Renal artery stenosis (RAS), an important cause of secondary hypertension in the elderly population, is frequently detected incidentally in patients with widespread peripheral artery disease or those undergoing coronary angiography. RAS may ultimately lead to end-stage renal disease and represents a sizeable fraction of new patients entering dialysis programs in the United States. Furthermore, patients with RAS have increased risk of death from cardiovascular events including myocardial infarction, stroke, and heart failure.

Renal revascularization by percutaneous transluminal renal angioplasty (PTRA) and stenting has been recommended commonly for patients with RAS, but recovery of glomerular filtration rate (GFR) after revascularization is inconsistent. Recent prospective randomized controlled trials comparing PTRA combined with medical therapy with medical therapy alone identified no differences in blood pressure and renal function at follow-up between the 2 treatment groups. This might reflect partly the difficulty in selecting patients whose renal function is likely to improve after revascularization. Considering the variable efficacy, cost, and potential complications of PTRA, identification of good candidates for revascularization beforehand is critical.

Pretreatment characteristics of the stenotic kidney (STK) likely determine its recovery potential better than the function of total renal mass, which the contralateral kidney (CLK) may mask. Renal flow and functional reserve...
Renal revascularization often shows little additive benefit over medical therapy for improvement in renal function in patients with renal artery stenosis. Identification of basal parameters to predict which specific patients would respond to revascularization could be invaluable.

**WHAT THE STUDY ADDS**

- This study shows that low basal stenotic-kidney glomerular filtration rate with preserved response to acetylecholine may predict benefit from revascularization.
- We also found that renal inflammation and robust stenotic kidney-R2* responses to furosemide (possibly reflecting avid tubular oxygen consumption) are associated with less-favorable outcomes.
- These tools may be potentially clinically applicable for the identification of patients likely to improve renal function after revascularization.

(responses to vasoactive challenge) may provide indices of STK viability. The degree of histopathologic STK tissue damage correlates with clinical outcomes in RAS patients, and might be partially mediated by inflammation, which magnifies parenchymal damage in RAS. Thus, the assessment of post-STK inflammation may constitute an important tool for evaluating RAS patients before vascular intervention.

The current study was therefore designed to test the hypothesis that STK inflammation and reduced functional reserve are associated with attenuated renal functional recovery after PTRA in a well-established swine model of RAS. For this purpose, we correlated basal hemodynamics, function, oxygenation, inflammation, and functional reserve in the STK and CLK with the change in STK-GFR after revascularization (ΔGFR).

**Methods**

**Experimental Design**

Twenty-nine female domestic pigs (40–55 kg) were studied after approval of the Institutional Animal Care and Use Committee. At baseline, RAS was induced in 22 animals, whereas 7 others (normal) underwent a sham procedure. Six weeks later, single-kidney function, oxygenation, and inflammatory marker release were measured, followed immediately by either a sham procedure (7 normal and 7 sham-RAS) or PTRA+stenoting (15 RAS+PTRA). Single-kidney function was then measured again 4 weeks later.

**Induction of RAS**

Pigs were anesthetized with intramuscular telazol (5 mg/kg) and xylazine (2 mg/kg), intubated, and anesthesia maintained with intravenous ketamine (0.2 mg/kg/min) and xylazine (0.03 mg/kg/min). The left femoral artery was catheterized, followed by a heparin bolus (5000 U). Under fluoroscopic guidance, a 7-mm balloon catheter wrapped with an irritant copper coil was placed into the proximal-middle right renal artery of RAS pigs and inflated to high pressure. The balloon was then deflated and removed, leaving the coil embedded in the vessel wall, as previously shown. Normal animals underwent a sham procedure. A telemetry transducer (Data Sciences International, Arden Hills, MN) was implanted in the femoral artery to continuously measure mean arterial pressure (MAP) until study completion. The animals were then allowed to recover.

