Urate-induced acute renal failure and chronic inflammation in liver-specific Glut9 knockout mice

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Preitner F, Laverriere-Loss A, Metref S, Costa AD, Moret C, Rotman S, Bazin D, Daudon M, Sandt C, Dessombz A, Thorens B. Urate-induced acute renal failure and chronic inflammation in liver-specific Glut9 knockout mice. Am J Physiol Renal Physiol 305: F786–F795, 2013. First published June 26, 2013; doi:10.1152/ajprenal.00083.2013.—Plasma urate levels are higher in humans than rodents (240–360 mg/dl vs. ~30 mg/dl) because humans lack the liver enzyme uricase. High uricemia in humans may protect against oxidative stress, but hyperuricemia also associates with the metabolic syndrome, and urate and uric acid can crystalize to cause gout and renal dysfunctions. Thus, hyperuricemic animal models to study urate-induced pathologies are needed. We recently generated mice with liver-specific ablation of Glut9, a urate transporter providing access of urate to uricase (Lg9KO mice). Lg9KO mice had moderately high uricemia (~120 mg/dl). To further increase their uricemia, here we gavaged Lg9KO mice for 3 days with inosine, a urate precursor; this treatment was applied in both chow- and high-fat-fed mice. In chow-fed Lg9KO mice, uricemia peaked at 300 mg/dl 2 h after the first gavage and normalized 24 h after the last gavage. In contrast, in high-fat-fed Lg9KO mice, uricemia further rose to 500 mg/dl. Plasma creatinine strongly increased, indicating acute renal failure. Kidneys showed tubule dilation, macrophage infiltration, and urate and uric acid crystals, associated with a more acidic urine. Six weeks after inosine gavage, plasma urate and creatinine had normalized. However, renal inflammation, fibrosis, and organ remodeling had developed despite the disappearance of urate and uric acid crystals. Thus, hyperuricemia and high-fat diet feeding combined to induce acute renal failure. Furthermore, a sterile inflammation caused by the initial crystal-induced lesions developed despite the disappearance of urate and uric acid crystals.

SLC2A9; glucose transporter 9; urate; crystal; acute renal failure; sterile inflammation

URATE IS THE METABOLIC DEGRADATION end product of purines in humans and great apes, whereas in lower species it is further degraded to the more soluble allantoin by the hepatic enzyme uricase. Humans, who lost uricase expression during evolution, exhibit high plasma urate levels (240–360 mg/dl) compared with mice (30–60 mg/dl). Evolutionary advantage of high urate levels is still controversial. Although urate is an antioxidant in vitro, it is still debated whether it is physiologically pro- or antioxidant. Urate is neuroprotective in vitro, and high plasma urate levels are associated with a reduced risk of developing Parkinson’s disease (41). Moreover, longevity among primates is highly correlated to serum and brain urate levels (4). On the other hand, high urate is a risk marker for cardiovascular and renal diseases in patients with hypertension, diabetes, and heart failure (8, 11, 25). A potential causal role of urate remains controversial.

Urate is relatively water-insoluble. Thus, saturation is often physiologically approached in human plasma and urine (20), and hyperuricemia increases the risk for urate precipitation in joints to cause gout or in kidney to cause uric acid nephrolithiasis and nephropathies. Acute uric acid nephropathy occurs in the setting of chemotherapy-induced tumor lysis that leads to an acute overproduction of urate, obstructive uric acid nephrolithiasis, and renal failure (31). Some patients with chronic hyperuricemia and gout present with a form of nephropathy characterized by tubulointerstitial fibrosis and glomerulosclerosis (14). However, chronic hyperuricemia alone is usually not sufficient to induce chronic interstitial nephritis and progressive kidney failure, and additional genetic/environmental factors are often involved (13, 20). Thus, features of the metabolic syndrome, including obesity, are associated with uric acid nephrolithiasis (20). Notably, a common etiology in the obese/diabetic uric acid stone formers is an overly acidic urine (20).

