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Abstract: Chronic kidney disease (CKD) research is limited by the lack of convenient inducible models mimicking human CKD and its complications in experimental animals. We demonstrate that a soluble oxalate-rich diet induces stable stages of CKD in male and female C57BL/6 mice. Renal histology is characterized by tubular damage, remnant atubular glomeruli, interstitial inflammation, and fibrosis with the extent of tissue involvement depending on the duration of oxalate feeding. Expression profiling of markers and magnetic resonance imaging findings established to reflect inflammation and fibrosis parallel the histological changes. Within 3 weeks the mice reproducibly develop normochromic anemia, metabolic acidosis, hyperkalemia, FGF23 activation, hyperphosphatemia and hyperparathyroidism. In addition, the model is characterized by profound arterial hypertension as well as cardiac fibrosis that persist following the switch to a control diet. Together, this new model of inducible CKD overcomes a number of previous experimental limitations and should serve useful in research related to CKD and its complications.

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Oxalate-induced chronic kidney disease with its uremic and cardiovascular complications in C57BL/6 mice

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Abstract

Chronic kidney disease (CKD) research is limited by the lack of convenient inducible models mimicking human CKD and its complications in experimental animals. We demonstrate that a soluble oxalate-rich diet induces stable stages of CKD in male and female C57BL/6 mice. Renal histology is characterized by tubular damage, remnant atubular glomeruli, interstitial inflammation, and fibrosis with the extent of tissue involvement depending on the duration of oxalate feeding. Expression profiling of markers and magnetic resonance imaging findings established to reflect inflammation and fibrosis parallel the histological changes. Within 3 weeks the mice reproducibly develop normochromic anemia, metabolic acidosis, hyperkalemia, FGF23 activation, hyperphosphatemia and hyperparathyroidism. In addition, the model is characterized by profound arterial hypertension as well as cardiac fibrosis that persist following the switch to a control diet. Together, this new model of inducible CKD overcomes a number of previous experimental limitations and should serve useful in research related to CKD and its complications.

Key words: renal failure, oxalate, CKD-MBD, hypertension, cardiovascular
**Introduction**

Translational research in the area of chronic kidney disease (CKD) requires experimental models that (1) can be induced in C57BL/6 mice as it represents the most commonly used genetic background for gene manipulations, (2) reliably generate stable CKD, (3) result in CKD within a relatively short time to limit the burden for animals and reduce housing costs, (4) do not require surgery in order to minimize animal distress and the use of anesthetic drugs or painkillers with potentially confounding effects. In addition, an ideal model should (5) work in male and female mice with low inter-individual variability to allow sex-based comparisons in response to interventions and minimize animal use, (6) not reduce renal mass, which limits the tissue available for analysis, (7) reduce glomerular filtration rate (GFR) by more than 50% in order to allow detection of the changes in the clinical biomarkers plasma blood urea nitrogen (BUN) and creatinine, and (8) be associated with typical complications associated with CKD in humans, such as normochromic anemia, hyperphosphatemia, hyperparathyroidism, hyperkalemia, acidosis, hypertension, and cardiovascular disease. The present work reviews the most commonly used murine models of inducible CKD (1, 3, 12, 16) and contrasts them with a novel oxalate-induced model of stable CKD.

**Methods**

*Animal studies and analytical methods*

Male and female 8-12 week old gender-matched C57BL/6 mice were obtained from Charles River Laboratories (Sulzfeld, Germany), housed in groups of five mice in standard housing conditions with unlimited access to food and water. Oxalate diet was prepared by
adding 50 μmol/g sodium oxalate to a calcium-free standard diet (Ssniff, Soest, Germany) as previously described (15). Removal of calcium from the diet increases the amount of soluble oxalate available for absorption as previously shown (15). Oxalate- and calcium-free diet (control diet) was given for 3 days to eliminate any residual intestinal calcium before switching to high soluble oxalate diet and used after ending the oxalate feeding period. All experimental procedures were approved by the local government authorities.

