Regional decreases in renal oxygenation during graded acute renal arterial stenosis: a case for renal ischemia

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Warner L, Gomez SI, Boltermann R, Haas JA, Bentley MD, Lerman LO, Romero JC. Regional decreases in renal oxygenation during graded acute renal arterial stenosis: a case for renal ischemia. Am J Physiol Regul Integr Comp Physiol 296: R67–R71, 2009. First published October 29, 2008; doi:10.1152/ajpregu.90677.2008.—Ischemic nephropathy describes progressive renal failure, defined by significantly reduced glomerular filtration rate, and may be due to renal artery stenosis (RAS), a narrowing of the renal artery. It is unclear whether ischemia is present during RAS since a decrease in renal blood flow (RBF), O2 delivery, and O2 consumption occurs. The present study tests the hypothesis that despite proportional changes in whole kidney O2 delivery and consumption, acute progressive RAS leads to decreases in regional renal tissue O2. Unilateral acute RAS was induced in eight pigs with an extravascular cuff. RBF was measured with an ultrasound flow probe. Cortical and medullary tissue oxygen (PtO2) of the stenotic kidney was measured continuously with sensors during baseline, three sequentially graded decreases in tissue oxygen was induced in eight pigs with an extravascular cuff. RBF was measured with an ultrasound flow probe. Cortical and medullary tissue oxygen (PtO2) of the stenotic kidney was measured continuously with sensors during baseline, three sequentially graded decreases in RBF, and recovery. O2 consumption decreased proportionally to O2 delivery during the graded stenosis (19 ± 10.8, 48.2 ± 9.1, 58.9 ± 4.7 vs. 15.1 ± 5, 35.4 ± 3.5, 57 ± 2.3%, respectively) while arterial venous O2 differences were unchanged. Acute RAS produces a sharp reduction in O2 efficiency for sodium reabsorption (P < 0.01). Cortical (PtO2) decreases are exceeded by medullary decreases during stenosis (34.8 ± 1.3%). Decreases in tissue oxygenation, more pronounced in the medulla than the cortex, occur despite proportional reductions in O2 delivery and consumption. This demonstrates for the first time that hypoxia is present in the early stages of RAS and suggests a role for hypoxia in the pathophysiology of this disease. Furthermore, the notion that arteriovenous shunting and increased stoichiometric energy requirements are potential contributors toward ensuing hypoxia with graded and progressive acute RAS cannot be excluded.

ischemia; renal tissue oxygenation; renal blood flow; pig

THE TERM “ISCHEMIC NEPHROPATHY” has been used to describe progressive renal failure, defined by a significantly reduced glomerular filtration rate (GFR) or loss of renal parenchyma due to renal artery stenosis, a narrowing of one or more of the renal arteries. Ischemia, however, results from a rate of blood flow that is insufficient to satisfy metabolic demands, thereby leading to tissue hypoxia. There is little evidence to suggest that ischemic nephropathy is accompanied by renal tissue hypoxia (24). In fact, the kidney has a high blood flow relative to its weight (3), which results in very small arterial-venous differences in oxygenation, suggesting a large O2 supply and limited O2 consumption. Importantly, Nielsen et al. (15) found in patients with significant unilateral renal artery stenosis that O2 consumption decreased with limited blood flow, suggesting that a decrease in O2 supply is not enough to cause ischemic renal disease (22). Moreover, the reduced O2 supply and demand suggest the term ischemic renal disease is inappropriately applied. In fact, there have been recent suggestions to rename ischemic nephropathy with a more representative term and also to exclude processes initiated by tissue hypoxia in exploring its pathophysiology (24).

