Comparative Medicine
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Vol 67, No 1
February 2017
Pages 56–66

Original Research

Assessment of Acute Kidney Injury and Renal Fibrosis after Renal Ischemia Protocols in Cats

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In an attempt to identify a feline model of acute or chronic kidney disease, this study was designed to evaluate the effects of 15 or 30 min of bilateral renal ischemia (RI) and 60 min of unilateral RI with delayed contralateral nephrectomy as models of acute kidney injury and chronic interstitial fibrosis in cats. Adult, purpose-bred, USDA Class A cats (*n* = 14) were randomly assigned to receive bilateral RI for 15 min (*n* = 3) or 30 min (*n* = 3), unilateral RI for 60 min with a delayed (2 wk) contralateral nephrectomy (*n* = 5), or sham unilateral RI with a delayed contralateral nephrectomy (*n* = 3). Serum creatinine concentration, urine specific gravity, and plasma clearance of iohexol were assessed at several time points throughout the study. Renal interstitial inflammatory cell counts and descriptive histopathology were acquired in all cats. Histomorphometry was used to quantify renal interstitial fibrosis and collagen at 120 d after RI in cats undergoing unilateral RI. Renal histopathology was evaluated at 21 and 120 d after bilateral and unilateral RI, respectively. Neither duration of bilateral RI resulted in appreciable histologic renal damage at 21 d after ischemia. At 120 d after ischemia, variable amounts of renal fibrosis were noted after 60 min of unilateral RI with delayed contralateral nephrectomy. Neither of the tested methods is a suitable model of consistent renal interstitial fibrosis in cats. Healthy cats appear able to sustain bilateral RI for as long as 30 min with no apparent effects on renal morphology or function at 21 d after ischemia.

Abbreviations: AKI, acute kidney injury; CKD, chronic kidney disease; GFR, glomerular filtration rate, PASH, periodic acid–Schiff and hematoxylin; RI, renal ischemia; SCr, serum creatinine concentration; UPC, urine protein:creatinine ratio; USG, urine specific gravity

Surgically induced kidney injury has been used in multiple species as a model of kidney disease.[28] Surgical models of renal ischemia (RI) are useful in veterinary medicine, because they mimic the acute kidney injury (AKI) due to ischemia–reperfusion that may be encountered during procedures such as renal transplantation, nephrotomy, or partial nephrectomy.[28] Animal models of RI also are useful as translational models of human disease, in which AKI due to perioperative ischemia is an important complication after cardiac and other vascular surgeries.[30] It is increasingly clear that AKI can result in chronic, progressive tubulointerstitial fibrosis in humans[4] and companion animals;[16] therefore, surgical models of RI may not only provide models of AKI but also chronic fibrotic lesions that are very similar to those characteristic of chronic kidney disease (CKD).[16,25]

Within the literature and across species, reported methods for establishing RI vary. Temporary occlusion of the renal artery only or of both the artery and vein of one or both kidneys is the surgical technique described most frequently.[28] Clamping of both the artery and vein may be ideal for achieving consistent, maximal injury in models of renal disease.[20] Susceptibility of the model species to RI and the duration of ischemia are common variables among reported studies. The duration of ischemia required to achieve irreversible renal damage typically is longer in larger animal models (such as dogs and pigs) than in rodent models.[21] This marked variation in tolerance to RI makes it difficult to translate model design across species. An ideal model of RI balances optimal renal injury with acceptable morbidity and mortality. Prolonged ischemia is associated with more consistent damage than are shorter times; therefore during model development, investigators often seek the maximal tolerable duration of ischemia for the species of interest.[29]

Bilateral models of RI more accurately reflect most natural renal insults and simplify measuring systemic renal biomarkers for outcome assessment. However, ischemia of sufficient duration to induce irreversible chronic renal damage is often associated with unacceptable acute morbidity when applied bilaterally in a single procedure, as reported with 60-min bilateral ischemia in cats.[26] In addition, variation in the response to RI between individual kidneys may be a confounding factor in bilateral models of RI in other species.[29]

To eliminate these complications, investigators have used unilateral RI with or without a preemptive or simultaneous contralateral nephrectomy.[25,28,29] Unilateral RI without immediate nephrectomy allows the maintenance of normal systemic homeostasis by the unaffected contralateral kidney, thereby permitting longer RI times with minimal to no postoperative morbidity. In cats, 60-min unilateral RI is well tolerated, with evidence of renal interstitial fibrosis at 70 d after ischemia.[25] However, standard biomarkers of renal injury (for example serum creatinine concentration (SCr), urine specific gravity (USG), and plasma clearance of iohexol) are difficult to interpret in unilateral models of RI

Received: 22 Feb 2016. Revision requested: 21 Jun 2016. Accepted: 14 Jul 2016.
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without contralateral nephrectomy, because the remaining normal kidney maintains these biomarkers within normal limits.[25] The presence of the uninjured kidney makes interpretation of data difficult not only in the acute phase but also when monitoring trends during the recovery phase, because of compensatory hypertrophy. To simplify monitoring of renal function, unilateral RI models often incorporate a delayed contralateral nephrectomy.[29] Delaying the contralateral nephrectomy provides the ischemic kidney an opportunity to heal before it becomes the sole kidney. The optimal delay between injury and contralateral nephrectomy is unknown and is dictated by the degree of injury and the temporal condition that investigators plan to study.

An ideal model of RI in cats has not been identified. However, the development of a model of feline renal disease would be an invaluable tool, given the persistently poor prognosis for cats with AKI and the prevalence and insidious nature of CKD. The purposes of the current study were 1) to evaluate the degree of AKI and consequential acute systemic morbidity associated with 15- and 30-min bilateral RI in cats, to determine whether a shorter duration of RI results in sufficient injury with lower morbidity as compared with historical data after 60 min of bilateral RI, and 2) to investigate the efficacy of 60-min unilateral RI with contralateral nephrectomy at 2 wk after RI as a model of chronic renal fibrosis in cats. We hypothesized that 1) shorter bilateral RI would result in less morbidity yet induce renal injury at 21 d after ischemia, and 2) 60-min unilateral RI with delayed contralateral nephrectomy would allow maintenance of normal homeostasis during acute healing, resulting in minimal morbidity and significant renal interstitial fibrosis at 120 d after ischemia.

Materials and Methods

Animals. All studies were approved by the University of Georgia IACUC and followed AALAS guidelines for Humane Care and Use of Laboratory Animals. Adult, purpose-bred, USDA Class A cats (n = 14) were vaccinated by the supplier with a combination vaccine against feline rhinotracheitis, calicivirus, and panleukopenia every 6 wk until 16 wk of age. Rabies vaccination was performed on arrival at the University of Georgia. All cats from the supplier are negative for feline leukemia virus and feline immunodeficiency virus. All cats were allowed a minimum of 7 d to acclimate before study enrollment. Cats had unlimited access to standard cat chow and water, were housed individually to allow monitoring of individual food consumption and in groups of 2 or 3 per enclosure during the evening to allow socialization, and were socialized with human interaction at least once daily. For 10 d postoperatively or while jugular catheters were in place, cats were housed individually to minimize self- or peer-induced complications.