Assessment of renal injury

Mice kidney sections of 2 μm were stained with periodic acid-Schiff (PAS) reagent. Tubular injury was scored by assessing the percentage of necrotic tubules and presence of tubular casts. F4/80+ve positive macrophages, CD3+ve T cells (both Serotec, Oxford, UK) were identified by immunostaining and were counted in 15 high power fields (hpf) per section using Image J software. Similar approach was also used to quantify Silver stains. Fibrotic areas were identified by Silver stain, Masson Trichrome stain & Collagen 1a1 and were quantified using Image J software. Serial sections of the kidney were stained with Tetragonolobus lectin to observe atubular glomeruli. All assessments were performed by an observer blinded to the experimental condition.

RNA preparation and real-time quantitative -PCR

Total RNA was isolated from kidneys using a Qiagen RNA extraction kit (Qiagen, Germany) following the manufacturer’s instructions. After quantification RNA quality was assessed using agarose gels. From isolated RNA, cDNA was prepared using reverse transcriptase (Superscript II)
Real time RT-PCR was performed using SYBRGreen PCR master mix and was analyzed with a Light Cycler 480 (Roche, Germany). All gene expression values were normalized using 18s RNA as a housekeeping gene. All primers used for amplification were from Metabion (Martinsried, Germany) and are listed in Table 1.

**Hematological analysis**

Hematological analysis was performed using prefilled Sysmex capillary tubes (Sysmex Deutschland GmbH, Norderstedt, Germany). An aliquot of 50µl EDTA-blood was determined by using a 50µl end-to-end capillary and was diluted 1:5 in 200µl prefilled Sysmex Cellpack buffer. Diluted samples were stored at room temperature until analysis. Complete peripheral blood counts, differential leukocyte counts and reticulocyte counts were determined with a Sysmex XT2000iV hematology analyzer (Sysmex Deutschland GmbH, Norderstedt, Germany) in the “capillary blood” mode using the option “CBC DIFF RET” as described previously (23) with the following parameters included:

- Total white blood cell count (WBC), total red blood cell count (RBC), platelet count (PLT), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin content (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width – coefficient of variance (RDW-CV), mean platelet volume (MPV), platelet distribution width (PDW), platelet large cell ratio (P-LCR), plateletcrit (PCT), absolute cell counts and proportions of WBC for lymphocytes (LYMPH), monocytes (MONO) as well as neutrophil (NEUT), eosinophil (EO) and basophil (BASO) granulocytes, and absolute cell counts and proportion of RDW of reticulocytes (RET), including proportions of reticulocyte maturation
states as determined by fluorescence intensity, mature low fluorescent reticulocytes (LFR), less mature medium fluorescent reticulocytes (MFR) and very immature highly fluorescent reticulocytes (HFR), as well as the sum of MFR and HFR as immature reticulocyte fraction (IRF).

Clinical chemistry analyses

Inorganic phosphate (Pi) was analyzed using an AU480 Clinical chemistry analyzer (Beckman-Coulter GmbH, Krefeld, Germany) and adapted test kits provided by Beckman Coulter in either undiluted or 1:2 with deionized water diluted EDTA-treated plasma samples as described previously (24). Heparinized capillaries were filled with 100 µL of whole blood and subjected to measurement of potassium and pH using a blood gas analyzer (ABL 835 Flex, Radiometer GmbH, Willich, Germany).

Blood pressure measurements

Blood pressure was measured continuously in male 10-12 week old C57BL/6 mice using an intra-arterial telemetric transmitter system as previously described (3). In brief, under isoflurane anesthesia a transmitter device (PhysioTel™ TA11PA-C10; Data Sciences International DSI, St. Paul, Minnesota, USA) was surgically implanted into the left common carotid artery one week prior to data sampling. The transmitter was placed subcutaneously over the abdominal area. All animals were then housed in open cages and allowed to recover for two weeks during which mice received unlimited access to regular chow (Ssniff, Soest, Germany) and tap water. Following recovery, the diet was changed to synthetic calcium-and oxalate-free diet (0% Ca 0% Oxalate, TD. 95027, Ssniff) for one week, followed by high oxalate diet (0% Ca 0.67% Oxalate,
TD. 95027, Ssniff) for 8 days and thereafter control diet for another 10 days. Blood pressure was
detected and recorded continuously for 5 weeks by the Dataquest A.R.T. System (Data Sciences
International DSI, St. Paul, Minnesota, USA). Systolic and diastolic blood pressure values were
calculated as average of the last 24h-day of each diet period by the Dataquest ART 4.1 software
(DSI). Mean arterial pressure (MAP) was calculated from diastolic and systolic pressure values
using a standard equation.

