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Diffusive oxygen shunting between vessels in the preglomerular renal vasculature: anatomic observations and computational modeling

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1School of Computer Science and Software Engineering, The University of Western Australia, Perth, Australia; 2Department of Physiology, Monash University, Melbourne, Australia; 3Department of Pharmacology, Monash University, Melbourne, Australia; and 4Department of Anatomy and Developmental Biology, Monash University, Melbourne, Australia

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Gardiner BS, Thompson SL, Ngo JP, Smith DW, Abdelkader A, Broughton BR, Bertram JF, Evans RG. Diffusive oxygen shunting between vessels in the preglomerular renal vasculature: anatomic observations and computational modeling. Am J Physiol Renal Physiol 303: F605–F618, 2012. First published June 6, 2012; doi:10.1152/ajprenal.00186.2012.—To understand how geometric factors affect arterial-to-venous (AV) oxygen shunting, a mathematical model of diffusive oxygen transport in the renal cortex was developed. Preglomerular vascular geometry was investigated using light microscopy (providing vessel shape, AV separation, and capillary density near arteries) and published micro-computed tomography (CT) data (providing vessel size and AV separation; Nordsletten DA, Blackett S, Bentley MD, Ritman EL, Smith NP. IUPS Physiome Project. http://www.physiome.org.nz/publications/nordsletten_blackett_ritman_bentley_smith_2005/folder_contents). A “U-shaped” relationship was observed between the arterial radius and the distance between the arterial and venous lumens. Veins were found to partially wrap around the artery more consistently for larger rather than smaller arteries. Intraarterial arteries were surrounded by an area of fibrous tissue, lacking capillaries, the thickness of which increased from ~5 μm for the smallest arteries (<16-μm diameter) to ~20 μm for the largest arteries (>200-μm diameter). Capillary density was greater near smaller arteries than larger arteries. No capillaries were observed between wrapped AV vessel pairs. The computational model comprised a single AV pair in cross section. Geometric parameters critical in renal oxygen transport were altered according to variations observed by CT and light microscopy. Lumen separation and wrapping of the vein around the artery were found to be the critical geometric factors determining the amount of oxygen shunted between AV pairs. AV oxygen shunting increases both as lumen separation decreases and as the degree of wrapping increases. The model also predicts that capillaries not only deliver oxygen, but can also remove oxygen from the cortical parenchyma close to an AV pair. Thus the presence of oxygen sinks (capillaries or tubules) near arteries would reduce the effectiveness of AV oxygen shunting. Collectively, these data suggest that AV oxygen shunting would be favored in larger vessels common to the cortical and medullary circulations (i.e., arcuate and proximal interlobular arteries) rather than the smaller vessels specific to the cortical circulation (distal interlobular arteries and afferent arterioles).

Fifty years ago Levy et al. (17, 18) provided evidence that oxygen is transported more rapidly than erythrocytes through the renal circulation, suggesting the existence of diffusional oxygen shunting between intrarenal arteries and veins. Shunting reduces the delivery of oxygen to renal tissue (26, 27). In further support of the existence of arterial-to-venous (AV) oxygen shunting, Welch et al. (29) observed that the PO2 of renal venous blood exceeds that of blood in the efferent arterioles of the outer cortex. In common with other countercurrent systems in biology and chemical engineering, it is thought that the parallel architecture of the renal artery and vein facilitates countercurrent oxygen exchange. It has also been noted by several groups (8, 23, 24, 26) that the arteries and veins in the kidney are intimately associated, and we will later show that the available data suggest this intimacy varies with distance along the cortical circulation. Despite general acceptance of the existence of AV oxygen shunting in the kidney, little is known about the effects of AV oxygen shunting on renal parenchymal oxygenation or the quantity of oxygen shunted at the various levels of the renal circulation (e.g., interlobar, arcuate, and interlobular arteries). In this paper, we focus on a quantitative analysis of diffusive oxygen shunting between vessels in the preglomerular renal vasculature of the rat.

Gardiner et al. (14) developed a one-dimensional (1D) mathematical model of AV oxygen shunting along the preglomerular renal vasculature. This model has a hierarchy of 11 countercurrent systems in series, representing the various levels of branching of the preglomerular renal vasculature described by Nordsletten et al. (22, 23). Reactive-advection equations were solved at each branch level. The model predicted that AV oxygen shunting is quantitatively significant, being of the same order of magnitude as total kidney oxygen consumption. However, this model has several limitations, the most important being that the cross-sectional geometry of typical vessel pairs at each branch level was unknown. To overcome this lack of information, the model (14) used a single “shunting coefficient” between arteries and veins that was calibrated using the measurements made by Welch et al. (29) for PO2 within microdomains of the rat superficial cortex. Our current study focuses on clarifying how the cross-sectional geometry of typical vessel pairs at each branch level affects the amount of AV oxygen shunting occurring along the preglomerular renal vasculature.

Clearly, the cross-sectional geometry of the renal vasculature is of critical importance for the quantification of AV oxygen shunting. We hypothesized that the two critical geometric factors were the mean distance between the arterial and venous wall (so-called “lumen separation”) and the degree to
The model was then employed for a series of “computational experiments” to assess how variations, typically observed or expected in the kidney based on data from light microscopy and micro-CT, influence AV oxygen shunting. We tested the specific hypotheses that oxygen shunting in the preglomerular vasculature is critically dependent upon 1) the distance separating arteries and veins, 2) the degree to which the venous wall wraps around the arterial wall, and 3) the presence of capillaries and tubules, which act as oxygen sinks, around and between artery-vein pairs. We have quantified the relative oxygen flux from artery to vein vs. the flux from these vessels to the surrounding cortical parenchyma.

