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Effects of febuxostat on metabolic and renal alterations in rats with fructose-induced metabolic syndrome

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Departments of 1Nephrology and 2Pathology, Instituto Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico; 3Nephrology, Hypertension and, Transplantation, University of Florida, Gainesville, Florida; and 4TAP Pharmaceutical Products, Incorporated, Lake Forest, Illinois

Submitted 2 October 2007; accepted in final form 23 January 2008

Sánchez-Lozada LG, Tapia E, Bautista-García P, Soto V, Ávila-Casado C, Vega-Campos IP, Nakagawa T, Zhao L, Franco M, Johnson RJ. Effects of febuxostat on metabolic and renal alterations in rats with fructose-induced metabolic syndrome. Am J Physiol Renal Physiol 294: F710–F718, 2008. First published January 23, 2008; doi:10.1152/ajprenal.00454.2007.—Increased fructose consumption is associated with hyperuricemia, metabolic syndrome, and renal damage. This study evaluated whether febuxostat (Fx), an investigational nonpurine, and selective xanthine oxidase inhibitor, could alleviate the features of metabolic syndrome as well as the renal hemodynamic alterations and afferent arteriolar area relative to fructose (P). Body weight was measured daily. Systolic blood pressure and fasting plasma uric acid (UA), insulin, and triglycerides were measured at baseline and at 4 and 8 wk. Renal hemodynamics and histomorphology were evaluated at the end of the study. A high-fructose diet was associated with hyperuricemia, hypertension, as well as increased plasma triglycerides and insulin. Compared with fructose + P, fructose + Fx rats showed significantly lowered blood pressure, UA, triglycerides, and insulin (P < 0.05 for all comparisons). Moreover, fructose + Fx rats had significantly reduced glomerular pressure, renal vasoconstriction, and afferent arteriolar area relative to fructose + P rats. Fx treatment in rats on a normal diet had no significant effects. In conclusion, normalization of plasma UA with Fx in rats with metabolic syndrome alleviated both metabolic and glomerular hemodynamic and morphological alterations. These results provide further evidence for a pathogenic role of hyperuricemia in fructose-mediated metabolic syndrome.

ATP to ADP by fructokinase; fructose also decreases the renal excretion of uric acid (UA) through an increase in lactate synthesis (19).

Interestingly, epidemiological studies have demonstrated that the prevalence of metabolic syndrome shows a graded increase with increasing serum UA levels (6, 22, 26, 29, 56), suggesting that hyperuricemia could be included in the definition of metabolic syndrome (10, 44, 51). Moreover, serum UA concentration increases in parallel to the number of metabolic syndrome traits (11, 30, 56); thus serum UA concentration is positively correlated with blood pressure, waist-to-hip ratio, the homeostasis model assessment index (an index of insulin resistance), body mass index, and levels of fasting plasma glucose, insulin, triglycerides, C-reactive protein, and is inversely correlated with HDL-cholesterol levels (reviewed in Ref. 51). In addition, it has been found that serum UA levels may be a reliable predictor of premetabolic syndrome in obese youth (13).

There is evidence that the increased serum UA occurring with metabolic syndrome may not only be a consequence of hyperinsulinemia (33, 34), but it may actually promote or exacerbate features of metabolic syndrome. For example, UA may play an important role in the pathogenesis of metabolic syndrome due to its ability to inhibit nitric oxide (NO) bioavailability and thereby induce endothelial dysfunction, which is the hallmark of insulin resistance (25, 39). Supporting this contention is the observation that increasing amounts of fructose in the diet results in escalating levels of hyperuricemia, which correlates with increasing features of metabolic syndrome in rats (38). Additional experimental studies have shown that chronic administration of allopurinol [an inhibitor of xanthine oxidase (XO)] or benzbromarone (a uricosuric agent) can reduce fructose-induced hyperinsulinemia and hypertriglyceridemia (33). Another potential mechanism activated by UA that may contribute to the pathogenesis of metabolic syndrome is oxidative stress. In this regard, it was shown that soluble UA stimulated an increase in NADPH oxidase activity and the production of reactive oxygen species (ROS) in mature adipocytes, which resulted in activation of

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MAP kinases p38 and Erk1/2, reduction in NO bioavailability, and increased protein nitrosylation and lipid oxidation (43). Collectively, these studies suggest that increased levels of UA play a fundamental role in the pathogenesis of the metabolic syndrome.

