Structural basis of muscle O\textsubscript{2} diffusing capacity: evidence from muscle function in situ

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Hepple, Russell T., Michael C. Hogan, Creed Stary, Donald E. Bebout, Odile Mathieu-Costello, and Peter D. Wagner. Structural basis of muscle O\textsubscript{2} diffusing capacity: evidence from muscle function in situ. J. Appl. Physiol. 88: 560–566, 2000.—Although evidence for muscle O\textsubscript{2} diffusion limitation of maximal O\textsubscript{2} uptake has been found in the intact organism and isolated muscle, its relationship to diffusion distance has not been examined. Thus we studied six sets of three purpose-bred littermate dogs (aged 10–12 mo), with 1 dog per litter allocated to each of three groups: control (C), exercise trained for 8 wk (T), or left leg immobilized for 3 wk (I). The left gastrocnemius muscle from each animal was surgically isolated, pump-perfused, and electrically stimulated to peak O\textsubscript{2} uptake at three randomly applied levels of arterial oxygenation [normoxia, arterial O\textsubscript{2} (P\textsubscript{A\textsubscript{O\textsubscript{2}}} ) 77 ± 2 (SE) Torr; moderate hypoxia, P\textsubscript{A\textsubscript{O\textsubscript{2}}} : 33 ± 1 Torr; and severe hypoxia, P\textsubscript{A\textsubscript{O\textsubscript{2}}} : 22 ± 1 Torr]. O\textsubscript{2} delivery (ml·min\textsuperscript{-1}·100 g\textsuperscript{-1}) was kept constant among groups for each level of oxygenation, with O\textsubscript{2} delivery decreasing with decreasing P\textsubscript{A\textsubscript{O\textsubscript{2}}}. O\textsubscript{2} extraction (%) was lower in I than in T or C for each condition, but calculated muscle O\textsubscript{2} diffusing capacity (D\textsubscript{musO\textsubscript{2}}) per 100 grams of muscle was not different among groups. After the experiment, the muscle was perfusion fixed in situ, and a sample from the midbelly was processed for microscopy. Immobilized muscle showed a 45% reduction of muscle fiber cross-sectional area (P < 0.05), and a resulting 59% increase in capillary density (P < 0.05) but minimal reduction in capillary-to-fiber ratio (not significant). In contrast, capillarity was not significantly different in T vs. C muscle. The results show that a dramatically increased capillary density (and reduced diffusion distance) after short-term immobilization does not improve D\textsubscript{musO\textsubscript{2}} in heavily working skeletal muscle.

methods

Animal care. In accordance with University of California-San Diego (UCSD) Animal Subjects Committee approval, six sets of three purpose-bred littermate dogs were studied, with one dog from each litter allocated to each of three groups: control, exercise trained, and immobilized. The trained group was trained 5 days/wk for 8 wk on a motor-driven treadmill by using a similar program to that used previously (3). Briefly, the dogs ran freely for an initial duration of 30 min, working up to 60 min by the end of the first week, and remaining at 60 min for the remainder of the study. During these sessions, the speed and incline of the treadmill were regularly adjusted to

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elicit a training heart rate between 190 and 210 beats/min. The control and immobilized groups were maintained 1 animal/cage (dimensions: ~2 m long and 1.3 m wide) at the UCSD vivarium for 8 wk, with physical activity limited to this area. For the final 3 wk, the immobilized group had the left hind leg immobilized in a fiberglass cast, with the foot in plantar flexion and the knee at 90° flexion, to fix the gastrocnemius in a shortened position.

