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Western diet promotes renal injury, inflammation, and fibrosis in a murine model of Alström syndrome

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Abstract

Introduction: Alström syndrome is a rare recessive genetic disease caused by mutations in *ALMS1*, which encodes a protein that is related to cilia function and intracellular endosome trafficking. The syndrome has been linked to impaired glucose metabolism and chronic kidney disease (CKD). Polymorphisms in *Alms1* have likewise been linked to CKD, but little is known about the modification of the phenotype by environmental factors.

Methods: To gain further insights, the *fat aussie (foz)* mouse strain, a genetic murine model of Alström syndrome, was exposed to a normal chow (NC) or to a Western diet (WD, 20% fat, 34% sucrose by weight and 0.2% cholesterol) and renal outcomes were measured.

Results: Body weight and albuminuria were higher in *foz* vs wildtype mice (WT) on both diets but WD significantly increased the difference. Measurement of plasma creatinine and cystatin C indicated that GFR was preserved in *foz* vs WT independent of diet. Renal markers of injury, inflammation and fibrosis were similar in both genotypes on NC, but significantly greater in *foz* vs WT on WD. A glucose tolerance test performed in *foz* and WT on WD revealed similar basal blood glucose levels and subsequent blood glucose profiles.

Conclusions: Western diet sensitizes a murine model of Alström syndrome to kidney injury, inflammation and fibrosis, an effect that may not be solely due to effects on glucose metabolism. Polymorphisms in *Alms1* may induce CKD in part by modulating the deleterious effects of high dietary fat and sucrose on kidney outcome.

Keywords

Western diet; Alström syndrome; Chronic kidney disease; kidney injury

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Author Contributions

YCK, SG, DB, DD and VV designed study and interpreted data. YCK, SG, JN, BF, HZ and DD performed experiments and analyzed data. YCK and VV wrote manuscript.

Introduction

Alström syndrome is a rare recessive genetic disease caused by mutations in *ALMS1*, which encodes a protein that plays a role in the formation and maintenance of cilia [1, 2]. Because of its expression in various tissues, individuals with functional mutations of *ALMS1* develop a variety of disorders at infancy or childhood such as retinal degeneration, hearing loss, dilated cardiomyopathy, obesity, type 2 diabetes mellitus (T2DM), liver dysfunction and kidney dysfunction [3–5]. A 182-case study of patients with Alström syndrome found that 30% of patients were hypertensive and 49% had nephropathy including end-stage renal disease that caused the death of 7 patients [6]. Furthermore, over 90% of patients had obesity and hyperinsulinemia, and 68% of patients developed T2DM [6]. In a meta-analysis of genome-wide association data from 67,093 individuals, *ALMS1* was identified as a new gene locus associated with renal function and chronic kidney disease [7]. More recently, a comprehensive multisystem evaluation study on 38 patients with Alström syndrome reported that estimated glomerular filtration rate (eGFR) progressively decreased as patients aged and 18% of them had chronic kidney disease (eGFR < 60mL/min/1.73m^2 and proteinuria) [8]. Although there was significant association between markers of kidney disease and markers of metabolic disease such as T2DM, HbA1c, HOMA-IR, HDL cholesterol, triglycerides and hypertension, age adjustment negated the significance, suggesting that kidney disease is a primary manifestation of the Alström syndrome resulting from dysfunctional ALMS1 rather than just secondary to the metabolic dysregulation [8].

The *fat aussie (foz)* mouse strain is a murine model of Alström syndrome caused by a spontaneous 11-bp deletion in exon 8 of *Alms1* [9]. *Foz* mice develop obesity associated with hyperphagia, which results in hyperglycemia and diabetes with >10 mM fasting blood glucose and glycosuria at about 5 months of age. Consistent with a role in cilia formation, reduced hearing and male infertility due to defective spermatogenesis were also observed in *foz* mice [9]. Two other mouse models of Alström syndrome were established by N-ethyl-N-nitrosourea (ENU)-mediated mutagenesis at exon 10 and a gene-trap insertion downstream of exon 13 of *Alms1*, respectively [10, 2]. These two mouse strains similarly recapitulate pathologic phenotypes of Alström syndrome including evidence for renal dysfunction such as dilated proximal tubules and increased urinary protein excretion [10, 2]. According to single nuclear RNA-seq data in the healthy mouse kidney, *Alms1* mRNA is present in multiple tubular and collecting duct segments (http://humphreyslab.com/SingleCell/search.php) and the expression has been confirmed on the protein level in the thick ascending limb and collecting duct of the rat kidney [11]. Despite the proposed role of Alms1 in cilia formation and function, kidney cilia appear to be normal in the *Alms1* mutant mice except for the lack of cilia in some proximal tubules [10, 2]. Moreover, Alms1 has been proposed to interact with components of intracellular endosome trafficking [12], and a recent study indicated that Alms1 negatively regulates the trafficking of the renal $Na^+/K^+/2Cl^-$ cotransporter NKCC2; as a consequence, NKCC2-mediated NaCl transport in the thick ascending limb was increased in *Alms1* knockout (KO) rats, which led to increases in urine osmolarity as well as higher blood pressure on a normal salt diet and a stronger increase in response to high-salt intake [11].

The renal pathophysiology of murine models and humans with Alström syndrome remains incompletely understood, including the modification of the phenotype by environmental factors. The present study was performed to further characterize the renal phenotype of *foz* mice. More specifically, the effect of a Western diet on the renal phenotype was investigated. Study read-outs included plasma creatinine and cystatin C as an indirect measure of GFR, urinary albumin to creatinine ratios (UACR), and renal expression of kidney markers of injury, inflammatory and fibrosis, as well as kidney morphology.

Materials and Methods

Animals.

Foz/foz mice (*foz*) were kindly provided by Dr. Geoffrey C. Farrell (the Australian National University Medical School at The Canberra Hospital, Australia). Male *foz* mice [13] and WT littermates on a C57BL/6 background were maintained in a pathogen-free environment under a 12h light–dark cycle at the UCSD animal housing facility. At 6 weeks of age, mice were placed on either a standard chow diet or a Western diet (WD, AIN-76A; 20% fat, 0.2% Cholesterol and 34% sucrose by weight; energy $(Kcal/g)^2$ 4.49; Test Diet) for 3 months. The salt content in both diets was in the normal range for murine experiments, and slightly lower in the WD (0.24 vs 0.6%). Two additional groups of WT and *foz* mice were maintained on the WD for 6 months. All the experiments were approved by the University of California, San Diego Institutional Animal Care and Use Committee.

Glucose tolerance test.

Intraperitoneal glucose tolerance test was conducted after 3 months of WD. After a 6 h fast (9 am to 3 pm), mice received a glucose bolus (2 mg/g body weight i.p.) and tail-vein blood samples were taken at 0, 15, 30, 60 and 120 min for measurement of glucose using a glucometer (Accu-chek Active, Roche Diagnostics). The area under the blood glucose level curve was determined using Prism software v6.07 (Graphpad).

Plasma creatinine, cystatin C and leptin measurement.

Blood was collected under terminal anesthesia (5% isoflurane) via heart puncture at the time of euthanasia. Plasma creatinine concentration was determined by isotope dilution liquid chromatography tandem-mass spectrometry by the O'Brien Center for Acute Kidney Injury Research at the University of Alabama-Birmingham. Plasma cystatin C and leptin levels were determined using ELISA kits (MSCTC0; MOB00, R&D systems) according to the manufacturer's instruction.

