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Vascular geometry and oxygen diffusion in the vicinity of artery-vein pairs in the kidney

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Renal arterial-to-venous (AV) oxygen shunting limits oxygen delivery to renal tissue. To better understand how oxygen in arterial blood can bypass renal tissue, we quantified the radial geometry of AV pairs and how it differs according to arterial diameter and anatomic location. We then estimated diffusion of oxygen in the vicinity of arteries of typical geometry using a computational model. The kidneys of six rats were perfusion fixed, and the vasculature was filled with silicone rubber (Microfil). A single section was chosen from each kidney, and all arteries (n = 1,628) were identified. Intrasrenal arteries were largely divisible into two “types,” characterized by the presence or absence of a close physical relationship with a paired vein. Arteries with a close physical relationship with a paired vein were more likely to have a larger rather than smaller diameter, and more likely to be in the inner-cortex than the mid- or outer cortex. Computational simulations indicated that direct diffusion of oxygen from an artery to a paired vein can only occur when the two vessels have a close physical relationship. However, even in the absence of this close relationship oxygen can diffuse from an artery to periarteriolar capillaries and venules. Thus AV oxygen shunting in the proximal preglomerular circulation is dominated by direct diffusion of oxygen to a paired vein. In the distal preglomerular circulation, it may be sustained by diffusion of oxygen from arteries to capillaries and venules close to the artery wall, which is subsequently transported to renal veins by convection.

arterial-to-venous oxygen shunting; arteries; blood vessels; countercurrent diffusion; hypoxia; oxygen transport to tissue

EVIDENCE FROM EXPERIMENTAL (25, 40, 47) and computational (14, 15) studies indicates that diffusional shunting of oxygen from arteries to veins acts to limit oxygen delivery to the renal cortex. The results of a one-dimensional computational model of arterial-to-venous (AV) oxygen shunting indicate that this phenomenon is quantitatively significant, being on the same order of magnitude as total oxygen consumption under normal physiological conditions (14). The adaptive feature of renal AV oxygen shunting may be that it acts as a structural antiox-
the relative quantities of oxygen shunting along the course of the preglomerular circulation.

In the current study, we performed a quantitative analysis of the spatial association of arteries and veins in the renal cortex. We determined how the four factors identified by computational modeling (15), that determine diffusion of oxygen from arteries to veins and the renal parenchyma, change along the course of the renal circulation. Specifically, we determined the diffusion (separation) distance between an artery and its closest vein, as well as the degree to which the venous wall surrounds the artery (termed wrapping throughout the paper). We then used a computational model to determine the pathways of oxygen diffusion in the vicinity of typical artery-vein pairs.

METHODS

Anatomic Observations

Animal ethics. All procedures involving animals were approved by the Animal Ethics Committee of the Monash University School of Biomedical Sciences and were in accordance with the Australian...
Preparation of tissues for morphometric analyses. Male Sprague-Dawley rats (250–300 g) were anesthetized with pentobarbital sodium (60 mg/kg ip). A large-bore catheter was inserted into the aorta below the level of the kidneys. Heparin (50 IU, Pfizer, West Ryde, NSW, Australia) was then administered, and the kidneys retrogradely perfused at physiological pressure. The kidneys were first cleared with phosphate buffer (0.2 M) containing sodium nitroprusside (0.24 mg/ml) and then perfused fixed with Karnovsky’s fixative (4% paraformaldehyde and 4% glutaraldehyde in 0.2 M phosphate buffer). Once fixed, the kidneys were filled with Microfil compound (MV-122; Flow Tech) at a rate of 1 ml/min. Once 10–12 ml of Microfil had been infused, the renal artery and vein were ligated and the kidneys removed and immersed in fixative overnight at 4°C.

Sampling of renal tissue. Following overnight fixation, both kidneys were decapsulated, weighed, and prepared for slicing. In brief, kidneys were embedded in 2% agarose gel and sliced into 1.25-mm slices using a slicing device (3, 18), generating 10–20 slices/kidney. Three kidneys were sliced perpendicular to the longitudinal axis in the sagittal plane. Another three kidneys were sliced along the longitudinal axis. Left and right kidneys were used in an alternating fashion so that all not right/left kidneys were sliced in the same direction. This approach minimized bias in our sampling method. Whole kidney volume was estimated from these slices using Cavalieri’s principle (32).