Clinical and experimental observations suggest that both metabolic syndrome and hyperuricemia confer a higher susceptibility to develop chronic kidney disease (9, 21). Clinical studies have shown that the greater the number of metabolic syndrome traits exhibited, the greater the risk of developing microalbuminuria (9). Moreover, a recent study suggests that increased metabolic risk in young healthy males is associated with a 6.9-fold increase in the likelihood of glomerular hyperfiltration occurring before the onset of overt cardiovascular disease (49). Hyperuricemia has also been associated with chronic renal damage. In a study of 6,400 subjects with normal renal function, a value of serum UA of >8.0 mg/dl was associated with a 2.9- and 10.0-fold increased risk for developing renal insufficiency within 2 yr in men and women, respectively (21). Increased serum UA has also been found to be a predictor of poor outcome in patients with IgA nephropathy (47). Recently, it was shown that allopurinol treatment for 12 mo in hyperuricemic patients with mild-to-moderate chronic renal disease significantly decreased serum UA and helped to preserve kidney function compared with patients receiving no allopurinol (46). However, even more compelling evidence of the contribution of hyperuricemia to the development and progression of renal damage comes from experimental studies. Rats made hyperuricemic by the administration of oxonic acid (inhibitor of uricase) developed systemic and glomerular hypertension, mild tubulointerstitial damage, arteriolaropathy of the afferent arteriole, and cortical vasoconstriction (32, 40, 41); these effects were prevented by reducing serum UA levels through the simultaneous administration of oxonic acid and allopurinol or benziodarone (32, 40, 41). Interestingly, the hyperuricemia induced by high fructose intake in rats produced renal effects similar to those seen in experimental hyperuricemia; a 60% fructose diet raised plasma UA and induced glomerular hypertension, cortical vasoconstriction, and afferent arteriole thickening (38).

Febuxostat, an investigational, nonpurine molecule, is being developed as an inhibitor of XO for the treatment of hyperuricemia in gout patients (3, 4). Febuxostat is different from allopurinol in that it does not inhibit other enzymes in purine and pyrimidine metabolism pathways (48). Moreover, studies have shown that febuxostat inhibits XO activity simply by obstructing substrate binding and that this inhibition is not influenced by changes in the redox status of the cofactor (36). Because it has been shown previously that administration of allopurinol, as a prophylactic agent or as treatment, prevented or reversed features of metabolic syndrome (33), the present study was undertaken to determine whether chronic treatment with febuxostat can alleviate the major traits of fructose-induced metabolic syndrome as well as the accompanying renal hemodynamic alterations and afferent arteriolopathy in rats.

METHODS

Experimental design. Four groups of male Sprague-Dawley rats (n = 10/group; 290–350 g) were studied over a period of 8 wk. Two groups (normal) received a regular diet and the other two groups (fructose) were fed a 60% fructose diet to induce development of the metabolic syndrome. Details on the composition of the diets are presented in Table 1. After 4 wk, febuxostat (Fx) was administered in the drinking water (50 mg/l; ~5–6 mg.kg⁻¹.day⁻¹) for an additional 4 wk in one normal-diet group (normal+Fx) and one fructose-diet group (fructose+Fx). Respectively placebo (P) control groups (normal+P and fructose+P) received no treatment, except for an additional amount of NaCl in the drinking water (5.84 mg/l, to maintain a salt concentration equivalent to that of the Fx-containing water) for 4 additional wk.

Two additional groups of fructose-fed rats (n = 5/group) were studied to assess the effects of a longer period (14 wk) of fructose feeding and Fx treatment on systemic blood pressure. Both groups received a 60% fructose diet for the entire 14 wk; one group received Fx (50 mg/l in the drinking water) for 10 wk (from week 5 to week 14), and the other group served as the placebo control. All experiments were approved by the Ethics Committee of the Instituto Nacional de Cardiologia Ignacio Chavez.