Surgical isolation of the gastrocnemius muscle. Dogs were anesthetized with 30 mg/kg pentobarbital sodium, subsequently intubated with a cuffed endotracheal tube and ventilated with a Harvard 613 ventilator. Esophageal temperature throughout the experiment was maintained near 37°C via heating pads. The left gastrocnemius and superficial digital flexor muscle complex (collectively referred to as the gastrocnemius for convenience) were surgically isolated, as described previously (9, 11). Briefly, an incision was made from the medial calf up the inside of the thigh to expose the gastrocnemius and Achilles tendon. The sartorius, gracilis, and semitendinosus and semimembranosus muscles, and their circulation, were doubly ligated and cut to expose the circulation of the gastrocnemius. All vessels connected to the left popliteal vein and artery were ligated to isolate the circulation to the gastrocnemius. The left popliteal vein was cannulated distal to the gastrocnemius and the venous return was diverted to the cannulated jugular vein. The right femoral artery was cannulated and connected, via a roller pump (Cole-Palmer, Chicago, IL), to the cannulated left femoral artery such that the left gastrocnemius and superficial digital flexor muscle complex (collectively referred to as the gastrocnemius for convenience) were surgically isolated, as described previously (9, 11). Briefly, an incision was made from the medial calf up the inside of the thigh to expose the gastrocnemius and Achilles tendon. The sartorius, gracilis, and semitendinosus and semimembranosus muscles, and their circulation, were doubly ligated and cut to expose the circulation of the gastrocnemius. All vessels connected to the left popliteal vein and artery were ligated to isolate the circulation to the gastrocnemius. The left popliteal vein was cannulated distal to the gastrocnemius, and the venous return was diverted to the cannulated jugular vein. The right femoral artery was cannulated and connected, via a roller pump (Cole-Palmer, Chicago, IL), to the cannulated left femoral artery such that the left gastrocnemius was now perfused by blood from the contralateral hind leg. Blood flow was regulated by systemic pressure at rest and by the roller pump during exercise periods. Systemic arterial pressure was continuously monitored by a pressure transducer in line with a cannula placed in a carotid artery. After surgery, 150 U/kg of heparin was given intravenously to the animals. The left sciatic nerve was isolated, ligated, and cut proximal to its innervation of the gastrocnemius, and then connected to an electrical stimulator (model S48D, Grass Medical Instruments) for eliciting muscle contractions. All exposed tissues were covered with saline-soaked gauze and Saran wrap.

The Achilles tendon was exposed down to the foot, cut away from the calcaneus, and secured to an isometric myograph (Interface Manufacturing, Scottsdale, AZ) with a clamp for measurement of muscle tension development. The left hind-limb was then fixed at the knee and ankle and attached to the myograph with struts to minimize movement.

Experimental protocol. Each dog was studied during electrically stimulated maximal contractions of the gastrocnemius at each of three levels of oxygenation (normoxia, moderate hypoxia, and severe hypoxia), separated by 30–45 min of recovery. The order of the treatments (oxygenation levels) was randomly chosen from a list of all possible combinations on the day of the experiment. Resting muscle length was adjusted to achieve the greatest contractile force before each contraction period. Pump perfusion was begun ~2–3 min before each contraction period to allow adequate time for blood flow to stabilize at the same levels observed during self-perfusion at rest. Isometric tetanic contractions were elicited by supramaximal stimulation of the sciatic nerve (tetanic train: 6–8 V, 0.2-ms stimuli for 200-ms duration at 50 Hz, once per second). Muscle mass was estimated, and blood flow was adjusted, to maintain a constant O2 delivery between animals for each level of inspired O2. Arterial and venous blood samples were drawn at rest before each contraction period and in the final 20 s of each contraction period, and immediately analyzed for PO2, PCO2, pH, O2 saturation, and Hb concentration ([Hb]) by a blood-gas analyzer and CO-oximeter (IL 813 and IL 282, respectively, Instrumentation Laboratories). Muscle blood flow was measured by timed collection in a graduated cylinder at the same time blood samples were drawn. Peak O2 uptake (\( \dot{V}O_2 \)) for each condition was calculated by the Fick equation.

