Allopurinol, rutin and quercetin attenuate hyperuricemia and renal dysfunction in rats induced by fructose intake: renal organic ion transporter involvement

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RUNNING TITLE: Association of organic ion transporters with urate and renal function

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Hu Q-H, Wang C, Li J-M, Zhang D-M, Kong L-D. Fructose consumption has been recently related to epidemic of metabolic syndrome and hyperuricemia plays a pathogenic role in fructose-induced metabolic syndrome. Fructose-fed rats showed hyperuricemia and renal dysfunction with reductions of urinary uric acid/creatinine ratio and fractional excretion of uric acid (FEUA), as well as other features of metabolic syndrome. Lowering serum uric acid levels with allopurinol, rutin and quercetin increased urinary uric acid/creatinine ratio and FEUA, and attenuated fructose-induced other metabolic abnormalities in rats, demonstrating that hyperuricemia contributed to the deficiency of renal uric acid excretion in this model. Furthermore, we found that fructose up-regulated the expression levels of rSLC2A9v2 and renal-specific transporter (rRST), down-regulated the expression levels of organic anion transporters (rOAT1 and rUAT) and organic cation transporters (rOCT1 and rOCT2), with the regulators prostaglandin E2 (PGE2) elevation and nitric oxide (NO) reduction in rat kidney. Allopurinol, rutin and quercetin reversed dysregulations of these transporters with PGE2 reduction and NO elevation in the kidney of fructose-fed rats. These results suggested that dysregulations of renal rSLC2A9v2, rRST, rOAT1, rUAT, rOCT1 and rOCT2 contributed to fructose-induced hyperuricemia and renal dysfunction. Therefore, these renal transporters may represent novel therapeutic targets for the treatment of hyperuricemia and renal dysfunction in fructose-induced metabolic syndrome.

high uric acid; rSLC2A9; rUAT; rSLC22A; hypouricemic agents
The epidemic of metabolic syndrome, a major public health problem, correlates with excessive dietary fructose consumption (43, 47, 69), suggesting a potential causal role of fructose in the development of metabolic syndrome (48). In fructose-fed rats, the urinary uric acid excretion is remarkably reduced (16, 47), which leads to hyperuricemia and plays a pathogenic role in renal dysfunction and metabolic syndrome (16, 47, 62, 63). Therefore, investigation of molecular mechanism for fructose-induced hyperuricemia is essential to understand the progression of renal dysfunction and metabolic syndrome.

The reabsorption and secretion of renal uric acid is governed by a complex system of classical polyspecific urate transporters including SLC2A and SLC22A family proteins in the proximal kidney tubule. SLC2A9 is firstly identified as a member of the SLC2A gene family of hexose transporters. In human, the short isoform of SLC2A9 (SLC2A9v2) is exclusively localized to the apical membrane of the renal tubules, a key site for urate handling in the kidney (33). Recent studies demonstrate that SLC2A9 is a transporter of uric acid that can be inhibited by uricosuric agents (12, 72). Mutations in SLC2A9 cause decreased urate reabsorption on both sides of the proximal renal tubules, which in turn results in reduction of serum uric acid concentrations and elevation of fractional excretion of uric acid (FEUA) (13, 39, 72). Renal-specific transporter (RST) identified in mouse and rat (28), is a homologue of the SLC22A12 protein (human urate transporter 1, URAT1). RST localize predominantly on the brush border membrane of the proximal renal tubule and is responsible for urate reabsorption (26). The renal urate reabsorption was diminished in RST-null mice (14). The organic anion transporters OAT1 (SLC22A6) and OAT3 (SLC22A8) are responsible for the uptake of organic anions from the blood across the basolateral membrane into proximal tubule cells, which are recognized as the first step of renal organic anion secretion (23, 29). Uricosurics probenecid and benzbromarone exhibited hypouricemic action by inhibiting OAT1-mediated urate secretion (29, 57). OAT1-knockout mice had reduced uric acid
secretion (50). Down-regulation of renal rOAT1 and rOAT3 was previously observed in hyperuricemic rats induced by combined administration of oxonic acid (uricase inhibitor) and uric acid (19, 20), indicating that OAT1 and OAT3 may play an important role in the pathogenesis of hyperuricemia (14). In addition, electrogenic urate transporter (UAT) is also involved in uric acid secretion across the luminal membrane in kidney (23, 34). Decreased UAT expression was found in renal cortical membrane vesicles of oxonate-induced hyperuricemic rats and rabbits (1, 2, 32). UAT-null proximal tubular cells had a defect in uric acid handling (14, 36). However, whether fructose-induced hyperuricemia in rats is caused by dysregulation of these renal urate transporters remains unknown.

Recent studies also demonstrate that SLC22A proteins play a pivotal role in renal dysfunction. The organic cation transporters OCT1 (SLC22A1) and OCT2 (SLC22A2) at the basolateral membrane of the proximal renal tubules cooperate with each other in renal excretion of organic cations, mediating the first step during secretion of organic cations in the proximal tubule. The expression level of rOCT2 in rat kidney was decreased not only in ischemia and reperfusion-induced acute kidney injury (40), but also in oxonic acid-induced hyperuricemia (19, 20). Reduction of renal rOCT1 and rOCT2 expressions was also reported in streptozotocin-induced diabetes of rats (17). The organic anion transporters OAT1 and OAT3 take up anion toxic from the blood into the proximal tubular cells and significantly contribute to nephrotoxicity (57). Therefore, it will be necessary to examine whether these renal transporters are involved in fructose-induced renal dysfunction in rats.