Measurements. Body weight and food intake were measured daily. Systolic blood pressure (SBP) was measured in conscious rats by a tail-cuff sphygmomanometer (XBP-1000 Kent Scientific, Torrington, CT). All animals were preconditioned for blood pressure measurements 1 wk before each experiment. Fasting plasma UA (Diagnostic Chem, Charlottetown, PEI, Canada), insulin (Crystal Chem, Downs Grove, IL), and triglycerides (Spinreact, Girona, Spain) were measured using commercial kits. SBP and all biochemical parameters were determined before the start of fructose feeding (i.e., baseline) and at the end of 4 and 8 wk.

For the two groups administered fructose for 14 wk, body weight was measured at baseline and weekly thereafter. SBP was measured at baseline and at the end of 4, 8, and 12 wk. No other measurements were conducted in these groups.

Micropuncture. Animals were anesthetized with pentobarbital sodium (30 mg/kg ip) and placed on a thermoregulated table to maintain body temperature at 37°C. Trachea, jugular veins, femoral arteries, and the left ureter were catheterized with polyethylene tubing (PE-240, PE-50, and PE-10). The left kidney was exposed, placed in a Lucite holder, sealed with agar, and covered with Ringer solution. Mean arterial pressure (MAP) was monitored with a pressure transducer (model p23 db, Gould, San Juan, PR) connected to the catheter in the femoral artery and recorded on a polygraph (Grass Instruments, Quincy, MA). Blood samples were taken periodically and replaced with blood from a donor rat. Rats were maintained under euolemic conditions by infusion of 10 ml/kg body weight of isotonic rat plasma during surgery, followed by an infusion of 25% polyfructosan at 2.2 ml/h (Inuteast, Fresenius Kabi, Linz, Austria). After 60 min, five to seven samples of proximal tubular fluid were obtained to determine flow rate and polyfructosan concentrations. Intratubular pressure under free-flow (FF) and stop-flow (SFP) conditions and peritubular capillary pressure (Pc) were measured in other proximal tubules with

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<tr>
<th>Table 1. Relevant nutrient content of rodent experimental diets</th>
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<td>Constituent</td>
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<tr>
<td>Energy, kcal/g</td>
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<tr>
<td>Starch, %</td>
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<td>Sucrose, %</td>
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<td>Fructose, %</td>
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<td>Protein, %</td>
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<td>Fat, %</td>
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<tr>
<td>Vitamins, %</td>
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<td>Minerals, %</td>
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Nutrient composition is presented as % of weight. Normal diet, catalog no. 2018, Teklad Global, 18% protein rodent diet; fructose diet: catalog no. TD 78463. Harlan Teklad, Madison, WI.
a servo-null device (Servo Nulling Pressure System, Instrumentation for Physiology and Medicine, San Diego, CA). Glomerular colloid osmotic pressure was estimated from protein concentrations obtained from blood of the femoral artery and surface efferent arterioles. Polyfructosan was measured in plasma and urine samples by the anthrone-based technique of Davidson and Sackner (12). The total glomerular filtration rate (GFR) was calculated using the following formula: \( GFR = \frac{U \times V}{P} \), where \( U \) is the polyfructosan concentration in urine, \( V \) is urine flow rate, and \( P \) is the polyfructosan concentration in plasma.

The volume of fluid collected from individual proximal tubules was estimated from the length of the fluid column in a constant-bore capillary tube of known internal diameter. The concentration of tubular polyfructosan was measured by the microfluorometric method of Vurek and Pegram (54). Single-nephron GFR (SNGFR) was calculated using the formula \( SNGFR = \frac{TF/P}{PF} \times V \), where \( PF \) is the concentration of polyfructosan in tubular fluid (TF) and plasma (P), and \( V \) is the tubular flow rate which is obtained by timing the collection of tubular fluid (2). The protein concentration in afferent and efferent samples was determined according to the method of Viets et al. (53). MAP, GFR, glomerular capillary hydrostatic pressure (PGC), single-nephron plasma flow (QA), afferent arteriole (AR), efferent arteriole (ER), and total (TR) resistances and ultrafiltration coefficient (\( K_f \)) were calculated according to equations previously reported (2).