Prior to entrance in the study, baseline data regarding weight, physical examination, serum chemistry profile, complete blood count, and urinalysis were obtained in all cats. The urine protein:creatinine ratio (UPC) was assessed in the unilateral RI study only. Three-point plasma clearance of iohexol was analyzed in all cats to estimate the global glomerular filtration rate (GFR), by using previously described methods.[26] Briefly, iohexol (300 mg I/kg) was administered through a peripheral intravenous catheter; blood samples were drawn from a jugular catheter at 2, 3, and 4 h after iohexol injection and sent to a commercial laboratory (Diagnostic Center for Population and Animal Health, Michigan State University, East Lansing, MI) for evaluation.

Experimental groups. Six cats (3 male and 3 female) were randomly assigned to receive either 15 min (n = 3) or 30 min (n = 3) of bilateral ischemia and had a 21-d survival time. Randomization was achieved by assigning each cat a number and using an online random number generator (www.randomizer.org) to randomize to groups.

Eight intact male cats were used to evaluate unilateral RI for 60 min followed by delayed contralateral nephrectomy. They were randomly assigned, as described earlier, to undergo either 60-min RI of the right kidney followed 2 wk later by left nephrectomy (n = 5) or a 60-min sham surgery followed 2 wk later by left nephrectomy (n = 3). All cats in the unilateral RI study had a 120-d survival time.

Surgery and anesthesia. All cats underwent general anesthesia for each surgical procedure. Cats were premedicated with ketamine (7 mg/kg IM), buprenorphine (0.02 mg/kg IM), and acepromazine (0.01 mg/kg, IM). After 15 min, a peripheral intravenous catheter was placed in a cephalic vein. Anesthesia was induced by isoflurane in 100% oxygen supplied by facemask to effect, followed by endotracheal intubation. Isoflurane was maintained at the lowest concentration necessary to maintain stage 3 general anesthesia. Isotonic crystalloid fluids were administered at 10 mL/kg/h during anesthesia. Doppler ultrasonography and sphygmomanometry were used to monitor pulse and systolic blood pressure during surgery. Body temperature was measured by using a digital thermometer inserted orally, as far caudally as possible, and recorded every 15 min throughout surgery. The ventral abdomen was shaved and aseptically prepared in all animals.

A ventral midline incision was performed for all procedures. Both kidneys were confirmed to be grossly normal prior to proceeding. In the bilateral RI study, the vascular pedicle of each kidney was isolated, and a pediatric nontraumatic vascular clamp (Cooley Pediatric Vascular Clamp, 14 cm, Surgipro, Shawnee, KS) was placed across the renal artery and vein of each kidney for either 15 or 30 min, as appropriate. For cats assigned to undergo unilateral RI, the right renal vascular pedicle was clamped for a total of 60 min. For cats in the sham group, the right renal vascular pedicle was clamped only long enough to obtain a cortical wedge biopsy.

Immediately after application of the vascular clamp, a wedge biopsy was obtained from the cortex of the right kidney in all cats. A no. 10 blade was used to cut a wedge into the renal cortex; the wedge was removed, and the defect was closed by using 2-0 polypropylene (Prolene, Ethicon, Somerville, NJ) in a horizontal mattress pattern. In the sham RI group, the vascular clamp was removed immediately after placement of the suture. After the renal biopsy, the abdomen was temporarily closed by placing towel clamps in the body wall for the duration of RI or sham surgery. The vascular clamps were removed immediately after the appropriate amount of time for RI (or sham), and the renal biopsy site was evaluated for hemorrhage; hemorrhage was addressed by the placement of additional sutures or digital pressure as needed. After hemostasis was achieved, the abdomen was closed routinely. After abdominal closure, all cats in the unilateral and sham RI groups were castrated through routine closed castration to facilitate group housing for the remainder of the study period.

All cats in the unilateral and sham RI groups underwent a routine left ureteronephrectomy at 2 wk after RI or sham surgery. A ventral midline incision was made directly through the previous incision site. The left kidney was isolated from the retroperitoneum. The renal artery and vein were isolated and double-ligated with 3-0 polypropylene. The ureter was isolated along its length and ligated with 3-0 polypropylene near its entrance to the urinary

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bladder. The left kidney and ureter were removed en bloc. The kidney was longitudinally divided with a no. 10 blade and placed in 10% formalin for fixation. Hemostasis was verified, and the abdomen was closed routinely.

Postoperative analgesia and pain scoring. A fentanyl patch (3 to 5 µg/kg/h) was placed on the lateral body wall of each cat prior to endotracheal extubation. Buprenorphine (0.02 mg/kg IM) was administered after extubation and every 8 h for the first 24 h postoperatively. Cats were assigned a pain score according to a previously described pain scale[26] at least twice daily for 7 d postoperatively. Any cat with a pain score of 8 or greater received supplemental analgesia (buprenorphine, 0.02 mg/kg IM).

Renal function monitoring. In all cats, day 1 was defined as the date of ischemia surgery. In bilateral RI cats, SCr and USG were evaluated (Clinical Pathology Laboratory, College of Veterinary Medicine, University of Georgia, Athens, GA) on days 0, 1 through 4, 6, 10, 14, 18, and 21. GFR was assessed on days 0, 6, and 21; samples were stored in a –80 °C freezer and shipped for analysis by a commercial laboratory (Diagnostic Center for Population and Animal Health, Michigan State University) at the end of the study.

For the unilateral and sham RI groups, SCr and USG were evaluated on days 0, 16, and 18; weekly (± 1 d) thereafter for 1 mo; and then monthly (± 1 d) until completion of the study. GFR (according to plasma clearance of iohexol) and UPC were evaluated on days 0, 60 (± 3 d), and 120 (± 3 d) in all groups.

Necropsy, histopathology, and histomorphometry. All cats in bilateral RI groups were euthanized on day 21; cats in the unilateral and sham RI groups were euthanized on day 120. Euthanasia was achieved through pentobarbital overdose (2 mL/kg IV). Necropsy was performed by a board-certified pathologist (CB or DR). The kidneys and samples of all other major organs were harvested and fixed in 10% neutral buffered formalin.

Sections (3 µm) of the kidneys were stained with hematoxylin and eosin, periodic acid–Schiff and hematoxylin (PASH), and Mason trichrome–PASH combination (PASH combo) stains.[7] All renal tissues were evaluated and scored independently by 2 pathologists (CB and DR) blinded to treatment group. Mean scores were recorded. Interstitial inflammatory cells within the kidneys were counted by using hematoxylin- and eosin-stained histologic sections of the kidneys. Inflammatory cells within the superficial cortex and at the corticomedullary junction were counted in 10 consecutive fields (magnification, 20×); data were recorded as the number of cells per 10 fields. Tubular epithelial mitoses, atrophic tubular profiles, and the number of tubules with evidence of ongoing necrosis were quantified for 10 fields (magnification, 20×) in each of these regions.

In the unilateral RI study, ischemic kidneys and the right kidney from the sham-operated controls were stained by using mouse monoclonal smooth muscle actin (1A4) antibody (catalog no. 202M-98, Cell Marque, Rocklin, CA) and PASH combo stain to quantify activated myofibroblasts (profibrotic activity) and interstitial collagen, respectively.[25] By using commercially available software (Image Pro Plus, Media Cybernetics, Rockville, MD), the percentage of positively stained tissue was calculated according to 10 individual photomicrographs (magnification, 20×) from 10 independent measurements for each region of the kidney (cortex, corticomedullary junction, and medulla) for each cat.

