Effect of Decreased Oxygen Supply on Skeletal Muscle Oxygenation and Oxygen Consumption during Sepsis: Role of Heterogeneous Capillary Spacing and Blood Flow

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RUNNING HEADLINE: Oxygen delivery and uptake in skeletal muscle during sepsis

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ABSTRACT

One of the main aspects of the initial phase of the septic inflammatory response to a bacterial infection is abnormal microvascular perfusion, including decreased functional capillary density (FCD) and increased blood flow heterogeneity. On the other hand, one of the most important phenomena observed in the later stages of sepsis is an increased dependence of tissue O$_2$ utilization on the convective O$_2$ supply. This “pathological supply dependency” is associated with organ failure and poor clinical outcomes. Here, a detailed theoretical model of capillary-tissue O$_2$ transport during sepsis is used to examine the origins of abnormal supply dependency. Using three-dimensional arrays of capillaries with heterogeneous spacing and blood flow, steady-state O$_2$ transport is simulated numerically during reductions in the O$_2$ supply. Increased supply dependency is shown to occur in sepsis for both hypoxic hypoxia (decreased hemoglobin O$_2$ saturation) and stagnant hypoxia (decreased blood flow). For stagnant hypoxia, it is found that a reduction in FCD with decreasing blood flow is necessary to obtain the observed increase in supply dependency. Our results imply that supply dependency observed under normal conditions does not have its origin at the level of individual capillaries. However, in sepsis diffusion limitation and shunting of O$_2$ by individual capillaries occur to a degree dependent on the heterogeneity of septic injury and the arrangement of capillary networks. Thus, heterogeneous stoppage of individual capillaries is a likely factor in pathological supply dependency.

KEYWORDS: inflammation, computational model, microcirculation, supply dependency, functional shunting
INTRODUCTION

Sepsis, in which a local bacterial infection produces a systemic inflammatory response, is a clinical disease that often leads to multiple organ failure and death (28). Despite some recent advances in the treatment of sepsis (6, 35), it remains one of the leading causes of mortality and many of its basic mechanisms are not fully understood (5, 13, 20, 26). One of the most well known features of sepsis (in both humans and animal models) is its effect on the microcirculation, including increased capillary stopped flow and decreased functional capillary density (FCD) (4, 14, 25), increased blood flow heterogeneity, and a loss of flow regulation resulting in blood flow maldistribution (14, 33, 41). Severe sepsis often results in a decreased ability of tissues to extract oxygen from the blood (12, 37, 39). Although the exact mechanism is not known, it has been suggested that decreased O\textsubscript{2} extraction in sepsis is related to the microcirculatory disturbances, e.g., to increased heterogeneity of capillary spacing and blood flow and the resulting increased heterogeneity of local O\textsubscript{2} supply (21, 22, 43, 44).

Based on experimental measurements of FCD, capillary hemodynamics, and oxyhemoglobin saturation (So\textsubscript{2}) in rat skeletal muscle (14), we have previously constructed a computational model to study the effect of changes in capillary O\textsubscript{2} supply parameters on tissue oxygenation and O\textsubscript{2} consumption under normal and sepsis conditions (16). Since stoppages of individual capillaries rather than entire networks are observed in this muscle during sepsis (14), the model uses a relatively large, three-dimensional (3D) array of parallel capillaries to simulate O\textsubscript{2} transport for normal (or control) and severe sepsis conditions. The key feature of the model is
that it allows study of steady-state O₂ transport under the conditions of heterogeneous capillary spacing and blood flow observed during sepsis (14), including possibly important effects such as diffusive interactions between capillaries, decreased O₂ consumption due to localized decreases in tissue oxygenation, and diffusion limitation of O₂ transport (31, 32). For measured baseline O₂ supply conditions, previous simulation results showed that the intrinsic (or maximum) O₂ consumption rate increases in sepsis and that tissue oxygenation decreases and becomes more heterogeneous, and that these effects increase with the degree and heterogeneity of capillary flow disturbances. Simulations also showed that capillaries with relatively fast flow, which increase in number in sepsis, act only partly as shunts and play a significant role in O₂ delivery. Although levels of tissue O₂ partial pressure (Po₂) low enough to decrease O₂ consumption (“dysoxia”) were not found, results indicated that in sepsis tissue would be more susceptible to dysoxia if the overall O₂ supply were decreased. This suggested an additional area of investigation, since cardiac output and respiratory function can both decrease in severe sepsis (3).