Renal histology and quantification of morphology. After the micropuncture study, kidneys were washed by perfusion with PBS and fixed with 4% paraformaldehyde. Renal biopsies were embedded in paraffin. Four-micrometer sections of fixed tissue were stained with periodic acid-Schiff (PAS) reagent. Arteriolar morphology was assessed by indirect peroxidase immunostaining for \( \alpha \)-smooth muscle actin (DAKO, Carpinteria, CA) (40, 41). Sections of kidney tissue incubated with normal rabbit serum were used as negative controls for immunostaining against \( \alpha \)-smooth muscle actin.

For each arteriole, and in 10 arterioles/biopsy, the outline of the vessel and its internal lumen (excluding the endothelium) were generated using computer-based analysis to calculate the total medial area (outline – inline). The media/lumen (M/L) ratio was calculated by the outline/inline relationship. Quantifications were performed blinded.

Statistical analysis. Values are expressed as means ± SE. Body weight, food intake, SBP, and the biochemical parameters measured over the course of the study were analyzed for treatment and diet effects, time effect, and treatment-by-time interaction using two-way ANOVA for repeated-measures test. Single-time measurements (MAP, whole-kidney GFR, glomerular hemodynamics, and histological parameters) were analyzed using standard two-way ANOVA. When the ANOVA \( P \) value was <0.05, posttest comparisons were made using a Bonferroni multiple-comparison test. The relationship between variables was assessed by correlation analysis. In the 14-wk fructose-feeding study, comparisons were made using an unpaired Student’s \( t \)-test.

RESULTS

Metabolic parameters, blood pressure, and body weight. Plasma UA increased in fructose-fed rats and was reversed with febuxostat treatment to normal levels (Fig. 1), while fructose-fed rats treated with placebo continued to show increased levels of plasma UA. Fasting triglyceride levels were

![Fig. 1. Effects of a high-fructose diet and febuxostat on plasma uric acid (UA), triglycerides (TG), and insulin (Ins) and on systolic blood pressure (SBP) in conscious rats (2-way ANOVA for repeated measurements; \( n = 10 \)/group). Fx, febuxostat; P, placebo. Results are mean arterial pressure (MAP) in anesthetized rats at week 8 (standard 2-way ANOVA; \( n = 8 \)/group, except for fructose diet+Fx group; \( n = 10 \)); 2-way ANOVA for repeated measurement: \( P < 0.0001 \) for treatment and time interaction for UA, TG, Ins, and SBP; and 2-way ANOVA: \( P = 0.003 \) for diet and treatment interaction in MAP. Bonferroni posttest comparisons: \( a,bP < 0.05 \).]
<100 mg/dl in all groups at baseline (Fig. 1). By week 4, rats fed a high-fructose diet had triglyceride levels that were twice those of rats fed a normal diet. Treatment with febuxostat during weeks 5–8 resulted in a significant decrease in triglyceride levels, while the levels in the untreated fructose-fed animals continued to rise. A positive correlation between UA concentrations and triglycerides at week 8 in all groups of rats was observed (UA vs. triglycerides: \( r = 0.69, P < 0.0001 \)).

Fasting plasma insulin levels rose with a high-fructose diet, becoming significantly elevated at week 8 (Fig. 1). Febuxostat treatment resulted in normalization of insulin levels, while continued administration of fructose alone caused a progressive increase. A positive correlation between UA and insulin concentrations in all groups of rats was present at week 8 (UA vs. insulin: \( r = 0.73, P < 0.0001 \)).