**Measurement of Kidney Oxygenation**

Six weeks later, animals were similarly induced, and anesthesia maintained with inhaled 1% to 2% isoflurane. Blood-oxygen-level–dependent (BOLD) 3-T MRI (Signa Echo Speed; GE Medical Systems, Milwaukee, WI) was performed in the normal, sham-RAS, and 9 of the 15 RAS+PTRA pigs, to assess renal oxygenation, as described. For data analysis (4–6 slices/kidney), regions of interest were manually traced in the cortex and medulla, and their average magnetic resonance signal computed. The BOLD signal (relaxivity index R2*) was then measured, which is directly proportional to the local concentration of deoxyhemoglobin. After baseline acquisition, furosemide (0.05 mL/kg IV) was administered, flushed with saline, and BOLD measurements repeated 15 minutes later. The degree of change in R2* in response to furosemide was calculated. These pigs underwent sample collection, measurement of kidney function, and PTRA or sham 2 to 3 days later.

**Sample Collection**

Before PTRA or sham, all pigs were again anesthetized, and under fluoroscopic guidance, catheters advanced into the STK and CLK renal vein to collect samples. Blood samples were also collected from the inferior vena cava for measurement of plasma renin activity and creatinine. Samples were centrifuged and plasma aliquots stored at −80°C until assay. Inferior vena cava and renal vein levels of e-Selectin (Biotang, P4988), tumor necrosis-factor (TNF)-α (Invitrogen, Cat# KSC011), interleukin (IL)-1β (R&D DY681), IL-10 (Invitrogen, Cat# KSC0101), IL-17 (Kingfisher Biotech, Cat# VS0206S-002), monocyte chemoattractant-protein (MCP)-1, and interferon (IF)-γ (Kingfisher, VS0259S-002) were measured by ELISA. Then, given the lack of arteriovenous gradient of inflammatory cytokines across the renal circulation, we estimated cytokine gradient (renal vein inferior vena cava) and net renal release (gradient×renal blood flow [RBF]) for each measured analyte, as recently shown. In addition, urine samples were collected and microalbumin concentration quantified by ELISA (Bethyl Laboratories).

**Measurement of Kidney Function**

Single-kidney volume, regional perfusion, RBF, and GFR were then evaluated using multidetector CT (MDCT, SOMOTOM Definition-64; Siemens, Forcheim, Germany), and again after a 10-minute suprarenal arterial infusion of acetylcolline (Ach, 5 mg/ kg/min). Ach induces endothelium-dependent microvascular vasodilation and diuresis, thereby increasing RBF and GFR. MDCT images were analyzed with Analyze (Biomedical Imaging Resource, Mayo Clinic, MN). Tissue time-attenuation curves obtained in regions of interest selected from the aorta, renal cortex, and medulla were fit by curve-fitting algorithms to obtain measures of renal function. Cortical and medullary volumes were calculated by planimetry, and RBF as the sum of the products of cortical and medullary perfusions and volumes. GFR was calculated from the cortical proximal-tubular curve. The degree of stenosis was assessed as the decrease in renal arterial luminal diameter and area, at its most stenotic compared with a disease-free segment, in images acquired at 6-mm slice thickness and 3-mm overlap, reconstructed with BioF convolution kernel. The stenosis length was measured at high magnification, using a computer-caliper program.
Revascularization

Immediately after MDCT scanning, a balloon catheter mounted with a standard tantalum stent was used for 15 RAS animals to undergo PTRA+stenting. The balloon was inflated to high pressure, and then deflated and removed.11,21,22 Renal artery patency was confirmed with repeat angiography. The other animals underwent a sham procedure with selective renal angiography.

Four weeks after revascularization, the pigs were similarly anesthetized, and MDCT studies repeated. We have previously demonstrated the reproducibility of single-kidney assessment of renal hemodynamics and function using MDCT over 1-month intervals.11

Statistical Analysis

Statistical analysis was performed using JMP software package version 8.0 (SAS Institute, Cary, NC). We used the Shapiro-Wilk test to evaluate deviation from normality. Comparisons within and among the groups were done using nonparametric tests (Wilcoxon and Kruskal Wallis, respectively). P values <0.05 were considered significant.

