Oxygen tension distribution in postcapillary venules in resting skeletal muscle

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Saltzman, Darin J., Andras Toth, Amy G. Tsai, Marcos Intaglietta, and Paul C. Johnson. Oxygen tension distribution in postcapillary venules in resting skeletal muscle. Am J Physiol Heart Circ Physiol 285: H1980–H1985, 2003.—We tested the hypothesis that blood flow is distributed among capillary networks in resting skeletal muscle in such a manner as to maintain uniform end-capillary PO2. Oxygen tension in venules draining two to five capillaries was obtained by using the phosphorescence decay methodolog- 

y in rat spinotrapezius muscle. For 64 postcapillary venules among 18 networks in 10 animals, the mean PO2 was 30.1 Torr (range, 9.7–43.5 Torr) with a coefficient of variation (CV; standard deviation/mean) of 0.26. Oxygen levels of postcapillary venules within a single network or single animal, however, displayed a much smaller CV (0.064 and 0.094, respectively). By comparison, the CV of blood flow in 57 postcapillary venules of 17 networks in 9 animals was 1.27 with a mean flow of 0.011 ± 0.004 nl/s and a range of 3.7 × 10−4 to 6.5 × 10−2 nl/s. Blood flow of postcapillary venules within single networks displayed a lower CV (mean, 0.51), whereas that in individual animals was 0.78. Results indicate that among venular networks, heterogeneity of oxygen tension is less than that of blood flow and within venular networks the heterogeneity of oxygen tension is much less than that of blood flow. In addition, postcapillary PO2 was independent of flow among venules in which both were measured. Results of this study may be attributable to three factors: 1) O2 diffusion between adjacent capillaries and venules, 2) structural remodeling in regions of lower PO2, and 3) O2-dependent local control mechanisms.

PREVIOUS INVESTIGATIONS HAVE revealed a substantial degree of spatial heterogeneity of blood flow indicated by a relatively large coefficient of variation (CV), defined as standard deviation/mean, among similar vessels in the microcirculation (6, 19). The spatial heterogeneity in capillary networks is typically in the range of 0.40 to 0.90 (19). In the cat sartorius muscle, the CV of volume flow was 0.63 in both fifth- and sixth-order venules (10), whereas in arterioles values of ~1.0 were seen (3). One possible explanation for this spatial variability in flow is that it relates to the oxygen requirements of the tissue region supplied. It is possible that local tissue regions with higher oxygen consumption are served by vessels with higher blood flow, thereby providing a balance between oxygen supply and oxygen demand. This concept has drawn support from evidence that flow heterogeneity in arterioles may be related, in part, to differences in local oxygen demand (21) and that flow heterogeneity within an isolated perfused muscle might also reflect local differences in oxygen consumption (32). If this is the case, then tissue and blood oxygen levels in the region of venous capillaries and postcapillary venules should be relatively uniform, despite differences in blood flow.

However, most of the data currently available do not support the suggestion that oxygen tension at the venous capillary and postcapillary level is relatively uniform. The CV of tissue PO2 in the vicinity of venous capillaries of cat sartorius muscle was 0.45 (3), and we calculate from data on rat intestinal muscle (4) that the CV in that tissue was 0.23. In studies on postcapillary venules of rat spinotrapezius muscle, values of 0.31 to 0.45 have been reported (20). Kergar et al. (14) obtained a value of 0.33 in larger venules in the hamster skinfold preparation of muscle. These CV values approach those cited above for blood flow. A possible explanation for this lack of uniformity in oxygen levels is that the studies pooled data from different animals and from different regions of the muscle in the same animal. In this case, the physiological state and experimental conditions may be sufficiently different among measurement sites so that local uniformity in PO2 was masked. The purpose of this study was to examine, in localized vascular beds, the hypothesis that oxygen tension in postcapillary venules is significantly more uniform than blood flow. The study used the microvascular bed of rat spinotrapezius muscle for which considerable data on oxygen tension are already available for comparison and to aid in interpretation. The study also examined uniformity of oxygen tension and blood flow among different regions in the same muscle and among muscles of different animals.

MATERIALS AND METHODS

Juvenile male Wistar rats (127 ± 13 g body wt) were prepared under pentobarbital sodium anesthesia (50 mg/kg) given intraperitoneally with additional booster doses given as needed. Animal use was in accord with guidelines of the

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Experimental protocol. In the first experimental series, data were obtained on $P_{O_2}$ in 64 venules among 18 networks in 10 animals. A microcirculatory region consisting of several collecting venules and their tributary postcapillary venules was selected for study on the basis of visibility of vessels of interest. The vascular network was recorded on videotape, and a preliminary map of the region was drawn by hand for reference during the experiment. A distal venular tree consisting of two to four postcapillary venules, each draining two to five capillaries in the region, was chosen for measurements. For each venular tree, oxygen tension was determined at each postcapillary venule in the manner described above. For the purpose of data analysis and comparisons, each venular tree was classified as an “individual network.” In some instances, it was possible to study more than one venular tree in a single muscle preparation. Data obtained from two or more individual networks in one animal were classified as “individual animals.” Measurements classified as “total population” consisted of all observations in all animals.