Mean capillary PO2, PCO2, for each condition was calculated numerically, as described previously (29). Briefly, a forward integration procedure was used to calculate the PCO2 from the observed venous and arterial PO2. This approach assumes that muscle O2 conductance between red cell and fiber mitochondria is constant along the capillary from arterial to venous end. Peak muscle \( \dot{V}O_2 \) for each treatment (normoxia, moderate hypoxia, and severe hypoxia) was plotted against PCO2, and the corresponding O2 conductance, DMusO2, was calculated as the slope of the line of best fit that passed through the origin. As stated previously (29), the calculation of DMusO2 assumes intracellular PO2 during these maximal contractions is close to 0 Torr. Although we recognize that this calculation oversimplifies a complicated diffusion process, it
is applied uniformly to all dogs at all inspiratory O2 fractions to calculate conductance values for comparative purposes.

Muscle perfusion-fixation and tissue preparation. At the end of the experiment, the left gastrocnemius was perfusion fixed in situ at a nonpulsatile pressure of 80–100 Torr (20). The vasculature was first perfused with saline until the venous exudate was clear of blood and then perfused with glutaraldehyde (GA) fixative (6.25% GA solution in 0.1 M sodium cacodylate buffer adjusted to 430 mosM with NaCl; total osmolarity 1,100 mosM, pH 7.4). Thin, longitudinal strips of muscle were obtained from the midbelly (midportion) of the gastrocnemius and processed for electron microscopy, as described previously (20). Eight blocks from each muscle were cut into four transverse and four longitudinal 1-µm-thick sections, using an LKB Ultratome III, and stained with 0.1% aqueous solution of toluidine blue. Sarcomere length was measured on each longitudinal section at a magnification of ×400 on a light microscope, with the data for this group are based on results from five animals.

Morphometry. Morphometry was performed with the identity of the muscle samples blinded to the observer, according to methods well established in our laboratory (20). Each section was subsampled systematically to yield as many nonoverlapping frames as possible, yielding ~200–300 fibers for each muscle. Capillary density was estimated in transverse sections by using a 100-point eyepiece square grid test at a magnification of ×400 on a light microscope, with the fibers used as the reference space (20). Briefly, using this approach, capillary density is expressed as the number of capillaries per square millimeter of fiber cross-sectional area to prevent errors due to inaccurate preservation of the intercellular spaces (20). The number of capillaries around a fiber and fiber cross-sectional area were measured by using an image analyzer (Videometric 150, American Innovision), in the same transverse sections used to estimate capillary density. Capillary density and fiber cross-sectional area were subsequently normalized to 1.9-µm sarcomere length because this value was close to that observed (range: 1.72–1.94 µm). Capillary-to-fiber ratio was calculated as the product of capillary density and fiber cross-sectional area.

Analysis and statistics. Data are expressed as means ± SE. Differences between groups were analyzed by using one-way ANOVA and the Bonferroni post hoc multiple-comparison test. The level of significance was set at α = 0.05.

RESULTS

One of the dogs from the immobilized group died during the initiation of surgical anesthesia, and thus the data for this group are based on results from five animals.

Muscle cardiovascular and metabolic function. Tables 1 and 2 present the O2 and hemodynamic data measured during the muscle function experiments. The venous O2 concentration (CvO2), venous PO2 (PvO2), and calculated PcO2 were significantly higher in the immobilized group than either control or trained groups only in normoxia (Table 2). Peak VO2 was significantly lower for the immobilized group compared with the trained group only in normoxia. O2 extraction (%) was significantly lower in the immobilized group than the trained or control groups in normoxia. Figure 2 shows peak VO2 plotted against PVO2 (A) and calculated Pco2 (B). Inter-