METHODS

Histological Studies

All experimental procedures were approved by the Animal Ethics Committee of the School of Biomedical Sciences, Monash University (reference 2009/84) and were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Sprague-Dawley rats (7 males and 1 female, 250–300 g) were anesthetized with pentobarbital sodium (60 mg/kg ip; Sigma, St Louis, MO). The kidneys of seven of these rats (including the female) were perfused via retrograde perfusion of the abdominal aorta at 150 mmHg. Perfusion commenced with a 0.1 M phosphate-buffered saline solution (pH 7.4) containing 0.2 mg/ml lidocaine (Xylocard 2000, Astra Pharmaceuticals, North Ryde, NSW, Australia). Once the kidneys were blanched (~60 s), they were perfused with ~300 ml of ice-cold 3% wt/vol parafformaldehyde in 0.1 M phosphate-buffered saline. In four rats, the vasculature was then filled with Microfil (MV-122; Flow Tech, Carver, MA) by slowly injecting 20–30 ml of the compound via the abdominal aorta. The 14 kidneys were decapsulated and postfixed in parafformaldehyde overnight before being placed in 70% vol/vol ethanol. In a single male rat, a hindlimb skeletal muscle (biceps femoris) was fixed by orthograde perfusion of the abdominal aorta, in an analogous manner to renal perfusion. The six kidneys in which Microfil was not injected were divided and embedded in paraffin, while the eight Microfil-treated kidneys were embedded in methacrylate (technovit 3040 resin). In all cases, the kidneys were embedded to provide cross sections (n = 81), indicating an image slice perpendicular to the

Table 1. Variables used in the formulation of the diffusive oxygen transport model

<table>
<thead>
<tr>
<th>Description</th>
<th>Name</th>
<th>Value/Expression</th>
<th>Units</th>
<th>Source/Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfusion constant</td>
<td>α&lt;sub&gt;cap&lt;/sub&gt;</td>
<td>−15</td>
<td>s&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td>Venous PO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>PO&lt;sub&gt;2&lt;/sub&gt;&lt;sub&gt;V&lt;/sub&gt;</td>
<td>52</td>
<td>mmHg</td>
<td>29</td>
</tr>
<tr>
<td>Tissue PO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>PO&lt;sub&gt;2&lt;/sub&gt;&lt;sub&gt;cap&lt;/sub&gt;</td>
<td>42</td>
<td>mmHg</td>
<td>29</td>
</tr>
<tr>
<td>Arterial PO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>PO&lt;sub&gt;2&lt;/sub&gt;&lt;sub&gt;A&lt;/sub&gt;</td>
<td>m&lt;sup&gt;3&lt;/sup&gt; PO&lt;sub&gt;2&lt;/sub&gt;&lt;sub&gt;V&lt;/sub&gt;</td>
<td>mmHg</td>
<td>29</td>
</tr>
<tr>
<td>Oxygen consumption rate</td>
<td>S&lt;sub&gt;O&lt;/sub&gt;</td>
<td>−0.1</td>
<td>mol·s&lt;sup&gt;−1&lt;/sup&gt;·m&lt;sup&gt;−3&lt;/sup&gt;</td>
<td>14</td>
</tr>
<tr>
<td>Diffusion coefficient of oxygen</td>
<td>D</td>
<td>2.8 × 10&lt;sup&gt;−9&lt;/sup&gt;</td>
<td>m&lt;sup&gt;2&lt;/sup&gt;/s&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>6</td>
</tr>
<tr>
<td>Arterial radius</td>
<td>R&lt;sub&gt;A&lt;/sub&gt;</td>
<td>75 × 10&lt;sup&gt;−6&lt;/sup&gt;</td>
<td>m</td>
<td>6</td>
</tr>
<tr>
<td>Solubility coefficient of oxygen</td>
<td>σ</td>
<td>1.34 × 10&lt;sup&gt;−3&lt;/sup&gt;</td>
<td>mol·m&lt;sup&gt;−3&lt;/sup&gt;·mmHg&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>14</td>
</tr>
<tr>
<td>Hill coefficient</td>
<td>n</td>
<td>4</td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>Ratio of arterial to venous PO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>m</td>
<td>1.6</td>
<td></td>
<td>29</td>
</tr>
</tbody>
</table>
arterial wall, for a quantitative analysis of the radial geometry of arteries and veins. The minimum distance between arterial and venous wall (lumen separation), in each case, was determined using ImageJ software (http://rsbweb.nih.gov/ij/).

In addition, for the eight Microfil-treated kidneys, the number of capillaries per unit image area (so-called “capillary density”) within four zones, defined by concentric circles around the center of the artery, was determined. The radii of these zones were increased with vessel size. That is, for arteries <16.0 μm in diameter (n = 12), these zones were defined by steps of 5 μm from the arterial wall, so that capillary density was determined at 0–5, 5–10, 10–15, and 15–20 μm from the arterial wall. For arteries 16.0–25.9 (n = 10), 26.0–50.9 (n = 8), 51.0–100.9 (n = 7), 101–200 (n = 9), and >200 μm (n = 3) in diameter, the zones were defined by steps of 10, 15, 20, 25, and 30 μm from the arterial wall, respectively. These data were analyzed by repeated measures analysis of variance (19). The between-subjects factor was vessel category, and the within-subjects factor was zone.

Analysis of Micro-CT Data Generated by Nordsletten and Colleagues

Nordsletten et al. (23) presented an analysis of the three-dimensional (3D) quantitative morphology of the rat renal circulation based on the Radial Dispersion of a Nonionic Contrast Agent Injected Intravenously (24). The study involved injecting a nonionic contrast agent into the renal artery and analyzing the radial dispersion of the agent using micro-CT imaging. The micrographs show different arrangements of arteries (A) and veins (V) in the renal cortex.
are the nodal (on 20-μm resolution CT data (Fig. 1). The 3D geometrical model generated by Nordsletten et al. (22) was obtained and analyzed in this study. This model consists of a series of points in 3D space (nodal points) connected by straight lines (elements). The nodal points belong to one of two networks, either the arterial tree or the venous tree. A Perl script was written so that for each nodal point in the arterial network, its closest neighbor in the venous network was found. Specifically, the distance between arterial and venous nodes, \(d\), is calculated according to Eq. 1

\[
d = \sqrt{(Ax - Vx)^2 + (Ay - Vy)^2 + (Az - Vz)^2}
\]

where \(d\) is distance between arterial and venous nodes, \((Ax, Ay, Az)\) are the nodal \((x, y, z)\) coordinates of the arterial node, and \((Vx, Vy, Vz)\) are the nodal \((x, y, z)\) coordinates of the venous node.