A high-fructose diet resulted in a significant rise in SBP at week 4, which persisted until the end of the study. Febuxostat tended to reduce blood pressure in rats fed a high-fructose diet, although the reduction was not statistically significant relative to the fructose+P group (Fig. 1). MAP measured by intrarterial measurement at week 8 was also increased in fructose-fed rats and was normalized by febuxostat treatment (\( P < 0.05 \)). A positive correlation between UA and MAP in all groups of rats was observed (\( r = 0.54, P = 0.001 \)). Febuxostat treatment in rats fed a normal diet had no significant effects on metabolic parameters and blood pressure.

Due to the discrepancy between the SBP and MAP data in the 8-wk study, an additional set of rats were fed a high-fructose diet for 14 wk (Fig. 2). Baseline values of SBP were similar between groups [fructose+P, 120 ± 4 mmHg; fructose+Fx, 117 ± 4 mmHg; \( P = \) not significant (NS)]. After four weeks of a high-fructose diet and before the start of placebo or febuxostat treatment, both groups developed hypertension (fructose+P, 153 ± 4 mmHg; fructose+Fx, 152 ± 4 mmHg; \( P = \) NS). SBP increased progressively with continued fructose feeding and reached a value of 163 ± 6 mmHg at 12 wk. Febuxostat treatment completely abrogated this increase in SBP (121 ± 4 mmHg at week 12, \( P = 0.0003 \)). This treatment with febuxostat for longer than 8 wk may be needed to enable detection of systemic blood pressure changes measured by the tail-cuff method in conscious rats.

Daily food intake and body weight measured during the course of the 8-wk study are presented in Fig. 3. The interaction between time and treatment was found to be significant for both parameters. However, the results of the Bonferroni post hoc test between treatments rendered statistical significance only at isolated time points, mostly at the beginning of the study when the rats’ diet was switched from normal to high fructose. Nonetheless, the overall tendency in food intake and body weight was for no significant difference among the four groups, indicating that treatment with febuxostat did not significantly affect food intake or body weight gain in these rats, regardless of the type of diet. In support of this contention are the results for body weight from the additional two groups of rats fed a high-fructose diet for 14 wk; febuxostat treatment during weeks 5–14 did not affect body weight gain (data not shown).

Glomerular hemodynamics. Glomerular hemodynamics results are depicted in Fig. 4 and Table 2. Two rats from each group of normal+P, normal+Fx, and fructose+P were removed due to complications during preparation of the micropuncture experiment. Fructose-fed rats developed renal cortical vasoconstriction, manifested as significantly lower SNGFR and elevated glomerular pressure compared with the normal+P group (Fig. 4). The reduction of SNGFR was caused by a significant decrement of ultrafiltration coefficient and glomerular plasma flow, despite the presence of increased glomerular pressure. Relative to rats on a normal diet, a lower renal cortical perfusion in fructose-fed rats (fructose+P) was the result of

Fig. 2. Effects of a high-fructose diet and Fx on systolic blood pressure in 2 additional groups of rats fed a high-fructose diet for 14 wk and treated with P or Fx beginning on week 5. \*\( P < 0.05 \) vs. fructose+P group by unpaired Student’s \( t \)-test.
numercially higher afferent and efferent arteriole resistances (Table 2). The glomerular hemodynamic changes accompanying the fructose-induced metabolic syndrome tended to improve with febuxostat treatment; glomerular hypertension was prevented and the ultrafiltration coefficient was normalized in the fructose+Fx group. Additionally, the values of SNGFR, glomerular plasma flow, AR, and ER in the fructose+Fx group were not different compared with the normal+P group. A positive correlation was shown to exist between plasma UA and glomerular pressure in all groups of rats (r = 0.54, P = 0.001).

Renal arteriolar morphology. Table 3 and Fig. 5 show results for renal arteriolar morphology. Fructose-fed rats (fructose+P) developed a thickening of the afferent arteriole as indicated by a significantly higher arteriolar area; however, in the fructose+Fx group, this alteration was reversed to a value similar to that in the normal+P group. In addition, UA level in all groups of rats correlated with arteriolar area (r = 0.58, P = 0.0003). There were no significant differences in M/L ratios among the various groups (Table 3). Febuxostat treatment in rats fed a normal diet had no significant effects on glomerular hemodynamics and renal arteriolar morphology.

