Renal functional decline and glomerulotubular injury are arrested but not restored by release of unilateral ureteral obstruction (UOO)

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W. Chaabane and F. Praddaude have developed the model and performed the surgery.
F. Praddaude and M. Buleon have performed the functional renal exploration
A. Jaafar was responsible for biological dosages.
M. Vallet and P. Rischmann have been involved in the interpretation of experimental data.
C.I. Galarreta and R.L. Chevalier have performed and interpreted renal histology.
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Running title: Renal repair of reversible unilateral ureteral obstruction
Abstract.

Murine unilateral ureteral obstruction (UUO), a major model of progressive kidney disease, causes loss of proximal tubular mass and formation of atubular glomeruli. Adult C57BL/6 mice underwent sham-operation or reversible UUO under anesthesia. In Group 1, kidneys were harvested after 7 days (7d). In Group 2, the obstruction was released after 7d and physiologic study of both kidneys was performed 30 days later. Renal blood flow (RBF), glomerular filtration rate (GFR), urine protein and albumin excretion were measured after ligation of either the left or right ureter. Glomerular volume (PAS), glomerulotubular integrity and proximal tubular mass (Lotus tetragonolobus lectin), and interstitial collagen (Sirius red) were measured by histomorphometry.

Obstructed kidney weight was reduced by 15% at 7d, but was not different from sham after 30d recovery. Glomerular volume and proximal tubular area of the obstructed kidney were reduced by 55% at 7d, but normalized after 30d. Interstitial collagen deposition increased 2.4-fold after 7 days of UUO, and normalized after release. However, GFR and RBF were reduced by 40% and urine albumin/protein ratio was increased 2.8-fold 30d after release of UUO. This was associated with 50% reduction in glomerulotubular integrity despite 30d recovery (p<0.05 for all data). We conclude that release of 7 days UUO can arrest progression, but does not restore normal function of the postobstructed kidney. Although remaining intact nephrons have hypertrophied, glomerular injury is revealed by albuminuria. These results suggest that glomerulotubular injury should become the primary target of slowing progressive kidney disease.

Keywords: glomerular filtration rate, albuminuria, renal hypertrophy, atubular glomeruli, proximal tubule, fibrosis.
Introduction:

Parenchymal loss and progressive interstitial fibrosis are common features of chronic kidney disease (23). Unilateral ureteral obstruction (UUO) is the animal model most widely used to study the development of tissue damage and fibrosis. In addition, release of obstruction permits examination of renal repair (for review see (5)). The renal impact of UUO varies with animal species and, until the past decade, rabbits, dogs and rats have been mostly used (21). However, most studies are currently performed in mice, which offer a variety of genetically engineered animals (9, 12, 16). Because of its technical difficulty, the use of reversible models of UUO in mice remains limited and little information is available regarding the renal functional impact of UUO and its relief. As in man, most species exhibit a compensatory renal growth of the non-obstructed kidney (5, 12) which undergoes a corresponding increase in glomerular filtration rate (GFR). Following release of obstruction, GFR is generally estimated by measurement of blood urea nitrogen following ureteral reimplantation (19). Proteinuria following ureteral reimplantation suggests that UUO induces irreversible glomerular damage. However, since proteinuria can result from either a glomerular leak of albumin or a decrease in reabsorption of small proteins at the proximal tubular level, the mechanism of proteinuria during reversible UUO needs to be confirmed. The purpose of the present work was to determine, in a new simplified model in adult mice, the consequences of reversible UUO (R-UUO) on renal structure and function in both kidneys following release of obstruction. Particular attention was paid to the relationship between delayed histological damage following UUO relief and persistent impact on renal function, including proteinuria.

Materials and Methods:

Animals

Adult female C57BL/6 mice were obtained from Harlan Laboratories and kept in a temperature-controlled room on a light-dark cycle (12h/12h). Animals were studied at 15 to 20 weeks of age, because sexual maturity is reached at 6 weeks of age, and lifespan is 2-3 years. As “young adults” they are fully mature, and recovery following release of obstruction reflects an adult response not limited by immaturity or senescence. They had free access to a standard diet and tap water. The animal study protocol was in accordance with the National
Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee.