Kidney slices were numbered in order and separated into “odd” slices and “even” slices. All morphometric analyses were carried out on the slices in the odd group. One slice from the odd group of each kidney was systematically chosen, processed, and embedded for morphometric analyses. For the kidneys sliced in the sagittal plane, the three options were odd slices numbered 1, 5, and 9. For the kidneys sliced along the longitudinal axis, the three choices were slices numbered 1, 3, and 5. Once a slice was chosen from one kidney, the same option (e.g., slice 1) was omitted from the other kidneys so that each slice number would not be chosen again. Thus morphometric analyses were carried out on sections obtained from one of three different areas of the kidney. That is, a slice near the edge of the kidney, a slice near the midline, and a slice in an intermediate position in each of the two planes, generating six slices in total.

Whole kidney slices were embedded in glycolmethacrylate (Technovit 7100; Heraeus Kulzer, Wehrheim, Germany), sectioned at 3 μm, and stained with hematoxylin and eosin. Sections were scanned with Aperio Scan Scope (Aperio, Vista, CA), providing a digital representation of the entire section. Representative images are shown in Fig. 1.

Morphometric studies. Morphometric studies were carried out using Image Scope (v. 11.2.0.780, Aperio Technologies), a digital slide viewer. All arteries within each section were identified and labeled. For each artery, we manually measured arterial diameter, the shortest diffusion distance from the artery to its nearest vein, the percentage of the arterial adventitia in direct contact with the vein wall (wrapping), the thickness of the adventitia around the artery (shortest width), the thickness of the arterial wall, the shortest diffusion distance from the artery to its nearest vein, and the diameter of this second artery.

Analysis of spatial relationships of artery-vein pairs by region of the kidney. In conjunction with our analyses by arterial diameter, we also analyzed the spatial geometry of artery-vein pairs in terms of their anatomic location within the kidney. Of the six kidney sections used in our morphometric analyses, four of these, which had a distinct medulla, were used in this analysis. The two kidney sections that contained only cortical tissue were not included.

For each of the four kidney sections, the corticomedullary junction was identified (22, 24). In brief, juxtamedullary glomeruli were identified as glomeruli located closest to the medulla. Using these glomeruli, as well as the large arteries situated nearby and the overall shape of the kidney section as a guide, a line of best fit was drawn manually. The region from this line to the surface of the cortex was then divided into thirds, outer cortex, midcortex, and inner cortex, while the region inward of this line was defined as the corticomedullary region. All arteries that were intersected by a line were assigned to the region that was closer to the medulla rather than the cortical surface.

Statistical methods. Continuous data were summarized as group means ± SE, together with group size (n). Continuous variables were analyzed by one-way ANOVA, followed by the Tukey-Kramer test for all possible pairwise contrasts (to control the type I error rate) (27). Categorical variables were analyzed by the exact Cochran-Armitage test for trends (2, 8). To protect against excessive type I error, P values were adjusted by the Ryan-Holm step-down Bonferroni procedure (26). Analyses were performed with either SYSTAT v.13 (Systat, San Jose, CA) or StatXact v.9 (Cytel Software, Cambridge, MA). Two-sided P ≤ 0.05 was considered statistically significant.

Fig. 2. Characteristic cross sections of tissue in the vicinity of an artery. The figure displays typical arrangements for a large “wrapped” artery [internal diameter (D_A) = 89 μm; A], a large “not-wrapped” artery (D_A = 133 μm; B), a small wrapped artery (D_A = 25 μm; C), and a small not-wrapped artery (D_A = 18 μm; D). A and V denote arteries and veins, respectively. The zones containing capillaries or venules are demarcated by red lines. The minimum lumen wall separation distances (diffusion distances) between the main artery and vein are 7 μm (A), 149 μm (B), 5 μm (C), and 71 μm (D).
**Computational Modeling**

**Utilization of anatomic information.** We have previously used computational methods to investigate the impact of the microanatomy of artery-vein pairs on AV oxygen shunting and oxygen transport in the parenchyma surrounding an artery-vein pair (15). However, the anatomic configurations of artery-vein pairs and the patterns of distribution of parenchymal oxygen sinks (tubules and capillaries) in the earlier study were highly idealized (15). In the current study, we generated more physiologically relevant information by simulating oxygen diffusion in an actual representation of the microanatomy of an artery-vein pair and its associated oxygen sinks. We selected four representative images (Fig. 2) on the basis that they represented large-wrapped (Fig. 2A), large not-wrapped (Fig. 2B), small-wrapped (Fig. 2C), and small not-wrapped arteries (Fig. 2D). An additional criterion was that the chosen arteries were nearly circular, indicating that the plane of section was perpendicular to the plane of the artery. The histological images clearly show the heterogeneous distribution of capillaries and venules (red boundaries) and tubule segments across the parenchyma.