DISCUSSION

In the present study, we examined whether the effect of reducing hyperuricemia with febuxostat during fructose-induced metabolic syndrome was associated with preservation of glomerular hemodynamic function and preglomerular vessel morphology. Administration of a 60% fructose diet to rats for 4 wk induced features of metabolic syndrome such as hypertension, hypertriglyceridemia, and hyperuricemia, as has been previously shown (33, 38). Two groups of rats were maintained on a high-fructose diet for an additional 4 wk, but only one group was then treated with the XO inhibitor febuxostat. Similar to what has been shown with allopurinol (33), febuxostat treatment reversed the hyperuricemia, the increases in both blood pressure and plasma triglycerides, and also prevented the increase in fasting plasma insulin. While the effect of febuxostat on SBP (measured by the tail-cuff method) was marginal at 8 wk, when a separate group of rats was fed a high-fructose diet for a longer time period (12 wk), a more pronounced effect of lowering UA on reducing blood pressure was observed. Moreover, glomerular hypertension, decreased ultrafiltration coefficient, and afferent arteriolopathy induced

Table 2. Effect of febuxostat on glomerular hemodynamics in normal and fructose-fed rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Diet + Placebo (n = 8)</th>
<th>Normal Diet + Febuxostat (n = 8)</th>
<th>Fructose Diet + Placebo (n = 8)</th>
<th>Fructose Diet + Febuxostat (n = 10)</th>
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<tbody>
<tr>
<td>Hct, %</td>
<td>0.47±0.01</td>
<td>0.48±0.01</td>
<td>0.50±0.01</td>
<td>0.51±0.01</td>
</tr>
<tr>
<td>GFR, ml/min</td>
<td>0.7±0.1</td>
<td>0.9±0.1</td>
<td>1.0±0.1</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>AR, dyn·s·cm⁻⁵</td>
<td>2.6±0.9</td>
<td>2.5±0.2</td>
<td>3.5±0.4</td>
<td>2.7±0.2</td>
</tr>
<tr>
<td>ER, dyn·s·cm⁻⁵</td>
<td>1.2±0.3</td>
<td>1.3±0.1</td>
<td>1.7±0.2</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>TR, dyn·s·cm⁻⁵</td>
<td>3.8±1.1</td>
<td>3.8±0.3</td>
<td>5.2±0.6</td>
<td>3.9±0.3</td>
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Values are means ± SE. Hct, hematocrit; GFR, glomerular filtration rate; AR, afferent arteriole resistance; ER, efferent arteriole resistance; TR, total resistance. Two-way ANOVA: diet was a significant factor for Hct; diet, treatment, and the interaction between diet and treatment were not significant in the other parameters.

Fig. 4. Glomerular hemodynamics in rats administered high-fructose or normal diets and treated with P or Fx [n = 8/group, except for fructose diet+Fx group (2-way ANOVA, n = 10)]. Results are single-nephron glomerular filtration rate (GFR); ANOVA: diet P = 0.002, treatment P = not significant (NS), interaction P = NS; glomerular pressure ANOVA: diet P = 0.04, treatment P = 0.04, interaction P = 0.02; glomerular plasma flow ANOVA: diet P = 0.02, treatment P = NS, interaction P = NS; and ultrafiltration coefficient ANOVA: diet P = NS, treatment P = NS, interaction P = 0.01. Bonferroni posttest comparisons: a,b P < 0.05.
by a high-fructose diet were also mitigated by febuxostat treatment. Therefore, the present results support a causal role for UA in the pathogenesis of fructose-induced metabolic syndrome and the accompanying renal damage.