Two groups of mice were used: in Group 1 (UUO 7 days), the left ureter was obstructed and kidney function and histology were studied 7 days later; in Group 2 (Release 30 days), the left ureter was obstructed, obstruction was released 7 days later, and recovery of the left renal kidney structure and function were explored 30 days after the release of UUO. A duration of 7 days obstruction was selected because previous reports have shown functional recovery after release of 6-10 days of UUO (8, 14, 17, 19). Although shorter duration of obstruction results in less severe injury (17), the effects of 7 days UUO optimizes the detection of accelerated recovery in future studies of mice of differing genetic background, selective knock out strains, or therapeutic interventions.

Reversible unilateral ureteral obstruction model

Unilateral ureteral obstruction or sham surgery was performed under general anaesthesia induced by an intra peritoneal Ketamine (250 mg/kg)-Xylazine (10 mg/kg). Mice were placed on a heated surgical table to maintain body temperature at 37° C. After an abdominal midline laparotomy, the perivesical fat and the bowel were gently displaced with a wet cotton swab. The left ureter was carefully dissected from vascular and fat tissue. A 4-0 monocryl wire was placed around the ureter. The two ends of the wire were introduced into a silicone catheter (length, 5mm, CH 7) and gently withdrawn (Fig. 1). Surgical knots were tied to hold the ureter loop in the device. The perivesical fat was then laid back in place. The muscle, fascia and skin were closed with 5-0 vicryl sutures. Prophylactic iodined polyvidone (Bétadine dermique 10%) was applied to the abdominal wound.

In Group 2, the device was removed 7 days later. It was easily identified and teased free of surrounding tissue, and the ureter loop was released by removing the surgical knots. To avoid ureteral damage, no further dissection was performed. The incision was closed with 5-0 vicryl sutures. Total surgery time was 15-20 minutes per animal for both procedures. During sham procedure, the left ureter was exposed, carefully dissected and repositioned. A piece of catheter was left intraperitoneally and removed 7 days later.

Methylene blue test

Ureteral obstruction was checked in two groups of 5 mice during preliminary experiments. In one group the folding device was set up on the left ureter and simultaneously the right ureter
was tied (Silk 5-0). Two hours following obstruction, an intra-abdominal injection of methylene blue (100 µL) was performed and mice were placed in a cage covered by blotting paper. Complete obstruction was confirmed in the 5 mice by the absence of urine for 12 hours and then mice were sacrificed. In the other group, the reversibility of the obstruction was confirmed by a positive methylene blue test 3 days after the removal of left UUO and immediately following contralateral right ureter ligature. In 4 of the 5 animals, methylene blue was detected in urine, indicating patency of the left ureter following UUO removal. To eliminate the inclusion of animals with significant residual partial ureteral obstruction, all mice with ureteral diameter more than two-fold that of the contralateral kidney were excluded from the study.

Proteinuria and albuminuria

Mice were placed in metabolic cages in order to collect 24-h urine. Urinary albumin excretion was determined with a mouse antigen specific ELISA (Mouse Albumin Quantitation Kit, Bethyl Laboratories®, USA). Urinary total proteins were quantified by the pyrogallol red/SDS colorimetric method. Urinary creatinine concentration was measured using an enzymatic colorimetric test based on sarcosine oxidase (reagent Beckman Coulter) on a Pentra 400 apparatus (Horiba Medical®). Urine protein and albumin excretions were factored by urine creatinine concentrations in order to take into account the differences in urinary flows. Finally, the albumin to protein ratio was calculated in order to define the glomerular or tubular origin of proteinuria.

Renal function studies

Renal function was measured as previously described (4). Briefly, mice were anesthetized with an intraperitoneal injection of 150 mg/kg of thiobutabarbital sodium (Inactin) and placed on a thermostatically controlled heating table. After tracheotomy, the left jugular vein was cannulated to infuse a Ringer lactate solution (Gelofusine) and a mixture of NaCl 0.9%, thiopental sodium (0.83 mg/kg/min, Pentothal), inulin (1.8 mg/kg/min) and p-aminohippurate (0.4 mg/kg/min). The rate of infusions was 0.1 mL/h. The left femoral artery was cannulated to monitor mean arterial blood pressure (MABP) and to obtain blood samples. Urine was collected through an intravesical catheter. One of the following procedures was then performed: 1) In order to investigate functional recovery of the left kidney, the right ureter was ligated; 2) In order to investigate functional adaptation of the right contralateral
kidney, the left ureter was ligated. At the end of surgery, a bolus of inulin and PAH (inulin: 100 mg/kg; PAH: 50 mg/kg) was perfused and mice were allowed to recover for 30 min. Renal function was evaluated for a 60-min clearance period.

