Fructose, but not dextrose, accelerates the progression of chronic kidney disease

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Gersch MS, Mu W, Cirillo P, Reungjui S, Zhang L, Roncal C, Sautin YY, Johnson RJ, Nakagawa T. Fructose, but not dextrose, accelerates the progression of chronic kidney disease. Am J Physiol Renal Physiol 293: F1256–F1261, 2007. First published August 1, 2007; doi:10.1152/ajprenal.00181.2007.—The metabolic syndrome has recently been recognized as a risk factor for kidney disease, but the mechanisms mediating this risk remain unclear. High fructose consumption by animals produces a model of the metabolic syndrome with hypertension, hyperlipidemia, and insulin resistance. The present study was conducted to test the hypothesis that consumption of a high-fructose diet could accelerate the progression of chronic kidney disease. Three groups of 14 male Sprague-Dawley rats were pair fed a specialized diet containing 60% fructose (FRU) or 60% dextrose (DEX) or standard rat chow (CON). After the animals were fed their assigned diet for 6 wk, five-sixths nephrectomy was performed, and the assigned diet was continued for 11 wk. Proteinuria was significantly increased and creatinine clearance was decreased in the FRU group compared with the CON and DEX groups, and blood urea nitrogen was higher in the FRU group than in the CON and DEX groups. Kidneys from the FRU group were markedly larger than kidneys from the CON and DEX groups. Glomerular sclerosis, tubular atrophy, tubular dilatation, and cellular infiltration appeared markedly worse in kidneys from the FRU group than in kidneys from the DEX and CON groups. Monocyte chemotactant protein-1 (MCP-1) was measured in renal tissue homogenate and found to be increased in the FRU group. In vitro studies were conducted to determine the mechanism for increased renal MCP-1, and fructose stimulation of proximal tubular cells resulted in production of MCP-1. In conclusion, consumption of a high-fructose diet greatly accelerates progression of chronic kidney disease in the rat remnant kidney model.

metabolic syndrome; high-fructose corn syrup; monocyte chemotactant protein-1

ACCORDING TO THE Third National Health and Nutrition Examination Survey data, 5,000,000-20,000,000 people in the United States have chronic kidney disease (CKD) (9). Physicians counsel these patients to follow various combinations of low-salt, low-fat, low-potassium, low-phosphate, and low-protein diets (23). However, patients are not routinely counseled regarding fructose consumption. Fructose has been recommended to diabetic patients, inasmuch as it does not elevate blood glucose levels (1, 6, 32). However, several researchers have raised the concern that fructose may contribute to the current epidemic of the metabolic syndrome (4, 17, 24, 28). A model commonly used in the literature to study the metabolic syndrome is fructose feeding. Rats that consume a high-fructose diet for 6 wk develop insulin resistance (11, 15), dyslipidemia (2, 8, 21, 22, 29, 37), hypertension (14, 15, 33), and hyperuricemia (27, 30). Fructose feeding in mice has been shown to lead to the development of obesity as well (18).

The metabolic syndrome is a well-established risk factor for diabetes, cardiovascular disease, and mortality. Recently, several studies have suggested that the metabolic syndrome may also contribute to the development of CKD. Using the Third National Health and Nutrition Examination Survey database, Chen et al. (7) showed an independent association of the metabolic syndrome with CKD. Expanding on this work, Kurella et al. (25) showed that the metabolic syndrome increased the risk of developing new-onset CKD in the Atherosclerosis Risk in Communities Study. Renal injury is also seen in other animal models of the metabolic syndrome, including Zucker fatty diabetic rats (10) and db/db mice (38).

Fructose consumption has steadily increased over the past 30 years in parallel to the growth of the obesity/metabolic syndrome epidemic, and fructose and high-fructose corn syrup are ingredients in many commercially produced food products (1). In fact, the average American now consumes 64 pounds of sweeteners every year (12). If we consider the animal and epidemiological data, it is reasonable to hypothesize that fructose consumption in our diet may be among the factors that contribute to the epidemic of the metabolic syndrome and, consequently, to the epidemic of chronic renal disease (1, 11, 17, 18, 27, 28). Importantly, fructose consumption by our CKD patients may actually be placing them at an increased risk for progression of their CKD. Despite the public health importance of this issue, little research exists on the long-term effects of fructose consumption in CKD patients. Thus we designed the present experiment to examine the effects of fructose feeding on the progression of chronic renal disease.

