Heterogeneity of Red Blood Cell Perfusion in Capillary Networks Supplied by a Single Arteriole in Resting Skeletal Muscle

C.G. Ellis, S.M. Wrigley, A.C. Groom

Abstract Flow heterogeneity within capillary beds may have two sources: (1) unequal distribution of red blood cell (RBC) supply among arterioles and (2) unique properties of RBC flow in branching networks of capillaries. Our aim was to investigate the capillary network as a source of both spatial and temporal heterogeneity of RBC flow. Five networks, each supplied by a single arteriole, were studied in frog sartorius muscle (one network per frog) by intravital video microscopy. Simultaneous data on RBC velocity (millimeters per second), lineal density (RBCs per millimeter), and supply rate (RBCs per second) were measured continuously (10 samples per second) from video recordings in 5 to 10 capillary segments per network for 10 minutes by use of automated computer analysis. To quantify heterogeneity, mean values from successive 10-second intervals were tabulated for each flow parameter in each capillary segment (ie, portion of capillary between successive bifurcations), and percent coefficient of variation (SD/mean · 100%) was calculated for (1) spatial heterogeneity among vessels (CVs) every 10 seconds and for the entire 10-minute sample and (2) temporal heterogeneity within vessels for every capillary segment and for the mean flow parameter. Analysis of these data indicates that (1) capillary networks are a significant source of both spatial and temporal flow heterogeneity, and (2) continuous redistributions of flow occur within networks, resulting in substantial temporal changes in CVs, although a persistent spatial heterogeneity of perfusion still exists on a 10-minute basis. In most networks, CVs decreased as supply rate within the network increased, thus indicating that rheology plays a significant role in determining the perfusion heterogeneity. (Circ Res. 1994;75:357-368.)

Key Words: spatial heterogeneity • temporal heterogeneity • microcirculation • capillary networks • flow redistribution

The most striking feature of the microcirculation of skeletal muscle, to the person who views it for the first time through a microscope, is the large variability in perfusion among individual capillaries. Some capillaries appear to have few red blood cells (RBCs) traveling at high velocities, whereas other vessels have a continuous train of RBCs traveling very slowly. Closer examination reveals that this apparent simple relation between RBC velocities and lineal densities (number of RBCs per millimeter) does not hold; there are vessels with almost any combination of low and high velocity and lineal density. This situation is further complicated by the fact that flow is not steady but fluctuates with time within each vessel.

For the past decade and a half there have been numerous studies that have attempted to quantify this heterogeneity of perfusion and to determine its physiological significance.1-5 Heterogeneity has been expressed in terms of the relative dispersion (SD/mean, ie, the coefficient of variation) of one or more hemodynamic parameters such as RBC velocity (millimeters per second), hematocrit (percent), RBC content (lineal density in RBCs per millimeter), or RBC supply rate (RBCs per second).6 The variability among vessels has been termed the spatial heterogeneity, and the variability with time within each vessel, the temporal heterogeneity.4 The physiological significance of spatial heterogeneity has been linked with the efficiency of tissue oxygenation and capillary exchange of diffusible solutes.5,7 Thus, one would expect that spatial heterogeneity should be under some form of regulatory control in response to changes in metabolic demand or oxygen delivery to the microvasculature. To date, research into this area has yielded contradictory results.1,8-11

The physiological significance of temporal heterogeneity has been less clear, although it likely affects the efficiency of microvascular exchange. Our understanding of the properties of the microvasculature that lead to flow heterogeneity and how these properties might be regulated is far from complete.12

To understand how the heterogeneity of perfusion might be regulated or altered, one needs to consider the potential sources of this heterogeneity. Although Krogh13 considered the capillary as the smallest independent controller of capillary perfusion, more recent studies have indicated that control lies entirely within the arteriolar tree.14 Even in passive microvascular networks, such as the mesentery, the level of perfusion within groups of capillaries reflects the distribution of perfusion among the arterioles feeding these capillary beds.15 Temporal heterogeneity has usually been associated with vasomotion,15 ie, the rhythmic contraction and relaxation of arterioles. Thus, the arteriolar tree appears to be a major component in determining both the spatial and temporal heterogeneity of capillary perfusion.

Another source of heterogeneity may be the capillary network. Conceptually, the microvasculature has been

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described as being composed of a number of parallel flow paths between artery and vein. However, the capillary bed is made up of networks of interconnected diverging and converging capillary segments. This arrangement provides numerous alternate routes for blood flow to follow, and as a consequence, the level of flow in individual capillary segments must be quite different. This type of spatial heterogeneity has been referred to as the longitudinal heterogeneity in capillary segment flow or between proximal and distal capillaries. We have also reported considerable dynamic variability in capillary flow, which is apparently unrelated to vasomotor activity and which we believe to have originated within the capillary network itself.

It is likely that all four aspects of heterogeneity (arteriolar, capillary network, spatial, and temporal) are interrelated. However, to date, no experimental studies have been designed to account for this possibility. Most experiments have studied spatial heterogeneity by using measurements of one or more flow parameters from a limited number of capillary segments selected at random. These studies cannot distinguish between the arteriolar and capillary network component of heterogeneity. Two studies in which data were collected from all arteriolar, capillary, and venular segments of the mesentery required up to 30 minutes for one data set. These experiments cannot distinguish between spatial and temporal components of the heterogeneity.

