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Research Article

Persistent subclinical renal injury in female rats following renal ischemia-reperfusion injury

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The incidence of acute kidney injury (AKI) continues to rise in both men and women. Although creatinine levels return to normal quicker in females following AKI than in males, it remains unclear whether subclinical renal injury persists in young females post-AKI. This study tested the hypothesis that AKI results in subclinical renal injury in females despite plasma creatinine returning to sham levels. For the present study, 12–13-week-old female Sprague-Dawley (SD) rats were randomized to sham or 45-minute warm bilateral ischemia-reperfusion surgery as an experimental model of ischemic AKI. Rats were euthanized 1, 3, 7, 14, or 30 days post-AKI/sham. Plasma creatinine, cystatin C, kidney injury molecule 1 (KIM-1), and NGAL were quantified via assay kits or immunoblotting. Kidneys were processed for histological analysis to assess tubular injury and fibrosis, and for electron microscopy to examine mitochondrial morphology. Immunoblots on kidney homogenates were performed to determine oxidative stress and apoptosis. Plasma creatinine levels were increased 24 hours post-AKI but returned to sham control levels three days post-AKI. However, cystatin C, KIM-1, and NGAL were increased 30 days post-AKI compared with sham. Tubular injury, tubulointerstitial fibrosis, and mitochondrial dysfunction were all increased in 30-day post-AKI rats compared with sham. Additionally, 30-day post-AKI rats had higher p-JNK expression and lower antioxidant enzyme glutathione peroxidase and catalase levels compared with sham. AKI resulted in higher expression of cleaved caspase 3, TUNEL+ cells, and caspase 9 than sham.
Despite the normalization of creatinine levels, our data support the hypothesis that subclinical renal injury persists following ischemia-reperfusion injury in young female rats.

Introduction

Acute kidney injury (AKI) is a sudden and often reversible reduction in kidney function. AKI is diagnosed by elevated serum creatinine levels or diminished urine output based on the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines [1]. The burden of AKI in the United States is significant, affecting over 1.2 million hospitalized patients annually [2–4]. Although women have been suggested to be protected from AKI relative to men, women account for 40% of AKI patients [5] and 50% of the global population [6]. Despite this, AKI in females remains understudied. This is problematic as incomplete maladaptive recovery from an incident of AKI is a contributing factor for the development and progression of chronic kidney disease (CKD) [7], and CKD is consistently more prevalent in women than in men, with recent data estimating a prevalence of 14% in women compared with 12% in men [8]. However, the mechanisms driving renal injury in the weeks following AKI in young women remain poorly understood. To improve health outcomes in women, it is vital to understand how AKI affects the female kidney, predisposing women to future disease [9,10].

Additionally, females have a unique physiology that distinguishes them from males [11]. The impact of a history of AKI on pregnancy outcomes in women of childbearing age was not appreciated until recently. Tangren et al. showed that women with a history of AKI present with higher rates of adverse maternal and fetal outcomes during pregnancy despite renal function returning to baseline levels before conception [12,13]. These adverse fetal and maternal pregnancy outcomes observed in women, despite complete clinical and laboratory recovery post-AKI, further highlight the need to study AKI in young females.

The mechanisms driving adverse pregnancy outcomes in women with a history of AKI have been poorly understood due, in part, to the absence of an appropriate animal model. Recently, Gillis et al. developed a novel rat model showing that female rats one-month post-AKI with 'normal' creatinine levels

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before conception develop adverse fetal and maternal outcomes in pregnancy [14]. These data align with findings in humans, suggesting lingering subclinical injury post-AKI in females despite 'recovery' based on clinical definitions [15]. However, little is known regarding how renal ischemia affects the female kidney or the mechanisms driving injury, specifically in females. The goal of the present study was to test the hypothesis that AKI induces subclinical renal injury in reproductive-aged females despite normal creatinine levels.

Materials and methods

Experimental animals: Experimental procedures and all protocols were approved by the Institutional Animal Care and Use Committee (IACUC protocol No. 2010-0141) at Augusta University. Female Sprague-Dawley (SD) rats were purchased from Envigo Laboratories (Indianapolis, IN) at 10–11 weeks of age and allowed 1–2 weeks of acclimatization before any experiments. The selection of SD rats for this study was based on our previous work showing that female SD rats with a history of AKI have adverse maternal and fetal pregnancy outcomes [14]. Rats were housed at room temperature (20–25°C), in humidity-controlled rooms (30–70%), and maintained on a 12/12-hour light/dark cycle with free access to standard rodent chow (Envigo Teklad, 2918) *ad libitum*.

Warm bilateral renal ischemia-reperfusion model for AKI: Female SD rats (n = 5–7) were randomized to sham or warm renal ischemia-reperfusion (IR) as previously described [14,16]. Briefly, rats were anesthetized with 2.5% isoflurane, and body temperature was maintained at 37°C using a heating pad and heating lamp (R40, Satco S4998). The right and left kidneys were accessed via dorsal flank incisions. Both renal arteries were isolated from the veins, cleaned using cotton tips, and clamped with microserrefines (Fine Science Tools, Foster City, CA). After 45 minutes, the clamps were released to allow for reperfusion, which was confirmed visually by a return to normal kidney coloration. For sham controls, the kidneys were exposed and arteries were isolated, but no clamps were applied. Sham controls were maintained under the same duration of anesthesia as the IR group. Flank incisions were closed using a 4-0 polypropylene suture, and the outside skin was closed using wound clips. Fluid loss was replenished using 1 mL of warm saline intraperitoneally, and buprenorphine (1.0 mg/kg) was administered subcutaneously as an analgesic after surgery. Subgroups of rats were allowed to recover for different time points (24 hours, 3 days, 7 days, 14 days, or 30 days) post-IR in conventional animal housing before being killed for tissue collection. Sham controls were killed 30 days post-sham surgery.

Tissue collection: All animals were put in metabolic cages for 24-hour urine collection before surgery for baseline urine collections and again 24 hours before they were killed. Animals were deeply anesthetized with 3% isoflurane when they were killed, and the depth of anesthesia was confirmed via tail pinch and pedal reflex. An abdominal midline incision was made, with a terminal blood sample obtained from the abdominal aorta before rapid exsanguination and thoracotomy. Kidneys were weighed. The right kidney was snap-frozen in liquid nitrogen and stored at −80°C for biochemical analyses. The left kidney was fixed in formalin for histology or glutaraldehyde fixative for electron microscopy.

Kits and assays to assess renal function and injury: Commercially available kits were used to measure plasma creatinine (BioAssay Systems, Hayward, CA; Quantichrome Creatinine Assay Kit, Cat# DICT-500) [14,16,17], plasma and urinary cystatin C (Cat# MSCTC0, R&D Systems, Minneapolis, MN), and urinary kidney injury molecule 1 (KIM-1) (Cat# RKM100, R&D Systems, Minneapolis, MN). The sensitivity for the cystatin C kit was 12.9 pg/mL with an assay range of 125–8,000 pg/mL, while the sensitivity of the KIM-1 kit was 2.74 pg/mL with an assay range of 7.8–500 pg/mL. Urinary protein excretion was assessed using the Bradford Assay (Bio-Rad, Hercules, CA) [14]. Each sample was run in duplicates, and all kits were run per manufacturer's instructions.

Assessment of tubular injury and renal fibrosis: Transverse sections of kidneys were made using a sharp razor blade, and sections were fixed in 10% formalin overnight and transferred to 70% ethanol before being embedded in paraffin wax. For assessment of renal injury and fibrosis, 5–6-μm-thick sections were stained for hematoxylin and eosin (H&E) or Masson's Trichrome (MT), respectively, according to manufacturer's instructions. Sirius red/fast green collagen staining was performed on renal sections as per the manufacturer's instructions to assess for collagen deposition (cat. No. 9046, Chondrex Inc, Woodinville, WA).

For each animal, five images were analyzed under an Olympus BX40 bright-field light microscope at 20× magnification for H&E and 10× for MT stains. Multiple non-overlapping fields were imaged for each section. Each image was assessed on a scale of 0–5, scoring for tubular injury, interstitial injury (H&E

staining), and fibrosis (MT staining) by an investigator blinded to the hypothesis and experimental groups. The scoring criteria were as follows: 0 = no injury observed; 1 = 1–10% of area affected; 2 = 11–25% of area affected; 3 = 26–50% of area affected; 4 = 51–75% of area affected; 5 = >75% of area affected. Scores were assigned based on tubular injury including the presence and extent of sloughed epithelial cells, debris, and hyalin casts in the tubules, and signs of tubular degeneration or dilation for tubular injury. Interstitial injury was assigned by the degree of interstitial expansion and the presence of inflammatory cells or debris. The fibrosis score was determined by the extent of blue staining in interstitial spaces. For each rat, scores from images were averaged yielding an averaged tubular injury, interstitial injury, and fibrosis score for analysis.

Transmission electron microscopy for mitochondrial imaging: Additional kidney sections were prepared for electron microscopy. Briefly, 1–2 mm sections of renal cortex were fixed overnight in freshly prepared 2% glutaraldehyde as previously described [18]. Samples were post-fixed in 2% osmium tetroxide for an hour on a rocker before dehydration in graded ethanol series. Samples were then embedded in resin and left overnight to polymerize. Sections were then trimmed and stained with uranyl acetate and lead citrate for 10 minutes. Tissues were imaged on a JEM 1400 Flash transmission electron microscope (JEOL USA, Peabody, MA, U.S.A.). Ten randomly selected proximal tubules were imaged per animal ($N = 3$), and three high-magnification images of the proximal tubule were taken for each animal to give about $n =$ 30 proximal tubule microphotographs per rat to evaluate the mitochondrial ultrastructural morphology. Mitochondrial area, circularity, density, and mitochondrial number per viewing area were measured using ImageJ software (NIH). Overall mitochondrial health scores from the microphotographs under the same viewing area were assessed on a scale of 1–5 based on density, the number of cristae, swelling, crista thickness, and the presence of spaces in between the cristae by an investigator blinded to the hypothesis and experimental groups. The scoring criteria was as follows: 1 = no changes observed, normal (healthy); 2 = 1–25% of mitochondria affected (minimal); 3 = 26–50% of mitochondria affected (moderate); 4 = 51–75% of mitochondria affected (severe); 5 = 76–100% of mitochondria affected (unhealthy).