Transcutaneous measurement of glomerular filtration rate (GFR) in conscious mice

For GFR measurement mice where anesthetized with isoflurane and a miniaturized imager
device built from two light-emitting diodes, a photodiode and a battery (Mannheim Pharma &
Diagnostics GmbH, Germany) was mounted via a double-sided adhesive tape onto the shaved
animal’s neck (26). For the duration of recording (approx. 1.5 h) each animal was conscious and
kept in a single cage. Prior to the intravenous injection of 150 mg/kg FITC-sinistrin (Mannheim
Pharma & Diagnostics GmbH, Germany), the skin’s background signal was recorded for 5 min.
After removing the imager device the data were analyzed using MPD Lab software (Mannheim
Pharma & Diagnostics GmbH, Germany). The GFR [µl/min] was calculated from the decrease of
fluorescence intensity over time (i.e. plasma half-life of FITC-sinistrin) using a two-compartment
model, body weight of mouse and an empirical conversion factor (26).

Measurement of intact FGF23, PTH and phosphorus

The plasma concentration of intact FGF23 (Kainos Laboratories, Japan or Immutopics
International, USA) and intact PTH (Immutopics International, USA) were measured by enzyme-
linked immunosorbent assays according to the manufacturers protocols. Phosphate was measured by the phosphomolybdate method (9, 10).

Magnetic resonance imaging (MRI)

Excised kidneys (n=12) from C57BL/6 male mice fed either high oxalate (n=6 kidneys) or control diet (n=6 kidneys) for 21 days were processed in 2% agarose and placed in a whole body coil for mice (Bruker, Ettlingen, Germany) of a dedicated small animal ultra-high-field MR scanner (ClinScan 7 Tesla, Bruker, Ettlingen, Germany). Standard sequences for morphology and mapping of T1-, T2- and T2*- relaxation times as well as of the apparent diffusion coefficient (ADC) (Siemens, Erlangen, Germany) were performed on kidneys in sagittal orientation (compare Fig. 5). By mapping of relaxation times, specific magnetic properties of tissues are quantified, which can be performed in multiple tissues and organs. Thereby an increase of T1 relaxation time may be associated with fibrosis, while prolonged T2 relaxation times are found in inflammation (25). Furthermore, calculation of the ADC by diffusion-weighted imaging captures the Brownian motion of water molecules that is locally altered in pathologic conditions like fibrosis, resulting in decreased water diffusion, and thus decreased ADC values (11). For post-processing of images, 3 regions of interest were placed in the cortex, medulla and transitional zone, respectively, to determine T1-, T2- and T2*- relaxation times as well as the ADC (Osirix, open-source software). Furthermore, the volume of the cortex was determined on morphological, T2 weighted images (Osirix, open-source software).