Moreover, intact endothelial function is assumed to provide a defense mechanism against progressive renal dysfunction. Fructose-induced hyperuricemia could reduce endothelial nitric oxide (NO) levels and possibly cause endothelial dysfunction (48, 54). And increased level of prostaglandin E2 (PGE2), a primary mediator of inflammation in the kidney, led to down-regulation of rOAT1 and rOAT3 expressions in rat proximal tubular cell line NRK-52E
Therefore, fructose-induced hyperuricemia may result in alternations in the level of PGE\textsubscript{2} and NO which could cause abnormal expressions of these organic ion transporters in rat kidney.

Hypouricemic action and nephroprotection remain the major challenges of pharmacotherapy to treat hyperuricemia and renal dysfunction of metabolic syndrome. Several drugs including natural origins show beneficial effects on the features of metabolic syndrome and renal dysfunction. Quercetin and its glycoside rutin are the most important flavonoids in herbal food, which significantly reduced serum uric acid levels in oxonate-induced hyperuricemic mice, exhibiting hypouricemic actions (44, 75). Quercetin attenuated the abnormalities in metabolic syndrome including obesity, insulin resistance, dyslipidemia and hypertension in obese Zucker rats (56). It also reduced serum creatinine and blood urea nitrogen (BUN) concentrations in ischemia/reperfusion-induced renal injury of rats (66) and decreased plasma creatinine levels in cadmium-induced nephrotoxicity (45), thus attenuating renal injury. Rutin decreased serum triglyceride levels in isoproterenol-treated rats (68), suppressed adipogenesis in 3T3-L1 adipocytes (22) and protected kidney from focal fatty infiltrate, inflammation and hemorrhage in streptozotocin-induced diabetic rats (30). In vitro study also showed that quercetin significantly inhibited cellular uptake of \[^{3}\text{H}]p\text{-aminohippurate}\mediated\ by hOAT1 and \[^{3}\text{H}]\text{-estrone sulfate}\mediated\ by hOAT3 in MDCK cells (25). In LLC-PK1 cells, quercetin and rutin, to a less extent, also reduced the cellular uptake of \[^{14}\text{C}]\text{-tetraethylammonium}\mediated\ by pOCT2 (51). Therefore, quercetin and rutin may modulate renal organic ion transporters to alleviate renal dysfunction. However, their exact role in fructose-induced hyperuricemia and renal dysfunction is not clear yet.

In the present study, we carefully examined the expression levels of renal organic ion transporters including rSLC2A9, rRST, rOAT1, rOAT3, rUAT which are responsible for the abnormal renal uric acid excretion involved in hyperuricemia and renal dysfunction in
fructose-fed rats. In the kidney of the same animals, we also examined changes for PGE\textsubscript{2} and NO, as well as rOCT1 and rOCT2, focusing on their potential mechanism(s) in renal dysfunction in fructose-fed rats. Furthermore, the effects of rutin and quercetin on these abnormalities induced by fructose consumption were also investigated in rats. Although allopurinol treatment for hyperuricemia and metabolic syndrome has been subject of extensive research (47), the knowledge about the mechanisms of its action on renal organic ion transporters is limited in fructose-fed rats. Therefore, allopurinol was employed to improve our understanding of the role of these transporters in hyperuricemia and renal dysfunction in fructose-induced metabolic syndrome in the present study.

MATERIALS AND METHODS

Reagents. Allopurinol, rutin and quercetin were purchased from Sigma Chemicals (St. Louis, MO, USA). Assay kits of total cholesterol (TC), triglyceride (TG), high density lipid (HDL-C), lower density lipid (LDL-C), creatinine, BUN and NO were obtained from Jiancheng Biotech (Nanjing, P. R. China), respectively. ELISA kit for insulin assay was purchased from Mercodia (Uppsala, Sweden). Glucose assay kit was purchased from Beckman Instruments (Irvine, CA, USA). ELISA kit for PGE\textsubscript{2} assay was purchased from R&D CO. (USA). TRIZOL reagent was obtained from Invitrogen and M-MLV reverse transcriptase was from Promega. All the primers (Table 1) were designed and synthesized by Shengxing Biotech (Nanjing, P. R. China). The antibodies of rSLC2A9 single nucleotide polymorphism (SNP) (NCBI accession No: XP_577349), rRST (NCBI accession No: NP_001030115), rOAT1 (NCBI accession No: NP_058920), rOAT3 (NCBI accession No: NP_112622), rUAT (NCBI accession No: AAB48591), rOCT1 (NCBI accession No: NP_036829) and rOCT2 (NCBI accession No: NP_113772) were supported by SaiChi
Biotech (Beijing, P. R. China). GAPDH and anti-rabbit IgG antibodies were purchased from Jingmei Biotech (Shanghai, P. R. China). Rat Na⁺-K⁺-ATPase antibody was supplied by Cell Signaling Technology, Inc. (Boston, MA, USA).

**Animals.** Male Sprague-Dawley rats were purchased from the Laboratory Animal Center (Hangzhou, Zhejiang Province, P. R. China) and housed in plastic cages with a 12-h light/12-h dark cycle at constant temperature of 22 - 24 °C. They were given standard chow *ad libitum* for the duration of the study and allowed 1 week to adapt the laboratory environment before experiments. All the procedures were in strict accordance with the P. R. China legislation on the use and care of laboratory animals and with the guidelines established by the Institute for Experimental Animals of Nanjing University.

**Animal Model and Drug Administration.** Animals were randomly divided into 12 groups. The initial body weight of rats was not significant different between control and fructose-fed groups. Rats were given drinking water (control rats) or 10 % fructose in drinking water (fructose-fed rats) with standard chow for 8 weeks (43, 70). Fresh drinking water was replaced every 2 days. After 4-week fructose feeding, fructose-fed rats were further divided into matched subgroups. Control animals were also divided. Different groups of animals (n = 7 in every group) were administered daily with vehicle (water 1 ml/kg), allopurinol (5 mg/kg, as a positive control group), rutin (50 and 100 mg/kg) and quercetin (50 and 100 mg/kg), respectively. All drugs were given orally once daily at 2:00 p.m.-3:00 p.m. for the subsequent 4 weeks. Body weight was recorded once a week during this time interval.

