Synergistic effect of uricase blockade plus physiological amounts of fructose-glucose on glomerular hypertension and oxidative stress in rats

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Tapia E, Cristóbal M, García-Arroyo FE, Soto V, Monroy-Sánchez F, Pacheco U, Lanaspa MA, Roncal-Jiménez CA, Cruz-Robles D, Ishimoto T, Madero M, Johnson RJ, Sánchez-Lozada LG. Synergistic effect of uricase blockade plus physiological amounts of fructose-glucose on glomerular hypertension and oxidative stress in rats. Am J Physiol Renal Physiol 304: F727–F736, 2013. First published January 9, 2012; doi:10.1152/ajprenal.00485.2012.—Fructose in sweetened beverages (SB) increases the risk for metabolic and cardiorenal disorders, and these effects are in part mediated by a secondary increment in uric acid (UA). Rodents have an active uricase, thus requiring large doses of fructose to increase plasma UA and to induce metabolic syndrome and renal hemodynamic changes. We therefore hypothesized that the effects of fructose in rats might be enhanced in the setting of uricase inhibition. Four groups of male Sprague-Dawley rats (n = 7/group) were studied during 8 wk: water + vehicle (V), water + oxonic acid (OA; 750 mg/kg BW), sweetened beverage (SB; 11% fructose-glucose combination) + V, and SB + OA. Systemic blood pressure, plasma UA, triglycerides (TG), glucose and insulin, glomerular hemodynamics, renal structural damage, renal cortex and liver UA, TG, markers of oxidative stress, mitoDNA, fructokinase, and fatty liver synthase protein expressions were evaluated at the end of the experiment. Chronic hyperuricemia and SB induced features of the metabolic syndrome, including hypertension, hyperuricemia, hyperglycemia, and systemic and hepatic TG accumulation. OA alone also induced glomerular hypertension, and SB alone induced insulin resistance. SB + OA induced a combined phenotype including metabolic and renal alterations induced by SB or OA alone and in addition also acted synergistically on systemic and glomerular pressure, plasma glucose, hepatic TG, and oxidative stress. These findings explain why high concentrations of fructose are required to induce greater metabolic changes and renal disease in rats whereas humans, who lack uricase, appear to be much more sensitive to the effects of fructose.

uric acid; insulin resistance; glomerular hypertension; liver steatosis

There is increasing experimental, clinical, and epidemiological evidence that sweetened beverage (SB) consumption increases the risk for obesity, metabolic syndrome, and cardiometabolic and renal consequences (7, 8, 11, 14, 16, 37). Ingestion of SB is also associated with increased risk for nonalcoholic liver disease (2, 4, 23).

Several potential mechanisms have been proposed to explain the mechanisms as how SB may increase the risk for metabolic syndrome, including its high glycemic content and its ability to encourage overnutrition (27, 35). However, some studies have suggested that the fructose component in SB may uniquely increase the risk for metabolic and cardiorenal disorders (26). For instance, data from our group demonstrated that the adverse impact of fructose on renal function and metabolic syndrome was directly related to the amount of fructose administered. (51). In this study, drinking water containing 10% fructose induced mild hypertension in the setting of preserved renal function. In contrast, a diet containing 60% fructose resulted in systemic and glomerular hypertension, renal vasoconstriction, and renal microvascular damage (50). Our group also documented that a high-fructose diet further aggravated renal disease in the 5/6 nephrectomy model of chronic renal damage (19). In addition, mice lacking ketohexokinase (KHK, fructokinase), the first enzyme in fructose metabolism, are also protected from developing metabolic syndrome when exposed to a high-fructose diet (22).

A particular feature of fructose metabolism is the generation of uric acid (UA) as a byproduct (20). While UA has often been viewed as simply a waste product in murine metabolism, there is increasing evidence that UA may have a contributory role in renal damage (29, 40, 49). Experimental studies have shown that, while UA can function as an antioxidant, it also can increase intracellular oxidative stress in various cell types (28, 32, 39). In turn, UA-induced oxidative stress decreases endothelial nitric oxide (NO) bioavailability (30, 69, 70). Rats made hyperuricemic by administration of the uricase inhibitor oxonic acid (OA) develop systemic and glomerular hypertension and renal cortical vasoconstriction, which is prevented by blocking oxidative stress or increasing NO substrate, maneuvers that also preserve the renal microvasculature (48, 53, 54).

