No development of hypertension in the hyperuricemic liver-Glut9 knockout mouse

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Urate is the metabolic end point of purines in humans. Although supra-physiological plasma urate levels are associated with obesity, insulin resistance, dyslipidemia, and hypertension, a causative role is debated. We previously established a mouse model of hyperuricemia by liver-specific deletion of Glut9, a urate transporter that provides urate to the hepatocyte enzyme uricase. These LG9 knockout mice show mild hyperuricemia (120 μmol/l), which can be further increased by the urate precursor inosine. Here, we explored the role of progressive hyperuricemia on the cardiovascular function. Arterial blood pressure and heart rate were periodically measured by telemetry over 6 months in LG9 knockout mice supplemented with incremental amounts of inosine in a normal chow diet. This long-term inosine treatment elicited a progressive increase in uricemia up to 300 μmol/l; however, it did not modify heart rate or mean arterial blood pressure in LG9 knockout compared with control mice. Inosine treatment did not alter cardiac morphology or function measured by ultrasound echocardiography. However, it did induce mild renal dysfunction as revealed by higher plasma creatinine levels, lower glomerular filtration rate, and histological signs of chronic inflammation and fibrosis. Thus, in LG9 knockout mice, inosine-induced hyperuricemia was not associated with hypertension despite partial renal deficiency. This does not support a direct role of urate in the control of blood pressure.

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Urate is a product of purine metabolism.[1,2] In most mammals, urate is further metabolized to allantoin by hepatic enzyme uricase, leading to low plasma urate levels (30–60 μmol/l in mice). By contrast, humans and great apes lost uricase expression during evolution and therefore have higher plasma urate levels. In humans, plasma urate levels gradually increased from ~200 μmol/l in the 1920s to 350 μmol/l in the 1970s, in parallel with the increased intake of foods known to increase urate levels, including fructose and purine-rich foods (meat, seafood, and beer).[3,4] The evolutionary advantage of high urate levels remains highly debated.

Urate is an antioxidant in cell-free systems. Thus, uricase mutations and higher uricemia were postulated to provide a survival advantage by reducing oxidative stress.[5] In line with this, longevity among primates highly correlates with serum and brain urate levels.[6] Urate and/or its precursors are neuroprotective *in vitro*[7] and *in vivo* in mice,[8,9] and the presence of high plasma urate levels in humans is predictive of a lower risk for Parkinson's disease.[7] Also, urate infusion in humans acutely improves endothelial function.[10,11] However, soluble urate causes intracellular oxidative stress *in vitro* in adipocytes and in vascular smooth cells.[12,13] Moreover, highly proinflammatory uric acid crystals can form in persistent clinical hyperuricemia that is often associated with gout, kidney stones, and nephropathies.[1,2]

Hyperuricemia is a risk marker for renal and cardiovascular diseases in patients with diabetes, hypertension, and heart failure.[14–16] However, *in vivo* evidence for causation remains scarce and highly controversial.[17,18] In rats, urate elevation (by oxonate-induced uricase inhibition) induced over weeks an elevation of blood pressure, a form of salt-sensitive hypertension, and renal injuries.[19,20] In a pilot clinical trial of 30 adolescents with primary hypertension and borderline hyperuricemia, blood pressure was decreased by the hypouricemiant drug allopurinol (that blocks the production of urate by xanthine oxidase (XO)).[21] However, these results may not be generalized to adult hypertensive patients.[17,22]

Genome-wide association studies recently identified polymorphisms in *SLC2A9* encoding the urate transporter Glut9 as strong determinants of urate levels in humans.[23–26]

Gene polymorphisms in *SLC2A9* could explain up to 5–6% of the variance in serum urate, and were associated with an increased risk for gout, but not for hypertension or other features of the metabolic syndrome, reviewed in Hediger and in So.[1,2]

Glut9 is highly expressed in liver and kidney.[1,2] Patients with inactivating mutations in *GLUT9* show idiopathic renal hypouricemia with high renal urate fractional excretion.[27] 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.[28] Systemic Glut9 knockout mice showed a very high urate fractional excretion reflecting a suppression of renal urate reabsorption. Moreover, both systemic and liver-specific Glut9 knockout mice were hyperuricemic and hyperuricosuric with a 20- to 30-fold higher urate excretion rate, reflecting the inability of plasma urate to enter hepatocytes and undergo degradation by uricase in the absence of the transporter.[28] Although elevated, plasma urate levels in liver-specific Glut9 knockout mice are still low compared with human values. Previously, we increased plasma urate levels acutely in liver-specific Glut9 knockout mice up to human levels by gavaging them with inosine, a metabolic precursor of urate.[29] We showed that hyperuricemia and high-fat diet feeding combine to induce extensive urate crystal deposition in the kidneys, acute renal failure, and long-term renal sterile inflammation. The consequence of the inosine treatment was much milder in mice fed a chow diet, with no acute renal failure and rare signs of inflammation.[29]

To study the development of urate-induced pathologies in mice, we supplemented inosine in a chow diet at incremental doses. Here we measured arterial blood pressure and heart rate in LG9 knockout (KO) mice with controlled hyperuricemia.