Moreover, it has been accepted that a high kidney perfusion, which is ~20% of cardiac output, results in an O2 delivery (DO2) in excess of metabolic demands. Interestingly, estimates suggest nearly 60% of O2 consumption occurs in the medullary thick ascending loop of Henle (TAL), where ~25% of sodium reabsorption occurs attributable to the 2Cl-Na-K cotransporter (2). This is in contrast to proximal tubules in the renal cortex, in which the bulk of sodium is reabsorbed isosmotically. Notably, reductions in renal blood flow (RBF) are known to decrease GFR, and tubular sodium reabsorption, thus decreasing O2 consumption. This effect has fostered the notion that the kidney compensates for reductions in RBF and thus O2 delivery by reducing O2 demand (2, 3, 8).

The notion that proportional changes in whole kidney O2 delivery and consumption accompany reductions in RBF suggests that renal tissue oxygenation is maintained in equilibrium. Nevertheless, prior work in our laboratory (7) suggests that renal tissue oxygenation (Pto2) may be impaired during acute renal arterial stenosis. Therefore, the present study was designed to test the hypothesis that despite proportional changes in whole kidney O2 delivery and consumption, acute progressive renal arterial stenosis (RAS) leads to decreases in regional renal tissue oxygenation. To test this hypothesis and determine whether stoichiometric energy requirements are altered, we assessed changes in renal O2 consumption, arteriovenous O2 differences, and sodium reabsorption, and we concurrently measured intrarenal tissue oxygenation directly with oxygen electrodes (17, 21, 25) during acute RAS.

MATERIALS AND METHODS

The experimental protocol was approved by the Mayo Clinic Institutional Animal Care and Use Committee. Eight domestic (Sus Scrofa) pigs (47 ± 2.8 kg) were anesthetized (ketamine 15.7 mg·kg⁻¹·h⁻¹ and xylazine 2.33 mg·kg⁻¹·h⁻¹). A catheter was introduced into the external left jugular vein for infusion of 2% inulin and 5% albumin in 0.9% saline (2.5 ml/min). A catheter was positioned in the left carotid artery for sampling arterial blood.

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Oxygen content and monitoring mean arterial pressure. Body temperature was monitored with a thermoprobe. The animal was kept warm with a warming blanket.

Surgical preparation. The right kidney was exposed through a right paramedian laparotomy. The kidney was freed of connective tissue, weighed, and placed in a lexan kidney holder (Mayo Clinic, Engineering Services) and held upright by a prism stand for the remainder of the experiment. The kidney was surrounded by cotton wool soaked in saline and mineral oil, and kept warm by a saline drip (37°C). A pneumatic vascular occluder (5–6 mm; Harvard Apparatus, Holliston, MA) was placed around the right renal artery, and an ultrasound flow probe (T206 Flowmeter; Transonic, Ithaca, NY) was placed in between the occluder and kidney hilus. Ureters were cannulated bilaterally for urine collection from both kidneys throughout the experiment. A bolus of insulin (60 ml) was followed by a continuous infusion of insulin (1 ml/min iv). Additionally, blood was collected for blood gas measurements from the right renal vein. P\textsubscript{\text{O}}\textsubscript{2} was measured by advancing Clark electrodes (100-μm diameter tip; Unisense, Aarhus, Denmark) into the renal cortex and the outer medulla. The tip of the electrode penetrated the right kidney capsule to depths between 0.5 and 0.8 cm and between 1 and 1.2 cm for cortex and outer medulla, respectively, as verified postprocedure by injection of India ink and dissection. Ventilation rate and tidal volume were adjusted to maintain arterial PO\textsubscript{2}, PCO\textsubscript{2}, and pH between 90 and 110 \text{mmHg}, 35 and 50 mmHg, and 7.3 and 7.5, respectively. A 45-min rest period preceded the start of urine collections and experimental maneuvers.