Nordsletten et al. (23) found that arteries are likely to pair with veins typically two orders higher; therefore, in this analysis a condition was set to find the closest vein within two Strahler orders of the artery. Once the closest vein had been found for each artery, the following parameters were extracted and calculated: the ratio of the arterial to venous radius, arterial diameter, venous diameter, lumen separation, and the ratio of the lumen separation to arterial diameter.

**Computational Model**

Here, we developed computational models of oxygen transport in the preglomerular renal vasculature based on the well-known diffusion equation (2). Parameters in the models such as the diffusion coefficient and oxygen consumption are appropriate for a volume of tissue contained in a low-magnification view of the renal parenchyma. That is, they represent a smoothing over of a small volume of tissue such that the discrete structures are averaged out. Vessel arrangements were based on the light microscope and micro-CT data obtained as described above. Each model consisted of a cross-sectional view of an artery and a vein lying parallel to each other. The arterial and venous radii corresponded to average vessel dimensions from analysis of micro-CT of vascular casts of the rat renal cortical circulation, reported by Nordsletten et al. (23).

Two dimensional (2D) steady-state oxygen transport by diffusion within a cross section of renal tissue (i.e., regions broadly characterized as everything other than blood vessels) can be described mathematically using the well-known diffusion equation

\[
D \left( \frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} \right) = S
\]

where, \(c\) is the concentration of free oxygen defined by \(c = \sigma \text{PO}_2\), where \(\sigma\) is the solubility coefficient of oxygen and \(\text{PO}_2\) is the partial pressure of oxygen. \(D\) is the effective “average” diffusion coefficient within this heterogeneous tissue, and \(S\) describes oxygen production or loss per volume of tissue (e.g., if \(S\) refers to an oxygen loss, then \(S < 0\)). The derivatives are with respect to spatial coordinates \((x, y)\). In this study, it was assumed that \(D\) is not a function of \((x, y)\) within any defined tissue regions (i.e., \(D\) is spatially constant).

It was assumed that there are two contributions to the term \(S\) in Eq. 2. The first is consumption of oxygen per volume of tissue, corresponding to kidney \(\dot{V}_O_2\), and was given the symbol \(S_0\). The second contribution comes from a potential source (or sink) of oxygen offered by capillaries, depending on any concentration difference between \(c\) and an assumed concentration within the capillaries, i.e., \(c_{\text{cap}}\). That is, oxygen may be lost or gained by the tissue through transport along the capillaries. We did not model this capillary transport explicitly. Rather, we treated it as a spatially homogenized contribution to \(S\) which is proportional to \((c_{\text{cap}} - c)\), with a proportionality constant \(\alpha_{\text{cap}}\). Specifically,

\[
S = S_0 - \alpha_{\text{cap}}(c_{\text{cap}} - c)
\]

**Boundary conditions.** Boundary conditions are required to solve Eq. 2 in each model. Here, it was assumed that oxygen concentration at the vessel wall is fixed, with \(c = c_{\text{wall}}\) on the arterial wall and \(c = c_{\text{v}}\) on the venous wall. That is, we assumed that oxygen is radially well mixed within each vessel such that there are no oxygen concentration gradients within each vessel. The vessel pair was assumed to be sitting at the center of a square of tissue, the boundaries of which were assigned a zero-flux boundary condition, consistent with a vessel-tissue organization that it spatially repeated. The width of the tissue under consideration was assumed to be 20 times the arterial radius, based on our light imaging results.

![Capillary density in concentric zones around arteries of various calibres.](http://ajprenal.physiology.org/)

The concentric zones were in steps of 5, 10, 15, 20, 25, and 30 μm from the arterial wall, respectively, for arteries <16 μm, 16–25.9 μm, 26–50.9 μm, 51–100.9 μm, 101–200 μm, and >200 μm in diameter. Values are means ± SE. The number of observations (\(n\)) for each arterial vessel class is shown in the figure. Statistical analysis of these data, using repeated measures analysis of variance (19), showed that capillary density around arteries varied significantly according to the arterial caliber (\(P_{\text{vessel}} = 0.003\)) and across the various concentric zones (\(P_{\text{zone}} = 0.03\)).
**Parameter selection**. Values used for the model parameters and their sources are shown in Table 1, unless otherwise stated. Welch et al. (29), using Clark-type microelectrodes, measured in vivo the renal venous and tissue PO₂. They reported a renal venous PO₂ of 52 mmHg for a known renal blood flow of ~5 ml/min with an arterial PO₂ of 85 mmHg. Furthermore, the average tissue PO₂ in the superficial cortex, the most oxygenated part of the kidney, was found to be 42 mmHg (referred to here as the tissue PO₂).

The diffusion coefficient of oxygen has been reported in the literature for a variety of mediums and tissues, from water to plasma, arterioles, and smooth muscle, etc. (4, 5, 9, 11, 20). Typically, the values fall within the range of 1.2 × 10⁻⁹ to 3.1 × 10⁻⁹ m²/s. Here, a value of 2.8 × 10⁻⁹ m²/s was chosen to be consistent with that used by Chen et al. (6) in their mathematical model of the rat outer medulla.

In the Wistar-Kyoto rat kidney, VO₂ per weight of renal tissue has been reported to be 7.6 μmol-min⁻¹·g⁻¹ (29). Assuming a tissue density of 1 g/m³, we then estimate S₉₀ to be ~0.1 mol·s⁻¹·m⁻³.

