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Sex differences in renal and metabolic responses to a high-fructose diet in mice

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Sex differences in renal and metabolic responses to a high-fructose diet in mice. Am J Physiol Renal Physiol 308: F400–F410, 2015. First published December 23, 2014; doi:10.1152/ajprenal.00403.2014.—High fructose intake has been associated with increased incidences of renal disease and hypertension, among other pathologies. Most fructose is cleared by the portal system and metabolized in the liver; however, systemic levels of fructose can rise with increased consumption. We tested whether there were sex differences in the renal responses to a high-fructose diet in mice. Two-month-old male and female C57BL/6J-129/SV mice (n = 6 mice per sex per treatment) were randomized to receive control or high-fructose (65% by weight) diets as pelleted chow ad libitum for 3 mo. Fructose feeding did not significantly affect body weight but led to a 19% and 10% increase in kidney weight in male and female mice, respectively. In male mice, fructose increased the expression of the Na\(^+/K^+\) cotransporter 2 in the thick ascending limb and aquaporin-2 in the collecting duct with fructose relative to male control mice, whereas male mice had no change. Overall, our results support greater proximal metabolism of fructose in male animals and greater distal tubule/collection duct (electrolyte homeostasis) alterations in female animals. These sex differences may be important determinants of the specific nature of pathologies that develop in association with high fructose consumption.

aquaporin-2; electrolyte; glucose transporter 5; ketohexokinase; proximal tubule

Fructose is a six-carbon monosaccharide that naturally exists in honey, fruits, and some vegetables. However, the development of high-fructose corn syrup, as a lower-cost sweetener, has led to a stark rise in the rate of its consumption. One estimate suggests that the average daily intake of fructose in the United States increased 32% between 1978 and 2004 (43). Fructose is ~1.4× sweeter than table sugar, which, in some respects, makes it a reasonable substitute, as the caloric content (4 kcal/g) is the same as that of glucose. Although fructose, at first pass, would seem a safe and natural sweetener, cellular metabolism and regulation by satiety hormones, such as insulin, are known to be substantially different from those of glucose. These metabolic differences, along with its high consumption level, make it a highly critical and timely research topic.

While most dietary absorbed fructose is cleared by the liver, and thus portal levels of fructose can be quite high (~1 mM), peripheral levels are elevated much less dramatically (~0.1 mM) (21). Even so, renal damage has been reported in response to high-fructose diets (36). However, whether these damaging changes are manifested as a direct effect of fructose metabolism within these cells or are indirectly caused by the elevated insulin, uric acid, or triglycerides in the circulation is not clear. The kidney expresses at least two major classes of proteins that transport fructose: 1) glucose transporters (GLUTs) or gene family name solute carrier (SLc)2a and 2) Na\(^+/K^+\)-coupled transporter (SLc5a) superfamilies (7, 38), each with a number of different isoforms. In the present study, we focus on the regulation of GLUT5. GLUT5 may be considered “insulin dependent” in that insulin is thought to elicit its trafficking from intracellular domains to the cell surface (29). Barone et al. (2) demonstrated that global knockout of GLUT5 abolished the hypertensive effects of dietary fructose.

In this respect, there have been several studies in humans (16, 42, 60) and animals (34) demonstrating sex differences in response to fructose feeding. Nearly all studies have shown that males are more sensitive. High-fructose feeding has been demonstrated to increase insulin resistance, blood pressure, and de novo triacylglycerol production to a greater extent in males. Unfortunately, few studies have tried to determine the mechanisms underlying differences in sensitivity between the sexes, nor have they focused on specific aspects of renal metabolism.

