The influence of radial RBC distribution, blood velocity profiles, and
glycocalyx on coupled NO/O₂ transport

Xuewen Chen, Dov Jaron, Kenneth A. Barbee, and Donald G. Buerk. The influence of radial RBC distribution, blood velocity profiles, and glycocalyx on coupled NO/O₂ transport. J Appl Physiol 100: 482–492, 2006. First published October 6, 2005; doi:10.1152/japplphysiol.00633.2005.—The purpose of this investigation was to study the effect of the presence of red blood cells (RBCs) in the plasma layer near the arteriole wall on nitric oxide (NO) and oxygen (O₂) transport. To this end, we extended a coupled NO and O₂ diffusion-reaction model in the arteriole, developed by our group, to include the effect of the presence of RBCs in the plasma layer and the effect of convection. Two blood flow velocity profiles (plug and parabolic) were tested. The average hematocrit in the bloodstream was assumed to be constant in the central core and decreasing to zero in the boundary layer next to the endothelial surface layer. The effect of the presence or absence of RBCs near the endothelium was studied while varying the endothelial surface layer and boundary layer thickness. With RBCs present in the boundary layer, the model predicts that 1) NO decreases significantly in the endothelium and vascular wall; 2) there is a very small increase in endothelial and vascular wall PO₂; 3) scavenging of NO by hemoglobin decreases with increasing thickness of the boundary layer; 4) the shape of the velocity profile influences both NO and PO₂ gradients in the bloodstream; and 5) the presence of RBCs in the boundary layer near the endothelium has a much larger effect on NO than on O₂ transport.

nitric oxide; oxygen; red blood cells; mass transport; mathematical model; diffusion; convection; endothelial surface layer

THE OBJECTIVE OF THIS INVESTIGATION was to study the effect of the presence of red blood cells (RBCs) in the plasma layer adjacent to the arteriole wall on coupled nitric oxide (NO) and oxygen (O₂) transport using computer simulations and to compare our model predictions with experimental data from the literature.

NO, produced by endothelial cells lining the vessel wall, diffuses both into the vessel lumen and to surrounding tissues. Most endothelium-derived NO is scavenged by hemoglobin in the RBCs in the bloodstream, and only a portion diffuses abuminally. The portion that diffuses into the vessel wall activates soluble guanylate cyclase (sGC), catalyzing the formation of cyclic 3',5'-guanosine monophosphate and activating cyclic 3',5'-guanosine monophosphate-dependent protein kinase. This results in a decrease of Ca²⁺ inside smooth muscle cells and dephosphorylation of contractile proteins, such as myosin light chain, causing relaxation of vascular smooth muscle (8, 24). Generation and transport of NO in vivo are closely linked to O₂, which diffuses from the vessel lumen into the vascular wall and tissue. O₂ and NO interact in several ways: O₂ is used during NO synthesis inside endothelial cells; at the same time, O₂ and NO compete for the binding site of cytochrome-c oxidase in mitochondria, affecting the respiratory chain. NO can also be autoxidized by O₂, although the reaction rate is very low and was ignored in this study (2).

There are certain questions that arise when constructing a model for NO transport. One is whether the amount of NO that reaches smooth muscle cells is high enough to produce vasodilation. Because deoxy- and oxyhemoglobin in the blood vessel lumen scavenge NO at a very rapid rate, early models found that the amount of NO reaching the abluminal smooth muscle was below the minimum concentration (80–250 nM) needed to activate sGC (21, 31, 34). Different theories have been proposed to resolve this problem, and research on this topic is continuing (19, 20, 32, 33, 34).

There are also some uncertainties regarding the intraluminal and extraluminal transport resistance to O₂. Experimentally measured PO₂ fluxes across the arteriolar wall have been reported to be one order of magnitude higher than those predicted by Fick’s law (14, 26). Pittman (25) showed that this discrepancy can be partly due to the use of low-O₂ permeability values in tissue. Hellums et al. (13) estimated the intraluminal O₂ gradient and found that using higher O₂ permeability values can decrease the extraluminal O₂ gradient, but not the intraluminal O₂ gradient. They also suggested that the luminal O₂ diffusion resistance, neglected in most previous models, should be included in future models. Tsai et al. (36) measured the O₂ tension in rat mesenteric arterioles and the surrounding tissue and suggested, using an O₂ transport model, that the vascular wall might be consuming significant amounts of O₂. In the model reported here, we address this issue using data from the literature to obtain both intraluminal and extraluminal O₂ distributions. We also consider O₂ requirements of the endothelium to produce NO, which might be a factor that contributes to the transmural PO₂ gradient.

Previous O₂ transport models reported in the literature treated blood as a homogeneous fluid, usually assuming plug flow with RBCs uniformly distributed in the bloodstream (13, 16, 17). It is also usually assumed that, except for diffusion and consumption, O₂ does not react with other chemical species in the bloodstream and tissue. Tsai et al. (36) used a phosphores-
cience quenching microscopy technique to measure PO2 values inside arterioles and surrounding tissues, regarding blood as a homogeneous hemoglobin solution in their model. In a recent review, Tsai et al. (37) commented that the nonuniform distribution of RBCs in the bloodstream due to the Fåhraeus effect might be an important factor for O2 delivery.