**Measurement of Systolic Blood Pressure (SBP).** SBP was measured in the morning using a tail-cuff method and recorded on an ALC-NIBP non-invasive blood pressure analysis system (Biosythesis Biotechnology Co., Ltd., Shanghai, P. R. China). All animals were preconditioned for SBP measurements 1 week before experiment and the mean value of three consecutive measurements was obtained.
Oral Glucose Tolerance Test (OGTT). The OGTT was performed according to the method described by Matsuda M (38). 1 h after drug administration, glucose (1.5 g/kg b. wt.) was orally administered to animals as a 50% glucose solution. Tail-vein blood samples were collected at 0, 30, 60, 90 and 120 min after glucose administration, respectively, and then centrifuged (3,000 × g) at 4 °C for 10 min to get serum for glucose and insulin assays. Glucose and insulin levels were measured with commercial kits, respectively. The insulin sensitivity index (ISI) was calculated using the following formula: 10000/square root of (fasting glucose × fasting insulin) × (mean glucose × mean insulin during OGTT).

Blood and Tissue Processing. After the OGTT test, animals were allowed 3 days to recover wounds, and then body weight was recorded at the end of week 8. To avoid the fluctuations of hormone levels due to circadian rhythms, animals were killed by decapitation at 09:00 a.m. to 10:00 a.m. after a 16 h fast, kidney tissues were dissected quickly on the ice and stored in liquid nitrogen for PGE2, NO, RT-PCR and Western blot analysis, respectively. Blood samples were centrifuged (3,000 × g) at 4 °C for 10 min to get serum frozen at -80 °C for uric acid, insulin and BUN assays.

Uric Acid Assay. Uric acid concentrations in serum (Sur) and urine (Uur) were determined by the phosphotungstic acid method (8), respectively.

Measurement of Creatinine and BUN. Creatinine and BUN levels in serum and urinary were measured using standard diagnostic kits purchased, respectively.

Semi-quantitative RT-PCR Analysis. Total RNA was extracted from rat kidney using TRIZOL reagent (Invitrogen Co, USA). The homogenate was mixed with 200 μl chloroform then centrifuged at 12,000 × g for 15 min. Aqueous phase (about 0.5 ml upper layer) was precipitated with equal volume of isopropanol and centrifuging at 12,000 × g for 10 min. The final RNA total pellet was resuspended in 20 μl of DEPC water.

Reverse transcription was performed with 1 μg RNA using M-MLV reverse transcriptase
Promega Co, USA) for cDNA synthesis. PCR amplification was carried out using
gene-specific PCR primers synthesized by Shengxing Biotech (Nanjing, Jiangsu, P. R. China).
The sequences of gene-specific PCR primers, the appropriate annealing temperature and the
length of production were summarized in Table 1. PCR products were electrophoresed on
1.2 % agarose gels, visualized with the Bio-Rad ChemiDoc XRS Gel Documentation system,
and then quantified using Bio-Rad Quantity One 1-D analysis software. Relative quantitation
for PCR products was calculated by normalization to the amount of beta-actin mRNA levels.

*Western Blot Analysis.* Tissue samples of rat kidney cortex were homogenized in 10 w/v
buffer (10 mM Tris-HCl, 1 mM EDTA and 250 mM sucrose, pH 7.4, containing 15 μg/ml
aprotinin, 5 μg/ml leupeptin and 0.1 mM phenylmethanesulfonyl fluoride, PMSF), using a
Polytron set and centrifuged at 3,000 × g for 15 min. The supernatant was centrifuged at
12,000 × g for 20 min. The final peptide was dissolved in a Tris-HCl buffer (pH 7.5)
containing 150 mM NaCl, 0.1 % SDS, 1 % NP-40 and 1 % PMSF. After resolution of 75 mg
of protein by 12 % SDS-PAGE using Power Pac Basic electrophoresis apparatus (Bio-Rad,
Hercules, CA), the protein samples were transferred onto PVDF membranes (Millipore,
Shanghai, P. R. China), which were then blocked with 5 % skim milk and subsequently
incubated with primary antibody and horseradish peroxidase-conjugated secondary antibody.
Immunoreactive bands were visualized by incubation with lumiGLO reagent (Cell Signaling,
Beverly, MA) and exposing to X-ray film (Kodak, New Haven, CT). Primary antibodies
included rabbit polyclonal antibodies against rOAT1 (diluted 1:2000), rOAT3 (1:2000), rUAT
(1:200), rOCT1 (1:3000), rOCT2 (1:200) and GAPDH (1:5000).

*Preparation of Renal Brush Border Membrane.* Renal cortical brush border membrane
vesicles were prepared from the kidneys of normal or fructose-fed rats by a modified
procedure protocol of Li et al, 2007 (35). The whole procedure was carried out at 4 °C.
Briefly, kidney cortex slices were homogenized for 2 min in 10 mM HEPES-Tris (pH 7.4)
buffer containing 300 mM D-mannitol, 5 mM EGTA and 12 mM MgCl₂ by a Polytron on setting 5. After 20 min, the homogenate was centrifuged at 2,400 × g for 15 min. The supernatant was collected and centrifuged at 30,000 × g for 30 min. The pellet was then resuspended in 5 mM HEPES-Tris (pH 7.4) buffer containing 150 mM D-mannitol, 2.5 mM EGTA and 12 mM MgCl₂ and homogenized on a glass-Teflon homogenizer for 20 min. The homogenate was centrifuged at 2,400 × g for 15 min. The supernatant was collected and centrifuged at 30,000 × g for 30 min. The final peptide was dissolved in a Tris-HCl buffer (pH 7.5) containing 150 mM NaCl, 0.1 % SDS, 1 % NP-40 and 1 % PMSF. Protein contents were determined by a Bradford method.