Urine analyses.

Urine was collected a week before euthanasia by grabbing the mice and stimulating spontaneous urination. Albumin was measured by a mouse albumin ELISA kit (Bethyl Laboratories) in urine samples diluted 1:500 (using sample diluent provided by kit). Urine creatinine concentration was determined by a kinetic modification of the Jaffe's reaction (TR35121; Infinity, Thermo Scientific). Urine osmolality was measured via a vapor pressure osmometer (Vapro 5520, Wescor). Urine glucose concentration was determined by the

hexokinase/glucose-6-phosphate dehydrogenase method (TR15421; Infinity, Thermo Scientific).

Quantitative RT-PCR.

Total RNA from snap-frozen half kidneys was isolated using RNeasy Plus Mini Kit (Qiagen), and cDNAs were synthesized with 5 μg of total RNA via SuperScript IV First-Strand Synthesis System (Life Technologies). Fifty ng of cDNA were mixed with TaqMan Universal PCR Master mix (Applied Biosystems) and TaqMan Gene Expression Assay primers (Thermo Fisher Scientific), and PCR was performed using Quant Studio 6 Flex Real-Time PCR system (Applied Biosystems). The relative expression level was determined using the $2^{-\Delta Ct}$ method ($\Delta Ct_{sample} = Ct_{sample} - Ct_{HPRT}$). Primers used in the study are listed in Table 1.

Immunohistochemistry.

Tissues were fixed in neutral buffered 10% formalin solution for overnight at room temperature. Immunostaining and quantification of renal CD68-positive cells were previously described [14]. CD68 is a lysosomal/endosomal glycoprotein highly expressed in macrophages and other types of mononuclear phagocytes [15, 16]. Briefly, paraffin-embedded renal tissue sections were hydrated and heat-induced epitope retrieval was performed in 10 mM Citrate buffer (pH 6.0). Endogenous peroxidase activity was quenched by incubating in 3% H_2O_2 for 5 min and blocking was done using 2.5% normal goat serum in PBS for 30 min. Tissues were then incubated with anti-CD68 antibodies (diluted 1:1,000; ab125212, Abcam) for overnight at 4°C as previously described. After washing, ImmPRESS secondary antibody HRP conjugated polymers (MP-7451; Vector Laboratories) were applied for 30 min at room temperature, and HRP activity was detected using a DAB substrate kit (SK-4100; Vector Laboratories). Stained slides were scanned using Axio Z1 slide scanner (Carl Zeiss), and the number of nuclei and CD68 positive cells were determined by QuPath and ImageJ.

Immunostaining of cilia was performed similarly as above using anti-acetyl-α-tubulin antibody (diluted 1:800; #5335; Cell Signaling Technology), and anti-rabbit IgG conjugated Alexa Fluor 647 antibody was applied together with Fluorescein-labeled lotus tetragonolobus lectin (LTL; FL-1321; Vector laboratories) to detect cilia and brush borders of proximal tubules. Ten tubules per each segment (total 50 tubules per kidney) that have identifiable luminal cilia were manually counted, and percentage of cilia positive cells was calculated by (number of cilia in tubule/number of cells in tubule using nuclear DAPI staining) ×100.

Histological analyses.

Masson’s trichrome staining was performed by Tissue Technology Shared Resource at the University of California-San Diego. Blue color stained area in the scanned image was determined by ImageJ. For tubular injury examination, tissue sections were stained using Periodic Acid-Schiff (PAS) kit (Sigma-Aldrich), and subsequently examined by a renal pathologist without knowing sample identity using the following score criteria: 0, no change;

1, up to 25%; 2, 25–50%; 3, >50% injury. Injury scoring was based on three commonly used features of injury: cast formation, tubular dilation, and tubular necrosis [17].

Statistical analysis.

Statistical analyses were performed by Sigma plot v14 (Systat). Outliers were identified (greater or lower than mean ± 2 × standard deviations of the group) and excluded from data analysis. For the analysis of 3-month regimen groups, two-way ANOVA was used to detect effects of Western diet or *foz* genotype. When the P value of interaction was below 0.05, multiple paired group comparisons were done with Holm-Sidak correction. For the analysis of the two 6-month WD groups, Student t-test was used. $P<0.05$ was considered statistically significant.

Results

Body weight and kidney weight were higher in foz mice on normal and Western diet.

Animals with mutant *Alms1* have been reported to have increased body weight compared with controls [10, 18, 9, 11]. Consistent with those studies, body weight of normal chow (NC)-fed *foz* mice was 50% higher than that of wildtype (WT) mice (Fig1 A). Applying the WD regimen for 3 months increased body weight in WT and *foz* mice, and the WD enhanced the difference between genotypes (Fig1 A). After feeding with WD for 6 months, body weight was 56% higher in *foz* mice than WT mice (Fig1 A). Similarly, kidney weight was higher in *foz* versus WT mice on NC or WD (Fig1 B). Because body weight and kidney weight were increased to a similar extent in *foz* versus WT mice, there was no significant difference between groups when kidney weight was normalized by body weight at the 3 month time point on NC or WD (Fig 1C). After 6 months on a WD, *foz* mice had modestly higher kidney to body weight ratios than WT mice ($p=0.0021$, Fig 1C).

Similar blood glucose levels in foz and WT mice on Western diet.

Previous studies showed that *Alms1* mutant mice on normal chow are hyperglycemic and hyperinsulinemic [10, 18, 9]. In comparison, an i.p. glucose tolerance test performed after 3 months on a WD revealed similar basal blood glucose levels (after 6 hrs of fasting) and subsequent blood glucose profiles in *foz* and WT mice (Fig1 D & E).

Increased plasma leptin levels in foz mice.

Plasma leptin levels were higher in *foz* vs WT mice on NC and after 6 months of WD (Fig1 F). Elevated serum leptin levels and leptin mRNA levels in fat tissue have previously been reported in chow-fed and high fat diet-fed *foz* vs WT mice [19]. Together with the well-documented hyperphagia in the *foz* mouse model [9], these findings point to a potential leptin resistance in *foz* mice.

Evidence for preserved GFR and increased glucosuria in foz mice on normal and Western diet.

In *Alms1* KO rats, glomerular filtration rate (GFR) was similar to WT rats [11]. To estimate GFR in foz mice, plasma creatinine and cystatin C concentrations were measured. The

current study revealed that plasma creatinine levels were decreased in foz mice vs WT mice in both 3-month NC and WD regimens (Fig2 A), suggesting that GFR was not reduced in foz mice. In accordance, plasma cystatin C levels were not different among groups (Fig2 B). These results suggested that GFR was preserved in foz mice regardless of diet. Nonetheless, glucosuria (measured as urine glucose to creatinine ratios) was slightly higher in foz mice compared with WT mice irrespective of NC or WD (Fig2 C).

Effects of mutant Alms1 on urine osmolality and renal renin mRNA expression.

It has been proposed that Alms1 regulates the trafficking of $Na^+/K^+/2Cl^-$ cotransporter (NKCC2) thereby enhancing NKCC2 activity and increasing urine osmolality and blood pressure in *Alms1* KO rats [11]. In the 3-month groups on NC, *foz* mice showed a trend of higher urine osmolality vs WT mice (Fig2 D), associated with a trend of lower renal renin mRNA expression in the former (Fig2 E), potentially reflecting enhanced renal fluid retention in *foz* mice. WD significantly decreased urine osmolality in both genotypes ($p<0.0001$).