Genomewide association studies recently identified polymorphisms in the SLC2A9 gene coding for the urate transporter Glut9 as strong determinants of urate levels in humans (5, 6, 19, 39). Glut9 is highly expressed in liver and kidney (13, 36). Patients with inactivating mutations in Glut9 show idio- pathetic renal hypouricemia with high renal urate fractional excretion (1). They are usually asymptomatic except for the occasional development of uric acid nephrolithiasis, chronic renal dysfunction, or strenuous exercise-induced acute renal failure. We recently generated mice with systemic or liver-specific deletion of Glut9 (29). Systemic Glut9 knockout mice showed a very high urate fractional excretion, whereas both systemic and liver-specific Glut9 knockout mice were hyperuricemic and hyperuricosuric with a 20- to 30-fold higher urine excretion rate, reflecting the inability of plasma urate to enter hepatocytes and undergo degradation by uricase in the absence of the transporter (29). Although elevated, plasma urate levels in liver-specific Glut9 knockout mice are still low compared with human values.
To study the development of urate-induced pathologies in mice, we sought to further increase plasma urate levels in liver-specific Glut9 knockout mice by gavaging them with inosine, a metabolic precursor of urate. Here we describe the renal consequences of the treatment in lean chow-fed and obese high-fat-fed liver-specific Glut9 knockout mice.

Materials and Methods

Mice

Alb-CreERT2;Glut9lox/lox (LG9KO), and Glut9lox/lox (control) mice were generated (29) by sequentially crossing Glut9lox/lox mice with Alb-CreERT2 (33), then with resulting Alb-CreERT2; Glut9lox/+; and finally Alb-CreERT2;Glut9lox/lox offsprings to produce 50% of both Alb-CreERT2;Glut9lox/lox and Glut9lox/lox mice. At age 5 wk, all mice received three daily intraperitoneal injections of 1 mg/mouse tamoxifen (100 μl of 10 mg/ml (1:10 ethanol-sunflower oil); Sigma-Aldrich). Tamoxifen-induced hepatic deletion of Glut9 in Alb-CreERT2;Glut9lox/lox (LG9KO) was systematically confirmed by measurement of high plasma urate levels compared with Glut9lox/lox (control) mice.

Mice were housed four to five per cage at 23°C, with a 12:12-h light-dark cycle and free access to water and food. All studies were performed at University of Lausanne, Switzerland, and the procedures were reviewed in advance and approved by the Service Vétérinaire du canton de Vaud, Switzerland.

Mouse Cohorts

Experiment 1 (“day 3”). Six-week-old control and LG9KO mice were either maintained on regular chow diet (3436; Provimi Kliba) or placed on a coconut oil-based high-fat diet (HFD, 58% calories from fat, cat. D12331; Research Diets) for 3 mo. Some groups of mice were euthanized for blood and tissue collection without further treatment while others were gavaged one time daily at 9:00–10:00 A.M. for 3 days (days 0, 1, and 2) with 4 g/kg inosine (500 mg/ml in 0.5% carboxymethylcellulose aqueous suspension; Sigma-Aldrich) and euthanized 24 h after the last inosine gavage (day 3). Kidneys were dissected, and one kidney was fixed in paraformaldehyde 4% while the other kidney was cut longitudinally and frozen for later detection of the macrophage marker F4/80 was performed using a rat anti-F4/80 primary antibody (1:800) and a horseradish peroxidase-conjugated goat anti-rat Ig secondary antibody (1:100) (Invitrogen). Cryosections of kidney were obtained from absolute ethanol-fixed kidneys to assess anisotropism of uric acid crystals under polarized light. Immunostaining images of full kidney sections were taken using a Nikon Coolscope image acquisition system.