An example of an individual network is shown in Fig. 1 together with $P_{O_2}$ data obtained from this network. Because it was a concern that temporal variations in $P_{O_2}$ could occur, a preliminary data set such as that shown in Fig. 1 was taken at each site and repeated 5 min later. Data from sites that...
showed significant temporal changes were not retained. Measurements were taken at least 1 min apart. Approximately 1–2 min elapsed when moving to the next postcapillary venule to be measured. Approximately 20 min were required to obtain measurements within one network. It took ~40–60 min to complete measurements in two or more networks in one animal.

In a second experimental series, data were obtained from nine animals on diameters and red blood cell velocities of 57 postcapillary venules in 17 individual networks. Velocity was determined with the dual-slit velocity technique (1, 33). Specifically, in this application, we used the Fiber Optic Photo Diode Pickup System (IPM; San Diego, CA) and the cross-correlation technique (model 102 B Velocity Tracker; IPM) (11). In eight networks, PO2 measurements were taken before velocity readings in the same vessels. Volume flow of blood was calculated from vessel diameters and dual-slit red blood cell velocity readings by using a correction factor of 1.3 to convert to mean velocities at diameters <10 μm and increasing by 0.06 per micrometer in the diameter range of 10–15 μm (12).

**Statistics.** Measured values were expressed in terms of mean, standard deviation, range, and CV defined as standard deviation/mean. Statistical analysis was performed by using a statistical software package (StatView; SAS, Cary, NC). The Mann-Whitney U-test (for nonparametric data) was used to test significance of PO2 values among individual networks. To determine the statistical significance of the correlation coefficient, an ANOVA table for the specified regression was generated. Statistical significance was assumed for \( P < 0.05. \)

## RESULTS

**Venular Po2.** Data were obtained from 64 postcapillary venules in 18 networks from 10 animals. Mean PO2 values for each network are presented in ascending order of PO2 in Fig. 2. As is apparent in this figure, the standard deviation in venules of the individual networks was small, averaging 2.0 Torr. The CV was small in individual networks, averaging 0.064, and was inversely related to mean network PO2, as shown in Fig. 3. The \( R^2 \) value is 0.31, indicating that 31% of the variation in CV may be related to the mean PO2. The slope is significantly different from zero, \( P < 0.05. \)

In six animals, we obtained data from more than one network as shown in Fig. 4. In animals A and B, three networks were studied, whereas in the remaining animals, data were obtained from two networks. Mean oxygen tension in these networks ranged from 24.1 to 38.0 Torr. In three animals, a significant difference was seen when mean PO2 values among networks were compared. The CV within animals was greater than for individual networks, averaging 0.94. When data from all collecting venules were pooled (total population), the mean PO2 was 30.1 ± 7.7 Torr. The CV for the total population was 0.26, which is greater by a factor of four than for individual networks and almost three times greater than for individual animals (Table 1).

**Venular volume flow.** Data on volume flow were obtained in 57 venules (mean diameter 8.68 ± 1.90 μm and mean red blood cell velocity 0.19 ± 0.27 mm/s) in 17 individual networks from nine animals as summarized in Table 2. As with the PO2 data, there was a progressive increase in CV from individual networks (0.51) to individual animals (0.78) to the total population (1.27). The CV for volume flow was much greater than that of PO2 at all levels, being eightfold greater
Thus it appears that variations in PO₂ levels among the blood collecting venules are unrelated to differences in vol-

works. This heterogeneity was greater within individ-

ations for each point = 3.2 ± 0.8) are shown. A relatively small CV for oxygen tension existed in individual net-

differences in PO₂ were unrelated to differences in

works. In nine individual networks, flow and PO₂ were mea-

ured in the same vessels (n = 23). In this study, no cor-

relation was evident between PO₂ and volume flow of

individual vessels (R = 0.04) as shown in Fig. 5. Thus it appears that variations in PO₂ levels among the collecting venules are unrelated to differences in vol-

ume flow.