Glomerular filtration rate (GFR) and effective renal plasma flow (RPF) were assessed by inulin and p-aminohippurate clearances, respectively. Renal blood flow (RBF) was calculated as the ratio of renal plasma flow to 1-haematocrit. Renal vascular resistance (RVR) was calculated as the ratio of mean arterial pressure to renal blood flow. The filtration fraction was calculated as the ratio of glomerular filtration rate to renal plasma flow. At the end of the process, blood and urine samples were stored for biochemical studies. Ureters and kidneys were systematically checked to confirm the resolution of hydronephrosis.

Renal histology and morphology

At the end of the renal function study, a catheter was introduced into the abdominal aorta. Both kidneys were washed with 10 mL of PBS, fixed by an infusion of 10 mL of 10 % pH 7.40 formalin, harvested, weighted, sectioned along the coronal axis and stored in formalin. Formalin fixed kidneys were embedded in methyl methacrylate. Sections of 3 µm thickness were stained with Sirius Red in order to perform fibrosis analysis, Periodic Acid Schiff (PAS) for glomerular morphology, or *Lotus tetragonolobus* lectin for tubular morphometry. Pictures were captured using a digital camera (Nikon D3, Japan) connected to a light microscope. For Sirius red stained sections, the overall kidney section was pictured at a magnification x100, and all pictures were assembled using Photoshop software (Adobe System, San Jose, CA) in order to provide a complete reconstructed section. Fibrosis was then quantified using the same software. The total area of the kidney section (excluding large vessels) was measured. The Sirius-positive structures were automatically selected using the properties of colour recognition of the software. The Sirius-positive area was expressed as a fraction of the total area of the section.

For PAS stained sections, pictures of 30 glomeruli were randomly chosen in the kidney section (for each mouse) and taken at magnification x 400, by moving the slide from the outer to the inner cortex to obtain non-crossing sample fields. Pictures were analysed using Photoshop software. As previously described (1), the total cortical area was examined. Glomerular tufts (including all the cellular and interstitial components of the glomerulus, including podocytes and capillaries) were encircled, and the enclosed area was copied to
create a new picture. The number of pixels (independent of their individual density) of this picture gave the surface area of the tuft. According to DeHoff’s equation for the measurement of spheroids of differing size, the harmonic mean of glomerular areas (Svm) was used to calculate the mean glomerular volume (GlmVm) for each animal, using the formula \( \text{GlmVm} = \frac{4}{3} \times \text{Svm}^{1.5} \). From the glomerular picture, the entire amount of PAS-positive material, except for the peripheral basement membranes, was selected automatically using the properties of colour recognition of the software and was manually completed by the inclusion of nuclei. The number of pixels in this area was considered to represent the mesangial area (i.e., mesangial cells and extracellular matrix areas) and was expressed as a fraction of the tuft surface area.

*Lotus tetragonolobus* lectin (Vector Laboratories, Burlingame, CA, USA) binds to proximal tubule epithelial cells in mouse and human kidney (11, 18). Kidney sections were treated by this staining procedure, which incorporated protease K enzymatic digestion prior to exposure to biotinylated *Lotus* lectin (1:50) with development by the ABC-DAB regimen. The fraction of nephrons with intact glomerulotubular junctions was determined in *Lotus* lectin-stained kidney sections as described previously (9). In the adult mouse, lectin-staining cuboidal epithelial cells normally extend from the proximal tubule around the urinary pole of Bowman's capsule. However, as a result of UUO, the glomerulotubular junction undergoes injury with tubular atrophy and formation of atubular glomeruli, which can be detected by altered morphology of the epithelial cells, which become flattened and lose *Lotus* lectin staining (9). Lotus-stained sections were examined from kidneys of 8 sham-operated, 4 obstructed, and 4 postobstructed mice, respectively. All glomeruli were counted and scored on the basis of the presence or absence of any *Lotus* staining of Bowman’s capsule. Regardless of the extent of staining, any *Lotus*-positive glomerular profiles were scored as positive, and those lacking visible staining were scored as negative. Results were expressed as the percent of lectin-positive glomeruli, which indicates the fraction of nephrons with normal glomerulotubular junctions. *Lotus* lectin-negative glomeruli are nonfunctional because they are either connected to atrophic proximal tubules or are atubular (9). The fractional contribution of *Lotus* lectin-staining proximal tubules to the subcapsular cortex was determined using a stereological approach as described previously (10). Ten fields were photographed at 400x magnification, and image analysis (ImagePro® Plus 5.1 [Media Cybernetics, Silver Spring, MD]), was used to measure proximal tubular area expressing cell staining with DAB reaction product, and the results are expressed as a percent parenchymal
area (volume fraction). This fraction reflects the functional proximal tubular mass of each kidney.

