Noninvasive Evaluation of a Novel Swine Model of Renal Artery Stenosis

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Abstract. Intrarenal hemodynamics and excretory function distal to renal artery stenosis are difficult to quantify noninvasively. In this study, a swine model of chronic unilateral renal artery stenosis, achieved by implantation of an intravascular device that leads to a gradual and progressive luminal area narrowing, was developed and evaluated. Bilateral cortical and medullary volumes, blood flows, and segmental tubular dynamics were assessed in the intact kidneys of seven pigs using electron-beam computerized tomography before and 1 mo after implantation of the device. Within 1 mo, a 66% angiographic stenosis was significantly correlated with a 25% increase in BP. The volume and blood flow were markedly lower in the stenotic compared with the contralateral kidney and cortex, while the medulla exhibited minimal changes. In the stenotic kidney, intratubular contrast content has decreased in all nephron segments, especially in the distal tubule, where it correlated with an increase in serum creatinine and stenosis severity. In the contralateral kidney, dilution of proximal tubular fluid correlated with the increase in BP, likely due to pressure-natriuresis. In conclusion, the swine model closely resembles human renovascular hypertension. In the stenotic kidney, the hemodynamic impairment of the cortex is dissociated from the relatively preserved renal medulla, and the earliest effect on excretory function is observed in the distal nephron, where the full in the amount of fluid reaching that segment is directly proportional to the renal arterial compromise. Electron-beam computerized tomography shows promise to noninvasively quantify, follow-up, and study changes in concurrent, in vivo intrarenal hemodynamics and segmental tubular function in renovascular hypertension.

Renal artery stenosis has long been known to be the major cause of renovascular hypertension in humans, and may result in target organ damage, ischemic nephropathy, and end-stage renal disease (1). Although potentially reversible, cure of either hypertension or ischemic nephropathy is not always achieved even with restoration of renal blood supply, partly due to long-term intrarenal modifications (2). There is currently no noninvasive tool capable of predicting which patients with ischemic nephropathy will have an improvement in renal function after intervention (2). Furthermore, despite intense investigation both in humans and in various animal models of renal artery stenosis, the mechanisms responsible for the progressive renal functional and structural alterations are still unclear, partly due to the lack of reliable, noninvasive techniques capable of quantifying renal regional hemodynamics and function in the intact kidney distal to a stenosis in the renal artery.

Experimental induction of renal artery stenosis (traditionally achieved by either surgical ligation of the renal artery or devices surgically placed around it) in animal models, such as the dog (3,4) and the rat (5,6), has indeed enabled extensive studies and greatly contributed to the understanding of mechanisms underlying consequences of renal hyperperfusion. Nevertheless, although the pig may represent a favorable model to study renal pathophysiology, with its renal anatomy and physiology comparable to those of humans (7), a well-established model of experimental swine renal artery stenosis has not been described, nor has it been resolved whether the pig would develop renovascular hypertension consequent to chronic renal artery stenosis. Furthermore, in porcine epicardial coronary arteries, stenoses have been successfully achieved through percutaneous deployment of intracoronary, balloon-expandable, local-irritant stents, which lead to a proliferative neointimal response and progressive luminal area narrowing (8,9). The progressive nature of the stenosis simulates development of a human vascular lesion more closely than an abrupt surgical constriction of the vessel. However, the feasibility and effectiveness of a percutaneous approach in producing a progressive stenosis in the renal artery have not been evaluated.

We have shown previously (10–12) that intrarenal hemodynamics (i.e., cortical and medullary volumes and blood flows) and concurrent segmental nephron dynamics (i.e., intratubular transit times and relative fluid concentrations) could be reliably quantified in the intact kidney, using electron-beam computerized tomography (EBCT). EBCT even allows discrimination of slightly different intrarenal perfusion patterns in patients with atherosclerotic compared to fibromuscular dysplastic renal ar-
tery stenosis (13). We have further demonstrated that even within the range of blood flow autoregulation, subtle alterations in renal perfusion (14) and segmental nephron function (15) consequent to changes in renal perfusion pressure were detectable in vivo with CT. This technique may now also provide novel insight into the effects of chronic unilateral renal artery stenosis on the regional concurrent hemodynamics and function of the hypoperfused kidney distal to the stenosis, as well as in the contralateral kidney. This methodology may thereby prove to be very useful in the evaluation of renal disease in general, and the renovascular hypertensive patient in particular.