The selected images were imported into an image-processing software (GIMP v2.8), and the boundaries of the arteries, veins, capillaries/venules and tubule segments were traced. The traced images were converted into a vector format to make them compatible with the finite-element computer software used for modeling the oxygen transport process (COMSOL Multiphysics, v. 4.2, COMSOL, Burlington, MA). Specifically, the vector format conversion was performed using a raster-to-vector conversion program (Magic Tracer V 2.0.012, Elgorithms, Pryor, OK). During the vector conversion process, the cross-sectional areas of putative capillaries (i.e., small vessels containing Microfil) <50 μm² were adjusted to 50 μm². Small vessels containing Microfil, but with cross-sectional areas >150 μm², were designated as venules due to their relatively high ratio of luminal diameter to vascular wall thickness, a characteristic that distinguishes venules from capillaries (39). The images imported into the computational domain were scaled to match the dimensions calculated from the scales in the captured photomicrographs (Fig. 2).

**Computational model.** Details of the 2D model of oxygen transport between artery-vein pairs have been described previously (15). Oxygen transport across a cross section of renal tissue located in a Cartesian 2D (x, y) plane is a combination of diffusion and consump-
ies and tubules increases the mass transfer distance of oxygen from the capillary to the tubular epithelium. The mass transfer distance increases with an increase in the aspect ratio of the tubules. In such cases, the capillaries captured in the sectioning plane only service a fraction of the tubular epithelium. The rest of the tubular epithelium is serviced by a capillary located outside the sectioning plane (green arrow in Fig. 3).

Allocating a fraction of \(V_O^2\) along the epithelium of oblique tubules corrects the net imbalance between the oxygen sources and sinks in a 2D representation of the actual 3D geometry. For perfectly horizontal sectioning planes, the capillary can service the entire tubular epithelium. Therefore, a capillary can service tubular epicapillary located at distances below or equal to the actual tubule diameter. The larger diameter of oblique tubular sections prevents oxygen in the capillary, captured in the 2D plane, servicing the entire tubular epithelium. The increase in the tubular cross-sectional diameter is dependent on the cosine of the angle \((O)\) of the sectioning plane relative to the horizontal. Under such circumstances, it is reasonable to assume that the capillary cross section captured in the 2D micrographs only contributes a fraction of the metabolic oxygen demand \((V_O^2)\) of the tubule, as shown in Fig. 3B. The tortuous segments of tubules and their servicing peritubular capillaries demonstrate a high degree of geometrical complexity in 3D (46). This makes it difficult to correlate the aspect ratio and \(V_O^2\) for tubules obtained by sectioning of tissue along the tortuous segments of the tubules. In such cases, we assume that the relationship between \(V_O^2\) and tubule aspect ratio in Fig. 3B is a reasonable approximation. For other elements, within the tissue (excluding the cross sections of tubules), \(S\) was set to zero. This decision was based on the fact that \(\sim 80\%\) of the oxygen consumed within the kidney under physiological conditions is used to power tubular sodium reabsorption (13). \(V_O^2\) (mol·m\(^{-2}\)·s\(^{-1}\)) thus represents the rate of oxygen consumption by the tubular epithelium.

**Boundary conditions.** A zero-flux condition was applied along the boundary of the tissue image (Fig. 2), assuming spatial repetition of the tissue geometry. The radius of each image in Fig. 2 was set at half the distance between arteries of that size and the nearest artery (Table 2). Therefore, each image approximates the area of the tissue region defined by the “physics-control” settings was used for all the simulations. This resulted in generation of 21,172, 73,208, 12,778, and 29,412 mesh elements for the computational domains generated from processing the photomicrographs represented in Fig. 2A, B, C, and D, respectively. The relative accuracy for all simulations was set at 0.0001.

### RESULTS

**Anatomical Observations.**

**Kidney weight and volume.** The weight of the six kidneys processed for morphometry averaged \(1.83 \pm 0.06\) g (range = 1.55–1.94 g). Their volume averaged \(1.671 \pm 94\) mm\(^3\) (range = 1.480–2.095 mm\(^3\)).

**Microfil as a means of identifying the renal vasculature.** Microfil could be seen as dark brown staining inside the lumen of most arteries, glomerular and peritubular capillaries, vasa recta, and venules (Fig. 1). Microfil was often not seen in larger veins, presumably reflecting incomplete filling of the vasculature. Nevertheless, these veins were easily identified because of their thin walls. Thus we were able to easily distinguish between the vascular and tubular elements in the renal cortex and medulla.