The Fåhraeus effect is the migration of RBCs to the axial core in vessels with diameters <0.3 mm (11, 12). This leads to the formation of a high-viscosity, RBC-rich core and a low-viscosity, mostly RBC-free plasma layer, causing a nonlinear blood flow velocity profile (32). Some previous NO models have included a RBC-free plasma layer. Butler et al. (4) considered a RBC-rich region with very high NO scavenging rate and a RBC-free region with a zero NO scavenging rate and concluded that NO produced by the endothelium may be significant enough for vascular dilation. In early coupled NO and O2 transport models developed by our group (3, 18), we also used a RBC-rich core with a high NO scavenging rate and a RBC-free plasma layer with negligible NO scavenging. We found that the peak NO in the endothelium for a given NO scavenging rate and a RBC-free plasma layer with negligible NO scavenging in tissue. However, neither of these models considered the effects of convection or the spatial distribution of RBCs in the lumen. Lamkin-Kennard et al. (19) assumed a constant value for blood PO2, with a NO scavenging rate in the central RBC-rich core, which varied as a function of the core radius and the discharge hematocrit. They assumed that RBCs were distributed uniformly in the core of the bloodstream with no RBCs in the plasma layer, but did not model convective transport.

While in smaller numbers than in the core, the presence of RBCs in the plasma layer near the endothelium may have a significant influence on NO synthesis and transport. Experimental observations of RBCs in the plasma layer can be found in the literature (15), although detailed information on hematocrit profiles is limited. Prakash and Singh (27) obtained erythrocyte distribution profiles at hematocrit values from 10 to 60% in a glass capillary of 200-μm diameter using axial tomographic and image velocimetry techniques. Their results showed that the profile becomes increasingly blunt as the average hematocrit rises to levels similar to those encountered physiologically. Long et al. (22) performed experiments in rat venules using fluorescent microparticle image velocimetry and calculated viscosity profiles in the vessel lumen. They showed that viscosity was constant across the center of the venule, but, near the vessel wall, it decreased approximately linearly. In later work, they related viscosity to blood hematocrit (unpublished observations).

One other factor may also affect O2 and NO transport. It has been shown that vascular endothelial cells have a layer of negatively charged glycosylated macromolecules attached on the extracellular membrane (7). Vink and Duling (41) reported a 0.4- to 0.5-μm-thick endothelial surface layer (ESL) and examined its barrier properties using combined fluorescence and bright-field intravital microscopy. They found that ESL permeability to anionic molecules depends on size and charge of the molecule. Barrier properties and mechanisms for penetration of protein molecules through the ESL are, however, unknown.

In the model reported here, we included the ESL along with RBCs in the plasma layer near the vessel wall and investigated their effects on coupled NO and O2 transport.

### Description of Mathematical Model

Mass transport equations for a cylindrical arteriolar segment were used to simulate NO and O2 distributions inside the lumen and surrounding tissue. Definitions for model parameters are listed in Table 1. The model geometry, shown in Fig. 1, consists of six concentric layers: RBC-rich core (0 < r < r1, where r is radius), RBC-poor plasma layer (r1 < r < r2), ESL (or glycocalyx, r2 < r < r3), endothelium (r3 < r < r4), vascular wall (r4 < r < r5), and surrounding tissue (r5 < r < r6). Blood flow in the vessel lumen was assumed to be steady. Hematocrit was assumed to be a continuous function related to the systemic hematocrit through conservation of mass (19). Three hematocrit profiles [H(r)] were used in the plasma layer: linear [H1(r)], parabolic [H2(r)], and step [H3(r)], the latter representing the absence of RBCs in plasma. For all three profiles, average hematocrit across the lumen was set to be the same. The ESL was assumed to be a stagnant layer surrounding the lumen with the same properties as plasma. Differences in convective transport for parabolic and plug velocity profiles were investigated to examine their effects on coupled NO and O2 transport.

### Governing Equations

**RBC-rich core (0 ≤ r < r1) and RBC-poor layer (r1 ≤ r < r2).** Luminal O2 transport was assumed to be governed by diffusion, convection, and reversible binding to hemoglobin (Eq. 1a). Luminal transport of NO was assumed to be by diffusion and convection, with irreversible scavenging by hemoglobin (Eq. 1b). Autooxidation of NO by O2 was neglected due to the very low reaction rate (2).

### Table 1. Values for parameters used in the model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel length (L)</td>
<td>250 μm</td>
<td></td>
</tr>
<tr>
<td>Outer radius of central blood stream (r1)</td>
<td>11.5–14.5 μm</td>
<td></td>
</tr>
<tr>
<td>Outer radius of plasma (r2)</td>
<td>11.5–15 μm</td>
<td></td>
</tr>
<tr>
<td>Thickness of plasma layer (δp)</td>
<td>0–2 μm</td>
<td></td>
</tr>
<tr>
<td>Thickness of glycocalyx (δg)</td>
<td>0–2 μm</td>
<td></td>
</tr>
<tr>
<td>Thickness of endothelium (δe)</td>
<td>1 μm</td>
<td></td>
</tr>
<tr>
<td>Outer radius of glycocalyx (r3)</td>
<td>15 μm</td>
<td></td>
</tr>
<tr>
<td>Outer radius of endothelium (r4)</td>
<td>19 μm</td>
<td></td>
</tr>
<tr>
<td>Outer radius of vascular wall (r5)</td>
<td>120 μm</td>
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</tr>
<tr>
<td>Systemic hematocrit (Hct)</td>
<td>45 %</td>
<td></td>
</tr>
<tr>
<td>O2 solubility (α)</td>
<td>1.34 M/Torr</td>
<td></td>
</tr>
<tr>
<td>O2 diffusion coefficient (DO2)</td>
<td>2.800 μm2/s</td>
<td></td>
</tr>
<tr>
<td>NO diffusion coefficient (DNO)</td>
<td>3.300 μm2/s</td>
<td></td>
</tr>
<tr>
<td>Maximum O2 consumption rate (Qmax)</td>
<td>1 μM/s</td>
<td></td>
</tr>
<tr>
<td>Vascular wall</td>
<td>1 μM/s</td>
<td></td>
</tr>
<tr>
<td>Tissue</td>
<td>20 μM/s</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin scavenging (λH) at 45% Hct</td>
<td>382.5 s⁻¹</td>
<td></td>
</tr>
<tr>
<td>Vascular wall scavenging (λV)</td>
<td>1 s⁻¹</td>
<td></td>
</tr>
<tr>
<td>Tissue scavenging (λT)</td>
<td>1 s⁻¹</td>
<td></td>
</tr>
<tr>
<td>Maximum NO production rate (RNO)</td>
<td>0–300 μM/s</td>
<td></td>
</tr>
<tr>
<td>Maximum O2 bound to hemoglobin (Cmax) at 45% Hct</td>
<td>8,000 μM</td>
<td></td>
</tr>
</tbody>
</table>