Synchrotron Analysis

Samples. Five micrometer kidney cryosections were deposited on low-e microscope slides (MirrIR; Kevley Technologies, Tienta Sciences). Synchrotron FTIR microspectroscopy measurements were carried out at SOLEIL Synchrotron (St. Aubin-Gif sur Yvette, France) on the SMIS beamline (7). The IR microspectroscopic mappings were collected in reflection mode using an Infrared microscope (Nicplan Thermo-Nicolet) coupled to a FTIR spectrometer (Magma 550-Thermo-Nicolet). The IR microscopes are equipped with a motorized sample stage (precision 1 μm) and a liquid nitrogen-cooled mercury cadmium telluride (MCT-250 μm) detector. Most of the analysis and maps presented here were achieved with a projected area on the sample of 6×6 μm² and a step size of 6 μm, and each spectrum was acquired after 64 accumulations at 8 cm⁻¹ spectral resolution. Data acquisition and processing was performed using Omnic software (version 7.4; Thermo-Scientific). The compounds were identified by comparing them with reference spectra (36).

RNA Extraction and Real-Time PCR

Total kidney RNA was prepared using TRIFAST PegGold (PEQLAB, Fareham, UK) and purified on RNeasy minicolumns (QIAGEN, Hombrechtikon, Switzerland). First-strand cDNA was synthesized from 2.5 μg of total RNA using random primers (Promega, Mannheim, Germany) and Superscript II reverse transcriptase (Invitrogen, Zug, Switzerland). Real-time PCR was performed using Power SYBR Green Master Mix (Applied Biosystems). All reactions were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) levels. Specific mouse primer pairs were used for the genes: α-smooth muscle actin: forward 5’-AAAACAGGAATACGACGAAG-3’, reverse 5’-CAGGAATGTATGGAAGAAGA-3’; F4/80: forward 5’-CCAGCATACTCCAGCAAGA-3’, reverse 5’-ACATCATGTTTC-CAGGAGACA-3’; GAPDH: forward 5’-CTCCACATCGGCAA-ATTCA-3’, reverse 5’-AAGATGGTGATGGGCTTCCC-3’; tumor necrosis factor (TNF-α): forward 5’-CCTCCTACACTAGCCTCT-3’, reverse 5’-GCTACAGACGGCTACAG-3’; IL-1β: forward 5’-GAACTCTGTTCTCAACTAC-3’, reverse 5’-ATCTTITGGG-GTCCGGTCAACT-3’.

Statistical Analyses

Statistical analyses were performed using R (version 2.11.1), on log-transformed data (to improve normality of distribution and to equalize variances among groups, as required for ANOVA and t-tests), using two-way ANOVA on factors genotype and diet, followed by Bonferroni-corrected post hoc t-tests with pooled SD, or paired t-tests to compare different time points, as indicated.

Results

Inosine Elevates Uricemia in LG9KO Mice

Male mice with a hepatic inactivation of Glut9 and controls were fed either chow diet or a HFD for 3 mo. Under HFD, mice became obese (controls 39 ± 1.4 g, LG9KO 39.3 ± 2.2 g) compared with chow-fed animals (controls 27.5 ± 0.9 g, LG9KO 27.9 ± 0.6 g). Plasma urate concentrations in LG9KO mice were elevated to similar levels under chow and HFD
feeding (Fig. 1, A and B). When fed either diet, LG9KO and control mice displayed normal kidney histology, no inflammation as assessed by staining for the macrophage marker F4/80 (Fig. 1, C and D), and no overt kidney dysfunction as revealed by normal plasma creatinine levels (Fig. 1, E and F).

In an attempt to further increase uricemia to human levels, we gavaged mice with the urate precursor inosine (4 g/kg daily for 3 days). This did not significantly alter uricemia in chow- or HFD-fed control mice (Fig. 1, A and B). In LG9KO mice fed chow, uricemia transiently rose 2 and 6 h after the first gavage and returned to their basal level 24 h after the last gavage (day 3) (Fig. 1A). In contrast, in HFD-fed LG9KO mice, inosine gavage induced a much higher hyperuricemia that reached 500 μM by day 3 (Fig. 1B).