Western blot analysis: A quantity of 30–50 mg of the whole kidney was homogenized in radioimmunoprecipitation assay buffer supplemented with protease and phosphatase inhibitors before being subjected to bicinchoninic acid assay for protein quantification as previously described [19,20]. A 35 µg of protein from each sample was resolved on 4–20% Tris-glycine-SDS gels (cat no. 4561094, Bio-Rad, Hercules, CA) before being transferred to a polyvinylidene difluoride (cat no. 1620177, Bio-Rad, Hercules, CA) membrane pre-activated with methanol. Membranes were probed with antibodies, and specific protein bands were detected using enhanced chemiluminescence (Cytiva Cat No. RPN2236) on the FlourChem-E system (cat no. 92-14860-00, Bio-Techne, Minneapolis, MN). Membranes were incubated overnight with primary antibodies to KIM-1 (cat# AF3689; 1:1000 dilution, R&D Systems, Minneapolis, MN, U.S.A.), NGAL (cat# sc-515876; 1:1000 dilution, Santa Cruz, U.S.A.), phosphorylated JNK/SAPK (cat# 9255; 1:1000 dilution, Cell Signaling Technology, U.S.A.), total JNK/SAPK (cat# 9252; 1:1000 dilution, Cell Signaling Technology, U.S.A.), phosphorylated Erk 1/2 (cat# 4370S; 1:1000 dilution, Cell Signaling Technology, U.S.A.), total Erk 1/2 (cat# 4695; 1:1000 dilution, Cell Signaling Technology, U.S.A.), phosphorylated P38 (cat# 4631; 1:1000 dilution, Cell Signaling Technology, U.S.A.), total P38 (cat# 9212; 1:1000 dilution, Cell Signaling Technology, U.S.A.), glutathione peroxidase (GPx1) (cat# ab22604; 1:1000 dilution, Abcam, U.S.A.), catalase (cat# AF3398; 1:1000 dilution, R&D Systems, Minneapolis, MN U.S.A.), SOD-2 (cat# 24127-1-AP; 1:25,000 dilution, Proteintech, U.S.A.), caspase 3 (cat# 9664; 1:1000 dilution, Cell Signaling Technology, U.S.A.), and caspase 9 (cat# 9508; 1:1000 dilution, Cell Signaling Technology, U.S.A.). The following morning, blots were washed and incubated for 1 hour with corresponding secondary antibodies including horse anti-mouse (cat# 7076; 1:1000 dilution, Cell Signaling Technology, U.S.A.), goat anti-rabbit (cat# 7074; 1:1000 dilution, Cell Signaling Technology, U.S.A.), or rabbit anti-goat (cat# HAF017; 1:1000 dilution, R&D Systems, Minneapolis, MN U.S.A.). Equal protein loading and normalization were confirmed using β-actin (cat# 4967S; 1:2000 dilution, Cell Signaling Technology, U.S.A.) or α-tubulin (cat# ab4074; 1:1000 dilution, Abcam, U.S.A.) depending on the molecular weight of the primary antibody. Band densitometry/intensity was analyzed using ImageJ software (NIH).

Immunohistochemistry (IHC) staining: Paraffin wax kidney sections were processed for IHC as previously described [21]. Briefly, sections were deparaffinized in xylene and rehydrated in graded ethanol solutions before being boiled in antigen retrieval using IHC-Tek Epitope retrieval (Cat No. NC0196883, IHC World, Ellicott City, MD) for 40 minutes and then cooled down to room temperature. Endogenous peroxidases were inactivated using 3% hydrogen peroxide for 15 minutes before being blocked in 10% horse serum in 0.1% phosphate-buffered saline with Tween (PBST) for 30 minutes. Slides were incubated

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overnight in primary antibodies to KIM-1 (cat# AF3689; 1:50 dilution, R&D Systems, Minneapolis, MN U.S.A.), NGAL (cat# sc-515876; 1:200 dilution, Santa Cruz, U.S.A.), alpha-smooth muscle actin (αSMA) (cat# M0851; 1:150 dilution, Agilent DAKO, U.S.A.), platelet-derived growth factor receptor-beta (PDGFRβ) (cat# 4564; 1:200 dilution, Cell signaling, U.S.A.), CD68 (cat# MCA1957GA; 1:200 dilution, AbD Serotec, U.S.A.), and CD3 (cat# ab-16669; 1:150 dilution, Abcam U.S.A.) in 10% horse serum in 0.1% PBST in a humidified chamber at 4°C. The next day, slides were washed in 0.1% PBST and incubated for 45 minutes in their corresponding secondary IgG horseradish peroxidase. Slides were then washed, and color was developed with diaminobenzidine (DAB; Cat No. 50-823-73, BioCare Medical; 32 μL DAB in 1.0 mL DAB substrate buffer). Slides were then dehydrated and cleared in xylene before being mounted with cytoseal medium (Cat No. 22-050-262) and imaged by an investigator blinded to the hypothesis utilizing bright-field settings using 20× magnification on a digital camera (Olympus DP12; Olympus America). The quantification of CD3+ and CD68+ cells and positive area percentage of α-SMA and PDGFRB were performed using ImageJ software (NIH).

Super oxide dismutase (SOD) activity measurement: Total SOD activity was measured on whole kidney homogenates by the SOD Assay kit (cat. no. 706002, Cayman Chemicals, Ann Arbor, MI) according to the manufacturer's instructions.

TUNEL staining: TUNEL-positive nuclei on renal tissue slides were identified according to the manufacturer's instructions using the ApopTag plus Peroxidation *in situ* Apoptosis Detection kit as previously described [16]. Slides were deparaffinized and rehydrated before a PBS wash and Proteinase K digestion for 15 minutes at room temperature. Endogenous peroxidase activity was quenched using 3% hydrogen peroxide in methanol and washed before terminal deoxynucleotidyl transferase labeling reaction at 37°C for 1 hour. Slides were washed and incubated with anti-digoxigenin peroxidase, and color development was performed using DAB peroxidase substrate solution. Methyl green was used as a counterstain before clearing in xylene and the slide covered with cytoseal medium (Cat No. 22-050-262) and a dry coverslip. An investigator blinded to the hypothesis and experimental groups quantified the number of brown nuclei indicative of TUNEL-positive nuclei cells in 10 fields acquired on 20× magnification per slide.

Statistical analysis: All data sets were expressed as mean ± SEM and analyzed using GraphPad Prism 10 (GraphPad Software Inc., La Jolla, CA). Student's unpaired *t*-test was used to analyze data between two groups after the determination of data distribution. Multiple comparisons were carried out using one-way ANOVA with Tukey post-hoc test. For all comparisons, P values < 0.05 were considered significant.

Results

Despite the return of plasma creatinine to sham levels, subclinical markers of renal injury persist post-AKI in female rats

Plasma creatinine and urinary protein were measured in female rats allowed to recover for 1, 3, 7, 14, or 30 days following 45 minutes of bilateral renal ischemia. Plasma creatinine was elevated relative to sham controls in rats recovered for 24 hours ($P = 0.004$). However, by three days post-AKI, plasma creatinine had returned to sham control levels (Figure 1A; $P = 0.9$). Similarly, urinary protein levels were greater in rats one day post-AKI compared with sham controls ($P = 0.006$) but returned to sham control levels by three days post-AKI ($P = 0.9$) (online supplementary figure S1). Of note, body weights were comparable in sham controls and 30 day post-AKI rats (260 ± 6 g vs. 249 ± 5 g; $P = 0.5$).

In contrast with plasma creatinine, plasma cystatin C levels remained elevated compared with sham controls 30 days post-AKI (Figure 1B; $P = 0.003$). Both urinary KIM-1 ($P = 0.02$) and urinary cystatin C ($P < 0.0001$) were greater one day post-AKI vs. sham; however, 30 days post-AKI, urinary excretion of KIM-1 ($P = 0.9$) and cystatin C ($P = 0.9$) was comparable with sham controls (online supplementary figure S2). Persistent injury following AKI was further confirmed by immunohistochemical and Western blot analysis, showing increased KIM-1 ($P = 0.04$) and NGAL ($P = 0.03$) levels in the 30-day post-AKI kidneys compared with sham controls (Figure 1C–F).

Tubular injury was assessed via H&E, and renal fibrosis was evaluated by staining with MT. AKI induced tubular injury starting one day post-AKI with sustained injury through day 30 compared with sham controls (Figure 2A; $P = 0.01$). Renal fibrosis was evident by seven days post-AKI with the greatest fibrosis score at day 30 compared with sham (Figure 2B; $P < 0.0002$). Fibrosis was further confirmed using Sirius red/fast green collagen staining and transdifferentiation of pericytes/fibroblasts to myofibroblasts by

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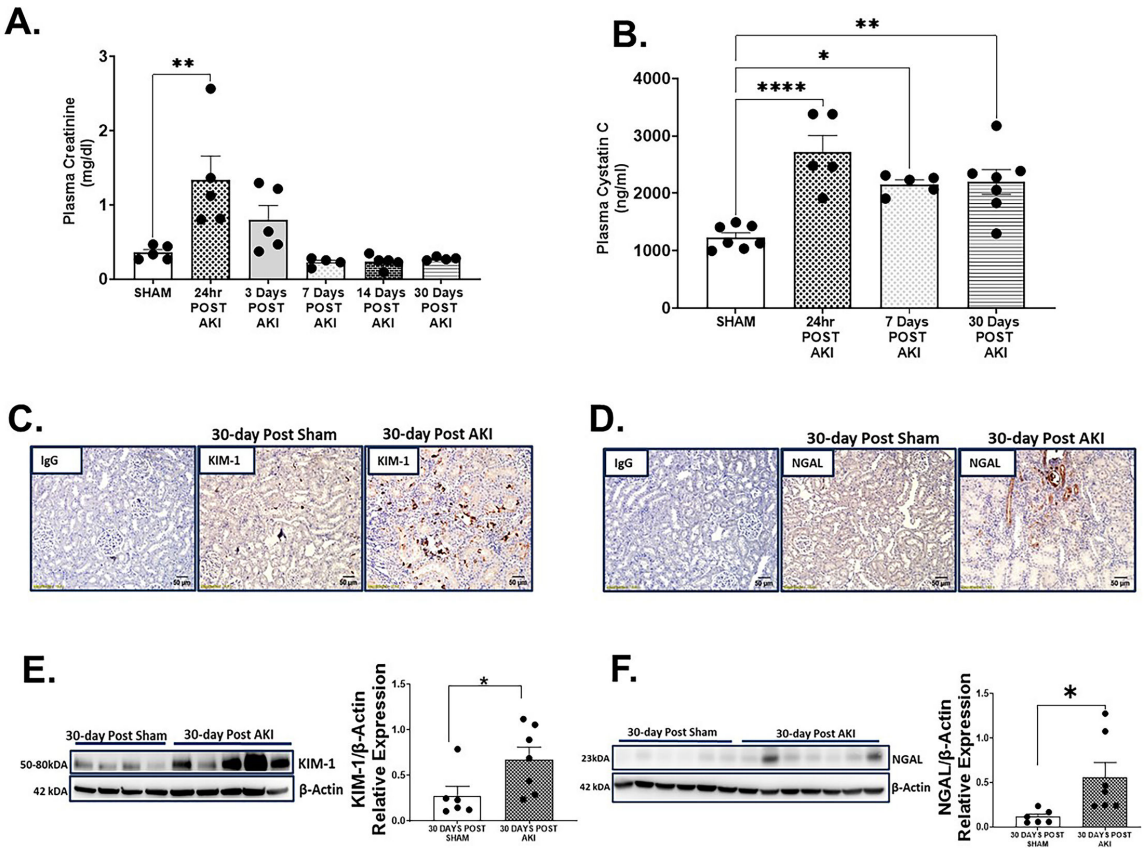

Figure 1: Female rats exhibit persistent subclinical renal injury 30 days post-AKI, despite resolution of plasma creatinine levels by three days post-AKI.