We therefore hypothesized that the effects of fructose to induce metabolic alterations and renal hemodynamic changes might be enhanced in the setting of uricase inhibition. To test this hypothesis, we administered OA with or without an 11% combination of fructose and glucose in drinking water to rats. The combined use of fructose and glucose was used to be more equivalent with the situation in humans in which fructose is usually delivered either as sucrose or high-fructose corn syrup.
METHODS

Experimental Design

Four groups of male Sprague-Dawley rats (n = 7/group) were studied over a period of 8 wk. Two groups received tap water and the other two groups were offered a SB containing 11% of simple sugars (7.15% fructose and 3.85% glucose, respectively) ad libitum. The proportion of fructose-glucose used in our study was based on recent evidence that major brands of soft drinks use this proportion of fructose-glucose in their products (60). The uricase inhibitor OA was administered by intragastric gavage (750 mg/kg BW, daily) in one tap water (water + OA) and one SB group (SB + OA). In addition, two vehicle (V) groups (water + V and SB + V) were studied (factorial design 2 × 2). For the gastric gavage procedure probes, made of soft polyethylene tube (PE-90, external diameter of 1.7 mm) attached to a Lucite holder, sealed with agar, and covered with Ringer’s solution.

Body weight was measured weekly. Mean total caloric intake was calculated from the amount of food and beverage consumed in each group of rats. Systolic blood pressure (SBP) was measured in conscious rats by a validated volume-based tail-cuff method (17) (XBPro-1000; Kent Scientific, Torrington, CT). All animals were preconditioned for blood pressure measurements 1 wk before each experiment. Fasting (16–18 h) glucose (Genzyme Diagnostics, Boston, MA), insulin (Chrystral Chem, Downers Grove, IL), nonfasting plasma UA (Amplex red; Life Technologies, Carlsbad, CA), and triglycerides (TG; Genzyme Diagnostics) were measured using commercial kits. Homeostasis model assessment-insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) were calculated from fasting glucose and insulin. HOMA-IR was calculated as the product of the fasting plasma glucose (FPG) and fasting plasma insulin (FPI) levels, divided by a constant, assuming that control young adult rats have an average HOMA-IR of 1, analogous to the assumptions applied in the development of HOMA-IR in humans (38). The equation was as follows HOMA-IR (FPG − FPI)/2.430, where FPI was in microunits per milliliter and FPG in milligram per deciliter. QUICKI was calculated according to the original formula (31) as the inverse log sum of fasting insulin in microunits per milliliter and fasting glucose in milligram per deciliter. QUICKI 1/log(FPG) − (1/ FPI)]. The equations have been found to be accurate in rats. (9). SBP and biochemical parameters were determined at the end of 8 wk. Proteinuria (Bradford method) and plasma and urinary sodium (Flame photometer; Instrumentation Laboratory, Lexington, MA) were measured at the end of the experiment in 16- to 18-h urine collections in metabolic cages. Fractional sodium excretion (FENA) was calculated using standard formulas.