**Characteristics of arteries.** A total of 1,628 arteries from 6 kidneys were analyzed. The lumen diameter of arteries in the juxtamedullary and inner cortical region tended to be \(>40\ \mu\text{m}\) (Table 2), consistent with a predominance of interlobar (\(>200\ \mu\text{m}\)), arcuate (\(>100\ \mu\text{m}\)), and proximal interlobular (\(>60\ \mu\text{m}\)) vessels in these regions (19, 42). The lumen diameter of arteries in the mid- and outer cortex tended to be \(<25\ \mu\text{m}\), consistent with the tapering of interlobular arteries as they extend toward the cortical surface, to a diameter of \(\sim 30\ \mu\text{m}\) at the terminal segment (19) and 10–25 \(\mu\text{m}\) at the level of afferent and efferent arterioles (10). As described previously (42), the thickness of the arterial wall and associated adventitia progressively diminished as vessel diameter reduced (Table 2). Consistent with the relatively thin arterial wall of all vessels studied (29), there was little evidence of vasa vasorum. The mean distance to the nearest artery varied according to both thin walls of the tubules and the small diameters of the peritubular capillaries, which are located outside the sectioning plane (green arrow in Fig. 3).

**Numerical solution.** The governing equations pertaining to the four models with the appropriate boundary conditions were solved numerically using COMSOL Multiphysics. The “fine” meshing scheme as defined by the “physics-control” settings was used for all the simulations. This resulted in generation of 21,172, 73,208, 12,778, and 29,412 mesh elements for the computational domains generated from processing the photomicrographs represented in Fig. 2A, B, C, and D, respectively. The relative accuracy for all simulations was set at 0.0001.

### Table 2. Characteristics of arteries according to internal diameter and anatomic region

<table>
<thead>
<tr>
<th>Region of the kidney</th>
<th>Total Wall Thickness (Arterial Wall and Adventitia), (\mu\text{m})</th>
<th>Distance to Nearest Artery, (\mu\text{m})</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM ((n = 147))</td>
<td>(57.46 \pm 6.46^a)</td>
<td>(167.20 \pm 16.75^a)</td>
</tr>
<tr>
<td>IC ((n = 226))</td>
<td>(41.15 \pm 2.04^b)</td>
<td>(198.30 \pm 12.50^b)</td>
</tr>
<tr>
<td>MC ((n = 429))</td>
<td>(21.45 \pm 0.70^b)</td>
<td>(172.40 \pm 6.61^b)</td>
</tr>
<tr>
<td>OC ((n = 243))</td>
<td>(16.26 \pm 0.51^d)</td>
<td>(191.10 \pm 10.39^d)</td>
</tr>
</tbody>
</table>

Values are means ± SE of mean arterial diameter, mean thickness of the arterial wall (media and endothelium only)*, and associated adventitia, as well as the mean distance to the nearest artery. Arteries were partitioned by vessel diameter and by anatomic region of the kidney. Letters represent bins of arterial diameter or region of the kidney for which the independent variables did not differ significantly (Tukey’s test). CM, corticomedullary; IC, inner cortex; MC, midcortex; OC, outer cortex.
arterial diameter and anatomic location (Table 2). Across all arteries, this distance averaged 164.6 ± 3.7 μm.

Spatial relationships between arteries and veins in the renal cortex. Visual inspection of the range of spatial arrangements of artery-vein pairs confirmed the finding of our previous qualitative analysis (15) that larger arteries tend to be more closely associated with their corresponding veins than are smaller arteries (Fig. 1). Capillaries or tubules were rarely found between these larger artery-vein pairs. In most cases, only adventitia separated the larger artery/vein pairs (Fig. 1B).

Smaller arteries were more often found at longer distances from their veins, with tubules and capillaries in between the artery-vein pair (Fig. 1C–F).

The scattergram plotting artery-vein diffusion distance against arterial diameter was L-shaped. That is, the diffusion distances for arteries of a diameter ≥100 μm were uniformly short (<100 μm) whereas there was considerable variation in the diffusion distances for smaller vessels (Fig. 4A). On average, large arteries had shorter diffusion distances than smaller arteries (Fig. 5A). The distribution of artery-vein diffusion distances progressively shifted to longer distances as arterial diameter reduced (Fig. 5B). For example, 89.4% of arteries ≥50 μm in diameter were closer than 50 μm to the nearest vein, whereas this was the case for only 28.3% of arteries <20 μm in diameter.