Western diet promotes renal injury, inflammation and fibrosis in foz mice.

Previous studies reported dilated proximal tubules, Ki67-positive proliferating tubular cells, and apoptotic kidney cells in 3- to 6- month old mice with *Alms1* mutation, suggesting renal injury in those mice [10, 2]. In the current study, the renal expression of tubular injury markers *Kim1* and *Ngal* was determine by RT-PCR. The tubular injury markers were higher in both 3-month and 6-month WD-fed *foz* versus WT mice but not in NC-fed mice (Fig3 A & B). Likewise, focal cortical acute epithelial tubular injury, defined as dilated tubules associated with loss of brush border or eosinophilic casts, were only observed in WD-fed *foz* kidneys (arrows, Fig3 C & D).

WD increased cortical tubular vacuolization in both WT and *foz* kidneys at the 3-month time point (arrowheads, Fig3 C and E), likely reflecting fat droplet and lysosome accumulation [20]. Tubular vacuolization may have been slightly increased in *foz* compared with WT mice fed a WD for 6 months (arrowheads, Fig3 C and E).

It was shown that 3- to 6-month old *Alms1* mutant mice are associated with increased urinary protein compared with WT mice [2]. Consistent with those findings, urinary albumin to creatinine ratio (UACR) was significantly increased in *foz* mice compared with WT mice on NC or WD with a significant interaction between genotype and WD, the latter further increasing the difference (Fig3 F).

Similar to the findings in kidney injury markers, RT-PCR analysis showed increased renal expression of pro-inflammatory genes (*Tnf*, *Il6*, *Il10*, and *Ccl2*) in WD-fed *foz* versus WT mice, whereas those inflammatory markers were not significantly upregulated in NC-fed mutant versus WT mice (Fig4 A–D).

WD feeding increased the number of CD68-positive kidney cells, a marker of macrophage infiltration, in 3-month regimen groups ($p=0.017$). *Foz* mice fed with WD for 6 months were associated with 3 times more CD68-positive cells in the kidney versus WT mice (Fig4 E &

F). Overall, the data indicate that Western diet promoted upregulation of renal markers of injury and inflammation in *foz* mice versus WT mice.

Subsequent analyses investigated whether increased renal injury and inflammation in WD-fed *foz* mice were associated with renal fibrosis. Renal expression of fibrosis markers was analyzed by quantitative RT-PCR. The fibrosis-associated genes *Col1A1* and *Timp1* were upregulated in WD-fed *foz* versus WT kidneys, with no significant difference between genotypes in NC-fed mice (Fig5 A–C). Moreover, the renal mRNA expression of Tgf-beta was higher in *foz* compared with WT mice, particularly on a WD (Fig5 C). Furthermore, Masson's trichrome staining of kidney tissues displayed increased collagen deposits in *foz* compared with WT mice only in WD-fed groups (Fig5 D & E). These results indicate that the WD promoted renal injury, inflammation and fibrosis in *foz* mice versus WT controls.

Fewer cilia positive cells in renal tubules in foz mice.

Alström syndrome is considered a ciliopathy inasmuch as Alms1 protein localizes to the basal body and mutant Alms1 has been associated with stunted cilia formation [1, 9, 2]. Furthermore, it has been proposed that defects in cilia formation and function may contribute to renal pathogenesis in patients with *ALMS1* mutation. Notably, a study in fibroblasts derived from an Alström syndrome patient indicated that ciliogenesis appears to be normal [12]. In order to determine whether cilia formation in renal tubules is affected in *foz* mice, the percentage of cilia positive cells was quantified via immunostaining in 5 groups of tubular and collecting duct segments: LTL positive proximal tubules in either cortex or outer stripe of outer medulla (OSOM), LTL negative other segments in either cortex or OSOM, and segments in the inner stripe of the outer medulla (ISOM) (Fig6 A). The percentage of cilia positive cells was significantly lower in *foz* vs WT mice in all groups of segments studied (Fig6 B–F). Moreover, significant interactions were observed between WD and genotype in the 3 months experiment in LTL negative OSOM and ISOM segments; this reflected an increased percentage in response to WD in these segments only in *foz* mice.

Discussion

Previous studies showed that *Alms1* mutant mice recapitulate Alström syndrome, a genetic disorder that affects multiple organs and metabolism and is associated with obesity [3, 9]. Although renal dysfunction is highly manifested in patients with the syndrome, the renal pathophysiology is not completely understood. Furthermore, little is known about the effects of environmental factors on the disease progression. The main finding of the current study was that a Western diet (WD), which is high in fat and sucrose, promoted renal injury, inflammation and fibrosis in *foz* versus WT control mice, and that this sensitization may not have resulted solely from effects on glucose metabolism.

Using a murine model of Alström syndrome, the *fat aussie* (*foz*) mouse, the current study characterized renal function in the absence of an intact *Alms1* gene. This was done in mice fed a normal chow (NC) as well as when the mice were metabolically challenged by a WD. Body weight, kidney weight, and glucosuria were higher whereas plasma creatinine was lower in *foz* mice, more or less irrespective of the diet. Lower plasma creatinine values can reflect an increase in GFR, but can also be due to reduced muscle formation of creatinine or

enhanced tubular secretion of creatinine; the latter contributing 40–50% of renal creatinine excretion in normal mice [21]. Since plasma levels of cystatin C, a GFR marker that is not subjected to tubular secretion, were not significantly different between *foz* mice and WT mice, the combined data suggested that GFR was well preserved in *foz* mice. In accordance, direct GFR measurements in conscious *Alms1* KO rats revealed similar values to WT rats [11].

Renal markers of injury and inflammation were not different in NC-fed *foz* versus WT mice, when assessed at 4.5 months of age. Feeding a WD for 3 or 6 months (initiated at 6 weeks of age), however, promoted renal injury, inflammation and fibrosis in *foz* versus WT control mice. These renal changes were not strong enough to increase plasma creatinine or cystatin C and thus likely were not associated with a decline in GFR. The WD, however, promoted a further increase in albuminuria and renal fibrosis in *foz* versus WT mice.

It is currently unknown how a WD sensitizes *foz* mice to renal injury, inflammation and fibrosis. Basal blood glucose levels (after 6 hrs of fasting) and the blood glucose profile after a glucose load were similar in *foz* and WT mice that had been fed a WD for 3 months. These results provided first experimental evidence that the WD-induced greater susceptibility of *foz* mice to renal injury, inflammation and fibrosis may not be solely due to a gluco-metabolic effect. Notably, also clinical studies suggested that kidney disease is a primary manifestation of the Alström syndrome resulting from dysfunctional ALMS1 rather than just being secondary to the metabolic dysregulation [8].

Previous studies in rats have linked a loss of Alms1 function to increased NKCC2 activity in the thick ascending limb (TAL) [11]. This was due to altered cellular NKCC2 trafficking, which increased NKCC2 expression in the luminal membrane with the resulting increase in net NaCl reabsorption in the TAL, potentially contributing to the observed increase in blood pressure in those rats [11]. Further studies are needed to determine whether enhanced renal NaCl retention and an increase in blood pressure contribute to kidney disease in patients with Alström syndrome.