The goal of the present study was to examine the spatial and temporal heterogeneity of RBC perfusion arising within the capillary network, independent of heterogeneity introduced by unequal flow distributions among arterioles. This was accomplished by simultaneously measuring RBC velocity, lineal density, and supply rate in individual capillary segments of networks supplied by a single arteriole. By studying such networks, we were able to demonstrate that the capillary network is a source of perfusion heterogeneity, and by measuring all three flow parameters, we were able to show that rheology plays a significant role in determining this heterogeneity.

Materials and Methods

Animal Preparation

Five mature frogs (Rana pipiens) weighing =50 g were anesthetized with a 20% urethane solution injected into the dorsal lymph sac (35 mg/10 g body wt). During the course of the experiment, anesthesia was maintained by 0.05-mL injections of urethane through a small cannula inserted under the ventral skin. With each frog, a sartorius muscle was exposed by removing the overlying skin. The transparent connective tissue surrounding the muscle was left intact to minimize surgical trauma to the muscle. The frog was placed in the supine position on the stage of a Wild Makskop (M420) and left at room temperature. The exposed muscle was covered with a plastic coverslip to isolate the tissue from the surrounding environment. The surface of the muscle was epi-illuminated using a Schott fiberoptic light source (KL1500) with both a green filter and a heat filter. Images of the microvasculature associated with the superficial muscle fibers were recorded on videotape with a Panasonic video camera (WV-1550) and a Panasonic videocassette recorder (WV-9240XD). The images were viewed on a Panasonic black and white monitor (WV-5410). After surgery, the flow in the microvasculature was allowed to stabilize for a period of up to 1 hour. For each muscle, a capillary network was chosen that permitted hemo-

dynamic analysis of at least five capillary segments in a network supplied by a single arteriole. A capillary segment was defined as the section of capillary between two branch points. A capillary network was defined as the group of capillary segments originating from the terminal end of a single arteriole (see Fig 1). Video images of the RBC flow in the network were recorded for at least 10 minutes. The size of the field of view was =0.8×0.6 mm, with the capillaries oriented vertically on the monitor.

Analysis of Video Images

RBC velocity (millimeters per second) and lineal density (content, RBCs per millimeter) were measured in each capillary segment from the video recordings by use of automated computer analysis routines. To ensure that the sampling of each capillary segment began at the same point in time, an audio cue was placed on one of the audio channels of the videotape. Sampling of the videotape began when the computer system detected the audio cue. Thus, by replaying the videotape, “simultaneous” data on RBC velocity and RBC lineal density were obtained for each capillary segment. RBC velocities were measured in a single capillary by use of a spatial correlation technique. The spatial correlation technique was able to measure positive, zero, and negative RBC velocities over the physiological range of velocities found in capillaries. RBC lineal densities were measured using a videodensitometric method. Since this technique did not attempt to detect individual cells, the lineal density could be measured in capillaries even with continuous trains of RBCs. Both velocity and lineal density were sampled every sixth video field (ie, rate of 10/s) for 10 minutes (total of 6000 samples per parameter per vessel). RBC supply rate was calculated for every sample interval from the product of velocity and lineal density. For the analysis presented in “Results,” the data are presented as 10-second mean values for successive 10-second time intervals (ie, each mean is computed from an independent set of data).

All statistical calculations were performed with Systat (Systat Inc). The schematic diagram was produced using CorelDRAW (Corel Inc). Graphs were plotted using SigmaPlot (Jandel Scientific) and arranged for publication using CorelDRAW. Tables were generated using Excel (Microsoft Inc).

Results

Video images of RBC flow in resting sartorius muscle were recorded from five capillary networks supplying superficial muscle fibers (one network per frog). Networks in which five or more capillary segments, with straight portions at least 140 μm long, could be included in a single field of view were selected at random. RBC velocity, lineal density, and supply rate were measured from the video recordings for 10 minutes in each capillary segment (number of segments per network: 9, 10, 5, 6, and 8). The architecture of a typical network is shown in Fig 1. The capillary segments were identified with a two-digit code, the first digit representing branching order (1, 2, 3 . . . ) and the second digit specifying the particular segment within each branching order. In the field of view represented by Fig 1, it was possible to record the flow simultaneously in all segments except 31 and 32.

The flow heterogeneity found in such a network may be appreciated visually from three-dimensional (3D) presentations of the flow data, as illustrated in Fig 2. In this figure, data for RBC velocity, lineal density, and supply rate are plotted as 10-second mean values for each capillary segment. To highlight the differences in
blood perfusion among capillaries, a line has been drawn at each time interval connecting the values for all capillary segments. The graph of RBC supply rate shows the considerable temporal and spatial fluctuations that exist within the network, and closer inspection reveals persistent differences between vessels (e.g., “mountain ranges” versus “valleys”). The contribution of velocity and lineal density to the heterogeneity of RBC supply rate can be easily recognized by comparing the 3D plots for all three parameters. For example, at time 0, capillary segments 23 and 25 show an almost 3-fold difference in RBC supply rate; this is due almost entirely to a 2.5-fold difference in lineal density and only a slight difference in velocity. In contrast, the difference in RBC supply rate between capillary segments 12 and 13 is due primarily to a difference in velocity. The vessels with the highest lineal densities, 24 and 25, arise from the most distal section of the arteriole. The graph of relative supply shows the supply rate for each segment relative to the total RBC supply rate to the network, i.e., relative to the estimated arteriolar input to the network. The 10-second mean arteriolar RBC supply rates were estimated from the measured RBC supply rates in individual capillary segments on the basis of a simple mass balance of RBCs; i.e., only one segment from each flow path between arteriole and venule was included in the summation. For example, in network 1 (Fig 1), the arteriolar input was estimated from capillary segments 11 and 20 through 25. When expressed as a relative supply rate, some vessels show a reduced temporal variability, whereas others do not (compare vessels 23 through 25 with vessel 11).