Arteries located in the corticomedullary and inner cortical regions tended to be relatively large and close to their corresponding veins. Arteries found in the mid- and outer cortex tended to be small and more distant to the closest vein (Fig. 4, A and B). For example, 63.3% of arteries found in the corticomedullary and inner cortical regions had diffusion distances of <50 μm, whereas this was the case for only 37.9% of arteries found in the mid- and outer cortex (Fig. 4C).

When analyzed by vessel caliber, arteries with diameters >200 μm consistently displayed a close physical relationship with their corresponding vein. That is, at least part of the arterial wall was wrapped by the corresponding vein (Fig. 6A). Consequently, the diffusion distance between the arterial and venous lumen was short. On average, the proportion of arterial wall wrapped by the vein progressively decreased from the larger to smaller vessels (Fig. 7A). Consistent with this finding, our frequency analysis showed that larger vessels were more likely to be wrapped by a vein than were smaller vessels (Fig. 7B). For example, 33.3% of arteries of diameter ≥100 μm had 30% or more of their wall wrapped by the vein, while this was the case for only 6.0% of arteries of diameter <50 μm.

**Fig. 4.** Distance between the arterial and venous lumens as a function of arterial diameter in various regions of the kidney. A: scattergram in which measurements are plotted against arterial diameter. The various colors represent the 4 different regions of the kidney: corticomedullary (CM), inner cortex (IC), midcortex (MC), outer cortex (OC). B: independent variable is bins of the 4 regions of the kidney. Values are means ± SE. C: relative frequencies of diffusion distances in each of the 4 regions of the kidney. The same lower case letters represent bins of anatomic region for which diffusion distance (Tukey’s test; B) or percentage frequency of categories of diffusion distance (Cochran-Armitage test for trends; C) did not differ significantly.

**Fig. 5.** Distance between the arterial and venous lumens as a function of arterial diameter. A: independent variable (arterial lumen diameter) is binned into 4 categories: <20, 20–49.99, 50–99.99, and ≥100 μm. Values are means ± SE. B: dependent variable is binned into ranges of diffusion distance. The statistical analysis is as for Fig. 4.
Arteries found at the corticomedullary and inner cortical regions had a greater proportion of their profile surrounded by the vein than did those in the mid- and outer cortex (Fig. 6). For example, 16.9% of arteries found in the corticomedullary and inner cortical regions had their profiles wrapped by 30% or more, while this was the case for only 4.3% of arteries in the mid- and outer cortex (Fig. 6).

We performed a separate analysis in which the data were partitioned according to whether any of the arterial profile was wrapped by a vein (Fig. 8). The arteriovenous diffusion distance was approximately 10-fold less for those arteries wrapped to any extent by a vein, than for “not wrapped” arteries. Furthermore, the relationship between arterial diameter and diffusion distance, seen when the data were amalgamated (Fig. 5), was completely lost when the data were partitioned into wrapped and not-wrapped vessels (Fig. 8, A and B). Similarly, the variation in diffusion distance by anatomic region (Fig. 6) was also largely lost when the data were partitioned in this way (Fig. 8, C and D). A scatterplot of arteriovenous diffusion distance against the proportion of wrapping for individual artery-vein pairs showed that the data clustered into two distinct categories of 1) wrapped vessels, which by definition have short arteriovenous diffusion distances; and 2) not-wrapped vessels with consistently long diffusion distances (Fig. 9). Both “types” of arteries can have large or small diameters, and can be present in any region of the cortex. However, the proportion of vessels of the two types varies with arterial diameter and cortical region.

**Computational Modeling**

**Spatial variations in renal tissue $PO_2$.** As would be expected, our simulations of $PO_2$ in the vicinity of the four typical artery-vein pairs shown in Fig. 2 demonstrated, in all cases, a region of high $PO_2$ (60–82 mmHg) around the artery (Fig. 10A). Oxygen diffuses from this region to nearby tubules, veins, venules, and capillaries. The $PO_2$ in the tubules outside the region of high $PO_2$ varied from 40 to 50 mmHg. The slightly lower $PO_2$ in the periphery of the field around the larger AV pairs is due to a lesser capillary density around the larger arteries, which we have previously quantified (15). The predicted $PO_2$ in the capillaries and their immediate vicinity was $\sim$50 mmHg. The range of tissue $PO_2$ predicted by our model is consistent with that measured experimentally (12, 47, 48).