Accumulating evidence dating back to the mid-1800s suggests that metabolic syndrome and obesity epidemics parallel sugar and fructose intake (23). Fructose is present in table sugar (sucrose), honey, high-fructose corn syrup (HFCS), and fruit. The widespread use of HFCS, coupled with the continued intake of table sugar, has led to a 30% increase in fructose intake over the last 20 years (14). Recent epidemiological analysis has found that greater intake of added sugars or sugar-sweetened drinks is associated with higher plasma UA concentration (18). Moreover, a graded positive association between concentrations of serum UA and the prevalence of metabolic syndrome traits has been described (11, 30); this relationship appears to apply to children and adolescents as well (17).

Recent evidence indicates that metabolic syndrome is related to the development of renal disease and is also a predictor of poor outcome in patients with chronic renal failure (9). However, because of the considerable overlap between clinical features of the metabolic syndrome and diabetes, a cause-effect relationship cannot be clearly established from clinical and epidemiological studies. One approach to identifying a distinction would be to study a model of primary metabolic syndrome as exemplified by the fructose-fed rat. The results of the present study support previous findings regarding the deleterious effect of metabolic syndrome induced by a high-fructose diet on renal structure and function (38). In addition, recent studies in humans have shown that metabolic syndrome is associated with an increased risk for a reduced GFR and microalbuminuria (9, 20). Interestingly, it has been reported that patients with essential hypertension and metabolic syndrome have glomerular hypertension and increased albumin excretion (52).

Our group recently demonstrated that treatment of fructose-fed rats with allopurinol, as well as with a uricosuric agent (benzodiarone), normalized plasma UA and triglycerides and significantly decreased SBP, suggesting that UA plays a causal role in the pathogenesis of fructose-induced metabolic syndrome (33). The primary importance of the current study is that we have now extended the studies to investigate the effects of XO inhibition on renal hemodynamics and structure associated with fructose ingestion. In addition, we also demonstrated the ability of a second XO inhibitor to improve features of the metabolic syndrome in this model; thus treatment with febuxostat also normalized plasma UA and reduced triglycerides, and the SBP measured in conscious animals showed a trend toward reduction, whereas MAP measured in anesthetized ani-

![Fig. 5. Afferent arteriolar morphology in normal and fructose-fed rats treated with P or Fx. Antibody anti-α-smooth muscle actin was counterstained with periodic acid-Schiff (×1,000).](http://ajprenal.physiology.org/)

<table>
<thead>
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<th>Normal Diet + Placebo (n = 8)</th>
<th>Normal Diet + Febuxostat (n = 8)</th>
<th>Fructose Diet + Placebo (n = 8)</th>
<th>Fructose Diet + Febuxostat (n = 10)</th>
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<tr>
<td>Arteriolar area, μm²</td>
<td>263.6±12.6</td>
<td>259.4±11.2</td>
<td>332.3±12.8*</td>
<td>248.5±10.3†</td>
</tr>
<tr>
<td>Media/lumen ratio</td>
<td>2.98±0.20</td>
<td>2.46±0.19</td>
<td>2.43±0.15</td>
<td>2.67±0.32</td>
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Values are means ± SE. Two-way ANOVA: diet, treatment, and the interaction between diet and treatment were significant in arteriolar area, but not in media/lumen ratio. Bonferroni posttest comparisons: *P < 0.001 vs. normal diet + placebo group. †P < 0.001 vs. fructose diet + placebo group.
mals at week 8 was significantly lower in febuxostat-treated animals on a high-fructose diet compared with the untreated rats.

In regard to the discrepancy between SBP and MAP in the 8-wk study, this phenomenon may derive from an inherent limitation of the tail-cuff method to detect small-to-moderate changes in blood pressure (28); by extending the time to 12 wk, when SBP differences could become greater, the tail-cuff method was able to detect significant differences (Fig. 2). Direct intra-arterial MAP, on the other hand, is more sensitive and is likely the reason we could detect the differences in blood pressure at 8 wk.