For this study, 12–13-week-old female SD rats were randomized to sham or 45-minute bilateral ischemia-reperfusion surgery (AKI) and allowed to recover 1, 3, 7, 14, or 30 days post-AKI. Plasma creatinine was measured at each timepoint (**A**), and plasma cystatin C was measured in samples collected 1, 7, and 30 days post AKI (**B**); $n = 5$–7. Data are expressed as mean ± SEM and compared via one-way ANOVA with Tukey post-hoc test for multiple comparisons, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$. KIM-1 (**C,E**) and NGAL (**D,F**) expression were visualized via immunohistochemical analysis: 20× magnification, 50 µm, and measured by Western blot analysis in rats 30 days post-sham or AKI surgery; $n = 6$–7. Data are expressed as mean ± SEM and compared via Student *t*-test, $^{*}P < 0.05$ vs. sham. AKI, acute kidney injury; SD, Sprague-Dawley.

staining for αSMA and PDGFRβ. Sirius red/fast green collagen staining showed greater collagen-positive areas in the 30-day post-AKI kidneys compared with sham controls ($P = 0.04$; online supplementary figure S3). Consistent with this, there was higher αSMA ($P < 0.0001$) and PDGFRβ ($P = 0.0033$) expression in kidneys 30 days post-AKI compared with sham controls (online supplementary figure S4).

Female rats with a history of AKI develop mitochondrial ultrastructural anomalies

Mitochondria are crucial for renal recovery post-AKI, with mitochondria dysfunction playing a central role in injury and repair [22]. We investigated the impact of AKI on renal mitochondrial ultrastructure via analysis of transmission electron microscope (TEM) images of kidneys from female rats 30 days post-sham or AKI surgery. Mitochondrial morphology was quantified by measuring the mitochondrial number, circularity, area, matrix density, and overall health score in proximal tubule mitochondria, as previously described [18,23]. Rats with a history of AKI exhibited mitochondrial ultrastructural anomalies compared with sham controls (Figure 3A). Higher magnification images of the proximal tubule revealed that 30 days post-AKI, female rats exhibited smaller mitochondrial areas (Figure 3B; $P = 0.04$), greater mitochondrial circularity (Figure 3C; $P = 0.009$), lower mitochondria numbers (Figure 3D; $P < 0.0001$), and lower mitochondrial density (Figure 3E; $P < 0.0001$) compared with sham controls. Scoring mitochondria for overall health score revealed that recovered AKI rats had worse mitochondrial health scores compared with sham controls (Figure 3F; $P < 0.0001$).

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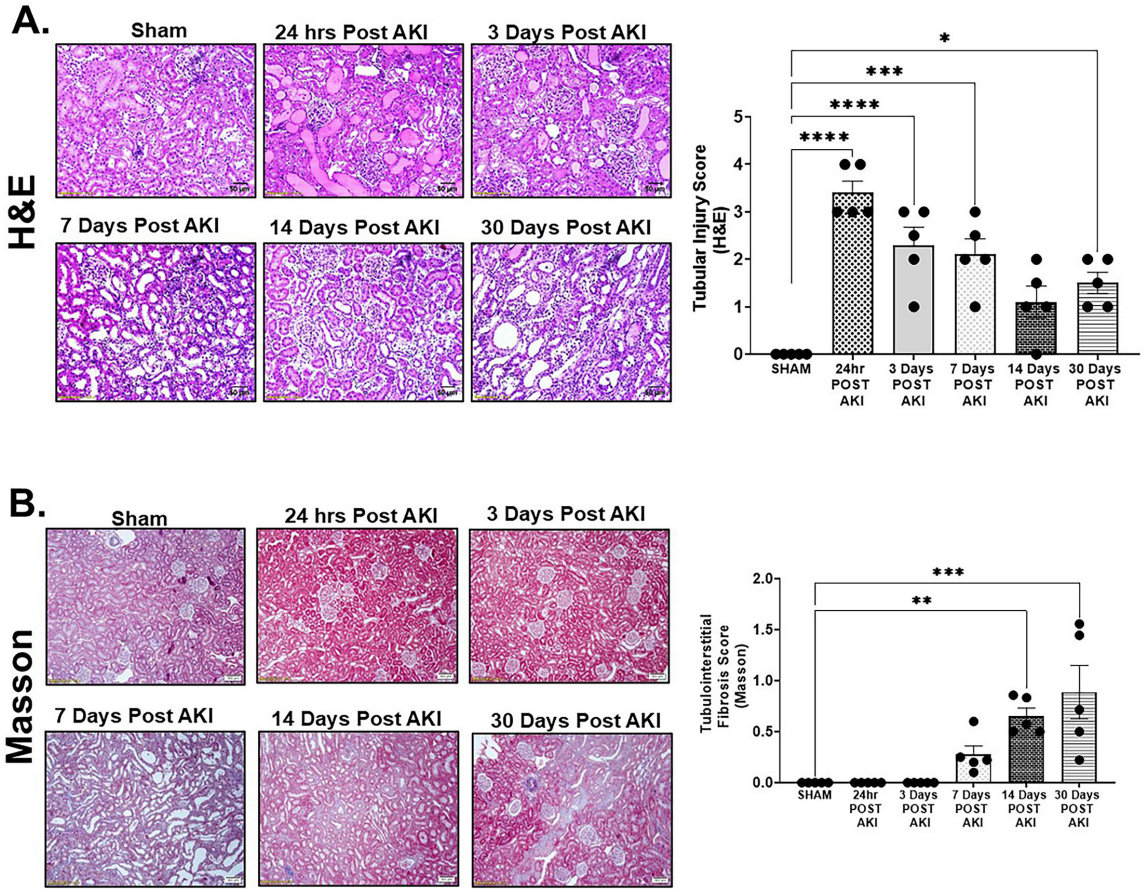

Figure 2: AKI induces sustained tubular injury and fibrosis in young female rats.

Renal histology was evaluated using H&E (**A**) and renal fibrosis was assessed via Masson's Trichrome (**B**) in 12–13-week old female SD rats randomized to sham or 45-minute bilateral ischemia-reperfusion surgery (AKI) and allowed to recover 1, 3, 7, 14, or 30 days post-AKI; $n = 5$. Shown are representative images with data expressed as mean ± SEM and compared via one-way ANOVA with Tukey post-hoc test for multiple comparisons, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ vs. sham. AKI, acute kidney injury; H&E, hematoxylin and eosin; SD, Sprague-Dawley.

Female rats with a history of AKI exhibit greater renal markers of reactive oxygen species activation

Increased oxidative stress and the production of reactive oxygen species (ROS) result in the activation of mitogen-activated protein kinases (MAPKs) ERKs, JNKs, and/or P38 [24]. We measured the protein expression of p-JNK/SAPK, p-ERK1/2, and p-P38 in whole kidney homogenates via Western blot analysis as indirect markers of ROS. As depicted in Figure 4A, rats with a history of AKI had higher expression of p-JNK/SAPK ($P = 0.049$) compared with sham controls. Neither p-ERK1/2 (Figure 4B; $P = 0.1321$) nor p-P38 (Figure 4C; $P = 0.1118$) was affected by a history of AKI.

Next, we examined protein levels and the activity of key antioxidant enzymes to determine whether a history of AKI altered antioxidant capability. GPx1, catalase, and SOD2 protein levels were measured by Western blot in whole kidney homogenates in female rats 30 days following sham or AKI surgery. Protein levels of GPx1 (Figure 5A; $P = 0.01$), catalase (Figure 5B; $P = 0.005$), and SOD2 (Figure 5C; $P = 0.058$) were lower in AKI compared with sham control rats. Despite lower SOD2 protein expression, total SOD activity was not different in AKI and sham rats 30 days post-surgery (Figure 5D; $P = 0.5$).

AKI induces renal apoptosis and inflammation in female rats with a history of AKI

To investigate the impact of a history of AKI in female rats on apoptotic cell death, renal levels of apoptotic-related markers caspase 9 and cleaved caspase-3 were measured via Western blotting and