Renal brush border membrane samples were applied for western blot analysis of rSLC2A9 and rRST protein levels as described before, and primary antibodies of rSLC2A9 SNP (1:1000), rRST (1:2000) and rat Na⁺-K⁺-ATPase (1:1000) were used, respectively.

Renal PGE₂ Assay. After weighing, the wet tissue of the rat kidney was homogenized in 10 volumes of 10 mM sodium pyrophosphate buffer (pH 7.4) containing 10 μg/ml of indomethacin to inhibit any additional PGE₂ synthesis. The resulting insoluble proteins were removed by centrifugation at 10,000 × g for 10 min. All procedures were performed at 4 °C. The supernatant was stored at -80 °C until PGE₂ assay. The amounts of PGE₂ were measured using ELISA kits.

Renal NO Assay. Kidney samples of rats were extracted by the method of Omer Bayrak (6). After weighing, the wet tissue of the kidney was homogenized in five volumes of ice-cold Tris-HCl buffer (50 mM, pH7.4) containing 0.50 ml/l Triton X-100 at 13,000 × g (4 °C) for 2 min. The lipid layer was carefully removed by centrifugation at 10,000 × g (4 °C) for 10 min, and the resulting supernatant fraction was stored at -80 °C until NO assay. The concentrations of renal NO were measured by kits.

Histological Analyses. Rat kidneys were removed and immediately fixed for 1 day at
room temperature in carnoy fixative (ethanol: chloroform: acetic acid = 6: 3: 1) and preserved in 70 % ethanol. Renal biopsies were dehydrated with a graded series of alcohol and embedded in paraffin. Specimens were cut in 7-μm-thick sections on a rotary microtome and mounted on APES-coated glass slides. Sections were deparaffinized in xylene, rehydrated in decreasing concentrations of alcohol in water, and stained with hematoxylin-eosin Reagent (Sigma Chemicals Co, USA). The slides were mounted with neutral balsam.

Food Intake, Fluid Intake and Urate Handling Investigation. Animal model and drug administration were conducted as above description. Food and fluid intakes were recorded daily during the 8th week when rats had free access to standard rat chow and tap water in metabolic cages, respectively. 24-h urine was also collected and the volume of which was recorded for each group. Urine samples were centrifuged at 2,000 × g for 10 min to remove the particulate contaminants and supernatant was used to uric acid and creatinine analysis. After urate handling investigation, animals were killed by decapitation and serum samples were collected as above description. Uric acid concentrations in urine (Uur) and serum (Sur), as well as creatinine concentrations in urine (Ucr) and serum (Scr) were measured as above description, respectively. Fractional excretion of uric acid (FEUA) was calculated using the formula: FEUA = (Uur×Scr)/(Sur×Ucr) ×100, expressed as percentage (52).

Postprandial TC, TG, HDL-C and LDL-C Assays. TC, TG, HDL-C and LDL-C levels were measured in serum samples collected after urate handling investigation using standard diagnostic kits.

Statistical Analysis. All data were expressed as mean ± S.E.M. and analyzed by unpaired Student’s t-test, a one-way analysis of variance (ANOVA), or a two-way AVONA. Where appropriate, post hoc comparisons among means were performed using the Tukey tests. A value of p < 0.05 was considered statistically significant. Figures were obtained by the Statistical Analysis System (GraphPad Prism 4, GraphPad Software, Inc., San Diego, CA).
RESULTS

Allopurinol, rutin and quercetin attenuate fructose-induced metabolic syndrome in rats.

As shown in Table 2 and Fig 1, in comparison to vehicle-fed rats, rats fed with 10% fructose in drinking water for 8 weeks exhibited clustering features of metabolic syndrome including elevated levels of serum uric acid, insulin, TC, TG and LDL-C, reduced levels of serum HDL-C, increased SBP and body weight. Four-week treatment with allopurinol reversed the elevated levels of serum uric acid, insulin, SBP and body weight, as well as serum lipid disorders in fructose-fed rats (Table 2 and Fig. 1A-1D). Treatment with rutin and quercetin significantly lowered serum uric acid and improved the features of metabolic syndrome in fructose-fed rats in dose-dependent manner (Table 2 and Fig. 1A-1D), but they had no significant effect in control rats. Furthermore, daily fluid intake was slightly increased and daily food intake was significantly reduced in fructose-fed rats, which were not affected by allopurinol, rutin and quercetin (Table 2).

In agreement with previous finding (47), although fructose-fed rats did not develop fasting or postprandial hyperglycemia (data not shown), they developed fasting hyperinsulinemia that was reversed by allopurinol, rutin and quercetin (Fig. 2A). Furthermore, postprandial hyperinsulinemia occurred in fructose-fed rats administrated an OGTT (Fig. 2A). Treatment with allopurinol, rutin and quercetin decreased insulin levels (Fig. 2A) and restored insulin sensitivity significantly (Fig. 2B).

Allopurinol, rutin and quercetin reverse fructose-induced reduction of urinary urate excretion and renal dysfunction in rats. Next, we examined the excretion of uric acid and renal function in fructose-fed rats. As shown in Table 3, the levels of urinary uric acid and creatinine were significantly decreased in fructose-fed rats compared to control-vehicle rats.
The Uur/Ucr ratio, as well as FEUA related renal uric acid handling parameters, was also significantly reduced in fructose-fed rats. Urine volume was increased in fructose-fed rats. In addition, fructose caused significant elevation in serum creatinine and BUN levels in rats. These data indicated that fructose consumption could cause urate underexcretion with impaired renal dysfunction in rats.