Response to revascularization was evaluated by the change in MAP (ΔMAP) and STK-GFR (ΔGFR). Then, RAS+PTA animals were classified as having improved (STK-GFR increased by ≥1 mL/min or by >15% versus baseline), deteriorated (STK-GFR decreased by ≥1 mL/min or by >15%), or stable (STK-GFR changed by <1 mL/min and <15%) GFR after revascularization, as described.3 A simple linear regression analysis was used to determine correlations between basal parameters and ΔGFR.

Results

Systemic Baseline Parameters and PTRA

Table 1 shows baseline parameters in normal, sham-RAS, and RAS+PTRA pigs with subsequent improved or deteriorated STK-GFR. Six weeks after induction, all RAS groups achieved similar hemodynamically significant decreases in the diameter and area of the stenotic renal artery (60%–99%, P=0.36, ANOVA), and equivalent length suggested comparable stenosis morphology. Basal MAP and serum creatinine levels were similarly elevated in RAS pigs compared with normal pigs, whereas urinary albumin and plasma renin activity were not different among the groups.

Subsequent PTRA was technically successful in all animals (0% stenosis 4 weeks later, Figure 1B). MAP levels remained elevated in sham-RAS pigs (P<0.05 versus normal), but decreased to baseline levels in PTRA-treated pigs (Figure 1C).

Baseline STK-GFR was lower in sham-RAS (38.7±7.3 mL/min) and RAS+PTRA (51.6±16.7 mL/min) pigs compared with normal. Ten of 15 pigs showed improved STK-GFR after PTRA (ΔGFR=+22.0±8.5 mL/min, P<0.0001 versus baseline), whereas in the other 5, GFR deteriorated ΔGFR =−12.1±3.4 mL/min, P=0.001 versus baseline; (Figure 1D); none sustained stable STK-GFR. STK-GFR decreased in sham-RAS pigs (ΔGFR=−12.4±4.3, P<0.05 versus normal). No significant change in single-kidney GFR was observed in sham-normal animals (ΔGFR=+2.6±1.3 mL/min, P=0.36).

Renal Function

Basal STK cortical volume, perfusion, and RBF were lower in RAS compared with normal, but not different among RAS groups (Table 2). They were also lower than their own CLK, except for cortical perfusion in pigs with deteriorated STK-GFR after PTRA. Notably, basal STK-GFR was lower in sham-RAS and pigs in which STK-GFR subsequently improved compared with those with subsequently deteriorated STK-GFR, yet those with subsequently improved STK-GFR showed more robust RBF and GFR responses to Ach compared with sham-RAS and to those with subsequently deteriorated STK-GFR (Figure 2A–B). In contrast, CLK-GFR was reduced in pigs with deteriorated STK-GFR compared with the other 2 RAS groups, but neither CLK-GFR nor RBF responses to Ach were different among the groups. Notably, Bonferroni-adjusted basal STK-RBF, STK-GFR, and their responses to Ach remained significantly different among these groups (all P<0.0001).

Table 1. Baseline Parameters (Mean±SD) in Normal, Sham-Treated Pigs With Unilateral RAS, and RAS Pigs That Improved or Deteriorated STK-GFR After Revascularization