The Alström syndrome is considered a ciliopathy [22, 23, 8] and the results of the current study are consistent with a reduced number and/or length of cilia in various segments of the *foz* mouse kidney, including proximal tubule segments in the cortex and OSOM. Interestingly, WD increased the percentage of cilia positive cells in *foz* mice, but not WT mice, in LTL negative (non-proximal tubules) segments in the OSOM as well as in ISOM segments. Previous studies showed that renal injury can increase cilia length in tubules, and that the increase in cilia length, in general, is greater in distal tubules and collecting ducts, which physiologically have longer cilia than proximal tubules [24–26]. Therefore, it seems possible that WD-induced tubular injury in *foz* mice may have increased cilia length and thereby enhanced the detectability of cilia positive cells in non-proximal tubule segments in OSOM and ISOM. The contribution of changes in cilia number and length in *foz* mice to the renal pathogenesis is unknown, and further studies are warranted.

Alström syndrome is a rare recessive genetic disease, but single nucleotide polymorphisms in the gene are associated with incident CKD [27]. Moreover, a meta-analysis of genome-

wide association data identified *ALMS1* as a new gene locus associated with renal function and chronic kidney disease [7]. Little is known, however, about the extent to which these polymorphisms affect target functions, like NKCC2 or other kidney-relevant genes, in vivo. The current study leads us to hypothesize that polymorphisms in *ALMS1* induce CKD in the general population in part by enhancing the deleterious effects of high dietary fat and sucrose on kidney outcome.

In conclusion, the current study shows that a Western diet, high in fat and sucrose, promoted renal injury, inflammation and fibrosis in mice with a loss of function mutation in *Alms1* versus WT control mice. This sensitization may not have solely resulted from effects on glucose metabolism. Further studies are needed to determine whether loss of function mutations or relevant polymorphisms in *ALMS1* sensitize the human kidney to the deleterious effects of high dietary intake of fat or sucrose.

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Disclosure Statement

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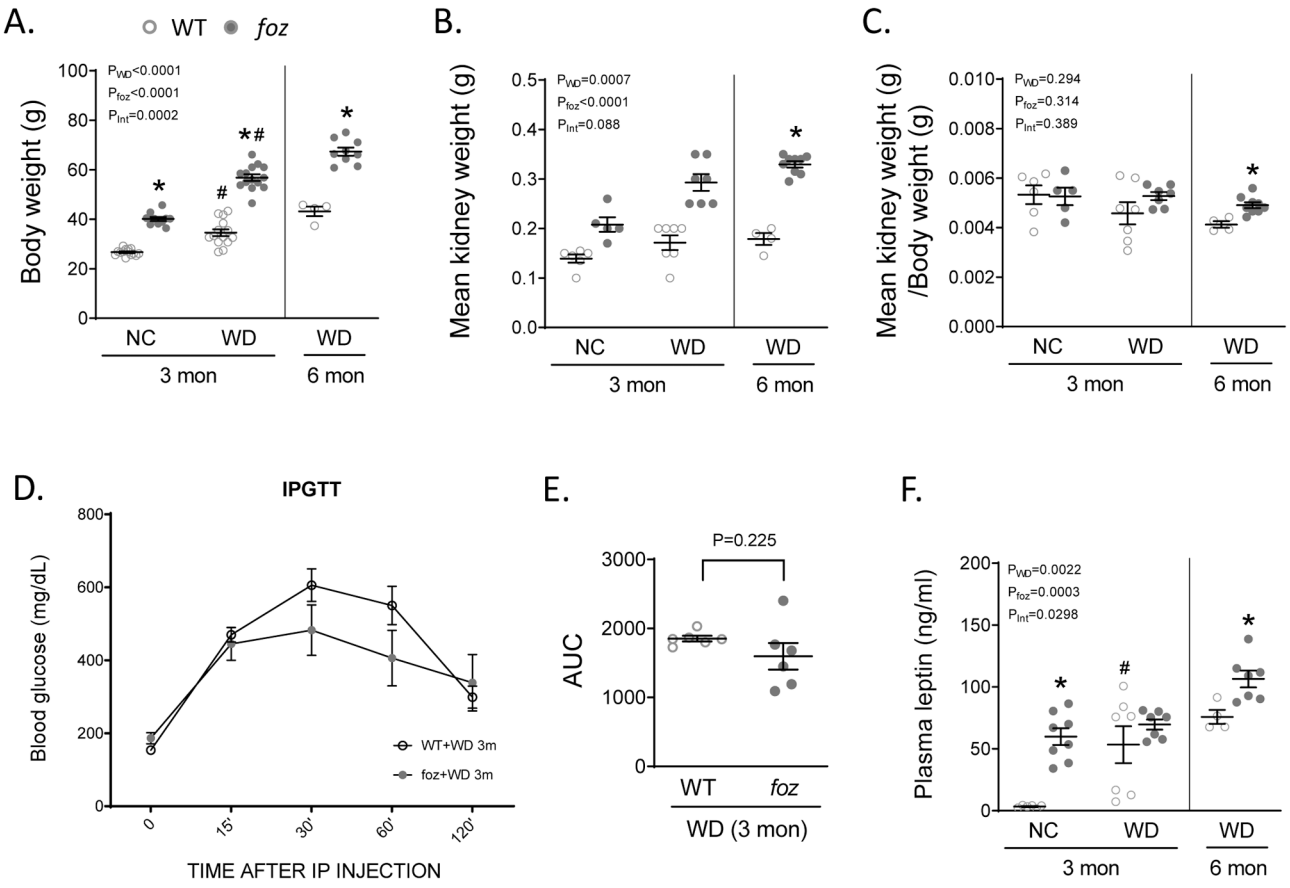


Figure 1. Body and kidney weight, glucose tolerance and plasma leptin in *foz* mice.

A. Mean body weight was higher in *foz* versus WT mice on normal chow (NC), and Western diet (WD) increased body weight in both genotypes and significantly enhanced the difference between genotypes. **B.** WD increased kidney weight in both WT and *foz* mice, and kidney weight was higher in *foz* versus WT mice regardless of diet. **C.** Due to parallel increases in kidney and body weight, there was no significant difference between WT and *foz* mice in the kidney to body weight ratio for the 3-month time point; slightly higher values were found in *foz* versus WT mice fed a WD for 6 months. **D & E.** Intraperitoneal glucose tolerance test (IPGTT): WT and *foz* mice fed a WD for 3 months received 2 mg glucose/g body weight intraperitoneally. Blood glucose was measured before (after 6 hour fast) and after the glucose load and the area under the curve (AUC) was determined. Basal blood glucose and AUC were not different between genotypes. **F.** Plasma leptin levels were higher in NC-fed *foz* mice vs NC-fed WT mice. WD increased plasma leptin levels in WT mice, and *foz* mice have significantly higher leptin levels compared with WT mice on WD for 6 months. Values represent means ± SE; n=4–14 per group. 3-month regimen: two-way ANOVA analysis was performed to probe for a significant effect of WD (P_{WD}), *foz* genotype (P_{foz}), or the interaction between the two factors (P_{int}). If interaction was statistically significant, then a pair-wise multiple comparison procedure (Holm-Sidak method) identified the significant effects; # P<0.05 vs. NC; * P<0.05 vs. WT. 6-month regimen: Student's t-test; * P<0.05 vs. WT.