One of the main characteristics of advanced or late stage sepsis is a decreased ability of tissues to extract oxygen from the blood (7). In healthy individuals, O₂ extraction (or total O₂ consumption for a given tissue volume, \( \dot{V}_{O_2} \)) is essentially independent of the O₂ supply rate (\( \dot{Q}_{O_2} \)) down to a critical \( \dot{Q}_{O_2, crit} \), below which \( \dot{V}_{O_2} \) begins to fall. That is, above the critical O₂ supply rate (\( \dot{Q}_{O_2, crit} \)) there is an “aerobic plateau,” and below, \( \dot{V}_{O_2} \) is “supply dependent.” Since the tissue is unable to extract 100% of the O₂ supply, the measured slope of \( \dot{V}_{O_2} \) vs. \( \dot{Q}_{O_2} \) during O₂ supply dependency (\( m_{sd} \)) is \( \sim 0.75 \). Septic patients have a similar \( \dot{V}_{O_2} \) vs. \( \dot{Q}_{O_2} \) relationship except, compared to non-septic individuals with the same baseline \( \dot{V}_{O_2} \), they have a much higher
\( \dot{Q}_{O_2}^{crit} \). They also extract a smaller proportion of the O\(_2\) supplied, resulting in a lower slope during supply dependency (\( m_{sd} \approx 0.6 \)) and a larger positive slope for the aerobic plateau. The higher \( \dot{Q}_{O_2}^{crit} \) and decreased ability to extract O\(_2\) in sepsis have led to the concept of “pathological supply dependency.” Pathological supply dependency in sepsis has been found in humans (11, 43) and in animal models (21, 30), and in various tissues including muscle (9).

Part of the motivation for the present study was to examine how changes in FCD and heterogeneity of capillary blood flow in sepsis are related to increased supply dependency. A previous model study using statistical theory has shown that increased heterogeneity in microvascular perfusion, leading to a mismatch between local \( O_2 \) supply and \( O_2 \) demand, can result in an increased \( \dot{Q}_{O_2}^{crit} \) and a decreased \( m_{sd} \) (44). However, this work did not directly address the source of the relevant increases in heterogeneity and, in particular, whether these increases are primarily due to individual capillary stoppages or loss of flow regulation at the arteriolar level.

In the present study, we use our computational model of spatially heterogeneous capillary \( O_2 \) transport in skeletal muscle to calculate how decreasing \( \dot{Q}_{O_2} \) from baseline values affects tissue oxygenation and \( \dot{V}_{O_2} \) under control and severe sepsis conditions. We present results for tissue \( O_2 \) distributions, minimum tissue \( P_{O_2} \), tissue fraction at risk for dysoxia, and \( \dot{V}_{O_2} \), including two different methods for decreasing \( \dot{Q}_{O_2} \) : stagnant hypoxia (reduced flow velocity) and hypoxic hypoxia (reduced capillary entrance \( S_{O_2} \)). We discuss the differences between the control and sepsis cases, and make comparisons to a simpler, single-capillary (modified Krogh) model.
Finally, we interpret our results in terms of the importance of capillary stopped flow in producing the observed increases in supply dependency during sepsis.

**MATHEMATICAL MODEL**

_Capillary Geometry and Blood Flow_

We have previously described a 3D spatially heterogeneous capillary array model for the rat extensor digitorum longus (EDL) skeletal muscle that incorporates observed changes in both FCD and capillary blood flow velocity distributions during sepsis (16). Based on experimental data for a 24-hour sepsis model (14) and a tissue region that is 400 µm long and approximately 17,500 µm² in cross-sectional area, distributions of normal/fast/stopped vessels of 16/8/8 and 7/7/13 are used to represent the observed control and (severe) sepsis cases, respectively. Parallel capillaries are placed in the tissue domain randomly using a muscle-fiber construction that produces a realistic degree of spatial heterogeneity (18). Fig. 1a shows how the 24 concurrently flowing capillaries for the control case are distributed in the cross-section, where the fast and normal capillaries are selected randomly. For the sepsis case (Fig. 1b), fast, normal, and stopped flow capillaries are selected from the same set used for the control case, but fast vessels are constrained to one quadrant to simulate observed increases in flow heterogeneity. This “clustering” of fast-flow capillaries is designed to simulate the most heterogeneous septic injury that is observed at the capillary level and one that would be most likely to result in increased O₂ supply dependency.
The tube hematocrit (HT) is fixed at 0.25, the average experimental value (14), and the discharge hematocrit (HD) is then obtained from an empirically-derived formula (34). For both control and sepsis cases, baseline red blood cell (RBC) velocities (vRBC) are initially fixed at 130 µm/s for normal flow capillaries (vnorm) (14) and 500 µm/s for fast flow capillaries (vfast) (16). Since there is evidence that total flow decreases in the EDL during sepsis (25), global adjustments are made in RBC velocity to obtain a baseline sepsis case with 64% of the RBC flow in the baseline control case.

**Capillary-Tissue Oxygen Transport**

The oxygen transport model (15-19) describes transport of O2 in both blood and tissue, including O2 bound to hemoglobin (Hb) and dissolved in the blood, as well as the effect of tissue myoglobin (Mb). In the capillaries, O2 transport is given by a time-dependent equation for So2:

\[
\frac{\partial S}{\partial t} = \left[HTC_{Hb} + \alpha_b \frac{dP_b}{dS}\right]^{-1} \left\{ -v_b \left[ H_pC_{Hb} + \tilde{\alpha}_b \frac{dP_p}{dS} \right] \frac{\partial S}{\partial \xi} - \frac{1}{\pi R} \oint j \cdot d\theta \right\}
\]  

(1)

where \(v_b\) is the mean blood velocity, \(R\) is the vessel radius, and \(\xi\) is the local axial coordinate. The two terms in square brackets represent the volume- and flow-weighted oxygen-carrying capacities of blood, \(\alpha_b\) and \(\tilde{\alpha}_b\) are the volume- and flow-weighted blood oxygen solubilities, and \(C_{Hb}\) is the O2 binding capacity of the Hb solution inside RBCs. \(P_b(S) = P_{50}[S/(1-S)]^{1/n}\) is the blood Po2 obtained by inverting the Hill equation for the Hb-O2 equilibrium binding curve. The blood-tissue O2 flux is given by:

\[
j = k(P_b - P_w)
\]

(2)
where $P_w$ is the tissue $\mathrm{Po}_2$ at the vessel surface and $k$ is a mass transfer coefficient that depends on hematocrit (18).

