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

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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, 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 the molecular mechanism for fructose-induced hyperuricemia is essential to an understanding of the progression of renal dysfunction and metabolic syndrome.

The reabsorption and secretion of renal uric acid are governed by a complex system of classic polyspecific urate transporters including SLC2A and SLC22A family proteins in the proximal kidney tubule. SLC2A9 is first identified as a member of the SLC2A gene family of hexose transporters. In humans, 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 the reduction of serum uric acid concentrations and elevation of the fractional excretion of uric acid (FE_{ur}) (13, 39, 72). The renal-specific transporter (RST) identified in the mouse and rat (28) is a homolog of SLC22A12 protein (human urate transporter 1). RST localizes predominantly on the brush-border membrane of the proximal renal tubule and is responsible for urate reabsorption (26). 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 benz bromarone exhibited hypouricemic action by inhibiting OAT1-mediated urate secretion (29, 57). OAT1-knockout mice had reduced uric acid secretion (50). Downregulation of renal OAT1 and OAT3 was previously observed in hyperuricemic rats induced by combined administration of oxonate (uricase inhibitor) and uric acid (19, 20), indicating that OAT1 and OAT3 play an important role in the pathogenesis of hyperuricemia (14). In addition, the electronegative urate transporter (UAT) is also involved in uric acid secretion across the luminal membrane in the 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 OCT2 in the rat kidney was decreased not only in ischemia- and reperfusion-induced acute kidney injury (40) but also in oxonic acid-induced hyperuricemia (19, 20). The reduction of renal rOCT1 and rOCT2 expression was also reported in streptozotocin-induced diabetic 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). Also, increased levels of prostaglandin E2 (PGE2), a primary mediator of inflammation in the kidney, led to downregulation of rOAT1 and rOAT3 expressions in the rat proximal tubular cell line NRK-52E (64). Therefore, fructose-induced hyperuricemia may result in alternations in the level of rOCT1 and rOCT2, focusing on their potential role 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 the subject of extensive research (47), the knowledge about the mechanisms of its action on renal organic ion transporters in fructose-fed rats is limited. Therefore, in the present study allopurinol was employed to improve our understanding of the role of these transporters in hyperuricemia and renal dysfunction in fructose-induced metabolic syndrome.

MATERIALS AND METHODS

Reagents. Allopurinol, rutin, and quercetin were purchased from Sigma (St. Louis, MO). 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, China), respectively. An ELISA kit for insulin assay was purchased from Mercodia (Uppsala, Sweden). A glucose assay kit was purchased from Beckman Instruments (Irvine, CA). An ELISA kit for PGE2 assay was purchased from R&D. TRizol reagent was obtained from Invitrogen, and Moloney murine leukemia virus reverse transcriptase was from Promega. All the primers were designed and synthesized by Shengxing Biotech (Nanjing, China). The antibodies of rSLC2A9 single-nucleotide polymorphism (SNP) (NCBI accession no. XP_577349), rRST (NCBI accession no. NP_01030115), 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, China). GAPDH and anti-rabbit IgG antibodies were purchased from Jingmei Biotech (Shanghai, China).

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>
<thead>
<tr>
<th>Primer</th>
<th>Annealing Temperature, °C</th>
<th>Length of Production, bp</th>
<th>NCBI Accession No.</th>
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<tr>
<td>rSLC2A9 SNP</td>
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<td>380 bp</td>
<td>XM_577349</td>
</tr>
<tr>
<td>rSLC2A9</td>
<td>55°C</td>
<td>380 bp</td>
<td>XM_577349</td>
</tr>
<tr>
<td>rRST</td>
<td>57°C</td>
<td>427 bp</td>
<td>NM_001034943</td>
</tr>
<tr>
<td>rOAT1</td>
<td>56°C</td>
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<td>rOAT3</td>
<td>56°C</td>
<td>394 bp</td>
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</tr>
<tr>
<td>rUAT</td>
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<td>414 bp</td>
<td>U67958</td>
</tr>
<tr>
<td>rOCT1</td>
<td>58°C</td>
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</tr>
<tr>
<td>rOCT2</td>
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</tr>
<tr>
<td>β-Actin</td>
<td>56°C</td>
<td>310 bp</td>
<td>NM_031144</td>
</tr>
</tbody>
</table>

OAT, organic anion transporter; OCT, organic cation transporter; UAT, urate transporter.
Rat Na\(^+\)-K\(^+\)-ATPase antibody was supplied by Cell Signaling Technology (Boston, MA).