Hence, the purpose of this study was to first develop and characterize a swine model of unilateral renal artery stenosis using percutaneous deployment of an intravascular stent in the renal artery, and to then assess intrarenal regional hemodynamics and function distal to a stenosis in the renal artery. The effectiveness of this approach to induce renal artery stenosis, and the feasibility of achieving and quantifying renal hypoperfusion, renal dysfunction, and renovascular hypertension in a pig model, were evaluated by defining: (1) degree of stenosis (angiographic appearance) of the renal artery; (2) daily changes in arterial pressure; (3) in vivo, bilateral renal hemodynamics and excretory function (e.g., renal volume, blood flow, and tubular dynamics) assessed with EBCT before and after implantation of stent; and (4) ex vivo histopathologic consequences to the renal artery and parenchyma. Finally, to demonstrate the renovascular origin of the change in BP, the renal arterial stenosis was revascularized in one pig by balloon angioplasty, and BP was monitored for temporally related changes.

Materials and Methods

This study was performed according to institutional guidelines for the care and use of laboratory animals. Seven domestic female pigs were studied with EBCT at baseline, and again 1 mo (an average of 4.6 wk) after placement of an intravascular stent in the renal artery.

Experimental Protocol

Animal Preparation. On the day of each “acute” study, each animal was anesthetized with 0.5 g of intramuscular ketamine, intubated, and mechanically ventilated with room air. Anesthesia was maintained with a mixture of 30 mg/kg ketamine and 3 mg/kg xylazine in normal saline, administered via an ear vein cannula at a rate of 1 to 2 ml/min. Under sterile conditions, an 8F arterial guide was advanced through a left carotid arterial sheath and positioned under fluoroscopic guidance in the upper abdominal aorta, to be used for performance of renal angiography, and saline infusion (1.5 ml/min) was initiated. Mean arterial pressure was monitored on-line during each (“acute”) study through a side arm of this catheter. Another similar guide catheter was advanced through a left jugular vein vascular sheath to the right and left renal veins, and then inferior vena cava, for collection of blood, after which it was replaced with a “pigtail” catheter positioned in the superior vena cava, to be used for contrast media injections. During repeat studies, the femoral vessels were used for vascular access, so that the right carotid artery and jugular vein remained intact. Finally, a supra-pubic 18-gauge angiocath was inserted in the urinary bladder for collection of urine during the “acute” study. Ureteral catheterization was not performed to minimize potential complications. The animal was then transferred and positioned in the EBCT scanning gantry for performance of in vivo studies.

Urine was collected from the urinary bladder catheter during a 10-min control period before the EBCT flow study, and peripheral blood was collected in the middle of this period. After the EBCT studies, the animal was transferred back to the animal surgery laboratory for performance of a renal angiogram. To minimize the risk of perturbing renal function, only non-ionic, low-osmolar contrast media were used during all imaging processes.

Renal angiography was performed after each EBCT study by further advancing the aortic catheter to the level of the renal arteries. At baseline studies, the intravascular stent was implanted at this stage, while in repeat studies selective contrast injections were performed to visualize the lumen of the arteries. The degree of stenosis was determined from the images off-line by assessing the decrease in luminal diameter of the renal artery compared to a stenosis-free segment, using a quantitative coronary angiography system (16). After completion of the “acute” study, all catheters were removed, the access vessels were ligated, and the incision was closed. At baseline studies, a permanent pressure transmitter was also implanted in the left carotid artery (vida infra). The pig received a standard regimen of postoperative analgesic and antibiotic treatment.

Induction of Renal Artery Stenosis. After the baseline EBCT study, heparin (5000 U) was given intravenously. A 5.0-mm percutaneous transluminal coronary angioplasty (PTCA) balloon containing 23-gauge copper wire coils wrapped around the deflated balloon was advanced through the aortic guide into the left renal artery over a 0.014-inch PTCA guide wire, and engaged in the proximal-middle section of the renal artery under fluoroscopic guidance. The balloon was inflated once to high pressure (14 atm), resulting in expansion of the coil to full balloon diameter, and then deflated and removed, leaving the copper coil embedded in the vascular wall. Another bolus of heparin was given, and fluoroscopy with selective renal angiography was performed 15 min later to ensure vessel patency and coil location. The copper coil was selected because of its characteristic generation of local irritation, leading to a progressive proliferative response and stenosis (17).

BP Measurement. Continuous (“chronic”) BP recording was obtained using a Physiotel® telemetry system (Data Sciences, St. Paul, MN), which allows for remote recording of BP in the chronically instrumented animal. After removal of the vascular sheath from the left carotid artery during the baseline study, an antithrombogenic telemetry catheter was guided into the puncture site, advanced 2 cm into the vessel, and sutured in place. The transmitter body was secured in a cervical pouch, and the incision was closed with sutures. A single bolus of heparin (5000 U) was administered, followed later by a small dose of penicillin to prevent infection, and the animal was returned to a cage containing a telemetry receiver, for continuous BP monitoring. Systolic, diastolic, and mean BP were recorded thereafter in the conscious pig at 5-min intervals, and averaged for each 24-h period. The levels reported (Table 1) were measured for 2 d before any consistent increase in BP (baseline), and for 2 d before the second study (1 mo).