Experimental protocol. The experiment comprised six sequential maneuvers. The 15-min maneuver period allowed for urine and systemic, right renal vein blood collections. Single-kidney insulin clearance was determined from these collections. Systemic arterial and right renal vein samples were collected for blood gas analysis (Instrumentation Laboratory GEM Premier 3000). After a baseline control period, the occluder was initially inflated progressively with a step-wise control period, the occluder was initially inflated progressively with maneuvers. The 15-min maneuver period allowed for urine and blood gas measurements from the right renal vein. P\textsubscript{\text{O}}\textsubscript{2} was measured by advancing Clark electrodes (100-μm diameter tip; Unisense, Aarhus, Denmark) into the renal cortex and the outer medulla. The tip of the electrode penetrated the right kidney capsule to depths between 0.5 and 0.8 cm and between 1 and 1.2 cm for cortex and outer medulla, respectively, as verified postprocedure by injection of India ink and dissection. Ventilation rate and tidal volume were adjusted to maintain arterial PO\textsubscript{2}, PCO\textsubscript{2}, and pH between 90 and 110 \text{mmHg}, 35 and 50 mmHg, and 7.3 and 7.5, respectively. A 45-min rest period preceded the start of urine collections and experimental maneuvers.

Statistical analysis. Repeated-measures analysis of variance (R-ANOVA) was used to test the changes due to interventions with post hoc comparisons to baseline conducted with Dunnet’s test. Differences between parameters measured on the occluded and contralateral kidneys (Table 1 and 2) were tested with a paired t-test. Results are reported as means ± SE with statistical significance (P < 0.05), unless otherwise noted.

RESULTS

Renal hemodynamics and function. Plasma and urine insulin concentrations (9) for each clearance period were measured using a standard colorimetric method. GFR was estimated by the clearance of insulin as GFR = U\text{ins}/V\text{ins}, where U\text{ins} is the urinary concentration of insulin, V is the urine flow rate (ml/min) and P\text{ins} is the plasma concentration of insulin. Plasma and urine sodium concentrations (P\text{Na} and U\text{Na}) were measured with a flame photometer (IL943; Instrumentation Laboratory, Monza, Italy). The amount of sodium reabsorbed was calculated as T\text{Na} = P\text{Na} \times GFR - U\text{Na}, V. Percent changes reported reflect the averaged outcome from calculations for each maneuver with respect to baseline.

Calculation of renal oxygen delivery, consumption, and extraction ratios. Renal oxygen consumption, \( V_o2 \) (ml·min\(^{-1}\)·100 g\(^{-1}\)), was calculated as the product of RBF and the arterial-venous oxygen difference in O\text{2} content and normalized for renal weight (7, 8). The arterial oxygen content (C\text{a}) was calculated by C\text{a} = (1.31·H\text{b}·S\text{a}O2) + (0.003·P\text{a}O2), and the renal venous oxygen content (C\text{v}) was calculated as C\text{v} = (1.31·H\text{b}·S\text{v}O2)/(0.003·P\text{v}O2), where H\text{b} is hemoglobin concentration (mg/dl), S\text{O}2 is O\text{2} saturation (%) as determined by blood gas analysis in the artery (\( a \) or vein (\( v \)), and P\text{a}O2 and P\text{v}O2 are the arterial (\( a \)) or renal vein (\( v \)) oxygen tension (mmHg). Renal oxygen delivery, DO\text{2}, was calculated as DO\text{2} (ml·min\(^{-1}·100\) g\(^{-1}\)) = RBF·C\text{a} and similarly normalized for renal weight. Renal oxygen extraction ratio, O\text{2}ER, was calculated as O\text{2}ER (%) = V\text{a}O2/DO\text{2}, while the O\text{2} efficiency for tubular sodium reabsorption was determined by the ratio of tubular sodium reabsorption (T\text{Na}) to V\text{o}2.

Tissue oxygenation. The Clark electrodes were connected to A/D converters, OxyMeter (Unisense). Digitized data were collected in 1-s intervals for the duration of the experiment with Sensor Trace Basic v1.3 (Unisense), and each probe’s data were averaged for the representative maneuver period. The sensors were calibrated in a 21% oxygenated lactate Ringer solution and an anoxic sodium bisulfide solution at 37°C. The criterion for probe calibration was a successful validation (±2 mmHg) with two samples of arterial and venous blood (20 cc) having different P\text{O}2 values (95 ± 2.7 and 44 ± 3.0 mmHg) measured prior to experimental maneuvers.