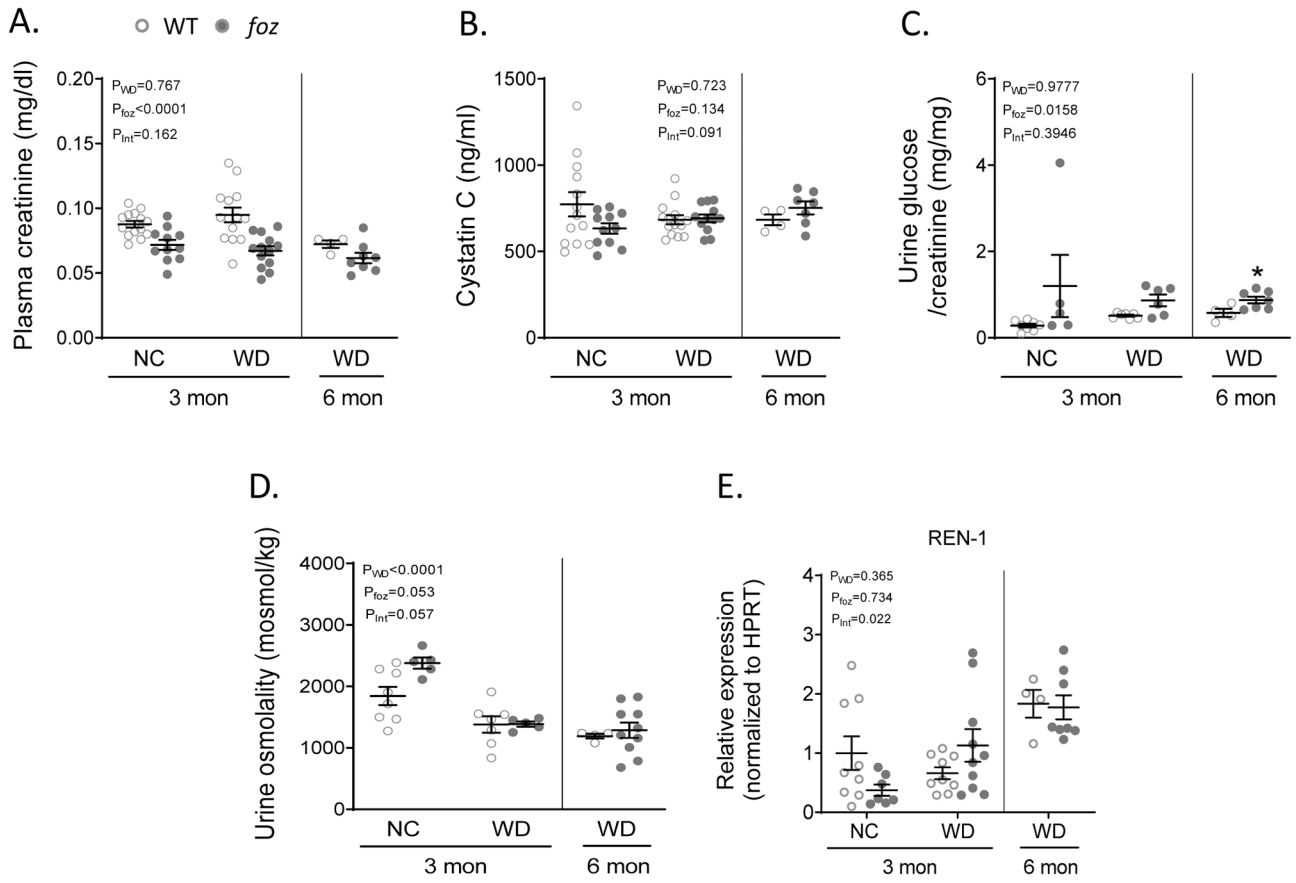


Figure 2. Kidney function in *foz* mice.

A-B. Measurements of plasma creatinine and cystatin C concentrations suggested that GFR was well preserved in *foz* mice compared with WT mice. **C.** Urine glucose to creatinine ratio was higher in *foz* versus WT mice. **D-E.** There was a trend for higher urine osmolality and lower renal renin mRNA expression in normal chow (NC)-fed *foz* versus WT mice. Urine osmolality and renal renin mRNA in mice on a Western diet (WD) were similar in both genotypes. Values represent means ± SE; n=4–14 per group. 3-month regimen: two-way ANOVA analysis was performed to probe for a significant effect of WD (P_{WD}), *foz* genotype (P_{foz}), or the interaction between the two factors (P_{int}). If interaction was statistically significant, then a pair-wise multiple comparison procedure (Holm-Sidak method) identified the significant effects; # P<0.05 vs. NC; * P<0.05 vs. WT. 6-month regimen: Student's t-test; * P<0.05 vs. WT.