EBCT Studies. After surgical preparation, the animals were transferred and positioned in the EBCT (Imatron C-150; Imatron, South San Francisco, CA) scanning gantry. After a 1-h recovery period, EBCT studies (one to determine renal volume, and one for simultaneous measurement of intrarenal vascular and tubular flow) were performed during respiratory suspension at end-expiration. Using abdominal localization scans, all tomographic levels containing
both kidneys were identified, and two mid-hilar tomographic levels demonstrating both kidneys were selected for performance of a flow study.

Renal Hemodynamics and Tubular Dynamics. For the study of renal perfusion and tubular dynamics, the kidneys were scanned in the standard resolution (50 ms/image), multislice flow mode, resulting in two contiguous 7-mm-thick tomographic sections through the hilar regions of both kidneys. Forty consecutive scans were performed over two contiguous 7-mm-thick tomographic sections through the hilar level for subsequent measurement of renal volume.

Table 1. Description of a group of seven domestic pigs at baseline and 1 mo after implantation of a stent in the left renal artery.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>1 Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>24.5 ± 1.9</td>
<td>34.3 ± 1.6b</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>112.0 ± 4.6</td>
<td>140.6 ± 11.3b</td>
</tr>
<tr>
<td>Plasma sodium (mEq/L)</td>
<td>135.1 ± 1.4</td>
<td>132.8 ± 0.7</td>
</tr>
<tr>
<td>Urine sodium (mEq/L)</td>
<td>47.2 ± 14.9</td>
<td>56.4 ± 7.6</td>
</tr>
<tr>
<td>Plasma potassium (mEq/L)</td>
<td>4.7 ± 0.1</td>
<td>5.5 ± 0.6</td>
</tr>
<tr>
<td>Urine potassium (mEq/L)</td>
<td>60.2 ± 23.7</td>
<td>40.2 ± 7.8</td>
</tr>
<tr>
<td>Serum creatinine (μmol/L)</td>
<td>79.6 ± 8.8</td>
<td>114.9 ± 8.8b</td>
</tr>
<tr>
<td>Urine creatinine (mol/L)</td>
<td>2.9 ± 1.0</td>
<td>2.5 ± 0.7</td>
</tr>
<tr>
<td>Plasma renin activity (ng/ml per h)</td>
<td>0.4 ± 0.1</td>
<td>6.7 ± 4.7</td>
</tr>
<tr>
<td>Serum aldosterone (pg/ml)</td>
<td>65.0 ± 26.6</td>
<td>75.5 ± 30.0</td>
</tr>
</tbody>
</table>

a Data are mean ± SEM. b P ≤ 0.05 compared with baseline.

density of each sampled region in each of the 40 images was then calculated and recorded by the software, and the data were transferred to a personal computer to generate for each region distinctive time–density curves, describing the change in tissue density consequent to transit of contrast in that region.

For each peak observed in the cortical, medullary, and papillary regions of interest, the area under the curve, its peak height, and mean transit time (18) were calculated (12), using a data analysis/graphics computer program (KaleidaGraph™). The parameters obtained from the first peak in each region (which results from vascular transit of contrast) were used to calculate regional perfusion (Figure 1). All subsequent peaks resulted from transit of contrast in the tubular compartment, as described previously (12). Unlike the canine kidney, the porcine kidney has a heterogeneous distribution of loops of Henle, most of which are short (7); therefore, descending or ascending limbs could not be distinguished.

Regional perfusion, which refers to blood flow normalized per unit tissue (ml of blood/min per cm³ tissue), was calculated directly from the vascular peak of each time–density curve (19) as:

\[
\text{Perfusion} = \frac{\text{Area under tissue curve}}{\text{Area under aortic curve}^{-1} \times \text{Mean transit time}^{-1} \times 60}
\]

Intratubular contrast concentration relative to pure blood (i.e., degree of concentration or dilution of tubular fluid, analogous to tubular fluid-to-plasma ratio) was calculated for each nephron segment (identified in the time–density curves) as the ratio of its area to that under the aortic curve (12).

Renal Volume. After identification of the renal cortex and medulla on each tomographic level obtained in the volume study, volumes were calculated using a statistical point-counting volume estimation program implemented with ANALYZE™ (10,20). Papillary volume was not independently determined, since the papilla could be distinguished from the medulla at the vascular phase, during which a volume scan was performed. Whole kidney volume was calculated as the sum of cortical and medullary volumes.