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Sham RAS</th>
<th>Improved STK-GFR</th>
<th>Deteriorated STK-GFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>46.4±3.6</td>
<td>49.1±4.0</td>
<td>43.7±5.4</td>
<td>47.4±3.0</td>
</tr>
<tr>
<td>Decrease in lumen diameter, %</td>
<td>0</td>
<td>69.7±14.7*</td>
<td>73.4±13.4*</td>
<td>68.1±5.0*</td>
</tr>
<tr>
<td>Area of stenosis, mm²</td>
<td>0</td>
<td>89.8±10.2*</td>
<td>90.8±15.0*</td>
<td>89.9±11.1*</td>
</tr>
<tr>
<td>Length of stenosis, mm</td>
<td>0</td>
<td>6.4±1.8*</td>
<td>6.6±2.0*</td>
<td>6.5±1.8*</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>113.0±8.2</td>
<td>144.6±24.2*</td>
<td>146.5±20.2*</td>
<td>156.2±4.5*</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>85.3±6.9</td>
<td>116.0±24.1*</td>
<td>104.9±24.6*</td>
<td>118.4±20.8*</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>94.6±6.8</td>
<td>125.5±23.9*</td>
<td>118.7±22.4*</td>
<td>124.4±19.5*</td>
</tr>
<tr>
<td>Serum creatinine, mg/dL</td>
<td>1.2±0.1</td>
<td>1.8±0.3*</td>
<td>1.8±0.2*</td>
<td>1.9±0.4*</td>
</tr>
<tr>
<td>Urinary albumin, µg/mL</td>
<td>13.9±8.9</td>
<td>25.3±14.8</td>
<td>23.4±27.8</td>
<td>23.7±12.2</td>
</tr>
<tr>
<td>PRA IVC, ng/mL/h</td>
<td>0.26±0.09</td>
<td>0.22±0.21</td>
<td>0.33±0.49</td>
<td>0.25±0.20</td>
</tr>
<tr>
<td>PRA STK, ng/mL/h</td>
<td>0.27±0.10</td>
<td>0.24±0.11</td>
<td>0.22±0.17</td>
<td>0.22±0.17</td>
</tr>
</tbody>
</table>

ΔGFR indicates renal artery stenosis; STK-GFR, stenotic kidney glomerular filtration; PRA, plasma renin activity; IVC, inferior vena cava; STK, stenotic kidney; and CLK, contralateral kidney.

*P<0.05 vs normal, †P<0.05 vs sham RAS, ‡P<0.05 vs improved STK-GFR (none observed for # and † comparisons).
Correlations Between Baseline Renal Function and ΔGFR

ΔGFR correlated inversely with basal STK-GFR (Figure 2C), but directly with basal CLK-GFR (Figure 2D), STK-RBF, and GFR responses to Ach (Figure 2E–F). No correlations were found between ΔGFR and either CLK-RBF or CLK-GFR responses to Ach (both, P > 0.05).

Renal Oxygenation and Response to Furosemide

STK and CLK cortical R2* values were similar in all groups. Medullary R2* values were higher in all STK compared with normal and CLK, suggesting decreased oxygenation, but did not differ among RAS groups (Table 2). Basal STK medullary R2* responses to furosemide were reduced in STK compared with normal and CLK, but were more attenuated in pigs with subsequent improved STK-GFR compared with sham-RAS and with pigs with deteriorated STK-GFR after revascularization (P=0.03, Figure 3A–B). CLK medullary R2* values and their responses to furosemide were comparable between RAS groups. Furthermore, basal STK (but not CLK) medullary R2* response to furosemide inversely correlated with ΔGFR (Figure 3C).

Renal Inflammation

STK-net release of e-Selectin, TNF-α, MCP-1, IL-1β, IL-17, and IF-γ were elevated, whereas release of the anti-inflammatory IL-10 was reduced in all RAS groups compared with normal and CLK (Table 2). Moreover, STK release of e-Selectin, TNF-α, and MCP-1 were lower and IL-10 higher in pigs with improved STK-GFR compared with sham-RAS and deteriorating RAS. CLK release of only e-Selectin and MCP-1 was higher in pigs with deteriorated STK-GFR after revascularization compared with those that improved. Bonferroni-adjusted STK-net release of e-Selectin, TNF-α, and MCP-1 remained highly significant (all P<0.0001).

ΔGFR correlated inversely with basal STK release of e-Selectin, TNF-α, and MCP-1 (Figure 4 A–C), directly with STK IL-10 release (Figure 4D), and inversely with CLK release of e-Selectin and MCP-1 (Figure 4 E–F). Furthermore, STK-GFR responses to Ach inversely correlated with STK-net release of TNF-α and MCP-1 (Figure 4G–H). However, no correlation was found between either STK or CLK release of IL-1β, IL-17 or IF-γ, and ΔGFR.