MATERIALS AND METHODS

All animal protocols were approved by the University of Florida Institutional Animal Care and Use Committee. Forty-two 150-g male Sprague-Dawley rats (Charles Rivers Laboratories, Wilmington, MA) were randomized to receive a diet of 60% fructose, 60% dextrose, or the standard rat chow, which contains complex carbohydrates, instead of purified sugars (Harlan-Teklad, Madison, WI). The animals were pair fed to eliminate animal weight as a variable in the study. Weights were obtained weekly. Animals were maintained in temperature- and humidity-controlled specific pathogen-free conditions on a 12:12-h light-dark cycle. After 6 wk, the animals were anesthetized with isoflurane, and a left nephrectomy and two-thirds right nephrectomy

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were performed by the polenectomy method, as previously described (19). Four animals died from complications related to the surgical procedure within 2 wk of the surgery and were excluded from the analysis. The assigned diets were continued for 11 wk, and then the animals were killed. Fasting blood samples for analysis were obtained by tail vein sampling 1 wk before remnant kidney (RK) surgery and 2, 7, and 11 wk after RK surgery. Metabolic cages were used for collection of urine samples 8 wk after RK surgery. Blood pressure was measured by the tail cuff method 8 wk after RK surgery (15).

Renal histology. Tissue was fixed in methyl-Carnoy solution, dehydrated in alcohols, and embedded in paraffin (Sigma-Aldrich, St. Louis, MO), and 2-μm sections were obtained. Tissue was stained with periodic acid-Schiff (PAS) stain, as previously described (19). Glomerular sclerosis and tubulointerstitial injury were scored using PAS-stained sections. All glomeruli in each section were counted. The percentage of glomeruli demonstrating sclerosis, defined as accumulation of hyaline material and collapse of the capillary loops, was calculated. Tubular interstitial injury was scored on a scale of 0–5 as follows (see Table 4). α-Smooth muscle actin (α-SMA) was stained as previously described (19) with an antibody obtained from Southern Biotechnology (Birmingham, AL). α-SMA, collagen, and osteopontin staining were quantified after examination of ≥40 fields at ×400 magnification by a scoring method similar to that used for tubulointerstitial injury (see Table 4), as previously described (19). Macrophages were stained with ED-1 (Serotec, Indianapolis, IN). ED-1-positive cells were counted in 40 nonconsecutive fields at ×400 magnification. Areas with extensive scarring from surgery were avoided. Biochemistries were tested with the VetAcc machine (Alpha Wasserman, West Caldwell, NJ).

ELISA for monocytic chemotactant protein-1. Tissue homogenates were prepared by mechanical homogenization after addition of fresh tissue to RIPA buffer (Sigma-Aldrich) to which Compete (Roche, Mannheim, Germany) protease inhibitors were added. Samples were incubated at 4°C for 1 h and then centrifuged at 4°C at 4,000 g for 60 min. The supernatant was removed, and the protein concentration was standardized at 8 mg/ml after total protein measurement (Pierce, Rockford, IL). Rat monocytic chemotactant protein-1 (MCP-1) protein was measured on these homogenates by ELISA with a kit (BD Biosciences Pharmingen, San Diego, CA) according to the manufacturer’s directions.

Cell culture. Human kidney proximal tubular (HK-2) cells, an immortalized proximal tubular epithelial cell line from normal adult human kidney, were obtained from American Type Culture Collection (Rockville, MD). Cells were grown to confluence in DMEM-nutrient mix (50:50) F-12 (DMEM-F-12) with l-glutamine and HEPES buffer (Invitrogen, Carlsbad, CA) supplemented with 10% FBS, 100 U/ml penicillin, 100 g/ml streptomycin, and 1% insulin-transferrin-serum (Invitrogen). Cells were plated in 24-well plates and cultured at 37°C in 95% air-5% CO2 up to 70–75% of confluence and then treated with forskolin (Invitrogen). Cells were treated with fructose or dextrose 6 wk before RK surgery was performed. The assigned diets were continued for 11 wk, and then the animals were killed. Fasting blood samples for analysis were obtained by tail vein sampling 1 wk before remnant kidney (RK) surgery and 2, 7, and 11 wk after RK surgery. Blood pressure was measured by the tail cuff method 8 wk after RK surgery (15).

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Statistics. Values are means ± SE. Statistics were analyzed with one-way ANOVA and Student’s t-test as appropriate. Significance defined as P < 0.05.

RESULTS

During the study, three animals in the fructose group died as a result of progressive kidney disease, whereas no animals in the normal or dextrose groups died. Renal function of animals treated with fructose or dextrose 6 wk before RK surgery was not different from renal function of animals fed the normal diet (Table 1). However, by 11 wk after RK surgery, renal function, as measured by serum blood urea nitrogen (BUN) and a trend, which did not quite reach statistical significance, toward an increased creatinine level, deteriorated significantly in fructose-treated, but not in dextrose-treated, animals (P = 0.09, normal vs. dextrose; Table 2). By 8 wk after the RK procedure, proteinuria was significantly increased and measured creatinine clearance was significantly decreased in fructose-fed animals (Table 3).