Statistics. Data were analyzed using standard statistical software (Prism, version 6, GraphPad Software, La Jolla, CA). For the bilateral RI study, standard descriptive statistics were reported as mean ± 1 SD or median and range as appropriate. The mean age, weight, and renal interstitial inflammatory cell counts were compared using an unpaired Student *t* test; 2-way repeated-measures ANOVA was used to compare changes in SCr, USG, and GFR over time within and between groups.

For the unilateral RI study, standard descriptive statistics were performed, with mean ± 1 SD reported. In addition, 2-way repeated measures ANOVA was used to assess differences in weight, SCr, USG, and UPC between the unilateral and sham RI groups over time, followed by a post-hoc Tukey test when appropriate. Two-way ANOVA was used to assess percentage positive staining for smooth muscle actin or PASH–combo stain between groups and region of the kidney; post-hoc Fisher least significant difference testing was used where appropriate. Individual unpaired Student *t* tests were used to compare kidney weight (as a percentage of total body weight) between the unilateral and sham RI groups. Histologic scoring parameters in each region of the kidney assessed were compared between the unilateral and sham RI groups by using individual unpaired Student *t* tests. Significance was set at a P value of less than 0.05.

Results

Animals. For the bilateral RI protocols, 3 sexually intact male and 3 intact female cats were used. Mean body weight was 4.08 ± 1.84 kg and 4.49 ± 1.27 kg and mean age was 10.0 ± 2.8 mo and 11.3 ± 2.9 mo in the 15- and 30-min groups, respectively, and did not differ significantly between groups. All cats had a normal baseline physical examination.

All 8 cats in the unilateral RI study were intact males. Mean body weight was 4.27 ± 0.53 kg and 3.60 ± 0.41 kg and mean age was 7.1 ± 0.2 mo and 6.9 ± 0.2 mo for the unilateral and sham IR groups, respectively, and did not differ significantly between groups. All cats had a normal baseline physical examination.

Surgery. In the bilateral RI study, 1 cat in the 15-min group died due to cardiac arrest shortly after extubation. Necropsy examination revealed no free fluid in the abdomen, intact renal vascular pedicles, and no obvious gross cause of death. Histopathology showed subacute, moderate to severe fibrinous endomyocarditis and multifocal neutrophilic and histiocytic interstitial pneumonia. The cat's death was attributed to cardiopulmonary complications associated with these lesions and was deemed unrelated to the RI surgery. Data from this cat were excluded from the study. All remaining cats in the bilateral RI study survived surgery without complication, and none required rescue analgesia.

In the unilateral RI study, all cats survived unilateral or sham RI followed by nephrectomy surgery without complication. One cat each in the unilateral and sham RI groups received a single dose of supplemental analgesic within the first 7 d postoperatively. One cat in the unilateral RI group and 2 cats in the sham IR group received supplemental analgesic once on the day after nephrectomy.

Renal function monitoring. Data from individual cats in the bilateral RI study (Table 1) showed no significant difference in mean SCr between or within the 15- and 30-min RI groups at any time point (Figure 1 A). In the 30-min group, USG on day 1 (1.046 ± 0.009) differed from that on day 3 (1.060 ± 0.006, P = 0.029), and USG on day 3 differed from that on days 14 (1.044 ± 0.009, P = 0.010) and 18 (1.045 ± 0.016, P = 0.016; Figure 1 B). There were no other significant differences in USG between or within groups over time. In the 15-min group, GFR was significantly greater on day 0 (5.531 ± 3.037 mL/kg/min) compared with day

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Table 1. Renal function parameters from individual cats in the bilateral RI study

	15-min group						30-min group								
	Cat 15-1			Cat 15-2			Cat 30-1			Cat 30-2			Cat 30-3		
Day	SCr	USG	GFR	SCr	USG	GFR	SCr	USG	GFR	SCr	USG	GFR	SCr	USG	GFR
0	0.9	1.058	7.678	1.4	1.044	3.384	1.3	1.04	2.772	1	1.052	2.346	1.4	1.054	2.465
1	0.7	1.060		1	1.052		1.7	1.035		1	1.052		1.6	1.050	
2	1.1	1.060		0.7	1.052		1.3	1.046		0.8	1.055		1.4	1.054	
3	0.7	1.072		1.2	1.053		1.2	1.055		0.7	1.067		1.3	1.058	
4	0.6	1.069		1.1	1.054		1.3	1.044		0.6	1.060		1.5	1.049	
6	0.8	1.060	4.705	1.3	1.060	2.293	1.4	1.043	2.948	0.8	1.050	3.474	1.5	1.054	1.993
10	0.8	1.055		1.2	1.052		1.4	1.045		0.8	1.051		1.5	1.053	
14	0.9	1.053		1.3	1.051		1.3	1.037		0.8	1.054		1.6	1.041	
18	0.9	1.058		1.3	1.050		1.4	1.029		0.9	1.060		1.6	1.045	
21	0.9	1.060	2.607	1.3	1.060	1.906	1.3	1.035	2.416	0.8	1.060	3.471	1.2	1.046	2.328

Serum creatinine (SCr, mg/dL), urine specific gravity (USG), and glomerular filtration rate (GFR [estimated according to plasma clearance of iohexol], mL/kg/min) are reported.