**Oxygen flux in the vicinity of artery-vein pairs.** Consistent with an area of relatively high $PO_2$ around all four typical artery-vein pairs, there was significant flux of oxygen from the artery in all cases (Fig. 10B). The oxygen flux from the artery was greatest when there were nearby veins, venules, and capillaries, as shown by the presence of red (high flux) and pale blue (moderate flux) “sparks” around the artery against the dark blue (low flux) background of the renal parenchyma (Fig. 10C). The oxygen flux from wrapped arteries was mainly to the closely associated vein. The oxygen flux from not-wrapped arteries was mainly to capillaries and venules in the immediate vicinity of the artery (compare Figs. 2 and 10, B and C).
Consistent with the predictions from our earlier simulations of oxygen fluxes from idealized artery-vein pairs (15), a significant flux of oxygen from an artery to its closest vein (i.e., AV oxygen shunting) was only observed when at least some of the wall of the artery was wrapped by the wall of the vein (Fig. 11A).

The analysis described above has an important limitation. The results of the simulation shown in Fig. 11A are based on the (unrealistic) assumption that all of the oxygen that diffuses from arteries to nearby capillaries and venules will diffuse back into the tissue further downstream. However, it is likely that some proportion of the oxygen that diffuses from arteries to capillaries, and an even larger proportion of the oxygen that diffuses to venules, will instead be transported to veins by convection. The results of the simulation shown in Fig. 11B are based on the (unrealistic) assumption that all of the oxygen that diffuses from arteries to nearby capillaries and venules is transported to veins by convection. Under these theoretical conditions, wrapped and not-wrapped arteries show similar patterns and magnitudes of oxygen flux (Fig. 11B). The real answer must lie somewhere between these two extreme examples.

Our analysis also indicates that AV oxygen flux is greater (~2-fold), and oxygen flux from vein to tissue is greater (~5-fold, although still negligible) for larger (50–150 μm) than smaller (<50 μm) arteries. The oxygen flux from arteries to the tissue is similarly greater (~2-to 5-fold) for larger (50–150 μm) than smaller (<50 μm) arteries.

DISCUSSION

Previously, we developed a 2D computational model of oxygen flux between artery-vein pairs of idealized geometry (15). Simulations derived from this model provided evidence that diffusion of oxygen from an artery to its paired vein can only be of significant magnitude if the arteriovenous diffusion distance is short (i.e., <50 μm) and at least some of the arterial wall is wrapped by the venous wall (15).

Our current anatomic observations indicate that artery-vein pairs with these characteristics are present throughout the preglomerular circulation. However, such close physical relationships between arteries and veins were observed in a much greater proportion of larger arteries than smaller arteries, and arteries in the inner cortex and juxtamedullary region rather than the mid- or outer cortex. Thus conditions required for oxygen to diffuse directly from an artery to its associated vein appear to be predominantly observed in segments of the preglomerular circulation proximal to the divergence of the medullary and cortical circulations. However, our current model simulations provide evidence of another route via which oxygen in renal arterial blood could be shunted to renal venous blood, even in the absence of a close physical relationship between artery-vein pairs. That is, in both small and large arteries that are not wrapped by a vein of similar size, oxygen appears to diffuse to peritubular capillaries and venules in the vicinity of the artery. Some of this oxygen is likely to be delivered to veins by convection. The distinction between wrapped and not-wrapped arteries may therefore not

Fig. 8. Diffusion distance for not-wrapped (A and C) and wrapped (B and D) arteries of various sizes and regions of the kidney. A and B: data binned by arterial diameter. C and D: data binned by anatomic region: CM, IC, MC, and OC. Values are means ± SE. The statistical analysis is as for Fig. 4 (Tukey’s test).

Fig. 9. Scattergram of diffusion distance against proportion of wrapping (%) for arteries in various regions of the kidney.

http://ajprenal.physiology.org/ by 10.220.33.4 on August 28, 2017
be as important as we initially proposed (15). Thus anatomic conditions favorable to AV oxygen shunting may be present throughout the cortical circulation. We propose that in more proximal vessels, mainly those common to the cortical and medullary circulations, AV oxygen shunting may be dominated by direct diffusion of oxygen from an artery to its closely associated vein. In more distal parts of the cortical circulation, mainly after its divergence from the medullary circulation, oxygen shunting may be dominated by diffusion of oxygen from arteries to capillaries and venules.