Fig. 1. Inosine gavage elevates uricemia in LG9KO mice. A and B: plasma urate levels at basal, 2 and 6 h after the first inosine gavage, and 24 h after the last gavage (day 3) in control and LG9KO mice fed either a chow diet (A) or a high-fat diet (HFD) for 3 mo (B). C and D: histological analysis of the kidneys of control and LG9KO mice fed a chow diet (C) or a HFD (D) without inosine treatment. H&E, hematoxylin and eosin staining; F4/80, immunodetection of macrophage marker F4/80. E and F: plasma creatinine levels in control and LG9KO mice fed a chow diet (E) or a HFD (F) without inosine treatment. Data are means ± SE; n = 6–8 experiments. Plasma urate at day 3: interaction genotype × diet, P < 0.0003 (ANOVA); *largest P value = 0.0003 vs. control (Bonferroni); §largest P value = 0.008, £P = 0.03 between time points (paired t-test).
This strikingly high hyperuricemia suggested induction of renal failure. Plasma creatinine levels were normal in inosine-gavaged, chow-fed (Chow + Ino) control and LG9KO mice at day 3 (Fig. 2A). In contrast, in inosine-gavaged, HFD-fed (HFD + Ino) LG9KO, plasma creatinine levels were increased fivefold compared with HFD + Ino control mice (Fig. 2B), indicating renal failure.

Kidneys in Chow + Ino controls appeared normal, whereas LG9KO showed some tubular dilation but no macrophage infiltration (Fig. 2, C and E). Kidneys of HFD + Ino control mice were normal (Fig. 2, D and F). In contrast, kidneys of HFD + Ino LG9KO mice showed both tubular dilation and macrophage infiltration (Fig. 2D), with a higher renal mRNA expression of both F4/80 (3.5-fold, Fig. 2F) and proinflammatory cytokines TNF-α (12.8-fold, \( P = 0.001 \)) and IL-1β (6.5-fold, \( P = 0.023 \)). Thus, combining inosine treatment and HFD feeding in LG9KO mice elicits hyperuricemic levels similar to those in humans, acute renal failure, and kidney inflammation.

**Acute Renal Failure in HFD-Fed LG9KO Mice is Associated with Renal Urate and Uric Acid Crystal Deposition**

Urate-induced acute renal failure usually results from the occlusion of renal tubules by highly proinflammatory urate and uric acid crystals (3). Analysis of kidney sections from Chow + Ino LG9KO mice at day 3 revealed few small birefringent crystals (Fig. 3A). In contrast, crystal deposits were much larger and more abundant in kidneys from HFD + Ino LG9KO mice (Fig. 3A). No crystal was found in Chow + Ino or HFD + Ino control mice (data not shown).

Identity of crystals was determined by synchrotron analysis (Fig. 3, B–E). Infrared absorption spectra of intratubular crystals in the medulla (Fig. 3B) and of annular crystals in the...
Fig. 3. Inosine gavage induces urate crystal deposition in the kidneys of HFD-fed LG9KO mice. A: ethanol-fixed kidney sections observed under direct and polarized light show anisotropic urate crystals in renal tubules. Magnification is ×40 or ×400. B–E: analysis of renal crystals by infrared microspectroscopy using synchrotron radiation in kidney sections from inosine-gavaged LG9KO mice. B: medullary, intratubular crystals; C: papillary, annular crystals. D: infrared spectra of medullary crystals revealed mainly ammonium urate (peaks at 1,338, 1,133, 1,041, 1,003, 768, and 727 cm\(^{-1}\)); E: in papilla, crystals consisted mainly of uric acid (with typical absorption peaks at 1,123 and 993 cm\(^{-1}\)), in either the dihydrate (predominant), amorphous, or anhydrous forms. The distinction between anhydrous and dihydrate forms is based on the influence of water molecules on some vibrations of the purine cycle in the 1,350–1,300 and 1,100–1,000 cm\(^{-1}\) areas where peaks at 1,350, 1,306, and 1,026 cm\(^{-1}\) are characteristic for the anhydrous phase while peaks at 1,327 and 1,037 cm\(^{-1}\) correspond to the dihydrate form. In D and E, absorption peaks around 1,650 and 1,540 cm\(^{-1}\) correspond to the amide bands of proteins from the tissue.
NH4Cl supplementation (urine pH 5.70) generally, urine acidification in Chow- and HFD-fed LG9KO mice (Fig. 5).