As reported (47, 54), allopurinol significantly prevented fructose-induced reduction of uric acid excretion in this model. Accordingly, both rutin and quercetin prevented fructose-induced urinary uric acid and creatinine reduction in rats (Table 3). Rutin and quercetin at 100 mg/kg significantly elevated Uur/Ucr ratio in fructose-fed rats (Table 3). Rutin and quercetin at 50 and 100 mg/kg reversed fructose-induced FEUA reduction in rats. Treatment with allopurinol, rutin and quercetin failed to reverse fructose-induced elevated urine volume of rats (Table 3). In addition, increased serum creatinine and BUN levels in fructose-fed rats were attenuated by allopurinol, quercetin and rutin except that 50 mg/kg rutin failed to alter BUN levels (Table 3). No significant changes were observed in control rats treated with allopurinol, rutin or quercetin (Data not shown).

Fructose induces dysregulation of renal organic ion transporters and attenuation by allopurinol, rutin and quercetin. To evaluate whether expression alterations of renal organic ion transporters are the major mechanisms for urate underexcretion and renal dysfunction in fructose-induced metabolic syndrome, we examined expressions of renal rSLC2A9, rRST, rOAT1, rOAT3, rUAT, rOCT1 and rOCT2 in fructose-fed rats.

A single nucleotide polymorphism (SNP) (Val253Ile, rs16890979) in SLC2A9 regarded as conservative region is most strongly associated with uric acid levels (5, 42). Thus, we used this SNP coding sequence to examine rSLC2A9 expression in rat kidney. As shown in Fig. 3A, a single band at 60 kDa represented rSLC2A9v2 protein in apical membrane of the proximal tubules (4). Significant increases in renal rSLC2A9v2 and rRST protein levels were observed
in fructose-fed rats compared to control-vehicle rats (Fig. 3A and 3B), which was likely due
to the up-regulation of their mRNA levels (Fig. 4A and 4B). Fructose also induced significant
decreases of renal rOAT1, rUAT, rOCT1 and rOCT2 at both protein (Fig. 3C-3G) and mRNA
levels (Fig. 4C-4G) in rats. However, rOAT3 expression was not altered by fructose
consumption (Fig. 3D and Fig. 4D). Allopurinol, rutin and quercetin significantly decreased
renal rSLC2A9v2 and rRST protein and mRNA levels, increased renal rOAT1, rUAT, rOCT1
and rOCT2 protein and mRNA levels, and had no effect on rOAT3 expression in fructose-fed
rats (Fig. 3A-3G and Fig. 4A-4G). Allopurinol, rutin and quercetin did not affect protein and
mRNA levels of these transporters in control rats (Data not shown).

Fructose enhances renal PGE2 secretion and reduces NO production in rats and
improvement by allopurinol, rutin and quercetin. Since PGE2 and NO play the important roles
in organic ion transporter regulation and renal function (16, 22, 24, 48, 54, 62, 64, 65), we
then determined renal PGE2 and NO in fructose-fed rats. Fructose consumption caused
increased PGE2 levels and decreased NO levels in the kidney of rats compared to
control-vehicle animals (Fig. 5A and 5B). Allopurinol, rutin and quercetin significantly
attenuated fructose-induced renal PGE2 and NO alteration in rats (Fig. 5A and 5B), and did
not change PGE2 and NO concentrations in control rats (Data not shown).

Allopurinol, rutin and quercetin prevent fructose-induced morphological changes in rats.
Results from histological analyses showed that renal inflammatory cells were infiltrated into
interstitium in fructose-fed rats (Fig. 6). This tubulointerstitial pathology was disappeared
after treatment with allopurinol, rutin and quercetin, respectively. Moreover, the kidney of
control rats receiving allopurinol, rutin and quercetin showed no obvious morphological
changes compared to vehicle normal states (Data not shown).

DISCUSSION
The present study confirmed that fructose consumption caused reductions of urinary uric acid and creatinine concentrations, Uur/Ucr ratio and FEUA, eliciting urinary uric acid underexcretion and serum uric acid elevation in fructose-fed rats. More importantly, hypouricemic agents allopurinal, rutin and quercetin attenuated these abnormalities in fructose-fed rats (Table 2). Nakagawa et al (47) also reported reduced uric acid excretion in fructose-fed rats and their reversal by allopurinol. Our current study further provided the evidence for the reduction of uric acid excretion in fructose-induced hyperuricemia in rats. Therefore, hypouricemic effects of allopurinal, rutin and quercetin might, at least partly, correlated with their abilities to enhance renal excretion of urate in fructose-fed rats.

Urinary excretion of uric acid involving uric acid reabsorption and secretion largely occurs in overlapping segments of the proximal tubular cell via urate transporters (14). In the present study, we for the first time demonstrated that fructose consumption up-regulated the expression levels of rSLC2A9v2 and rRST, down-regulated the expression levels of rOAT1, rUAT, rOCT1 and rOCT2 in rat kidney, accompanying with hyperuricemia, renal dysfunction and other features of metabolic syndrome. Since rSLC2A9v2 and rRST control the reabsorption of urate (28, 72), and rOAT1 and rUAT are responsible for urate secretion (23, 29, 34), dysregulation of these renal transporters should be responsible for reduced uric acid excretion and increased serum uric acid in fructose-fed rats, thereby playing a significant role in the pathogenesis of hyperuricemia in fructose-induced metabolic syndrome of rats. Moreover, fructose consumption elevated PGE2 and reduced NO in rat kidney, which might be associated with dysregulation of these transporters. The increased excretion of uric acid produced by allopurinol, rutin and quercetin might be mediated by modulating the expression levels of renal specific transporters including rSLC2A9v2, rRSTs, rOAT1 and rUAT. Furthermore, down-regulators for rSLC2A9v2 and rRST and up-regulators for rOAT1 and
rUAT might predictably enhance urate excretion and have application in the treatment of fructose-induced hyperuricemia in rats.