## Table 1. Arterial blood data at peak VO2

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Trained</th>
<th>Immobilized</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO2, mmHg</td>
<td>74 ± 2</td>
<td>74 ± 5</td>
<td>84 ± 3</td>
</tr>
<tr>
<td>PaCO2, mmHg</td>
<td>40 ± 2</td>
<td>38 ± 2</td>
<td>37 ± 2</td>
</tr>
<tr>
<td>pH</td>
<td>7.37 ± 0.02</td>
<td>7.37 ± 0.02</td>
<td>7.33 ± 0.02</td>
</tr>
<tr>
<td>[Hb], g/dl</td>
<td>16.4 ± 0.6</td>
<td>16.7 ± 0.8</td>
<td>17.4 ± 4</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>146 ± 6</td>
<td>159 ± 9</td>
<td>161 ± 13</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 in control and trained groups and n = 5 in immobilized group. VO2 uptake; PaO2 and Pao2, partial pressure of O2 and CO2 in arterial blood, respectively; pHa, pH of arterial blood; [Hb], hemoglobin concentration; MAP, muscle arterial pressure. *P < 0.05 vs. other groups.

## Table 2. Oxygen utilization data at peak VO2

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th>Moderate Hypoxia</th>
<th>Severe Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaO2, ml/100 ml</td>
<td>20.6 ± 0.9</td>
<td>21.0 ± 0.8</td>
<td>22.3 ± 0.5</td>
</tr>
<tr>
<td>CvO2, ml/100 ml</td>
<td>7.8 ± 1.3</td>
<td>5.9 ± 0.7</td>
<td>12.5 ± 1.2*</td>
</tr>
<tr>
<td>PVO2, Torr</td>
<td>30 ± 2</td>
<td>25 ± 2</td>
<td>38 ± 3*</td>
</tr>
<tr>
<td>Blood flow</td>
<td>101 ± 4</td>
<td>102 ± 5</td>
<td>102 ± 7</td>
</tr>
<tr>
<td>QO2, ml·min⁻¹·100 g⁻¹</td>
<td>20.7 ± 1.2</td>
<td>21.4 ± 1.7</td>
<td>22.8 ± 1.7</td>
</tr>
<tr>
<td>Peak VO2</td>
<td>12.8 ± 0.7</td>
<td>15.4 ± 1.1</td>
<td>10.3 ± 2.0t</td>
</tr>
<tr>
<td>O2 extraction, %</td>
<td>63 ± 5</td>
<td>72 ± 3</td>
<td>44 ± 6*</td>
</tr>
<tr>
<td>Peak muscle force, N/100 g</td>
<td>59 ± 8</td>
<td>74 ± 13</td>
<td>65 ± 15</td>
</tr>
<tr>
<td>Pco2, Torr</td>
<td>47 ± 2</td>
<td>44 ± 1</td>
<td>55 ± 2*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 in control and trained groups and n = 5 in immobilized group. CaO2 and CvO2, arterial and venous concentration of O2, respectively; PVO2, partial pressure of O2 in venous blood; QO2, O2 delivery [=blood flow × (CaO2); Pco2, mean partial pressure of capillary O2. *P < 0.05 vs. other groups; †P < 0.05 vs. trained group.
Interestingly, the immobilized group demonstrated a disproportionately small increase in peak VO₂ between moderate hypoxia and normoxia, suggesting that factors other than diffusing capacity were constraining peak VO₂ in this condition. In this respect, one immobilized animal and one control animal in normoxia deviated from the linear relationship between peak VO₂ and PCO₂ observed in severe and moderate hypoxia, and another immobilized animal demonstrated no proportionality between these variables between treatments. Because the calculation of DmusO₂ requires that the relationship between VO₂ and PCO₂ be linear, these data points were omitted in calculating DmusO₂. As a result, DmusO₂ for one immobilized animal and one control animal are based only on data from moderate and severe hypoxia, and it was not possible to calculate DmusO₂ for another immobilized animal. Mean absolute DmusO₂ was significantly lower in the immobilized group (3.01 ± 0.11 ml·min⁻¹·kg⁻¹·Torr⁻¹) than trained (3.53 ± 0.32 ml·min⁻¹·kg⁻¹·Torr⁻¹) or immobilized (2.89 ± 0.61 ml·min⁻¹·kg⁻¹·Torr⁻¹; n = 4) groups.