Histological examination of cardiac fibrosis
Hearts were excised, snap frozen in liquid nitrogen and stored at -80 °C until further use. The lower base of the left ventricle was embedded in tissue tec, whereupon 10 µm thick cross-sections were prepared on a SLEE-MNT cryostat (Mainz, Germany). Ventricular sections were stained for fibrotic tissue using Masson’s trichrome staining (Sigma-Aldrich, Taufkirchen, Germany). Briefly, sections were fixed in 4% paraformaldehyde for 1 h at room temperature and refixed overnight in Bouin’s solution. The next day, slides were washed in distilled water and incubated for 5 min with hematoxylin. After washing the slides in distilled water for 10 min, sections were incubated for 5 min in biebrich scarlet acid fuchsin. Slides were washed 3 times in distilled water, where after they were incubated in phosphomolybdic acid 3 times each for 3 min. After 5 min of incubation with aniline blue, which leads to a blue staining of fibrotic tissue, slides were rinsed in distilled water and dehydrated in isopropanol and xylene, before they were mounted with roti-histokit (Carl Roth, Karlsruhe, Germany). Differentiation was performed by incubating the slides 2 min in ice-cold acetic acid. After a washing step in distilled water, slides were covered with mounting medium. Microscopic composite images of the complete cross-sections were obtained at x150 magnification on a light microscope (Olympus IX70, Hamburg, Germany) with an inverted CCD-camera (Nikon DXM1200, Düsseldorf, Germany). Blue-stained fibrotic area was manually quantified by an observer blinded to the experimental condition on three cross-sections per heart using Image J software. The endocardium and trabeculae carnae were excluded from quantification. After determination of the ventricular area, the mean fibrosis content was calculated by dividing the area of Trichrome-stained fibrotic fibers by total left ventricular area. For qualitative electron microscopic investigations of the hearts several semithin and ultrathin sections were prepared and stained with methylene blue or uranyl
acetate/lead citrate, respectively. Images of ultrathin sections were taken with a Zeiss electron
microscope LEO EM 910 (Zeiss, Oberkochen, Germany).

Preparation of femoral bones

Femoral bones were removed, weighed and fixed overnight using 1% (w/v) PFA buffered with
PBS pH 7.4. Bones were decalcified in 10% (w/v) EDTA solution pH 7.4 before they were
dehydrated and embedded in paraffin. Tissues were cut into 2-μm sections and stained with
hematoxylin and eosin (H & E). Images were taken using BX60 Olympus microscope equipped
with a XC 30 camera (Olympus BC, Hamburg, Germany).

Results

A diet high in soluble oxalate induces stable CKD

Previous work from our groups suggests that intraperitoneal oxalate can induce acute
kidney injury (22), and a high oxalate diet can cause progressive renal failure (15). In the present
study we first examined the effects of duration of feeding a soluble oxalate diet on renal
function in C57BL/6 mice. Providing an oxalate diet induced a decline in GFR and an increase in
plasma BUN and creatinine (Fig. 1A-C). Shorter duration of oxalate feeding followed by
switching to control diet induced less advanced stages of CKD as defined by GFR, plasma BUN
and creatinine (Fig. 1D-F). Serial monitoring documented that these parameters peaked at the
end of oxalate feeding followed by mild recovery over the following 2 week period. Urinary
oxalate increased following a switch to a soluble oxalate as compared to control diet (Fig. 1G)
but no renal or bladder stones were detected (images not shown). Similarly, we observed a
sharp increase in plasma oxalate levels followed by a reduction of plasma oxalate levels when mice were switched back to control diet (Fig. 1H). Therefore, while a diet high in soluble oxalate was found to induce a progressive decline in renal function as previously reported (13), these results show for the first time that it can also be used to induce stable CKD after dietary oxalate is discontinued.

Renal pathology of oxalate-induced CKD

As progressive scarring and fibrotic processes are a hallmark of CKD irrespective of the initiating disease, we next examined renal histology of mice fed a soluble oxalate diet for various durations. Periodic acid-Schiff (PAS) staining revealed progressive tubular atrophy (Fig. 2A) resulting in remnant atubular glomeruli (Fig. 2B) (2, 17). In addition, infiltration by macrophages/monocytes and T cells was observed, mainly in areas of interstitial fibrosis (Fig. 3A). Furthermore, progressive renal fibrosis was demonstrated as measured by Collagen I, Silver and Trichrome staining, and the duration of feeding allowed titration of the degree of parenchymal loss (Fig. 3A). Expression profiling for markers of kidney injury, inflammation and fibrosis were consistent with progressive kidney atrophy (Fig. 3B). Female C57BL/6 mice revealed similar findings with very low inter-individual variability (Fig. 4A-F). Given the increasing use of diagnostic imaging modalities to assess changes of renal morphology, we performed magnetic resonance imaging (MRI) of kidneys from mice. As compared to mice receiving a control diet, animals receiving an oxalate diet displayed an increase in kidney cortex volume, T1-, T2- and T2*- relaxation times as well as a reduction of the apparent diffusion coefficient in the cortex (Fig 5). These significant differences in the cortex are known to be
associated with inflammation (increased volume and T2-time) and fibrosis (increased T1-time and decreased diffusion), and are consistent with findings from histology and expression profiling (25). Taken together, these data indicate that a high oxalate diet induces CKD in male and female C57BL/6 mice characterized by progressive kidney atrophy and fibrosis.