Renal Blood Flow. Renal blood flow was subsequently calculated for each (right and left) cortex and medulla as the product of its perfusion (ml/min per cc tissue) and the corresponding regional volume (cm³), to yield absolute cortical and medullary blood flows in units of ml of blood/min. Blood flow of the whole kidney (renal blood flow) was calculated as the sum of cortical and medullary blood flows (11).

Post-Mortem Examination. After completion of studies, six of the animals were killed by Sleepaway® (Fort Dodge Laboratories, Fort Dodge, IA). Both kidneys were removed with their renal arteries attached and preserved in formalin 10% for a few days. Cross sections were obtained from each kidney, and the renal arteries were step-sectioned proximal, distal, and through the stenosis. These were processed routinely for light microscopy and stained with hematoxylin and eosin and Lawson’s elastic-van Gieson stains (17). The histopathologic features of the renal arterial wall lesion were studied, as well as intrarenal histopathologic changes. The degree of proliferative response within the vessel wall was graded by assessing the thickness (21) of the vascular adventitia, media, and neointima compared to adjacent, stent-free segments of the same vessel, and to the contralateral renal artery.

Renal Angioplasty. In one animal, on the day of the repeat study, balloon angioplasty was performed in the stenotic renal artery. In an attempt to normalize BP, a 4F PTCA balloon catheter was inflated at 4 atm for 30 s to partially dilate the stenosis in the stenotic renal artery from 79 to 48%, just below the severity considered hemodynamically significant. The reason for the lesion being only partially dilated at that point was to ensure that any re-stenosis would
render it hemodynamically significant, and thereby detectable by remote monitoring of BP. To determine objective change in luminal diameter, renal angiography was repeated after an additional 2 wk, during which BP recording continued.

**Statistical Analyses**

Results of the quantitative studies are expressed as means ± SEM. Statistical comparisons between different experimental periods or between kidneys were performed using *t* test. Regressions were calculated by the least-squares fit. Statistical significance was accepted at *P* ≤ 0.05.

**Results**

At baseline, both the right and left renal arteries of all of the pigs were patent and devoid of any evidence of a stenosis. Deployment of stent in the left renal artery was technically successful in all the animals. All arteries remained widely patent at 15 min after placement of stent, and the procedure was not associated with any postoperative mortality or symptomatic morbidity.

Mean arterial pressure started rising 11 ± 4 d after the procedure, continued rising over a period of 7 ± 1 d to reach a plateau, and by 1 mo after placement of stent had increased by 25% (Table 1) (*P* = 0.0001 compared to baseline). At that time, renal angiography revealed a 66 ± 10% reduction in luminal diameter in the left renal artery (Figure 2). This included two mild (<50%), two moderate (50 to 75%), and three severe (76 to 99%) stenoses. The length of time for appearance of hypertension was related to the eventual degree of stenosis, although this relationship was not statistically significant (*r* = 0.57, *P* = 0.14), and neither was the time it took to BP plateau (*r* = −0.07). On the other hand, the change in mean arterial pressure (*y*) correlated well with the angiographically determined degree of stenosis (*x*) in the left renal artery (*y* = −16 + 0.64*x*, *r* = 0.86, *P* = 0.006). Renal vein plasma renin activity lateralized to the side of the stenotic kidney in only three pigs; systemic plasma renin activity showed a 16-fold increase (Table 1), but in our seven pigs this change did not reach statistical significance due to large variability. Serum creatinine increased by 40.2 ± 14.7% (*P* = 0.01), while sodium and potassium remained unchanged (Table 1).

**Renal Hemodynamics**

At baseline, the right and left cortical, medullary, and whole renal volumes and blood flows, as measured with EBCT, were very similar (Table 2). Papillary volume, and consequently blood flow in ml/min, could not be determined separately from the medulla. One month after induction of stenosis, the volume and blood flow of the cortex contralateral to the stenosis was found significantly increased (Table 2) (*P* = 0.018 and *P* = 0.011, respectively), as was contralateral renal volume and blood flow (*P* = 0.016 and *P* = 0.012, respectively), whereas the volume and blood flow of the stenotic kidney and cortex failed to increase. The volume and blood flow of the stenotic cortex were at that time significantly lower (about one-half) than those of the contralateral cortex (Table 2) (*P* = 0.013 and *P* = 0.011, respectively), and stenotic whole renal volume and blood flow were also about one-half of those of the contralateral kidney (*P* = 0.017 and *P* = 0.010, respectively).