Muscle structure. Muscle structural data are presented in Table 3. The differences between groups are due in large part to the significant muscle atrophy in the immobilized group (Fig. 3). The mass of the gastrocnemius muscle was reduced by 32% after immobilization (55 ± 3 g) compared with control (79 ± 3 g, P < 0.05). Consistent with the atrophy evident at a whole muscle level, the immobilized group demonstrated a 45% reduction in fiber cross-sectional area (902 ± 66 µm²) compared with control (1,630 ± 169 µm²) and trained (1,836 ± 139 µm², P < 0.05) groups. Thus, despite an unchanged capillary-to-fiber ratio (Table 3), capillary density was significantly increased in the immobilized group (2,449 ± 142 capillaries/mm²) compared with control (1,537 ± 113 capillaries/mm²) and trained (1,305 ± 150 capillaries/mm², P < 0.05) groups. In contrast to immobilization, there were no significant changes in capillarity or fiber size in trained compared with control muscle. Collectively, these structural changes indicate a large reduction in diffusion distance in the immobilized group. In contrast, neither capillary density nor the capillary-to-fiber ratio was significantly affected by training.

Relationships between capillarity and DmusO₂. Figure 4 shows the plots of the mean values for the variables of primary interest. Figure 4A shows DmusO₂ plotted against capillary density and demonstrates that a greater capillary density did not improve DmusO₂. Figure 4B shows DmusO₂ vs. capillary-to-fiber ratio. There was no difference in capillary-to-fiber ratio among groups, and the quotient of DmusO₂ and capillary-to-fiber ratio in control (1.26 ± 0.09, n = 6), trained (1.54 ± 0.09, n = 6), and immobilized (1.34 ± 0.28, n = 4) muscles was similar.

DISCUSSION

The major finding of this study was that a dramatic increase in capillary density (reflecting a reduced diffusion distance due to reduced fiber size) did not increase DmusO₂, suggesting diffusion distance per se is not a primary determinant of DmusO₂. Because there was no difference in capillary-to-fiber ratio among groups, direct evaluation of the effect of a change in capillary surface area on DmusO₂ was not possible.

Table 3. Muscle structure data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Trained</th>
<th>Immobilized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle mass, g</td>
<td>79 ± 3</td>
<td>80 ± 2</td>
<td>55 ± 3*</td>
</tr>
<tr>
<td>Sarcomere length, µm</td>
<td>1.84 ± 0.02</td>
<td>1.85 ± 0.01</td>
<td>1.84 ± 0.04</td>
</tr>
<tr>
<td>Fiber cross-sectional area, µm²</td>
<td>1.630 ± 169</td>
<td>1.836 ± 139</td>
<td>902 ± 66*</td>
</tr>
<tr>
<td>Capillary density, mm⁻²</td>
<td>1.537 ± 113</td>
<td>1.305 ± 150</td>
<td>2,449 ± 142*</td>
</tr>
<tr>
<td>Number around a fiber</td>
<td>6.2 ± 0.6</td>
<td>6.5 ± 0.2</td>
<td>6.2 ± 0.1</td>
</tr>
<tr>
<td>Capillary-to-fiber ratio</td>
<td>2.41 ± 0.09</td>
<td>2.30 ± 0.16</td>
<td>2.17 ± 0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 for control and trained groups and n = 5 for immobilized group. Note: fiber cross-sectional area and capillary density have been normalized to 19-µm sarcomere length to adjust for the effect of sarcomere length on fiber cross-sectional area. *P < 0.05 vs. other groups.
Implicit in our comparisons of muscle capillary density among groups is that in stimulated muscles with blood flow sustained by roller pump and systemic pressure regulated to keep O₂ delivery constant, functional capillary density is maximal, i.e., can be represented by anatomic capillary density. This premise has not been directly evaluated previously; however, previous work by Honig and colleagues (13) and computer modeling by Fuglevand and Segal (4) suggest that the number of capillaries perfused is near maximal even during submaximal muscular work. In addition, previous results from our group showed that there was no increase in peak VO₂ after adenosine infusion, despite a significant reduction in muscle arterial resistance (18), suggesting no augmentation of the surface area available for diffusion (and thus, no further capillary recruitment) despite greater relaxation of the resistance vessels.