Our current anatomic observations confirm and extend those of our previous study (15). In that study, we measured arteriovenous diffusion distance by light microscopy in a relatively small sample of 82 artery-vein pairs. This approach was limited both in terms of the small sample size and by the fact that identification of veins was hindered by the lack of a method to specifically label all vascular elements. To overcome the limitation of sample size, our previous study also employed the data set generated by Nordsletten and colleagues (30) from microcomputed tomography of the vasculature of a rat kidney that had been filled with Microfil. This allowed us to determine arteriovenous diffusion distances across 5,260 vessel profiles. However, these data were limited both because of the potential confounding effects of shrinkage of Microfil (28), and because the radial geometry of this data set had been converted to circular profiles (15). To overcome these limitations, in the current study we were able to identify 1,628 artery-vein pairs from six different kidneys using light microscopy. Importantly, vascular elements were clearly labeled by the presence of Microfil. The size and resolution of these data set allow us to draw conclusions, regarding the characteristic arrangements of arteries and veins in the kidney, with considerable confidence.

The present findings demonstrate that the mean absolute diffusion distance, between arteries and veins, progressively increased as arterial diameter decreased. Furthermore, the mean percentage of the arterial wall wrapped by the wall of the corresponding vein progressively diminished as arterial diameter decreased. Taken at face value, these data might suggest that the intimacy of arteries and veins progressively decreases from proximal to distal segments of the preglomerular circulation. However, we believe these mean data may be potentially misleading. Rather, our interpretation is that the geometric relationships between arteries and veins in the kidney may be simplified into two types of vessel pairs, the relative abundance of which differs according to arterial diameter and anatomic region of the kidney. The first type is characterized by arteries that are at least partially wrapped by their corresponding vein and so by definition have short arteriovenous diffusion distances (~10 – 15 µm). The second type is characterized by arteries that are not wrapped by their corresponding vein and uniformly have much longer arteriovenous diffusion distances (~100 – 200 µm). Our argument relies on the following logic. First, even 13.8% of arteries of <20–µm diameter had >10% of their circumference wrapped by the corresponding vein. Consistent with this observation, the arteriovenous diffusion distance was <50 µm for 28.3% of arteries with a diameter of <20 µm. Second, when arteries were binned into those that were wrapped and those that were not, the relationship between arterial diameter and diffusion distance, and the relationship between anatomic region of the kidney and diffusion distance, that were clearly evident in the amalgamated dataset, were both largely lost. Third, a scatterplot of the relationship between diffusion distance and proportion of wrapping revealed two distinct clusters of data.
significant diffusion of oxygen from an artery to its associated paired vein only occurred if the vein wrapped at least part of the wall of the artery. This phenomenon was largely independent of arterial diameter, although oxygen flux from artery to vein was greater for larger than smaller vessels. We also observed significant diffusion of oxygen from not-wrapped arteries to capillaries and venules in the immediate vicinity of the arterial wall. This phenomenon has been observed experimentally in skeletal muscle (9, 23) and subjected to considerable scrutiny through development of computational models (16, 21, 38, 41). In the renal cortex, it may sustain oxygen shunting in smaller arteries in the mid- and outer cortex because 1) these are less likely to be wrapped by a vein than their larger counterparts in the inner cortex and corticomedullary border, and 2) capillary density in the immediate vicinity of renal arteries is greater for smaller than larger arteries (15). We are currently unable to quantify the contribution of this putative mechanism to AV oxygen shunting. This limitation arises because we cannot determine the relative proportions, of the oxygen that diffuses from arteries to capillaries and venules, that diffuse back into renal tissue vs. that which is transported by convection to renal veins.

We must acknowledge some further limitations of our current study. Our computational model is limited by the presence of some unknown quantities. For example, there is considerable controversy as to the level of oxygen consumption by the arterial wall and its consequent impact on the delivery of oxygen from arteries to surrounding vessels and tissue (36, 43–45). In the current study, we do not include oxygen consumption in the vascular wall. In supplementary analyses (data not shown), we examined the impact of oxygen consumption by the arterial wall on AV shunting and oxygen fluxes in the four tissue cross sections shown in Figs. 2 and 10. This was done by incorporating, in our model, the vascular wall oxygen consumption term from earlier computational studies of vascular oxygen transport (4, 7). Incorporation of arterial wall oxygen consumption had minimal impact on the distributions of tissue oxygen concentration and oxygen flux (<0.05% difference). It should also be noted that oxygen fluxes from arterioles that have been measured experimentally tend to be an order of magnitude greater than those generated by computational models (37). Furthermore, our model does not incorporate the potential for heterogeneity of oxygen diffusion coefficients across various renal tissues, due to lack of reliable experimental data. It has been suggested that tissue microstructure plays a crucial role in tissue oxygenation (17). We also assumed that oxygen is well mixed in the intravascular space, so our model neglects intraluminal resistance to oxygen transport (20). Additional analysis of intraluminal resistances to oxygen transport (data not shown) revealed negligible intraluminal gradients in \( P_{O_2} \) (~2% difference between the average luminal and vessel wall \( P_{O_2} \)). Finally, we cannot exclude the possibility of incomplete filling of capillaries and venules by Microfil, which may have led to underestimation of oxygen flux from arteries. Thus the oxygen fluxes computed in our current work, although they provide qualitative insights into the diffusion of oxygen in the vicinity of artery-vein pairs in the renal cortex, may not be quantitatively accurate.