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*J Appl Physiol • VOL 100 • FEBRUARY 2006 • www.jap.org*
In the above equations, \( \alpha_{O_2} \) is the \( O_2 \) solubility, \( D_{O_2} \) and \( D_{NO} \) are diffusion coefficients for \( O_2 \) and \( NO \) in the vessel lumen, respectively, \( PO_2 \) is \( O_2 \) partial pressure, \( C(r) \) is the \( O_2 \)-carrying capacity of blood, \( dS(PO_2)/d(PO_2) \) is the slope of the oxyhemoglobin equilibrium curve computed from a modified Easton model (1), \( C_{NO} \) is \( NO \) concentration, \( v \) is the blood velocity, \( z \) is the axial coordinate, and \( \lambda(r) \) is the \( NO \) scavenging rate by hemoglobin (5). 

\[
\lambda(r) = \lambda_b \frac{H(r)}{H_b}, \quad 0 < r < r_2
\]

\[
C(r) = C_b \frac{H(r)}{H_b}, \quad 0 < r < r_2
\]

where superscripts \( L, P, \) and \( S \) are linear, parabolic, and step hematocrit function, respectively. The discharge hematocrit (\( H_b \)) was assumed to be equal to the systemic blood hematocrit (45%), \( \lambda_b \) is the \( NO \) scavenging rate for blood at the systemic hematocrit, and \( C_b \) is the maximum \( O_2 \)-carrying capacity of blood at the systemic hematocrit. \( H_c \) is the hematocrit in the core of the bloodstream and was defined from the mass balance for RBCs:

\[
2\pi \int_0^{r_2} H(r)vrdr = H_2 2\pi \int_{r_1}^{r_2} vrdr
\]

Convective effects of two blood velocity profiles (\( v \)) were investigated: 1) parabolic flow:

\[
v = v_{max} \times [1 - (r/r_2)^2]
\]

2) plug flow:

\[
v = \begin{cases} v_{max} & 0 < r < r_1 \\ v_{max} \times (r - r_2)/(r_1 - r_2), & r_1 < r < r_2 \end{cases}
\]

where \( v_{max} \) is the maximum velocity at the centerline (\( r = 0 \)), and \( r_1 \) and \( r_2 \) are the outer radii of the RBC-rich core and the RBC-poor layer, respectively. The arteriole was assumed to have a uniform radius; therefore, radial velocity profiles did not vary with axial location along the vessel. 

**Glycocalyx layer** (\( r_2 \leq r < r_3 \)). The glycocalyx was assumed to be a stagnant layer with the same properties as blood plasma. Both \( NO \) and \( O_2 \) diffuse radially and axially through this layer.

\[
\frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial PO_2}{\partial r} \right) + \frac{\partial^2 PO_2}{\partial z^2} = 0
\]

\[
\frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial CNO}{\partial r} \right) + \frac{\partial^2 CNO}{\partial z^2} = 0
\]

**Endothelial layer** (\( r_3 \leq r < r_4 \)). Endothelial cells use \( O_2 \) to synthesize \( NO \), catalyzed by endothelial \( NO \) synthase.

\[
\frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial PO_2}{\partial r} \right) + \frac{\partial^2 PO_2}{\partial z^2} = -R_{NO} = 0
\]

where \( R_{NO} \) is the \( NO \) production rate (\( R_{NO} \)) by endothelial \( NO \) synthase is dependent on endothelial \( O_2 \) availability, and the amount of \( O_2 \) consumed is equal to the amount of \( NO \) synthesized, i.e., \( R_{NO} = R_{NO} \). The \( R_{NO} \) by the endothelium was described by Michaelis-Menten kinetics (2):

\[
R_{NO} = R_{NO} \times \frac{PO_2}{PO_2 + K_m}
\]

where \( R_{NO} \) is the maximum \( R_{NO} \) when the \( PO_2 \) is at half maximum (29).

**Vascular wall layer** (\( r_4 \leq r < r_5 \) and tissue layer (\( r_5 \leq r < r_6 \)). The distribution of \( NO \) and \( O_2 \) in the vascular wall and surrounding tissue is determined by:

\[
\frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial PO_2}{\partial r} \right) + \frac{\partial^2 PO_2}{\partial z^2} = -R_{NO} = 0
\]

NO consumption by the vascular wall and surrounding tissue was assumed to obey a first-order reaction with a reaction rate \( R_{NO} = \lambda \times C_{NO} \), where \( \lambda \) is a pseudo-first-order rate constant for \( NO \) scavenging by sGC. The consumption rates of \( NO \) by tissue (\( \lambda_t \)) and vascular wall (\( \lambda_v \)) were chosen here to be the same: \( \lambda = \lambda_t = \lambda_v = 1.0 \text{ s}^{-1} \).

