Knockout of the urate oxidase gene provides a stable mouse model of hyperuricemia associated with metabolic disorders

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The urate oxidase (*Uox*) gene encodes uricase that in the rodent liver degrades uric acid into allantoin, forming an obstacle for establishing stable mouse models of hyperuricemia. The loss of uricase in humans during primate evolution causes their vulnerability to hyperuricemia. Thus, we generated a *Uox*-knockout mouse model on a pure C57BL/6J background using the transcription activator-like effector nuclease (TALEN) technique. These *Uox*-knockout mice spontaneously developed hyperuricemia (over 420 μmol/l) with about 40% survival up to 62 weeks. Renal dysfunction (elevated serum creatinine and blood urea nitrogen) and glomerular/tubular lesions were observed in these *Uox*-knockout mice. Male *Uox*-knockout mice developed glycol-metabolic disorders associated with compromised insulin secretion and elevated vulnerability to streptozotocin-induced diabetes, whereas female mice developed hypertension accompanied by aberrant lipo-metabolism. Urate-lowering drugs reduced serum uric acid and improved hyperuricemia-induced disorders. Thus, uricase knockout provides a suitable mouse model to investigate hyperuricemia and associated disorders mimicking the human condition, suggesting that hyperuricemia has a causal role in the development of metabolic disorders and hypertension.

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Uric acid (UA) is the end product of purine metabolism in humans. Due to the evolutionary disruption of the *Uox* gene encoding urate oxidase (Uox) or uricase, humans are vulnerable to hyperuricemia (HUA).[1] HUA, a metabolic disorder of purine, contributes to the development of gout and renal disease, and also relates to multiple complications including hyperlipidemia, hypertension, and diabetes.[2–4] The prevalence of HUA has reached 21.4% (21.1% in men and 21.6% in women) in US adults according to the National Health and Nutrition Examination Survey (NHANES) 2007–2008.[5] In a systematic meta-analysis, the prevalence of hyperuricemia in mainland China was estimated to be 13.3% (19.4% in men and 7.9% in women).[6] Elevated serum UA (SUA) can lead to the formation of monosodium urate crystals in connective tissues, causing the pathogenesis of gout. Approximately 10% of HUA eventually develop into gout. HUA has also been identified to be an independent risk factor of cardiovascular diseases.[7,8]

Uricase expressed in the liver of rodents can further degrade UA into allantoin,[1] which has hindered the establishment of suitable rodent models for HUA. Currently, the mechanisms underlying the pernicious effects of HUA remain poorly understood, and the development of effective medications for HUA remains largely halted, partially due to the lack of an efficient and stable animal model of HUA. So far, drug induction using purine synthesis promoters (e.g., adenine) and/or uricase inhibitors (e.g., potassium oxonate) and injection of UA remain the primary strategies for establishing HUA mouse models.[9] However, UA levels often fluctuate by large margins in drug-induced HUA animals.[9] Alternatively, gene targeting in embryonic stem cells has been employed to generate *Uox*-deficient mice.[10] However, UA levels often reach lethal ranges in these mice, so few of them survive to maturity.[10]

In order to establish a stable HUA mouse model for long-term studies, we herein generated a *Uox*-knockout (KO) mouse on a pure C57BL/6J genetic background using the transcription activator-like effector nuclease (TALEN) technology, and examined the phenotypes of these mice in terms

of SUA, survival rate, renal function, glucose and lipid metabolism, cardiovascular function and dimensions, and response to multiple urate-lowering drugs (ULDs). This study characterized HUA-related phenotypes comprehensively for the first time. The successful generation of this *Uox*-KO mouse model with spontaneous HUA provides a suitable tool for HUA-related research.

RESULTS

Hepatic Uox expression was markedly reduced and SUA elevated in the *Uox*-KO mice

The knockout scheme was described in Methods (Figure 1a). Both uricase mRNA and protein dramatically decreased in the *Uox*-KO mouse liver compared to wild-type (WT) mice as determined by quantitative reverse-transcription polymerase chain reaction (RT-PCR) and Western blot (Figure 1b and c). Kaplan-Meier analysis of survival rate over a 62-week observation period showed that roughly 40% of the *Uox*-KO mice deceased within 5 weeks after birth, and the survival rate stabilized at approximately 40% from 10 weeks after birth (Figure 1d). Genotyping at 3 to 4 weeks after birth showed that the distribution of homogeneous, heterogeneous *Uox*-KO mice and WT mice had a different plot from the Mendelian inheritance law, at 15.94% (55 of 345), 51.60% (178 of 345), and 32.46% (112 of 345), respectively, suggesting loss of the *Uox* gene might lead to embryonic or neonatal death. *Uox*-KO mice had significantly higher SUA levels compared with WT mice (Figure 1e). Notably, male *Uox*-KO mice had higher SUA levels than did females (Figure 1e), displaying a pattern similar to that of humans.[5] The SUA stabilized at elevated levels ($>$ 420 μmol/l) in the *Uox*-KO mice from 4 to 30 weeks of age (Figure 1f).

