Effect of a maldistribution of microvascular blood flow on capillary O₂ extraction in sepsis

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SEPTIC PATIENTS AND ANIMAL models of sepsis have demonstrated an impaired capacity to increase tissue O₂ extraction (O₂ ER) in skeletal muscle (2, 3, 22) even in the presence of normal or elevated levels of systemic O₂ delivery (1, 21). The apparent paradox of hyperdynamic sepsis is, despite an increase in cardiac output (CO) and a decrease in systemic vascular resistance, O₂ ER actually decreases (20). One proposed explanation is impairment of mitochondrial respiration (24, 28), whereby there is a reduction of O₂ ER because of a decrease in O₂ utilization. An alternative theory proposes an increase in the heterogeneity of capillary flow (29) secondary to a microvascular injury, which results in a maldistribution of O₂ delivery; some capillary beds receive an inadequate supply of O₂, whereas others are oversupplied. Whether the impaired O₂ ER is the result of mitochondrial injury or microvascular dysfunction is uncertain.

Sepsis causes several dysfunctions of the microvascular bed that result in significant disturbances in capillary perfusion in vivo. These include changes in arteriolar diameter (6), an increased number of stopped-flow and high-velocity capillaries (16), and an increase in the heterogeneity of capillary transit time (14) and spatial distribution of perfused capillaries (16). There is no conclusive evidence, however, that these perfusion disturbances cause a maldistribution of O₂ delivery.

Tissue O₂ tension measurements have yielded contradictory results in similar animal models of sepsis, supporting either defective cellular O₂ metabolism (22) or an O₂ transport dysfunction (23). Tissue O₂ tension measurements are difficult to interpret without additional data on the nature of the microvascular perfusion disturbance that may have occurred in these studies; for example, does high tissue PO₂ indicate cell death, mitochondrial injury, increased O₂ delivery, or microvascular arteriole-to-venule convective shunting? To address this limitation, we have developed a spectrophotometric functional imaging system (7, 8) based on intravital video microscopy that provides quantitative in vivo data on functional capillary density and on the convective transport of O₂ at the individual capillary level. Using this technique, we are able to measure the local O₂ saturation (SO₂) gradient across individual capillaries, i.e., from the capillary entrance (arteriolar end) to the capillary exit (venular end).

The goal of our study was to investigate skeletal muscle capillary O₂ transport in a fluid-resuscitated,
normotensive, peritonitis model of sepsis [cecal ligation and perforation (CLP)] in rats and to determine whether the \( O_2 \) transport evidence supported a mitochondrial injury or a maldistribution of \( O_2 \) delivery. We hypothesized that, by studying capillary \( O_2 \) transport in capillaries that maintained normal velocities in the septic animals, we would be able to distinguish between mitochondrial dysfunction and impaired \( O_2 \) delivery. These capillaries retain normal hemodynamic parameters and provide evidence of local \( O_2 \) gradients in the context of the heterogeneous microvascular injury. If there was impaired mitochondrial function then there should be a decrease in \( O_2 \) consumption, a reduced fall in the \( S_O_2 \) gradient from the arteriolar to venular end of these capillaries, and a decrease in the \( O_2 \) \( ER \) ratio across these capillaries compared with sham animals. Conversely, if a microvascular dysfunction were affecting \( O_2 \) delivery (and mitochondrial function was not impaired), then there should be evidence of a greater fall in the \( S_O_2 \) gradient along these capillaries and an increased \( O_2 \) \( ER \) ratio, since the remaining functional capillaries must supply a larger volume of tissue because of the loss of functional capillary density during sepsis.

We report for the first time that, 24 h after induction of peritonitis, venular-end \( S_O_2 \) levels decreased in normally perfused skeletal muscle capillaries, leading to a threefold increase in the capillary \( O_2 \) \( ER \) ratio. There was no change in capillary arteriolar-end \( S_O_2 \) level between septic and sham animals. This decrease in venular-end capillary \( S_O_2 \) was strongly related to an increase in the number of stopped-flow capillaries. Because the tissues that received capillary blood flow were clearly capable of extracting \( O_2 \), functional mitochondrial injury does not appear to have occurred to any significant extent, supporting the hypothesis that, in early stage sepsis, \( O_2 \) transport is compromised by a maldistribution of \( O_2 \) delivery. This is manifested by an oversupply of \( O_2 \) to some regions and an undersupply of \( O_2 \) to others, approaching the critical \( O_2 \) \( ER \) point.