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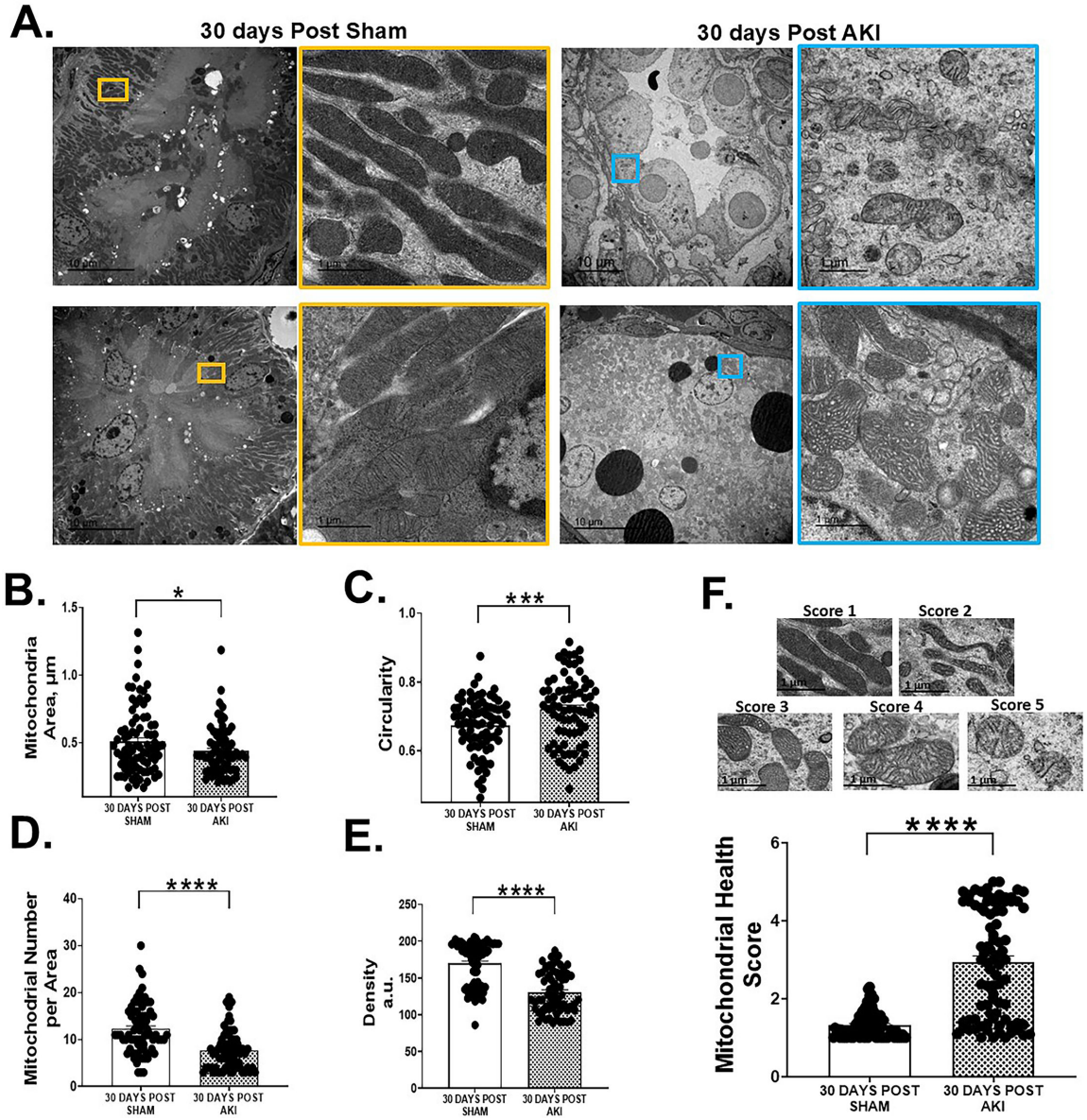

Figure 3: AKI female rats exhibit mitochondrial ultrastructural anomalies compared with sham controls 30 days post-AKI.

For this study, 12–13-week-old female SD rats were randomized to sham or 45-minute bilateral ischemia-reperfusion surgery (AKI) and allowed to recover 30 days post-AKI; $n = 3$ rats, $n = 75–85$ images analyzed/rat with each data point representing an average for all mitochondria analyzed. Shown are representative EM microphotographs (**A**), mitochondrial area (**B**), mitochondrial circularity (**C**), mitochondrial numbers (**D**), mitochondrial density (**E**), and mitochondrial health scores (**F**). Data are expressed as mean ± SEM and compared via a Student's unpaired *t*-test, $^{*}P < 0.05$, $^{***}P < 0.001$, $^{****}P < 0.0001$ vs. sham. AKI, acute kidney injury; SD, Sprague-Dawley.

TUNEL staining 30 days post-sham or AKI surgery. Rats with a history of AKI had higher protein levels of cleaved caspase-3 (Figure 6A; $P = 0.04$), caspase 9 (Figure 6B; $P = 0.0006$), and more TUNEL-positive cells compared with sham controls (Figure 6C; $P = 0.04$). Inflammation was assessed by staining 30-day post-AKI and sham kidneys for CD3 (pan T cell marker) and CD68 (pan macrophage marker). There was an increased renal infiltration of CD3 ($P = 0.0001$) and CD68 ($P < 0.0001$) in the 30-day post-AKI rats compared with sham controls (online supplementary figure S5).

Discussion

In the present study, the main finding is that despite evidence of renal functional recovery, as assessed by plasma creatinine levels, subclinical injury persists in young female rats following AKI. Thirty days

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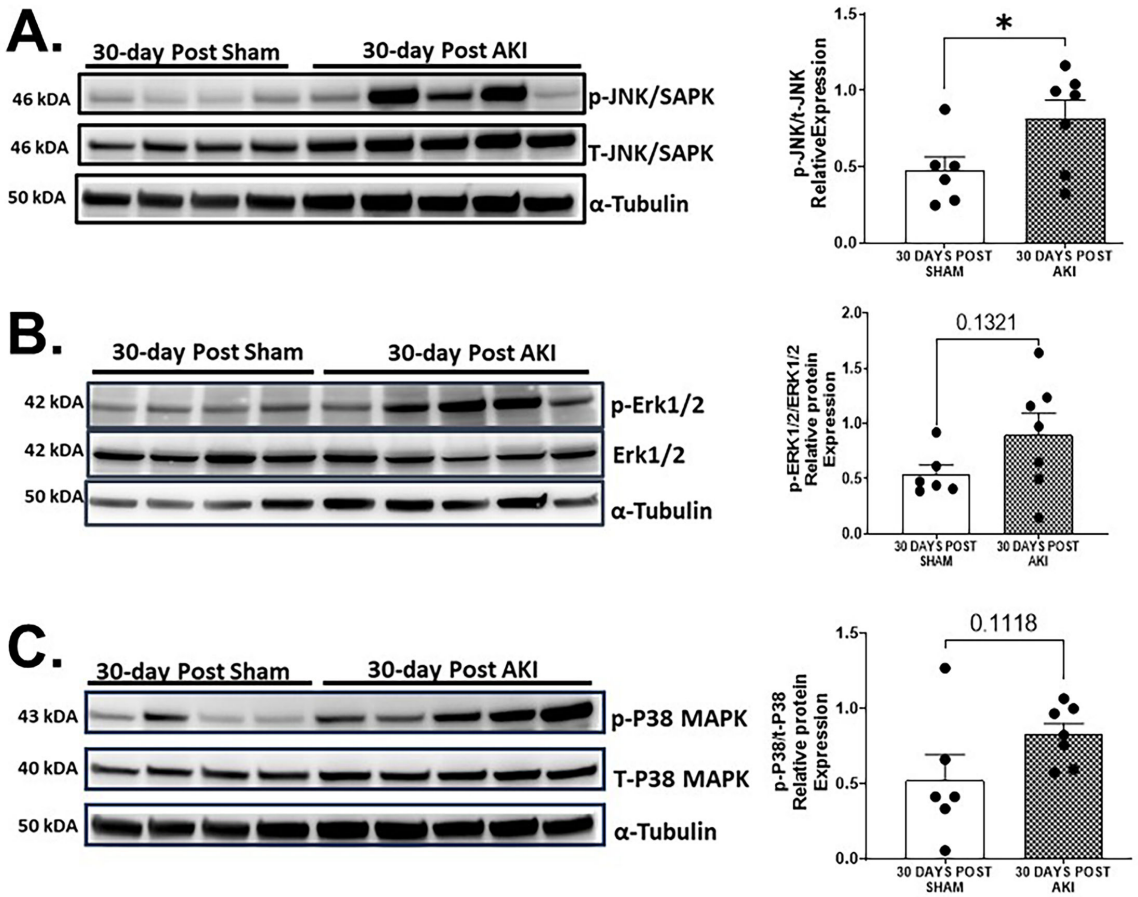

Figure 4: Female rats with a history of AKI have greater renal protein levels of MAPKs, markers of oxidative stress than sham controls.

Protein levels of p-JNK/SAPK, p-ERK1/2, and p-P38 were measured via Western blot analysis in whole kidney homogenates from 12- to 13-week-old female SD rats randomized to sham or 45-minute bilateral ischemia-reperfusion surgery (AKI) and allowed to recover 30 days post-AKI; $n = 6–7$. Representative images and densitometric analysis of protein expression are shown for p-JNK/SAPK (**A**), p-ERK1/2 (**B**), and p-P38 (**C**). Data are expressed as mean ± SEM and compared via Student's unpaired *t*-test, $*P < 0.05$ vs. sham. AKI, acute kidney injury; MAPKs, mitogen-activated protein kinases; SD, Sprague-Dawley.

following a single renal ischemic insult, renal injury markers such as KIM-1, NGAL, and cystatin C remain elevated above sham controls with evidence of increases in oxidative stress and mitochondrial dysfunction. This study has important implications for reproductive-aged women who have experienced AKI and who demonstrate clinical recovery, as assessed by the KDIGO guidelines. Our data indicate that a return of plasma creatinine to normal levels is insufficient to indicate complete recovery as it is not a reflective measure of underlying renal injury.

The current diagnosis of AKI relies on increases in serum creatinine levels or a reduction in urine output, assessed over defined time points according to the KDIGO criteria [25,26]. Multiple reports show that serum creatinine is not sensitive enough to detect small changes in renal function and, therefore, is not a sensitive measure of renal injury [27]. As a result, there remains interest in 'subclinical' AKI, which is defined as kidney damage without loss of kidney function or any clinical manifestation [28,29]. KIM-1, NGAL, and cystatin-C have emerged as key markers of subclinical AKI [29,30]. However, these are not routinely assessed clinically in patients post-AKI. KIM-1 is a transmembrane glycoprotein up-regulated following AKI, making KIM-1 a sensitive indicator of renal tubular injury [30]. Similarly, NGAL (lipocalin-2) is released by damaged distal tubules following tubular injury and is a good predictor of initial renal injury [31,32]. Cystatin C, produced by all nucleated cells, is freely filtered by the glomerulus and reabsorbed/metabolized by proximal tubular cells, with elevated levels indicating tubular injury [32,33]. Our study is the first to show KIM1/NGAL/cystatin C elevation in 'recovered' female rats 30 days after AKI, confirming persistent subclinical renal injury despite normal plasma creatinine levels. These data collectively support the hypothesis that female rats, similar to males [34], develop subclinical renal injury