In the stenotic kidney, the angiographically determined degree of renal artery stenosis correlated well with the change in volume and blood flow of the cortex (Figure 3) and whole kidney (*r* = 0.92, *P* = 0.002, and *r* = 0.92, *P* = 0.003, respectively). Medullary volume, on the other hand, remained similar between the contralateral and the stenotic kidneys (*P* =
0.08), although its blood flow was slightly lower in the stenotic kidney than in the contralateral (\( P = 0.049 \)). Furthermore, compared with the cortex and whole kidney, the change in medullary volume and blood flow exhibited a weaker correlation with severity of stenosis (\( r = 0.61 \) and \( r = 0.76 \), respectively).

In the contralateral kidney, changes in volume and flow showed no correlation with either changes in BP or with the degree of stenosis in the stenotic kidney. By 1 mo after the procedure, however, a small increase in perfusion of the cortex (i.e., blood flow normalized per unit renal volume or weight, as calculated directly from the time–density curves) was observed, and was associated with an increase in papillary perfusion (from 2.97 ± 0.52 to 4.20 ± 0.68 ml/min per cm\(^3\) tissue, \( P = 0.008 \)). Cortical and papillary perfusions of the stenotic kidney were by then significantly lower than in the contralateral kidney (\( P = 0.02 \) and \( P = 0.03 \), respectively), whereas medullary perfusion was similar.

**Renal Tubular Function**

At baseline, EBCT-derived segmental nephron intratubular fluid (contrast media) concentration was very similar between the contralateral and the stenotic kidneys (Table 3). When the study was repeated after development of stenosis, renal intratubular fluid concentration in the stenotic kidney had decreased in the proximal tubule, loop of Henle, and collecting duct (\( P = 0.04 \), \( P = 0.05 \), and \( P = 0.02 \), respectively). In the distal tubule of the stenotic kidney, a 20% decrease in tubular fluid concentration did not achieve statistical significance due to large variability. However, compared with the contralateral kidney, the intratubular fluid concentration was significantly lower only in the distal tubule and collecting duct (\( P = 0.04 \), \( P =

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**Figure 2.** Renal angiogram of a pig 1 mo after placement of an intravascular stent in the left renal artery. The arrow points to a high-grade stenosis in the middle portion of the left renal artery, followed more distally with post-stenotic dilation.

**Table 2.** Renal volume and blood flow, quantified using electron-beam computed tomography, at baseline and 1 mo after placement of a stent in the left renal artery of six pigs\(^a\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cortex</th>
<th>Medulla</th>
<th>Whole Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>Volume (cc)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>63.2 ± 8.2</td>
<td>61.0 ± 6.8</td>
<td>15.2 ± 1.6</td>
</tr>
<tr>
<td>1 mo</td>
<td>52.4 ± 11.4(^b)</td>
<td>101 ± 15.3(^c)</td>
<td>16.7 ± 4.9</td>
</tr>
<tr>
<td>Blood flow (ml/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>294.2 ± 40.3</td>
<td>276.4 ± 29.6</td>
<td>50.4 ± 8.6</td>
</tr>
<tr>
<td>1 mo</td>
<td>234 ± 69(^b)</td>
<td>510 ± 87(^c)</td>
<td>45 ± 19(^c)</td>
</tr>
</tbody>
</table>

\(^a\) The stent causes a progressive stenosis in the renal artery, which by 1 mo was angiographically evident.

\(^b\) \( P \leq 0.05 \) compared with right kidney.

\(^c\) \( P \leq 0.05 \) compared with baseline.
Moreover, of all nephron segments, the best inverse correlation between the changes in intratubular fluid concentration and the increase in serum creatinine was observed in the distal tubule ($r = 0.71$, $P = 0.05$), which also correlated very well with the angiographic degree of stenosis (Figure 4) ($r = 0.90$, $P = 0.006$).

In contrast, in the contralateral kidney tubular fluid concentration in all nephron segments remained unchanged compared with the baseline study. Nevertheless, the change (from the baseline study) in proximal tubular contrast concentration correlated inversely and significantly with both the severity of stenosis ($r = -0.76$, $P = 0.049$) and with the increase in mean arterial pressure ($r = -0.8$, $P = 0.03$). The changes in contrast concentration in the distal tubule, the loop of Henle, and the collecting duct did not show a statistically significant correlation with the severity of stenosis or with mean arterial pressure.

No significant change was observed in mean tubular transit times in any segment in either kidney. The change in cortical or medullary perfusion, or in renal tubular dynamics, did not show a consistent relationship with lateralization of renins.