**Animals.** Male Sprague-Dawley rats were purchased from the Laboratory Animal Center (Hangzhou, Zhejiang Province, China) and housed in plastic cages with a 12:12-h light-dark cycle at a constant temperature of 22–24°C. They were given standard chow ad libitum for the duration of the study and allowed 1 wk to adapt to the laboratory environment before experiments. All the procedures were in strict accordance with Chinese 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 the 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 wk (43, 70). Fresh drinking water was replaced every 2 days. After 4-wk fructose feeding, fructose-fed rats were further divided into matched subgroups. Control animals were also divided. Different groups of animals (n = 7 in each 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 wk. Body weight was recorded once a week during this time interval.

**Measurement of systolic blood pressure.** Systolic blood pressure (SBP) was measured in the morning using a tail-cuff method and recorded using an ALC-NIBP noninvasive blood pressure analysis system (Biosythesis Biotechnology, Shanghai, China). All animals were preconditioned for SBP measurements 1 wk before the experiment, and the mean value of three consecutive measurements was obtained.

**Oral glucose tolerance test.** The oral glucose tolerance test (OGTT) was performed according to the method described by Matsuda (38). One hour after drug administration, glucose (1.5 g/kg body 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 (3,000g) 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: 10,000/square root of (fasting glucose × fasting insulin) × (mean glucose × mean insulin during OGTT).

**Blood and tissue processing.** After the OGTT, animals were allowed 3 days to recover, and then their 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 9:00–10:00 a.m. after a 16-h fast, kidney tissues were dissected quickly on ice, and stored in liquid nitrogen for PGE2, NO, RT-PCR, and Western blot analysis, respectively. Blood samples were centrifuged (3,000g) 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 and urine were determined by the phosphotungstic acid method (8), respectively.

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

**Semiquantitative RT-PCR analysis.** Total RNA was extracted from rat kidney using TRIzol reagent (Invitrogen). The homogenate was mixed with 200 μl chloroform and then centrifuged at 12,000g for 15 min. The aqueous phase (~0.5 ml of the upper layer) was precipitated with an equal volume of isopropanol and centrifuging at 12,000g for 10 min. The final total RNA pellet was resuspended in 20 μl of DEPC water.

Reverse transcription was performed with 1 μg RNA using Moloney murine leukemia virus reverse transcriptase (Promega) for cDNA synthesis. PCR amplification was carried out using gene-specific PCR primers synthesized by Shengxing Biotech (Nanjing, Jiangsu, China). The sequences of gene-specific PCR primers, the appropriate annealing temperature, and the length of production are 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 β-actin mRNA levels.

**Western blot analysis.** Tissue samples of rat kidney cortex were homogenized in 10 (wt/vol) 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 PMSF), using a Polytron set and centrifuged at 3,000g for 15 min. The supernatant was centrifuged at 12,000g 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 2D-SDS-PAGE using the Power Pac Basic electrophoresis apparatus (Bio-Rad, Hercules, CA), the protein samples were transferred onto polyvinylidene difluoride membranes (Millipore, Shanghai, China), which were then blocked with 5% skim milk and subsequently incubated with primary antibodies and horseradish peroxidase-conjugated secondary antibody. Immuno-reactive bands were visualized by incubation with lumiGLO reagent (Cell Signaling, Beverly, MA) and exposure to X-ray film (Kodak, New Haven, CT). Primary antibodies included rabbit polyclonal antibodies against rOAT1 (diluted 1:2,000), rOAT3 (1:2,000), rUTA1 (1:200), rOCT1 (1:3,000), OCT2 (1:200), and GAPDH (1:5,000).

**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. (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 o-mannitol, 5 mM EGTA, and 12 mM MgCl2 by a Polytron on setting 5. After 20 min, the homogenate was centrifuged at 2,400g for 15 min. The supernatant was collected and centrifuged at 30,000g for 30 min. The pellet was then resuspended in 5 mM HEPES-Tris (pH 7.4) buffer containing 150 mM o-mannitol, 2.5 mM EGTA, and 12 mM MgCl2 and homogenized using a glass-Teflon homogenizer for 20 min. The homogenate was centrifuged at 2,400g for 15 min. The supernatant was collected and centrifuged at 30,000g for 30 min. The final pellet 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 the 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 SNAP (1:1,000), rRST (1:2,000), and rat Na\(^+\)-K\(^+\)-ATPase (1:1,000) were used, respectively.