**METHODS**

**Animal preparation.** After approval from the University of Western Ontario’s Animal Care and Use Committee, male Sprague-Dawley rats (100–150 g) were acclimatized within our animal storage facility. After 1 wk, providing their weight was between 150 and 200 g, animals were randomized (sealed envelope) to CLP or sham laparotomy groups. They underwent preparatory surgery using inhalation anesthesia [halothane (1–1.5%) entrained in an \( O_2 \) and \( N_2 \) mixture yielding an inspired \( O_2 \) level of 30%]. The inspired \( O_2 \) concentration was monitored with a calibrated fuel cell \( O_2 \) analyzer (Critikon Oxychek). Under direct vision, a 0.28-mm Silastic catheter (Dow Corning) was advanced in the superior venca cava via the right internal jugular vein. An arterial catheter (polyethylene-50; Clay Adams) and a fine thermocouple probe (IT-21; Physitemp, Clifton, NJ) were advanced in the ascending aorta via the left carotid artery. The catheters were tunneled subcutaneously to the interscapular region and attached to a swivel harness (Harvard Apparatus, St. Laurent, Quebec, Canada).

Adequate fluid resuscitation in both groups was maintained by administering 2 ml/100 g of 0.9% saline during the operative procedure through the jugular vein catheter (~1 h duration). The animals then underwent abdominal surgery through a midline incision. The CLP procedure consisted of identification, exteriorization, devascularization, and ligation of the cecum just distal to the ileocecal valve to ensure bowel continuity. The cecum was punctured two times with an 18-gauge needle and was returned to the peritoneal cavity. The sham procedure consisted of identification, exteriorization, and then replacement of the cecum within the peritoneal cavity. The incision in both cases was closed in two layers. At the end of the surgical procedure, the rats were allowed to recover from anesthesia with free access to rat chow and water. After surgery, 0.9% saline with fentanyl (1 \( \mu g/ml \)) was continuously infused (intravenously) at a rate of 1 ml/100 g. Arterial line patency was maintained by continuous infusion through a flush valve that delivered ~0.5 ml/h of heparinized saline.

**Experimental protocol.** After the CLP or sham procedure was performed (2 h), animals were reviewed for clinical signs of the development of sepsis. All CLP animals were lethargic; showed lack of interest in their environment, food, and hygiene; displayed piloerection; and had crusty exudates around their eyes. In contrast, the sham animals were healthy, moving freely, and eating. After this, CO was measured by thermodilution using a 200-\( \mu l \) 0.9% saline bolus at room temperature injected in the venous catheter; the thermocouple output was monitored by a CO computer (Cardiotherm 500; Columbus Instruments, Columbus, OH). Mean arterial blood pressure and heart rate were recorded via the left carotid line (disposable Baxter flow transducers; Digi-Med). Respiratory rate was counted by observing chest movements; temperature was recorded from the thermistor probe.

After discontinuation of the fentanyl infusion, the animals were reanesthetized with isoflurane (1.5% sham, 0.9% CLP) in a mixture of 30% \( O_2 \) (remainder \( N_2 \)) and were allowed to breathe spontaneously via a Bain circuit. The extensor digitorum longus (EDL) muscle of the right hindlimb was then prepared for intravital microscopy. The EDL muscle was exposed as described by Tyml and Budreau (26). Following the procedure of Sullivan and Pittman (25) for muscle transillumination, the distal tendon was identified, and a ligature was placed around it before excision. The animal was then transferred to the microscope stage and placed in the right lateral decubitus position. The muscle was reflected along its length, perpendicular to the limb, on the microscope stage, moistened with warm saline, and immediately covered with Saran Wrap and a glass cover slip. The core temperature of the animal was monitored via the central thermistor and was maintained at 37.5–38°C with a radiant heat lamp. On the stage, the muscle was covered with a transparent film of Saran Wrap (Dow Corning) to prevent desiccation and to isolate the tissue from \( O_2 \) in the surrounding atmosphere. After transfer to the microscope stage, the preparation was allowed to stabilize for 30 min before any data collection.