Renal function and histological integrity were compromised in the *Uox*-KO mice

To examine the effects of HUA on the kidney, renal function and kidney histology were determined in the *Uox*-KO mice. Compared with WT controls, serum creatinine and blood urea nitrogen (BUN) were significantly elevated in both male and female *Uox*-KO mice (Figure 2a), suggesting that glomerular filtration was compromised. Kidney histology was examined from 2 to 8 weeks of age (Supplementary Figure S1). Lesions in kidney tissues initially occurred at 6 weeks of age in the *Uox*-KO mice. Histological impairments in the kidneys of 8-week-old *Uox*-KO mice included dilated Bowman's spaces and tubules, collapsed and necrotic nephrons, and focal tubulointerstitial fibrosis (Figure 2b and c). Severe nonspecific chronic corticomedullar inflammation was observed as manifested by substantial lymphocyte ($CD3^+$) and macrophage ($CD68^+$) infiltration (Figure 2d and e). Urate crystals were detected in kidney interstices under polarized light (Figure 2f). In addition, mRNA expression of inflammatory cytokines F4/80 and interleukin-1β (IL-1β) were significantly elevated in the kidneys of *Uox*-KO mice compared with WT mice (Figure 2g and h).

Female *Uox*-KO mice developed high blood pressure

To examine the effects of HUA on the cardiovascular system, blood pressure (BP) and cardiac function were determined by sphygmomanometer and echocardiography. Notably, 8-week-old female *Uox*-KO mice developed significantly higher systolic BP (SBP) and diastolic BP (DBP) than WT controls, while male *Uox*-KO showed no differences from their WT counterparts (Figure 3). Echocardiography was performed on 26-week-old *Uox*-KO mice to examine the effects of long-term HUA on cardiac dimensions and function. Neither dimensional (i.e., mass, volume, inner diameter, and wall thickness of the left ventricle) nor functional parameters (i.e., cardiac output, stroke volume, fractional shortening, and ejection fraction) were significantly altered in the *Uox*-KO mice compared with WT controls (Supplementary Table S1).

Lipometabolism were disordered in the *Uox*-KO mice

Analysis of lipid profiles revealed that, compared with WT counterparts, total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol were significantly elevated in female *Uox*-KO mice, whereas TG was significantly lower in male *Uox*-KO mice (Table 1).

Glycometabolism were disordered in the *Uox*-KO mice

Neither fasting insulin nor fasting glucose was significantly changed in the *Uox*-KO mice compared with WT controls (Figure 4a and b). No apparent histological lesions were detected in the pancreatic islets of the *Uox*-KO mice either (Supplementary Figure S2). However, upon i.p. glucose tolerance test (IPGTT), blood glucose at 15 and 30 minutes after initiation was significantly higher in male *Uox*-KO mice than in male WT controls (Figure 4c, left panel). Consistently, calculation of the area under the curve (AUC) showed it was significantly larger for male *Uox*-KO mice than male WT mice (Figure 4c, right panel). Upon insulin tolerance test (ITT), both sexes of *Uox*-KO mice displayed a similar pattern to WT controls (Figure 4d), suggesting that insulin resistance was unlikely to contribute to the aberrant glycometabolism in the *Uox*-KO mice.

Insulin secretion was compromised in the *Uox*-KO mice

Islet function was determined by glucose-stimulated insulin secretion (GSIS) performed *in vitro* and *in vivo*. Although isolated islets derived from 8-week-old male *Uox*-KO mice secreted a similar amount of insulin to WT islets at baseline glucose concentration (3.3 mmol/l), insulin secretion was decreased by 20.56% in *Uox*-KO islets at an elevated glucose concentration (16.7 mmol/l), as shown in Figure 4e. Furthermore, after a glucose injection (2 g/kg), 8-week-old male *Uox*-KO mice secreted a significantly lower amount of insulin at 15 and 30 minutes (Figure 4f), consistent with the IPGTT results above.

Vulnerability to diabetes and islet β cell apoptosis were elevated in the *Uox*-KO mice

By induction with multiple injections of low-dose streptozotocin (STZ; 40 mg/kg/d, consecutively for 5 days),