DISCUSSION

The principal finding in this study was the demon-

stration of a low heterogeneity of oxygen tension among postcapillary venules within individual networks. This heterogeneity was greater within individ-

ual animals and much greater for all animals. In ad-

dition, the degree of heterogeneity in venular PO₂ was related to mean network PO₂, being greater in net-

works with low PO₂. Volume flow in venules was more heterogeneus than PO₂ but showed the same trend of being lowest in individual networks and highest in the total population. Also, within a group of venules in which both oxygen tension and flow were measured, differences in PO₂ were unrelated to differences in blood flow. Comparison of PO₂ and volume flow data among individual networks, individual animals, and all animals suggests that pooling data from a group of animals may mask trends within individual networks and animals.

Previous studies of oxygen tension in venules. Mean values of PO₂ ranging from 22.9 to 30.0 Torr have been reported for end-capillary values in hamster retractor

Table 1. Postcapillary venule PO₂ and coefficient of variation

<table>
<thead>
<tr>
<th></th>
<th>PO₂, Torr</th>
<th>PO₂ CV</th>
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<tbody>
<tr>
<td>Total population</td>
<td>30.1 ± 7.7</td>
<td>0.260</td>
</tr>
<tr>
<td>Individual animals</td>
<td>0.094</td>
<td></td>
</tr>
<tr>
<td>Individual networks</td>
<td>0.064</td>
<td></td>
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</tbody>
</table>

Mean postcapillary venule PO₂ (±SD) and coefficient of variation (CV) for the entire population [total population (n = 64)], for animals that had more than one network observed [within animals (average observations for each point = 18 ± 5.0)], and for individual networks (average observations for each point = 3.2 ± 0.8) are shown. A relatively small CV for oxygen tension existed in individual networks.

than PO₂ in individual networks and individual ani-
mals and six times that of PO₂ for the total population.

In nine individual networks, flow and PO₂ were mea-

ured in the same vessels (n = 23). In this study, no cor-

relation was evident between PO₂ and volume flow of

individual vessels (R = 0.04) as shown in Fig. 5. Thus it appears that variations in PO₂ levels among the collecting venules are unrelated to differences in vol-

ume flow.

In our study, the spatial variation in volume

flow was lowest in individual networks, intermediate among venules in the same animal and highest in the total population (Table 2).

The lower variation in volume flow within individual networks could indicate that local flow regulatory mechanisms are involved. Sarelius (21) observed that muscle and collecting venules of hamster skinfold, respectively (25, 13, 14). A mean PO₂ of 33 Torr in fourth-order venules (22 μm diameter) was obtained in the cremaster muscle of rats breathing 30% O₂ in N₂ (15). The mean value of PO₂ we obtained in postcapillary (fifth order) venules (30.1 ± 7.7 Torr) is somewhat higher than previously reported from our laboratory, 17.7 ± 5.4 Torr by Richmond et al. (20) in 20-μm venules and 21 ± 9 Torr by Shonat et al. (22) for postcapillary venules in the same muscle but a differ-

ent strain of rat (Sprague-Dawley) and maintenance anesthetic (α-choralose-urethane) than we used. The CV for all animals in those studies was 0.31 and 0.45, respectively, compared with our value of 0.26. How-

ever, whereas Shonat et al. (22) did not compare PO₂ in fifth-order venules from the same network, they did examine consecutive orders of venules in the same network and found only small differences. Their find-
ings is consistent with the present study and would not be expected if there were large differences in PO₂ among postcapillary venules in the same network. They also observed substantial differences among net-

works in all orders of venules, which is consistent with our findings.

Heterogeneity of microcirculatory volume flow. Previ-

ous investigators have reported heterogeneity of vol-

ume flow and red blood cell flux at the microcirculatory level. The values we obtained for CV of volume flow in venules for all animals (1.27) is somewhat greater than that found by others for red blood cell velocity or flux in capillaries and volume flow in arterioles and venules (0.42–1.03) (3, 10, 19). There has not, to our knowl-

edge, been a direct comparison previously of flow het-

erogeneity within and among networks and animals.

In our study, the spatial variation in volume flow was lowest in individual networks, intermediate among venules in the same animal and highest in the total population (Table 2).

Table 2. Postcapillary venule flow and coefficient of variation

<table>
<thead>
<tr>
<th>Flow, ml/s</th>
<th>Flow CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total population</td>
<td>0.011 ± 0.014</td>
</tr>
<tr>
<td>Individual animals</td>
<td>0.78</td>
</tr>
<tr>
<td>Individual networks</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Mean postcapillary venule flow (±SD) and CV for the entire population [total population (n = 57)], in animals that had more than one network observed [within animals (average number of observations for each point = 6.3 ± 2.0)], and within individual networks (average number of observations for each point = 3.2 ± 0.9) are shown.
local flow regulatory mechanisms tend to reduce the heterogeneity of flow among terminal arterioles. However, because, as discussed below, local metabolic requirements may vary, differences in flow rate are not unexpected in comparing venules in the same or different regions of one muscle. When comparing microcirculatory flow in muscles from different animals, a number of factors such as surgical preparation and anesthetic level could increase the variability and obscure underlying similarities.