Table 1. Whole kidney hemodynamic response of the acutely stenotic kidney to progressive acute renal arterial stenosis at baseline, autoregulation, 20%, 40%, and 60% decrease in RBF, and recovery

<table>
<thead>
<tr>
<th></th>
<th>GFR, ml/min</th>
<th>T\text{Na}, mol/min</th>
<th>DO\text{2}, ml·min(^{-1})·100 g tissue(^{-1})</th>
<th>V\text{o}2, ml O\text{2}·min(^{-1})·100 g tissue(^{-1})</th>
<th>T\text{Na}/V\text{o}2, mol Na/ml O\text{2}</th>
<th>A-V O\text{2} O\text{2} mol O\text{2}·min(^{-1})·100 g tissue(^{-1})</th>
<th>P\text{v}O2, mmHg</th>
<th>O\text{2}ER, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL</td>
<td>34.9±4.6</td>
<td>4.9±0.6</td>
<td>32.1±5.7</td>
<td>6.1±0.5</td>
<td>0.92±0.1</td>
<td>2.67±0.3</td>
<td>48.8±1.9</td>
<td>18.7±1.9</td>
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<tr>
<td>AR</td>
<td>33.5±4.4</td>
<td>4.3±0.5</td>
<td>31.2±4.7</td>
<td>5.8±0.4</td>
<td>0.94±0.1</td>
<td>2.2±0.2</td>
<td>49.3±1.4</td>
<td>16.4±1.4</td>
</tr>
<tr>
<td>20</td>
<td>21.2±4.5</td>
<td>2.6±0.6</td>
<td>25.6±2.4</td>
<td>4.5±.5</td>
<td>0.63±0.1</td>
<td>2.23±0.2</td>
<td>51.5±2.1</td>
<td>16.2±1.8</td>
</tr>
<tr>
<td>40</td>
<td>15.2±7.6*</td>
<td>1.9±0.8</td>
<td>19.7±3.3</td>
<td>2.7±3.0</td>
<td>0.58±0.2</td>
<td>2.14±0.2</td>
<td>52.2±2.0</td>
<td>15.2±1.7</td>
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<tr>
<td>60</td>
<td>4.9±2.6*</td>
<td>0.58±0.3*</td>
<td>13±1.8*</td>
<td>2.3±3.0</td>
<td>0.26±0.1*</td>
<td>2.18±0.14</td>
<td>47.8±1.5</td>
<td>15.5±1.0</td>
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<tr>
<td>REC</td>
<td>40.7±11.5</td>
<td>5.2±1.4</td>
<td>28.3±5.5</td>
<td>5.4±3</td>
<td>0.65±0.2</td>
<td>3.13±0.26</td>
<td>49.1±19</td>
<td>21.7±2.1</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. BL, baseline; AR, autoregulation; 20%, 40%, 40%; 60, 60%; REC, recovery; GFR, glomerular filtration rate; T\text{Na}, tubular sodium reabsorption; DO\text{2}, oxygen delivery; V\text{o}2, renal oxygen consumption measured from arterial venous differences; T\text{Na}/V\text{o}2, O2 efficiency for sodium reabsorption; A-V O\text{2}, arteriovenous oxygen differences; P\text{v}O2, renal vein O2 tension; O\text{2}ER, oxygen extraction ratio in the stenotic kidney. *P < 0.01 compared to baseline.

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GFR, or $T_{Na}$ in the contralateral kidney was observed during these maneuvers. (Table 2).