Previous studies suggested insulin resistance or hyperinsulinemia enhanced urate reabsorption and reduced urate excretion in the kidney, leading to elevated serum uric acid concentrations (15, 46, 59). Lowering uric acid levels with either a xanthine oxidase inhibitor or a uricosuric agent improved insulin sensitivity in fructose-induced metabolic syndrome (47). Dyslipidemia with elevated lipoprotein lipase was involved in the retarded uric acid clearance, resulting in elevation of serum uric acid levels (10). Treatment of hypertriglyceridemia with finofibrate or atorvastatin could reduce serum uric acid concentrations (47). Thus, upon high-fructose feeding, hyperinsulinemia (Table 2) and dyslipidemia (Fig. 1) might be partly responsible for dysregulations of renal rSLC2A9v2, rRST, rOAT1 and rUAT in rats.

Recent epidemiological evidence indicated that hyperuricemia might be a risk factor for renal dysfunction (31, 41, 49, 55, 58, 63). Besides reductions of urinary uric acid and creatinine, significant elevations of serum creatinine and BUN concentrations were found (Table 3), indicating renal dysfunction in fructose-fed rats. Interstitial inflammation and tubular damage, one of the earliest manifestations of nephropathy preceding the development of hyperfiltration or glomerular lesions (71), was also observed in the kidneys of fructose-fed rats (Fig. 6). OATs are expressed in the renal epithelial cells to regulate the excretion of endogenous and exogenous organic anions. It was reported that hOAT1 mRNA expression was reduced in the kidney of patients with renal diseases compared with that in normal controls (60). OCTs are also important for the renal homeostasis of a number of physiologically important endogenous cations. Down-regulation of rOATs, rUAT, rOCT1 and rOCT2 (Fig. 3-4) might lead to excessive accumulation of endogenous and exogenous toxin, resulting in renal damage in fructose-fed rats. Regulations of allopurinol, rutin and quercetin
on these organic ion transporters effectively improved renal dysfunction and prevented interstitial inflammation and tubular damages in the kidney pathohistological sections of fructose-fed rats. The observation that these hypouricemic agents improved fructose-induced renal dysfunction was consistent with other reports in fructose-fed rodent (3, 11, 16), in oxonic acid-induced hyperuricemia remnant kidney rats (25, 41) and in patients with chronic renal disease (67).

Mechanisms underlying the altered renal organic ion transporter expression in fructose-induced metabolic syndrome are unclear. Stimulation of the basolateral organic anion uptake by PGE2 is a widespread regulatory mechanism. PGE2 inhibited its own renal transport by down-regulation of rOAT1 in NRK-52E cells (64, 65). In addition, prostaglandins are full anions at physiological pH, a recent study demonstrated efficient transport of PGE2 by OCT1 and OCT2 (22). NO is responsible for a down-regulation of renal rOCT1 and rOCT2 in LPS-treated Wistar Hannover rats (24). Moreover, high uric acid levels potently reduced endothelial NO bioavailability in fructose-induced metabolic syndrome of rats (47). In the present study, fructose induced the elevation of PGE2 and the reduction of NO in rat kidney associated with the impairment of renal function, further confirming that fructose-induced hyperuricemia was responsible for renal dysfunction through PGE2 and NO (16, 61, 62). Therefore, altered PGE2 and NO in kidney might be relative to down-regulation of renal rOAT1, rUAT, rOCT1 and rOCT2, which make significant contribution to high serum urate levels and renal dysfunction in fructose-induced metabolic syndrome of rats. Moreover, lowering uric acid levels with allopurinol, rutin and quercetin normalized PGE2 and NO levels and improved renal function impairment, which were correlated with restored expression of renal rSLC2A9v2, rRST, rOAT1, rUAT, rOCT1 and rOCT2 in fructose-fed rats. Rutin potently inhibited rabbit renal conversion of PGI2 and PGF2 alpha to 6-oxoPGE1 and PGE2, respectively (45) and PGE2 production in human neutrophils (7). Quercetin inhibited activated
PGE₂ production in lipopolysaccharide–induced human gingival fibroblasts (18), endotoxin-stimulated murine macrophages (73) and human neutrophils (7). Thus, allopurinol, rutin and quercetin might modulate renal organic ion transporters partly by regulating renal PGE₂ and NO levels in fructose-fed rats.

Our study provides solid evidence that dysregulation of renal organic ion transporters is in parallel with urinary urate underexcretion and renal dysfunction in fructose-induced hyperuricemia and metabolic syndrome in rats. hOAT1 and hUAT showed high identity to rOAT1 and rUAT (94 and 73 %) (9, 27, 37, 53), and hOCT1 and hOCT2 also exhibited high identity to rOCT1 and rOCT2 (78 and 64 %) (74). If indeed the findings in rats can be extrapolated to humans, this model of fructose-fed rats will also provide a powerful tool for predicting drug efficacy in metabolic syndrome patients with hyperuricemia and renal dysfunction. In addition, renal organic ion transporters play an important role in distribution and excretion of anion and cation drugs, result in the aggregation of exogenous and endogenetic noxious substances in the kidney, which further cause renal dysfunction. Expression of these renal transporters renders cells sensitive to drugs and noxious substance. In this regard, it should be noted that disorders of renal rSLC2A9v2, rRST, rOAT1, rUAT, rOCT1 and rOCT2 expression associated with hyperuricemia and renal dysfunction may increase the body’s exposure to drugs in clinic, and could cause unwanted side effects of drugs during high fructose consumption.