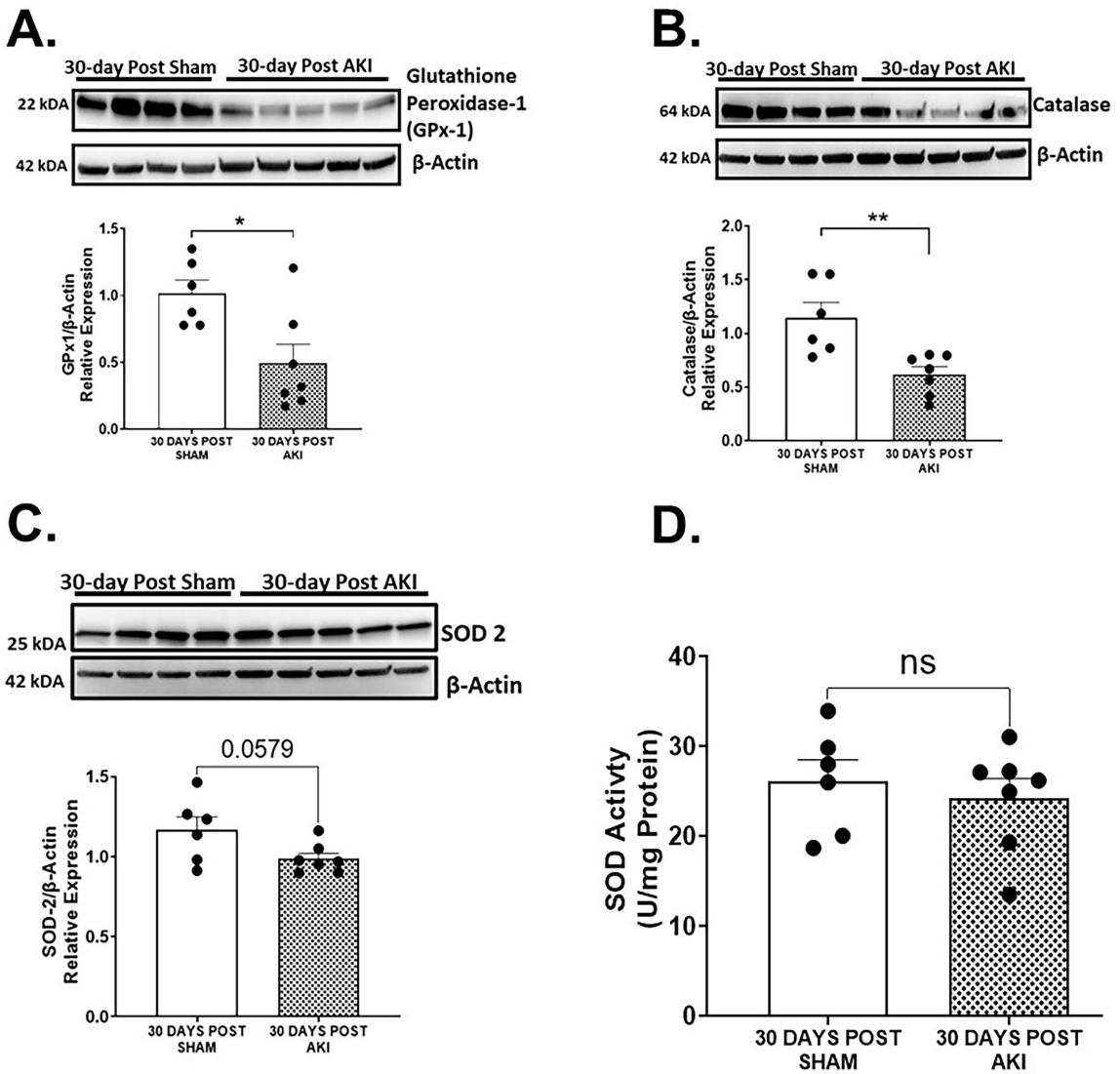

Figure 5: Female rats with a history of AKI have less GPx1 and catalase protein levels vs. sham controls.

Antioxidant enzyme protein levels were measured via Western blot analysis in whole kidney protein homogenates from 12- to 13-week-old female SD rats randomized to sham or 45-minute bilateral ischemia-reperfusion surgery (AKI) and allowed to recover 30 days post-AKI; $n = 6–7$. Representative images and densitometric analysis of protein expression are shown show for GPx1 (**A**), catalase (**B**), and SOD2 (**C**). Total SOD activity was measured via SOD Assay kit according to the manufacturer's instructions (**D**). Data are expressed as mean ± SEM and compared via a Student's unpaired *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$ vs. sham. AKI, acute kidney injury; GPx1, glutathione peroxidase; SD, Sprague-Dawley; SOD, super oxide dismutase.

progressively and support the need for clinical verification of these markers in young women to understand the long-lasting implications of AKI better.

Our study also provides evidence of the development of mild fibrosis 14 days post-AKI, which could be critical to the transition to CKD in females. Our data show features of chronic injury such as tubular dilation, hyalin casts, and inflammatory cell infiltration in kidney sections from females starting three days post-AKI and worsening by day 30. AKI to CKD transition following IR injury has extensively been studied in male rodent models [35–44]. The reliance on the use of male rodent models in the study of AKI to CKD transition is probably based on the finding that females are less susceptible to AKI vs. males [7,42,45]. However, ~40% of AKI patients are women [5], and CKD is more prevalent in women compared with men [9,10,46]; therefore, female subjects need to be included more in AKI-to-CKD research studies. Consistent with our findings, others have shown evidence of fibrosis one month post-AKI in female rats [47], yet more studies are needed to mechanistically assess AKI injury and recovery in female subjects.

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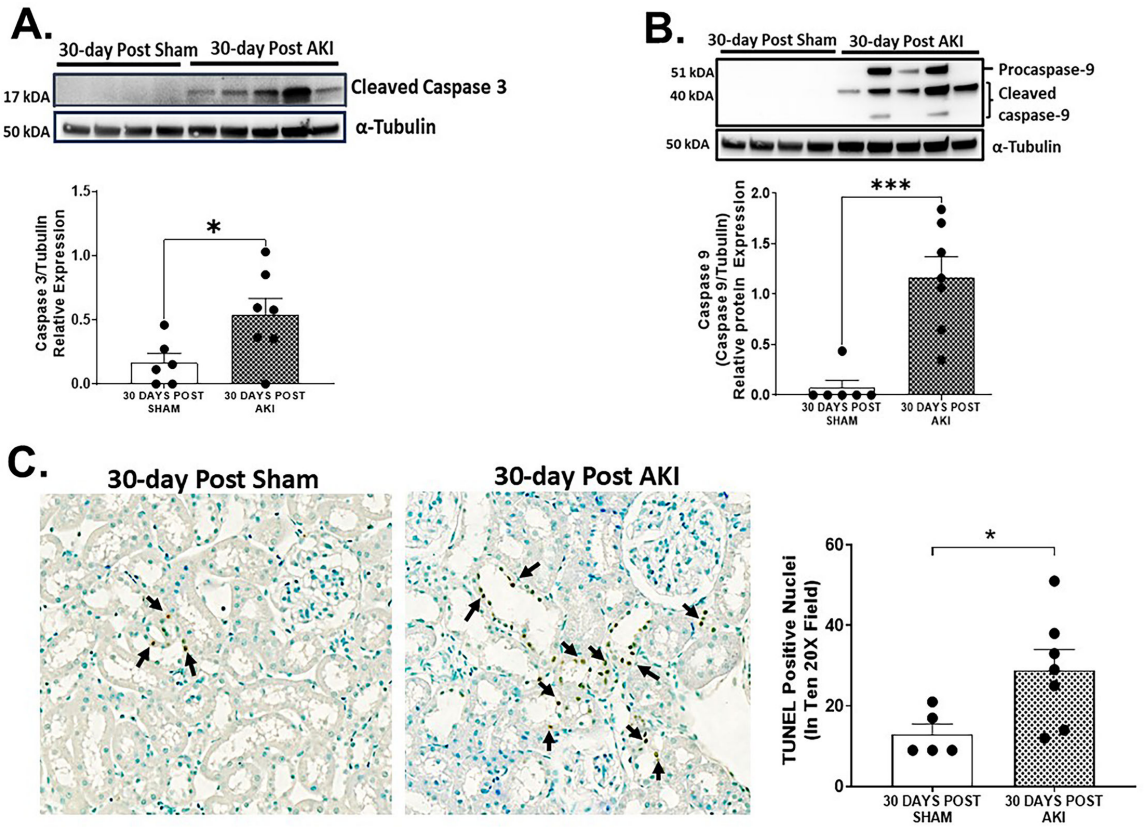

Figure 6: AKI induces renal apoptosis in female rats.

Markers of renal cell death were measured via Western blot analysis in whole kidney protein homogenates from 12- to 13-week-old female SD rats randomized to sham or 45-minute bilateral ischemia-reperfusion surgery (AKI) and allowed to recover 30 days post-AKI; $n = 6–7$. Representative images and densitometric analysis of protein expression are shown for cleaved caspase-3 (**A**) and caspase-9 (**B**). TUNEL-positive nuclei were assessed using the ApopTag plus Peroxidation *in situ* Apoptosis Detection kit (**C**). Shown are representative images and mean data. Data are expressed as mean ± SEM and compared via a Student's unpaired *t*-test, $^{*}P < 0.05$, $^{***}P < 0.001$ vs. sham. AKI, acute kidney injury; SD, Sprague-Dawley.

Proximal tubule cells have a high number of mitochondria [48,49] which are critical to the recovery of renal function post-AKI [22,50]. Indeed, mitochondrial dysfunction is central in the injury and repair process of AKI [22,51,52], and mitochondrial morphology is highly dynamic to allow adaptations to energetic demands [53,54]. Interestingly, we report for the first time that renal mitochondria remain impaired at least 30 days post-AKI in females. Consistent with our findings, male mice have lower mitochondria numbers, more circular mitochondria profiles, smaller mitochondria area, and more degenerate mitochondria post-AKI compared with healthy male controls [55,56]. While most studies designed to increase our understanding of mitochondrial involvement in AKI have been in male subjects [57–66], our data show that mitochondria are also impaired following AKI in females. Therefore, the potential to target mitochondria therapeutically may improve recovery following AKI in both males and females. Future studies will determine the contribution of mitochondria to IR injury and repair in females.

Closely related to mitochondrial dysfunction and an essential mediator of renal dysfunction is oxidative stress [67]. Very few AKI studies have focused on females in the literature; however, many AKI studies in males show that mitochondrial dysfunction following AKI is a potential source of oxidative stress [68–72]. Here, we report evidence of oxidative stress in female rats following AKI. It is worth noting that we observed no change in total SOD activity despite lower SOD2 protein expression levels. It is possible that despite lower SOD2 protein levels, total SOD activity may remain unchanged due to compensation from other SOD isoforms [73]. In the present study, we focused on MAPKs as indirect markers of oxidative stress but did not directly measure levels of ROS to identify the source of ROS. The pathophysiology of AKI is complex and multifactorial [74] potentially involving various pathways, including the immune system, all of which may contribute to ROS production [75]. Future studies will be designed to identify the source

ROS and assess the contribution of oxidative stress to persistent IR injury in females to determine whether antioxidants or ROS-sequestering agents would benefit females post-AKI.