### Histopathologic Examination

#### Renal Arteries.

The most striking result implantation of the copper stent produced in the renal artery was a fibroproliferative intimal response surrounding areas where the internal elastic lamina was disrupted (Figure 5). The neointima contained numerous spindle-shaped cells within an abundant collagenous stroma, and there was marked luminal compromise with reduction of the original vascular lumen. The copper stents were located within the media of the renal arteries. Adjacent to the stent, an intense inflammatory infiltrate was identified consisting predominantly of neutrophils surrounded by lymphocytes and macrophages, with dystrophic calcium deposits frequently identified adjacent to the stent. The media surrounding the stent was disorganized with localized neovascularization and fibrosis, and extensive disruption of both the internal and external elastic lamina. Lymphocytic infiltrates were identified within the adventitia, most prominent adjacent to the copper stents. There was prominent adventitial neovascularization; these vessels showed prominent medial hypertrophy. Contralateral vessels were unremarkable with only mild focal intimal thickening. The internal and external elastic laminae were intact.

#### Renal Parenchyma.

The stenosed kidneys of the animals with the most severe stenoses showed severe interstitial fibrosis with associated tubular atrophy. Diffuse mononuclear inflammatory infiltrates were identified within the expanded interstitial regions. There were localized areas of ischemic collapse, which contained dystrophic calcium deposits. Glomeruli showed extensive segmental and global glomerulosclerosis. In the contralateral kidney, there was no significant segmental or global glomerulosclerosis. The tubules were back-to-back with minimal interstitial fibrosis, tubular atrophy, or interstitial inflammation. Interlobular size arteries and arterioles showed no significant intimal sclerosis.

Both stenotic and contralateral kidneys obtained from the rest of the animals showed minimal light microscopic alterations (interstitial fibrosis, tubular atrophy, inflammation, or glomerulosclerosis), with no significant sclerosis of intrarenal arteries or arterioles.

#### Renal Angioplasty.

In the animal that underwent balloon angioplasty, a 79% stenosis in the left renal artery was dilated to a residual stenosis of 48%, which is just below the degree considered hemodynamically significant (22). This procedure was followed by a decrease in BP (apart from a short postoperative increase) to baseline levels (Figure 6). However, 1 wk after the balloon dilation procedure, systemic BP was observed again to be on the rise (Figure 6). Renal angiography repeated 1 wk later revealed re-stenosis of the lesion to an 85% stenosis.
Table 3. Relative intratubular concentration of contrast media (%), assessed using electron-beam computed tomography, at baseline and 1 mo after placement of a stent in the left renal artery of six pigs

<table>
<thead>
<tr>
<th>Time</th>
<th>Proximal Tubule</th>
<th>Loop of Henle</th>
<th>Distal Tubule</th>
<th>Collecting Duct</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>Baseline</td>
<td>1.07 ± 0.1a</td>
<td>1.03 ± 0.1a</td>
<td>1.7 ± 0.2</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>1 mo</td>
<td>0.7 ± 0.1a</td>
<td>0.99 ± 0.1</td>
<td>1.3 ± 0.3</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.9 ± 0.7</td>
<td>2.9 ± 0.6</td>
</tr>
</tbody>
</table>

a P ≤ 0.05 compared with baseline.
b P ≤ 0.05 compared with right kidney.

Figure 4. Correlation between the angiographically determined degree of renal artery stenosis, and the change (from baseline to 1 mo) in distal tubular fluid concentration distal to the stenosis, as determined in vivo with electron-beam computerized tomography. These correlated as: y = 107 – 1.9x, r = 0.90, P = 0.006.

Discussion

This study demonstrates that chronic, progressive renal artery stenosis of various grades can be induced percutaneously in the pig. This model develops many pathophysiologic characteristics of human renal artery stenosis, including renovascular hypertension, unilateral decreases in cortical and whole kidney (but relative maintenance of medullary) volume and blood flow, impairment in renal function, lateralization of plasma vein renins, and a vascular lesion in the renal arterial wall. Furthermore, this study shows that regional alterations in renal hemodynamics and segmental nephron function distal to the stenosis are detectable in vivo with EBCT, with the most pronounced functional alteration observed in the distal nephron.

Continuous measurement of systemic BP in our chronically instrumented pigs enabled us to monitor the progression of the stenosis in the renal artery. As reported previously, the duration of blood flow compromise was sufficient to induce detectable functional changes (5,6,23). As would be expected, significant renal artery stenosis led to an increase in mean arterial pressure directly and significantly related to the severity of the stenosis in the renal artery. Our experimental group of adult pigs (24,25) with fully developed renal function (26,27) had normal baseline pressures (25), and the high levels of arterial pressure obtained in the repeat 1-mo study (Table 1) were much higher than normal for the adult pig (24,25). Moreover, the normalization of BP subsequent to revascularization of a stenotic renal artery, and reinstatement of hypertension upon re-stenosis, lends support to the conclusion that the increase in BP was renovascular in origin. Nonetheless, in line with a high false-negative rate observed in renovascular hypertensive humans (28), lateralization of plasma vein renins was not a consistent finding, and did not correlate with hemodynamic or functional alterations. Yet a significant unilateral stenosis was clearly associated with renal hemodynamic and functional derangements.