For all simulations, $S$ is fixed in all inlet segments by the parameter $S_a$. Although in reality $S_a$ is not the same for all capillaries, this is a simplifying assumption that allows us to focus on heterogeneities in capillary spacing and blood flow and could be justified for capillaries supplied by the same arteriole. For baseline cases (control and sepsis), the blood velocities and hematocrits needed in Eq. 1 are assigned based on experimental data as described above. For the cases with reduced $\mathrm{O}_2$ supply, $v_{\text{RBC}}$ and $S_a$ are each decreased separately from baseline with all other $\mathrm{O}_2$ transport parameters (e.g., hematocrit) fixed at baseline values. This procedure does not explore the full range of possible $\mathrm{O}_2$ transport parameters; however, it does cover the relevant range for sepsis for the cases corresponding to separate decreases in cardiac output and respiration.

For reductions in $v_{\text{RBC}}$, two cases are studied: constant functional capillary density (FCD) and decreasing FCD. The latter case represents the de-recruitment observed experimentally as the RBC supply to a capillary bed is reduced. Based on experiments by Lindbom and Arfors (27), FCD is decreased by first specifying uniform distributions of normal (90-170 $\mu$m/s) and fast (300-700 $\mu$m/s) velocities and then using a threshold velocity (40 $\mu$m/s for control, 80 $\mu$m/s for sepsis) below which red cell flow is assumed to stop. The numbers of flowing capillaries for several reductions in $v_{\text{RBC}}$ are shown in Table 1. In general, decreasing FCD with $v_{\text{RBC}}$ will
decrease the efficiency of O\textsubscript{2} transport and therefore decrease O\textsubscript{2} extraction, with higher threshold velocities producing greater decreases in O\textsubscript{2} extraction.

Oxygen transport in the tissue is given by a time-dependent equation for P\textsubscript{O\textsubscript{2}}:

\[
\frac{\partial P}{\partial t} = \left[ 1 + \frac{c_{Mb}}{\alpha} \frac{dS_{Mb}}{dP} \right]^{-1} \left\{ -\frac{1}{\alpha} M(P) + D
\nabla^2 P + \frac{1}{\alpha} D_{Mb} c_{Mb} \nabla \cdot \left( \frac{dS_{Mb}}{dP} \nabla P \right) \right\}
\]

(3)

where \( \alpha \) and \( D \) are the tissue oxygen solubility and diffusivity and \( c_{Mb}, D_{Mb}, \) and \( S_{Mb} \) are the concentration, diffusivity, and oxygen saturation of Mb. Michaelis-Menten consumption kinetics are used to describe the dependence of O\textsubscript{2} consumption on P\textsubscript{O\textsubscript{2}}, \( M(P) = M_0 P / (P + P_c) \), and the equilibrium Mb saturation is given by \( S_{Mb}(P) = P / (P + P_{50,Mb}) \). At the vessel-tissue interface a flux boundary condition on \( P \) is applied using Eq. 2 and no-flux boundary conditions are applied on the outer tissue surfaces in the \( z \) direction.

On the outer tissue surfaces in the \( x \) and \( y \) directions, periodic (16, 19) or no-flux (18) boundary conditions are specified to represent two extremes of the effect of neighboring capillary networks on the oxygen distribution within the computational domain, i.e., the region being modeled. With periodic boundary conditions, the neighboring tissue is effectively tiled with identical copies of the computational domain. In the sepsis case, as shown in Fig. 2a, the groups of seven fast capillaries are evenly spaced in the surrounding tissue. With no-flux boundary conditions, the neighboring tissue is tiled with multiple versions of the computational domain reflected at the domain boundaries. Regions containing the group of seven fast capillaries are thus clustered in groups of four (Fig. 2b). The difference in effective capillary spacing between the periodic and zero flux boundary conditions is due to the corner location of the group of fast capillaries, i.e., to
the heterogeneity of capillary spacing in the network being considered. Once the boundary conditions are specified, steady-state solutions to the $O_2$ transport equations (1)-(3) are calculated numerically using a finite-difference method (15).

**Model Parameters**

Most of the parameters used in our oxygen transport calculations, shown in Table 2, are the same as used previously (16). As before, the tissue $O_2$ consumption rate ($M_0$) is estimated by comparing measured capillary $O_2$ extraction ratios (14) to numerical calculations of the extraction ratios for several values of $M_0$. For control, this yields the same $M_0$ as used previously, $1.5 \cdot 10^{-4}$ ml $O_2$/ml/s. For sepsis, $M_0$ was previously estimated to be $5.26 \cdot 10^{-4}$ ml $O_2$/ml/s under the assumption that baseline $O_2$ supply did not decrease (16). However, in the present work we have assumed a 36% decrease in baseline $O_2$ supply in sepsis (25). Therefore, to be consistent with measured $O_2$ extraction ratios (14), it was necessary to use a reduced value, $M_0=3.98 \cdot 10^{-4}$ ml $O_2$/ml/s.