To minimize the consequences of blood loss in such small animals, a limited amount of arterial blood (100 \( \mu l \)) was collected at 24 h to measure hemoglobin (OSM2 Hemoximeter). Mean arterial pressure, heart rate, and temperature were monitored continuously throughout the experiment. At the end of the experiment, arterial \( P_{O_2} \) was measured (ABL3-2 Blood Gas Analyzer), and the animals were killed with intravenous pentobarbital sodium and underwent post-mortem examination.
In vivo red blood cell oxygenation and red blood cell dynamics: functional imaging system. The theoretical basis for the spectrophotometric functional imaging system used in this study is that the hemoglobin SO$_2$ level of an individual red blood cell (RBC) is linearly related to the ratio of the cell’s optical density (OD) at 431 nm divided by its OD at 420 nm (11). In these experiments, RBC oxygenation and RBC dynamic information was obtained in vivo using a dual video densitometric image analysis system, as described elsewhere (7, 8). In brief, the EDL preparation was transilluminated (75W Xenon lamp) and observed using a Zeiss ACM microscope (Leitz) at ×25/0.35 (Fluotar). Two silicon-intensified target cameras (model 66, Dage-MTI), fitted separately with 420- and 431-nm interference filters, were configured to provide in-focus superimposable images. In this way, each camera viewed the identical microscope image at one of the wavelengths required to measure hemoglobin SO$_2$. Images were captured on S-VHS videotape via two high-resolution closed-circuit video systems (video monitor, model WV-5410, and video cassette recorder, model AG-7300, Panasonic) for later off-line analysis. An audio cue (Telecom Research, Burlington, Ontario, Canada) was simultaneously placed on both tapes to provide precise timing information. The system was calibrated in vivo against 0 and 100% SO$_2$ levels as previously described (8).

An interactive microcomputer system with graphics display was used in the off-line analysis of the 420- and 431-nm videotapes, as previously described (7, 8). In brief, light intensity information was obtained from each capillary selected for analysis using a microcomputer with a 12-bit analog-to-digital (A/D) converter (Compaq Deskpro 386/25, A/D board, model RTI-815-A; Analog Devices) to digitize the output of a sample window aligned with the capillary (model 321; Colorado Video Analyzer). A 100-pixel segment of capillary (i.e., 93 μm long) was sampled for 60 s at the video-framing rate of 30 Hz (i.e., 1,801 light intensity values at each spatial location along the capillary). The same time sequence was obtained from both videotapes using the audio cue to synchronize the two separate light intensity samples. The computer used this light intensity data to calculate RBC SO$_2$ and dynamics (velocity, lineal density, and supply rate) for each capillary over the 60-s sample period.

SO$_2$ values were calculated from the ratio of OD values for RBCs obtained at the two wavelengths. OD data were calculated using Beer’s Law, OD = log(I$_0$/I), where I$_0$ is the intensity of the incident light reaching the RBC and I is the intensity of the light that has passed through the RBC. I$_0$ was determined from the light intensity of plasma gaps between the RBCs and I from the light intensity of the RBCs. The SO$_2$ values were calculated from mean RBC OD values for every 10-s interval. RBC velocity (V; mm/s) was calculated frame-by-frame using a spatial correlation technique (i.e., displacement of the light intensity profile along the capillary segment from one video frame to the next, which yields the maximum correlation coefficient between the two profiles). RBC lineal density (LD; RBC/mm) was calculated by detecting the number of RBCs in the capillary segment in each video frame (both velocity and lineal density from 420-nm data). RBCs were detected based on the OD data. Any object with an OD above a threshold level for a RBC that was longer than 5 μm in length was considered to be either a single RBC or a train of overlapping or abutting cells. The number of RBCs in a train was determined by the shape of the OD profile (8). Supply rate (SR; RBC/s) was calculated as the product of the velocity and lineal density. Each 60-s sample yielded 1,800 velocity, lineal density, and supply rate values.

Sampled light intensity data were also graphically displayed as an image of the 100 pixels along the capillary sampled over 1,800 video frames, i.e., as a space-time image. A space-time image shows changing light intensity patterns within the sample window over time that depict the movement of individual RBCs through a sampled capillary segment. Dark banding patterns trace the movement of RBCs over time, whereas white spaces indicate plasma gaps (Fig. 1). Although the space-time image was primarily used to
verify the quality of the light intensity sample, it was also used to provide a simple visual interpretation of the RBC flow through the capillary. The slope of a dark band yields the RBC velocity; the number of bands intersecting a line perpendicular to the x-axis (time) yields the lineal density; the number of bands intersecting a line perpendicular to the y-axis (position) yields the supply rate. The quality of the data obtained from the automated analysis routines was verified routinely by direct comparison with the visual interpretation of the space-time image.

Analysis strategy for quantifying O2 delivery, utilization, and O2 extraction ratio in the microcirculation. In whole organ studies of O2 transport, O2 ER is computed from the total O2 consumption or utilization (\(V_O2\)) and the total O2 delivery to the organ (\(QO2\))

\[
O2\ ER = \frac{V_O2}{QO2} \tag{1}
\]

In most cases, \(V_O2\) is computed from the product of the arterial-to-venous \(SO2\) gradient (\(SAO2 - SVO2\)) times the blood flow to the organ (\(Qh\)), and \(QO2\) is computed from the product of the arterial \(SO2\) times the blood flow

\[
O2\ ER = \frac{(SAO2 - SVO2) \times Qh}{(SO2 \times Qh) / SAO2} = \frac{(SAO2 - SVO2)}{SO2} \tag{2}
\]