**Statistical analysis**

Since the number of animals did not fit a Gaussian distribution for all parameters, comparisons between experimental groups used the non-parametric Kruskal-Wallis test. When this test indicated a significant difference, a post-hoc test (Dunn’s multiple comparison test) was performed using GraphPad Prism 4.0 (GraphPad Software, USA). $P<0.05$ was considered statistically different.

**Results**

**Technical success rate**

The efficiency of the folding device for complete UUO was 100%. Reversibility (i.e. ureteral patency) after 7 days of UUO was obtained in 62%, allowing resolution of hydronephrosis and the collection of urine again. All cases of failure were related to the development of fibrosis around the folded ureter. Only mice with demonstrated ureteral patency were used for study. None of the mice from the sham group exhibited hydronephrosis. Procedure duration was similar for both UUO and UUO release with a mean between 15 and 20 min.

For metabolic and histologic studies, to avoid including animals with partial obstruction, the ureter was inspected at the time of study and all animals with ureteral diameter more than two-fold that of the contralateral kidney were excluded.

**Renal morphology and histopathology**

Seven days of UUO induced a significant atrophy of the obstructed kidney (Fig. 2A), as shown by the ratio between kidney weight and body weight that was significantly decreased when compared to sham ($5.56 \pm 0.22$ vs $6.75 \pm 0.35$ mg/g of body weight, $P<0.05$). Sham-operated kidneys showed normal histologic architecture, with distinct cortex, medulla, and renal papilla. The obstructed kidney was characterized by thinning of renal parenchyma, particularly with an involution of the papilla and outer medulla (Fig. 3A and 3C). Cortical damage was evidenced by a 55% decrease in glomerular volume (UUO 7 days: $1.38 \times 10^7 \pm 0.02 \times 10^7$ vs sham: $3.08 \times 10^7 \pm 0.06 \times 10^7 \mu m^3$, $P<0.05$, Fig. 2B). However, relative mesangial area was not altered (Fig. 2C and 3E). Whereas the median fraction of *Lotus*-stained
glomeruli in sham-operated mice was 32%, the fraction was 16% for obstructed kidneys and 34% for contralateral kidneys (Fig. 4). When compared to the contralateral kidney, glomerulotubular integrity (fraction of Lotus-staining glomeruli) was decreased after 7 days of obstruction \( (P<0.05, \text{Fig. 4}) \). In addition, the volume fraction of proximal tubular mass was reduced by 50% following 7 days of UUO (Fig. 2D, 3A and B). Tissue staining by Sirius red (Fig. 2E and 3D), an index of collagen accumulation, showed a significant increase in cellular matrix when compared to sham \( (3.18 \pm 0.35 \text{ vs } 1.38 \pm 0.15 \% , \ P<0.05) \). This increase was mostly located in the inner medulla and papilla.

Release of UUO resulted in partial restoration of renal tissue architecture at 30 days (Fig. 2 and 3). The ratio of kidney weight to body weight remained suppressed compared to that of sham (UUO release 30 days: 5.76 ± 0.45; Sham 30 days: 7.11 ± 0.28 mg/g of body weight, \( P<0.05, \text{Fig. 2A}) \). Notably, a significant number of glomeruli exhibited capsular lesions with glomerular tuft adhering to capsular membrane, and glomerulotubular integrity remained decreased 30 days after release of UUO \( (P<0.05) \) (Fig. 3B and 4). Whereas the median fraction of Lotus-stained glomeruli in sham-operated mice was 26%, the fraction was 22% for obstructed kidneys and 38% for contralateral kidneys (Fig. 4). By contrast, glomerular volume increased following release of ureteral obstruction (Fig. 2B), and this was paralleled by an increase in the volume fraction of proximal tubular mass after release of obstruction (Fig. 2D and 3A). The release of UUO also resulted in a significant decrease in extracellular matrix (UUO release 30 days: 1.65 ± 0.17 % versus UUO 7 days: 3.18 ± 0.35 %, \( P<0.05) \) with a value close to that of sham (Fig. 2E). Sirius red staining did not show any significant periglomerular fibrosis when compared to sham (Fig. 3D).