**RESULTS**

For each case studied, $O_2$ delivery parameters and the $P_{O_2}$ boundary conditions in the $x$ and $y$ directions are fixed and numerical calculations are performed to obtain the steady-state distributions of $O_2$ in the capillaries and muscle tissue. Both periodic and no-flux boundary conditions are considered to simulate the extremes of the effect of neighboring capillary networks on the $O_2$ distributions in the tissue surrounding the capillary network being
considered. Fig. 3 shows the tissue O\textsubscript{2} distributions at baseline O\textsubscript{2} supply (\(\dot{Q}_{O_2}\)) for control (Fig. 3a) and sepsis (Fig. 3b) cases with periodic boundary conditions. At baseline, the sepsis case already has lower minimum tissue Po\textsubscript{2} (P\textsubscript{min}, 17.9 vs. 40.0 mmHg for control), higher spatial heterogeneity of tissue Po\textsubscript{2} (standard deviation/mean, CV(Po\textsubscript{2}), of 0.22 vs. 0.04 for control), and higher total O\textsubscript{2} consumption (\(\dot{V}_{O_2}\) of 4.04\times10^{-9} ml O\textsubscript{2}/s vs. 1.54\times10^{-9} for control). Using no-flux boundary conditions has little effect on the control case, but decreases minimum tissue Po\textsubscript{2} and increases spatial heterogeneity for the sepsis case (Fig. 4). Note that the difference between results for periodic and no-flux boundary conditions depends on the heterogeneity of the capillary arrangement inside the computational domain. This is one reason why the sepsis case, with fast flow capillaries clustered in one corner, is more sensitive to the imposed boundary conditions.

Figs. 3-4 also show the effect of decreasing \(\dot{Q}_{O_2}\) via \(v_{RBC}\), where FCD is held constant. For control, Po\textsubscript{2} distributions become broader as \(\dot{Q}_{O_2}\) decreases, but P\textsubscript{min} does not reach zero, and hence \(\dot{V}_{O_2}\) remains nearly constant, until \(\dot{Q}_{O_2}\) becomes very small (~10% of baseline). For sepsis (Fig. 3b), Po\textsubscript{2} distributions also become broader as \(\dot{Q}_{O_2}\) decreases, but P\textsubscript{min} decreases more quickly than for control and becomes almost zero when \(\dot{Q}_{O_2}\) is only 50% of baseline (75% for no-flux boundary conditions). This indicates that, for sepsis, \(\dot{V}_{O_2}\) begins to decrease sharply at a much higher relative \(\dot{Q}_{O_2}\) than for control (50-75% of baseline as compared to ~10%).

Fig. 5 compares calculated curves of \(\dot{V}_{O_2}\) vs. \(\dot{Q}_{O_2}\) for control (Fig. 5a) and sepsis (Figs. 5b) with periodic boundary conditions for: i) stagnant hypoxia with constant FCD, ii) stagnant hypoxia
with decreasing FCD, and iii) hypoxic hypoxia. For all three types of hypoxia, $\dot{V}_{O_2}$ is initially flat for control and then drops off sharply as $\dot{Q}_{O_2}$ approaches zero, while for sepsis $\dot{V}_{O_2}$ has a faster initial decrease and begins to drop sharply at a lower relative $\dot{Q}_{O_2}$. Fig. 6 shows calculated $\dot{V}_{O_2}$-$\dot{Q}_{O_2}$ curves for control (Fig. 6a) and sepsis (Fig. 6b) with no-flux boundary conditions. It can be seen that no-flux boundary conditions increase the slope of the aerobic plateau, cause supply dependency to begin at a higher $O_2$ supply, and decrease the slope in the supply-dependent regime.