Thus whole organ O2 ER can be estimated from the measured \(SO2\) levels. In a similar manner, one can calculate microvascular O2 ER (\(ER_m\)) using the arteriolar (\(SAO2\))-to-venular (\(SVO2\)) saturation gradient

\[
O2\ ER_m = \frac{(SAO2 - SVO2)}{SO2} \tag{3}
\]

To calculate a true capillary O2 ER ratio on the basis of differing capillary \(SO2\) gradients, however, one must account for the differences in flow between the vessels. This is because the situation at the capillary level is complicated by the heterogeneity in RBC supply rate among capillaries in the same muscle and even within the same microvascular unit (9). As well, from in vivo measurements in healthy hamster retractor muscle (12), we also know that there is considerable heterogeneity in \(SAO2\) and in the arteriovenous saturation gradient.

The \(O2\) flow rate in individual capillaries (\(qO2\)) is defined as

\[
qO2 = \kappa \times \ SR \times SO2 \tag{4}
\]

where \(\kappa\) is the total \(O2\)-carrying capacity of a single RBC at 100% saturation (0.0362 pl \(O2/RBC\)), \(SR\) is the RBC supply rate (RBC/min) in individual capillaries, and \(SO2\) is the \(SO2\) in individual capillaries. One would anticipate that there would be considerable variation in \(O2\) flow rate among individual capillaries at both the arteriolar \((qO2_{arteriolar})\) and venous \((qO2_{venular})\) ends of the capillary bed because of the variability of supply rate and \(SO2\) among capillaries.

To account for the heterogeneity in \(O2\) transport parameters at the capillary level, the O2 ER ratio in the capillary bed (\(O2\ ER_c\)) can best be calculated from the total O2 extracted from all capillaries divided by the total O2 delivered to these capillaries

\[
O2\ ER_c = \frac{[\Sigma(qO2_{arteriolar}) - \Sigma(qO2_{venular})]}{\Sigma(qO2_{arteriolar})} \tag{5}
\]

or

\[
O2\ ER_c = \frac{[\kappa \times \Sigma(SR \times SO2_{arteriolar}) - \kappa \times \Sigma(SR \times SO2_{venular})]}{\Sigma(SR \times SO2_{arteriolar})} \tag{6}
\]

Canceling the \(O2\) content term and simplifying

\[
O2\ ER_c = \frac{[\Sigma(SR \times SO2_{arteriolar}) - \Sigma(SR \times SO2_{venular})]}{\Sigma(SR \times SO2_{arteriolar})} \tag{7}
\]

Thus the capillary O2 ER ratio is computed from the RBC supply rate weighted \(SO2\) values in individual capillaries. These weighted values also give us the best estimate of the mean \(SO2\) levels for the population of the capillaries sampled, since they take into account the heterogeneity of capillary perfusion. In this study, we calculated capillary O2 extraction for both sham and CLP animals in normally perfused capillaries (\(O2\ ER_{cn}\)) on the basis of capillary arteriolar-end and venular-end flow-weighted \(SO2\) levels.

Video recording protocol. To investigate O2 delivery and O2 utilization at the capillary level, \(O2\) transport levels (\(O2\)) must be quantified at the entrance (\(Qa\)) and exit (\(Qv\)) of the capillary. To accomplish this, video recordings were made in three to four randomly chosen capillary networks per animal. Five-minute continuous recordings were made at the arteriolar entrance of each capillary followed by an additional 5-min recording at the venular end. Video recording began 30 min after the animal was placed on the microscope stage and continued for ~3 h.

Functional capillary density analysis. Functional capillary density in the EDL muscle was determined using the method described by Lam et al. (16). A transparency with a single horizontal reference line drawn perpendicular to the muscle fibers was placed over the monitor screen, and capillaries with clearly distinguishable RBCs were counted. The depth of the tissue was ~20 \(\mu\)m.

Capillaries that intersected the reference line were classified over a 30-s time interval as being either 1) continuous (cont), 2) intermittent (int), in which case capillary flow either came to arrest at least once or flow reversed during the observation period, or 3) stopped (stop). Continuously perfused capillaries were further subdivided as being either normal or fast flow, where the former was defined for resting muscle as having RBC velocities within a range of 20–325 \(\mu\)m/s (10). Vessels exhibiting vasomotion, or rhythmic flow variations, were included as normal. Only capillaries containing RBCs were counted. Empty capillaries were difficult to identify and were considered to be nonfunctional with respect to RBC supply rate. Functional capillary density information (for example, \(CD_{stop}\)) was calculated as the number of capillaries per millimeter test line (caps/mm) and was expressed as a percentage of the total capillary density (%\(CD_{stop}\)).