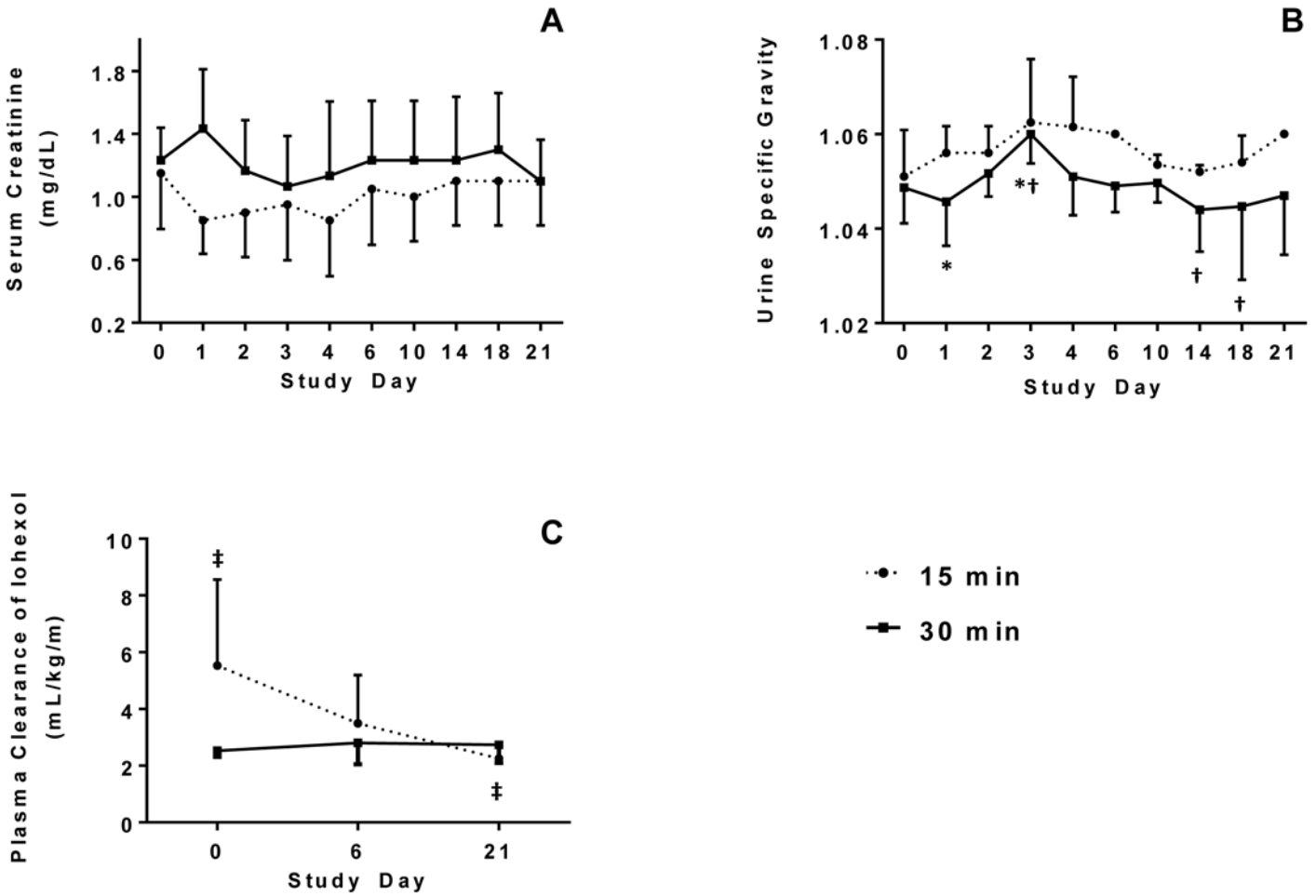

Figure 1. (A) Serum creatinine concentration, (B) urine specific gravity, and (C) plasma clearance of iohexol (a marker for glomerular filtration rate) in cats undergoing 15 (n = 3) or 30 (n = 3) min of bilateral renal ischemia. (B) There was a significant difference in USG on day 1 (1.046 ± 0.009) compared with day 3 (1.060 ± 0.006, P = 0.029; *) and on day 3 compared with days 14 (1.044 ± 0.009, P = 0.010) and 18 (1.045 ± 0.016, P = 0.016; †) in the 30-min group. (C) Iohexol clearance was significantly greater on day 0 (5.531 ± 3.037 mL/kg/min) compared with day 21 (2.257 ± 0.496 mL/kg/min; ‡) in the 15-min group. There were no other significant differences within or between groups for any parameter.

21 (2.257 ± 0.496 mL/kg/min, P = 0.019); otherwise there were no significant differences in GFR between or within groups over time (Figure 1 C).

Individual cat data for the unilateral RI study are reported in Tables 2 and 3. Cats in the unilateral RI group were transiently azotemic after nephrectomy, with a peak in SCr on day

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Table 2. Renal function parameters from individual unilateral RI cats in the unilateral RI study

	60-min unilateral RI group														
	Cat 60-1			Cat 60-2			Cat 60-3			Cat 60-4			Cat 60-5		
Day	SCr	USG	GFR	SCr	USG	GFR	SCr	USG	GFR	SCr	USG	GFR	SCr	USG	GFR
0	0.9	1.049		0.8	1.054		0.8	1.049		0.9	1.049		0.9	1.036	
16	1.4	1.057		1.5	1.047		2.5	1.042		2.5	1.039		3.7	1.020	
18	1.5	1.060		1.3	1.056		2.5	1.045		2.3	1.035		3.5	1.017	
25	1.5	1.053		1.4	1.055		1.9	1.047		1.6	1.047		2.1	1.027	
32	1.3	1.060		1.1	1.053		1.8	1.051		1.7	1.045		1.5	1.034	
39	1.5	1.060		1.4	1.058		1.9	1.060		1.5	1.045		1.7	1.041	
46	1.5	1.060		1.4	1.059		1.8	1.055		1.7	1.045		1.7	1.046	
60	1.2	1.055		1.3	1.058		1.6	1.051		1.5	1.040		1.5	1.048	
74	1.6	1.059		1.5	1.060		2.0	1.059		1.9	1.055		1.7	1.055	
102	1.5	1.056		1.5	1.061		1.7	1.060		1.6	1.051		1.6	1.056	
120	1.1	1.041	1.787	1.0	1.045	1.828	1.6	1.053	1.629	1.3	1.053	1.805	1.3	1.051	2.480

Serum creatinine (SCr, mg/dL), urine specific gravity (USG), and glomerular filtration rate (GFR [estimated according to plasma clearance of iohexol], mL/kg/min) are reported.

Table 3. Renal function parameters from individual sham RI cats in the unilateral RI study

	Cat Sham-1			Cat Sham-2			Cat Sham-3		
Day	SCr	USG	GFR	SCr	USG	GFR	SCr	USG	GFR
0	0.9	1.053		0.9	1.051		1.0	1.049	
16	1.2	1.060		1.4	1.060		1.6	0.060	
18	1.3	1.057		1.2	1.060		1.2	1.060	
25	1.2	1.056		1.4	1.056		1.2	1.060	
32	1.4	1.058		1.7	1.044		1.4	1.060	
39	1.1	1.058		1.2	1.058		1.1	1.059	
46	1.5	1.060		1.7	1.056		1.4	1.060	
60	1.5	1.050		1.6	1.044		1.3	1.038	
74	1.4	1.055		1.4	1.053		1.5	1.060	
102	1.6	1.059		1.6	1.058		1.6	1.060	
120	1.2	1.043	2.521	1.3	1.039	2.505	1.1	1.060	2.153

Serum creatinine (SCr, mg/dL), urine specific gravity (USG), and glomerular filtration rate (GFR [estimated according to plasma clearance of iohexol], mL/kg/min) are reported.

17 (2.32 ± 1.16 mg/dL, reference range 0.6 to 1.8). In addition, SCr was significantly higher in the unilateral RI group compared with the sham RI group on days 16 (2.32 ± 0.94 compared with 1.40 ± 0.20 mg/dL, $P = 0.008$) and 18 (2.22 ± 0.89 compared with 1.23 ± 0.06 mg/dL, $P = 0.003$; Figure 2 A). In contrast, USG was significantly lower in the unilateral RI group compared with the sham RI group on day 16 (1.041 ± 0.014 compared with 1.060 ± 0, $P = 0.0255$; Figure 2 B). Furthermore UPC was mildly elevated at baseline in some of the male cats in our study population, most likely related to their intact sexual status and increased urinary levels of the protein cauxin.[18] Whereas UPC was significantly higher on day 0 compared with days 60 and 120 in both groups individually, there was no significant difference in UPC between groups at any time point, and all cats had normal UPC values on days 60 and 120 (Figure 2 C).

Cats were inadvertently fed prior to sample collection for analysis of plasma clearance of iohexol on days 0 and 60 in the unilateral RI group and on day 0 in the sham RI group; the majority of these results were markedly elevated. Because recent meals are associated with false elevation of GFR in people,[6] these data were excluded from the study. The mean GFR on day 120 was 1.92 ± 0.32 mL/kg/min in the unilateral RI group and 2.33 ± 0.25 mL/kg/min in the sham IR group, with no statistically significant difference between them ($P = 0.23$).