\( O_2 \) consumption by the vascular wall and tissue was inhibited by \( NO \), assuming modified Michaelis-Menten kinetics (2):

\[
R_{O_2} = Q_{max} \frac{PO_2}{PO_2 + appK_m}
\]

where \( Q_{max} \) is the maximum \( O_2 \) consumption rate of the vascular wall or surrounding tissue, and \( appK_m = 1.0 \times (1 + CNO/0.027) \). In the absence of \( NO \), \( appK_m = 1 \text{ Torr} \). Values for \( Q_{max} \) were chosen to be 1.0 and 20.0 \( \mu \text{M/s} \) in the vascular wall and tissue, respectively. \( Q_{max} \) in the vascular wall was close to the overall average estimated from a review of experimental results in the literature by Vadapalli et al. (39), and \( Q_{max} \) in tissue was close to that estimated by Tsai et al. (36).
Boundary Conditions

We assumed zero mass flux at the centerline of the arteriole and at the outermost surface of the tissue cylinder for both O$_2$ and NO, except at the inlet of the bloodstream:

\[
\begin{align*}
\frac{\partial \text{O}_2}{\partial r} &= 0, \quad \text{at } (z = 0, r_2 < r < r_6), \quad (0 < z < L, \ r = 0) \\
\frac{\partial \text{NO}}{\partial r} &= 0, \quad \text{at } (0 < z < L, \ r = r_6), \quad (z = L, \ 0 < r < r_6)
\end{align*}
\]

(15)

where L is length.

To eliminate effects of an abrupt boundary change in the numerical computations, a parabolic \( \text{PO}_2 \) and an exponential \( \text{CNO} \) radial profile were assumed at the inlet of the bloodstream,

\[
\begin{align*}
\text{PO}_2 &_{z=0, 0<r<r_2} = P_{\text{max}} - a \times (r/r_1)^2 \\
\text{CNO} &_{z=0, 0<r<r_2} = a_1 + a_2 \times \exp[-a_3(r_1 - r)]
\end{align*}
\]

(16)

where \( a, a_1, a_2, \) and \( a_3 \) are arbitrary constants, which are iteratively determined under the assumption that the steady-state profiles for NO and \( \text{PO}_2 \) at the boundary should be very similar in shape to that at the 5% axial distance, and \( P_{\text{max}} \) is the maximum \( \text{PO}_2 \) at the entrance. A value of 50 Torr was
assigned to $P_{\text{max}}$ at $r = 0, z = 0$ for all simulations to provide a similar O$_2$ supply to the system.

At the interface between layers, NO and O$_2$ partial pressures were assumed to be continuous, with equal mass fluxes for both species entering and leaving each interface:

\[
\begin{align*}
\frac{\partial \text{PO}_2}{\partial r} |_{r=r_i} &= \frac{\partial \text{PO}_2}{\partial r} |_{r=r_f}, \\
\frac{\partial \text{CNO}}{\partial r} |_{r=r_i} &= \frac{\partial \text{CNO}}{\partial r} |_{r=r_f}, \\
\left\{ \text{PO}_2 |_{r=r_i} = \text{PO}_2 |_{r=r_f}, \\
\text{CNO} |_{r=r_i} = \text{CNO} |_{r=r_f}. \right. \\
\end{align*}
\]

where $i = 2, 3, \ldots 5$ (17)

The set of coupled nonlinear partial differential equations for the model were solved by a finite element method using commercial software (FlexPDE 4, PDESolutions, Antioch, CA). The cubic element type was chosen, with mesh densities adaptively refined by the program to ensure a relative accuracy of 0.0001 for the numerical solutions.

METHODS

We compared NO and O$_2$ transport in the absence and presence of RBCs in the plasma layer, represented by linear, parabolic, and step profiles. For the linear and step profiles, we used two blood velocity profiles: parabolic and plug. For each velocity profile, we performed simulations at different plasma layer thicknesses ($\delta_p$) and $R_{\text{NO}}$. For parabolic hematocrit profile, we performed simulations with the $\delta_p$ of 1.5 μm and endothelial $R_{\text{NO}}$ values of 300 μM/s using parabolic blood velocity profile. We also performed simulations for varying glyocalyx (ESL) thicknesses.

In addition, we performed a dimensional analysis. Averaged values of blood flow velocity and the NO scavenging rate in blood were used to estimate their effects on the luminal NO distribution. The dimensionless governing equation for the luminal NO transport in the vessel lumen is derived below, starting with the averaged form of Eq. 1b,

\[
\frac{\text{DNO}}{r^2} \frac{\partial (\bar{\text{CNO}})}{\partial r} + \frac{\partial \text{CNO}}{\partial z} - \bar{v} \frac{\partial (\text{CNO})}{\partial z} - \bar{\lambda} \text{CNO} = 0 \quad (18)
\]

where $\bar{v}$ is the radially averaged blood flow velocity determined from,

\[
\frac{1}{2 \pi} \int_0^{2\pi} v(r) r dr / \pi r^2
\]

and $\bar{\lambda}$ is the radially averaged blood scavenging NO rate, determined from

\[
\frac{1}{2 \pi} \int_0^{2\pi} \lambda(r) r dr / \pi r^2
\]

For the parameters used in this simulation (Table 1), $\bar{v} = 750$ μm/s and $\bar{\lambda} = 333.77$ s$^{-1}$.