Heterogeneity of venular PO2. Heterogeneity of venular PO2 was considerably less than that of volume flow, most strikingly within individual networks and individual animals, as is apparent from comparison of data in Tables 1 and 2. Within networks, this may be due, in part, to diffusion of oxygen among adjacent capillaries and venules. Because of the complexity of the venular network in the postcapillary region, it is not feasible to estimate this effect. However, the CV for individual animals was only 50% greater than that for individual networks, suggesting that factors other than diffusion must be involved. One such factor could be a matching of blood flow to oxygen consumption within localized regions. The spinotrapezius consists of different fiber types, which are reported in other muscles to have different oxygen consumption at rest (26) and differing degrees of capillary-fiber contact (27). Thus it is possible that some of the heterogeneity in flow within muscles is due to oxygen requirements of the different fiber types and would not be reflected in the PO2 of postcapillary venules. If the oxygen consumption in a region drained by a postcapillary venule is matched to volume flow in that region, then it would be expected that venular PO2 would not be a function of volume flow. This is, in fact, our finding as shown in Fig. 5, which adds support to this hypothesis. Our finding may not be representative of microcirculation in all muscles, because Swain and Pittman (28) found that oxyhemoglobin saturation in the hamster cremaster muscle was higher in venules with higher volume flow.

Microcirculatory flow and tissue oxygen levels. If local flow is matched to local metabolic requirements in our preparation, one underlying mechanism could be structural modification according to local oxygen levels, because there is evidence that angiogenesis is stimulated by low oxygen tension (8). This could set a lower limit on the PO2 in the capillary and venular network. A more precise adjustment of flow may be affected by arteriolar tone, which has been shown to be dependent on tissue oxygen levels in the vicinity of the arterioles and the capillary network (21). It has been reported that hemoglobin deoxygenation leads to release of ATP from the red blood cell (24), and it has been shown that ATP causes release from the venular endothelium of prostanoids that could diffuse to adjacent arterioles (10). Finally, there is evidence that a conducted vasodilator response from the capillary network to the arterioles is involved in the hyperemia of exercising muscle (2). Thus available data from other vascular beds suggest that there may be localized feedback mechanisms that would tend to maintain a constant PO2 among postcapillary venules despite wide differences in volume flow as shown in Fig. 5.

Possible contribution of nonmetabolic factors to heterogeneity. Despite the ample evidence cited above of oxygen-related mechanisms for flow regulation in the skeletal muscle microcirculation, we found a small but significantly greater heterogeneity of PO2 in networks having lower PO2 levels (Fig. 3). This appears to be inconsistent with a preeminent role for oxygen in local flow regulation, because, intuitively, one would expect lower, rather than greater, heterogeneity at low PO2 in this instance. However, other mechanisms of flow regulation unrelated to oxygen are also present in the microcirculation and may contribute to regulation of flow, especially in resting muscle. These mechanisms include neural regulation (17) and the myogenic response (18) as well as shear-stress-dependent release of endothelium-derived relaxing factor from the endothelium (16). It is possible that blood flow in regions in which PO2 is low is under greater influence of nonmetabolic factors. Consistent with this is the evidence that flow in capillary networks is more heterogeneous at low flow rates in resting muscle than when metabolic rate is increased during muscle contraction (30).

Heterogeneity in total population compared with individual networks and animals. As seen in Tables 1 and 2, heterogeneity was greater in the total population than in individual networks and animals especially with respect to oxygen tension. Whether this increased heterogeneity is due to day-to-day differences in muscle surgical preparation, variability in conditions of the study, or intrinsic differences among the animals themselves is not clear. Because the pH of venular blood is lower than systemic levels (15), the relationship between PO2 and hemoglobin oxygen saturation may be subject to local metabolic conditions. Whatever the reason for the differences among animals, this finding suggests that pooled data from a number of animals tend to overestimate the variability of oxygen tension and volume flow in a local venular network.

In conclusion, this study demonstrates that heterogeneity of venular PO2 in the rat spinotrapezius muscle is lower in localized networks than in individual animals and considerably lower than in the total population of animals. Heterogeneity of venular PO2 is also considerably less than that of blood flow at all levels. The lower heterogeneity of Po2 compared with volume flow may reflect the effects of diffusion among adjacent vessels as well as mechanisms that would match blood flow to oxygen demand of the tissues in the short term and in the long term. Our finding that PO2 is unrelated to volume flow among venules in which both are measured is consistent with this interpretation. Finally, our findings show that the pooled data from different animals overestimate the variability of venular PO2 and volume flow likely to be found in a local network or an individual animal.
REFERENCES