Similar to humans, we found that the decrease in whole kidney and cortical volumes in our group of pigs was directly related to the severity of stenosis. Progression of a significant stenosis in the renal artery is often accompanied by a decrease in the size of the affected kidney, in association with deterioration of renal function (29). In fact, a significant difference in size between the two kidneys—and especially a decrease in cortical volume (30)—is considered a clue for the existence of a unilateral renal artery stenosis (31), although in bilateral renal artery stenosis renal atrophy is not consistently observed (13,32). In the contralateral kidney, an increase in renal size and blood flow probably resulted from both an increase in the body size of the pigs (24) and the high perfusion pressure, since kidneys contralateral to renal artery stenosis may also appear hypertrophied (32).

In our pig model, blood flow to the whole kidney was lower in the stenosed compared with the contralateral kidney, as observed in humans with unilateral renal artery stenosis (33,34). In our experimental group, even blood flow normalized to renal size (cortical and papillary perfusions) was different between the two kidneys. We have shown before that in patients with fibromuscular dysplasia (analogous to Goldblatt hypertension), this reduction in renal and cortical volumes and blood flows correlated with the severity of the stenosis (13). Interestingly, this study supports previous reports from our laboratory (13) and others (35,36) of relative maintenance of medullary perfusion in renovascular hypertensive patients with a chronic reduction in renal blood flow. This redistribution of blood flow, demonstrating a decrease in cortical perfusion in
association with maintenance of good medullary tissue oxygenation, seems to represent a general renal protective mechanism \((32,37,38)\). In contrast, in animal models with an acute decrease in renal perfusion pressure, a marked decrease in either medullary \((39)\) or papillary \((14,40)\) perfusion is found, possibly reflecting differences between an acutely induced as opposed to a chronic stenosis. This observation underscores the need to investigate consequences of human renal artery stenosis in experimental models having a chronic reduction in renal perfusion pressure, since a gradual reduction may produce functional and morphologic consequences different from those observed with an acute ischemic injury \((37)\).

The decrease in cortical blood flow observed in this study probably resulted from a combination of decrements in both its perfusion (which correlated significantly with severity of stenosis) and volume. The initial decrease in volume may be partly an adaptive mechanism, aimed at preserving tissue perfusion by realignment of oxygen demand to match the lower oxygen supply, as suggested previously \((32)\). Consequently, the decrease in cortical blood flow was more marked than a change in its perfusion. Moreover, a considerable fraction of the volume of the \textit{in vivo} kidney is dependent on BP, as well

\textbf{Figure 5.} Cross section of the left (A) and right (B) renal arteries 1 mo after implantation of an intravascular copper stent in the left renal artery. An intimal fibroproliferative response is evident in the left renal artery, with localized disruption and fragmentation of the internal elastic lamina (arrows). The right renal artery demonstrates minimal intimal thickening.

\textbf{Blood pressure (mmHg)}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{blood_pressure_graph.png}
\caption{Daily changes in systolic (▲), mean (●), and diastolic (♦) BP in a pig during development of severe renal artery stenosis. After revascularization, BP normalized, but by the time of the repeat angiogram (arrow), hypertension resumed due to restenosis of the renal artery.}
\end{figure}
as the blood, filtrate, and urine contents of the kidney (10). Notably, we have found marked functional but minimal morphologic intrarenal alterations in many of the stenotic kidneys in this group of pigs, except for the most severely hypoperfused kidneys, which showed definite signs of morphologic damage. This suggests that more severe or at least more prolonged decrements in renal perfusion are necessary to eventuate in renal scarring (36,41).

Renal function in these pigs has also shown signs of deterioration, reflected in a mild increase in serum creatinine. Furthermore, utilization of EBCT enabled unique quantification of intrarenal, segmental nephron dynamics both in the stenotic and contralateral kidneys. The changes we found in intratubular dynamics were therefore more marked than blood or urine tests, since our measurements were taken distal to the stenosis and were uncompensated for by the contralateral kidney.