Nondimensionalized variables (designated by *) are defined as:

\[
\begin{align*}
\bar{r} &= \frac{r}{r_2}, \\
\bar{z} &= \frac{z}{L}, \\
\bar{\text{CNO}} &= \frac{\text{CNO}}{\text{CNO}^*}, \\
\bar{v} &= \frac{\bar{v}}{v}, \\
\bar{\lambda} &= \frac{\bar{\lambda}}{\lambda}, \\
\bar{r}_2 &= \frac{r_2}{L}.
\end{align*}
\]

where $r_2$ is the outer radius of the lumen, $L$ is the arterial length, and $\text{CNO}^*$ is 500 μM.

Introducing variables defined in Eq. 19 into Eq. 18, we obtain:

\[
\begin{align*}
\frac{1}{r^2} \frac{\partial (\bar{\text{CNO}}^*)}{\partial r} + \frac{r_2}{L} \frac{\partial \bar{\text{CNO}}^*}{\partial z} + \frac{\text{Pe} \bar{\lambda}^*}{\bar{c}_\text{NO}^*} \bar{\text{CNO}}^* = 0
\end{align*}
\]

The above dimensionless governing equation shows that NO inside the vessel lumen is determined by three coefficients: $(r_2/L)^2$, Peclet number (Pe), and $\lambda^*$. Using parameters in Table 1, $(r_2/L)^2 = 0.0036$, $\text{Pe} = -0.20$, and $\lambda^* = -22.7$.

RESULTS

Results shown in Figs. 2–7 were computed at the radial cross section in the middle of the vessel ($z = 125$ μm).

Figure 2 depicts effects of increasing endothelial $R_{\text{NO}}$ (0, 150, 300 μM/s) on NO and O$_2$ coupled transport. Note that increasing $R_{\text{NO}}$ enhances NO levels in tissue. Peak NO concentration always appears in the endothelium for all $R_{\text{NO}}$ values. Figure 2A also shows that increasing NO elevates tissue O$_2$, due to inhibition of O$_2$ consumption by NO. $P_{\text{O}_2}$ on the outermost surface of the tissue cylinder ($r = 120$ μm) at its half length falls below 0.5 Torr for $R_{\text{NO}} = 300$ μM/s.

Figure 3 shows how the presence or absence of RBCs in the plasma layer affects O$_2$ and NO distributions in surrounding tissue. The $\delta_p$ was varied from 1.0 to 2.0 μm in 0.5-μm increments (35). The radial $P_{\text{O}_2}$ profile adjacent to the lumen boundary ($13 < r < 20$ μm) and the radial NO profile ($0 < r < 120$ μm) with RBCs in the plasma layer are shown in Fig. 3, B and D, and without RBCs in Fig. 3, A and C. Note that $P_{\text{O}_2}$ levels in Fig. 3A are only slightly lower than in Fig. 3B, while NO levels in Fig. 3C are significantly higher than in Fig. 3D at each $\delta_p$. Figure 3 demonstrates that an increase in $\delta_p$ raises NO in the surrounding tissue and decreases $P_{\text{O}_2}$ adjacent to the vascular wall. We also calculated O$_2$ gradients across the endothelium and vascular wall as summarized in Table 2, using data from Fig. 3. These results show that O$_2$ gradients across the endothelium and vascular wall (15 μm < $r < 19$ μm) for all simulations are $-1.2$ Torr/μm. However, O$_2$ gradients from simulations with RBCs are slightly higher than without RBCs in the plasma layer.

Figure 4 demonstrates how mean $P_{\text{O}_2}$ and peak NO in the endothelium are affected by the presence or absence of RBCs in the plasma layer, by changes with the boundary thicknesses and $R_{\text{NO}}$, and by different blood velocity profiles. The mean endothelial $P_{\text{O}_2}$ is higher, but the peak endothelial NO is lower for simulations with RBCs compared with corresponding simulations in the absence of RBCs in the plasma layer. An increase in $\delta_p$ elevates mean endothelial $P_{\text{O}_2}$ and reduces peak endothelial NO. However, the decrease in NO is more pronounced than the rise in $P_{\text{O}_2}$. Raising $R_{\text{NO}}$ from 0 to 150 μM/s and further to 300 μM/s increases both $P_{\text{O}_2}$ and NO levels in the endothelium and plasma layer for all simulations. Mean endothelial $P_{\text{O}_2}$ is at least 1.0 Torr higher for plug flow than for parabolic flow simulations. However, peak endothelial NO obtained with the parabolic velocity profile simulations is nearly the same as the plug velocity profile simulations.

Figure 5, A and B, depicts the effect of varying $\delta_p$ on mean $P_{\text{O}_2}$ and peak NO in the endothelium for parabolic flow when $R_{\text{NO}} = 150$ μM/s. The glyocalyx was set to 0.5 μm. The $\delta_p$ was varied from 0 to 2.0 μm in 0.5-μm increments, keeping the sum of the $\delta_p$ and radius of the RBC-rich core constant. When the $\delta_p$ was set to zero, RBCs were assumed to be distributed uniformly inside the vessel lumen. This extreme case resulted in the highest mean $P_{\text{O}_2}$ and smallest peak NO compared with all other simulations. In all cases, the mean endothelial $P_{\text{O}_2}$ was higher for simulations with RBCs present than with RBCs absent in the plasma layer, and the presence of
RBCs reduces peak endothelial NO. We also performed a sensitivity analysis (Fig. 5C) to examine the effect of varying δₚ. Peak NO in the endothelial layer increased with thicker plasma layers, but mean PO₂ in the endothelial layer decreased only slightly. These results suggest that the NO distribution is much more sensitive than PO₂ to changes in the δₚ. Note that the presence of RBCs in the plasma layer attenuates the sensitivity of NO and O₂ distributions to variations in the δₚ.