In conclusion, the present study demonstrated that hyperuricemia and renal dysfunction were mainly related to reduction of urinary urate excretion in fructose-induced metabolic syndrome of rats. Furthermore, we found that fructose increased rSLC2A9v2 and rRST mRNA and protein levels, decreased rOAT1, rUAT, rOCT1 and rOCT2 mRNA and protein levels, with PGE₂ elevation and NO reduction in the kidney of rats. The dysregulation of renal rSLC2A9v2, rRST, rOAT1 and rUAT might be responsible for reduced uric acid excretion.
involved in hyperuricemia. The alterations of renal rOCT1 and rOCT2 might be crucial for renal dysfunction in fructose-fed rats. Allopurinol, rutin and quercetin attenuated fructose-induced dysregulation of rSLC2A9v2, rRST, rOAT1 and rUAT expression, lowered serum uric acid levels and improved other abnormalities in rats. The regulatory mechanisms of renal PGE$_2$ and NO on these renal organic ion transporters were also in relevance of fructose-induced hyperuricemia and renal dysfunction. These findings that hypouricemic agents down-regulated renal rSLC2A9v2 and rRST and up-regulated renal rOAT1, rUAT, rOCT1 and rOCT2 in fructose-fed rats might provide novel therapeutic targets for hyperuricemia and renal dysfunction in metabolic syndrome.

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DISCLOSURES

The authors declare that there is no duality of interest associated with this manuscript.

REFERENCES


2. Abramson RG, King VF, Reif MC, Leal-Pinto E, Baruch SB. Urate uptake in membrane vesicles of rat renal cortex: effect of copper. *Am J Physiol Renal Physiol* 242:


37. Lu R, Chan BS, Schuster VL. Cloning of the human kidney PAH transporter: narrow


44. **Mo SF, Zhou F, Lv YZ, Hu QH, Zhang DM, Kong LD.** Hypouricemic action of


52. Perez-Ruiz F, Calabozo M, Erauskin GG, Ruibal A, Herrero-Beites AM. Renal underexcretion of uric acid is present in patients with apparent high urinary uric acid


**FIGURE LEGENDS**
Fig. 1. Effects of rutin, quercetin and allopurinol on dyslipidemia in fructose-fed rats (Fr). Rut, rutin; Que, quercetin; AP, allopurinol. A: Serum TC levels were significantly increased in fructose-fed rats and attenuated by rutin (50 and 100 mg/kg), quercetin (100 mg/kg) and allopurinol (5 mg/kg). B: Fructose caused an apparent decrease in serum TG levels and reversed by rutin (50 and 100 mg/kg), quercetin (50 and 100 mg/kg) and allopurinol (5 mg/kg). C: Lower serum HDL-C levels were observed in fructose-fed rats, which could be attenuated by rutin (50 and 100 mg/kg), quercetin (50 and 100 mg/kg) and allopurinol (5 mg/kg). D: Serum LDL-C levels were increased in fructose-fed rats and attenuated by rutin (50 and 100 mg/kg), quercetin (50 and 100 mg/kg) and allopurinol (5 mg/kg). Results represented mean ± S.E.M., n = 7 for each group. Statistical analyses were performed by a one-way ANOVA. *P<0.05, **P<0.01, ***P<0.001 vs. fructose-fed rats treated with vehicle. ##P<0.01, ###P<0.001 vs. control rats treated with vehicle.

Fig. 2. Effects of rutin, quercetin and allopurinol on glucose metabolism in fructose-fed rats (Fr) in OGTT test. Rut, rutin; Que, quercetin; AP, allopurinol. A: Fructose-fed rats treated with vehicle, rutin (50 and 100 mg/kg), quercetin (50 and 100 mg/kg) and allopurinol (5mg/kg) were examined for glucose response to and insulin response to OGTT test. B: ISI calculation revealed that fructose induced a significant decrease in insulin sensitivity that was improved by rutin, quercetin and allopurinol, respectively. Results represented mean ± S.E.M., n = 7 for each group. Statistical analyses were performed by a two-way ANOVA for insulin response to the OGTT test and by Student's t-test for ISI. *P<0.05, **P<0.01, ***P<0.001 vs. fructose-fed rats treated with vehicle. #P<0.05, ###P<0.001 vs. control rats treated with vehicle.

Fig. 3. Effects of rutin, quercetin and allopurinol on dysregulations of renal rSLC2A9v2, rRST, rOAT1, rOAT3, rUAT, rOCT1 and rOCT2 protein levels in fructose-fed rats (Fr). Rut,
rutin; Que, quercetin; AP, allopurinol. A-G: The densitometric ratios of renal brush border membrane rRST and rSLC2A9v2 proteins to rat Na\(^+\)-K\(^+\)-ATPase protein, renal cortex rOAT1, rOAT3, rLAT, rOCT1 and rOCT2 proteins to rat GAPDH protein detected by Western blot, in control rats treated with vehicle, fructose-fed rats treated with vehicle (Fr), fructose-fed rats treated with rutin (50 and 100 mg/kg), quercetin (50 and 100 mg/kg) and allopurinol (5 mg/kg), respectively. Data represented mean ± S.E.M., n = 4 for each group. Statistical analyses were performed by Student's \(t\)-test for the protein levels. *P<0.05, **P<0.01, ***P<0.001 vs. fructose-fed rats treated with vehicle. ##P<0.01, ###P<0.001 vs. control rats treated with vehicle.