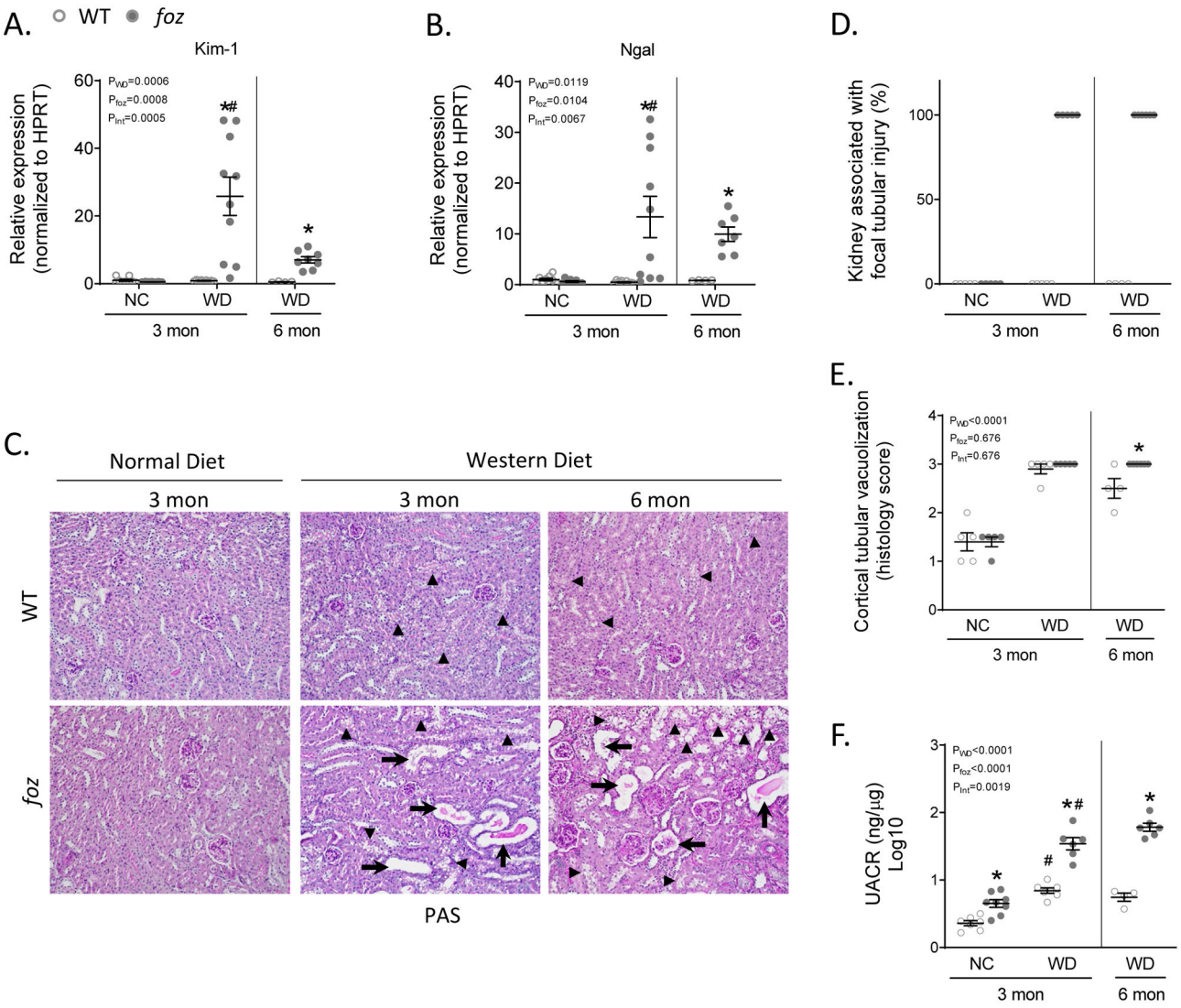


Figure 3. Western diet promoted renal injury in *foz* mice.

A & B. Expression of kidney injury marker genes, *Kim1* and *Ngal* was determined by quantitative RT-PCR. Expression of the tubular injury markers were increased in Western diet (WD)-fed *foz* vs WT mice (NC, normal chow). **C.** Histopathological analysis of Periodic acid-Schiff (PAS) stained kidney tissues. Focal acute cortical tubular injury (arrow) was only observed in the WD-fed *foz* kidneys. WD induced tubular vacuolization (arrowhead) in both WT and *foz* mice. Representative images are shown. **D.** All examined WD-fed *foz* kidneys were associated with focal tubular injury, but no injury was observed in NC-fed *foz* and WT kidneys. **E.** Histological scoring of cortical tubular vacuolization. WD increased tubular vacuolization in both genotypes, and slightly higher vacuolization was found in *foz* vs WT mice fed with WD for 6 months. **F.** Urinary albumin to creatinine ratio (UACR) was higher in *foz* versus WT mice on NC and WD. WD increased UACR in both genotypes. The significant interaction between genotype and WD indicated that the WD further increased the difference in albuminuria between genotypes. Values represent means ± SE; n=4–10 per group. 3-month regimen: two-way ANOVA analysis was performed to probe

for a significant effect of WD (P_{WD}), *foz* genotype (P_{foz}), or the interaction between the two factors (P_{int}). If interaction was statistically significant, then a pair-wise multiple comparison procedure (Holm-Sidak method) identified the significant effects; # $P<0.05$ vs. NC; * $P<0.05$ vs. WT. 6-month regimen: Student's t-test; * $P<0.05$ vs. WT.

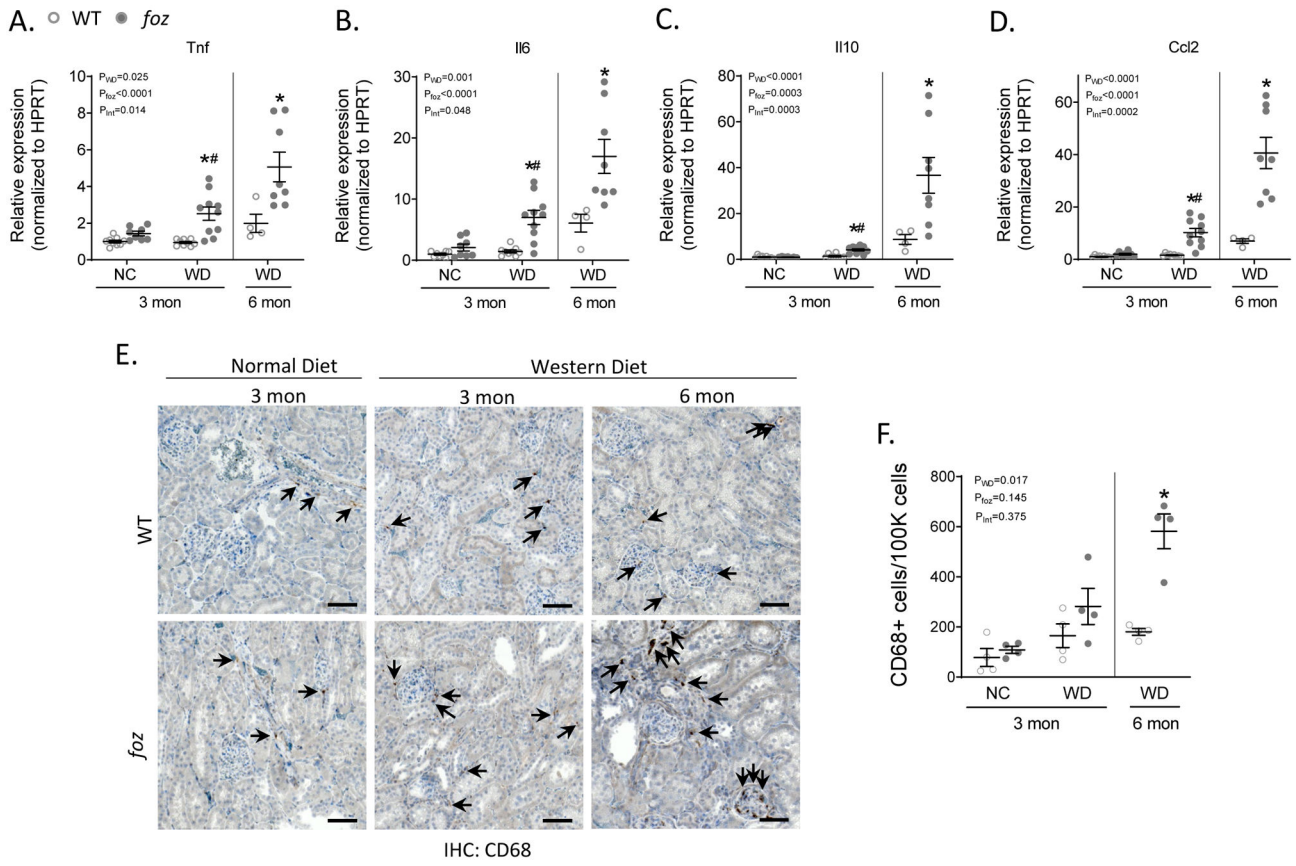


Figure 4. Western diet promoted renal inflammation in *foz* mice.

A-D. Quantitative RT-PCR analysis of renal expression of pro-inflammatory genes. Western diet (WD)-fed *foz* mice showed higher renal expression of pro-inflammatory markers (NC, normal chow). **E.** Immunohistochemistry of kidney tissues using anti-CD68 antibody. Representative images are shown. Scale bar, 50 μm. **F.** Quantification of CD68-positive cells (arrow) in kidney tissue indicated that WD increased the infiltration of CD68-positive cells in the 3-month regimen in both genotypes, and that the infiltration was significantly higher in *foz* vs WT mice fed a WD for 6 months. Values represent means ± SE; n=4–10 per group. 3-month regimen: two-way ANOVA analysis was performed to probe for a significant effect of WD (P_{WD}), *foz* genotype (P_{foz}), or the interaction between the two factors (P_{int}). If interaction was statistically significant, then a pair-wise multiple comparison procedure (Holm-Sidak method) identified the significant effects; # P<0.05 vs. NC; * P<0.05 vs. WT. 6-month regimen: Student's t-test; * P<0.05 vs. WT.