Renal $O_2$ delivery, extraction ratio ($O_2$ER) and transport efficiency. Renal $O_2$ delivery was not altered during the decrease of RBF within the range of autoregulation and following a 20% reduction in RBF (Fig. 1D), but subsequent decrements in RBF were paralleled by decrements in $O_2$ delivery and consumption (Fig. 1E), suggestive of a balance that should maintain tissue oxygenation. During the stepwise progressive stenosis (by 40% and 60% of RBF), total renal $O_2$ consumption decreased significantly from the control period in the stenotic kidney (Table 1) ($-48.2 \pm 9.1$ and $-58.9 \pm 4.7\%$, respectively, $P < 0.01$) matched by comparable decreases in GFR ($-63.6 \pm 14.6$, and $-88.5 \pm 4.8\%$, $P < 0.01$, Fig. 1B) and $T_{Na}$ ($-65.9 \pm 13.9$ and $-89.5 \pm 4.3\%$, $P < 0.01$, Fig. 1C), the major determinant of $O_2$ consumption, and recovered to control levels during recovery. The arterial-venous $O_2$ differences ($2.7 \pm 0.3$ ml $O_2$/min) and $O_2$ER ($18.7 \pm 1.9\%$) did not change from baseline (Table 1) with reductions in RBF. However, less sodium was reabsorbed per mole of $O_2$ with reduced RBF, as evidenced by the reduced $O_2$ efficiency for sodium reabsorption. (Table 1) The $O_2$ efficiency for sodium reabsorption returned to baseline values with recovery.

Table 2. Renal hemodynamic responses of the contralateral kidney to progressive acute renal arterial stenosis

<table>
<thead>
<tr>
<th></th>
<th>GFR, ml/min</th>
<th>$T_{Na}$, mol/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL</td>
<td>39.1±5.6</td>
<td>5.4±0.8</td>
</tr>
<tr>
<td>AR</td>
<td>40.2±5.3</td>
<td>5.3±0.7</td>
</tr>
<tr>
<td>20</td>
<td>43.5±6.1</td>
<td>5.1±0.9</td>
</tr>
<tr>
<td>40</td>
<td>53.3±8.2</td>
<td>5.7±1.0</td>
</tr>
<tr>
<td>60</td>
<td>40.1±5.6</td>
<td>5.0±0.7</td>
</tr>
<tr>
<td>REC</td>
<td>48±7.6</td>
<td>5.9±1.0</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE.
increase in RBF, O₂ tensions fell (Fig. 3) despite O₂ delivery and consumption decreases (Fig. 1, D and E).

DISCUSSION

The main new finding from this study is the decrease in directly measured regional renal tissue oxygenation (more pronounced in the medulla than the cortex) during graded acute renal arterial stenosis. The decreased tissue Po₂ during acute and progressive renal arterial stenosis supports the notion that tissue hypoxia occurs despite the concurrent fall in whole kidney O₂ delivery and consumption. An important observation from this study indicates for the first time that renal ischemia occurs during acute progressive renal arterial stenosis despite conventional measures failing to indicate the presence of ischemia. The decreases in renal tissue oxygenation implicate factors contributing to hypoxia that may alter local tissue O₂ delivery and consumption, as opposed to whole kidney alterations.

Interestingly, in a chronic 2 kidney, 1 clip rat model of Goldblatt hypertension, Palm et al. (20) demonstrated reduced cortical Po₂ in the clipped kidney, underscoring the present findings. Similarly, Johannes et al. (6) found a decrease in cortical and medullary tissue Po₂, in venous Po₂, and a widening of the venous/tissue Po₂ gap during acute normovolemic hemodilutions. Further, their study showed an aggressively amplified O₂ extraction ratio, which counterbalanced their conclusion that arteriovenous (a-v) O₂ shunting was increased. Nevertheless, the present study extends the findings of Palm et al. and conclusions of Johannes et al. by concurrently demonstrating an accelerated decline in medullary PₐO₂ with respect to cortex, while in the presence of stable O₂ extraction ratios and a-v O₂ differences. Furthermore, this study demonstrates decreases in O₂ delivery concurrently with O₂ consumption.