Figs. 5-6 show that for sepsis, for both periodic and no-flux boundary conditions, the curves for hypoxic hypoxia and stagnant hypoxia with decreasing FCD are nearly identical, while the curve for stagnant hypoxia with constant FCD is slightly higher. Since, experimentally, the type of hypoxia imposed has not been observed to affect the shape of $\dot{V}_{O_2}$-$\dot{Q}_{O_2}$ curves (38), the result here indicates that decreasing rather than constant FCD is the appropriate assumption for stagnant hypoxia. Therefore, for sepsis with stagnant hypoxia only decreasing FCD is considered below. For control, on the other hand, constant (rather than decreasing) FCD gives a better match between the $\dot{V}_{O_2}$-$\dot{Q}_{O_2}$ curves for stagnant and hypoxic hypoxia. However, since the curve for hypoxic hypoxia lies between the two stagnant hypoxia cases, the conclusion is that the threshold velocity chosen for control (40 $\mu$m/s) was too large. Rather than iterate to find a self-consistent threshold velocity, we consider stagnant hypoxia with constant FCD to be a reasonable approximation for the control case.
For sepsis, calculations show that hypoxic hypoxia has a greater effect on $P_{\text{min}}$ than stagnant hypoxia (4.3 vs. 7.2 mmHg for periodic conditions with a 35% decrease in $\dot{Q}_{O_2}$). Hypoxic hypoxia also produces a higher fraction of tissue with $P_{O_2}<1$ mmHg (0.18 vs. 0.15 for periodic conditions with a 60% decrease in $\dot{Q}_{O_2}$ and 0.20 vs. 0.17 for no-flux conditions with a 50% decrease in $\dot{Q}_{O_2}$). The criterion $P_{O_2}<1$ mmHg is used to identify tissue at risk for dysoxia, since in our Michaelis-Menten description of $O_2$ consumption kinetics a critical $P_{O_2}$ of 0.5 mmHg is assumed. Calculations of $CV(P_{O_2})$ indicate that hypoxic hypoxia results in slightly less heterogeneity of tissue $P_{O_2}$ than stagnant hypoxia (0.606 vs. 0.614 for periodic conditions and 0.816 vs. 0.900 for no-flux conditions, both with a 50% decrease in $\dot{Q}_{O_2}$).

In addition to the above results, the following parameters of the $\dot{V}_{O_2}$-$\dot{Q}_{O_2}$ curves are shown in Table 3 for control and sepsis cases: the slope of the aerobic plateau ($m_{\text{plateau}}$), the slope of the supply-dependent portion of the curve ($m_{\text{sd}}$), and the critical $O_2$ extraction ratio ($O_2ER_{\text{crit}} = \dot{V}_{O_2,\text{crit}}/\dot{Q}_{O_2,\text{crit}}$). These parameters are derived by performing dual-line regressions on the calculated data points (44) as shown in Fig. 7. It can be seen that these parameters are mainly affected by the physiological conditions (control vs. sepsis) and in sepsis by the boundary conditions (periodic vs. no-flux), and do not vary greatly between the two types of hypoxia considered.
DISCUSSION

Our experiment-based simulations of O$_2$ transport in the EDL show the importance of increased O$_2$ consumption, decreased overall O$_2$ supply, and increased O$_2$ supply heterogeneity during sepsis. At baseline, i.e., under average measured conditions, $\dot{Q}_{O_2}$ is 36% lower and $M_0$ is 165% higher in sepsis vs. control, leading to a greater susceptibility to hypoxia. This susceptibility is increased by the loss of FCD and greater blood flow heterogeneity in sepsis. By incorporating these factors into our computational model, we have shown that increased capillary-level heterogeneity during sepsis can lead to an increase in supply dependency that is similar to what is often observed during sepsis (9, 21, 30, 43).

Since local O$_2$ consumption depends on Po$_2$, the supply dependency found here in sepsis is a direct result of insufficient O$_2$ delivery to certain tissue regions. The role of heterogeneity in sepsis can be seen in the fact that when $\dot{Q}_{O_2}$ was decreased by 50% (stagnant hypoxia), $P_{min}$ decreased by 83% but mean tissue Po$_2$ only decreased by 31%. In control, a 50% decrease in $\dot{Q}_{O_2}$ resulted in an 11% decrease in $P_{min}$ and a 6% decrease in mean P$_{O2}$. Another measure of O$_2$ delivery heterogeneity is CV(Po$_2$), which for sepsis increased 136% when $\dot{Q}_{O_2}$ was reduced by 50%, in contrast to a 75% increase for control. The differences between results for periodic and no-flux boundary conditions also show the importance of heterogeneity in sepsis. In particular, when the fast-flow capillaries are clustered to represent a heterogeneous septic injury, tissue oxygenation is impaired, and this effect is increased when nearby capillary networks are not able to deliver oxygen to regions containing only stopped and normal-flow capillaries. For stagnant
hypoxia with no-flux boundary conditions, Fig. 8 shows the change in the spatial distribution of Po$_2$ at the venous end of the tissue domain during the development of supply dependency in sepsis.

Our simulation results for sepsis also show that tissue PO$_2$ is somewhat affected by the way in which $\dot{Q}_{O_2}$ is decreased. In particular, given the same overall reduction in $\dot{Q}_{O_2}$, changes in $S_a$ have a greater effect on muscle Po$_2$ than changes in $v_{RBC}$. The implication of these results is that $S_a$ is the most important variable to maintain near baseline in order to maximize tissue O$_2$ delivery during sepsis.