The mass transfer coefficient αₜₐ₉ for oxygen transport through the capillary wall is unknown but can be estimated. For tissue far from the capillary wall, the oxygen consumption by the tissue must be provided by the capillaries. In this case, from Eq. 3, \( S_0 = \alpha_{\text{cap}} (c - c_\text{cap}) \). If the difference between the PO₂ in the capillary and the tissue is in the range 1 to 10 mmHg, then this corresponds to \( \alpha_{\text{cap}} \) varying between ~80 and ~8 s⁻¹, respectively. Here, a value of \( \alpha_{\text{cap}} = -15 \text{ s}^{-1} \) was chosen and corresponds to a PO₂ difference of 5 mmHg between the capillary and the average value in the tissue.

Arterial diameter was selected based on our analysis of the model of Nordsletten et al. (22), in which an artery of Strahler order 5, typically considered an arcuate artery (23), had an average diameter of 150 μm.

All models were implemented in the commercial simulation package COMSOL (version 4.2) and solved as steady-state problems using a "parameter sweep" on a Dell PC with an Intel Core 2.67-GHz CPU running Windows 7 Enterprise. Diffusive transport in the renal cortex was solved as steady-state problems with a "parameter sweep" on a Dell PC with an Intel Core 2.67-GHz CPU running Windows 7 Enterprise. For the capillary network, the mass transfer coefficient \( \alpha_{\text{cap}} \) was chosen and corresponds to a PO₂ difference of 5 mmHg between the capillary and the average value in the tissue.

**RESULTS**

**Light Microscopy**

A total of 200–250 intrarenal arteries were surveyed. The relationships between arteries and veins varied markedly throughout the rat kidney. In the case of larger arteries (e.g., interlobar arteries ~200–300 μm in diameter), the corresponding vein tended to surround a considerable proportion of the total arterial circumference, with a typical lumen separation of <100 μm (Fig. 2A). A similar arrangement was observed for vessels presumed to be arcuate arteries (~200-μm diameter; Fig. 2B). In both cases (interlobar and arcuate arteries), the space between the artery and vein walls was mostly filled with loose connective tissue, with a notable absence of capillaries and tubules. In the case of smaller artery-vein pairings (<150-μm diameter), presumed to be mainly interlobular arteries and glomerular arterioles, a less intimate relationship was often observed (Fig. 2, C–F). That is, veins less often “wrapped” around the artery, and the space between the artery and vein was often filled by tubular elements and/or capillaries. However, there were some exceptions in which smaller arteries were found to be closely associated with veins, as for larger vessels. In biceps femoris, although arteries and veins were sometimes found in close proximity, veins were never found to wrap arteries (Fig. 2, G and H).

For these quantitative analyses, all artery-vein cross sections in which the artery approached a circular shape, indicating a section approximately perpendicular to the artery, were included, while those in which the artery appeared oval shaped were excluded. Of the 81 profiles examined from kidney tissue, in all but 3 the distance between the artery and vein wall was <200 μm (Fig. 3A). Forty percent of the profiles showed wrapping of the vein around the artery. The ratio of lumen separation to arterial radius averaged 0.5 ± 0.1 in the wrapped vessels, compared with 5.7 ± 1.0 in the nonwrapped vessels pairs, indicating that oxygen diffusion should be favored between wrapped vessels rather than those where wrapping was not present.

In many of the light microscope images, an area of loose connective tissue surrounded each artery, as has been described previously by Frank and Kriz (13) (Fig. 2). The minimum thickness of this area varied with arterial diameter, being 6.6 ± 0.7 μm for arteries <16.0 μm in diameter, 6.8 ± 0.9 μm for arteries 16.0–25.9 μm in diameter, 8.1 ± 1.0 μm for arteries 26.0–50.9 μm in diameter, 11.1 ± 1.4 μm for arteries 51.0–100.9 μm in diameter, 18.5 ± 1.5 μm for arteries 101–200 μm in diameter, and 22.2 ± 2.9 μm for arteries >200 μm in diameter.

Peritubular capillary density in the zones surrounding the arterial wall progressively reduced with increasing arterial diameter (Pvessel = 0.003). Peritubular capillary density also appeared to be less in the zones immediately surrounding vessels compared with those further away, at least in the case of vessels <16 μm, 26.0–50.9 μm, 101–200 μm, and >200 μm (Pzone = 0.03). The pattern of changes in capillary density, across the four zones, differed significantly according to vessel caliber (Pvessel+zone < 0.001) (Fig. 4).

**Table 2. Results from the mathematical analysis of the 3D (micro-CT) model of rat renal vasculature of Nordsletten et al. (22)**

<table>
<thead>
<tr>
<th>Strahler</th>
<th>Dₐ, μm</th>
<th>LS, μm</th>
<th>Rᵥ/RA</th>
<th>LS/RA</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>58.1</td>
<td>474.0</td>
<td>1.8</td>
<td>13.6</td>
<td>2.1</td>
<td>14.8</td>
<td>18.2</td>
<td>19.6</td>
<td>32</td>
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<tr>
<td>3</td>
<td>80.4</td>
<td>177.0</td>
<td>1.8</td>
<td>6.4</td>
<td>0.2</td>
<td>2.5</td>
<td>8.3</td>
<td>14.7</td>
<td>1,803</td>
</tr>
<tr>
<td>4</td>
<td>102.8</td>
<td>90.4</td>
<td>1.8</td>
<td>3.8</td>
<td>-0.1</td>
<td>1.1</td>
<td>3.9</td>
<td>10.5</td>
<td>2,103</td>
</tr>
<tr>
<td>5</td>
<td>150.7</td>
<td>45.3</td>
<td>1.8</td>
<td>0.9</td>
<td>-0.3</td>
<td>0.4</td>
<td>0.9</td>
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<td>768</td>
</tr>
<tr>
<td>6</td>
<td>263.7</td>
<td>44.1</td>
<td>1.8</td>
<td>0.4</td>
<td>-0.2</td>
<td>0.2</td>
<td>0.5</td>
<td>1.3</td>
<td>429</td>
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<tr>
<td>7</td>
<td>325.6</td>
<td>115.3</td>
<td>2.7</td>
<td>0.9</td>
<td>0.0</td>
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<td>1.0</td>
<td>2.3</td>
<td>116</td>
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<tr>
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<td>317.4</td>
<td>189.4</td>
<td>4.4</td>
<td>1.3</td>
<td>0.0</td>
<td>0.6</td>
<td>1.4</td>
<td>2.6</td>
<td>9</td>
</tr>
</tbody>
</table>