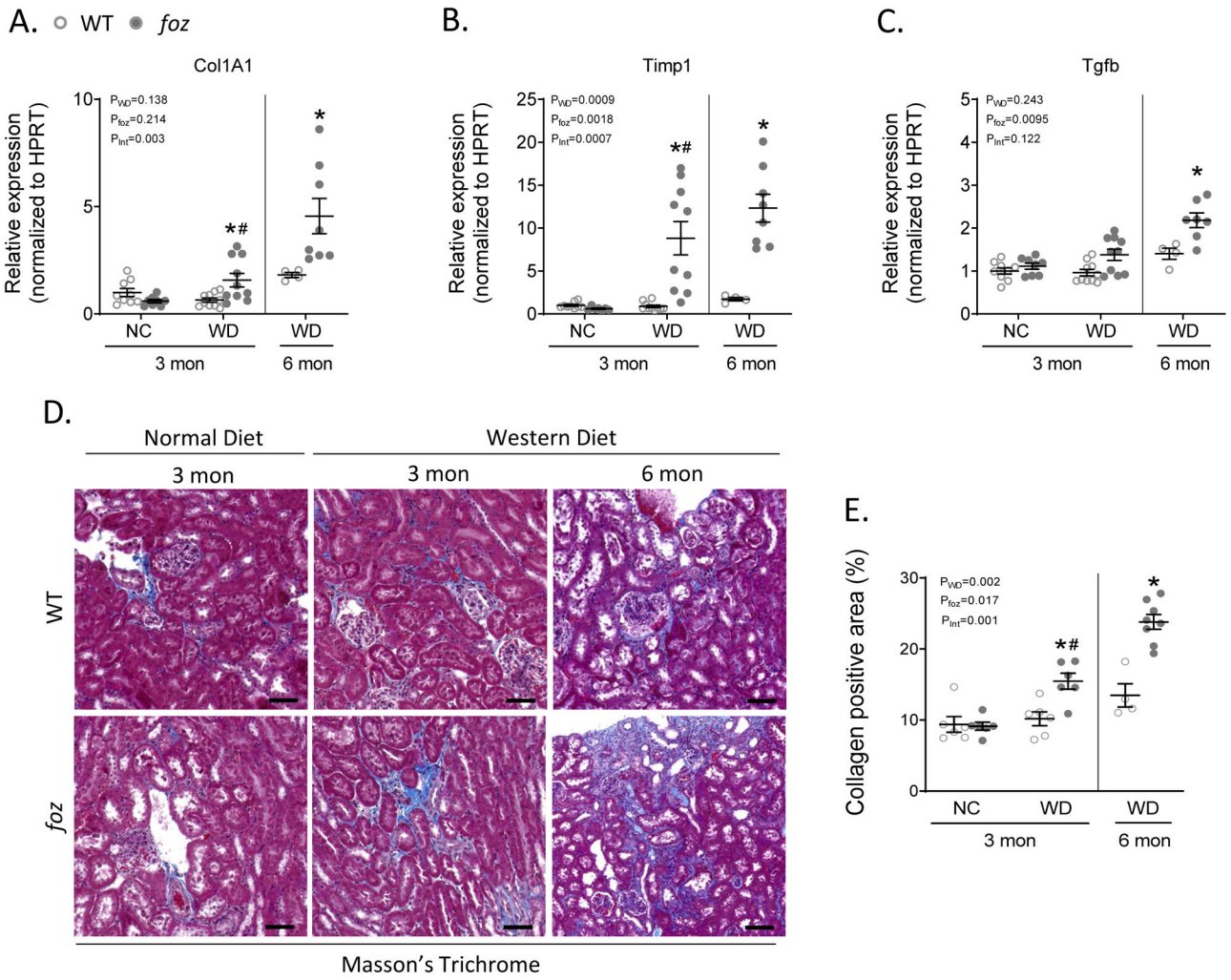
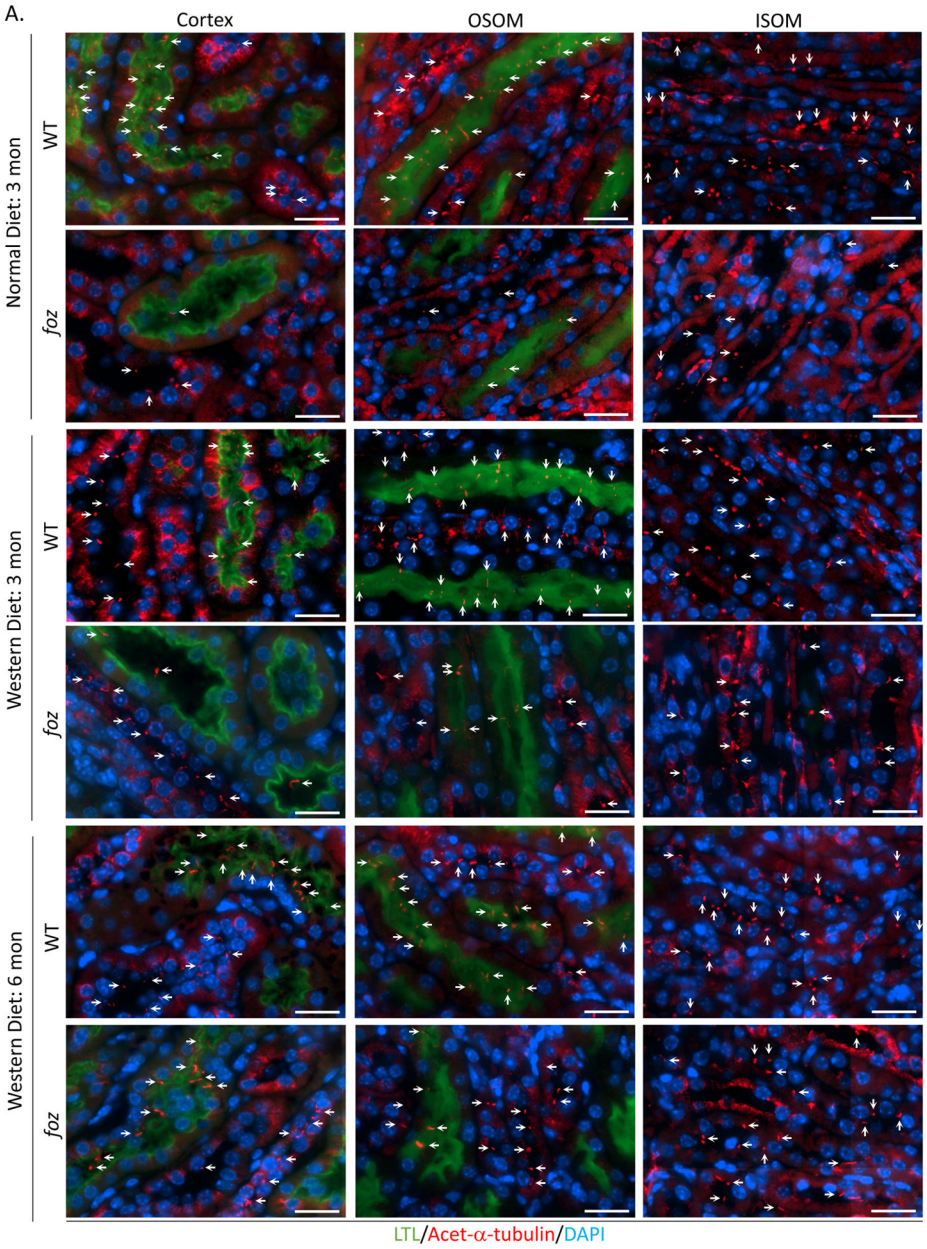


Figure 5. Western diet promoted renal fibrosis in *foz* mice.