In addition to subclinical renal injury, we also show evidence of apoptotic cell death in young females following AKI, suggesting maladaptive repair processes. Recovery from AKI is characterized, in part, by aberrant cell death processes, including apoptosis [76]. As shown in males [16,77–79], we show that female rats post-AKI have greater apoptotic markers (caspase 9, caspase 3, and TUNEL + cells) compared with sham controls. The current study focused on apoptotic cell death following AKI, yet many cell death modalities have been reported in AKI [80]. Whether other forms of cell death are present, or which cell death pathways predominate could be important in understanding injury in females post-AKI. Future studies will be designed to interrogate other forms of cell death and whether cell death can be targeted to limit AKI in females.

Our study provides important translational insights to human studies where women with a history of AKI present with higher rates of adverse maternal and fetal outcomes during pregnancy, despite normal pre-pregnancy serum creatinine levels [12,81]. We have previously shown that creatinine levels are not different in female rats 30 days after sham or AKI surgery before conception [14], suggesting 'recovery' before pregnancy. Here, we provide the first evidence of subclinical renal injury 30 days post-AKI in female rats. During pregnancy, renal demands are increased [82,83], and the present work extends our findings to show that subclinical renal injury persists in 'recovered' AKI female kidneys, meaning they will enter pregnancy with lower renal thresholds compared with healthy kidneys. The existence of subclinical renal injury before pregnancy could potentially mediate the adverse pregnancy outcomes in women with a history of clinically recovered AKI [12,13]. Therefore, young females of childbearing age with a history of AKI need careful monitoring and may require additional supportive care during pregnancy. Our data support a need to measure subclinical renal injury markers in addition to the traditional renal function markers in the assessment of baseline renal health in women before pregnancy, especially those who have had a history of AKI.

There are some considerations with our study. While not the focus of this study, we have previously shown that female SD rats are protected from IR injury relative to males. Using a variety of renal injury markers, including plasma creatinine, blood urea nitrogen, and urine protein-to-creatinine ratio, we demonstrated that male SD rats exhibit greater IR-induced injury compared with female SD rats, one day post-AKI [19,21]. Our previous findings are consistent with others, suggesting that females are protected from IR injury compared with males [84–86]. However, both males and females develop injury post-AKI, and understanding the mechanisms driving injury in both sexes is important. Sex differences in IR-induced injury have been suggested to be linked to hormones, as estrogen has been shown to be renoprotective [87,88]. Of note, in the present study, female rats were randomly assigned to sham or AKI groups without an assessment of hormones or stage of the cycle. Future studies will assess the impact of female sex hormones using surgical gonadectomy. Moreover, the 45-minute IR in female rats results in moderate injury and the scope of this study was limited to 30 days. Therefore, the ability to make conclusions regarding the 'long-term' consequences of AKI on overall health needs to be cautioned. It is possible that with a more extended recovery period, indicators of subclinical injury in females will resolve.

In conclusion, our study provides the first evidence of subclinical renal injury, mitochondrial dysfunction, and imbalances in ROS/oxidative stress in young female rats post-AKI. With the rising numbers of women with a history of AKI, persistent subclinical renal injury could be responsible for adverse outcomes later in life. Therefore, future studies targeting these pathways could potentially be efficient strategies to limit these adverse outcomes associated with persistent subclinical renal injury, especially in females.

Clinical perspectives

- Acute kidney injury (AKI) incidence continues to rise in both men and women. Following AKI, females show a quicker normalization of creatinine levels. However, it remains unclear whether subclinical injury persists.
- Despite creatinine levels returning to a normal range, subclinical injury persists following AKI in young females. Mitochondrial health, renal morphology, and oxidative stress are all impaired in females with histories of AKI in young females.

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- Taken together, our findings suggest that targeting the mechanisms mediating subclinical injury could offer potential therapeutic strategies to address the adverse outcomes associated with a history of AKI in young females.

Data Availability

All relevant data for this study are included in the main manuscript file or supplement.

Competing Interests

The authors declare no conflicts of interest associated with the manuscript.

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CRediT Author Contribution

DM: Writing - original draft and Data curation, HG: Data curation, VA: Writing - original draft and Data curation, EM: Data curation, ME: Data curation, GC: Data curation, RM: Data curation, SO: Data curation, EG: Conceptualization, JLF: Writing - review & editing, Conceptualization, and Data curation, JCS: Writing - review & editing and Conceptualization.

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Abbreviations

AKI, acute kidney injury; GPx1, glutathione peroxidase; H&E, hematoxylin and eosin; MAPKs, mitogen-activated protein kinases; PBST, phosphate-buffered saline with Tween; ROS, reactive oxygen species; SD, Sprague-Dawley; SOD, super oxide dismutase.

References

1 Yaqub, S., Hashmi, S., Kazmi, M.K., Aziz Ali, A., Dawood, T. and Sharif, H. (2022) A comparison of AKIN, KDIGO, and RIFLE definitions to diagnose acute kidney injury and predict the outcomes after cardiac surgery in a South Asian cohort. *Cardiorenal Med.* **12**, 29–38 https://doi.org/10.1159/000523828
2 Lewington, A.J.P., Cerdá, J. and Mehta, R.L. (2013) Raising awareness of acute kidney injury: a global perspective of a silent killer. *Kidney Int.* **84**, 457–467 https://doi.org/10.1038/ki.2013.153
3 Chawla, L.S., Eggers, P.W., Star, R.A. and Kimmel, P.L. (2014) Acute kidney injury and chronic kidney disease as interconnected syndromes. *N. Engl. J. Med.* **371**, 58–66 https://doi.org/10.1056/NEJMra1214243
4 Ide, S., Ide, K., Abe, K., Kobayashi, Y., Kitai, H., McKey, J, et al. (2022) Sex differences in resilience to ferroptosis underlie sexual dimorphism in kidney injury and repair. *Cell Rep.* **41**, 111610 https://doi.org/10.1016/j.celrep.2022.111610
5 Güzel, C., Yeşiltaş, S., Daşkaya, H., Uysal, H., Sümer, I. and Türkay, M. (2019) The effect of gender on acute kidney injury developing in the intensive care unit. *Hippokratia* **23**, 126–130
6 Taylor, C.M., Pritschet, L. and Jacobs, E.G. (2021) The scientific body of knowledge - whose body does it serve? A spotlight on oral contraceptives and women's health factors in neuroimaging. *Front. Neuroendocrinol.* **60**, 100874 https://doi.org/10.1016/j.yfrne.2020.100874
7 Curtis, L.M. (2024) Sex and gender differences in AKI. *Kidney360* **5**, 160–167 https://doi.org/10.34067/KID.0000000000000321
8 Vosters, T.G., Kingma, F.M., Stel, V.S., Van der Born, B.H., Huisman, B.J.M.V., Van Ittersum, F.J, et al. (2024) Sex differences in CKD risk factors across ethnic groups. *Nephrol. Dial. Transplant.* **39**, 1194–1197 https://doi.org/10.1093/ndt/gfae038
9 Lewandowski, M.J., Krenn, S., Kurnikowski, A., Bretschneider, P., Sattler, M., Schwaiger, E. et al. (2023) Chronic kidney disease is more prevalent among women but more men than women are under nephrological care: analysis from six outpatient clinics in Austria 2019. *Wien. Klin. Wochenschr.* **135**, 89–96 https://doi.org/10.1007/s00508-022-02074-3
10 Melsom, T., Norvik, J.V., Enoksen, I.T., Stefansson, V., Mathisen, U.D., Fuskevåg, O.M. et al. (2022) Sex differences in age-related loss of kidney function. *J. Am. Soc. Nephrol.* **33**, 1891–1902 https://doi.org/10.1681/ASN.2022030323
11 Regitz-Zagrosek, V. (2012) Sex and gender differences in health. Science & society series on sex and science. *EMBO Rep.* **13**, 596–603 https://doi.org/10.1038/embor.2012.87