To quantify flow heterogeneity, 10-second mean values for each flow parameter were tabulated for all capillary segments in each network, as illustrated in Table 1 for RBC supply rate. From these data, one can compute the mean±SD values for the entire network at each 10-second interval (rows) and for each capillary over the entire 10-minute period (columns). The 10-second mean arteriolar RBC supply rates were also included in the supply rate tables for calculation of the relative supply rates. For RBC velocity and lineal density, similar tables were constructed, excluding all reference to the supplying arteriole.

The heterogeneity for each capillary flow parameter can be quantified in terms of the coefficient of variation (CV% = SD/mean • 100%). This heterogeneity can be resolved into two components, spatial (among capillaries, CVs) and temporal (CVt). In Table 1, the spatial heterogeneity has been computed for each 10-second interval (individual rows) as well as for the entire 10-minute data set (row of 10-minute mean values). The 10-second CVs values indicate how spatial heterogeneity varied with time, whereas the 10-minute CVt value indicates the persistent spatial heterogeneity within the network. The temporal heterogeneity has been computed for each capillary (individual columns) as well as for the mean capillary flow in the entire network (column of 10-second mean values). The 10-minute CVs values for capillaries indicate how the temporal heterogeneity varied among capillaries, whereas the 10-minute CVt value for the mean capillary flow indicates how the mean perfusion of the network varied with time. The overall heterogeneity of capillary flow (CVo) is computed from the entire set of values in the table (rows and columns). CVo indicates the variability of capillary perfusion due to both spatial and temporal factors. Repeated random samples of capillaries at different points in time during the 10-minute interval would approximate CVo.

Results from the analysis of the 10-second data from all five networks are summarized in Table 2, in terms of 10-minute mean values and their corresponding CV

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**Fig 1.** Schematic of capillary network 1. Direction of flow is indicated by the arrows. Each segment has been identified with a two-digit code, the first digit indicating whether it is a first-, second-, or third-order vessel and the second digit specifying the particular segment within each branching order.

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**Fig 2.** Three-dimensional plots of velocity, lineal density, supply rate, and relative supply vs capillary segment ID vs time for capillary network 1. RBC indicates red blood cells. ID refers to the two-digit code identifying each capillary segment (see Fig 1 legend).
TABLE 1. Mean Values of Red Blood Cell Supply Rate Over Successive 10-Second Intervals for Each Capillary of the Network Throughout a 10-Minute Period

<table>
<thead>
<tr>
<th>Estimated Arteriolar Input, RBCs/s</th>
<th>RBC Supply Rate in Capillary Segments, RBCs/s</th>
<th>10-Second Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time, s</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>0</td>
<td>10.2</td>
<td>1.9</td>
</tr>
<tr>
<td>10</td>
<td>13.2</td>
<td>3.1</td>
</tr>
<tr>
<td>20</td>
<td>13.6</td>
<td>2.1</td>
</tr>
<tr>
<td>30</td>
<td>12.9</td>
<td>2.1</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>560</td>
<td>15.5</td>
<td>2.4</td>
</tr>
<tr>
<td>570</td>
<td>14.8</td>
<td>2.6</td>
</tr>
<tr>
<td>580</td>
<td>16.8</td>
<td>3.4</td>
</tr>
<tr>
<td>590</td>
<td>17.9</td>
<td>3.5</td>
</tr>
</tbody>
</table>

RBC indicates red blood cell; CV, coefficient of variation; subscript o, the overall statistic computed from the data as a whole, both rows and columns; subscript s, the spatial statistic calculated from the row of 10-minute mean values; and subscript t, the temporal statistic calculated from the column of 10-second mean values for all capillaries.

The arteriolar supply of RBCs to the network is estimated from individual capillary supply rates based on a mass balance of RBCs. Mean, SD, and CV are computed for each column yielding the 10-minute mean and 10-minute temporal CV values for the arteriolar input and for each capillary. The same calculations are performed on each row of capillary data yielding the successive 10-second mean and 10-second spatial CV values. The seven values in the lower right corner (values with subscripts) are statistics calculated from the entire 10-minute data set.

values (temporal, spatial, and overall) for the three flow parameters and for the estimated arteriolar input. In all cases, the temporal CV values were much less than the spatial values, so that spatial and overall CV values were similar. However, a two-way ANOVA of the data for each of the five networks (arranged as in Table 1, time versus capillary number) showed that temporal variations, based on 10-second intervals, made a small but statistically significant ($P<.005$) contribution to the overall heterogeneity of capillary network perfusion. This reflects the presence of a degree of temporal variation that was consistent across the entire network. The statistics compiled from the mean data for all five networks (not weighted with respect to the number of capillaries in a network) indicates the variability in these parameters among networks.