A-C. Quantitative RT-PCR analysis of fibrosis markers. Renal expression of *Col1A1, Timp1* and *Tgf-beta* was higher in Western diet (WD)-fed *foz* vs WT mice (NC, normal chow). **D.** Masson's trichrome staining of kidney tissues. Representative images are shown. Scale bar, 50 μm. **E.** Quantification of collagen-positive areas showed increased renal fibrosis in WD-fed *foz* vs WT mice. Values represent means ± SE; n=4–10 per group. 3-month regimen: two-way ANOVA analysis was performed to probe for a significant effect of WD (P_{WD}), *foz* genotype (P_{foz}), or the interaction between the two factors (P_{int}). If interaction was statistically multiple comparison procedure (Holm-Sidak method) identified the significant effects; # P<0.05 vs. NC; * P<0.05 vs. WT. 6-month regimen: Student's t-test; * P<0.05 vs. WT.



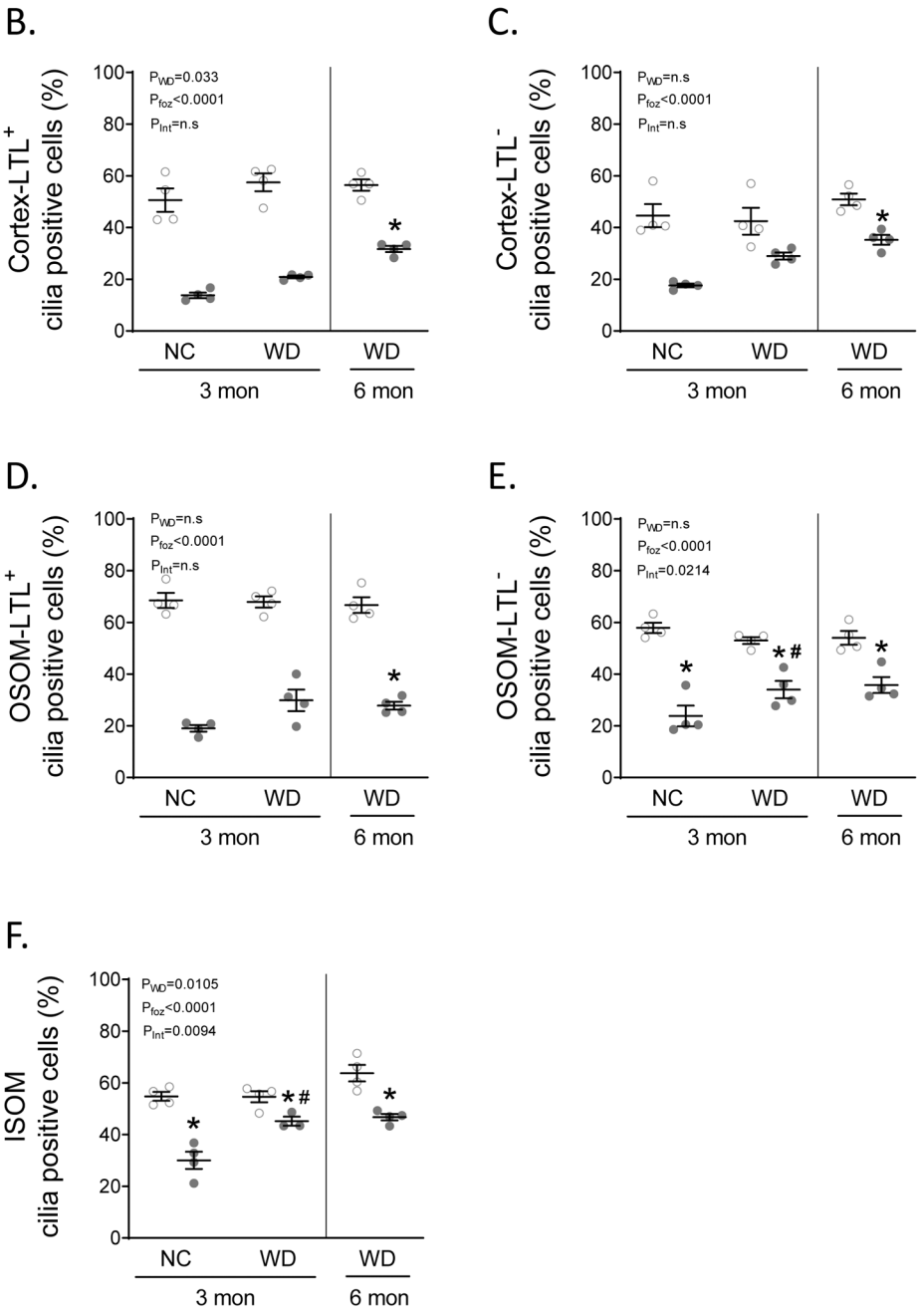


Figure 6. Fewer cilia positive tubular cells in *foz* mice.

A. Immunostaining of cilia and brush border using anti-acetyl-α-tubulin antibody and LTL, respectively. Arrows indicate luminal primary cilia. Scale bars, 20 μm. **B-F.** Quantification of cilia positive cells in cortex, outer stripe of outer medulla (OSOM), and inner stripe of outer medulla (ISOM) indicated significantly fewer cilia positive tubular cells in *foz* mice vs WT mice. Only luminal cilia were counted (from 10 tubules per segment), and the percentage of cilia positive cells was calculated [(number of cilia in tubule segment / number of cells in tubule segment using nuclear DAPI staining) × 100]. Values represent means ± SE; n=4–3 per group. 3-month regimen: two-way ANOVA analysis was performed to probe

for a significant effect of WD (P_{WD}), *foz* genotype (P_{foz}), or the interaction between the two factors (P_{int}). If interaction was statistically significant, then a pair-wise multiple comparison procedure (Holm-Sidak method) identified the significant effects; # $P<0.05$ vs. NC; * $P<0.05$ vs. WT. 6-month regimen: Student's t-test; * $P<0.05$ vs. WT.

Table1.

RT-PCR Primer list

Target gene	Assay ID
Col1a1	Mm00801666_g1
Ccl2/MCP1	Mm00441242_m1
HPRT	Mm00446968_m1
Il6	Mm00446190_m1
Il10	Mm00439614_m1
KIM1/Havcr1	Mm00506686_m1
Ngal/LCN2	Mm 01324470_m 1
Ren1	Mm02342889_g1
Tgfb1	Mm01178820_m1
Timp1	Mm00441818_m1
Tnf	M m00443258_m1