Renal Outcomes

Micropuncture. Animals were anesthetized with pentobarbital sodium (30 mg/kg ip) and placed on a thermoderegulated 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’s solution. Mean arterial pressure (MAP) was monitored with a pressure transducer (model p23 db; Gould, San Juan, Puerto Rico) 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 the erythrocytes recovered after centrifugation and mixed with isotonic BSA (5 mg/dl) to substitute the plasma volume. Rats were maintained under euvolemic conditions by infusion of 10 ml/kg of body wt of isotonic BSA (5 mg/dl) during surgery, followed by an infusion of 25% polyfructosan, at 2.2 ml/h (Inutest; 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 and stop-flow conditions and peritubular capillary pressure were measured in other proximal tubules with a servo-null device (Servo Nulling Pressure System; Instrumentation for Physiology and Medicine, San Diego, CA). Glomerular colloid osmotic pressure was estimated from blood concentrations obtained from blood of the femoral artery and surface effluent arterioles. Polyfructosan was measured in plasma and urine samples by the anthrone-based technique of Davidson and Sackner (15). Total glomerular filtration rate (GFR) was calculated using the following formula: GFR = (U × 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 (63). Single nephron glomerular filtration rate (SNGFR) was calculated using the formula: SNGFR = (TF/P)PF × V, where PF is the concentration of polyfructosan in tubular fluid (TF) and plasma (P) and V is the tubular fluid rate that is obtained by timing the collection of tubular fluid (2). Protein concentration in afferent and efferent samples was determined according to the method of Viets et al. (61). MAP, GFR; glomerular capillary hydrostatic pressure (PGC); single-nephron plasma flow; afferent arteriole, efferent arteriole, and total resistances; and ultrafiltration coefficient (Kf) were calculated according to equations previously reported (6). After the micropuncture study, the left kidney was fixed with 4% paraformaldehyde and the right kidney was divided in cortex and medulla and snap frozen in liquid nitrogen until posterior processing.

Renal histology and quantification of morphology. Fixed renal tissue was embedded in paraffin and processed accordingly. Evaluation and quantifications were performed blinded.

Microvascular Damage. Two-micrometer sections of fixed tissue were stained with periodic acid Schiff reagent. Arteriolar morphology was assessed by indirect peroxidase immunostaining for α-smooth-muscle actin (DAKO, Carpinteria, CA) (59). Sections of kidney tissue incubated with normal rabbit serum were used as negative controls for immunostaining against α-smooth-muscle actin. For each arteriole, the outline of the vessel and its internal lumen (excluding the endothelium) were generated using computer analysis (Image Pro Plus 7.0; Media Cybernetics) to calculate the total arteriolar medial area (outline − outline/interstitial fibrosis. Sections were stained with Masson’s trichrome. Ten noncrossed fields of cortex (640 × 477 mm, 10×) per biopsy were analyzed by light microscopy (Olympus BX51; Olympus American, Melville, NY) and captured with a digital camera (VF Evolution; Media Cybernetics, Silver Spring, MD). Positive blue color areas (excluding glomeruli and vessels) were analyzed in Image Pro Plus (Media Cybernetics).

GLOMERULAR SCLEROSIS. Masson’s trichrome-stained renal cortical sections were divided in four quadrants. Segmental and global scle-roded glomeruli were reported as a percentage of the total number of glomeruli counted in one quadrant.
GLomerular Volume. In periodic acid Schiff-stained sections the cross-sectional area (A) of 30 representative superficial glomeruli was analyzed; only glomeruli in which a vascular pole was present were included. The individual radius (r) of the glomeruli was determined by r = (4A/πV). The mean glomerular volume (V) was estimated by the following formula: V = 4/3πr³ (39).

Liver Outcomes

At the end of micropuncture experiment, the right lobe of the liver was excised and snap frozen in liquid nitrogen until subsequent analysis for UA, TG, markers of oxidative stress, and Western blotting.

Evaluation of tissue UA, TG, markers of oxidative stress, mitDNA quantification, KHK, and fatty acid synthase protein expressions. Tissue UA. UA was extracted as previously described (10). In brief, 20 mg of renal cortex or liver were homogenized in buffer (25 mM HEPES, 100 mM KCl, 1 mM DTT, and 0.1 mM EDTA, pH 7.1). Homogenates were frozen in liquid N₂ and unfrozen three times. UA was measured in supernatants obtained after centrifuging using Amplex red assay kit (Life Technologies). Fluorescence was measured on a Synergy H1 hybrid multimode microplate reader using Gen5 analysis software (Biotek Instruments, Winooski, VT). Values of UA were normalized by protein concentration.