The observed hypoxic response to acute RBF reductions suggests for the first time that not only is the term ischemia appropriate but further indicates that renal arterial stenosis involves hypoxia to a greater extent in the medulla compared with the cortex. This is in agreement with clinical observations of medullary vulnerability to abrupt decreases in renal perfusion (4, 5, 23). Hypoxia occurs despite the lack of an overall O₂ delivery and consumption mismatch, thus implying that global measures of renal hemodynamics are insufficient to fully explain regional differences.

While our present findings cannot fully explain the increased hypoxia, several possibilities merit mention. For example, reduced perfusion may selectively increase O₂ consumption by facilitating reabsorption activities in TAL (1). The possibility of redistribution in sodium reabsorption along the medullary TAL warrants further investigation. The reduced efficiency or ratio of TNa to O₂ consumption may suggest basal metabolic consumption has an increased impact on the diminished local supply of O₂. Moreover, the reduced O₂ efficiency for sodium reabsorption may reflect the increased impact of medullary O₂ consumption occurring due to a shifting of reabsorption from paracellular to transcellular pathways, resulting in increased stoichiometric energy requirements. Furthermore, arteriovenous shunting (12, 18, 25) may also occur during progressive renal arterial stenosis and warrants further investigation.

Because of the parallel arrangement of descending and ascending vasa recta, important for the concentrating mechanism, the kidney is subjected to “shifting” or shunting of arterial O₂ to the venous side (11, 16, 25). Shunting accounts for both the higher O₂ concentration in the renal vein with respect to the superficial cortex and for the very low O₂ concentration in the renal papilla (3, 25). The contribution of this mechanism to renal hypoxia, however, is unclear. Nevertheless, the notion that a-v shunting occurs during progressive renal arterial stenosis and the added possibility that stoichiometric energy requirements are increased, due to shifting reabsorption from paracellular to transcellular pathways, cannot be excluded as potential contributors to tissue hypoxia.

The present study demonstrates that despite the lack of an O₂ delivery and consumption mismatch, regional ischemia may exist during acute renal arterial stenosis, and it suggests that hypoxia may play a role in pathophysiology. While the present study demonstrates variations in intrarenal PₐO₂ associated with healthy kidneys in an acute situation, future studies will need to
explore changes in tissue $PO_2$ within chronic disease. The Clark type $O_2$ electrodes measure $O_2$ through the consumption of $O_2$ at the tip of the electrode (11, 19, 20, 25) and are limited by the need for penetration of the kidney capsule; however, they are considered to be a reference standard for assessment of tissue oxygenation (10). Recent advances in polymer biomaterials may offer a promising coating that may improve future sensor biocompatibility. (10) Our observations in swine have particular clinical relevance because the pig kidney is anatomically and physiologically comparable to the human kidney (13, 14). Future studies should also examine renal tissue $PO_2$ in conjunction with chronic renal arterial stenosis.

**Perspectives and Significance**

Importantly, in this study we measured the concomitant changes in both cortical and medullary tissue $O_2$ and demonstrated that medullary losses in tissue oxygenation exceed those of the cortex during RBF, $O_2$ delivery and consumption reductions. Overall, our findings underscore the complex relationship between many hemodynamic variables, and highlights that global renal supply and demand may not be representative of local conditions and may mask regional disparity. Although conventional methods may downplay the existence of renal ischemia in acute renal arterial stenosis, the present study provides convincing evidence that hypoxia may be present in the early stages of acute renal arterial stenosis. Moreover, the evidence for renal ischemia during acute progressive renal arterial stenosis may provide important support for the role that hypoxia may play in the pathophysiology of this disease. The current findings may also implicate factors such as redistribution of sodium reabsorption along the medullary TAL, the shifting of reabsorption from paracellular to transcellular pathways possibly increasing stoichiometric energy requirements, increased impact of basal metabolic demands upon a diminished supply, and enhanced renal arteriovenous oxygen shunting as potential mediators of microvascular and glomerular disease that precede direct and detectable effects upon the kidney.

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