We attempted to exploit the adaptive response in skeletal muscle structure to exercise training and short-term immobilization to gain insights into the effect of diffusion distance vs. capillary per fiber number on DmusO₂. Whereas immobilization caused a significant increase in capillary density secondary to fiber atrophy with little or no reduction in capillary-to-fiber ratio, training had no effect on either capillary density or capillary-to-fiber ratio in this model. An increased capillary-to-fiber ratio after endurance training is well-known in both humans (e.g., 2, 15) and animal models such as rodents (e.g., 1). Thus it was surprising that 8 wk of endurance training did not induce an increase in capillary-to-fiber ratio in the canine gastrocnemius, particularly since a similar training protocol has been shown to increase systemic maximal VO₂ and blood flow to the gastrocnemius muscle in canines (23). It is likely that a more intense and/or longer duration training stimulus is required to induce an increase in capillary-to-fiber ratio in the canine gastrocnemius due to the high vascularization evident in this muscle in the control (untrained) state.

Evidence of a muscle O₂ diffusing limitation. Although much evidence has been gathered supporting a muscle diffusion limitation to maximal VO₂ (e.g., 10, 28), it has been noted that shunt and/or mismatching between metabolic demand (VO₂) and blood flow (VO₂/Qmus mismatch) could explain the residual O₂ in venous effluent (24, 29). In this regard, blood flow heterogeneity per se has been shown to have little influence on DmusO₂, except under very-low-blood-flow conditions (e.g., ischemia) (17). In addition, changes in peak VO₂ with altered Hb-O₂ affinity (10, 28) are inconsistent with a large contribution of VO₂/Qmus heterogeneity or shunt to the residual O₂ in venous blood. Rather, the effect on muscle peak VO₂ in these experiments is consistent with an altered capillary PO₂ at a constant muscle diffusing capacity, whereas VO₂/Qmus heterogeneity is unchanged (35).

Muscle O₂ conductance and peak VO₂. One of the interpretations of the linear relationship usually observed between peak VO₂ and PCO₂ is that maximal mitochondrial capacity is not limiting peak muscle VO₂ (34). Under these conditions, the slope of this relationship reflects DmusO₂. Conversely, deviation from linearity (i.e., a decreasing slope) with increasing PCO₂ suggests that factors other than diffusing capacity are constraining peak VO₂, and thus the slope of the line in this region does not represent DmusO₂. It is clear from Fig. 2 that the slope of the line between severe and moderate hypoxia, DmusO₂, was similar among groups. In contrast, Fig. 2 also shows that in normoxia the...
immobilized group demonstrated an increase in peak VO\textsubscript{2} that was disproportionately smaller than the increase in PC\textsubscript{O\textsubscript{2}} observed between moderate hypoxia and normoxia. This suggests that in normoxia there was a surplus of O\textsubscript{2} being delivered to the muscle in the immobilized group and that other factors (such as maximal mitochondrial respiratory capacity) were limiting further increases in VO\textsubscript{2}. This is similar to what was observed recently in sedentary humans during dynamic knee-extensor exercise (36) and illustrates that the relative importance of the various factors that interact to determine maximal VO\textsubscript{2} can be altered by inactivity. An important issue relevant to the study design, therefore, is that by calculating D\textsubscript{musO\textsubscript{2}} only on the basis of the linear region of the relationship between peak VO\textsubscript{2} and PC\textsubscript{O\textsubscript{2}} (see RESULTS), the limits of maximal mitochondrial respiration are not masking any possible beneficial effect of a reduced diffusion distance on D\textsubscript{musO\textsubscript{2}} in the immobilized muscles.