Fig. 4. Effects of rutin, quercetin and allopurinol on dysregulations of renal rSLC2A9, rRST, rOAT1, rOAT3, rLAT, rOCT1 and rOCT2 mRNA expressions in fructose-fed rats (Fr). Rut, rutin; Que, quercetin; AP, allopurinol. A-G: The densitometric ratios of renal cortex rSLC2A9, rRST, rOAT1, rOAT3, rLAT, rOCT1 and rOCT2 mRNA levels to rat beta-actin mRNA level detected by RT-PCR, in control rats treated with vehicle, fructose-fed rats treated with vehicle (Fr), fructose-fed rats treated with rutin (50 and 100 mg/kg), quercetin (50 and 100 mg/kg) and allopurinol (5 mg/kg), respectively. Data represented mean ± S.E.M., n = 4 for each group. Statistical analyses were performed by Student's \(t\)-test for the mRNA levels. *P<0.05, **P<0.01, ***P<0.001 vs. fructose-fed rats treated with vehicle. ##P<0.01, ###P<0.001 vs. control rats treated with vehicle.

Fig. 5. Effects of rutin, quercetin and allopurinol on elevated PGE\(_2\) formation and suppressed NO production in rat kidney of fructose-fed rats (Fr). Rut, rutin; Que, quercetin; AP, allopurinol. A: PGE\(_2\) levels in rat kidney were increased by fructose consumption and reversed by rutin (50 and 100 mg/kg), quercetin (50 and 100 mg/kg) and allopurinol (5
mg/kg). B: A drastic reduction of NO levels in the kidney was observed in fructose-treated rats, which was restored by rutin (50 and 100 mg/kg), quercetin (50 and 100 mg/kg) and allopurinol (5 mg/kg). Results represented mean ± S.E.M., n = 7 for each group. Statistical analyses were performed by a one-way ANOVA. *P<0.05, **P<0.01, ***P<0.001 vs. fructose-fed rats treated with vehicle. ##P<0.01, ###P<0.001 vs. control rats treated with vehicle.

Fig. 6. Effects of rutin, quercetin and allopurinol on morphological changes in kidneys of fructose-fed rats (Fr). Rut, rutin; Que, quercetin; AP, allopurinol. Inflammatory cells were also infiltrated into the interstitium in fructose-fed rats. This tubulointerstitial pathology was disappeared after treatment with rutin, quercetin and allopurinol. Moreover, kidneys of control rat receiving rutin, quercetin and allopurinol, showed no any morphological changes compared to vehicle normal states.

Table 1. Summary of the sequences of gene-specific PCR primers, the appropriate annealing temperature and the length of production used in the experiments.

Table 2. Effects of rutin, quercetin and allopurinol on general parameters of metabolic syndrome and food/fluid intake in fructose-fed rats. Notes: Results represented mean ± S.E.M., n = 7 for each group. Statistical analyses were performed by Student's t-test. *p<0.05, **p<0.01, ***p<0.001 vs. fructose-fed rats treated with vehicle. ##p<0.05, ###p<0.01, ####p<0.001 vs. control rats treated with vehicle.

Table 3. Effects of rutin, quercetin and allopurinol on parameters of impaired uric acid excretion and renal dysfunction induced by fructose in rats. Notes: Results represented mean...
± S.E.M., n = 7 for each group. Statistical analyses were performed by Student's *t*-test.

*p*<0.05, **p*<0.01, ***p*<0.001 vs. fructose-fed rats treated with vehicle. #*p*<0.05, ##*p*<0.01, ###*p*<0.001 vs. control rats treated with vehicle.
Fig. 1.
Fig. 2.
Fig. 3.
Fig. 4.
Normal Control  Fructose-fed Control

Fructose + Rutin (50 mg/kg)  Fructose + Rutin (100 mg/kg)

Fructose + Quercetin (50 mg/kg)  Fructose + Quercetin (100 mg/kg)

Fructose + Allopurinol (5 mg/kg)

Fig. 6.
<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer Sequence</th>
<th>Annealing temperature</th>
<th>Length of production</th>
<th>NCBI accession No.</th>
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<td>5’-CGGAGGAATTGAGACGCTAG-3’ 5’-TTGTGGCAAGACGAGGA-3’</td>
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<td>rOAT3</td>
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<td>rUAT</td>
<td>5’-AAAGCAGAATGGGAAGTGG-3’ 5’-ACGCCTGATATGGATGGGA-3’</td>
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<td>rOCT1</td>
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<td>rOCT2</td>
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Table 2.

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<th>SBP (mmHg)</th>
<th>Serum insulin (pmol/l)</th>
<th>Body weight (g)</th>
<th>Food intake (g/24h)</th>
<th>Fluid intake (ml/24h)</th>
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<td>1.96±0.04</td>
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<td>64.77±2.11</td>
<td>379.7±14.6</td>
<td>27.15±1.01</td>
<td>63.25±3.30</td>
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<td>62.14±4.89</td>
<td>377.3±9.8</td>
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<td>26.78±1.09</td>
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<td>26.28±1.11</td>
<td>69.02±5.78</td>
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<td>1.51±0.08***</td>
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<td>67.33±4.01</td>
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<tr>
<td>Fructose</td>
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<td>121.9±2.5***</td>
<td>104.15±3.63***</td>
<td>432.1±8.0***</td>
<td>23.19±1.22**</td>
<td>71.41±7.61</td>
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<td>71.08±5.03**</td>
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<td>361.0±20.9**</td>
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<td>68.36±4.08***</td>
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<td>372.3±24.5*</td>
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<td>Serum creatinine (mg/dl)</td>
<td>BUN (mg/dl)</td>
<td>Urine volume (ml/24h)</td>
<td>Urinary uric acid (mg/24h)</td>
<td>Urinary creatinine (mg/24h)</td>
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<td>188.44±15.35</td>
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