**Renal PGE2 assay.** After weighing, the wet tissue of the rat kidney was homogenized in 10 vol 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 10,000g 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 Bayrak (6). After weighing, the wet tissue of the kidney was homogenized in 5 vol of ice-cold Tris·HCl buffer (50 mM, pH 7.4) containing 0.5% Triton X-100 and 13,000g (4°C) for 2 min. The lipid layer was carefully removed by centrifugation at 10,000g (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’s 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
The slides were mounted with neutral balsam. Deparaffinized in xylene, rehydrated in decreasing concentrations of ethanol in water, and mounted on APES-coated glass slides. Sections were stained with hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS). The slides were covered with APES-coated glass coverslips. Cell count was performed using ImageJ software.

Table 2. Effects of rutin, quercetin, and allopurinol on general parameters of metabolic syndrome and food/fluid intake in fructose-fed rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose, mg/kg</th>
<th>Serum Uric Acid, mg/dl</th>
<th>SBP, mmHg</th>
<th>Serum Insulin, pmol/l</th>
<th>Body Weight, g</th>
<th>Food Intake, g/24 h</th>
<th>Fluid Intake, ml/24 h</th>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>1.96±0.04</td>
<td>107.5±1.0</td>
<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>Control+ rutin</td>
<td>2.15±0.14</td>
<td>105.0±2.4</td>
<td>62.14±4.89</td>
<td>377.3±9.8</td>
<td>28.00±1.22</td>
<td>64.90±4.68</td>
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<tr>
<td></td>
<td>2.09±0.06</td>
<td>102.6±2.2</td>
<td>61.61±4.11</td>
<td>361.2±16.7</td>
<td>27.39±1.15</td>
<td>60.20±6.78</td>
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</tr>
<tr>
<td>Control+ quercetin</td>
<td>2.06±0.09</td>
<td>105.1±3.0</td>
<td>66.63±2.72</td>
<td>409.0±15.1</td>
<td>26.78±1.09</td>
<td>65.82±3.92</td>
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<td></td>
<td>1.96±0.07</td>
<td>106.3±3.1</td>
<td>65.90±2.87</td>
<td>404.3±15.3</td>
<td>26.28±1.11</td>
<td>69.02±5.78</td>
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<td>Control+ allopurinol</td>
<td>1.51±0.08</td>
<td>106.6±2.2</td>
<td>60.68±2.97</td>
<td>352.9±23.8</td>
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<td>Fructose</td>
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<tr>
<td></td>
<td>2.83±0.13</td>
<td>121.9±2.5</td>
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<td>372.3±24.5</td>
<td>24.79±1.96</td>
<td>74.50±7.51</td>
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Values are means ± SE; n = 7/group. SBP, systolic blood pressure. Statistical analyses were performed by Student’s t-test, *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.

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

RESULTS

Allopurinol, rutin, and quercetin attenuate fructose-induced metabolic syndrome in rats. As shown in Table 2 and Fig. 1, compared with vehicle-fed rats, rats fed 10% fructose in the drinking water for 8 wk 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, and 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. 1).

Fig. 1. Effects of rutin, quercetin, and allopurinol on dyslipidemia in fructose-fed rats (Fr). Rut, rutin; Que, quercetin; AP, allopurinol. A: serum total cholesterol (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 triglyceride (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). Values are means ± SE; n = 7/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.
Metabolic syndrome in fructose-fed rats in a dose-dependent manner lowered serum uric acid and improved the features of dysregulated glucose metabolism in these rats. The Uur/Ucr ratio, as well as FEur-related renal uric acid excretion, increased in fructose-fed rats compared with control vehicle.)

In agreement with previous findings (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 administered 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 with control vehicle rats. The Uur/Ucr ratio, as well as FEur-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 a 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 urinary 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 the Uur/Ucr ratio in fructose-fed rats (Table 3). Rutin and quercetin at 50 and 100 mg/kg reversed fructose-induced FEur reduction in rats. Treatment with allopurinol, rutin, and quercetin failed to reverse fructose-induced elevated urine volume in 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 ion transporters are the major mechanisms for urate underexcretion and renal dysfunction in fructose-induced metabolic syndrome, we examined expressions of renal transporters in rats. As reported (47, 54), allopurinol significantly prevented fructose-induced reduction of urinary 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 the Uur/Ucr ratio in fructose-fed rats (Table 3). Rutin and quercetin at 50 and 100 mg/kg reversed fructose-induced FEur reduction in rats. Treatment with allopurinol, rutin, and quercetin failed to reverse fructose-induced elevated urine volume in 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).

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rSLC2A9, rRST, rOAT1, rOAT3, rUAT, rOCT1, and rOCT2 in fructose-fed rats.