Tissue TG. Twenty milligrams of renal cortex or liver were homogenized in a 1-mL solution containing 5% Triton X-100 in water, then heated slowly to 90°C in a water bath for 2–5 min or until Triton X-100 became cloudy, and then slowly cooled down to room temperature (34). This procedure was repeated one more time. Samples were centrifuged for 5 min (top speed in microcentrifuge) to remove insoluble materials. Samples were diluted 10-fold with water before assay using a lipase based colorimetric kit (Genzyme Diagnostics). Absorbance was measured on a Synergy H1 hybrid multimode microplate reader using Gen5 analysis software (Biotek Instruments). TG concentration was calculated by interpolating the values of samples absorbance in a standard curve and normalized by protein concentration.

Markers of Oxidative Stress. Tissue homogenates were prepared in potassium phosphate buffer (20 mM) containing BHT (0.5 M) and a proteases cocktail (Halt protease inhibitor; Thermo Scientific, Waltham, MA), pH 7.0. For protein carbonyl content, homogenates were incubated overnight with streptomycin sulfate to remove nucleic acids. Later, homogenates were treated with 2,4-dinitrophenyldihydrzone and HCl and finally with guanidine hydrochloride. Assessment of carbonyl formation was done on the basis of formation of protein hydrazone by reaction with 2,4-dinitrophenyldihydrzone. The absorbance was measured at 370 nm (46). Protein carbonyl content was expressed as nanomoles of carbonyl per milligrams of protein. Lipid peroxidation was assessed by measuring 4-hydroxynonenal (4-HNE) using a standard curve of tetramethoxypropane. A fluorescence spectra were continuously monitored by the ABI-Prism 4000 Sequence Detection System (Applied Biosystems, Carlsbad, CA) with sequence detection software version 1.3.1. Data analysis was based on measurement of the cycle threshold (Cₜ). As a measure of the relative expression of D-Loop mitDNA among groups we took the ΔCₜ mean value of control group as calibrator and obtained the ΔΔCₜ values. Results are reported as the relative mitDNA copy number (calculated as 2⁻ΔΔCₜ) (42).

KHK and Fatty Acid Synthase Protein Expression by Western Blot Analysis. Hepatic and renal cortex proteins were extracted using a MAP kinase lysis buffer, as previously described (47). Thirty to forty micrograms of liver homogenate samples were resolved on Ready Gel Tris-HCl precast gels (12% or 4 to 15%; Bio-Rad Laboratories, Hercules, CA) and transferred to polyvinylidenedifluoride membranes by electroblotting. Each primary antibody was incubated at 4°C overnight. Anti-KHK (GeneTex, Irvine, CA; liver and renal cortex), anti-fatty acid synthase (anti-FAS; Cell Signaling, Danvers, MA; liver), and anti-β-actin antibody (Cell Signaling; liver and renal cortex). After being washed with Tween-TBS, the membrane was rocked with secondary antibody (anti-rabbit IgG, horseradish peroxidase-linked antibody; Cell Signaling). Blots were then developed using the Immun-Star HRP chemiluminescence kit (Bio-Rad Laboratories). Chemiluminescence was recorded and quantified using the ID image-analysis system software (Kodak Digital Science, Rochester, NY).

Statistical Analysis

Values are expressed as means ± SD. Significant differences between treatment groups were determined by 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. Statistical analysis was performed with Prism version 5.04 (Graph Pad Software, San Diego, CA).

Results

Effect of Uricase Inhibition With or Without SB on Metabolic Alterations

OA and SB treatments induced the development of metabolic alterations. As we reported previously, OA induced a significant increment of plasma UA in water groups (+400%). The effect of SB alone on plasma UA was also significant but milder compared with OA (+173%). The cotreatment of SB + OA also significantly increased UA plasma levels (+400%); this rise was comparable to the W + OA group (Table 1).

Rats with OA-induced hyperuricemia and rats with chronic SB consumption both demonstrated a significant and similar increment of postprandial plasma TG (OA +39% and SB +60%) at 8 wk. The combination of both treatments had a tendency for a further increase plasma TG with respect to control animals (+200%) but was not significant different from SB alone (+25%; Table 1). We found a significant effect of treatment and beverage in fasting (16–18 h) glucose (Table 1). In addition, fasting plasma glucose was significantly raised by the cotreatment inducing a synergistic effect and causing a further increase in glucose compared with SB alone (+44%). We found a significant effect of SB alone on plasma insulin concentration, HOMA-IR, and QUICKI compared with both groups receiving tap water (Table 1).