The CV values for RBC supply within each network of capillaries ($CV_{cap}$) were comparable in magnitude to those of their arteriolar input ($CV_{input}$) (Table 2). However, analysis of the 10-second data on the basis of individual capillaries showed that although some vessels exhibited similar temporal heterogeneity to the input ($CV_{cap}/CV_{input} = 1$), others displayed much greater heterogeneity ($CV_{cap}/CV_{input} > 1$).

Fig 3 shows that the capillaries with a greater heterogeneity were primarily those with RBC supply rates <20% of the input supply rate. The capillary segment order is also shown in Fig 3, and there appears to be no relation between the location of the segment in the network and the degree of temporal heterogeneity. The question arises whether the capillaries having a high temporal heterogeneity are those with a low RBC velocity, a low RBC lineal density, or a combination of both. This issue is explored in Fig 4, which shows a 3D plot of the relative temporal heterogeneity $CV_{cap}/CV_{input}$ versus velocity and lineal density. It is clear that the highest relative temporal heterogeneity occurred in those capillaries having a combination of low RBC velocity and low RBC lineal density. Conversely, the lowest relative temporal heterogeneity ($CV_{cap}/CV_{input}$ was found in vessels with the highest velocity and lineal density. A nonlinear regression analysis ($CV_{cap}/CV_{input} = a_0 + a_1 \cdot v + a_2 \cdot n$) showed that there is a significant relation between relative temporal heterogeneity and both velocity ($P<.001$) and lineal density ($P=.011$, with $r=.693$). The relative heterogeneity fell more rapidly with increasing velocity than with increasing lineal density.

The spatial heterogeneity of perfusion for each 10-second interval (10-second CV,) showed considerable variability during the 10-minute sample interval. Fig 5 (upper panels) shows histograms of 10-second CV, for velocity, lineal density, and supply rate in capillary network 1. The source of the variability in spatial heterogeneity was explored by testing the correlation between the 10-second CV, and the 10-second data on arteriolar input, mean velocity, mean lineal density, and
mean supply rate. The lower panels in Fig 5 show the correlation of the 10-second CV for velocity, lineal density, and supply rate with the arteriolar input. The results for all five networks have been summarized in Table 3. Each network was found to have a considerable dispersion of 10-second CV values. Three of the five networks showed a modest correlation between the spatial heterogeneity of RBC supply rate within the network and the estimated arteriolar input to the network. Two networks showed a correlation between the heterogeneity of velocity and of lineal density with the input as well. In all of these cases, the heterogeneity of perfusion decreased as the input to the network increased. Since RBC supply rate is determined both by velocity and lineal density, we tested to see whether the heterogeneity of RBC supply rate correlated with these two parameters by use of multilinear regression analysis [10-second CV_{rate} = a_0 + a_1 (10-second mean velocity) + a_2 (10-second mean lineal density)]. The results of this analysis are given in Table 3. Only networks 1 and 2 showed a significant correlation.

Network 5 demonstrated very unique behavior compared with the networks studied. During the first 400 seconds (Fig 6) the spatial heterogeneity showed the same behavior as the other three networks (spatial heterogeneity was negatively correlated with arteriolar input), but during the last 200 seconds the behavior was

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### TABLE 2. Summary of Statistics for Red Blood Cell Velocity, Lineal Density, and Supply Rate for the Entire 10-Minute Sample Interval for All Five Capillary Networks and for the Estimated Arteriolar Input to Each Network

<table>
<thead>
<tr>
<th>Capillary RBC supply parameter</th>
<th>Network</th>
<th>Statistics Compiled From All Five Networks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Number of capillary segments analyzed</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Velocity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, mm/s</td>
<td>0.15</td>
<td>0.12</td>
</tr>
<tr>
<td>CV, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temporal</td>
<td>6.4</td>
<td>12.6</td>
</tr>
<tr>
<td>Spatial</td>
<td>38.8</td>
<td>41.9</td>
</tr>
<tr>
<td>Overall</td>
<td>40.2</td>
<td>44.9</td>
</tr>
<tr>
<td>Lineal density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, mm/s</td>
<td>14.9</td>
<td>18.3</td>
</tr>
<tr>
<td>CV, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temporal</td>
<td>14.0</td>
<td>14.0</td>
</tr>
<tr>
<td>Spatial</td>
<td>33.3</td>
<td>42.2</td>
</tr>
<tr>
<td>Overall</td>
<td>38.3</td>
<td>51.0</td>
</tr>
<tr>
<td>Supply rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, mm/s</td>
<td>2.3</td>
<td>2.5</td>
</tr>
<tr>
<td>CV, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temporal</td>
<td>10.7</td>
<td>18.3</td>
</tr>
<tr>
<td>Spatial</td>
<td>40.2</td>
<td>67.6</td>
</tr>
<tr>
<td>Overall</td>
<td>43.0</td>
<td>71.2</td>
</tr>
</tbody>
</table>

RBC indicates red blood cell; CV, coefficient of variation.
reversed (the heterogeneity followed the change in arteriolar input [positive correlation]). The spatial heterogeneities of all three flow parameters during the final 200 seconds were correlated with the arteriolar input and the mean value of each parameter (Table 4).

Discussion

Several strategies could be used to evaluate different aspects of the spatial heterogeneity of RBC flow in a capillary bed. For example, one could focus on the distribution of perfusion among capillary segments intersecting a cross section of the muscle or on the dispersion of flow based on capillary segments yielding a mass balance. Alternatively, one could estimate the heterogeneity of perfusion in the entire population of capillary segments at the surface of the muscle, as most previous investigators have done. We have chosen a different strategy, one that allowed us to distinguish between potential sources of heterogeneity present in these previous studies. We have restricted our observation to the population of capillary segments within a network supplied by a single arteriole.