The contralateral non-obstructed kidney was not affected by seven days of UUO, as indicated by stable values of kidney to body weight ratio, percentage of extracellular matrix and relative mesangial area (Fig. 2A, C and E). The glomerular volume tended to increase without reaching statistical significance (Fig. 2B). By contrast, 30 days after the removal of left UUO, right kidney weight to body weight ratio (Fig. 2A) was significantly increased when compared to sham (UUO release 30 days: 8.47 ± 0.44 vs sham: 7.21 ± 0.42 mg/g of body weight, \( P<0.05) \). Interstitial extracellular matrix, glomerular volume and mesangial area remained unchanged.
Renal function and hemodynamics

Thirty days after release of obstruction, there was no significant difference between sham and obstructed animals regarding body weight, blood pressure, urinary flow and haematocrit (Table 1). Thirty days after UUO release, left kidney GFR was partly restored (up to 59 %) but remained significantly lower than that of sham (UUO release 30 days: 70.9 ± 11.5 vs sham: 119 ± 12 µL/min/65cm², \( P<0.05 \)) (Fig. 5A). Renal Blood Flow (RBF) of the obstructed kidney was partly restored (up to 56 %) without reaching sham values (UUO release 30 days: 370 ± 54 vs sham: 656 ± 58 µL/min/65cm², \( P<0.05 \)) (Fig. 5B). As a result, the filtration fraction (FF) in postobstructed kidneys remained similar to sham (UUO release 30 days: 33.6 ± 1.7 vs sham: 32.3 ± 2.9 %) (Fig. 5D). Since mean arterial blood pressure was similar in both groups (Table 1), renal vascular resistance (RVR) was significantly increased in R-UUO when compared to sham (UUO release 30 days: 227 ± 30 vs sham: 125 ± 19 mmHg/ml/min/65cm², \( P<0.05 \)) (Fig. 5C).

Seven days after obstruction, renal hemodynamics of the non obstructed right kidney (Fig. 5E) were altered, with a 37% increase in GFR (210.4 ± 20.9 µL/min/65cm²) when compared to sham (154.4 ± 9.9 µL/min/65cm²), indicating an adaptation to contralateral UUO. Thirty days after left kidney obstruction removal, right kidney GFR was no longer significantly different from sham.

Protein and albumin excretion

Total proteinuria was not different between groups (Fig. 6A). However, albumin content in the urine of postobstructed animals was three-fold greater than that of sham-operated mice \( (P<0.01) \), with an albumin to protein ratio increased in the same proportion indicating a glomerular leak of albumin rather than decrease in tubular reabsorption of protein (Fig. 6B and C).

Discussion

The ureteral obstruction model has become widely used to study chronic kidney disease and renal fibrosis. Surgical models of reversible UUO provide the great advantage of allowing the study of renal recovery following the relief of obstruction, but few mouse models have been described. Independent of the technical challenge resulting from the small size of these...
animals, the technique of reversible UUO in mice has been limited by the high frequency of irreversible ureteral damage (5). Cochrane described a reversible UUO model using a vascular clamp, allowing separate assessment of renal function in post-obstructed and contralateral kidneys (used as control) (8). Nevertheless, despite a large number of animals, this report describes functional studies in only four animals, with heterogeneous results. Tapmeier and colleagues re-established ureteral continuity by uretero-vesical reimplantation to ensure reversibility (19). This procedure, completed by a contralateral nephrectomy after ureteral reimplantation, was associated with high mortality. Moreover, renal function was only assessed by BUN. Using a model of ureteral clamping, Puri and colleagues (17) proposed replacing the vascular clamp every 2 days after demonstrating that longer continuous clamping impairs the reversibility of UUO. However, the requirement for repeated induction of anaesthesia could impact renal haemodynamics by inducing recurrent hypotension and hypoxemia. Unfortunately, renal haemodynamics were not studied in this work. The present study also relies on two surgical procedures, the first to create the ureteral obstruction (duration 15 to 20 minutes), and the second to remove it (duration 10 to 15 minutes). As with previously published reports of reversible UUO, it is possible that anaesthesia, through temporary renal hemodynamic disruption, may have contributed to renal injury. However, no injury was detected in the sham group despite similar surgical procedures and anaesthesia.

