actions of endogenous adenosine.

Perspectives and Significance

Although many effects of both acute and chronic hypoxia on the circulation are well characterized, the distribution and regulation of blood flow heterogeneity in skeletal muscle during systemic hypoxia is not well elucidated in humans. In this study, we report several major findings. First, during small muscle mass exercise in moderate systemic hypoxia, muscle capillary blood flow is increased to a larger extent than oxygen...
R78 SKELETAL MUSCLE BLOOD FLOW REGULATION

extractions to compensate for the lower arterial oxygen content. It is shown here for the first time in humans that this increased blood flow is confined only to the exercising muscle, and flow heterogeneity per se is not affected by hypoxia. The hypoxia-induced increase in muscle capillary blood flow appeared not to be mediated by adenosine, which supports recent whole limb blood flow findings, and exercise intensity rather than the oxygen content of the blood determines the level of capillary recruitment of the muscle in this exercise model. Finally, the results also suggest that the observed increase in blood flow heterogeneity in noncontracting human muscles reflects sympathetic nervous constraints to the muscular vasculature to blunt blood flow increments elsewhere than in working skeletal muscles, but which are not yet potentiated in this moderate level of hypoxia. We propose that, when oxygen delivery to a given mass of activated muscle can be maintained by an increase in muscle blood flow, no alterations in muscle recruitment occur, the muscle is not in a situation of ischemia, and adenosine is not a mediator of the flow increase. Of interest is how flow distribution and its regulation may be altered during exercise with a larger muscle mass where a central flow limitation may occur at high intensities of exercise.

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DISCLOSURES

No conflicts of interest are declared by the authors.

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