Both OA treatment and SB were associated with a significant increment in systolic blood pressure compared with control (OA +16% and SB +12%); combination of both treatments induced a further and significant increase of 7% respect to SB...
OA further increased MAP compared with SB alone (\(P < 0.0001\)). In contrast, SB alone had a mild, but significant, effect on MAP that was measured using a validated tail-cuff method (Fig. 2). OA-treated rats showed a 17% reduction in whole GFR, while in SB + V animals GFR was similar to control group. The concomitant treatment of SB + OA significantly reduced GFR vs. SB + V (\(-27\%\)).

Figure 2 depicts the glomerular hemodynamic data. We confirmed a significant renal vasoconstrictive effect mediated by OA; therefore, single nephron GFR (\(-10\%\)), glomerular plasma flow (\(-20\%\)) and ultrafiltration coefficient (\(-47\%\)) were significantly reduced and coupled with higher afferent (+61%) and efferent (+72%) arteriolar resistances compared with the control group receiving water alone. In contrast, administration of a SB + V for 8 wk showed minimal changes in corticomedullary glomerular function as evaluated by renal micropuncture, single nephron GFR, glomerular plasma flow, ultrafiltration filtration coefficient, glomerular pressure, as well as afferent and efferent resistances were comparable to control rats (Fig. 2). The cotreatment of SB + OA did not cause further changes in those parameters compared with W + OA (Fig. 2).

**Renal Outcomes**

Twenty-four hour urinary protein excretion was not modified by OA treatment nor SB after 8 wk of follow-up. On the other hand, SB severely reduced urinary sodium excretion in V- and OA-treated animals. Both SB groups (V or OA treated) excreted \(-30\%\) of the sodium relative to V- or OA-treated rats receiving water (Table 1). OA-induced hyperuricemia has a primary role in causing renal vasoconstriction. MAP measured during micropuncture in anesthetized rats receiving OA was increased compared with water-treated rats (+20%) and mirrored the changes noted by SBP that was measured using a validated tail-cuff method (Fig. 2). In contrast, SB alone had a mild, but significant, effect on MAP compared with control rats (+8%). The coadministration of SB + OA further increased MAP compared with SB alone (+18%) and vs. OA alone (+6%). OA-treated rats showed a 17% reduction in whole GFR, while in SB + V animals GFR was similar to control group. The concomitant treatment of SB + OA significantly reduced GFR vs. SB + V (\(-27\%\)).

![Figure 1. Weekly body weight gain in rats receiving tap water with or without the uricase inhibitor oxonic acid during 8 wk. Body weight gain was similar among the 4 experimental groups. W, water; Veh, vehicle; OA, oxonic acid; SB, sweetened beverage.](https://www.ajprenal.physiology.org/)