A single nucleotide polymorphism (SNP; Val253Ile, rs16890979) in SLC2A9 regarded as a conservative region is most strongly associated with uric acid levels (5, 42). Thus we used this SNP coding sequence to examine rSLC2A9 expression in the rat kidney. As shown in Fig. 3A, a single band at 60 kDa represented rSLC2A9v2 protein in the apical membrane of the proximal tubules (4). Significant increases in renal rSLC2A9v2 and rRST protein levels were observed in fructose-fed rats compared with control-vehicle rats (Fig. 3, A and B), which was likely due to the upregulation of their mRNA levels (Fig. 4, A and B). Fructose also induced significant decreases in renal rOAT1, rUAT, rOCT1, and rOCT2 at both protein (Fig. 3, C-G) and mRNA levels (Fig. 4, C-G) in rats. However, rOAT3 expression was not altered by fructose consumption (Figs. 3D and 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 (Figs. 3, A-G, and 4, A-G). 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 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 with control-vehicle animals (Fig. 5, A and B). Allopurinol, rutin, and quercetin significantly attenuated fructose-induced renal PGE2 and NO alteration in rats (Fig. 5, A and B) 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 the interstitium in fructose-fed rats (Fig. 6). This tubulointerstitial pathology 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 with vehicle normal states (data not shown).

DISCUSSION

The present study confirmed that fructose consumption caused reductions of Ur, Ucr, the Ur/Uc ratio, and FExr, eliciting urinary uric acid underexcretion and serum uric acid elevation in fructose-fed rats. More importantly, the hypouricemic agents allopurinol, 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 allopurinol, rutin, and quercetin might, at least partly, correlate 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 upregulated the expression levels of rSLC2A9v2 and rRST, downregulated the expression levels of rOAT1, rUAT, rOCT1, and rOCT2 in the rat kidney, accompanying 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 in rats. Moreover, fructose consumption elevated PGE2 and reduced NO in the 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, downregulators of rSLC2A9v2 and rRST and upregulators of 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 kidneys 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. Downregulation of rOATs, rUAT, rOCT1, and rOCT2 (Figs. 3 and 4) might lead to excessive accumulation of endogenous and exogenous toxin, resulting in renal damage in fructose-fed rats. Regulation of allopurinol, rutin, and
Fig. 3. Effects of rutin, quercetin, and allopurinol on dysregulations of renal rSLC2A9v2, renal-specific transporter (rRST), organic anion transporter rOAT1, rOAT3, rUAT, rOCT1, and rOCT2 protein levels in fructose-fed rats (Fr). A–G: densitometric ratios of renal brush border membrane rRST and rSLC2A9v2 proteins to rat Na⁺-K⁺-ATPase protein, renal cortex rOAT1, rOAT3, rUAT, rOCT1, and rOCT2 proteins to rat GAPDH protein detected by Western blotting in control rats treated with vehicle, fructose-fed rats treated with vehicle (Fr), and fructose-fed rats treated with rutin (50 and 100 mg/kg), quercetin (50 and 100 mg/kg), and allopurinol (5 mg/kg), respectively. Values are means ± SE; n = 4/group. Statistical analyses were performed by Student’s t-test for 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, rUAT, rOCT1, and rOCT2 mRNA expressions in fructose-fed rats (Fr). A–G: densitometric ratios of renal cortex rSLC2A9, rRST, rOAT1, rOAT3, rUAT, rOCT1, and rOCT2 mRNA levels to rat β-actin mRNA level detected by RT-PCR in control rats treated with vehicle, fructose-fed rats treated with vehicle (Fr), and fructose-fed rats treated with rutin (50 and 100 mg/kg), quercetin (50 and 100 mg/kg), and allopurinol (5 mg/kg), respectively. Values are means ± SE; n = 4/group. Statistical analyses were performed by Student’s t-test for 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.
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 rodents (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 basolateral organic anion uptake by PGE2 is a widespread regulatory mechanism. PGE2 inhibited its own renal transport by downregulation 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 downregulation 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 in rats (47). In the present study, fructose induced the elevation of PGE2 and the reduction of NO in the 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 the kidney might be relative to downregulation of renal rOAT1, rUAT, rOCT1, and rOCT2, which make significant contributions to high serum urate levels and renal dysfunction in fructose-induced metabolic syndrome in 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 PGF2α to 6-oxo-PGE1 and PGE2, respectively (45), and PGE2 production in human neutrophils (7). Quercetin inhibited activated PGE2 production in LPS-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, resulting in the aggregation of exogenous and endogenous noxious substances in the kidney, which further cause renal dysfunction. Expression of these renal transporters renders cells sensitive to drugs and noxious substances.

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 in 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 kidneys 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 PGE2 and NO on these renal organic ion transporters were also relevant to fructose-induced hyperuricemia and renal dysfunction. These findings that hypouricemic agents downregulated renal rSLC2A9v2 and rRST and upregulated 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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