Oxalate-induced CKD in C57BL/6 mice demonstrates classical CKD complications

We next examined whether the mouse model displays human CKD complications. Oxalate diet induced normochromic anemia (Fig. 6A) without affecting other blood cell lines (Supplementary Table 1). CKD-related mineral bone disease (CKD-MBD) was demonstrated by elevated plasma FGF23 and PTH levels as well as hyperphosphatemia and hyperkalemia (Fig. 6B-E) in addition to metabolic acidosis (Fig. 6F). Bone marrow of femoral bones demonstrated no oxalate crystal deposition of mice receiving an oxalate diet (Fig. 6G). Furthermore, oxalate diet induced arterial hypertension (Fig. 7A and B), which persisted after cessation of the oxalate-rich diet (Fig. 7C and D). In addition, oxalate feeding induced cardiac fibrosis (Fig. 7E and F), another serious cardiovascular complication of CKD. Electron microscopy demonstrated fibroblast activation in mice receiving an oxalate diet, as demonstrated by an increase in size and vacuolization of fibroblasts as compared with mice receiving a control diet (Fig. 7G; white arrows). Similar to our observation in femoral bones, we could not detect any oxalate crystal deposition in heart tissue arguing against systemic oxalosis to explain the observed phenotype.

Thus, this model of oxalate diet-induced CKD presents with a wide spectrum of clinically established CKD complications.
It is of interest to compare the features of this oxalate-induced model of CKD with previous models of CKD, as summarized in Table 2.

Discussion

The model of unilateral ureteral obstruction (UUO) has become a widely used model to study CKD and fibrosis (3). While the model causes extensive parenchymal damage, functional compensation by the intact contralateral kidney prevents a significant change of renal function and the development of CKD complications (29). Absence of urine from the damaged kidney precludes biomarker discovery studies. In contrast, the oxalate-diet induced CKD model enables study of renal fibrosis while providing a functional endpoint of renal function as changes in GFR, serum BUN and creatinine can be measured. It also allows the collection of urine and demonstrates established CKD complications.

The renal mass reduction models have been a mainstay of CKD studies (16). Unilateral nephrectomy is followed by either partial infarction or amputation of the poles of the remaining kidney resulting in glomerular and tubule-interstitial injury. A strong limitation of the model is the need for two surgical interventions requiring microsurgery training, confounding effects related to anesthesia as well as surgical procedures, and the model’s reproducibility and variability (16). In addition, performing 5/6 nephrectomy limits the tissue available for analysis.

After 16 weeks, the C57BL/6 mice demonstrate increased albuminuria without increased blood pressure and without developing cardiac fibrosis unless angiotensin II is administered (18). The oxalate diet induced-model requires no microsurgery training/intervention, maintains sufficient renal tissue for analysis, and reliably induces CKD complications.
The adenine-induced model of CKD - unlike UUO or renal mass reduction – does not require surgery. It has largely been established in rats (32) as it is not easily adapted to mice given their reluctance to consume adenine. Addition of casein appears to blunt the smell and taste of adenine in order to apply the approach to mice (13). Male mice present a more severe renal phenotype as compared with female mice presumably secondary to gender differences in the metabolism of adenine (7, 27). In addition, the model requires diets of different adenine content (13) or induction for 6 weeks (28). Most strikingly, the mice develop profound hypotension compared with mice receiving a control diet (19). In contrast, oxalate is readily eaten by mice; the amount of dietary intake can be precisely monitored by measuring urinary oxalate excretion. Additional benefits include the short time frame of 1-3 weeks for the induction of moderate to advanced CKD, which accelerates scientific workflow and reduces housing costs. Most strikingly, the oxalate model induces reliable and profound hypertension, an established complication of CKD in humans.