### Table 1. Effect of the single treatments and in combination on metabolic syndrome traits

<table>
<thead>
<tr>
<th></th>
<th>W + V</th>
<th>W + OA</th>
<th>SB + V</th>
<th>SB + OA</th>
<th>Treatment</th>
<th>Beverage</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric acid, mg/dl</td>
<td>0.19 ± 0.02</td>
<td>1.03 ± 0.12‡</td>
<td>0.52 ± 0.17</td>
<td>1.01 ± 0.08‡</td>
<td>&lt;0.0001</td>
<td>&lt;0.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>78 ± 24</td>
<td>109 ± 42</td>
<td>125 ± 35</td>
<td>156 ± 43</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>79 ± 23</td>
<td>88 ± 28</td>
<td>105 ± 26</td>
<td>151 ± 45†</td>
<td>&lt;0.001</td>
<td>&lt;0.0001</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>0.47 ± 21</td>
<td>0.48 ± 0.86</td>
<td>0.65 ± 0.33</td>
<td>0.58 ± 0.34</td>
<td>NS</td>
<td>&lt;0.03</td>
<td>NS</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.2 ± 1.3</td>
<td>2.6 ± 1.4</td>
<td>4.2 ± 2.7</td>
<td>4.6 ± 1.7</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.69 ± 0.13</td>
<td>0.66 ± 0.13</td>
<td>0.58 ± 0.9</td>
<td>0.58 ± 0.34</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>122 ± 8</td>
<td>141 ± 11†</td>
<td>147 ± 5</td>
<td>147 ± 2*</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>BW, g</td>
<td>342 ± 17</td>
<td>327 ± 22</td>
<td>337 ± 19</td>
<td>353 ± 33</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Uprot, mg/day</td>
<td>7 ± 2</td>
<td>9 ± 3</td>
<td>4 ± 4</td>
<td>9 ± 10</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>FENa, %</td>
<td>0.49 ± 0.17</td>
<td>0.72 ± 0.34</td>
<td>0.26 ± 0.13</td>
<td>0.29 ± 0.26</td>
<td>&lt;0.0001</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. W, water; V, vehicle; OA, oxonic acid; SB, sweetened beverage; TG, triglycerides; HOMA-I, homeostasis model assessment-immunoreactivity; QUICKI, quantitative insulin sensitivity check index; SBP, systolic blood pressure; FENa, fractional sodium excretion; BW, body weight; Uprot, urinary protein; NS, nonsignificant. *\(P < 0.01\) vs. V. †\(P < 0.001\) vs. V. ‡\(P < 0.0001\) vs. V.
with SB increased the M/L ratio by 30% more vs. SB alone, but this difference did not reach statistical significance (Table 2).

OA-induced hyperuricemia results in higher renal cortex UA levels and increased oxidative stress. Treatment with OA significantly increased renal cortical UA concentrations in both W and SB groups (+250 and +61% compared with their counterparts, respectively; Fig. 3). Compared with SB alone, SB + OA increased renal UA content by 85%. SB in tap water drinking rats had a milder and nonsignificant effect on renal UA content and increased it by 80% compared with W + V.

Table 2. Effect of the single treatments and in combination on renal histology

<table>
<thead>
<tr>
<th></th>
<th>W + V</th>
<th>W + OA</th>
<th>SB + V</th>
<th>SB + OA</th>
<th>Treatment</th>
<th>Beverage</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular volume, μm³</td>
<td>319 ± 13</td>
<td>363 ± 38</td>
<td>565 ± 32</td>
<td>605 ± 53</td>
<td>&lt;0.05</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td>Arteriolar wall area, μm²</td>
<td>197 ± 21</td>
<td>193 ± 38</td>
<td>302 ± 39</td>
<td>320 ± 41</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td>M/L ratio</td>
<td>1.52 ± 0.16</td>
<td>2.59 ± 0.60*</td>
<td>2.62 ± 0.31</td>
<td>3.41 ± 0.91</td>
<td>&lt;0.001</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td>Glomerulosclerosis, %</td>
<td>0.68 ± 0.79</td>
<td>3.90 ± 2.18</td>
<td>6.23 ± 5.99</td>
<td>7.04 ± 3.69</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>NS</td>
</tr>
<tr>
<td>TI fibrosis, %</td>
<td>2.50 ± 2.43</td>
<td>4.71 ± 4.61</td>
<td>8.67 ± 20</td>
<td>11.86 ± 6.57</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. M/L, media/lumen; TI, tubulointerstitial. *P < 0.01 vs. V.
Renal UA levels correlated with values of plasma UA ($r = 0.84; P < 0.0001$).

Both OA treatment alone and SB treatment alone increased protein carbonylation and lipid peroxidation (4-HNE) in the renal cortex compared with the W + V group. In addition, a significant synergistic effect in lipid peroxidation was observed in rats administered OA and SB.

**SB increases KHK protein expression.** Administration of SB in V- and OA-treated groups induced a significant and similar increment in kidney cortex KHK (Fig. 3).

**Liver Effects**

**OA and SB increase hepatic UA and TG.** Both OA treatment alone, and SB alone, increased liver UA content, with OA causing a greater increment than SB (Fig. 4). Coadministration of SB + OA did not further increase UA hepatic concentration compared with W + OA animals. Likewise, OA and SB alone significantly increased liver TG; however, OA alone had a more robust effect compared with SB alone. Combination of both treatments had a synergistic effect in the SB + OA group, and liver TG doubled their concentration compared with OA animals receiving tap water.