12 Tangren, J.S., Wan Md Adnan, W.A.H., Powe, C.E., Ecker, J., Bramham, K., Hladunewich, M.A. et al. (2018) Risk of preeclampsia and pregnancy complications in women with a history of acute kidney injury. *Hypertension* **72**, 451–459 https://doi.org/10.1161/HYPERTENSIONAHA.118.11161
13 Tangren, J.S., Powe, C.E., Ankers, E., Ecker, J., Bramham, K., Hladunewich, M.A. et al. (2017) Pregnancy outcomes after clinical recovery from AKI. *J. Am. Soc. Nephrol.* **28**, 1566–1574 https://doi.org/10.1681/ASN.2016070806
14 Gillis, E.E., Brands, M.W. and Sullivan, J.C. (2021) Adverse maternal and fetal outcomes in a novel experimental model of pregnancy after recovery from renal ischemia-reperfusion injury. *J. Am. Soc. Nephrol.* **32**, 375–384 https://doi.org/10.1681/ASN.2020020127
15 Boutin, L., Latosinska, A., Mischak, H., Deniau, B., Asakage, A., Legrand, M. et al. (2023) Subclinical and clinical acute kidney injury share similar urinary peptide signatures and prognosis. *Intensive Care Med.* **49**, 1191–1202 https://doi.org/10.1007/s00134-023-07198-2
16 Mohamed, R. and Sullivan, JC. (2023) Sustained activation of 12/15 lipoxygenase (12/15 LOX) contributes to impaired renal recovery post ischemic injury in male SHR compared to females. *Mol. Med.* **29**, 163 https://doi.org/10.1186/s10020-023-00762-y
17 Crislip, G.R., O'Connor, P.M., Wei, Q. and Sullivan, JC. (2017) Vasa recta pericyte density is negatively associated with vascular congestion in the renal medulla following ischemia reperfusion in rats. *Am. J. Physiol. Renal Physiol.* **313**, F1097–F1105 https://doi.org/10.1152/ajprenal.00261.2017
18 Domondon, M., Polina, I., Nikiforova, A.B., Sultanova, R.F., Kruger, C., Vasileva, V.Y. et al. (2019) Renal glomerular mitochondria function in salt-sensitive hypertension. *Front. Physiol.* **10**, 1588 https://doi.org/10.3389/fphys.2019.01588
19 Mohamed, R., Crislip, G.R., McLarnon, S., Wei, Q., O'Connor, P.M. and Sullivan, J.C. (2022) Persistent vascular congestion in male spontaneously hypertensive rats contributes to delayed recovery of renal function following renal ischemia perfusion compared with females. *Clin. Sci. (Lond)* **136**, 825–840 https://doi.org/10.1042/CS20220002
20 Moronge, D., Ayulo, V., Elgazzaz, M., Mellott, E., Ogbi, S. and Faulkner, J.L. (2024) Both endothelial mineralocorticoid receptor expression and hyperleptinemia are required for clinical characteristics of placental ischemia in mice. *Am. J. Physiol. Heart Circ. Physiol.* **327**, H118–H130 https://doi.org/10.1152/ajpheart.00188.2024
21 Crislip, G.R., Patel, B., Mohamed, R., Ray, S.C., Wei, Q., Sun, J. et al. (2020) Ultrasound measurement of change in kidney volume is a sensitive indicator of severity of renal parenchymal injury. *Am. J. Physiol. Renal Physiol.* **319**, F447–F457 https://doi.org/10.1152/ajprenal.00221.2020
22 Zhang, L., Miao, M., Xu, X., Bai, M., Wu, M. and Zhang, A. (2023) From physiology to pathology: the role of mitochondria in acute kidney injuries and chronic kidney diseases. *Kidney Dis. (Basel).* **9**, 342–357 https://doi.org/10.1159/000530485
23 McCrimmon, A., Cahill, K.M., Kruger, C., Mangelli, M.E., Bouffard, E., Dobroski, T. et al. (2022) Intact mitochondrial substrate efflux is essential for prevention of tubular injury in a sex-dependent manner. *JCI Insight* **7**, e150696 https://doi.org/10.1172/jci.insight.150696
24 Son, Y., Cheong, Y.K., Kim, N.H., Chung, H.T., Kang, D.G. and Pae, H.O. (2011) Mitogen-activated protein kinases and reactive oxygen species: how can ROS activate MAPK pathways? *J. Signal Transduct.* **2011**, 792639 https://doi.org/10.1155/2011/792639
25 Saygili, S., Canpolat, N., Cicek, R.Y., Agbas, A., Yilmaz, E.K., Sakalli, A.A.K. et al. (2023) Clinical and subclinical acute kidney injury in children with mild-to-moderate COVID-19. *Pediatr. Res.* **93**, 654–660 https://doi.org/10.1038/s41390-022-02124-6
26 Ivica, J., Sanmugalingham, G. and Selvaratnam, R. (2022) Alerting to acute kidney injury - challenges, benefits, and strategies. *Pract. Lab. Med.* **30**, e00270 https://doi.org/10.1016/j.plabm.2022.e00270
27 Heinzl, M.W., Resl, M., Klammer, C., Fellinger, P., Schinagl, L., Obendorf, F. et al. (2022) Subclinical kidney injury is caused by a moderate single inflammatory event. *Shock* **58**, 14–19 https://doi.org/10.1097/SHK.0000000000001942
28 Turgut, F., Awad, A.S. and Abdel-Rahman, E.M. (2023) Acute kidney injury: medical causes and pathogenesis. *J. Clin. Med.* **12**, 375 https://doi.org/10.3390/jcm12010375
29 Zarbock, A., Koyner, J.L., Hoste, E.A.J. and Kellum, J.A. (2018) Update on perioperative acute kidney injury. *Anesth. Analg.* **127**, 1236–1245 https://doi.org/10.1213/ANE.0000000000003741
30 Bonventre, J.V. (2008) Kidney injury molecule-1 (KIM-1): a specific and sensitive biomarker of kidney injury. *Scand. J. Clin. Lab. Invest. Suppl.* **241**, 78–83 https://doi.org/10.1080/00365510802145059
31 Albert, C., Zapf, A., Haase, M., Röver, C., Pickering, J.W., Albert, A, et al. (2020) Neutrophil gelatinase-associated lipocalin measured on clinical laboratory platforms for the prediction of acute kidney injury and the associated need for dialysis therapy: a systematic review and meta-analysis. *Am. J. Kidney Dis.* **76**, 826–841 https://doi.org/10.1053/j.ajkd.2020.05.015
32 Antonelli, A., Allinovi, M., Cocci, A., Russo, G.I., Schiavina, R., Rocco, B, et al. (2020) The predictive role of biomarkers for the detection of acute kidney injury after partial or radical nephrectomy: a systematic review of the literature. *Eur. Urol. Focus* **6**, 344–353 https://doi.org/10.1016/j.euf.2018.09.020
33 Zou, C., Wang, C. and Lu, L. (2022) Advances in the study of subclinical AKI biomarkers. *Front. Physiol.* **13**, 960059 https://doi.org/10.3389/fphys.2022.960059
34 Dong, Y., Zhang, Q., Wen, J., Chen, T., He, L., Wang, Y. et al. (2019) Ischemic duration and frequency determines AKI-to-CKD progression monitored by dynamic changes of tubular biomarkers in IRI mice. *Front. Physiol.* **10**, 153 https://doi.org/10.3389/fphys.2019.00153
35 Rodríguez-Romo, R., Benítez, K., Barrera-Chimal, J., Pérez-Villalva, R., Gómez, A., Aguilar-León, D. et al. (2016) AT1 receptor antagonism before ischemia prevents the transition of acute kidney injury to chronic kidney disease. *Kidney Int.* **89**, 363–373 https://doi.org/10.1038/ki.2015.320
36 Barrera-Chimal, J., Pérez-Villalva, R., Rodríguez-Romo, R., Reyna, J., Uribe, N., Gamba, G. et al. (2013) Spironolactone prevents chronic kidney disease caused by ischemic acute kidney injury. *Kidney Int.* **83**, 93–103 https://doi.org/10.1038/ki.2012.352
37 Barrera-Chimal, J., Perez-Villalva, R., Ortega, J.A., Sánchez, A., Rodríguez-Romo, R., Durand, M, et al. (2015) Mild ischemic injury leads to long-term alterations in the kidney: amelioration by spironolactone administration. *Int. J. Biol. Sci.* **11**, 892–900 https://doi.org/10.7150/ijbs.11729
38 Basile, D.P., Donohoe, D., Roethe, K. and Osborn, J.L. (2001) Renal ischemic injury results in permanent damage to peritubular capillaries and influences long-term function. *Am. J. Physiol. Renal Physiol.* **281**, F887–F899 https://doi.org/10.1152/ajprenal.2001.281.5.F887
39 Yang, L., Besschetnova, T.Y., Brooks, C.R., Shah, J.V. and Bonventre, JV. (2010) Epithelial cell cycle arrest in G2/M mediates kidney fibrosis after injury. *Nat. Med.* **16**, 535–543, https://doi.org/10.1038/nm.2144
40 Gong, S., Zhang, A., Yao, M., Xin, W., Guan, X., Qin, S. et al. (2023) REST contributes to AKI-to-CKD transition through inducing ferroptosis in renal tubular epithelial cells. *JCI Insight* **8**, e166001 https://doi.org/10.1172/jci.insight.166001