3D Plots

The first goal of the present study was to determine whether the capillary network is a source of spatial and temporal heterogeneity of RBC flow. The 3D plots in Fig 2 provide a simple means of visualizing the heterogeneity and attempt to answer this question. The obvious heterogeneity of perfusion in these plots cannot be due to differences in RBC supply among individual arteriolar networks, since the capillary network analyzed for these graphs originated from a single arteriole. The presence of a persistent spatial heterogeneity among the capillaries within the network can easily be appreciated from the distinct “mountain ranges and valleys.” The temporal heterogeneity within individual vessels can be appreciated from the “peaks” in the mountain ranges and the “dips” in the valleys.

Did this spatial heterogeneity originate in the capillary network? For two pairs of second-order capillaries, segments 20/21 and 22/23, the answer is clearly yes, since in each case the flow from the parent vessel was divided very unequally between the daughter branches. For the other capillaries, the answer is not as simple, since this network contained four inputs from the supplying arteriole, ie, four first-order capillary segments (Fig 1, segments 11 through 13 and the parent of vessels 24 and 25). Several of these vessels show distinct differences: segment 13 had the highest velocity; segments 24 and 25, the highest lineal density; and segments 13 and 25, the highest supply rates. From the 3D plots we cannot tell whether these differences were a passive result of the geometry of the network and of the rheology or whether the flow was being actively controlled by the supplying arteriole to each individual first-order vessel, ie, the activity of capillary “sphincters.” More detailed analysis of the data given below will help to answer this question.

Did the temporal heterogeneity originate in the capillary network? We can attempt to answer this question by comparing the temporal heterogeneity in the network with that in the supplying arteriole. This can be done only in terms of RBC supply rate, since RBC velocities and lineal densities in the arteriole could not be estimated from the capillary data. Moreover, we were unable to measure these parameters directly.
Comparison of the 3D plots of absolute (RBCs per second) versus relative (percent input) RBC supply rates (Fig 2) can be made on the basis of the following premise. The plot of relative supply was constructed on the assumption that consistent temporal fluctuations in flow across the network, which most likely originated in the supplying arteriole, would be removed when the data were plotted as a percentage of the input. Fluctuations originating within the network would not be coordinated and hence would be unaffected by normalizing the data in this manner. There are four segments, 13 and 23 through 25, in which the dynamic variations in supply have been obviously reduced, and there are at least three capillary segments, 11, 12, and 22, which had large temporal variations in supply rate that have not been appreciably affected (the responses in other vessels are hidden by those with the highest flow). Thus, some, but not all, of the temporal heterogeneity of RBC supply did have its origins in the network.

The 3D plots, when used together with the schematic of the network layout, can provide useful insights into the heterogeneity of capillary flow. However, to inves-
tigate the factors affecting the heterogeneity, more
detailed analysis of the data, such as presented in Table
1, is required. This analysis yields quantitative values for
the spatial and temporal components of the heteroge-
neity, which then can be correlated with the flow
parameters.

Analysis of Spatial Heterogeneity

The hemodynamic data have been presented as mean
values for successive 10-second intervals. This interval
was selected for a number of reasons. First, since each
10-second CV value is computed from the mean ± SD of
100 "instantaneous" data values per capillary, we can
have considerable confidence in the validity of spatial
heterogeneity estimates. Second, since this interval
exceeded the mean RBC transit time through a capil-
lar segment, each successive time period corresponded
to a new set of RBCs within the segment. Thus,
temporal variations due to the heartbeat and to discrete
events such as the passage of RBCs through bifurcations
or capillary constrictions were removed. Furthermore,
this averaging interval corresponds to that frequently
reported by others in the literature (note that the
analysis of 10-second mean data for the 10 minutes may
be considered to be equivalent to 59 sequential analyses
of a single capillary network). We have shown previ-
ously that using 10-second mean data reduces the
temporal CV for a single vessel to between 30% and
60% of the value obtained from instantaneous data.2

Calculation of the spatial CV of network perfusion
for successive 10-second intervals allows one to follow
changes in spatial heterogeneity over prolonged periods
of time (eg, 10 minutes in the present study). Interest-
ingly, even under resting conditions there existed up to
a threefold variation in spatial CV for RBC velocity and
lineal density and up to a twofold variation for RBC
supply rate. Thus, as we expected, within the network
there exists a continuous redistribution of RBC flow
among capillaries. However, the fact that each 10-
minute CV value fell within the range of its correspond-

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>0 to 400 Seconds</th>
<th>400 to 590 Seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Independent Variables</td>
<td>r</td>
<td>Slope</td>
</tr>
<tr>
<td>10-Second spatial CV of velocity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Input supply rate</td>
<td>0.67</td>
<td>-ve</td>
</tr>
<tr>
<td>Mean velocity</td>
<td>0.43</td>
<td>-ve</td>
</tr>
<tr>
<td>10-Second spatial CV of lineal density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Input supply rate</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Mean lineal density</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>10-Second spatial CV of supply rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Input supply rate</td>
<td>0.38</td>
<td>-ve</td>
</tr>
<tr>
<td>Mean supply rate</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Both mean velocity and mean lineal density</td>
<td>0.62</td>
<td>+ve, -ve</td>
</tr>
</tbody>
</table>

CV indicates coefficient of variation; -ve, negative; and +ve, positive.
Regression coefficient and sign of slope reported for significant correlations (P < .01).

ing 10-second values confirms that an inherent spatial
heterogeneity of perfusion persists within each capillary
network. Although this inherent heterogeneity cannot
be abolished, it can be diminished to some extent by
flow redistribution, as evidenced by the fact that some
of the 10-second CV values were considerably smaller
than the 10-minute value. The lower limit of the histo-
grams in Fig 5 (minimum CV values listed in Table 3)
may represent the maximum degree of homogeneity
that can be achieved.