**OA and SB increase liver oxidative stress.** OA and SB treatments increased hepatic protein carbonylation and lipid peroxidation in tap water drinking groups (Fig. 4). SB + OA-treated rats showed a greater effect in oxidized proteins than SB treatment alone. Lipid peroxidation was also increased in OA- and SB-treated rats and the combination showed a synergistic effect. In addition, we found significant correlations between: liver UA content and protein carbonyls ($r = 0.72; P < 0.0001$), liver UA and 4-HNE ($r = 0.69; P < 0.0001$), and liver TG and 4-HNE ($r = 0.92; P < 0.0001$). Neither treatment nor SB induced alterations in hepatic mitDNA relative copy number (W + V 1.02 ± 0.50; W + OA 1.20 ± 0.82; SB + V 1.45 ± 0.79; and SB + OA 1.46 ± 0.75).

**SB increases KHK and FAS protein expression.** Administration of SB in V- and OA-treated groups induced a significant and similar increment in liver KHK and FAS expressions (Fig. 4).

**DISCUSSION**

The present study has several important findings. First, we demonstrate that chronic hyperuricemia alone extended for 8 wk can induce renal and metabolic alterations, including hyperuricemia, postprandial hypertriglyceridemia, systemic and...
glomerular hypertension, and hepatic TG accumulation. Second, we found that SB containing clinically relevant concentrations of fructose and glucose could also induce mild hyperuricemia and similar metabolic manifestations as animals receiving OA alone. Finally, we demonstrated that SB plus uricase inhibition induced a combined phenotype including all the deleterious changes induced by OA and SB independently and, in addition, also acted synergistically on systemic and glomerular pressure, plasma glucose, hepatic TG, and renal and liver lipid peroxidation. There was also a tendency for the combination of SB and OA to induce a greater rise in plasma TG. Thus the novelty of our study relies on the demonstration that inhibiting uricase increases the effect of fructose to enhance hepatic fat stores, increase serum glucose, increase systemic blood pressure, and produce renal damage. We believe the clinical implication may be explained by the fact that humans who lack uricase are likely more sensitive to the effects of fructose than mammals that do not express uricase. In turn, this can explain why high concentrations of fructose are required to induce greater metabolic changes and renal disease in rats whereas humans appear to be much more sensitive to the effects of fructose than mammals that do not express uricase.

Renal Alterations Associated with SB Intake With or Without Uricase Inhibition

A key finding in these studies was the observation that SB + OA produced a significant rise in glomerular hypertension to levels comparable to what is observed in models of more extensive chronic renal damage such as the 5/6 nephrectomy model (59). Indeed, the levels of glomerular pressure induced by SB + OA were significantly higher vs. SB alone (33%) and vs. OA alone (+14%). In contrast, in SB + V animals we observed minimal changes in cortical glomerular hemodynamics, as evaluated by renal micropuncture; thus single nephron GFR, glomerular plasma flow, ultrafiltration filtration coefficient, glomerular pressure, as well as afferent and efferent resistances were comparable to control rats (Fig. 2). In addition, oxidative stress (lipid peroxidation) was significantly increased by the combination of SB + OA compared with each treatment alone. On the other hand, despite no evident glomerular hemodynamics alterations, SB per se did produce glomerular hypertrophy, arteriolopathy, glomerulosclerosis, and tubulointerstitial fibrosis. It is likely that additional mechanisms stimulated by SB, besides UA increment, act to induce mild...
levels can predict the development of metabolic syndrome in high doses of uricase inhibitor used. This may need to be operative in humans, who lack the expression of uricase. The documentation that uricase inhibition can increase hepatic fat accumulation in liver (HepG2) cells via a mechanism that involves mitochondrial oxidative stress (33). In the present studies we did not observe an increment on hepatic fructokinase (KHK) and FAS levels. However, we cannot rule out that an increase in enzyme activities and likely other mechanisms are involved in the liver and plasma increase of TG observed in these animals. Many years ago Wexler (65) and Wexler and Greenberg (66) also reported that chronic uricase inhibition could induce features of metabolic syndrome, but these findings were largely ignored because of the high doses of uricase inhibitor used. This may need to be revised in light of recent studies showing that high serum UA levels can predict the development of metabolic syndrome in humans, and the increasing experimental evidence that UA may have a role in metabolic syndrome (25).