We must also concede that our measures of vascular geometry could be biased. The model does not use any specific geometrical approach to account for the complex 3D capillary

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**Fig. 11. Quantification of oxygen diffusion in the vicinity of the 4 typical arteries shown in Figs 2 and 10.**

**A:** effects of typical geometries of artery-vein pairs on the flux of oxygen from artery to vein (AV), vein to tissue (VT), and artery to tissue (AT), when all the capillaries and venules in the computational domain are a part of the renal tissue for post-process calculations of oxygen fluxes.

**B:** predicted oxygen fluxes, when in addition to the representative vein, the capillaries and venules receiving high oxygen fluxes from the representative artery are considered as part of the representative venous vasculature for post-process calculations of oxygen fluxes.
networks that service the tortuous sections of the tubules represented in 2D. Instead, it relies on a relationship between \( VO_2 \) and tubule aspect ratio shown in Fig. 3B. This relationship is based on a simple geometrical arrangement of a parallel capillary and tubule to account for the imbalance in oxygen source and sink in a 2D representation of the actual 3D geometry. Ideally, we would have used 3D reconstructions of the entire circulation of the rat kidney to quantify the intimacy of artery-vein pairs. This would enable us to follow the circulation and be certain of which vein corresponds to which artery. Furthermore, we would not be limited by plane of section as we are with light microscopy. Such information could, at least theoretically, be obtained from microcomputed tomography of the renal vasculature after perfusion with Microfil. Our ongoing studies are aimed at generating this information using synchrotron radiation. Nevertheless, such 3D reconstructions of the rat renal circulation would be limited to only the vasculature; parenchymal elements such as tubules would not be visualized. Thus, to model oxygen flux between vessel pairs, as well as oxygen flux from vessels to renal tissue, we required information regarding the spatial arrangement of tubular elements, which is best generated by light microscopy.

In conclusion, our current findings suggest that the close physical relationship between arteries and veins in the kidney, that is obligatory for direct diffusion of oxygen from arterial to venous blood, is far more prominent in the proximal than distal segments of the renal circulation. These proximal segments likely include arteries that supply blood to the renal medulla (i.e., interlobar, arcuate, and proximal interlobular arteries). Quantitatively significant AV oxygen shunting in the proximal preglomerular circulation could explain the phenomenon of medullary hypoxia during cortical ischemia, even when medullary perfusion is well maintained (35). However, our current findings also suggest that, in the absence of a close physical relationship between an artery and a paired vein, oxygen can diffuse from arterial blood to surrounding peritubular capillaries and venules. Some of this oxygen will diffuse back into renal tissue, but some will likely be transported to veins by convection and so contribute to AV oxygen shunting. Our current and previous (15) anatomic observations indicate that this phenomenon is likely to be most prominent in the smaller arteries in the distal cortical circulation, after the divergence of the medullary circulation (e.g., distal interlobular arteries and perhaps afferent and/or efferent arterioles). Our findings do not allow us to estimate the relative quantities of oxygen that are shunted by these two related mechanisms, or their impact on renal oxygenation under physiological and pathologic conditions. However, they have allowed us to develop a simplified understanding of the nature of the relationships between afferent (arteries) and efferent (veins, venules, and peritubular capillaries) vessels in the kidney. That is, arteries appear to be largely divisible into two “types,” characterized by the presence or absence of a close physical relationship with a paired vein. Our current findings show how the relative proportions of these vessel types change throughout the renal circulation. They also provide an assessment of diffusive oxygen transport in the vicinity of arteries of these types. Armed with this information, and a 1D model of oxygen convection and diffusion in the preglomerular circulation that we developed previously (14), we can now turn our attention to the construction of a pseudo-3D computational model of oxygen diffusion from arteries in the rat kidney. Such a model should allow quantification of AV oxygen shunting along the course of the preglomerular circulation and estimation of how this changes under physiological and pathophysiological conditions.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


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