If the spatial heterogeneity seen in the network is due
to network geometry and rheological mechanisms, then
the distribution of RBC flow within the network should
be influenced by factors such as the RBC input to the
network and by the number and velocity of RBCs within
the network. Fig 5 presents convincing data that this
was the case for network 1. As the RBC input to the network
increased, the spatial heterogeneity (CVi) of RBC
supply rate decreased. Two other networks showed
similar results (Table 3). The spatial heterogeneity of
velocity and of lineal density also was reduced in two
networks as the RBC input increased. Furthermore,
only one network (3) did not show a correlation with at
least one of the flow parameters. The level of RBC
input to the networks in the present study only ac-
counted for 10% to 50% of the variability in the
10-second CV values. The remaining variability may
have been due to other factors, such as transient white
blood cell plugging of individual flow paths or changes
in vascular resistance or pressure on the venous side of
the network. Or it may have been due to a limitation in
the measurements themselves. The arteriolar input was
estimated from the supply rates in individual capillary
segments. Since it requires a finite time for cells to move
from one location to the next, our estimate of the
dynamics of the cell input to the network may be
distorted. However, we did observe that some vessels
(eg, segment 13, network 1) that were not included in
the calculation of the input supply rate did correlate
well with the dynamics of the estimated input. The
estimated arteriolar input to the network should not be confused with the total RBC supply rate in the arteriole. The supplying arteriole delivers RBCs to other capillary networks as well. The correlations of spatial heterogeneity with mean velocity, mean lineal density, and mean supply rate should be valid, since these values represent events that were measured within the network.

Why did not all of the networks show the same behavior? Network 3 showed no correlation between the measures of heterogeneity and the rheological parameters that might affect spatial heterogeneity. This network had the fewest capillary segments (five), composed of two groups of capillaries with no interconnections between them. Other than differences in geometry, there was no obvious reason for the lack of correlation. Network 5 did show the same correlation as the other three networks during the first 400 seconds and the opposite correlation during the final 200 seconds. The combination of behaviors led to the lack of correlation for the data set as a whole. Inspection of a set of 3D plots for this network, of the type shown in Fig 2, revealed that one first-order segment, and thus the segments downstream from it, received a greater proportion of the input to the network than the other vessels during the last 200 seconds of the sample. This “imbalance” in perfusion led to the positive correlation shown in Fig 6 and summarized in Table 4. The imbalance was not due to a higher than usual supply of RBCs to the network, since the input did not rise above earlier levels (Fig 6) and it did not appear to steal flow from the other first-order capillaries downstream, since these vessels maintained approximately the same level of flow during this period of time. The change in RBC supply also occurred without a change in velocity and was entirely due to an increase in lineal density. We may speculate that the supplying arteriole actively diverted additional RBCs to this first-order segment, for a brief period of time, in response to local metabolic demand or that a passive event such as the transient plugging of another capillary out of the field of view caused RBCs to enter this vessel.

Changes in mean RBC velocity for a network should reflect changes in driving pressure relative to the hemodynamic resistance across the network. It is interesting that the values of 10-second CV for velocity were not correlated with the mean velocity in any of the networks (except for network 5 during the flow imbalance, Table 3). This can be interpreted as indicating that changes in driving pressure and/or hemodynamic resistance across the network did not significantly alter the heterogeneity of pressure gradients and/or resistance within the network. In contrast, increasing the number of RBCs entering the network did decrease the spatial heterogeneity of velocity. We interpret this to mean that rheological mechanisms associated with the passage of an increased number of RBCs through the network resulted in a more uniform distribution of segmental driving pressures relative to flow resistance.

Our previous estimate of spatial heterogeneity of RBC supply rate included interarteriolar and animal-to-animal variation, whereas in our present estimate these variations have been excluded. Nevertheless, the mean network spatial CV of 47% (Table 2) reported in the present study amounted to more than two thirds of our previous global estimate of 66% for frog sartorius (and 67% for rat gracilis) muscle. The implication is that the capillary network itself is a significant source of the spatial heterogeneity of RBC supply in capillaries across the frog sartorius muscle.