Some infiltrates (lymphocytes and macrophages; Fig. 5, C) associated with tubular dilation, tubular atrophy (Fig. 5, C), focal interstitial fibrosis as revealed by fuchsin acid orange staining (Fig. 5, C) or uric acid (amorphous, dehydrate, dihydrate, polymorph ammonium, Fig. 3, D) or uric acid crystal formation in LG9KO mice. While in chow-fed LG9KO mice urine pH tended toward lower values (Fig. 4, B), in HFD-fed LG9KO mice, urine pH was 5.6, i.e., ~0.4 pH units more acidic than in control mice. Interestingly, urine acidification in Chow + Ino LG9KO mice by NH4Cl supplementation (urine pH 5.70 ± 0.01) elicited an increase of plasma creatinine (Fig. 4C) comparable to the effect of HFD to potentiate inosine-induced acute renal failure.

Thus, inosine-induced hyperuricemia leads to tubular urate and uric acid crystal formation. The more extensive crystal deposition in LG9KO mice on a HFD may be caused by their more acidic urine on that diet.

Renal Failure Reverses but Sterile Inflammation Progresses after Cessation of Inosine Gavage

To assess the possible progression of the renal condition, we studied mice 6 wk after the end of the 3-day inosine treatment. Body weights of HFD + Ino-fed mice were 37.5 ± 1.2 g in controls and 35.4 ± 2.1 g in LG9KO. In LG9KO mice, plasma urate and creatinine strikingly normalized (Fig. 5, A and B), indicating recovery from renal failure. However, histological analysis revealed renal shrinkage, with a lobular pattern associated with tubular dilation, tubular atrophy (Fig. 5C), focal interstitial fibrosis as revealed by fuchsin acid orange staining and qRT-PCR analysis of smooth muscle actin mRNA expression (Fig. 5, D and G), and extensive presence of mononuclear infiltrates (lymphocytes and macrophages; Fig. 5, C, E, and F) as revealed by immunohistochemical detection of CD3 and F4/80, respectively. No crystal was detected in kidneys of either control or LG9KO mice (Fig. 5H).

In Chow + Ino lean controls (29.5 ± 0.8 g), kidney morphology was normal, whereas, in Chow + Ino LG9KO mice (28.5 ± 0.7 g), it was only minimally altered at week 6 (Fig. 6A). Although rare foci of inflammation were apparent (Fig. 6B), LG9KO kidneys were mostly devoid of inflammation (Fig. 6, B and D) and fibrosis (Fig. 6, C and E).

DISCUSSION

Here we describe a model of controlled hyperuricemia in LG9KO mice acutely treated with inosine; we show that hyperuricemia and HFD feeding combine to provoke acute renal failure associated with obstructive tubular urate and uric acid crystal formation. Initial crystal-induced renal lesions elicit a rapid sterile inflammation that develops extensively beyond the acute inosine treatment to generate fibrosis and organ remodeling, despite the disappearance of urate crystals.

Using a genetic mouse model of impaired access of plasma urate to hepatic uricase (29), the LG9KO mice, we show that inosine gavage induced hyperuricemia comparable to the normal-to-high range in humans. To evaluate kidney function, we measured plasma creatinine levels, which have been widely used in studies of acute renal failure in mice (12, 15, 18, 24, 35, 37) as a convenient alternative to a more formal determination of glomerular filtration rate by inulin clearance test (28). As previously reported, chow-fed hyperuricemic LG9KO mice have normal plasma creatinine levels as well as renal morphology (29). Strikingly, 3 days of inosine gavage elicited an acute renal failure in HFD + Ino LG9KO mice, with four- to fivefold higher plasma creatinine levels, tubular dilation, and inflammation. In contrast, inosine treatment in Chow + Ino LG9KO mice elicited much milder effects with some tubular dilation but unchanged plasma creatinine and no inflammation. Thus, inosine-induced hyperuricemia and HFD feeding combine to induce acute renal failure in LG9KO mice.