The aristolochic acid model represents an additional tool to investigate mechanisms of CKD progression and to access potential interventions (6, 12). Aristolochic acid I (AAI) is the active ingredient implicated in Chinese herb nephropathy. Intraperitoneal injection of AAI weekly has been shown to lead to progressive fibrosis and kidney failure that is more severe in male than female mice (6, 12), similar to observations made in the adenine model. No significant hypertension ensues (12). Contrary to the AAI model, the oxalate diet-induced model avoids direct handling and injections of mice. As the oxalate model works in male and female mice, it allows examination of gender differences in response to pharmaceutical interventions to treat CKD, providing a platform to use both male and female mice in preclinical studies as
requested by the National Institute of Health (4). In addition, gender-restricted models produce excess pups, which are avoided when both males and females can be used for experimentation, and the low inter-individual variability allows smaller group sizes.

The folic acid nephropathy model induces interstitial fibrosis following i.p. injection of high dosage of folic acid. The advantage of the model as compared with the UUO model is that renal function can be assessed. However, the model requires i.p. injections as compared with the oxalate feeding model, is somewhat variable and yet to be characterized in regards to gender differences and CKD complications (8, 30, 31).

Despite these advantages of the oxalate model over previous CKD models, a number of limitations have to be considered. Oxalate crystals primarily induce tubular injury (20-22), while many forms of human CKD are primarily driven by podocyte loss (5). The mechanism of crystal-induced progression may not translate to all forms of CKD. However, the extensively studied adenine and folic acid model are similarly crystal-induced (19). Likewise, one may argue that UUO as the benchmark model of fibrosis associated with an increase of vascular resistance and a reduced blood flow following surgery provides a mechanism of fibrosis not mimicking the pathophysiology of common forms of CKD (14).

Nevertheless, several elements of the oxalate model including tubular atrophy, renal inflammation, and fibrosis are common to many forms of CKD. In addition, one of the major strengths of the model is the reproducibility of CKD complications thereby allowing study of the mechanisms of FGF23 activation, hyperphosphatemia, hyperparathyroidism, hypertension and
cardiac fibrosis. Moreover, increasing the number of CKD models available may help to validate important findings and improve standards.

In conclusion, feeding a soluble oxalate diet represents a simple, reproducible and technically undemanding CKD model providing functional data in male as well as female C57BL/6 mice. Moreover, the duration of feeding enables titrated induction of various stages of CKD associated with numerous clinically important CKD complications. Thus, the oxalate diet-induced CKD model offers novel opportunities for CKD research.

Acknowledgements

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Disclosure

The authors have nothing to disclose.
References


Figure legends

Figure 1. A diet high in soluble oxalate induces progressive or stable CKD. A-C: C57BL/6 male mice were fed either high oxalate or control diet for 21 days and (A) GFR, (B) plasma BUN, and (C) plasma creatinine were measured at baseline, days 7, 14 and 21. D-F: C57BL/6 male mice received high oxalate diet for 0, 7 or 14 days respectively and thereafter control diet for 14 days. (D) GFR, (E) plasma BUN, (F) plasma creatinine, (G) urine oxalate and (H) plasma oxalate were measured at indicated time points. Data are mean ± SEM from 5 mice in each group. *p<0.05, **p<0.01 and ***p<0.001 versus control group.

Figure 2. Oxalate-induced CKD shows atubular glomeruli. C57BL/6 male mice were fed either high oxalate or control diet for 21 days. (A) PAS staining at various time points and quantification, (B) Serial sections of the kidney were stained with Tetragonolobus lectin to observe atubular glomeruli. Note the atubular glomerulus (clearly separated by a membrane) is surrounded by tubules containing crystals. Data are mean ± SEM from 7-8 mice in each group. **p<0.01 and ***p<0.001 versus control group.