Analysis of Temporal Heterogeneity

One might expect the global temporal variability of RBC perfusion within the network to arise from variations in the arteriolar supply. This expectation was confirmed by the similarity of temporal CVs for the entire network to those for the estimated arteriolar input and by the ANOVA, which indicated consistent temporal variations throughout the capillary network. However, the spatial heterogeneity results also indicated another source of heterogeneity, the temporal redistributions of RBC flow among capillaries. This was seen particularly with capillaries that carried <20% of the total RBC input, for these vessels showed temporal CV values up to four times that of the arteriolar supply. Vessels with a high relative heterogeneity of perfusion were characterized by low velocity and low lineal density of RBCs (Fig 4). However, the magnitude of the relative heterogeneity appeared to depend more on velocity than on lineal density. The underlying mechanism for this behavior may depend on a number of factors. Flow of RBCs in capillary segments with a low driving pressure across them (as indicated by a low velocity of flow) will be sensitive to irregularities in capillary luminal cross section that are due to the fine energy required for the deformation of RBCs. Moreover, at each capillary bifurcation the distribution of cells to vessels with a low velocity may be highly variable compared with the vessel with the higher velocity.

Summary

How are we able to explain the degree of spatial and temporal heterogeneity we found in these networks? We believe that the topology of the networks is one important factor. For example, at a diverging capillary bifurcation, the blood flow in the parent capillary must be divided between the daughter vessels. In terms of RBC supply rate, the sum of the supply rates in the two daughter vessels must equal that of the parent. A homogeneous distribution of cells between the two daughter vessels would reduce the supply rate in these segments to half of that in the parent. The level of RBC velocity in the daughter vessels relative to the parent is more difficult to predict, since this is determined by the relative cross-sectional areas of the daughter vessels to that of the parent (and by the relative velocity of the RBCs to that of the blood flow as a whole). The RBC lineal density in the daughter vessels is then determined by the supply rate and by the velocity in each vessel: lineal density (RBCs per millimeter)=supply rate (RBCs per second)/velocity (millimeters per second). There are situations when a daughter vessel can have either a much lower or much higher lineal density than the parent. The distribution of RBCs at branch points is further exaggerated by the phase separation of plasma and RBCs at bifurcations. The daughter vessel with the higher blood flow rate tends to receive an even greater proportion of the RBCs than one would predict simply from the blood flow distribution. Thus, even if the arteriolar supply to all first-order capillary segments (ie,
capillaries branching directly from the arteriole) were homogeneous, one would measure a spatial heterogeneity of perfusion from either a random sample of the capillary bed or from a network analysis.

These arguments did not take into account variations in vascular resistance due to the geometry of the network that would further contribute to the spatial heterogeneity. The geometry of the capillary network in frog sartorius muscle has been studied by Pyley et al. Analysis of the fundamental structure of the diverging and converging vessels revealed that the number of branch points per capillary path, from arteriole to venule, formed a Poisson distribution and that the capillary segment lengths formed an exponential distribution. Both findings indicate that branch points are distributed randomly along each capillary path. Furthermore, for this same muscle Safranyos et al. have reported a wide range of capillary diameters, and Ellis and colleagues have demonstrated considerable changes in luminal cross-sectional area along capillaries. One would expect that the complexity of such a capillary network must lead to an inherent spatial heterogeneity of vascular resistances and thus of flow among vessels, even for a simple Newtonian fluid. In addition, for networks perfused by blood, we would predict the spatial heterogeneity to be dynamic because of the two-phase nature of capillary blood flow. As RBCs enter and leave a capillary segment, they alter the resistance to flow within that segment, and this phenomenon would lead to a redistribution of blood flow and of RBCs between daughter branches at diverging bifurcations. This is the most likely explanation of temporal changes in the spatial heterogeneity of blood flow that we observed.

This explanation of the spatial and temporal heterogeneity of RBC flow derives solely from the passive rheological properties of blood in capillary networks. This concept is supported by the dynamic simulation of Kiani et al. for blood flow in capillary networks, which demonstrates that flow within a given vessel varies with time even when the driving pressure across the network is fixed.

Discussion of the Present Study Related to Others

The approach to studying flow heterogeneity has varied considerably among different research groups. In most cases, experiments have been designed to study the spatial heterogeneity of perfusion (variation in flow among capillaries) rather than the temporal heterogeneity (variation in flow with time in individual capillaries). To obtain a representative measure of the distribution of blood flow among capillaries, RBC velocities have been sampled randomly from capillaries on the muscle surface. Most velocity measurements are reported to represent approximately a 10-second mean value for each vessel. In some of these studies, care has been taken to determine the "true" spatial heterogeneity by measuring the velocity distribution at one fixed point in time within one muscle region; others have pooled data that have been sampled at different times and from different muscles. Since these two studies obtained contradictory results, it has been proposed that their disagreement was due to this fundamental difference in sampling strategy. Another approach has been to study entire capillary networks and to measure the distribution of RBC velocities, content, and supply rate within these networks. In these studies, the spatial distributions of flow parameters were measured simultaneously in all vessels. More recently, entire microvascular networks of arterioles, capillaries, and venules (with up to 1000 vascular segments) have been analyzed in rat mesentery under normal conditions and after isovolemic hemodilution. Because of the vast size of the mesenteric networks, hemodynamic data could not be sampled simultaneously in all vascular segments (data collection required up to 30 minutes with 300 individual fields of view). The fact that the network analysis of the effect of hemodilution on spatial heterogeneity disagreed with almost all previous studies based on a random sample of capillaries highlights the important issue of how to obtain representative data to properly investigate flow heterogeneity under a variety of conditions.