Statistical analysis. All values are reported as means \(\pm SE\) unless otherwise stated. For all tests of significance, \(P\) values <0.05 were considered statistically significant. All analysis of cardiorespiratory variables was performed using SAS for Windows, version 6.08 (SAS User’s Guide: Statistics; SAS Institute, Cary, NC). The comparison of continuous variables was performed using Student’s \(t\)-test under the assumption of normality. Regression analysis (version 2.0; Sigma Stat) was used to test the significance of the relationship between flow-weighted \(SVO2\) and the microvascular \(O2\) ER ratio against stopped-flow capillaries.

RESULTS

Animals. Twelve rats were randomized to either the sham (n = 6, 169 \(\pm\) 7.9 g) or the CLP group (n = 6, 168 \(\pm\) 6.6 g). Postmortem examination revealed that a septic focus had developed within the peritoneum of all
CLP animals. Sham animals showed no signs of peritonitis.

Cardiorespiratory parameters. At 24 h postsham laparotomy or CLP, there were no significant differences (CLP vs. sham) in mean arterial pressure (108 ± 3.8 vs. 109 ± 3.8 mmHg), CO (260 ± 55 vs. 236 ± 45 ml/min), respiratory rate (95.8 ± 4.2 vs. 95.3 ± 4 breaths/min), or hemoglobin (10.2 ± 1.3 vs. 11.4 ± 1.2 g/100 g). Heart rate increased in CLP animals (450 ± 15 vs. 403 ± 15.1 beats/min, \( P = 0.008 \)) and temperature decreased in CLP animals (36.1 ± 0.1 vs. 37 ± 0.2°C, \( P < 0.05 \)) compared with sham. There was no difference in arterial PO2 (CLP, 140.6 ± 36.1 vs. sham, 130 ± 24.7 mmHg) at the end of the experiment.

Intravital video microscopy of EDL. In total, 60 fields of view (0.2 × 0.2 mm) of the EDL microvasculature were examined, 28 in sham and 32 in CLP animals. The total numbers of capillaries (continuous, intermittent, and stopped) identified were 445 (sham 173 capillaries, CLP 272 capillaries). Of these, 40 capillaries were sampled at both the arteriolar and venular ends of the capillary bed for SO2 and hemodynamic parameters, 17 in sham and 23 in CLP. The capillary length between sample sites was 0.34 ± 0.48 mm in sham and 0.40 ± 0.41 mm in CLP animals.

Functional capillary density. Figure 2 expresses functional capillary density as a percentage of total capillary density (%CDtotal). In CLP animals, there were significant decreases in continuous flow (CDcont, 62 ± 8.6 vs. 84.1 ± 11.0%, \( P < 0.05 \)) and normal flow (CDnorm, 28 ± 11.5 vs. 60 ± 11%, \( P < 0.05 \)) and a significant increase in stopped flow (CDstop, 38 ± 4.5 vs. 10 ± 3.7%, \( P < 0.05 \)) compared with sham animals. No significant changes in the percentage of capillaries with either intermittent flow or fast flow were detected between the two groups; however, the proportion of fast-flow to normal-flow capillaries increased 3.4-fold in CLP animals (median value of 0.7 in sham vs. median value of 2.4 in CLP, \( P < 0.05 \)) compared with sham animals.

Capillary hemodynamics, SO2, and O2 ERcn. The measured SO2 values at the arteriolar end of the capillaries were 65.8 ± 11.6% in CLP vs. 67.7 ± 7.1% in sham and at the venular end of capillaries 38.2 ± 21.8% in CLP vs. 56.7 ± 21.0% in sham (\( P < 0.05 \)). Average hemodynamic data for all animals is shown in Fig. 3. There were no significant differences in mean values of RBC velocity, lineal density, or supply rate in normally perfused capillaries between sham and CLP animals. Flow-weighted SO2 values (Fig. 4) were similar at the arteriolar end of capillaries (66.0 ± 11.3% in CLP vs. 67.6 ± 7.6% in sham) but were significantly decreased at the venular end in CLP animals (38.5 ± 21.2% in CLP vs. 58.4 ± 19.8% in sham, \( P < 0.05 \)). In CLP animals, flow-weighted capillary O2 ER (Fig. 4) was found to have increased threefold in normally

Fig. 3. Average hemodynamic parameters. RBC velocity (V), lineal density (LD), and supply rate (SR) are shown for normally perfused capillaries (RBC velocity: 20–325 μm/s). No differences in these parameters were detected.