Histology. Results of gross necropsies and evaluation of preischemia cortical wedge biopsies were normal for all cats in the bilateral RI study. At 21 d after RI, 1 cat in the 15-min group had no histopathologic changes, and the other had rare streaks of tubular atrophy, mild inflammation, and fibrosis. In the 30-min RI group, 1 cat had no histopathologic changes, and another had a small number of fibrotic streaks associated with mild inflammation (Figure 3). similar to those seen in one cat from the 15-min group. The third cat in the 30-min group had moderate, predominantly medullary fibrosis and mild inflammation, collecting duct epithelial hyperplasia, and distortion of the papilla. These findings were interpreted to be consistent with the presence of a preexisting renal disease, such as previous pyelonephritis or papillary necrosis. These medullary changes were not identified in the pre-RI biopsy, because only superficial cortex was sampled.

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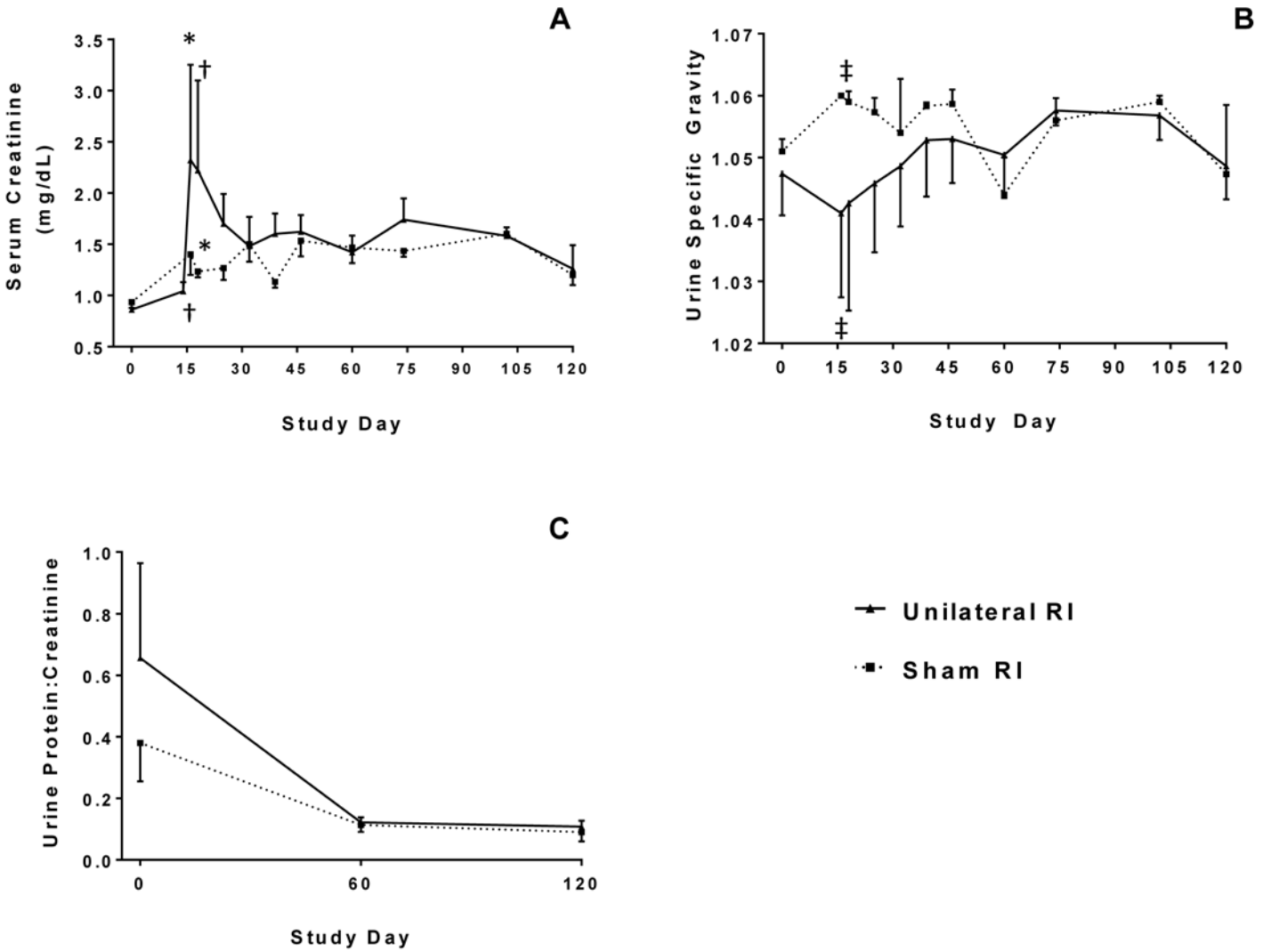

Figure 2. (A) Serum creatinine concentration, (B) urine specific gravity, and (C) urine protein:creatinine ratio (C) in cats undergoing 60-min unilateral renal ischemia with delayed contralateral nephrectomy (n = 5) or sham RI with delayed nephrectomy (n = 3). (A) Serum creatinine was significantly higher in the unilateral IR group compared with the sham IR group on days 16 (2.32 ± 0.94 compared with 1.40 ± 0.20 mg/dL; *, P = 0.008) and 18 (2.22 ± 0.89 compared with 1.23 ± 0.06 mg/dL; †, P = 0.003). (B) Urine specific gravity was significantly lower in the unilateral IR group compared with the sham IR group on day 16 (1.041 ± 0.014 compared with 1.060 ± 0; ‡, P = 0.0255). (C) Urine protein:creatinine ratio did not between groups at any time point, and all cats had ratios on days 60 and 120. There were no other significant differences within or between groups for any other parameter.

Tubular necrosis, tubular atrophy, eosinophils, plasma cells, neutrophils, monocytes, and mitotic figures were minimal or absent in kidneys from both bilateral RI groups. Rare tubular epithelial cell attenuation and anisokaryosis, indicative of regeneration after epithelial cell necrosis, was present in the corticomedullary junction of 1 cat in the 30-min group (Figure 4). This cat also had rare foci of granulomatous inflammation with free lipid, indicative of ischemic tubulorrhexis. Tubular necrosis at the corticomedullary junction was not present in any other cat. Neither the number of renal interstitial inflammatory cells nor degree of tubular damage differed between bilateral RI groups (Tables 1 and 2).

All cats in the unilateral RI study had normal preischemia biopsies. No gross abnormalities were noted on necropsy in either group. Although histologic lesions of tubular atrophy and inflammation were present in all previously ischemic kidneys from this group, the lesions varied in severity among cats. One of the 5 cats in the unilateral RI group had moderate multifocal linear streaks of interstitial fibrosis and tubular atrophy (Figure 5). Lesions of similar character were considered mild in 2 of 5 cats in the unilateral RI group. The 3 remaining cats had minimal tubular atrophy and inflammation (Figure 6). Neutrophils were not noted in any ischemic kidneys; tubular epithelial degeneration and mitotic figures were rare. Renal interstitial inflammatory scores did not differ significantly between RI or sham groups in any region of either kidney (Tables 4 and 5).

Body temperature during surgery was associated with the presence of significant renal lesions on histology in the unilateral RI study. The 3 cats with detectable microscopic lesions had a significantly higher mean body temperature during RI surgery (99.1 ± 0.2 °F) than did cats with mild lesions (96.4 ± 0.3 °F, $P < 0.001$) in the unilateral RI group.