The present study employs a simple, inexpensive and reproducible mouse model of reversible UUO that leads to 62% ureteral patency. This model allowed us to study both renal histomorphology and unilateral renal function in mice, and should be useful in elucidating mechanisms of tissue regeneration after release of UUO (13, 15). Little attention has been paid to the glomerular impact of temporary UUO. In the present work, after seven days of UUO, glomerular volume was decreased by 50% in the obstructed kidney. Importantly, these changes in glomerular volume were reversed 30 days following obstruction relief. Based on mesangial volume, there was no evidence for the development of progressive glomerulosclerosis, but scattered glomeruli remained contracted in the R-UUO group. This is presumably a reflection of the reduced fraction of nephrons with intact glomerulotubular junctions that persists even after release of the obstruction (9, 20). In the absence of significant fibrosis, the resulting decreased number of functional glomeruli could explain reduced GFR 30 days following release of ureteral obstruction. Although statistically significant, renal interstitial collagen accumulation seven days after UUO was less than 1% of the parenchymal area and returned to normal levels after release of UUO. Release of temporary UUO in the neonatal rat attenuates, but does not completely reverse interstitial
collagen accumulation (6). Although there are reports of reversible fibrotic lesions (2-3), the increase in renal collagen concentration due to UUO correlates with a decrease in renal tissue dry weight (8).

A previous study of renal hemodynamic changes following R-UUO in mice reported a slight but not significant decrease in GFR in four mice (8). However, GFR in the postobstructed kidney was expressed as a percentage of the GFR of the contralateral kidney and no data were available regarding renal blood flow and vascular resistance. Thus, it is not possible to determine whether this change resulted from a decrease in postobstructed kidney GFR or from an adaptive increase of the contralateral kidney GFR. In the present work, 30 days after release of UUO, ureteral patency was assessed by disappearance of dilation and by methylene blue tests. Following release of UUO, kidneys only partly recovered GFR and RBF, with a stable filtration fraction. The reduction in GFR in the postobstructed kidney at 30 days can be explained by a proportionate decrease in the fraction of nephrons with intact glomerulotubular junctions. Renal vascular resistance was significantly increased in postobstructed kidneys, exposing them to long-term histological and functional damage, as described in adult rats after temporary neonatal UUO (7). This hemodynamic impact and the capsular lesions observed in some glomeruli following R-UUO could contribute to the increase in albuminuria that we observed. Indeed, increased albuminuria without significant change in proteinuria indicates a glomerular leak rather than a decrease in proximal tubular reabsorption of proteins. Recovery of a normal fractional proximal tubular volume in the postobstructed kidney provides a morphologic explanation for the functional data.

Seven days after UUO, contralateral kidney weight remained unchanged. By contrast, 30 days after obstruction relief, contralateral kidney weight was significantly increased, indicating adaptive renal growth. Such growth of the contralateral kidney could result from a delayed response to temporary contralateral UUO, or a persistent stimulus due to decrease in GFR of the obstructed kidney. Unfortunately, little is known regarding the mechanisms that promote contralateral renal hypertrophy during UUO (22). Examination of the contralateral kidney after R-UUO is of interest since it reflects adaptation to the recovery of the previously obstructed kidney. In addition to comparison of the postobstructed kidney with the contralateral kidney, comparison of kidney structure and function with a sham-operated group is a necessary control (5). After 7 days of UUO, GFR of the contralateral kidney was significantly increased, indicating an early functional adaptation. However, 30 days after R-
UO, GFR of the contralateral kidney was no longer different from that of the sham-operated kidney, indicating the reversibility of hyperfiltration resulting from R-UUO.