3D, three-dimensional; CT, computed tomography; Dₐ, median arterial diameter; LS, median lumen separation; Rᵥ/RA, ratio of venous to arterial radius; LS/RA, average ratio of lumen separation to arterial radius defined over all vessels within an order (Overall) and over each quartile in an order (Q1–Q4); n is the number of observations at each order. See text for further details.
Quantification of Spatial Variations in the Renal Preglomerular Vasculature

A mathematical analysis was conducted of the micro-CT model of the rat renal vasculature (Table 2) by Nordsletten et al. (22). The center-to-center distance between arterial and venous vessels in the model, along with lumen separation, was calculated. First, across each Strahler order, the lumen separation of an artery-vein pair decreased with increasing diameter, to a point (Strahler order 7) where it then started to increase. Prima facie, this suggests that the quantity of AV shunting per vessel pair will vary along the various levels of branching. This U-shaped relationship was also observed with the light microscopic data set (Fig. 3). This relationship points to a structural variation in which vessel pairs with an intermediate arterial diameter of 150–350 μm tend to consistently have a smaller lumen separation than larger or smaller vessels. It is noteworthy that some data points (Fig. 3B) have a negative value for lumen separation. This is because the data we used in this analysis were not raw data but instead came from an existing model where an equivalent vessel radius was assigned to each vessel node according to the vessel cross-sectional area observed in micro-CT images. Importantly, the negative values indicate that there was some degree of overlap or wrapping between the vessels.

The next step was to look more closely at the distribution within each Strahler order. For each Strahler order, the median, lower, and upper quartiles for lumen separation were determined. This analysis demonstrated that although vessels in the first three quarters of orders 5–7 had a wide range of size (median arterial diameter 150.7–325.6 μm) and separation (median lumen separation 44.1–115.3 μm), they had an average lumen separation to arterial radius ratio of 1.0 or less (Table 2).

Computational Results

Idealized geometries. The imaging studies conducted here have quantified the spatial associations of arteries and veins at...
various discrete locations within the preglomerular network. A number of different vessel arrangements were observed in the histological images, including circular veins, elliptical veins, and veins that partially wrap around the artery they paired with. Our analysis provided information on vessel pair separation for a range of vessel sizes. Based on these two sources of information, we created two representative configurations of vessel pairs for our subsequent computational modeling. The first contains a circular artery and vein (“circular” geometry) with the venous radius set to 1.8 times that of the arterial radius, as determined from our analysis of Nordsletten’s micro-CT model (Table 2). The wrapped geometry comprised a circular artery with a vein wrapped around 50% of its circumference. Due to the difficulty in defining the radius of this wrapped vein, its perimeter was set to be equal to that of the vein in the idealized circular geometry. Lumen separation for both models was initially set at nine-tenths of the arterial radius, as this was the average lumen separation calculated for a 5th order artery (Table 2). From a qualitative analysis of histological images, it was noted that the separation between adjacent AV pairs was <20 times the arterial radius ($R_A$). Thus the representative volume in which a single artery-vein pair was considered had length of $20R_A$. Unless otherwise stated, all simulations used these geometric parameters.

The diffusion equation was solved for the two idealized geometries to predict the steady-state distribution of oxygen through the tissue. The concentration of oxygen dropped off rapidly with distance from the vessels (Fig. 5). The diffusive flux normal to the surface of each vessel was numerically integrated around the boundary of each vessel and used to calculate AV flux (shunting), artery-to-tissue (AT) flux, and vein-to-tissue (VT) flux. Specifically, total oxygen flux directed into the vein provides AV flux. The oxygen flux directed out of the vein was VT flux. Subtracting the AV flux from the total oxygen flux from the artery provides the AT flux. The idealized models showed that AT flux (circular = 128 nmol·m$^{-1}$·min$^{-1}$, wrapped = 149 nmol·m$^{-1}$·min$^{-1}$) was about twice that of VT flux (circular = 66 nmol·m$^{-1}$·min$^{-1}$, wrapped = 78 nmol·m$^{-1}$·min$^{-1}$), but that no shunting was predicted to occur in either configuration (AV flux = 0 nmol·m$^{-1}$·min$^{-1}$) (Fig. 5). That is, these standard configurations, based on median values observed in the images predict that AV shunting is not significant. Assuming that AV shunting is significant, this result suggests that the majority of shunting is occurring in a subset of vessels in which the conditions (e.g., degree of wrapping, lumen separation) are more favorable and/or the computational model is poorly parameterized or incomplete. Therefore, our attention turned to the impact of the observed absence of capillaries and tubules between and around the AV pair.

Oxygen sinks around intrarenal arteries. Our histological analysis demonstrated that an area of loose connective tissue, lacking capillaries or tubules, immediately surrounds intrarenal arteries. Furthermore, in vessel pairs in which the vein partially wrapped the artery, neither capillaries nor tubules were seen in the space between the two vessels. In addition, peritubular capillary density around arteries $\geq 26 \mu$m in diameter was relatively low. This indicates that there is a zone around arteries, and particularly between closely associated AV pairs (the “exclusion zone”) relatively devoid of oxygen sinks. From our simulations (Fig. 5, A and B), we know that $P_{O_2}$ is relatively high between and near the AV pair. In development, hypoxia is known to induce vasculogenesis and angiogenesis (7), whereas hyperoxia inhibits angiogenesis. We therefore made the model assumption that the observed lack of capillaries is related to $P_{O_2}$ and simulated this zone using a numerical “switch” in the oxygen source/sink term, $S$, of Eq. 2. The switch operated so that when the oxygen concentration of tissue is high (such as in the immediate vicinity of the blood vessels), oxygen removal (either by metabolic consumption or by capillaries) is shut down, creating the exclusion zone. Further from the vessels, the oxygen concentration decreases, and this effect is inhibited, allowing capillaries to supply/remove oxygen in addition to the metabolic consumption of oxygen from kidney $V_{O_2}$.