Figure 6 shows the effect of changing glycocalyx thickness while holding the δₚ constant. Regardless of the presence or absence of RBCs in the plasma layer, an increase in glycocalyx thickness leads to a decrease in mean PO₂ (Fig. 6A) and increase in peak NO in the endothelial layer (Fig. 6B). Changes in mean PO₂ and peak NO vs. changes in the glycocalyx layer thickness are linear. Also note that the NO distribution is affected more by variations in glycocalyx thickness than the PO₂ distribution.

Figure 7 demonstrates how PO₂ and NO concentrations are affected by changes in the hematocrit profile. Although average hematocrit across the lumen for the three profiles was set to be the same, a change in the number of RBCs in the plasma layer affects O₂ and NO transport. The greater the number of RBCs in the plasma, the higher the O₂ and the lower the NO in tissue. The change in O₂ is less pronounced than that in NO.

**Fig. 2.** The influence of varying nitric oxide (NO) production rate (RNO) on radial PO₂ (A) and NO (B) in the middle of the 250-μm-long arteriole, with RBCs in a 2-μm plasma layer. The hematocrit profile is linear. The glycocalyx thickness is 0.5 μm, and plasma layer thickness is 2 μm. The blood flow velocity profile is parabolic, with a maximum magnitude of 1,500 μm/s at the vessel centerline. *Inset* in A: enlarged view of PO₂ near the outer radius. Vertical lines represent the interface between the plasma, glycocalyx, endothelium, vascular wall, and tissue.

**Fig. 3.** The effect of the presence (B and D) or absence (A and C) of RBCs in the plasma layer represented by linear and step hematocrit profiles on O₂ (A and B) and NO (C and D) transport when the plasma layer thickness was varied from 1.0 to 2.0 μm. Parameters used in the simulations were as follows: parabolic blood flow profile with maximum magnitude at centerline (1,500 μm/s); endothelial RNO = 300 μM/s; glycocalyx thickness = 0.5 μm. *Insets* in C and D: magnified view of tissue NO near the outer radius. The vertical lines represent the interface between the plasma, glycocalyx, endothelium, vascular wall, and tissue.
DISCUSSION

Our numerical simulations demonstrate the effect of nonuniform luminal RBC distribution, blood velocity profiles, and the effect of the glycocalyx (ESL) on NO and O2 concentration profiles inside and around an arteriole. The distribution of luminal RBCs is directly related to the O2-releasing ability of blood, NO scavenging rates in the blood, and blood velocity profiles. Our model quantifies how the presence of RBCs in the plasma layer increases release of O2 from blood and decreases NO in surrounding tissue. It also demonstrates how a blunter blood velocity profile enhances O2 delivery to tissue without causing noticeable disturbance to NO transport. Our computations also show how the glycocalyx influences NO and O2 transport.

Effect of Diffusion, Convection, and Consumption on Luminal NO Transport

Most endothelial-derived NO reaching the bloodstream is scavenged by RBCs, and the remainder is transported in the bloodstream. This process can be described by Eq. 20, in which the first term on the left represents radial diffusion, the second term axial diffusion, the third term convection, and the last term NO scavenging by RBCs. Examination of each term reveals that \( r^2/L^2 \) is small. Therefore, axial diffusion can be neglected, and Eq. 20 can be simplified to:

\[
\frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial C_{NO}^*}{\partial r} \right) - \frac{\partial}{\partial z} \left( \frac{\partial C_{NO}^*}{\partial z} \right) - \lambda C_{NO}^* = 0 \quad (21)
\]

The coefficient

\[
Pe = \frac{v/L}{D_{NO}/r^2}
\]

is the Pe, representing the ratio of axial convective to radial diffusive NO transport. The relative value of Pe suggests that the rate of axial convection of NO is five times lower than the rate of radial diffusion.

Influence of Blood Velocity Profiles on O2 Transport and NO

To estimate the effect of blood velocity profiles on NO/O2 transport, we compared two markedly different blood flow velocity profiles: one was an ideal parabolic shape (Poiseuille flow), and the other was a flat profile. Our results (Fig. 4) suggest that a blunter velocity profile can significantly increase O2 delivery to tissue, but has a negligible effect on NO transport. Because shear stress is determined by the blood velocity profile, the shape of the profile can affect NO synthesis by endothelial cells. Measurements by Mashour and Boock (23) from human endothelial cells cultured in an artificial capillary system suggest that there is a linear relationship

<table>
<thead>
<tr>
<th>Plasma Layer Thickness, ( \mu \text{m} )</th>
<th>Oxygen Gradient Across Endothelium and Vascular Wall, Torr/( \mu \text{m} ) Without RBCs</th>
<th>With RBCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>−1.193</td>
<td>−1.210</td>
</tr>
<tr>
<td>1.5</td>
<td>−1.173</td>
<td>−1.196</td>
</tr>
<tr>
<td>2.0</td>
<td>−1.153</td>
<td>−1.184</td>
</tr>
</tbody>
</table>

Blood flow is parabolic with maximum velocity = 1,500 \( \mu \text{m/s} \) at the centerline. Maximum nitric oxide production rate in the endothelial layer = 300 \( \mu \text{M/s} \). Glycocalyx thickness = 0.5 \( \mu \text{m} \), with the plasma layer thickness varying from 1.0 to 2.0 \( \mu \text{m} \). Hematocrit profile is linear in the plasma layer. RBC, red blood cell.
between NO production and shear stress. Shear stress-dependent production of NO was not considered in the present study, but should be investigated in future refinements of our model.