The main cause of urate-induced acute renal failure is the precipitation of urate and uric acid crystals in urine that mechanically occlude renal tubules and provoke highly proinflammatory lesions (3). Inosine treatment of LG9KO mice induced the renal deposition of ammonium urate and uric acid crystals. These were of similar composition under both chow and HFD but were much more abundant under HFD.

Fig. 4. HFD-fed LG9KO mice have low urine pH, and low urine pH is a major determinant of inosine-induced acute renal failure. A and B: urine urate concentration in chow- and HFD-fed control and LG9KO mice, not treated with inosine (A), and pH of fresh urine spots (B) (n = 6–10). C: chow-fed LG9KO mice received NH4Cl 0.3 M in drinking water 24 h before the first inosine gavage, and throughout the inosine treatment. Plasma creatinine measured at basal and at day 3 (n = 5). Urine pH, genotype P < 0.05 (ANOVA); **; lowest P value = 1.4−6 vs. control (Bonferroni); *P = 0.02 vs. control (t-test) EP = 1.5−3 vs. basal (paired t-test).
humans, hyperuricemia per se is usually insufficient to cause uric acid stones. Uric acid nephrolithiasis is more common among patients with type 2 diabetes, obesity, and/or the metabolic syndrome, as a result of overly acidic urines that cause the precipitation of uric acid crystals (20). Here, we show that urine pH was more acidic in HFD-fed LG9KO mice than HFD-fed controls and that urine acidification in chow-fed LG9KO mice induced acute renal failure. Uric acid crystals are
favored at low pH (<5.35), whereas ammonium urate precipitates at pH >5.7. The distinct distribution of ammonium urate crystals in the medulla and of uric acid crystals in the papillum likely relates to local renal differences in ammonium concentration and/or in pH. Overall, low urine pH in HFD-fed LG9KO mice may be the important factor that combines with hyperuricemia to induce acute renal failure. Importantly, HFD-fed LG9KO mice were aciduric without inosine treatment (Fig. 4). Thus aciduria precedes inosine-induced uric acid crystal deposition. Urine pH was not acidified by HFD feeding in control mice, in line with other studies (40). Conversely, urine pH was lowered by the combination of HFD feeding and hepatic deletion of Glut9. Thus, increasing urine pH in HFD-LG9KO mice should protect from inosine-induced acute renal failure. However, usual protocols to alkalinize urine in chow-fed mice (citrate or bicarbonate supplementation) unexpectedly failed in HFD-fed mice.

An interesting finding is the long-term effect of an acute inosine treatment. At week 6, i.e., >5 wk after the last inosine gavage, HFD + Ino LG9KO mice displayed severe kidney remodeling with massive chronic inflammation and fibrosis, despite the disappearance of the initial stressor, i.e., urate and uric acid crystals. The initial crystal-induced inflammation, attested by elevated mRNA levels for F4/80, IL-1β, and TNF-α, developed after disappearance of the obstructive urate and uric acid crystal deposition, possibly as a result of both tissue lesions and persistent moderate hyperuricemia in LG9KO mice. Indeed, monosodium urate crystals of submicroscopic structure, as well as necrotic cells, are potent activators of the NALP3/NLRP3 inflammasome/IL-1β pathway (21, 34) and may therefore sustain a sterile inflammation.

Persistent hyperuricemia and hyperuricosuria might also affect kidney function by crystal-independent mechanisms, via activation of the intrarenal renin-angiotensin axis (22) and
induction of oxidative stress (2), which stimulate vascular smooth muscle cell proliferation and induce endothelial dysfunction. Interestingly, both pathways are also activated in obesity (10, 38), thus providing a possible additional link between hyperuricemia, HFD feeding, and sterile inflammation.