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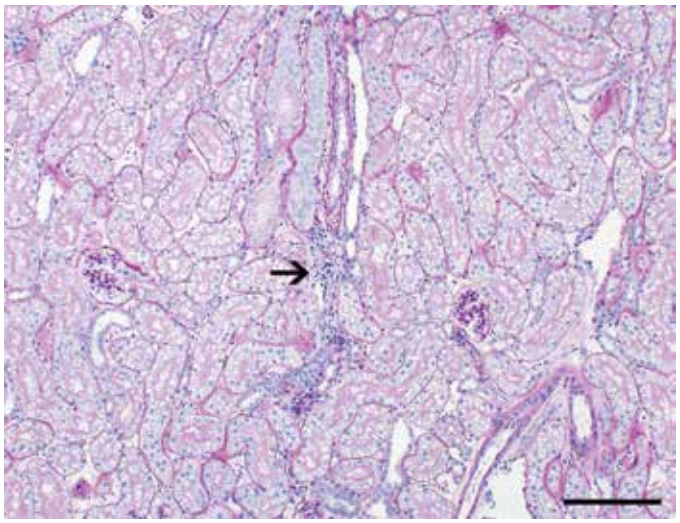

Figure 3. Photomicrograph of a cat kidney at 21 d after 30 min of bilateral RI (bar, 500 µm) contains rare streaks of interstitial inflammation and tubular atrophy (arrow) in renal cortex.

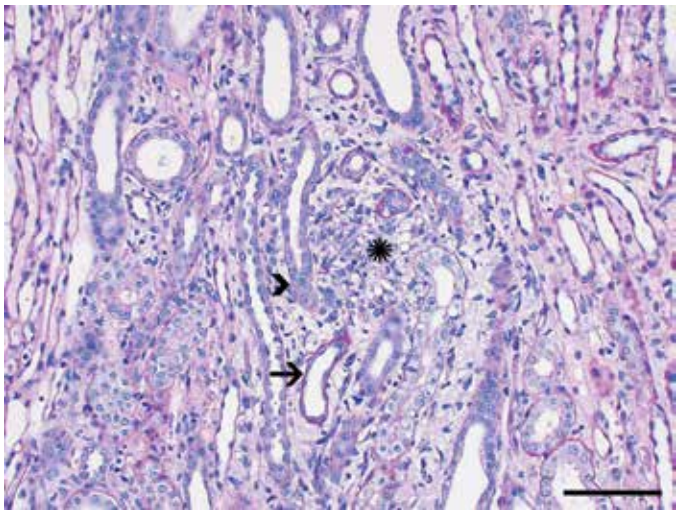

Figure 4. Photomicrograph of a cat kidney at 21 d after 30 min of bilateral RI (bar, 100 µm). Tubular epithelial cell attenuation (arrow) and anisokaryosis (arrowhead) are present in the corticomedullary junction. Note the small focus of granulomatous inflammation with free lipid (*).

Histomorphometry. For PASH-combo staining of the renal medulla, positive staining was significantly greater in the ischemic kidneys (1.16% ± 0.79%) compared with the sham kidneys (0.06% ± 0.04%, $P = 0.005$; Figure 7 A). Otherwise, there were no statistically significant differences for PASH combo staining. Smooth muscle actin staining did not differ between groups in any region of the kidney (Figure 7 B).

Discussion

Development of a model of AKI and renal fibrosis in cats would facilitate the evaluation of novel treatments for AKI and CKD. In the current study, only mild evidence of renal injury was histologically apparent at 21 d after 15 or 30 min of bilateral RI in some but not all cats. Although USG differed statistically between groups at some time points, the clinical significance is likely minimal given that urine remained acceptably concentrated in most cats. These RI durations are thus inadequate for modeling AKI or chronic fibrosis. Variable injury was seen at 120 d after 60 min unilateral RI combined with contralateral nephrectomy 2 wk after the ischemia induction, suggesting this protocol for inducing RI may be approaching a threshold, such that the RI-induced renal injury does not consistently result in the desired degree of subsequent chronic fibrosis. Given the transient rise in SCr and decrease in USG after nephrectomy, the unilateral RI model presented here has potential value as a model of AKI in cats. Importantly, unlike previously described models of AKI in cats,[26] none of our animals demonstrated any clinical morbidity after any of the RI durations tested.

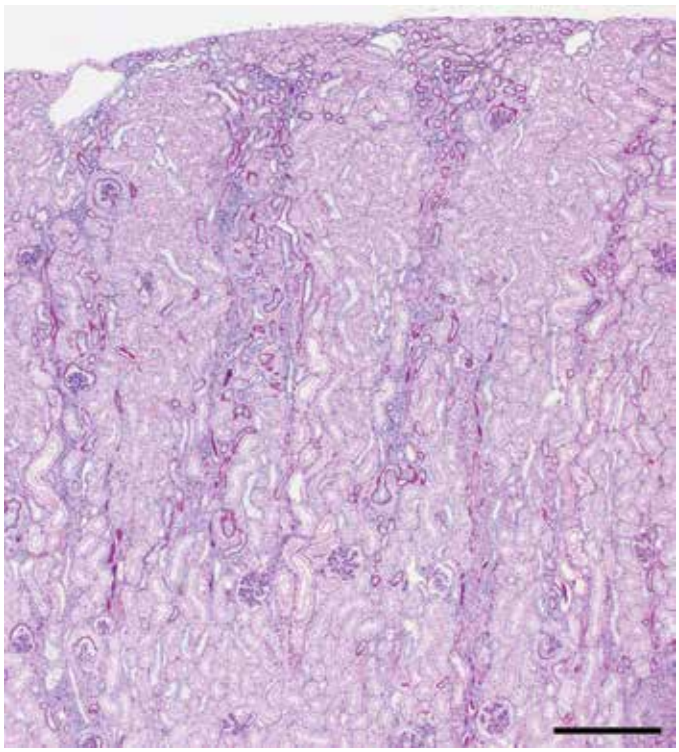

Figure 5. Photomicrograph from the right kidney of a cat at 120 d after 60 min of unilateral (right) RI combined with contralateral nephrectomy at 2 wk after RI (bar, 1 mm). Multiple prominent linear streaks of cortical inflammation, interstitial fibrosis, and tubular atrophy are present.