Diffusion Resistance of Glycocalyx to NO and O2 Transport

The ESL has been shown to affect blood flow resistance (28), mass transport (42), shear stress, and hematocrit. We evaluated how changes in the thickness of the ESL affect NO and O2 transport. An increase in ESL thickness for a vessel with a fixed radius reduces the lumen diameter and thus increases the tube hematocrit, enhancing NO scavenging in the central bloodstream. On the other hand, an increase in ESL thickness also increases resistance for NO diffusion toward the center of the lumen. The net result of these opposing effects is to decrease the amount of NO reaching the bloodstream, permitting more NO to diffuse into tissue. Currently, there are no experimental data to determine the permeability of NO and O2 in the ESL. Because we assumed that the ESL diffusion coefficients and gas solubilities are the same as in plasma, the effect may be more pronounced if ESL properties are different.

Comparison of O2 Transport Resistance in the Vascular Wall with Reports in the Literature

The O2 transport resistance of the microvascular wall has been studied by a number of investigators. Widely varying values of O2 gradients across the vascular wall have been reported. Using recessed-tip O2 microelectrodes, Duling et al. (9) measured luminal O2 tension and tissue O2 tension in the vicinity of the wall (vessel diameters from 22 to 230 μm) and reported a vascular wall PO2 gradient of 1.0 Torr/μm. Using arteriolar O2 flux values in the literature (5.69 × 10^{-5} ml O2·cm^{-2}·s^{-1}) and Fick’s law with DO2 = 1.390 μm²/s and α = 1.12 μM/Torr, Popel et al. (26) computed a value of 15 Torr/μm for the PO2 gradient across the wall. Tsai et al. (36) used a phosphorescence optical technology to measure axial PO2 in the vicinity of the vascular wall in an arteriole with a diameter of 23 μm and blood velocity of 1.5 mm/s and calculated a vascular wall PO2 gradient of 7 Torr/μm with an O2 flux of 2.7 × 10^{-5} ml O2·cm^{-2}·s^{-1}.

Our prediction for the vascular wall O2 gradient agrees with the value reported by Duling et al. (9), and our calculations of O2 flux from blood toward the vascular wall are in the same range as values from the literature tabulated by Vadapalli et al. (39). For a parabolic velocity profile in the presence of RBCs in the plasma (δp = 2 μm, glycocalyx thickness = 0.5 μm, and RNO = 150 μM/s), we calculated the O2 flux to be 1.34 × 10^{-5} ml O2·cm^{-2}·s^{-1} and O2 gradient in the middle of the vessel (z = 125 μm) to be 1.24 Torr/μm. This O2 gradient differs significantly from values reported by Popel et al. (26). One possible reason for this disagreement is the higher permeability
in PO2 across the vessel lumen, from 48.6 at the centerline to 0.5 m/s at the vessel centerline. The dash and dotted line represents the linear hematocrit profile in the plasma layer; the solid line presents the parabolic hematocrit profile in the plasma layer; and the dashed line presents no RBCs in the plasma layer.

Our current model is in agreement with Tsoukias and Popel (36) and Tsai et al. (36). Our model also predicts a large drop between our modeling results and those reported by Popel et al. (26) in the glycocalyx thickness profile in the plasma layer; and the dashed line presents no RBCs in the plasma layer.

Most previous models have not considered the effect of the nonuniform RBC profiles on NO and O2 transport (4, 21). The only approach that considered this phenomenon was a two-dimensional NO transport model proposed by El-Farra et al. (10) that evaluated four different scenarios. In the first scenario, RBCs were modeled as a homogeneous hemoglobin solution; in the second and third scenarios, RBCs were assumed to be discrete cells, distributed randomly in the lumen. The RBC membrane was assumed to allow NO to pass freely in the second scenario and to resist NO diffusion in the third scenario; the last scenario kept the same assumption as in the third scenario but added a RBC-free plasma layer near the wall with a thickness of ~5% of the vessel radius. The random distribution of RBCs in their model is similar to a uniform distribution when the hematocrit equals 45%, because “the NO distribution is essentially independent of the RBC placement due to the resulting tight packing of the RBCs” (10). Their results for the third and fourth scenarios suggested that the presence of RBCs reduced peak NO concentration in the endothelium by ~18%.

Our current model is in agreement with Tsoukias and Popel (38), who concluded that the difference in NO uptake between RBCs and free hemoglobin is small. We assumed that blood is a nonuniform hemoglobin solution with the NO scavenging in blood related to the hematocrit function. Our results from simulations with a similar δp as used by El-Farra et al. (10) show that the drop of peak NO due to the presence of RBCs in the plasma layer is not more than 10% for the linear hematocrit profile (Fig. 5). One possible reason for this difference may be our assumption that the number of RBCs in the plasma near the

Fig. 7. The influence of varying hematocrit profiles on radial PO2 (A) and NO (B) in the middle of the 250-μm-long arteriole. Endothelial RNO = 300 μM/s, the glycocalyx thickness = 0.5 μm, and plasma layer thickness = 1.5 μm. The blood flow velocity profile is parabolic with a maximum magnitude of 1,500 μm/s at the vessel centerline. The dash and dotted line represents the linear hematocrit profile in the plasma layer; the solid line presents the parabolic hematocrit profile in the plasma layer; and the dashed line presents no RBCs in the plasma layer. B, inset: magnified view of hematocrit profiles near the vessel wall. A, inset: magnified view of PO2 near the outer radius. Vertical lines represent the interface between the plasma, glycocalyx, endothelium, vascular wall, and tissue.