Urate is a potent antioxidant that contributes to 60% of total plasma antioxidant capacity (27). However, in conditions of endothelial redox stress, urate can undergo a paradoxical antioxidant-prooxidant switch (11). High urate levels within cells can become prooxidant (32) and induce oxidative stress and inflammation (2). In damaged tissues, the elevated nucleotide turnover from RNA-DNA breakdown increases the activity of xanthine oxidase to produce both urate and reactive oxygen species that can, in high amounts, increase tissue lesions and inflammation (11). These mechanisms may contribute to the development of inflammation.

The effect of acute inosine treatment in adult HFD + Ino LG9KO mice is reminiscent of the phenotype of chow-fed systemic Glut9 knockout (Glut9KO) mice that acutely develop an obstructive intratubular urate and uric acid crystal deposition, hydronephrosis, and inflammation at 2 wk of age, when mice naturally display a transient postnatal peak in urate urinary excretion (16) and a low urine pH (29). At 6 wk of age and beyond, when renal crystals have disappeared, the inflammatory reaction and fibrosis spread in Glut9KO kidneys, leading to severe organ remodeling and moderate chronic renal insufficiency (29). Not surprisingly, uricase knockout mice develop postnatal uric acid nephropathy and die from renal failure before weaning (42) unless rescued by injection of a long-lived form of the enzyme in circulation (16).

The inosine effect in HFD + Ino LG9KO mice is also reminiscent of the obstructive acute renal failure that developed in rats in which a 6- to 10-fold increase in serum urate (500 μM and above) was induced by chronic treatment with the uricase inhibitor oxonate and cosupplementation of urate (9, 17). In the absence of urate complementation, a 7-wk oxonate treatment induced only mild hyperuricemia (100–120 μM), which induced low-grade renal inflammation, fibrosis, subtle tissue injuries, impaired renal function, and systemic hypertension despite the absence of intrarenal crystal deposition (8, 22, 23). This is in contrast with chow-fed LG9KO mice where similar levels of hyperuricemia induced no renal inflammation, mild alterations in kidney function (lower urine concentration capacity), but no alterations in plasma creatinine or kidney histology (29). Discrepancies between these genetic and pharmacological models of hyperuricemia may stem from the different experimental approaches and species used. Moreover, the existence of chronic uric acid nephropathy is still debated (26).

In summary, we show that different levels of hyperuricemia can be obtained in mice by combining liver-specific genetic inactivation of Glut9 and inosine administration. Strikingly, hyperuricemia combines with HFD to induce obstructive acute renal failure, likely as a result of HFD-induced urine acidification leading to the formation of urate crystals. The renal failure can be reversed by cessation of inosine supplementation; this, however, does not prevent further development of sterile inflammation and fibrosis. Combining genetic ablation of Glut9 in liver with low-dose inosine supplementation should provide a novel rodent model compatible with long-term investigations of the role of hyperuricemia in the development of cardiometabolic diseases, including hypertension, insulin resistance, and atherosclerosis.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


2. Baldwin W, McRae S, Marek G, Wymer D, Pannu V, Baylis C, Baldwin W, McRae S, Marek G, Wymer D, Pannu V, Baylis C, Anzai N, Ichida K, Jutabha P, Kimura T, Babu E, Jin CJ, Srivastava S, Kitamura K, Hisatome I, Endou H, Sakurai H. Urate is a potent antioxidant that contributes to 60% of total plasma antioxidant capacity (27). However, in conditions of endothelial redox stress, urate can undergo a paradoxical antioxidant-prooxidant switch (11). High urate levels within cells can become prooxidant (32) and induce oxidative stress and inflammation (2). In damaged tissues, the elevated nucleotide turnover from RNA-DNA breakdown increases the activity of xanthine oxidase to produce both urate and reactive oxygen species that can, in high amounts, increase tissue lesions and inflammation (11). These mechanisms may contribute to the development of inflammation.