A previous study in dogs that evaluated unilateral RI up to 6 h in duration with a simultaneous contralateral nephrectomy found no mortality after 120 min of RI, with a sharp increase in mortality with greater RI times.[12] A recent study in dogs saw no mortality after 90 min unilateral renal artery occlusion with simultaneous contralateral nephrectomy, with return to 75% of baseline renal function at 3 wk after RI.[21] Porcine models have used 120 min of unilateral RI with simultaneous contralateral nephrectomy, with a mortality rate of 10%;[19] a second study in pigs found no mortality due to 90 min of RI.[3] In comparison, 82% mortality was observed after 90 min of RI in rats.[13] In mice, 30 min of RI is commonly used because of unacceptable mortality with longer durations.[31] Although none of the cited studies evaluated the long term effects of RI on renal histologic findings, these previous results do suggest that the threshold for tolerance of RI is greater in large animal models compared with rats and mice. Differences between

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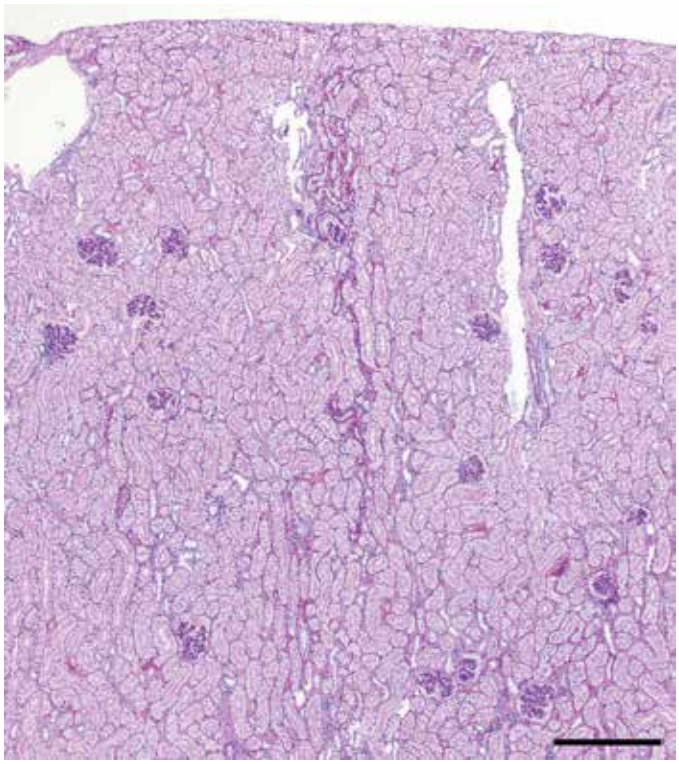

Figure 6. Photomicrograph from the right kidney of a cat at 120 d after 60 min of sham unilateral (right) RI combined with contralateral nephrectomy at 2 wk after sham RI (1 bar = 1 mm). Rare thin streaks of cortical inflammation and tubular atrophy (center) are surrounded by normal renal cortical parenchyma.

species highlight the importance of determining the threshold for recovery from RI that is specific to cats.

The duration of RI is an important variable not only in terms of degree of renal recovery but also in regard to the degree of consequential fibrosis. A study in mice showed that an RI duration of 26 min induced chronic fibrotic injury in only 50% to 60% of mice, whereas 30 min of RI induced injury and fibrosis in all mice.[29] In another murine study, renal tubular necrosis and residual morphologic damage at 30 d after RI were seen in 81% of kidneys after 60 min of RI and in only 18% and 5% of kidneys after 45 and 30 min of RI, respectively.[14] These findings likely are directly related to renal recovery threshold after RI, given that shorter durations of RI led to increased recovery and ultimately decreased histologic evidence of chronic renal fibrosis. These findings stress the importance of determining an appropriate duration of RI in cats to improve the utility of the model.

In cats, 60 min of unilateral RI without a contralateral nephrectomy has been studied.[25] In that study, renal histology and histomorphometry were assessed at several time points ranging from 1 to 70 d after RI. In general, epithelial necrosis and regeneration were present until day 21 after RI, when tubular atrophy and interstitial fibrosis predominated. Inflammation was present at all post-RI time points, with greater numbers of inflammatory cells in the ischemic kidneys compared with contralateral control tissues. Interstitial profibrotic activity and collagen, assessed through histomorphometry, were more extensive in all regions of the ischemic kidney compared with contralateral control tissues. In addition, interstitial fibrosis was decreased on day 70 in comparison with day 42;[25] this finding may have been secondary to continued repair within the kidney, thus reducing interstitial fibrosis. This decrease in interstitial fibrosis on day 70 in the cited study,[25] coupled with CKD being defined as injury present for 3 mo or longer, was our reason for choosing a 120-d survival time in the unilateral ischemia study. Certainly evaluation of renal histology at various time points after ischemia is expected to yield different pathology, consistent with the phase of healing at a given time.

Although our current study of unilateral RI yielded few statistically significant differences in percentage interstitial collagen or smooth muscle actin, cats in the unilateral RI group had increased staining with both stains in all regions of the kidney compared with that in samples from the sham RI group. Larger sample sizes might achieve a statistically significant difference. This technique shows promise as an objective tool to quantify renal injury, and future research to evaluate histomorphometry in cats with naturally occurring CKD is indicated.

Lesion severity after 60 min of unilateral RI combined with delayed contralateral nephrectomy was highly variable. A potential contributing factor to this variability may be body temperature during RI surgery; body temperature during 60 min of unilateral RI was significantly higher in those cats that had more severe renal lesions at 120 d after RI. The effect of temperature on cellular injury after ischemia has been studied extensively in other species, especially in the context of organ transplantation. In humans that have undergone partial nephrectomy, warm ischemia has been associated with an increased risk of renal dysfunction, compared with cold ischemia.[1,11] Increased body temperature, even within the physiologic range, is associated with increased injury after RI in rats.[9] Increased renal injury after warm compared with cold RI may be related to the negative effects of high temperature on cell membranes as well as to the decreased ability of warmer tubular cells to tolerate decreases in ATP concentration and increases in the production of free radicals.[9] In our current study, the temperature recorded may not have been reflective of the temperature of the kidney itself. In addition, temperatures were measured by using an oral thermometer, which has not been validated in cats. Further studies are required to elucidate causation, and future RI model studies should pay particular attention to maintenance of body temperature.

Although the underlying cause of CKD in cats is frequently unknown at the time of diagnosis, renal tubulointerstitial fibrosis is the outcome, independent of etiology.[16] The progression of AKI to CKD is multifactorial; overall, chronic inflammation within the kidney is believed to promote fibrosis through the production of profibrotic cytokines and reactive oxygen species and the modulation of myofibroblast activity.[15,16,24] Anemia, hypoxia, and aging also have been implicated in the progression of interstitial fibrosis. Although future studies that compare lesions resulting from surgical ischemia models with those seen in naturally occurring feline CKD are needed, the lesions seen in studies to date are very similar.[25]

The small number of cats used may have limited the power of the study, especially in the bilateral ischemia study. Although statistical significance was achieved only for USG at a few time points throughout the study, SCr appeared to increase and USG to decrease in the 30-min group compared with the 15-min group. A larger sample size might have realized a statistically

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Table 4. Enumeration of tubular necrosis (TN), atrophic tubules (AT), eosinophils (Eos), plasma cells (PC), mononuclear cells (Mono), polymorphic neutrophils (PMN) and mitotic figures (Mito) in 10 superficial cortical fields (magnification, 20×)

	TN	AT	Eos	PC	Mono	PMN	Mito
Bilateral RI							
15 min (n = 3)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
30 min (n = 3)	0 ± 0	2.00 ± 2.00	1.00 ± 1.73	1.67 ± 2.08	3.33 ± 5.77	0.33 ± 0.58	0 ± 0
Unilateral RI							
60 min unilateral RI (n = 5)	0.20 ± 0.45	46.60 ± 51.51	16.40 ± 28.58	4.60 ± 6.39	8.40 ± 13.15	0 ± 0	0.20 ± 0.45
ShamRI (n = 3)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Table 5. Enumeration of tubular necrosis (TN), atrophic tubules (AT), eosinophils (Eos), plasma cells (PC), mononuclear cells (Mono), polymorphic neutrophils (PMN) and mitotic figures (Mito) in 10 corticomedullary fields (magnification, 20×)