![Fig. 7. Effect of applying (CS) or not applying (NCS) the capillary switch on arterial-to-venous (AV) shunting (A), vein-to-tissue (VT) flux (B), and arterial-to-tissue (AT) flux (C) is shown as arterial radius changes in the circular and wrapped idealized configurations. Note different scales for flux.](http://ajprenal.physiology.org/)
In Eq. 4, \(c_{\text{cap}}\) is the oxygen concentration at which the oxygen sink has been reduced by 50%, and the parameter \(n\) changes the oxygen sensitivity of the switch. We set \(n = 4\), to have a relatively sharp change in the oxygen sink, reflecting the observed distinct region of connective tissue devoid of capillaries and tubules. We also assumed that \(c_{\text{cap}} = 0.056 \text{ mol/m}^3\) (corresponding to a \(\text{PO}_2\) of 42 mmHg) to have the oxygen sink transition to occur at the average tissue \(\text{PO}_2\) recorded by Welch et al. (29) of 42 mmHg. Qualitatively, we can see how this capillary switch (CS) creates an exclusion zone around the vessels (Fig. 6). In both the wrapped and circular geometries, the effect of including the CS was compared with the idealized scenario in which consumption is always active, i.e., no capillary switch (NCS). It was found that AV shunting was greater over a wider range of arterial radii when the exclusion zone was incorporated, by use of the CS, than in the idealized NCS geometries (Fig. 7). That is, a lack of oxygen consumption between the AV pair aided shunting efficiency.

These findings, from both imaging and computational predictions, led to the incorporation of the CS into the model for both circular and wrapped geometries. Parametric studies varying lumen separation, \(\text{PO}_2\), arterial radius, and wrapping were then conducted. The geometries used in these analyses, along with corresponding fields of \(\text{PO}_2\) and oxygen flux, are shown in Figs. 8 and 9. Note that the geometries we used in the parametric analysis included more than just the typical geometries observed by light microscopy, so that we could examine the impact of specific features of vessel geometry (e.g., degree of wrapping, vein aspect ratio, etc.). The results of the parametric studies are discussed below.

**Length scale.** Simulations were performed for the circular and wrapped geometries for a range of arterial radii, while keeping the relative proportions (e.g., vein radius and lumen separation) constant to evaluate the influence of length scale on various fluxes (Figs. 10 and 11; also, see Fig. 13). In all cases, AV shunting decreased and VT flux and AT flux increased, as the length scale \((R_A)\) increased. The gradient in oxygen concentration was less steep for smaller length scales than larger (see Fig. 8, \(K-M\)), with oxygen moving further into the tissue before being removed by the oxygen sink.

**Lumen separation.** Due to the large variation observed in lumen separation (Table 2), it was important to determine how this parameter affected AV shunting. We found that when the lumen separation between the artery and vein was reduced to one-tenth of the arterial radius, the flux between the artery and vein increased, particularly in the wrapped configuration (Figs.

\[
\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} = \left( \frac{S}{D} \right) \left( \frac{1}{1 + \left( \frac{c}{c_{\text{cap}}} \right)^n} \right)
\]
Conversely, the flux from the vessels to the tissue (VT flux and AT flux) decreased as lumen separation was reduced (Fig. 10, B, C, E, and F). AV shunting was compared between the circular and wrapped configurations across a range of lumen separations. Results show the same qualitative patterns of change in flux between the two configurations. However, in the wrapped configuration AV shunting was about five times greater than the circular configuration across all lumen separations. This was due to a longer “contact” surface area between the artery and the vein in the wrapped configuration compared with the circular configuration. This finding indicates that the closeness of the vessels as well as their spatial arrangement is critically important in facilitating AV shunting. For the smallest lumen separation, AV shunting was largely independent of length scale, particularly in the case of wrapping. This is because the 2D diffusion begins to be approximated by a 1D diffusion model, particularly in the case of wrapping, and the arterial radius dependence of vessel surface area is cancelled by the arterial radius dependence of separation distance, such that the AV flux is independent of the arterial radius and length scale.

9, C–F, and 10, A and D). The mathematical analysis of Nordsletten’s micro-CT model (22) showed that the ratio of the venous to arterial radius ($R_v/R_A$) increases with vessel size. Therefore, $R_v/R_A$ was altered in the circular configuration, with constant arterial radius, to determine the effect of this ratio (Fig. 11). Increasing $R_v/R_A$ increased AV shunting in the model and VT flux, but had little effect on AT flux.

Radius. We hypothesized that the degree to which the vein wall wraps around the arterial wall profile greatly influences the amount of AV shunting. Therefore, diffusion was solved in six different vessel configurations where the spatial association between artery and vein changed (Fig. 12). The first orientation was a vein wrapped 75% around an artery (Fig. 9 J). The second orientation was one of our idealized geometries with the vein wrapped 50% around the artery (Fig. 9 E). The third orientation was similar to the first but with a lesser degree of wrapping; the vein only wrapped around 25% of the artery (Fig. 9 I). The fourth orientation had an elliptical vein, with its long edge parallel to the circular artery (Fig. 9 H). The fifth orientation also had an elliptical vein, but with its short edge parallel to the circular artery (Fig. 9 G). The sixth orientation was a circular vein and artery (Fig. 9 C). All configurations were investigated with a 0.1$R_A$ lumen separation and with an arterial radius of 75 $\mu$m. When moving from the wrapped to circular arrangement (i.e., as the degree of wrapping decreased), AV shunting decreased...
significantly, while AT flux and VT flux increased slightly (Fig. 12). AV shunting in the 25% wrapped vessel was about one-third that in the 75% wrapped vessel, indicating a roughly linear relationship between the proportion of wrapping and AV shunting.