Fig. 4. Flow-weighted O2 saturation (SO2) values and capillary O2 extraction ratio (O2 ERcn). To account for the heterogeneity of RBC supply rates in individual capillaries, flow-weighted SO2 values were calculated at the entrance [arteriolar-end [SO2(ca)]] and exit (venular-end [SO2(cv)]) of all capillaries used in the study. Although there was no change in SO2cv values, there was a significant decrease in SO2ca levels in CLP animals (\( P < 0.05 \)) relative to sham. Based on this decrease in SO2, the calculated O2 ERcn was found to increase 3-fold (\( P < 0.05 \)) in normally perfused capillaries (RBC velocity: 20–325 μm/s).
perfusion capillaries relative to sham animals (O₂ ER_{cn} 0.44 ± 0.23 in CLP vs. 0.15 ± 0.22 in sham, P < 0.05).

Relationship between capillary venular-end SO₂, O₂ ER_{cn}, and CD_{stop}. As shown in the scatter plots of capillary venular-end SO₂ and O₂ ER_{cn} vs. %CD_{stop} (Fig. 5, A and B, respectively), as %CD_{stop} increased in CLP animals, there was a linear decrease in the flow-weighted venous-end SO₂ of normally perfused capillaries (regression analysis, P < 0.05) and a linear increase in the capillary O₂ extraction ratio (regression analysis, P < 0.05). In the sham group, no relationship existed between these parameters.

**DISCUSSION**

In this 24-h animal study, we demonstrated that normotensive sepsis caused a maldistribution of blood flow in the EDL microcirculation. This was manifested in CLP animals by an increase in the percentage of stopped-flow capillaries and an increase in the proportion of fast-flow to normal-flow capillaries. Although normally perfused capillaries had no detectable change in either RBC dynamics or RBC SO₂ levels at the arteriolar end of capillaries, there was a significant decrease in the flow-weighted RBC SO₂ level at the venular end in CLP animals compared with sham animals. The resulting increase in O₂ ER from these capillaries was directly related to the extent of stopped flow in the skeletal muscle of CLP animals and indicated that O₂ transport had been compromised by a maldistribution of O₂ delivery.

**Functional imaging of EDL capillaries.** The advantage of our spectrophotometric intravital video microscopy system is its ability to measure both RBC SO₂ levels and RBC dynamics in individual capillaries and hence calculate local O₂ delivery and extraction. In combination with regional information on changes in capillary density, this O₂ transport data enables us to draw conclusions about the mechanism(s) responsible for changes in local O₂ extraction or local O₂ levels. For example, we can determine whether a measured change in venular-end capillary SO₂ is the result of a change in capillary entrance O₂ levels, capillary hemodynamics, tissue O₂ consumption, or capillary density. Other approaches for studying O₂ transport, such as tissue Po₂ measurements (22, 23) or NADH fluorescence (15), cannot be used to draw mechanistic conclusions without additional microvascular O₂ transport data. However, tissue Po₂ and NADH fluorescence measurements have the advantage of being able to establish if regions of the tissue are hypoxic, which we cannot determine with our system. Ideally, one would like to be able to measure all of these O₂ transport parameters to determine whether an O₂ transport dysfunction has directly contributed to the pathology of a disease. In the context of this study, we can determine with our functional imaging system whether the loss of perfused capillaries in sepsis was compensated for by another source of O₂ (e.g., O₂ from fast-flow capillaries or redistribution of O₂ delivery by arterioles), but we cannot determine if the loss of perfused capillaries has caused any of the tissue to become anoxic.

Other limitations of the current system were discussed previously (8). Of importance for this study, capillary velocities were limited to <400 μm/s and lineal density to <110 RBC/mm. Only capillaries in the normal range of velocities were analyzed, and thus the O₂ transport results presented here are only representative of this population of vessels and should not be assumed to represent O₂ transport across the entire capillary bed.

**Animal model.** The 24-h fluid-resuscitated peritonitis model of sepsis used in this study has been validated (17). Consistent with the development of sepsis, CLP rats showed signs of intraperitoneal infection at postmortem and had an unhealthy body habitus, a reduced body temperature, and an elevated heart rate. CLP animals were also normotensive during the exper-
iment, thus satisfying criteria for the clinical diagnosis of chronic resuscitated sepsis (4). Our CLP model closely approximates the clinical situation in fluid-resuscitated septic patients who maintain normal blood pressure yet progress to remote organ failure.