(9.54 × 10^{-10} \text{ml}\cdot\text{s}^{-1}\cdot\text{Torr}^{-1}\cdot\text{cm}^{-1}) used in our model compared with the value used by Popel et al. (4.2–6.8 × 10^{-10} \text{ml}\cdot\text{s}^{-1}\cdot\text{Torr}^{-1}\cdot\text{cm}^{-1}) (26). The presence of RBCs in plasma in our model, and coupled interaction between NO and O2, neither of which was included in previous models, are contributing factors that may account for these differences between our modeling results and those reported by Popel et al. (26) and Tsai et al. (36). Our model also predicts a large drop in PO2 across the vessel lumen, from 48.6 at the centerline to 33.6 Torr at the glycocalyx-endothelium interface. PO2 cannot be assumed to be uniform across the lumen, and thus measurements or calculations of blood/tissue PO2 gradients could be overestimated using an averaged intraluminal PO2.

Influence of Nonuniform RBC Profiles on NO and O2 Transport

RBCs in the bloodstream are the most important NO scavenger (2); therefore, the RBC profile may have a key influence on NO transport. Factors affecting the distribution of RBCs, for which there is paucity of information, include species, organs, as well as time. An important observation by Kobayashi and Takizawa (15) was that the RBC distribution in the vessel lumen is uneven. They noted that the edge close to the wall may contain RBCs. This agrees with the erythrocyte distribution curve by Prakash and Singh (27), who found that the erythrocyte cell population is almost constant in the center of a glass tube and decreases to zero almost linearly when the average hematocrit is high. Similarly, blood viscosity profiles obtained by Long et al. (22) in rat venules were approximately constant in the center of the vessel and decreased in an almost linear fashion near the vessel wall. They proposed a relationship between viscosity and hematocrit, which would imply a hematocrit profile similar to the viscosity profile across the vessel lumen (unpublished observations). Based on this information, we assumed a hematocrit function in our model to be constant in the RBC-rich region and to decrease in the plasma layer, reaching zero in the vessel wall.

We tested a linear and a parabolic hematocrit function for the plasma layer (inset in Fig. 7B). For the linear function, the average hematocrit is ~50% of that in the center of the vessel and is lower (~32%) for the parabolic function. As observed in Fig. 7, the concentration of NO in tissue is greater with the parabolic hematocrit profile than with the linear profile due to the reduced NO scavenging with a smaller number of RBCs in the plasma layer. Experimental results in the literature, while sparse, suggest a linear decrease in the distribution of RBCs near the vessel wall. Thus our linear profile may more closely mimic in vivo conditions.

A
vessel wall is less than in the lumen core. Another possible reason is that our model included coupled NO/O2 transport. The presence of RBCs in the plasma layer near the endothelium has significant effects on both O2 and NO transport. They provide an additional source of O2, with a small increase in O2 delivery from blood to tissue. Table 2 shows that the O2 gradient across the vascular wall can increase by 1.4 to 2.7% with the linear hematocrit profile in the plasma layer. Figure 4, A and B, also illustrates that the mean endothelial P02 for simulations with RBCs in the plasma layer is higher than without RBCs. The elevated availability of O2 in the endothelium also increases NO production. However, RBCs in the plasma layer also act as NO scavengers, with the net result that there is a significant decrease in tissue NO bioavailability (Fig. 3, C and D, and Fig. 4, C and D). However, NO in the vessel wall predicted from our simulations is still high enough to perform its physiological role, although it is reduced due to the presence of RBCs in the plasma layer. Mean NO in the vascular wall for RNO = 150 μM/s with RBCs in the plasma layer is ~180 nM, higher than the minimum concentration to activate sGC (30, 34). Our model also demonstrates that the higher concentration of RBCs in the lumen impairs diffusion of NO from the endothelium to the bloodstream and enhances diffusion of O2 from the bloodstream to tissue. For parabolic blood flow, RNO = 300 μM/s and δp = 2.0 μm (Fig. 4, C and D), peak NO in the endothelium decreased by 79.4 nM (21.5%), while mean P02 increased by only 0.6% in simulations with the presence of RBCs compared with the absence of RBCs in the plasma layer. In conclusion, NO scavenging by RBCs and convective transport in blood are the dominant factors determining radial NO distributions in blood and tissue, while axial diffusion is negligible. Whereas varying the shape of blood flow velocity profiles has a negligible effect on NO transport, a blunter NO distributions in blood and tissue, while axial diffusion is negligible. Whereas varying the shape of blood flow velocity profiles has a negligible effect on NO transport, a blunter NO scavenging by RBCs compared with the absence of RBCs in the plasma layer.

In conclusion, NO scavenging by RBCs and convective transport in blood are the dominant factors determining radial NO distributions in blood and tissue, while axial diffusion is negligible. Whereas varying the shape of blood flow velocity profiles has a negligible effect on NO transport, a blunter velocity profile can significantly enhance O2 delivery to tissue. Our results illustrate that the presence of RBCs in the plasma layer near the vessel wall has a significant effect on both NO and O2 transport. Our computer simulations demonstrate that the presence of RBCs in the plasma layer decreases NO availability, but enhances O2 availability to tissue, and that the effect on NO transport is much more pronounced than that on O2 transport.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant HL-068164 and National Science Foundation Grant BES 0301446.

REFERENCES