**Comparison to Modified Krogh Model**

The above results differ somewhat from those that would be obtained using a Krogh-type, single-capillary model of oxygen transport (24), which does not take into account the spatial heterogeneity of capillary spacing and blood flow. To show this, we use a modified Krogh model (c.f. (29)) that includes Po$_2$-dependent consumption, intravascular resistance, and the appropriate values of $M_0$, average tissue cylinder radius, and average blood flow per capillary for our control and sepsis cases. Myoglobin, which has been shown to have a minimal effect under steady-state conditions (29), is not included. For the control case, Po$_2$ distributions obtained with the modified Krogh model are similar to those obtained with the 3D capillary array model, implying effectively little supply heterogeneity. However, for sepsis (Fig. 9) the Krogh model gives narrower distributions and higher minimum Po$_2$ than the 3D model, especially when no-flux boundary conditions are used, implying a greater role for supply heterogeneity. The Krogh model predicts increased supply dependency for sepsis; however, it does not capture the
pathological supply dependency found in the 3D model with no-flux boundary conditions (Fig. 10) and gives a slope of approximately 1 for the supply-dependent portion of the $\dot{V}_{O_2}$-$\dot{Q}_{O_2}$ curves. The Krogh model also predicts smaller slopes for the aerobic plateaus. For stagnant hypoxia, the Krogh model gives values for $m_{\text{plateau}}$ of 0.00065 and 0.0056 for control and sepsis, respectively, while the 3D model gives 0.0054 and 0.061 for periodic boundary conditions and 0.0058 and 0.126 for no-flux conditions. Comparing these results to the measurements of Humer et al. (21), which gave $m_{\text{plateau}}$=0.002 for control and $m_{\text{plateau}}$=0.039 for endotoxemia, suggests that the results of the 3D model are more consistent with experiment.

**Capillary-Level Disturbances and Pathological Supply Dependency**

The results of our 3D model for baseline control and sepsis conditions have been shown (16) to be in basic agreement with other measurements in skeletal muscle, such as those of Astiz et al., Anning et al., Sair et al., and Vallet et al. (1, 2, 36, 42). Using a modified Krogh model similar to the one described above, Schumacker and Samsel (38) showed that under normal conditions capillary spacing ($d_K$, diameter of Krogh tissue cylinder) was a key parameter in determining the nature of supply dependency. In particular, it was shown that for $d_K$$\leq$80 $\mu$m the supply-dependent slope of the $\dot{V}_{O_2}$-$\dot{Q}_{O_2}$ curve was approximately 1 and that supply dependency was not sensitive to the type of hypoxia imposed, as is also the case experimentally ((10); see also (38)). In order to match experimental findings for $O_2ER_{\text{crit}}$ (typically 0.6-0.75), it was necessary to postulate a functional arteriovenous shunt of $\sim$30% (38).
Walley (44) investigated supply dependency using a statistical model in which distributions of local oxygen demand/supply ratios \((\dot{d}o_2/q_o2)\) were used to construct \(\dot{V}_{O2}-\dot{Q}_{O2}\) curves. Using data on gut capillary transit time distributions in healthy and endotoxemic pigs (21), Walley was able to obtain \(O_2ER_{crit}\) values close to those measured experimentally. Although its functional origin (i.e., whether it occurs at the capillary or arteriolar level) was not discussed, Walley’s assumed \(\dot{d}o_2/q_o2\) distributions imply a degree of arteriovenous shunting of \(O_2\) (23). Usually, shunting of \(O_2\) indicates a direct flow from an arteriole to a venule that bypasses the capillaries; however, within the capillary bed functional shunting can also occur if individual capillaries carry \(O_2\) through to the venous side instead of delivering \(O_2\) to where it is needed in surrounding tissue (e.g., due to diffusion limitation).

In the present study, the average distance to the nearest normal or fast flow capillary \((\sim d_{K}/2)\) is approximately 14 \(\mu m\) for control and 22 \(\mu m\) for sepsis, with maximum distances of 39 and 67 \(\mu m\) for control and sepsis, respectively. Using a classic Krogh analysis, it can be shown that diffusion limitation begins for \(d_{K}/2\) approximately equal to 125 \(\mu m\) and 81 \(\mu m\) for control and sepsis, respectively. Thus, while for the control case shunting is highly unlikely at the capillary level, for sepsis capillary shunting of \(O_2\) can occur if there is a slight increase in \(d_{K}/2\), as is the case for no-flux boundary conditions. Thus, for periodic boundary conditions, despite the imposed heterogeneity in capillary arrangement and the loss of FCD due to sepsis, we find a supply-dependent slope close to 1 (i.e. \(\sim 100\%\) of the \(O_2\) supplied is consumed), in agreement with the results of Schumacker and Samsel. The fast-flow capillaries provide some functional shunting of \(O_2\); however, this was not enough to affect \(m_{sd}\) or \(O_2ER_{crit}\) which we estimate as \(\sim 1\) for both control and sepsis. Therefore, since \(m_{sd}\) and \(O_2ER_{crit}\) do not decrease during sepsis in
the present model, classic pathological supply dependency did not occur for periodic boundary conditions.

In contrast to the preceding results, for no-flux boundary conditions, which effectively double the maximum capillary-tissue distance (see Fig. 2), the fast-flow capillaries did provide enough functional shunting in sepsis to decrease \( m_{sd} \) and \( O_2ER_{crit} \) well below 1. Therefore, for no-flux boundary conditions pathological supply dependency did occur in sepsis. It should be noted that the present study assumed a 36% decrease in baseline blood flow in sepsis, which resulted in a lower intrinsic tissue \( O_2 \) consumption rate \( (M_0) \) and therefore less potential for capillary shunting of \( O_2 \) due to diffusion limitation. If baseline blood flow remains the same in sepsis, there would likely be more supply dependency than found here. Two other characteristics of increased supply dependency in sepsis were found for both periodic and no-flux boundary conditions: increased \( m_{plateau} \) and decreased \( Q_{O2, crit} \). This suggests that these two quantities are directly related to increased \( O_2 \) transport heterogeneity at the individual capillary level, while \( m_{sd} \) and \( O_2ER_{crit} \) depend both on individual capillary stoppages and the arrangement and relative \( O_2 \) supply of surrounding microvascular units.