Differences in capillary perfusion. One of the most striking features of the CLP animals, and consistent with experimental models of sepsis (16, 19), was the change in EDL capillary perfusion (summarized in Fig. 2). In sham animals, all areas of the muscle had a relatively uniform distribution of capillaries perfused with RBCs in an apparently steady pattern from arteriole to venule; there were very few vessels with stopped RBCs or with intermittent perfusion. This pattern of spatial and temporal heterogeneity is characteristic of normal capillary perfusion (10, 9). However, in the septic animals, there were numerous capillaries throughout the muscle that had stopped RBCs, and the population of continuously perfused vessels showed a much greater proportion of capillaries with high velocities. Capillaries with intermittent flow were much more evident in sepsis, even though their numbers did not increase, as many of these vessels exhibited large low-frequency flow reversals, i.e., oscillations about zero velocity (Fig. 1C). Flow reversals of this type were rarely seen in sham animals.

O$_2$ transport in the microcirculation. Although significant changes in perfusion patterns occurred in the CLP animals, there were no significant differences in capillary hemodynamics (RBC velocity, lineal density, and supply rate) for the perfused capillaries in the normal range compared with sham animals. Mean flow-weighted SO$_2$ levels at the entrance to the capillary bed were unchanged in CLP animals (66 ± 11% for CLP animals and 68 ± 7% for sham), indicating that O$_2$ ER along the arteriolar tree in CLP animals was similar to sham animals. These capillary bed entrance SO$_2$ levels were consistent with data from the hamster retractor muscle of 61 ± 11% (12). The slightly higher values in our sham animals compared with the hamster data were expected, since the animals in our study were breathing 30% O$_2$ (to avoid the potential problem of low arterial SO$_2$ levels in septic animals), whereas the hamsters were breathing room air (12). Breathing 30% O$_2$ likely increased SO$_2$ levels throughout the microvasculature by ~5%, which would correspond to an approximate increase in PO$_2$ levels of only 4 mmHg, insufficient to have a significant impact on tissue oxygenation.

Important findings from this study were related to the effect of sepsis on the SO$_2$ levels at the venular end of capillaries with normal velocities and on the calculated O$_2$ ER$_{cn}$ from these vessels (Fig. 4). If sepsis had caused a mitochondrial dysfunction by 24 h, leading to decreased O$_2$ utilization, then the venular-end SO$_2$ values in this group of capillaries should have been higher than in similar capillaries in the sham group. In CLP animals, however, the mean flow-weighted SO$_2$ levels at the exit of these capillaries were lower at 38 ± 21% compared with 58 ± 20% in sham animals. This resulted in a threefold increase in O$_2$ ER$_{cn}$ (Fig. 4), 0.44 in CLP compared with 0.15 in the sham animals. Factors that might have been expected to affect the O$_2$ ER$_{cn}$, such as RBC velocity, lineal density, and supply rate, entrance SO$_2$ levels, or capillary path length from arteriole to venule, were not significantly different between the groups in the capillaries sampled and thus were not the reason for the increased O$_2$ extraction.

Relationship between venular-end SO$_2$ and the percentage of stopped-flow capillaries. If the increased O$_2$ extraction in the CLP animals was the result of the increase in stopped-flow vessels, then there should be a relationship between the percentage of stopped-flow capillaries and both the venular-end SO$_2$ and O$_2$ ER$_{cn}$. Figure 5, A and B, shows that linear relationships existed between these parameters in the CLP group but not in the sham animals. As the percentage of stopped-flow vessels increased from 20 to 45%, the venous-end SO$_2$ decreased to below 20%, and the O$_2$ ER$_{cn}$ increased to ~75%, i.e., five times greater than the mean O$_2$ ER$_{cn}$ for sham animals. Assuming that O$_2$ consumption did not change with sepsis, a fivefold increase in O$_2$ extraction corresponds to a fivefold increase in the volume of tissue supplied with O$_2$ from these capillaries, i.e., an increase in diffusion distance of $\sqrt{5}$ or 2.2 times, assuming a cylindrical geometry. An approximate twofold increase in diffusion distance is consistent with the loss of ~50% of perfused capillaries. From these results, we conclude that the impact on tissue oxygenation of an increase in stopped-flow capillaries was not compensated by O$_2$ delivery from fast-flow capillaries or from arterioles. The increased O$_2$ extraction does indicate a functional increase in O$_2$ diffusion distance consistent with the loss of perfused capillaries.