**AV PO2 ratio.** The measurements of PO2 within microdomains of the superficial cortex by Welch et al. (29) indicate that AV PO2 must vary along the course of the renal preglomerular circulation. Unfortunately, it is not technically feasible to directly measure PO2 at specific locations within the arterial and venous vessel networks, only at the entry/exit of the kidney. Therefore, in the majority of these simulations we have used arterial and venous PO2 values measured at the renal artery/vein level. To assess how variations in PO2 expected at the different representative levels of the vasculature may affect shunting, the AV PO2 ratio was varied. As the AV PO2 ratio was increased from 1.2 to 2, by changing the arterial PO2, AV shunting in both the wrapped and circular vessels increased, with a much steeper gradient of increase in the former (Fig. 13, A and D). For example, AV shunting at an arterial radius of 50 μm was nine times greater when the AV PO2 ratio was set at 2 than when it was set at 1.2. AT shunting steadily increased as the AV PO2 ratio was increased in both the wrapped and circular geometries (Fig. 13, C and F). In the experiment of Welch et al. (29), the AV PO2 ratio was ~1.6 (85/52 mmHg) for the main renal artery/vein pair. If we assume equilibration of tissue PO2 with venular PO2, the ratio is likely to be ~1.1 (45/42) at the level of efferent arterioles and venules. Thus the change in the AV PO2 ratio along the course of the renal circulation is likely to favor shunting in larger vessels.

**DISCUSSION**

The factors controlling oxygen shunting in the kidney are not well understood. It is assumed that the parallel architecture of the renal circulation facilitates countercurrent oxygen exchange. Experimental data have shown that renal venous PO2 exceeds PO2 in the efferent arterioles (29), which means that some of the oxygen in the renal arteries diffuses to adjacent veins. It is not known whether this occurs throughout all levels of the vasculature in the kidney or only in certain regions. This question is not just of academic interest. In particular, knowledge of the impact of AV oxygen shunting on oxygen delivery to the medullary circulation has important implications for our understanding of the causes of medullary hypoxia in ischemic acute kidney injury (25). It has been assumed, since the seminal work of Schureck (28), that most AV oxygen shunting occurs in vessels downstream from the divergence of the cortical and medullary circulation. This conclusion was based on the fact that, in a branching vascular network such as that in the renal cortex, the overall surface area for diffusion of oxygen from arteries increases geometrically at each order of
branching (23). But this conclusion is based on the assumption that the driving force for oxygen shunting (i.e., AV Po2 difference) and the barriers to AV diffusion (e.g., lumen separation, wrapping) do not change appreciably across the course of the preglomerular circulation. Our current findings indicate that such an assumption is unwarranted.

We identified four factors that have a critical impact on the quantity of oxygen shunted between individual artery and vein vessel pairs. These are 1) the presence of oxygen sinks (capillaries or tubules) between the artery and the vein walls; 2) the distance separating the lumen of the artery and vein; 3) the spatial geometry of AV pairs, especially the degree to which the vein wall wraps around the wall of the artery; and 4) the ratio of Po2 of blood in the artery and vein. In all four cases, these factors favor AV oxygen shunting in vessels that are likely common to the cortical and medullary circulations.

Our model predicts that the efficiency of oxygen shunting from arteries to veins is significantly enhanced and that the flux of oxygen from arteries to tissue and veins to tissue is reduced, if the kidney parenchyma is not treated as an homogeneous oxygen sink. That is, AV oxygen shunting is facilitated by the absence of oxygen sinks, in the form of capillaries and tubules, between and around artery and vein pairs. As previously shown by Frank and Kriz (13), there is a zone in the immediate vicinity of intrarenal arteries of loose connective tissue in which neither capillaries nor tubules are present. Capillaries not only deliver oxygen but may also remove oxygen from tissue if they are in close vicinity to arteries or arterioles where oxygen concentrations are above those in nearby capillaries (16). Renal tubules account for the vast majority of oxygen consumption within the kidney through the utilization of ATP to drive sodium reabsorption (10). Thus capillaries and tubules, if they were to appear between the AV pair would “rob” oxygen that might have otherwise contributed to AV shunting. In short, our modeling shows that AV oxygen shunting could not operate if there were any kind of oxygen sink removing significant quantities of oxygen from the region between the artery and vein.

Importantly, we found a virtually complete absence of capillaries and tubules in the space between arteries and veins in which the vein partially wrapped the artery, and a reduced capillary density in the vicinity of larger (>26-μm diameter) compared with smaller (<26-μm diameter) arteries. Furthermore, while capillaries and/or tubules were rarely observed between large artery and vein pairs, they were frequently observed between and around smaller arteries and veins, presumably corresponding to distal interlobular arteries and glomerular arterioles. This arrangement would be expected to reduce AV oxygen shunting in these smaller vessels. Interestingly, the lack of capillaries and tubules we observed around large arteries and veins also serves to reduce the oxygen transport from the artery to the tissue. Fick’s 1st law (2) states that oxygen diffusive flux is driven by the oxygen concentration gradient. The reduction in the strength of the oxygen sinks close to the vessel wall reduces the oxygen gradient near the wall and the oxygen flux from the vessel to the tissue.