It should be noted that the present model does not consider the influence of \( CO_2 \) or \( pH \), which could be expected to alter somewhat \( O_2 \) transport in sepsis. In addition, the role of \( NO \) is not explicitly considered. \( NO \) is known to be upregulated in sepsis and to inhibit mitochondrial respiration, although its production also consumes \( O_2 \). At a minimum \( NO \), like \( CO_2 \) and \( pH \), might be expected to alter the spatial distribution of \( O_2 \) in tissue during sepsis. An examination
of the idea that NO overproduction during sepsis could eventually lead to inhibition of mitochondrial respiration and a decrease in $O_2$ extraction (8, 40) is planned for future modeling efforts. It should also be noted that additional heterogeneities in capillary hematocrits and inlet saturations, not considered in the present model, might serve to further decrease $O_2$ extraction and increase supply dependency in sepsis.

**Conclusions**

Using a detailed, experiment-based model of $O_2$ transport in muscle during control and sepsis conditions, we have explored the effect on tissue oxygenation and oxygen extraction of reducing the overall $O_2$ supply. We found more sensitivity to $O_2$ supply in sepsis vs. control, due to increased $O_2$ consumption and decreased functional capillary density. We also found that in sepsis tissue oxygenation is affected more by hypoxic hypoxia than stagnant hypoxia.

We have also used the model to explore the role of heterogeneities in capillary spacing and blood flow in producing supply dependency in muscle. Our results imply that the supply dependency observed under normal experimental conditions, with $< 100\%$ $O_2$ extraction, does not have its origin at the level of individual capillaries, since the necessary functional $O_2$ shunting cannot occur due to the absence of diffusion limitation (even when no-flux boundary conditions are imposed). Although our results show little functional shunting during sepsis for periodic boundary conditions, they show that diffusion limitation and shunting of $O_2$ by individual capillaries can occur for no-flux boundary conditions. The most physiologically appropriate boundary conditions will depend on the local arrangement of microvascular units and their
relative $O_2$ supplies. However, the overall effect of nearby microvascular units should lie between those seen here for periodic and no-flux conditions. Therefore, it is expected that in many cases the pathological supply dependency seen for no-flux conditions will be relevant. In summary, we have shown that pathological supply dependency can occur within individual capillary networks and that loss of individual capillaries in sepsis likely plays a role. To fully determine the relative importance of individual capillary stoppages and impairment of local blood flow regulation will require further modeling based on new experimental data, which we plan to pursue in future work.
ACKNOWLEDGMENT

This work was supported by the Whitaker Foundation (DG), by Canadian Institutes of Health Research Grant MOP-49416 (CGE), and by a Heart and Stroke Postdoctoral Fellowship (RMB).
REFERENCES


Table 1. Decreases in number of flowing capillaries with $v_{RBC}$.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Sepsis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%$v_{RBC}$</td>
<td>N$_{flowing}$</td>
</tr>
<tr>
<td>100</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

Number of flowing capillaries vs. percent of baseline $v_{RBC}$. Control: 100%, 50%, 25%, 10%.
Sepsis: 100%, 75%, 50%, 25%.
Table 2. Biophysical parameters for O₂ transport.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>$3.89 \times 10^{-5}$ ml O₂·ml⁻¹·mmHg⁻¹</td>
</tr>
<tr>
<td>D</td>
<td>$2.41 \times 10^{-5}$ cm²·s⁻¹</td>
</tr>
<tr>
<td>C_Hb</td>
<td>0.52 ml O₂·ml⁻¹</td>
</tr>
<tr>
<td>P₅₀</td>
<td>37 mmHg</td>
</tr>
<tr>
<td>n</td>
<td>2.7</td>
</tr>
<tr>
<td>P_c</td>
<td>0.5 mmHg</td>
</tr>
<tr>
<td>αₕ</td>
<td>$2.92 \times 10^{-5}$ ml O₂·ml⁻¹·mmHg⁻¹</td>
</tr>
<tr>
<td>α̅ₕ</td>
<td>$2.96 \times 10^{-5}$ ml O₂·ml⁻¹·mmHg⁻¹</td>
</tr>
<tr>
<td>C_Mb</td>
<td>$2 \times 10^{-3}$ ml O₂·ml⁻¹</td>
</tr>
<tr>
<td>P₅₀,Mb</td>
<td>5.3 mmHg</td>
</tr>
<tr>
<td>D_Mb</td>
<td>$3 \times 10^{-7}$ cm²·s⁻¹</td>
</tr>
</tbody>
</table>

For descriptions and other parameters, see the text.
Table 3. Parameters of $\dot{V}_O_2$-$\dot{Q}_O_2$ curve for stagnant and hypoxic hypoxia.