There is evidence that ROS contribute to the pathophysiology of metabolic syndrome. In this regard, fructose intake has been associated with oxidative stress mediated by several different pathways: 1) reduction of copper intestinal reabsorption and the resulting decrease in the activity of Cu-Zn SOD (8, 37); 2) diminished mRNA expression of catalase, Cu-Zn SOD as well as Mn-SOD in several tissues (8); and 3) increased activity of NADPH oxidase via angiotensin II type 1 receptor activation (35, 45). Fructose also increases XO activity (which is why it increases UA) and hence increases XO-associated oxidants (5). However, in aortic vessels of fructose-fed rats only NADPH oxidase inhibitors such as apocynin and diphenylene iodonium were able to abrogate the increased synthesis of superoxide; in contrast, the XO blocker oxy- purinol did not show such an inhibitory effect (45). These findings suggest a primary role of NADPH oxidase overactivation in the pathogenesis of fructose-induced metabolic syndrome.

Interestingly, UA may have a role in NADPH oxidase activation. Recently, our group reported that soluble UA stimulated an increase in NADPH oxidase activity and ROS production in mature adipocytes but not in preadipocytes (43). The stimulation of NADPH oxidase-dependent ROS by UA resulted in activation of MAP kinases p38 and ERK1/2, a decrease in NO bioavailability, and an increase in protein nitrosylation and lipid oxidation. In fructose-fed rats, the primary sites metabolizing fructose (liver, intestine, kidney, and adipocytes) might therefore increase UA levels that could affect other (vascular and endothelial) sites.

Previously, we demonstrated that elevated UA levels induced by a 60% fructose diet in rats may be partially responsible for the glomerular hypertension and cortical vasoconstriction induced by metabolic syndrome (38). In that study, we did not find evidence of glomerular or tubulointerstitial structural damage, nor was any fructose-induced albuminuria detected. These observations suggest that, at least at 8 wk, the primary renal abnormalities induced by fructose are hemodynamic in nature, with renal structural changes limited to the afferent arteriole. This generalization is supported by the fact that normalization of plasma UA with febuxostat in the present study was associated with normal values of glomerular pressure and conserved ultrafiltration coefficient and afferent arteriolar morphology.

The mechanisms that contribute to renal damage during metabolic syndrome are not completely understood. However, there exists clinical and/or experimental evidence suggesting that endothelial dysfunction, oxidative stress, serum lipid abnormalities, and inflammatory cytokines synthesized by adipose tissue may play a role (1, 27, 42, 50, 55). In this respect, there are studies suggesting that UA participates in the development of these alterations. Thus increased UA decreases NO levels and induces endothelial dysfunction (24, 25); UA stimulates NADPH oxidase with oxidative generation, reduction in NO levels, and the formation of peroxynitrite in cultured adipocytes (43); and finally, hyperuricemia closely correlates with hypertriglyceridemia (15, 16, 33) and predicts the development of obesity (31).

In summary, hyperuricemia induced by a high-fructose diet was associated with hypertension, hypertriglyceridemia, and hyperinsulinemia as well as glomerular hypertension, renal cortical arteriole vasoconstriction, and preglomerular arteriolar-opathy. Normalization of plasma UA with febuxostat in rats with fructose-induced metabolic syndrome alleviated both metabolic and glomerular hemodynamic alterations; these results support a pathogenic role of hyperuricemia in fructose-mediated metabolic syndrome and renal damage. Although more studies are needed, the results provide a possible explanation by which metabolic syndrome is associated with chronic renal disease.

ACKNOWLEDGMENTS

We thank Jose Santamaria, Magdalena Cristobal, and Benito Chavez-Renteria for technical assistance. We also acknowledge and are grateful to the late Dr. Jaime Herrera-Acosta for contributions and stimulating discussions during the conception of this project.

DISCLOSURES

This study was supported by a grant provided by TAP Pharmaceutical Products, Lake Forest, IL. L. Zhao is an employee of TAP Pharmaceutical Products. R. I. Johnson is listed as an inventor on several patent applications by the University of Florida and the University of Washington related to the lowering of uric acid or the inhibition of fructose as a means to prevent or treat cardiorenal and obesity-related diseases and will also have a book on fructose and obesity published in April 2008 (Rodale Press). T. Nakagawa is listed as inventor on patent applications by the University of Florida related to the role of fructose in hypertension and metabolic syndrome.

REFERENCES