There was no statistically significant difference in blood pressure between the three groups at 9 wk or at the end of the study; however, there was a trend that did not reach statistical significance for higher blood pressure in the fructose-fed animals: 158 ± 1, 156 ± 2, and 166 ± 3 mmHg in normal, dextrose-fed, and fructose-fed groups, respectively (P = 0.3). Throughout the study, there were no significant differences in the animals’ weights between the groups at any of the time points (data not shown).

Grossly, kidneys from the fructose-fed animals were markedly larger than those from the normal or dextrose-fed animals: 3.4 ± 0.03, 2.6 ± 0.2, and 4.9 ± 0.3 g kidney/kg body wt in normal, dextrose-fed, and fructose-fed animals, respectively (P < 0.05; Fig. 1). PAS staining showed significantly more glomerulosclerosis and tubulointerstitial injury in the fructose-fed group (Table 4, Fig. 2). Additionally, more tubular damage, such as dilatation, atrophy, and epithelial cell proliferation, was observed in fructose-fed animals. These damaged tubules ex-
pressed osteopontin (Table 4, Fig. 2). On the other hand, myofibroblast formation from interstitial fibroblasts was also induced in fructose-treated animals, as evidenced by α-SMA staining (Table 4, Fig. 2). Interstitial collagen deposition was also increased in animals fed fructose compared with animals fed dextrose or a normal diet (Table 4). More macrophage infiltration, as evidenced by infiltration of ED-1-positive cells, was observed in fructose-fed animals (Table 4, Fig. 2).

To explain the increased numbers of macrophages in the kidneys from the fructose-treated animals, MCP-1 levels were measured in renal homogenates. MCP-1 was significantly increased in the fructose-fed animals compared with animals fed the normal or the dextrose diet (Fig. 3).

To determine whether the increase in renal MCP-1 was due to fructose exposure or differences in the underlying kidney disease, HK-2 cells were stimulated with fructose in vitro. Stimulation of HK-2 cells with low doses of fructose resulted in a dose-dependent increase in production of MCP-1 normalized to cell number by total DNA content of the cells (Fig. 4). Stimulation of HK-2 cells with fructose did not result in HK-2 cell proliferation or increased cell death (data not shown).

**DISCUSSION**

In the present study, we have shown that fructose consumption accelerates the progression of CKD in the rodent RK model. Fructose-treated animals had greater protein excretion, higher serum BUN and creatinine, decreased creatinine clearance, more renal hypertrophy, and higher mortality. The difference in serum creatinine did not reach statistical significance; however, serum creatinine is not a very sensitive marker of renal damage (13). Histologically, the kidneys from the fructose-treated animals showed more glomerulosclerosis, tubular dilatation, tubular atrophy, interstitial inflammation, myofibroblasts, tubular osteopontin, interstitial collagen, and ED-1-positive cells.

This is the first study showing that fructose consumption can accelerate the progression of chronic renal disease. Previously, fructose was reported to cause glomerular hypertrophy and subtle renal damage (3). More recently, it was reported that consumption of fructose induces glomerular hypertension and sclerosis of the afferent arteriole (30). In the present study, the dextrose-fed animals did not develop worse renal disease than the control animals, because glucose consumption did not result in systemic hyperglycemia in these nondiabetic animals.

Indirectly, fructose consumption could lead to the progression of CKD by causing hypertension. However, in the present study, all the RK animals developed hypertension, and there was no statistically significant difference in blood pressure between groups. Thus the increased renal injury in the fructose-treated group was unlikely to be primarily a blood pressure effect, although it is possible that the trend toward a higher blood pressure in the fructose-fed animals contributed to some of the progression of kidney disease. The hypertensive effects of fructose feeding may have been masked by the underlying hypertension induced by the RK surgery.

Several mechanisms explain how the metabolic syndrome could contribute to the development of CKD: induction of endothelial dysfunction, oxidative stress, hemodynamic alterations, proinflammatory environment, lipotoxicity, and hyperuricemia (31). Hyperinsulinemia may have also played a role in progression of kidney disease in this model. In the present study, insulin levels were elevated, and it is known that treatment with troglitazone to correct hyperinsulinemia slows progression of kidney disease (35). Lipotoxicity is an attractive mechanism, inasmuch as the cholesterol derangements in fructose feeding are striking, and treatment with statins has been shown to slow the progression of kidney disease in the RK model (20). However, fructose may also have direct toxic effects on the kidney.