Finally, ΔMAP did not correlate with the stenosis characteristics, serum creatinine levels, or with STK or CLK basal hemodynamics, oxygenation, or inflammatory marker release.
Discussion

This study suggests that preserved endothelial function associated with lower basal STK-GFR may be linked to preserved recovery capacity of renal function after revascularization in RAS. Furthermore, lower release of inflammatory markers from both kidneys is associated with more favorable functional outcomes after PTRA. In contrast, robust STK BOLD MRI responses to furosemide (possibly reflecting ongoing tubular oxygen consumption) are associated with blunted improvement of renal function.

RAS remains an important cause of chronic renal disease for which therapeutic strategies remain controversial. In the current study, 4 weeks after revascularization blood pressure was restored to normal levels in all animals, whereas renal function was improved in only two thirds, underscoring the greater recovery capacity of renal function after revascularization.26

In our study, morphological characteristics of the stenotic lesion (length, decrease in luminal diameter or area) did not distinguish pigs with different PTRA response; this finding is consistent with previous reports that parenchymal injury predicts PTRA success better than the degree of proximal narrowing.23 Indeed, selection of patients for revascularization based on angiographic parameters primarily does not guarantee improvement in renal function, whereas lower basal GFR is associated with favorable renal outcomes in RAS patients.34

In our study, morphological characteristics of the stenotic lesion (length, decrease in luminal diameter or area) did not distinguish pigs with different PTRA response; this finding is consistent with previous reports that parenchymal injury predicts PTRA success better than the degree of proximal narrowing.23 Indeed, selection of patients for revascularization based on angiographic parameters primarily does not guarantee improvement in renal function, whereas lower basal GFR is associated with favorable renal outcomes in RAS patients.34

Because rapid deterioration in renal function25 predicts favorable outcomes after PTRA, progressive parenchymal damage may reflect the hemodynamic significance of the stenosis. Our findings extend previous observations, and emphasize that although lower basal GFR is associated with a measurable improvement after revascularization, basal RBF and GFR reserve to Ach in these kidneys needs to be preserved. STK-RBF, STK-GFR, and their responses to Ach emerged as dominant hemodynamic determinants of ΔGFR. In agreement, attenuated endothelium-dependent vasodilation blunts improvement after percutaneous coronary interventions.35 In our study, medullary volume and perfusion did
not correlate with renal response to PTRA, possibly because of compensatory mechanisms that ensure their preservation in chronic renovascular disease. Interestingly, STK-GFR, but not serum creatinine levels, negatively correlated with response to PTRA, implying greater sensitivity of selective STK assessment to ultimate recovery. This is likely secondary to the compensatory effect of the CLK, which was supported by a modest direct correlation between basal CLK-GFR and $\Delta$GFR.

BOLD MRI allows assessment of renal oxygen content in the cortex and medulla. Furthermore, their responses to furosemide offer the opportunity to assess the functional tubular mass, as blunted changes in medullary R2* imply reduced prevailing oxygen consumption related to tubular sodium transport. We observed that basal intrarenal oxygenation (R2*) did not predict response to PTRA. Similarly, basal R2* was similar in human RAS kidneys that improved after PTRA and those that did not, although their ratio with STK-GFR better-predicted renal functional outcome, possibly because STK-GFR was somewhat lower in subjects with subsequently improved STK-GFR. Indeed, the combination of R2* and STK-GFR might be a powerful index of renal recovery potential. Interestingly, we found that blunted response to furosemide was associated with better renal function after intervention, as were robust responses to Ach. In some respects, the ability to vasodilate in response to acetylcholine seems contradictory to the reduced tubular furosemide response. This ostensible discrepancy between the responses to vascular and tubular functional challenges perhaps attributable to the decreased basal STK-GFR in pigs that subsequently improved after PTRA, which in turn decreased the basal filtrate volume and response to furosemide. This poststenotic hibernating kidney decreases its workload and oxygen consumption, thus adequate recruitment of this protective mechanism may portend better outcomes. Therefore, although blunted responses to Ach (endothelial dysfunction) reflect microvascular injury and are associated with little benefit from revascularization, decreased medullary R2* responses to furosemide might imply decreased tubular oxygen consumption that preserved tubular viability.