![Fig. 11. Effect of R_A/R_V ratio on AV shunting (A), VT flux (B), and AT flux (C) in the circular configuration.](http://ajprenal.physiology.org/)

![Fig. 12. Effect of vessel orientation on flux. W75%, W50%, and W25% refer to veins surrounding 75, 50, and 25%, respectively, of the arterial circumference. Long and short refer to elliptical veins with either their long or short axis in close contact with the paired artery. Columns show oxygen flux from artery to vein (AV; black), vein to tissue (VT; white), and artery to tissue (AT; grey).](http://ajprenal.physiology.org/)
As might be expected from first principles (i.e., from Fick’s 1st law), our model predicts that AV oxygen shunting diminishes as the distance between the lumens of adjacent arteries and veins increases. Interestingly, our analysis of Nordsletten’s micro-CT model suggests that there is a U-shaped relationship between arterial diameter (or Strahler order) and lumen separation. The smallest separation distances were found for vessels of Strahler orders 5 and 6, and a proportion of vessels of Strahler orders 4 and 7, roughly corresponding to arteries of 130- to 300-μm diameter. Larger (>300 μm) and smaller (<130 μm) arteries tended to be associated with more distant veins. The findings of this analysis were supported by a smaller data set of observations from light microscopy. It is not possible to definitively assign anatomic classifications to arteries based on lumen diameter or level of branching. Nevertheless, it is generally accepted that arteries of 130- to 300-μm diameter or Strahler orders 4 – 7 would include interlobar, arcuate, and interlobular arteries (12, 23). The interlobular arteries taper as they radiate out from the juxtamedullary cortex, so an interlobular artery of 130-μm diameter is likely to be proximal rather than distal (12), and so potentially before the divergence of the cortical and medullary circulations.

Our model predicts that the ratio of PO2 in the blood of AV pairs (AV PO2 gradient) is critical in determining AV flux. The AV PO2 gradient is generated by tissue oxygen consumption, but it is also modulated by diffusive transport of oxygen among the arterial, venous, and tissue compartments (including AV oxygen shunting). The current model does not allow us to definitively predict the precise AV PO2 ratio at each point along the preglomerular circulation. However, it must fall from a value of ~1.6 at an arterial PO2 of 85 mmHg at the main renal artery-vein pair to a value much closer to unity (~1.1) at the level of the efferent arteriole and smallest venules. Thus the driving force for AV oxygen shunting diminishes along the course of the preglomerular circulation.

Fig. 13. Effect of the ratio of arterial to venous PO2 on AV shunting (A and D), VT flux (B and E), and AT flux (C and F) in circular (A–C) and wrapped (D–F) configurations.
Our previous model provided a conservative estimate of the quantity of oxygen shunted between arteries and veins in the rat preglomerular circulation (14), but it provided no insight into the sites within the preglomerular circulation where shunting occurs. Our current study has identified four factors which should promote AV oxygen shunting in preglomerular vessels common to the cortical and medullary circulation (see above). These findings challenge the accepted view that most AV oxygen shunting occurs in distal vascular elements. However, there is another critical factor, not assessed in the current model, which would be expected to promote AV oxygen shunting in more distal cortical vessels, downstream from the divergence of the cortical and medullary circulation. That is, because of the branching nature of the renal circulation, the total surface area for diffusion of oxygen from arteries increases geometrically at each branch level. How, then, might we make quantitative predictions regarding the amount of oxygen shunted between arteries and veins at each representative level of the renal preglomerular circulation? To do this, we require information regarding the lengths and calibers of vessels at each representative level [as provided by the micro-CT model of Nordsletten and colleagues (22, 23)]. However, our current study shows that we also require quantitative information about the radial geometry of AV pairs at each representative level, and knowledge of the density of oxygen sinks (tubules and capillaries) in the vicinity of these vessels. Unfortunately, the data set generated by Nordsletten et al. (23) does not have the level of resolution required for provision of such information. Nevertheless, generation of high-resolution data is feasible (21), and our current findings provide justification for such studies.

In its current form, our mathematical model only deals with diffusion of oxygen in discrete cross sections of tissue. An important area for model improvement would be to combine the results from this oxygen transport model with the Gardiner et al. (14) model of countercurrent exchange of oxygen to create a 3D model of diffusive and advective oxygen transport in the kidney. In the model of Gardiner et al. (14), the diffusion barrier to oxygen shunting was assumed to be constant throughout the kidney. Relaxing this assumption, to incorporate the variation in AV spatial intimacy along the preglomerular renal vasculature, will help estimate the degree to which shunting occurs in the vessels common to the cortical and medullary circulation, and how variations in oxygen delivery and VO₂ alter this shunting along the length of the vasculature. Development of such a model will require precise quantitative information regarding the radial geometry of arteries and veins at each branching level of the renal circulation, rather than the mostly qualitative analysis we have provided herein. However, once such data are available it will be possible to model changes in kidney oxygen transport in disease states associated with, for example, renal fibrosis. Our current model also does not account for the impact of PCO₂ on the affinity of hemoglobin for oxygen, or for local variations in parenchymal oxygen consumption, both of which are known to vary across various regions of the renal cortex (1, 15).

**Perspectives**

The susceptibility of the renal medulla to hypoxia is thought to be a critical factor in the pathogenesis of acute kidney injury of multiple etiology (3). It is known to arise from multiple factors. These factors include the countercurrent shunting of oxygen between descending and ascending vasa recta (6). Furthermore, the predominant tubular elements in the outer medulla, the thick ascending limbs of Henle’s loop, are highly metabolically active, yet their main oxygen supply is from the relatively deoxygenated blood within ascending vasa recta (3). Our current findings raise the possibility that an additional factor, AV oxygen shunting in the renal preglomerular circulation, may also limit oxygen delivery to the medullary circulation, since multiple factors appear to favor oxygen shunting in vessels upstream from the point where the cortical and medullary circulations diverge. This proposition is supported by our experimental findings in anesthetized rabbits and rats, in which we found that cortical ischemia can induce medullary hypoxia even when medullary perfusion is maintained (25). It is not currently feasible to measure the PO₂ or oxygen content of blood in arteries deep within the renal cortex. However, our current and previous (14, 25) findings predict that when such methods become available, the PO₂ of blood in the arcuate and proximal interlobular arteries, some of which is destined for the medullary circulation, may be found to be considerably lower than that of arterial blood. The loss of oxygen from arterial blood before it reaches the medullary circulation is likely exacerbated by cortical ischemia.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


**REFERENCES**