In conclusion, this model of reversible UUO permits the study of functional and histological recovery following release of ureteral obstruction. Following 7 days of obstruction, functional renal responses are correlated with glomerular and proximal tubular damage. These results suggest that studies of glomerular and proximal tubular injury and repair may be more fruitful than measurement of interstitial fibrosis as a target of intervention in chronic kidney disease.

References


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**Legends**

**Fig. 1. Reversible Unilateral Ureteral Obstruction method.** A) Diagram of the left ureter with the catheter and the monocryl wire. B) The left ureter was carefully dissected from vascular and fat tissue and a 4-0 monocryl wire was placed around the ureter. C and D) The two ends of the wire were introduced into a silicone catheter (length, 5mm, CH 7) and gently tracted. E) Surgical knots were placed to hold the ureteral loop in the device.

**Fig. 2. Renal histological and morphological parameters in both kidneys after 7 days of UUO and 30 days after UUO release.** A) Ratio of kidney weight to body weight; B) Glomerular volume; C) Relative mesangial area analysed in PAS stained sections; D) Fraction of subcapsular cortex constituting *Lotus*-positive staining proximal tubules; E) Fraction of total renal parenchyma stained with Sirius red (collagen).

OLK: Obstructed Left Kidney, RK: Righ Kidney (contralateral); *P<0.05; **P<0.01 for the indicated comparison.

*For A and B : Sham 7 days: n=7, UUO 7 days: n=11, Sham 30 days: n=15, UUO Release 30 days: n=8. For C, D and E: n=4 in each group.*

**Fig. 3. Representative histologic sections of sham-operated, obstructed (7 days) and kidneys 30 days after release of obstruction.** A) Sections stained with *Lotus tetragonolobus* lectin (brown) showing the distribution of functional proximal tubules. Compared to sham-operated kidneys, proximal tubular volume fraction was reduced after 7 days of ipsilateral UUO. In postobstructed kidneys 30 days following release of obstruction proximal tubular volume fraction was increased. B) Detail of *Lotus* lectin-stained kidneys, showing glomeruli with contiguous *Lotus*-staining proximal tubule (arrowhead) and glomeruli lacking *Lotus* staining (*). C) Left kidney section stained with Sirius red (magnification x100) in sham mice, after 7 days of UUO, and 30 days after UUO release. D) Glomeruli stained with Sirius Red (magnification x400). E) Glomeruli stained with PAS (magnification x400).

**Fig. 4. Glomerulotubular integrity: fraction of *Lotus*-staining glomeruli in kidneys from each group of mice.** Each point represents mean data for one animal. UUO = kidney with ipsilateral unilateral ureteral obstruction; CL = kidney from mouse with contralateral UUO; UUO release = kidney 30 days following release of ipsilateral obstruction; CL release = kidney from mouse 30 days after release of contralateral UUO. *p<0.05.
Fig. 5. A-D) Renal hemodynamics of left sham-operated kidney or postobstructed kidney 30 days after obstruction release. A) GFR: glomerular filtration rate; B) RBF: renal blood flow; C) RVR: renal vascular resistance and D) Filtration fraction. E) Glomerular filtration rate of right contralateral kidney * P<0.05; **P<0.01 for the indicated comparison (Mann-Whitney test). Sham: n=15; UUO Release: n=8

Fig. 6. A) Urine protein excretion (mg/g creatinine); B) Urinary albumin excretion (mg/g creatinine) and C) Albuminuria / proteinuria (%); 30 days after UUO release. *P<0.05; **P<0.01 for the indicated comparison. Sham: n=8, UUO: n=8.
Figure 2.
Table 1. Clinical parameters 30 days after UUO release

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham (30 days)</th>
<th>UUO (30 days after)</th>
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<tbody>
<tr>
<td>n = 17</td>
<td>n = 8</td>
<td></td>
</tr>
<tr>
<td>Body Weight (g)</td>
<td>21.3 ± 0.3</td>
<td>21.4 ± 0.5</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>75.2 ± 2.7</td>
<td>74.3 ± 3.4</td>
</tr>
<tr>
<td>Urinary Flow (µL/min)</td>
<td>0.54 ± 0.06</td>
<td>0.38 ± 0.02</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>43.2 ± 0.5</td>
<td>44.1 ± 0.2</td>
</tr>
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Values are means ± SEM. No statistical difference was found between groups. MABP: Mean Arterial Blood Pressure.
Figure 5
Figure 6