	TN	AT	Eos	PC	Mono	PMN	Mito
Bilateral RI							
15 min (n = 3)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
30 min (n = 3)	67 ± 1.16	3.00 ± 2.65	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Unilateral RI							
60 min unilateral RI (n = 5)	0.20 ± 0.45	13.00 ± 13.00	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.40 ± 0.55
ShamRI (n = 3)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

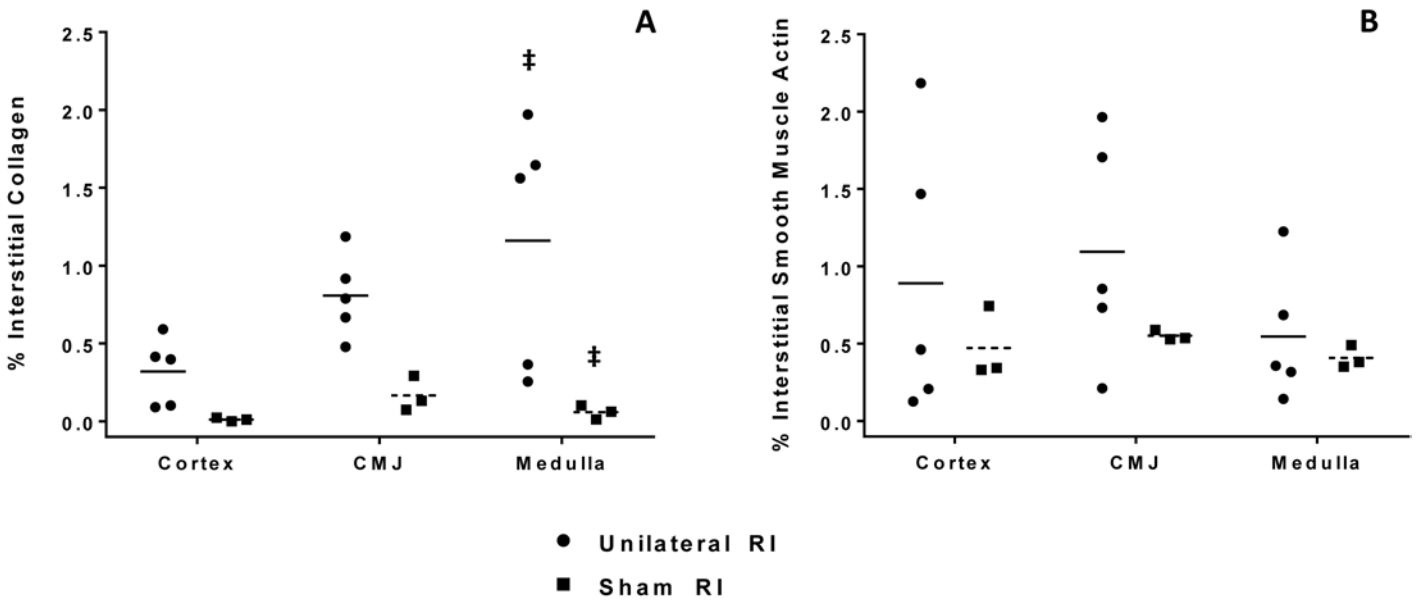

Figure 7. Percentage positive staining for (A) interstitial collagen (PASH-combo stain) and (B) interstitial smooth muscle actin at 120 d after unilateral or sham RI followed by contralateral nephrectomy 2 wk later. The mean of 10 photomicrographs from each region of the kidney is reported for each cat. Individual cats (n = 5) in the unilateral RI group are represented by circles (overall mean indicated with a solid line), whereas individual sham RI cats (n = 3) are represented by squares (overall mean indicated with a dashed line). There was a statistically significant difference in the percentage of interstitial collagen in the medulla (‡), with 1.16% ± 0.79% positive staining in the ischemic kidneys compared with 0.06% ± 0.04% in the sham kidneys. No other significant differences between groups occurred with either stain. CMJ, corticomedullary junction.

significant difference. As such, 30-min bilateral RI may have potential as a model of AKI. However, given the absent to minimal histologic lesions noted on day 21 and lack of azotemia in any cat, we believe that neither of the bilateral models we assessed here has potential as a model of CKD. Lack of a control group in the bilateral study further hinders interpretation of the utility of these models.

Aging has been associated with decreased capacity for renal healing in other species, secondary to changes in renal growth factor profiles, alterations in stem cells function and number, decreased renal epithelial cell proliferation, and maladaptive apoptosis.[27] In cats, increasing age has been associated with changes in antioxidant defenses and shortened telomere length[23] and has been implicated as a predisposing factor in animals with CKD.[8]

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The young age of the cats in our current study may have enhanced their ability to recover after RI, especially relative to that of the typical middle-aged to geriatric cat with CKD. Future studies should use cats that are at least 12 mo of age to eliminate this concern.

The effect of sex or castration status in cats on tolerance of RI is unknown. In rodents and in people, female sex is protective against RI.[2,22] In one study, intact female mice were able to survive 28-min unilateral RI with immediate contralateral nephrectomy, whereas all male mice required humane euthanasia within 48 h of surgery.[2] In that same study, castration 5 wk prior to ischemia surgery significantly improved tolerance to RI.[2] In humans, male sex is a predictor of AKI[5] and is moderately associated with delayed graft function in patients undergoing renal transplantation surgery.[2] A retrospective study evaluating 70 cats with AKI did not identify sex as risk factor for AKI nonsurvival.[17] Likewise, sex was not identified as a risk factor for the development of CKD in a retrospective study of 148 geriatric cats.[10] Nevertheless, our current findings must be interpreted in light of the unknown effects of sex and castration status on recovery from RI surgery in cats.

Several alterations to the models presented here should be considered. Longer durations of unilateral RI, with or without delayed contralateral nephrectomy, should be evaluated to determine the threshold for recovery in cats and to improve the repeatability of renal histologic changes, particularly fibrosis. Although the effect of body temperature on renal cellular injury during surgically induced RI has not been fully evaluated in cats, avoiding fluctuations in body temperature, particularly hypothermia, may improve the consistency of renal injury in surgical RI models.

Limitations of the current study include its small sample size, lack of a control group in the bilateral study, and the variability in renal fibrosis. Although the extrapolation of experimental models to clinical disease should always be done with caution, our findings suggest that healthy feline kidneys can undergo as long as 30 min of bilateral ischemia without evidence of renal injury 21 d after RI. This propensity may be germane to a variety of clinical situations, including partial nephrectomy, nephrotomy, renal transplantation, and systemic hypotension.

In conclusion, the unilateral model of RI presented here may be useful for studies of AKI but is inadequate for modeling renal fibrosis in cats at 120 d after RI. Future studies should evaluate longer durations of unilateral RI, with particular attention to accurate monitoring and management of body temperature during surgery.

Acknowledgments

The authors acknowledge Latisha Moody for her invaluable assistance throughout this study.

Funding was provided by Elanco Animal Health, a division of Eli Lilly and Company.

This study was presented in part at the American College of Veterinary Surgery Summit, Nashville, TN, 22 October 2015.

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