RESULTS

Inosine elevates uricemia in LG9KO mice

Alb-CreERT2; Glut9lox/lox and Glut9lox/lox (control) mice ($N = 8$) fed a normal chow diet were implanted with indwelling carotid artery pressure telemetric devices at 8 weeks of age. One month post surgery, before induction of the hepatic deletion of Glut9, plasma urate was similar in both groups (Figure 1a). Tamoxifen-induced hepatic deletion of Glut9 in Alb-CreERT2; Glut9lox/lox (LG9KO) mice raised plasma urate levels to 170 µmol/l—i.e., 70% higher as compared with Glut9lox/lox (control) mice (Figure 1a and c).

To further increase uricemia, we supplemented chow diet with incremental amounts of the urate precursor inosine (0, 7.5, 10, and 15 g inosine/kg chow diet, INO7.5, INO10, and INO15, respectively). The treatment progressively increased plasma urate up to 300 µmol/l (Figure 1a). Interestingly, in control mice inosine decreased plasma urate instead.

The inosine treatment did not significantly alter plasma creatinine in LG9KO mice despite a consistent trend for elevated levels compared with controls (Figure 1b, $P = 0.11$, repeated measure analysis of variance).

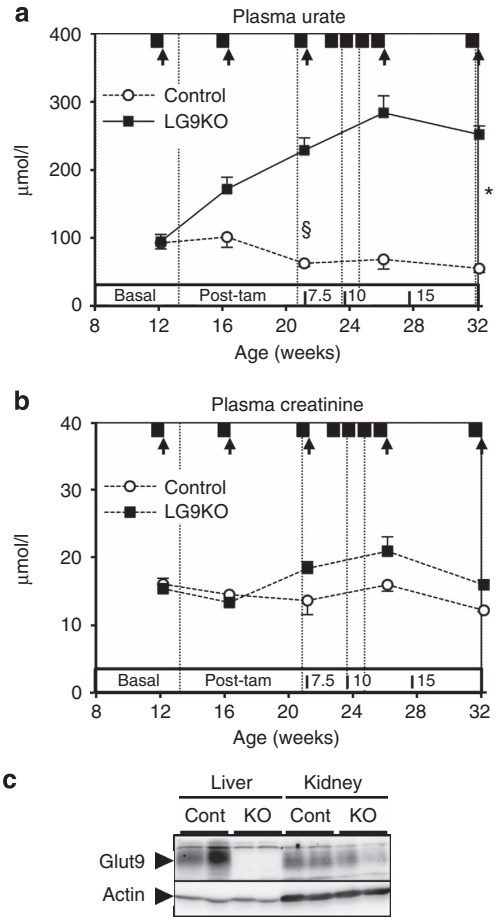

Figure 1 | Inosine gavage elevates uricemia in LG9 knockout (KO) mice. Evolution of (**a**) plasma urate and (**b**) creatinine levels measured at five time points during the longitudinal study. Indications for both age and sequence of treatments (tamoxifen, diets) are shown on the graph. (**c**) Western blot detection of Glut9 protein showing tamoxifen-induced selective deletion in the liver. Cont, controls; KO, LG9KO. $N = 7$–8. Plasma urate, $^{\S}P = 0.02$ (paired *t*-test, Controls 'l7.5' vs. 'post-tam'); $^{*}P = 6E-8$ (repeated measure analysis of variance (ANOVA), 'post-tam' to week 32, LG9KO vs. control). Plasma creatinine, not significant (NS; $P = 0.11$, repeated measure ANOVA, 'post-tam' to week 32, LG9KO vs. control).

Inosine supplementation does not increase heart rate or blood pressure in LG9KO mice

In order to assess the potential effect of inosine-induced hyperuricemia on the cardiovascular function, we measured heart rate and arterial blood pressure by telemetry during 8 sessions spread over the course of the study, either during a treatment transition or further into a treatment.

Figure 2a shows a representative telemetric measurement of blood pressure, during the transition from chow diet to chow diet supplemented with 7.5 g/kg inosine (INO7.5). The data show that arterial blood pressure (systolic, diastolic, and mean) was not altered in LG9KO mice as compared with controls.

Longitudinal analysis of the evolution of mean arterial blood pressure over the course of the study was performed for each session on 24 h averages of data acquired during Saturday night and Sunday morning, which was observed to