It is interesting that the overall CV in the present study was very similar to the 10-minute spatial CV, even though the overall CV included the temporal variability as well. Does this mean that a random-sample strategy at different points in time is as valid as a strategy that samples at a specific point in time? No, because of the redistribution of flow within the network, each value sampled at a different point in time would represent a value from a different state of heterogeneity. The resulting data would be very difficult to interpret. However, there are also problems with the CV calculated at a specific point in time, since it represents only one state of the muscle. A number of values should be calculated to get a better estimate of the true mean spatial heterogeneity for a given situation in a specific muscle.

The network analysis of the mesenteric microvasculature required at least 30 minutes and 300 individual fields of view to complete, i.e., 6 seconds per field, which is slightly less than the 10-second interval used in the present study. From our analysis, we would predict that even a passive network such as the mesentery would show dynamic redistributions of flow over the course of the 30-minute sample. The montage of images may actually represent a series of snapshots of the microvascular network at various stages of flow distribution. The fact that hemodilution had such a profound effect on the hematocrit distribution and the spatial heterogeneity of perfusion of the mesenteric network indicates that passive rheological events do have the potential to cause dynamic redistributions of flow within this microvasculature.

In the present study, an increase in RBC supply to the network was accompanied, in most cases, by a decrease in the spatial heterogeneity of RBC perfusion. This result may be interpreted to indicate that the fractional distribution of RBCs at capillary bifurcations depends not only on the flow distribution, as reported in the literature, but also on the magnitude of the RBC supply in the parent vessel. During periods of low RBC input to the network, RBCs would be found primarily in preferential flow paths through the network, whereas during periods of high RBC input, the distribution would be more uniform among all flow paths.

Would we expect to see the same situation in mammalian muscle? From our previous comparative study between frog and rat, we found a surprisingly close
agreement between the mean values of RBC velocity and supply rate. The difference in lineal density was almost entirely accounted for by differences in RBC size. Furthermore, the heterogeneity of the flow parameters was also very similar. Since the flow and the architecture of capillary networks in rat skeletal muscle appears to be similar to that in frog, we would expect the above conclusions to be valid for mammalian muscle also. However, we do not have enough data as yet to determine how important the precise network geometry is in determining the spatial and temporal heterogeneity. Also, significant arteriolar activity, such as arteriolar vasomotion, which is seen more often in mammalian muscle,15 may easily dominate both the spatial and temporal heterogeneity described in the present study.

**Heterogeneity and Microvascular Exchange**

Spatial heterogeneity in the literature is associated with the efficiency of exchange processes. Do our measurements of spatial heterogeneity accurately reflect the ability of the microvasculature to deliver oxygen and exchange diffusible solutes with the tissue? If exchange occurs only between a capillary and the tissue volume surrounding it, then the measures of spatial heterogeneity used in the present study are likely very important. However, if diffusive exchange occurs among capillaries such that a number of capillary segments are collectively responsible for exchange with a volume of tissue, then the measures of spatial heterogeneity used in the present study and in the literature will be misleading. Ellsworth and colleagues28,29 have presented results indicating that oxygen exchange in resting skeletal muscle occurs among capillaries28 and between arterioles and capillaries.29 Thus, a resting muscle may be able to tolerate what appears to be a very significant degree of spatial heterogeneity, because it is the integrated supply of a group of capillaries that determines the tissue oxygenation, not the supply in each capillary independent of its neighbors. However, if the oxygen demands of the tissue increase, then the degree of diffusive exchange among microvessels will decrease, and spatial heterogeneity will become important. The level of heterogeneity that the tissue was able to tolerate at rest may now result in local regions of ischemia.

In organs that actively regulate their blood supply in response to tissue demands, such as skeletal muscle, one would predict that the heterogeneity of perfusion due to the arteriolar tree can be actively regulated. Although the heterogeneity arising within the capillary network likely cannot be directly regulated, it can be modified as indicated above. Thus, the increase in RBC supply to a capillary network that would occur with an increase in blood flow should reduce the heterogeneity within each network; ie, the distribution of 10-second spatial CV values should shift toward some minimum value. However, even under conditions of high arteriolar supply, not all of the heterogeneity can be removed because of the basic geometric structure of a branching network.

Finally, the vessels that could be recruited were the capillaries with the greatest dynamic variability relative to the input. These vessels were also found to have the lowest RBC velocities. A low RBC velocity indicates that the driving pressure relative to the hemodynamic resistance across these segments must be small. Thus, these capillaries will be the most susceptible to plugging by leukocytes or stiffened RBCs20 at low flow rates. If these vessels are lost, then the ability of the network to reduce its heterogeneity and increase its efficiency for exchange will be impaired, and in fact the heterogeneity will shift toward higher values.

**Conclusions**

The key concepts that can be derived from this analysis are as follows: (1) Capillary networks are a significant source of both spatial and temporal heterogeneity of capillary perfusion. (2) Redistribution of RBC flow occurs within the capillary network, resulting in a wide range of spatial heterogeneity values, although persistent spatial heterogeneity does remain because of the network geometry. (3) In most networks, the spatial heterogeneity decreased as the input of RBCs to the network increased, indicating the importance of the rheological properties of blood in capillary networks.

(4) There are preferential flow paths within each network that follow the dynamics of the arteriole, and there are other pathways or individual segments that have the lowest percentage of the input supply rate when the supply is low but can be recruited to carry more RBCs when the supply to the network increases. (5) These vessels, which can be recruited, have the greatest dynamic variability.

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Heterogeneity of red blood cell perfusion in capillary networks supplied by a single arteriole in resting skeletal muscle.
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