41 Matsushita, K., Saritas, T., Eiwaz, M.B., McClellan, N., Coe, I., Zhu, W, et al. (2020) The acute kidney injury to chronic kidney disease transition in a mouse model of acute cardiorenal syndrome emphasizes the role of inflammation. *Kidney Int.* **97**, 95–105 https://doi.org/10.1016/j.kint.2019.06.022
42 Xu, L., Guo, J., Moledina, D.G. and Cantley, L.G. (2022) Immune-mediated tubule atrophy promotes acute kidney injury to chronic kidney disease transition. *Nat. Commun.* **13**, 4892 https://doi.org/10.1038/s41467-022-32634-0
43 Xiao, L., Zhou, D., Tan, R.J., Fu, H., Zhou, L., Hou, F.F. et al. (2016) Sustained activation of Wnt/β-catenin signaling drives AKI to CKD progression. *J. Am. Soc. Nephrol.* **27**, 1727–1740 https://doi.org/10.1681/ASN.2015040449
44 Hörbelt, M., Lee, S.-Y., Mang, H.E., Knipe, N.L., Sado, Y., Kribben, A. et al. (2007) Acute and chronic microvascular alterations in a mouse model of ischemic acute kidney injury. *Am. J. Physiol. Renal Physiol.* **293**, F688–F695 https://doi.org/10.1152/ajprenal.00452.2006
45 Park, K.M., Kim, J.I., Ahn, Y., Bonventre, A.J. and Bonventre, JV. (2004) Testosterone is responsible for enhanced susceptibility of males to ischemic renal injury. *J. Biol. Chem.* **279**, 52282–52292 https://doi.org/10.1074/jbc.M407629200
46 Harris, R.C. and Zhang, MZ. (2020) The role of gender disparities in kidney injury. *Ann. Transl. Med.* **8**, 514 https://doi.org/10.21037/atm.2020.01.23
47 Lima-Posada, I., Portas-Cortés, C., Pérez-Villalva, R., Fontana, F., Rodríguez-Romo, R., Prieto, R. et al. (2017) Gender differences in the acute kidney injury to chronic kidney disease transition. *Sci. Rep.* **7**, 12270 https://doi.org/10.1038/s41598-017-09630-2
48 Handy, D.E. and Loscalzo, J. (2012) Redox regulation of mitochondrial function. *Antioxid. Redox Signal.* **16**, 1323–1367 https://doi.org/10.1089/ars.2011.4123
49 Kang, J. and Pervaiz, S. (2012) Mitochondria: redox metabolism and dysfunction. *Biochem. Res. Int.* **2012**, 896751 https://doi.org/10.1155/2012/896751
50 Ralto, K.M. and Parikh, S.M. (2016) Mitochondria in acute kidney injury. *Semin. Nephrol.* **36**, 8–16 https://doi.org/10.1016/j.semnephrol.2016.01.005
51 Jiang, M., Bai, M., Lei, J., Xie, Y., Xu, S., Jia, Z. et al. (2020) Mitochondrial dysfunction and the AKI-to-CKD transition. *Am. J. Physiol. Renal Physiol.* **319**, F1105–F1116 https://doi.org/10.1152/ajprenal.00285.2020
52 Emma, F., Montini, G., Parikh, S.M. and Salviati, L. (2016) Mitochondrial dysfunction in inherited renal disease and acute kidney injury. *Nat. Rev. Nephrol.* **12**, 267–280 https://doi.org/10.1038/nrneph.2015.214
53 Zhan, M., Brooks, C., Liu, F., Sun, L. and Dong, Z. (2013) Mitochondrial dynamics: regulatory mechanisms and emerging role in renal pathophysiology. *Kidney Int.* **83**, 568–581 https://doi.org/10.1038/ki.2012.441
54 Tilokani, L., Nagashima, S., Paupe, V. and Prudent, J. (2018) Mitochondrial dynamics: overview of molecular mechanisms. *Essays Biochem.* **62**, 341–360 https://doi.org/10.1042/EBC20170104
55 Lan, R., Geng, H., Singha, P.K., Saikumar, P., Bottinger, E.P., Weinberg, J.M. et al. (2016) Mitochondrial pathology and glycolytic shift during proximal tubule atrophy after ischemic AKI. *J. Am. Soc. Nephrol.* **27**, 3356–3367 https://doi.org/10.1681/ASN.2015020177
56 Taguchi, K., Elias, B.C., Krystofiak, E., Qian, S., Sant, S., Yang, H. et al. (2021) Quantitative super-resolution microscopy reveals promoting mitochondrial interconnectivity protects against AKI. *Kidney360* **2**, 1892–1907 https://doi.org/10.34067/KID.0001602021
57 Wang, J., Sun, X., Wang, X., Cui, S., Liu, R., Liu, J. et al. (2021) Grb2 induces cardiorenal syndrome type 3: roles of IL-6, cardiomyocyte bioenergetics, and Akt/mTOR pathway. *Front. Cell Dev. Biol.* **9**, 630412 https://doi.org/10.3389/fcell.2021.630412
58 Gu, Z., Qiu, W., He, R., Zhang, Z. and Zhou, X. (1999) Selection of quantitative criteria for evaluating metastases of a highly metastatic salivary adenoid cystic carcinoma cell line to the lungs of nude mice. *Chin. J. Dent. Res.* **2**, 16–20
59 Xiao, X., Hu, Y., Quirós, P.M., Wei, Q., López-Otín, C. and Dong, Z. (2014) OMA1 mediates OPA1 proteolysis and mitochondrial fragmentation in experimental models of ischemic kidney injury. *Am. J. Physiol. Renal Physiol.* **306**, F1318–F1326 https://doi.org/10.1152/ajprenal.00036.2014
60 Funk, J.A. and Schnellmann, R.G. (2012) Persistent disruption of mitochondrial homeostasis after acute kidney injury. *Am. J. Physiol. Renal Physiol.* **302**, F853–F864 https://doi.org/10.1152/ajprenal.00035.2011
61 Brooks, C., Wei, Q., Cho, S.G. and Dong, Z. (2009) Regulation of mitochondrial dynamics in acute kidney injury in cell culture and rodent models. *J. Clin. Invest.* **119**, 1275–1285 https://doi.org/10.1172/JCI37829
62 Tran, M.T., Zsengeller, Z.K., Berg, A.H., Khankin, E.V., Bhasin, M.K., Kim, W. et al. (2016) PGC1α drives NAD biosynthesis linking oxidative metabolism to renal protection. *Nature* **531**, 528–532 https://doi.org/10.1038/nature17184
63 Szeto, H.H., Liu, S., Soong, Y., Seshan, S.V., Cohen-Gould, L., Manichev, V. et al. (2017) Mitochondria protection after acute ischemia prevents prolonged upregulation of IL-1β and IL-18 and arrests CKD. *J. Am. Soc. Nephrol.* **28**, 1437–1449 https://doi.org/10.1681/ASN.2016070761
64 Gibbs, W.S., Collier, J.B., Morris, M., Beeson, C.C., Megyesi, J. and Schnellmann, RG. (2018) 5-HT_{1F} receptor regulates mitochondrial homeostasis and its loss potentiates acute kidney injury and impairs renal recovery. *Am. J. Physiol. Renal Physiol.* **315**, F1119–F1128 https://doi.org/10.1152/ajprenal.00077.2018
65 Kim, S.-M., Kim, Y.G., Kim, D.-J., Park, S.H., Jeong, K.-H., Lee, Y.H. et al. (2018) Inflammasome-independent role of NLRP3 mediates mitochondrial regulation in renal injury. *Front. Immunol.* **9**, 2563 https://doi.org/10.3389/fimmu.2018.02563
66 Kong, M.J., Bak, S.H., Han, K.H., Kim, J.I., Park, J.W. and Park, K.M. (2019) Fragmentation of kidney epithelial cell primary cilia occurs by cisplatin and these cilia fragments are excreted into the urine. *Redox Biol.* **20**, 38–45 https://doi.org/10.1016/j.redox.2018.09.017
67 Zhao, Z.B., Marschner, J.A., Iwakura, T., Li, C., Motrapu, M., Kuang, M. et al. (2023) Tubular epithelial cell HMGB1 promotes AKI-CKD transition by sensitizing cycling tubular cells to oxidative stress: a rationale for targeting HMGB1 during AKI recovery. *J. Am. Soc. Nephrol.* **34**, 394–411 https://doi.org/10.1681/ASN.0000000000000024
68 Ding, W., Guo, H., Xu, C., Wang, B., Zhang, M. and Ding, F. (2016) Mitochondrial reactive oxygen species-mediated NLRP3 inflammasome activation contributes to aldosterone-induced renal tubular cells injury. *Oncotarget* **7**, 17479–17491 https://doi.org/10.18632/oncotarget.8243
69 Liu, D., Xu, M., Ding, L.-H., Lv, L.-L., Liu, H., Ma, K.-L, et al. (2014) Activation of the Nlrp3 inflammasome by mitochondrial reactive oxygen species: a novel mechanism of albumin-induced tubulointerstitial inflammation. *Int. J. Biochem. Cell Biol.* **57**, 7–19 https://doi.org/10.1016/j.biocel.2014.09.018
70 Zhao, W., Sui, M., Chen, R., Lu, H., Zhu, Y., Zhang, L, et al. (2021) SIRT3 protects kidneys from ischemia-reperfusion injury by modulating the DRP1 pathway to induce mitochondrial autophagy. *Life Sci.* **286**, 120005 https://doi.org/10.1016/j.lfs.2021.120005

71 Zhao, M., Wang, Y., Li, L., Liu, S., Wang, C., Yuan, Y. et al. (2021) Mitochondrial ROS promote mitochondrial dysfunction and inflammation in ischemic acute kidney injury by disrupting TFAM-mediated mtDNA maintenance. *Theranostics* **11**, 1845–1863 https://doi.org/10.7150/thno.50905
72 Yuan, L., Yuan, Y., Liu, F., Li, L., Liu, J., Chen, Y. et al. (2021) PGC-1α alleviates mitochondrial dysfunction via TFEB-mediated autophagy in cisplatin-induced acute kidney injury. *Aging* **13**, 8421–8439 https://doi.org/10.18632/aging.202653
73 Van Raamsdonk, J.M. and Hekimi, S. (2009) Deletion of the mitochondrial superoxide dismutase sod-2 extends lifespan in Caenorhabditis elegans. *PLoS Genet.* **5**, e1000361 https://doi.org/10.1371/journal.pgen.1000361
74 Makris, K. and Spanou, L. (2016) Acute kidney injury: definition, pathophysiology and clinical phenotypes. *Clin. Biochem. Rev.* **37**, 85–98
75 Khang, A.R., Kim, D.H., Kim, M.-J., Oh, C.J., Jeon, J.-H., Choi, S.H. et al. (2024) Reducing oxidative stress and inflammation by pyruvate dehydrogenase kinase 4 inhibition is important in prevention of renal ischemia-reperfusion injury in diabetic mice. *Diabetes Metab. J.* **48**, 405–417 https://doi.org/10.4093/dmj.2023.0196
76 Sanz, A.B., Sanchez-Niño, M.D., Ramos, A.M. and Ortiz, A. (2023) Regulated cell death pathways in kidney disease. *Nat. Rev. Nephrol.* **19**, 281–299 https://doi.org/10.1038/s41581-023-00694-0
77 Taguchi, K., Elias, B.C., Sugahara, S., Sant, S., Freedman, B.S., Waikar, S.S. et al. (2022) Cyclin G1 induces maladaptive proximal tubule cell dedifferentiation and renal fibrosis through CDK5 activation. *J. Clin. Invest.* **132**, e158096 https://doi.org/10.1172/JCI158096
78 Price, P.M. and Hodeify, R. (2012) A possible mechanism of renal cell death after ischemia/reperfusion. *Kidney Int.* **81**, 720–721 https://doi.org/10.1038/ki.2011.495
79 Schumer, M., Colombel, M.C., Sawczuk, I.S., Gobé, G., Connor, J., O'Toole, K.M. et al. (1992) Morphologic, biochemical, and molecular evidence of apoptosis during the reperfusion phase after brief periods of renal ischemia. *Am. J. Pathol.* **140**, 831–838
80 Noh, M.R. and Padanilam, B.J. (2024) Cell death induced by acute renal injury: a perspective on the contributions of accidental and programmed cell death. *Am. J. Physiol. Renal Physiol.* **327**, F4–F20 https://doi.org/10.1152/ajprenal.00275.2023
81 Tangren, J.S., Powe, C.E., Ankers, E., Ecker, J., Bramham, K., Hladunewich, M.A. et al. (2017) Pregnancy outcomes after clinical recovery from AKI. *J. Am. Soc. Nephrol.* **28**, 1566–1574 https://doi.org/10.1681/ASN.2016070806
82 Moronge, D., Sullivan, J.C. and Faulkner, J.L. (2023) Physiology of pregnancy-related acute kidney injury. *Compr. Physiol.* **13**, 4869–4878 https://doi.org/10.1002/cphy.c220026
83 Cheung, K.L. and Lafayette, R.A. (2013) Renal physiology of pregnancy. *Adv. Chronic Kidney Dis.* **20**, 209–214 https://doi.org/10.1053/j.ackd.2013.01.012
84 Aufhauser, D.D., Jr, Wang, Z., Murken, D.R., Bhatti, T.R., Wang, Y., Ge, G, et al. (2016) Improved renal ischemia tolerance in females influences kidney transplantation outcomes. *J. Clin. Invest.* **126**, 1968–1977 https://doi.org/10.1172/JCI84712
85 Hosszu, A., Fekete, A. and Szabo, AJ. (2020) Sex differences in renal ischemia-reperfusion injury. *Am. J. Physiol. Renal Physiol.* **319**, F149–F154 https://doi.org/10.1152/ajprenal.00099.2020
86 Neugarten, J. and Golestaneh, L. (2022) Sex differences in acute kidney injury. *Semin. Nephrol.* **42**, 208–218 https://doi.org/10.1016/j.semnephrol.2022.04.010
87 Lima-Posada, I. and Bobadilla, N.A. (2021) Understanding the opposite effects of sex hormones in mediating renal injury. *Nephrology (Carlton).* **26**, 217–226 https://doi.org/10.1111/nep.13806
88 Rodríguez, F., Nieto-Cerón, S., Fenoy, F.J., López, B., Hernández, I., Martinez, R.R. et al. (2010) Sex differences in nitrosative stress during renal ischemia. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **299**, R1387–R1395 https://doi.org/10.1152/ajpregu.00503.2009