<table>
<thead>
<tr>
<th></th>
<th>Periodic B.C.</th>
<th>No-Flux B.C.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stagnant</td>
<td>Hypoxic</td>
</tr>
<tr>
<td>$m_{plateau}$</td>
<td>0.00538</td>
<td>0.00560</td>
</tr>
<tr>
<td>$m_{sd}$</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>$O_2ER_{crit}$</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Periodic B.C.</th>
<th>No-Flux B.C.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stagnant</td>
<td>Hypoxic</td>
</tr>
<tr>
<td>$m_{plateau}$</td>
<td>0.0608</td>
<td>0.0632</td>
</tr>
<tr>
<td>$m_{sd}$</td>
<td>0.836</td>
<td>0.917</td>
</tr>
<tr>
<td>$O_2ER_{crit}$</td>
<td>0.961</td>
<td>0.965</td>
</tr>
</tbody>
</table>

Parameters shown: $m_{plateau}$, slope of aerobic plateau; $m_{sd}$, slope of supply-dependent region; $O_2ER_{crit}$, critical $O_2$ extraction ratio.
FIGURE LEGENDS

**Figure 1:** Distribution of capillaries in tissue cross-section. (a) control. (b) sepsis. Normal (open circles), fast (closed circles), and stopped (x’s) capillaries are as indicated.

**Figure 2:** Effect of boundary conditions in the x and y directions for the sepsis case. Shown for (a) periodic and (b) no-flux conditions are the effective arrangements of capillary networks in and around the computational domain, with the group of clustered fast-flow capillaries represented by a square. For periodic conditions, surrounding capillary networks are identical and the maximum distance between any tissue point and the fast capillaries is approximately 115µm. For no-flux conditions, surrounding networks are a reflection of the network considered and the maximum distance between any tissue point and the fast capillaries is increased by approximately 50%.

**Figure 3:** Tissue Po2 probability distribution functions for stagnant hypoxia with constant FCD and periodic boundary conditions, where V=vRBC. (a) control with baseline sepsis case shown for comparison. (b) sepsis.

**Figure 4:** Tissue Po2 probability distribution functions during sepsis for stagnant hypoxia with constant FCD and no-flux boundary conditions, where V=vRBC.

**Figure 5:** O2 extraction vs. O2 supply curves for stagnant and hypoxic hypoxia with periodic boundary conditions. (a) control. (b) sepsis.
Figure 6: O₂ extraction vs. O₂ supply curves for stagnant and hypoxic hypoxia with no-flux boundary conditions. (a) control. (b) sepsis.

Figure 7: Dual-line regression of the $\dot{V}_O_2$-$Q_{O_2}$ curve (O₂ extraction vs. O₂ supply) for sepsis during stagnant hypoxia with decreasing FCD calculated using the 3D model and no-flux boundary conditions. This procedure gives the slopes $m_{\text{plateau}}$ and $m_{\text{ad}}$, the value $\dot{Q}_{O_2,\text{crit}}$ at which supply dependency begins, and the critical O₂ extraction ratio, O₂ER$_{\text{crit}}$.

Figure 8: Spatial distribution of tissue Po₂ at the venular end of the tissue domain during the onset of supply dependency in sepsis. Shown for stagnant hypoxia with decreasing FCD and no-flux boundary conditions are O₂ supplies corresponding to 100% (A), 65% (B) and 40% (C) of baseline. As the O₂ supply decreases, the clustered fast-flow capillaries act as O₂ shunts, while the tissue supplied by normal and stopped capillaries becomes dysoxic and stops consuming O₂.

Figure 9: Comparison of tissue Po₂ probability distributions during sepsis for various degrees of stagnant hypoxia with decreasing FCD calculated using the modified Krogh (K) and full capillary array (3D) models.

Figure 10: Comparison of curves of O₂ extraction vs. O₂ supply for hypoxic hypoxia calculated with the modified Krogh model and the full 3D model.
Figure 1a
Figure 1b

Sepsis: normal, fast and stopped capillaries

- Normal
- Fast
- Stopped

x location (cm) vs y location (cm)
**Figure 2a**
Figure 2b
Figure 3a
Figure 3b
Figure 4
Figure 5a
Figure 5b

Graph showing the relationship between total O₂ extraction and total O₂ supply for different conditions:

- Stagnant/Constant FCD
- Stagnant/Decreasing FCD
- Hypoxic Hypoxia

The graph is labeled "Sepsis/Periodic" and includes a line of unity.
Figure 6a
Figure 6b
Figure 7

Sepsis
Stagnant Hypoxia with Decreasing FCD
No-Flux B.C.

critical O₂ supply
"aerobic plateau"

\[ y_{pl} = 0.12554x + 29.7286 \]

\[ y_{sd} = 0.40052x + 15.8377 \]

\[ E_{crit} = 0.71403 \]

Supply dependency

total O₂ extraction \((10^{-10} \text{ml} \text{O}_2 / \text{s})\)

total O₂ supply \((10^{-10} \text{ml} \text{O}_2 / \text{s})\)
Figure 8
Figure 9
Figure 10

![Graph showing oxygen extraction per capillary versus fraction of baseline oxygen supply for hypoxic hypoxia, sepsis, and control conditions.](image)