We have shown before using our technique that when renal perfusion pressure was decreased within the range of renal blood flow autoregulation, increases in intratubular concentration consequent to augmented tubular fluid reabsorption were detectable in the proximal and distal tubules, as well as in the loop of Henle (15). In the current study with a decrease in renal perfusion pressure below the range of autoregulation (evident by the clear decline in renal blood flow), possible increases in intratubular fluid reabsorption in the stenotic kidney were counterbalanced by the marked decrease in renal blood flow and probably GFR, and thereby lower contrast medium delivery to the kidney. In particular, changes in intratubular concentration in the distal tubule were inversely proportional to both the degree of stenosis and to an independent measure of renal function (a change in serum creatinine). It is possible that with marked decreases in renal perfusion pressure, the intratubular pressure gradient no longer suffices to efficiently drive all tubular fluid to the distal parts of the nephron (distal tubule and collecting duct). It is speculated that more prolonged or greater degrees of stenosis and hemodynamic impairments result in decreases in intratubular dynamics in more proximal segments of the nephron. In support of our findings, Navar et al. (42) and others (43,44), using invasive renal micropuncture and injections of lisamine green in single outer cortical nephrons, have shown that during acute decrements of renal perfusion pressure, functional alterations in the distal tubule both precede and exceed changes in the proximal tubule, which become evident only when renal perfusion pressure is further reduced. Our noninvasive observation is in agreement with these previous reports, and, for the first time, extends them to the total nephron population of the intact renal cortex during chronic decrements of renal perfusion pressure.

In the contralateral kidney, no changes in intratubular dynamics were detected. However, it is not unlikely that as opposed to the stenotic kidney, increased delivery of contrast to the contralateral kidney with the increase in blood flow could have been offset by dilution of intratubular fluid during the process of pressure-induced diuresis (45). This was especially evident in the proximal tubule, whose tubular fluid dilution (likely due to decreased tubular fluid reabsorption) correlated with the increase in mean arterial pressure. The increase in papillary perfusion in the contralateral kidney may also have been related to pressure-natriuresis (14), although a causal relationship could not be determined in this study.

Implantation of the local-irritant copper stent in the renal artery created a local response similar to that observed in the coronary artery (17), and led to an average luminal narrowing of 66%. The time for development of stenosis probably depended at least partly on the size-match between the renal artery and our balloon catheter at the time of implantation, and on intrinsic variability in the sensitivity of the renal arteries for the proliferative effect of the device. Originally designed for swine coronary arteries about 3 to 5 mm in diameter, an average coronary stenosis of 83% can be obtained when both the stent and balloon catheter are adjusted to vascular size (17). However, still larger devices may be needed for the renal arteries (which were often greater than 5 mm in diameter) to consistently achieve severe stenoses. Nevertheless, hemodynamically significant stenoses (>50% reduction in luminal diameter, equivalent to an approximately 70 to 75% decrease in cross-sectional area) were obtained in most of the pigs in the current study. Although the heterogeneity of our experimental group in terms of grades of renal artery stenosis may have made subtle changes from baseline less easily detectable, it nonetheless provided a wide range of alterations, thus enabling depiction of relationship between measured physiologic variables.

Moreover, the results of angioplasty performed in one of our pigs demonstrate that the renal arterial lesion may be reversible. The balloon dilation procedure performed in our pig was deliberately incomplete, and BP started rising again after about 1 wk due to restenosis (which was demonstrated in repeat angiography). Conceivably, with complete dilation of the lesion, restenosis would not be evident for a longer period of time, thus allowing studies of the effects of revascularization on the stenotic and contralateral kidneys.

In conclusion, the swine model of renal artery stenosis characterized in this study exhibited attributes resembling human renal artery stenosis. The methodology used to induce renal artery stenosis was minimally invasive and was associated with an intravascular wall lesion, which may be closer to human renal artery stenosis etiologies (e.g., atherosclerosis) than an extravascularly induced constriction of the renal artery. Furthermore, the stenosis may potentially be reversible, using minimally invasive percutaneous balloon dilation, and enable investigation of renal parenchymal viability with different grades and durations of luminal narrowing. The progressive nature of the stenosis may lead to intrarenal alterations similar to those occurring in humans, and inferences regarding mechanisms associated with this disorder and its consequences in the pig may potentially be applicable to humans. In addition to arterial hypertension and renal function, distinct changes in
regional renal volume, blood flow, and excretory function were detectable in this model using EBCT. Using intravenous contrast injections as performed in this study, this technology can be implemented in humans to simultaneously quantify synchronous intrarenal hemodynamics and function, and estimate the intrarenal consequences distal to the stenosis. Thus, EBCT may be useful to noninvasively assess, follow-up, and study in vivo changes in intrarenal hemodynamics and segmental tubular function in subjects with renal artery stenosis.

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References


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