Recent data indicate that renal inflammation is associated with deleterious processes in the STK, like fibrosis and microvascular damage. Indeed, our study illustrates that higher basal renal release of inflammatory markers was associated with poorer PTRA outcomes in RAS. STK-net release of e-Selectin, TNF-$\alpha$, and MCP-1 seem to be key determinants of improvement in GFR after revascularization. These cytokines, possibly secreted by inflammatory cells, may also impair endothelial functional reserve in the STK and its response to revascularization. In line with this postulation, we have shown

![Figure 2](http://circinterventions.ahajournals.org/Downloaded from)
that endothelial function improved in renovascular hypertensive pigs treated with an MCP-1 inhibitor. Furthermore, IL-6 and TNF-α levels in RAS patients are elevated shortly after PTRA, but decrease 1 month later. Importantly, our study showed an inverse relationship between release of proinflammatory markers and renal functional recovery, as well as with basal GFR responses to Ach. Therefore, inflammation may impair response to revascularization by ameliorating STK endothelial function and contribute to systemic inflammation by releasing inflammatory markers.

Importantly, both basal levels and responses to Ach of STK-RBF and STK-GFR, as well as STK-net release of e-Selectin, TNF-α, and MCP-1, remained significant predictors of ∆GFR upon Bonferroni adjustment, underscoring their predominant role as chief determinants of response to revascularization in swine ARAS.

Interestingly, CLK release of the inflammatory markers e-Selectin and MCP-1 also inversely correlated with ∆GFR. These findings extend previous studies in experimental and clinical RAS showing CLK inflammation and fibrosis. Furthermore, unilateral CLK nephrectomy is associated with postatrophic regeneration of the STK in experimental RAS, underscoring the contribution of the non-STK to the pathophysiology of RAS.

Our study is limited by the short duration of the disease, relatively small group of young animals, and lack of comorbidities like atherosclerosis, essential hypertension, or diabetes mellitus, which might modify parenchymal injury and revascularization outcomes. Hence, PTRA was more successful in restoring blood pressure control in our pigs than typically achieved in humans. Moreover, the majority of RAS patients are treated with statins and blockers of the renin-angiotensin-system, which may modulate both blood pressure and renal response to PTRA. The absence of correlation between urinary albumin and ∆GFR in our study may be related to the relatively early stage of the disease. Also, we cannot rule out the possibility that some aspects of the stenoses that were not fully captured by our measurements contributed to differences between the groups. In addition, BOLD MRI data were available only in 9 of the revascularized RAS animals. The statistical relationship between basal STK-GFR and its change in response to PTRA (∆GFR) should be interpreted with caution, because it might reflect mathematical artifacts. Although these limitations may affect the translational potential of our observations, our study represents an important first step in the identification of parameters to predict renal response to PTRA in human RAS. The extent of renal tissue injury and functional decline in our
model are similar to that in human kidneys. Furthermore, our assessment of single-kidney hemodynamics and function is clinically applicable, and provides a unique opportunity to evaluate parameters that may help predict response to PTRA in RAS. Future studies need to confirm these results in humans.

In conclusion, lower basal STK-GFR, tubular hibernation (decreased STK tubular oxygen consumption) reflected by diminished response to furosemide, and preserved endothelial functional reserve to acetylcholine may predict recovery of GFR after revascularization in RAS. Conversely, elevated release of inflammatory markers from both kidneys is associated with attenuated renal functional recovery after PTRA. These tools may be potentially clinically applicable for the identification of patients likely to improve renal function after revascularization.

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Disclosures
None.

References


Changes in Glomerular Filtration Rate After Renal Revascularization Correlate With Microvascular Hemodynamics and Inflammation in Swine Renal Artery Stenosis

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