Figure 3. Pathology of oxalate-induced CKD in male mice. C57BL/6 male mice were fed either high oxalate or control diet for 21 days. (A) F4/80, CD3, Collagen I, Silver, Masson Trichrome staining and their quantification, (B) Gene expression of kidney injury (TIMP-2, Kim-1), inflammation (RANTES, TNFα, IL-6) and fibrosis (Fibronectin, FSP-1, Col1a1) was analyzed using RT-PCR at different time points. Data are mean ± SEM from 7-8 mice in each group. *p<0.05,
**p<0.01 and ***p<0.001 versus control group. +p<0.05, ++p<0.01 and +++p<0.001 versus control group. ##p<0.01 and ###p<0.001 versus control group.

Figure 4. Oxalate-induced CKD in female mice. C57BL/6 female mice were fed either high oxalate or control diet for 21 days. Note that female mice develop comparable CKD to male mice. (A) Plasma BUN levels. (B) Plasma creatinine levels. (C) Quantification of tubular injury. (D) Quantification of CD3+ve cells. (E) Quantification of CaOx crystal deposits, F4/80+ve macrophages, Masson Trichrome, Silver and Collagen I+ve area. (F) mRNA expression of kidney injury markers, pro-inflammatory genes and pro-fibrotic genes. Data are mean ± SEM from 7-8 mice in each group. *p<0.05, **p<0.01 and ***p<0.001 versus control group.

Figure 5. Magnetic resonance imaging (MRI) of oxalate-induced CKD. MRI was performed on kidneys of C57BL/6 male mice fed either high oxalate or control diet for 21 days. (A, B) On representative morphological images, contrast characteristics of kidneys in the cortex, medulla and transitional zone could be visualized. (C) Quantitative parameters on total cortex volume, (D) T1 - relaxation time, (E) T2 - relaxation time, (F) T2* - relaxation time and (G) Apparent diffusion coefficient (ADC) showed significant differences between the groups in cortex, medulla or transitional zone. C, cortex; M, medulla; CM, transitional zone. Data are mean ± SEM from 7-8 mice in each group. *p<0.05, **p<0.01 and ***p<0.001 versus control group.

Figure 6. Oxalate-induced CKD demonstrates classical CKD complications. A-F: C57BL/6 male mice were fed either high oxalate or control diet for 21 days and different parameters for CKD
complications were analyzed. (A) Hemoglobin levels in blood. (B) Plasma FGF-23 levels. (C) Plasma parathyroid hormone levels. (D) Plasma phosphorous levels. (E) Plasma potassium levels (F). pH of blood. (G) HE staining of femoral bones. Data are mean ± SEM from 4-5 mice in each group. *p<0.05, **p<0.01 and ***p<0.001 versus control group.

Figure 7. Oxalate-induced CKD demonstrates hypertension and cardiac fibrosis. (A) Mean arterial blood pressure. (B) Heart Rate. C-D: C57Bl6 male mice received high oxalate diet for 8 days and thereafter control diet for 10 days. (C) Mean arterial blood pressure. (D) Heart Rate. E-F: Cardiac fibrosis was quantified (E) on Masson trichrome staining of heart cross-sections (F) at day 21 after high oxalate diet. (G) Electron microscopy of heart sections. Data are mean ± SEM from 4-5 mice in each group except for n=2 at day 18 (control diet fed group) in C-D. *p<0.05, **p<0.01 and ***p<0.001 versus control group.
### Table 1. Primers sequences