Impact on tissue oxygenation. Without direct measurements of tissue PO$_2$ or the functional status of mitochondria (e.g., NADH fluorescence), we cannot determine if tissue hypoxia did occur in these animals. However, based on our capillary SO$_2$ measurements, we can estimate that venular-end capillary PO$_2$ fell from 42 mmHg in sham animals to as low as 22 mmHg in septic animals (based on SO$_2$ = 58% in sham and SO$_2$ = 20% in CLP and Hill’s equation with p50 = 37 mmHg and Hill coefficient = 2.7). If, as we have concluded, the fall in capillary SO$_2$ in sepsis was a result of an increase in diffusion distance, then the fall in PO$_2$ in the tissue surrounding these capillaries must be greater than the 20-mmHg fall in capillary PO$_2$. Was this sufficient to contribute directly to the pathology of sepsis? Without additional data on local tissue PO$_2$, mitochondrial function, or cell viability, we cannot answer this question. However, we can speculate that, with a capillary PO$_2$ of 22 mmHg and double the normal diffusion distance, the tissue supplied by these vessels was vulnerable to any further decrease in perfused capillary density, decrease in O$_2$ delivery, or increase in tissue O$_2$ requirements. Our results support the hypothesis that the capacity of the tissue to increase tissue O$_2$ ER has been impaired because of a maldistribution of O$_2$ delivery and not because of an
inability to utilize O2. Furthermore, our evidence indicates that the degree of stopped capillary flow has physiological significance and may be considered as one index of the microvascular dysfunction.

Maldistribution of blood flow. From the change in the proportion of normal flow to fast flow in the CLP animals, we can calculate that 52% of the perfused capillaries carried fast flow in CLP animals compared with only 27% in the sham animals. The increased proportion of high-flow capillaries may not signify a substantial increase in muscle blood flow but rather may indicate that blood flow was diverted from stopped-flow capillaries and directed to fast-flow vessels. Although at present we have little direct data on these high-speed vessels, they likely function as convective arterial-venous shunts.

We speculate that this maldistribution of microvascular blood flow leads to a mismatching of O2 supply and demand, analogous to the mismatching of ventilation and perfusion in the lung in some disease states (18) and consistent with a recent model of the effect of heterogeneity of O2 delivery on O2 ER (29). The consequence of this flow maldistribution is that some local regions of tissue are oversupplied with O2, whereas other local regions are undersupplied, most notably where capillaries are no longer functional.

Impaired microvascular response. Why did the arteriolar tree not respond to a fall in So2 levels in the normal-velocity capillaries by diverting excess flow to these vessels to increase their O2 supply and thus compensate for the loss of perfused capillaries? The presence of low So2 levels in capillaries suggests that the microvasculature was unable to properly redistribute O2 delivery in response to the local metabolic requirements of the tissue, thus failing to correct for the maldistribution of blood flow and O2 supply. This conclusion is supported by recent findings showing that both the communication between capillaries and arterioles (27) and the vasoreactivity of arterioles are impaired in this 24-h peritonitis model of sepsis (13). We can speculate that, if the arterioles were unable to redistribute flow to restore capillaries with low So2 levels, then the microvasculature would also be unable to respond adequately to increased metabolic requirements. There is evidence that the hyperemic response after muscle stimulation was diminished in the same 24-h CLP model of sepsis in the rat (16). Furthermore, these results imply that increasing O2 delivery to supranormal levels may not improve tissue oxygenation if the increased O2 supply cannot be properly distributed. Perhaps these results offer insight into why clinical studies investigating the treatment of septic patients with supranormal levels of O2 delivery were unable to demonstrate improvement in patient outcomes (5).

The key findings of this study were 1) that within the EDL, skeletal muscle sepsis caused a decrease in flow-weighted So2 saturation levels at the venular ends of normally flowing capillaries, 2) that sepsis caused no change in flow-weighted So2 saturation levels at the arteriolar end of the same capillaries, and 3) that the extent of O2 ER from these capillaries was directly related to the degree of stopped flow within the capillary bed. These data demonstrate that the EDL muscle was consuming O2 24–27 h after the CLP injury and, based on the evidence of increased O2 extraction, impaired mitochondrial function does not appear to have occurred to any significant extent in this peritonitis model of sepsis. The most likely cause for the increased flux of O2 from these capillaries was the increased volume of tissue that each normally perfused capillary had to supply to compensate for the loss of surface area for gas exchange, given the threefold increase in stopped-flow capillaries. This study supports the hypothesis that microvascular injury resulting in a maldistribution of O2 delivery precedes any significant bioenergetic failure that may develop in sepsis. The O2 transport dysfunction in sepsis is not characterized by a uniform decrease in O2 delivery but rather by a patchy and disperse distribution of regions of the tissue that are undersupplied with O2. Both the loss of surface area for O2 exchange and the apparent inability of the microvasculature to redistribute O2 delivery likely play an important role in the impaired capacity to increase tissue O2 ER in skeletal muscle in sepsis. The implication from this study is that early treatment aimed at restoring uniform distribution of O2 within the tissue, before reaching critical O2 ER levels, may lead to improved outcomes for septic patients.

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