An intriguing finding was that we did not demonstrate an effect on insulin levels or insulin resistance (HOMA-IR and QUICKI) despite mild but significant effects on fasting glucose levels. In this regard, we have found that a high-sucrose diet induced pancreatic inflammation and mild islet injury and reduced insulin levels with the development of type 2 diabetes mellitus, effects that were partially mediated by UA (47). Although speculative, we believe that in SB + OA rats mild pancreatic damage could also occur with increased susceptibility to UA-mediated damage, therefore diminishing insulin synthesis and secretion, allowing fasting plasma glucose to increase despite almost normal insulin levels.

Some synergistic metabolic effects were observed when SB was administered to rats receiving OA. The combined treatment resulted in higher plasma glucose, hepatic TG, and hepatic lipid peroxidation. In this respect, chronic exposure to diets enriched with sucrose or fructose induces hepatic insulin resistance and increased hepatic gluconeogenesis (45, 64), effects partially attributed to an increased activation of JNK, which in turn interferes with proximal steps in the insulin-signaling pathway (3). Chronic oxidative stress and 4-HNE act synergistically to trigger JNK overactivation in hepatocytes (57). In this study we observed that OA substantially enhanced 4-HNE formation in SB drinking rats; therefore, we speculate that in liver this effect might contribute to increase liver insulin resistance, gluconeogenesis, and glycojenolysis, as suggested by the marked increase in fasting plasma glucose in these animals.

An intriguing finding was that SB + OA did not further increase UA concentrations in plasma or tissue compared with W + OA animals. This effect likely relates to the timing of the UA measurement. Thus Stavric et al. (58) reported that the acute administration of OA could dramatically enhance an acute rise in UA in response to fructose. It is known that the effects of OA induce a rise in UA that peaks at 6 h and then decreases over time; we dosed OA to rats during the morning while majority of SB consumption is at night, due to the nocturnal rat feeding habits, so it is possible that we missed the time point when this synergy would have been maximally demonstrated. In addition, it is also possible that the synergy observed with UA and fructose may decrease over time, either due to a compensatory increase in uricase that reduces the efficacy of the uricase inhibitor (68) or by the fact that UA and its precursors may feedback to block xanthine oxidase activity (9–10). Despite our inability to show a synergy of fructose and OA on UA levels in our study, we did show a synergy for oxidative stress in the kidney and liver (lipid peroxidation). In addition, we also found a synergy of fructose and OA on glomerular hydrostatic pressure.

In summary, we document the contribution of hyperuricemia to the deleterious effects induced by the increased consumption of added sugars on diet. These additive effects are likely fully operative in humans, who lack the expression of uricase. The documentation that uricase inhibition can increase hepatic fat content and serum TG is consistent with our hypothesis that the uricase mutation occurred in the Miocene provided a survival advantage to apes starving in Europe during a period of climatic cooling that resulted in food shortage due to a loss of fruits (24). Today, with a dramatic increase in fructose, the loss
of uricase may enhance the risk for humans to develop metabolic syndrome and cardiorenal disease.

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DISCLOSURES

R. J. Johnson is listed as an inventor on a patent from the University of Washington for use of allopurinol to treat hypertension and is also an inventor on patent applications from the University of Florida, Takeda, and University of Colorado for use of UA-lowering agents or agents that block fructose metabolism in the management of metabolic and renal disorders. R. J. Johnson is the author of two lay books on fructose, of Colorado for use of UA-lowering agents or agents that block fructose metabolism in the management of metabolic and renal disorders. R. J. Johnson is the author of two lay books on fructose, (mercola.com, 2012) and The Sugar Fix (Rodale, 2008). R. J. Johnson has also consulted for Ardea, Astellas, Biocryst, Danone, and Novartis and is on the Scientific Advisory Board for Amway.

AUTHOR CONTRIBUTIONS


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