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<tr>
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<td>KIM1</td>
<td>5’-TCAGCTCGGGAATGCACA -3’</td>
<td>5’-TGGTTGCCTTCCTCGTCTCT -3’</td>
</tr>
<tr>
<td>TIMP2</td>
<td>5’-CAGACGTAGTGATCAGAGC -3’</td>
<td>5’-ACTCGATGTCTTTTCGAGCTCC -3’</td>
</tr>
<tr>
<td>RANTES</td>
<td>5’- GTGCCACGTCAGAGGAGTAT -3’</td>
<td>5’-CCACTTCTTCTCTGGGTGG -3’</td>
</tr>
<tr>
<td>IL 6</td>
<td>5’- TGATGCACTTGCAGAAACAC -3’</td>
<td>5’- ACCAGAGGAAATTTCATAAGGC -3’</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>5’- GAGAGTGCACTGTCAACCTC -3’</td>
<td>5’-ACTGGGATGGGGGTGGAAT -3’</td>
</tr>
<tr>
<td>Collagen1a1</td>
<td>5’-ACATGTTTCAGGTTTGTGGACC -3’</td>
<td>5’-TAGGGCTTGTGTATGCA -3’</td>
</tr>
<tr>
<td>FSP-1</td>
<td>5’- CAGCACCTCTCTCTCTGG -3’</td>
<td>5’-TTTGTGGAAGGTTGGACACA -3’</td>
</tr>
<tr>
<td>TNF-alpha</td>
<td>5’- CCACCAGCTTTCTGTCTAC -3’</td>
<td>5’-AGGGTCTGGGCCATAGAACT -3’</td>
</tr>
<tr>
<td>Alpha-SMA</td>
<td>5’- ACTGGGACGACATGGAAAG -3’</td>
<td>5’-GTCAGTGCGTGCCTCTTG -3’</td>
</tr>
<tr>
<td>iNOS</td>
<td>5’- GAGACAGGGAAGTCTGAAGC -3’</td>
<td>5’- CCAGCAGTAGTTGCTCTTTC -3’</td>
</tr>
<tr>
<td>18s RNA</td>
<td>5’- GCAATTATTTCCCATGAACG -3’</td>
<td>5’-AGGGCCCTCACTAAACCATCC -3’</td>
</tr>
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</table>
### Table 2: Comparison of murine CKD models.

<table>
<thead>
<tr>
<th>CKD Animal Models</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unilateral ureteral obstruction (UUO)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(3, 29)</td>
</tr>
<tr>
<td>Surgical renal mass ablation (5/6 Nephrectomy)</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>(16, 18)</td>
</tr>
<tr>
<td>Adenine-induced CKD</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>(7, 13, 19, 27, 28, 32)</td>
</tr>
<tr>
<td>Aristolochic acid-induced CKD</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>(6, 12)</td>
</tr>
<tr>
<td>Folic acid-induced CKD</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>?</td>
<td>✓</td>
<td>✓</td>
<td>?</td>
<td>(8, 30, 31)</td>
</tr>
<tr>
<td>Oxalate-induced CKD</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>

### Criteria

1. Inducible in C57BL/6 mice
2. Reliable induction of stable CKD
3. Require relatively short time to induce CKD and, thus, limit the burden for animals, reduce housing costs
4. Avoid surgical interventions, thus, minimize animal distress and use of painkillers
5. Inducible in male and female mice with low inter-individual variability
6. Avoid reduction in renal mass
7. Reduction in GFR
8. CKD complications such as normochromic anemia, hyperphosphatemia, hyperparathyroidism, hyperkalemia, acidosis, hypertension, and cardiovascular disease.
Figure 4

A

Plasma Urea (mg/dl)

Control
Oxalate

Days of high oxalate diet

1 7 14 21

** **

B

Plasma Creatinine (mg/dl)

Control
Oxalate

Days of high oxalate diet

1 7 14 21

* ** ***

C

Tubular Injury Score [0-5]

Control
Oxalate

0 1 2 3 4 5

***

D

CD3+ cells [per hpf]

Control
Oxalate

0 20 40 60 80 100

***

E

% Area

CaOx
FibR.
Masson Trichrome
Silver
Collagen-1

0 20 40 60

***

F

Fold Increase

Kyn-1
IL-6
TNF-α
INOS
α-SMA
Collagen1-1
Fibronectin
FSP-1

0 2 4 6 8 10 12 14 16

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Figure 5

A. Localization of region of interest (ROI)

B. Images of control and Oxalate conditions.

C. Comparison of total cortex volume (cm³) between Control and Oxalate groups.

D. Bar chart showing T1 values for different regions: Cortex (C), CM, Medulla (M) for Control and Oxalate groups.

E. Bar chart showing T2 values for different regions: Cortex (C), CM, Medulla (M) for Control and Oxalate groups.

F. Bar chart showing T2* values for different regions: Cortex (C), CM, Medulla (M) for Oxalate group.

G. Bar chart showing ADC values (10⁻⁶ mm²/s) for different regions: Cortex (C), CM, Medulla (M) for Control and Oxalate groups.