It has been found previously that chronically low activity levels (e.g., as occurs with sedentary lifestyle) can result in skeletal muscle intracellular PO\textsubscript{2} during maximal exercise being significantly higher than in trained individuals on the basis of evidence from myoglobin spectroscopic measurements during human knee-extensor exercise (27). If this were also true of the immobilized muscles in the present study, D\textsubscript{musO\textsubscript{2}} would have been underestimated because calculation of D\textsubscript{musO\textsubscript{2}} relies on the assumption that intracellular PO\textsubscript{2} is near 0 Torr (29). Note that unlike the immobilized muscles in the present study, these sedentary human subjects showed no proportionality whatsoever between VO\textsubscript{2} and PC\textsubscript{O\textsubscript{2}} as the inspired fraction of O\textsubscript{2} was altered (27). Because we specifically calculated D\textsubscript{musO\textsubscript{2}} only over the linear region of the relationship between VO\textsubscript{2} and PC\textsubscript{O\textsubscript{2}} (see RESULTS), the limits of maximal mitochondrial respiration are not masking any possible beneficial effect of a reduced diffusion distance on D\textsubscript{musO\textsubscript{2}}.

The role of O\textsubscript{2} diffusion distance in determining D\textsubscript{musO\textsubscript{2}}. A classic view has been that diffusion distance plays an important role in determining the structural capacity for O\textsubscript{2} flux into muscle fibers. However, this view is not consistent with results indicating that there is a substantial gradient between blood and intramyocyte PO\textsubscript{2} (5, 26). Rather, these latter results suggest that the majority of the resistance to O\textsubscript{2} flux is in the short diffusion path through plasma and capillary wall to the sarcoplasm, and that, therefore, the capillary-to-fiber interface, rather than diffusion distance, is more critical to O\textsubscript{2} flux capacity (see review in Ref. 12). Indeed, since the pioneering work of Krogh (16), there has been contradictory structural evidence about the importance of diffusion distance.

For example, the reduction in fiber size and resulting increased capillary density seen in humans after chronic exposure to simulated high altitude (6, 19) or after high-altitude sojourns (14) suggested that reducing diffusion distance may be important when O\textsubscript{2} supply is compromised. In contrast, studies with animal models have predominantly shown that the higher capillary density often seen with chronic hypoxia is a consequence of the slower rates of growth in the hypoxic animals (30–32). Furthermore, it has been shown that adaptations to both increased activity (21, 25) and chronic hypoxia (8, 22) occur to match the size of the capillary-to-fiber interface to fiber mitochondrial volume rather than to minimize diffusion distances. For example, it was found that in both the flight (22) and leg (8) muscles of finches naturally living and flying at altitude, fiber size was not reduced compared with finches living at sea level, and thus radial diffusion distance was not reduced. Instead, capillary surface per fiber surface was greater in the high-altitude finches, in proportion to their larger fiber mitochondrial volume. The present results provide further evidence that diffusion distance per se does not directly determine the
structural capacity for O₂ flux into muscle fibers. In particular, the immobilized muscles, which had a dramatically reduced diffusion distance (due to a 45% reduction in fiber cross-sectional area and a resulting 59% increase in capillary density compared with control), did not have a higher DmusO₂ per unit mass of muscle.

In summary, the present results show that diffusion distance per se is not a primary determinant of DmusO₂ in maximally working skeletal muscle. In particular, a dramatic increase in capillary density due to muscle fiber atrophy with minimal change in capillary-to-fiber ratio, did not result in a greater DmusO₂ in the isolated perfused canine gastrocnemius muscle. In contrast, evaluation of the effects of altering capillary surface area on DmusO₂ will require further investigation.

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