**Table 3. Urinary protein and creatinine clearance assessment of acceleration of CKD progression by fructose feeding**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Proteinuria, mg/dl</th>
<th>Ccreatinine, ml/min</th>
<th>Ccreatinine/Kidney Wt</th>
<th>Ccreatinine/Body Wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>33±5.7</td>
<td>1.23±0.04</td>
<td>0.80±0.03</td>
<td>0.28±0.09</td>
</tr>
<tr>
<td>Dextrose</td>
<td>35±7.5</td>
<td>1.16±0.08</td>
<td>0.63±0.05</td>
<td>0.26±0.12</td>
</tr>
<tr>
<td>Fructose</td>
<td>73±15.4*</td>
<td>0.96±0.08*</td>
<td>0.35±0.03†</td>
<td>0.23±0.18†</td>
</tr>
</tbody>
</table>

Values (means ± SE) were measured 8 wk after RK surgery. Ccreatinine, creatinine clearance. *P < 0.05. †P < 0.01. ‡P < 0.0001.

**Fig. 1. Kidney weight (g) normalized to animal body weight (kg) in a remnant kidney (RK) model in which animals were fed 60% fructose, control (normal), or 60% dextrose diet. Fructose-fed animals developed renal hypertrophy.**

**Table 4. Renal histological scoring of CKD progression by fructose feeding**

<table>
<thead>
<tr>
<th>Injury Marker</th>
<th>Normal</th>
<th>Dextrose</th>
<th>Fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerulosclerosis, %</td>
<td>9±2.0</td>
<td>11±2.6</td>
<td>44±6.1†</td>
</tr>
<tr>
<td>Tubulointerstitial damage</td>
<td>1.7±0.3</td>
<td>1.5±0.2</td>
<td>4.1±0.4‡</td>
</tr>
<tr>
<td>α-SMA</td>
<td>0.5±0.2</td>
<td>0.4±0.2</td>
<td>3.5±0.4‡</td>
</tr>
<tr>
<td>ED-1-positive cells/400 field</td>
<td>8.5±1.2</td>
<td>6.2±1.6</td>
<td>18.7±2.6*</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>1.5±0.22</td>
<td>1.25±0.25</td>
<td>3±0.5*</td>
</tr>
<tr>
<td>Interstitial collagen type IV</td>
<td>0.5±0.34</td>
<td>0.75±0.48</td>
<td>3.9±0.48†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Tubulointerstitial damage, α-smooth muscle actin (α-SMA) staining, osteopontin staining, and interstitial collagen deposition were scored on a scale of 1–5 as follows: 0 = normal appearance, 1 = injury to <10% of tubules, 2 = injury to 10–25% of tubules, 3 = injury to 25–50% of tubules, 4 = injury to 51–75% of tubules, and 5 = injury to >75% of tubules. *P < 0.05. †P < 0.01. ‡P < 0.0001.
effects on the renal tubular cells, as evidenced by the marked tubular changes and α-SMA and osteopontin expression by tubular cells. Renal tubular cells are known to express the fructose transporter GLUT-5, as well as the enzymes necessary for glucose metabolism (26). Further studies are needed to determine mechanistically how fructose may lead to tubular cell damage.

An increase in ED-1-positive cells was noted in the renal cortex from animals treated with fructose. ED-1 antibody stains tissue monocytes and macrophages. Because monocytes were increased in the kidneys from fructose-treated animals, we examined the level of MCP-1 in the renal cortex. We found that MCP-1 was elevated in the fructose-treated animals. This led to the cell culture studies showing that fructose stimulated MCP-1 production in HK-2 cells. In these studies, MCP-1 production was normalized for cell number by the amount of DNA in the cells. This technique was preferred over measurement of protein content of the cell lysate, inasmuch as stimu-
lation with sugars can result in cell hypertrophy. Measurement of DNA content in the cells also allowed us to demonstrate that the fructose stimulation did not result in proliferation or death of the HK-2 cells.

Fructose feeding has been reported to result in hyperuricemia (33, 34); however, this was not detected in the present study. Because hyperuricemia is greatest 50–200 min after fructose consumption (5), this effect may be missed in fasting samples. Fasting samples were collected for measurement of fasting insulin and triglyceride concentrations.

The 60% fructose diet utilized in the present study is the classical model of fructose-induced metabolic syndrome described in the literature (2, 8, 14–16, 27). Although this is four times the amount of fructose that many people are consuming in their diet, in animal experiments, it is common practice to use higher-dose treatments to achieve a statistically significant result in a very limited time frame. This experiment was conducted over only a few months, whereas patients are more likely to have CKD for many years. Recently, Sanchez-Lozada et al. (30) reported the induction of the metabolic syndrome after 6 wk of feeding a diet of only 30% fructose. More studies are needed to determine whether lower doses of fructose, administered over a longer time period, also lead to the progression of CKD.

In conclusion, we have shown that consumption of a high-fructose diet accelerates the progression of CKD. Over the past 30 years, fructose has become a standard part of the American diet. It is possible the fructose consumption by our patients may be contributing to the development or progression of CKD. If this is the case, restriction of dietary fructose may slow the progression of CKD in these patients. More studies are needed to explore the pathophysiological mechanisms underlying this important public health issue.

ACKNOWLEDGMENTS

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REFERENCES