The first step in fructose metabolism is phosphorylation by fructokinase, also known as ketohexokinase (KHK), an enzyme that has been associated with rapid ATP depletion (14). Fructose-1-phosphate is cleaved by aldolase B to dihydroxyacetone phosphate and glyceraldehyde, which are substrates for triglyceride synthesis or gluconeogenesis, depending on the cell type. Rapid ATP depletion has been demonstrated to increase circulating levels of uric acid, a byproduct of ATP metabolism, in some individuals (5, 10, 62). Hyperuricemia has been associated with several of the pathological effects of fructose (10, 14, 62).

At the whole body level, high consumption of high levels of dietary fructose has been linked to the development of the metabolic syndrome, i.e., a constellation of disorders consist-

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ing of hyperinsulinemia, hypertension, visceral adiposity, and dyslipidemia (9). Some of these effects are simply a manifestation of increased calorie consumption, and, in this respect, any sugar will do. However, in this regard, fructose does not stimulate the release of pancreatic insulin, a major satiety hormone, which then can lead to overeating. While sex differences in these responses have not been extensively studied, one study (26) did show that fructose increased blood pressure and led to metabolic perturbations in male rats but not in female rats.

Sex differences exist in a number of renal activities including, but not limited to, the regulation of transport, including salt, water, and organic anion and cation reabsorption (40, 46, 52); metabolic activities, including the propensity toward oxidative stress (50); endocrine systems, such as the renin-angiotensin-aldosterone system (64) and endothelin (37); and drug disposition and clearance (52). Moreover, there are a number of metabolic differences between the sexes with regard to nutrient storage and utilization in different tissue beds. For example, men have higher rates of gluconeogenesis and women have higher rates of fatty acid oxidation. Men generally have a higher respiratory quotient and rely on carbohydrates preferentially as an energy source (51). Therefore, it would not be unexpected if renal responses to dietary fructose vary. Indeed, most studies examining the effects of fructose on the kidney have been done in male animals, in general, because they are thought, and in a few situations, found to be more sensitive, at least to the major cardiometabolic effects of fructose. Therefore, the aim of the present study was to characterize sex differences in basic renal responses to chronic dietary fructose that might affect and explain the sex-differential susceptibility to renal pathology, hypertension, and/or other water and electrolyte disturbances.

MATERIALS AND METHODS

Experimental animals. Mice (C57BL/6/129/SV mixed genetic background) were bred and raised at Georgetown University under approved protocols within the established guidelines of the Institutional Animal Care and Use Committee of Georgetown University. Two-month-old male and female mice (n = 6 mice per sex per treatment) were randomized to receive control (TD01457, Teklad) or high-fructose (65% by dry weight, TD01458, Teklad) diet as pelleted chow. In the control diet, cornstarch primarily replaced fructose as the high-fructose (65% by dry weight, TD01458, Teklad) diet as pelleted chow. Plasma glucose levels were measured using a one-step glucose oxidase-based method (Arkray G7200). Mice were fasted for 5 h and then injected intraperitoneally with 20% dextrose solution (2 g/kg body wt). Tail vein blood glucose was measured at 0, 15, 30, 45, 60, and 90 min. The area under the curve for glucose tolerance was calculated based on Tai’s model (56).

Histochemical analyses. The right kidney was embedded in paraffin blocks and sectioned by the Histology Core (Lombardi Cancer Center, Georgetown University). Sections from 3 mice/group were stained with Masson’s trichrome and analyzed for histological evidence of pathology. Sections were imaged with a Zeiss Axiosport 410 inverted microscope. Sections from 4 mice/group were semiquantitatively analyzed for GLUT5 subcellular localization using immunoperoxidase-based staining. Heat-induced target retrieval was performed on slides using citrate buffer (pH 6, DakoCytomation, Carpenteria, CA) to unmask antigenic sites. Endogenous peroxidase activity was removed by incubation with 3% H2O2 for 10 min and blockade with avidin/biotin (Vector) and 10% normal goat serum. Sections were incubated with GLUT5 primary antibody (PA1737, Boster Immunoleader, 1:2,000) overnight at 4°C. Secondary antibody was biotinated goat anti-rabbit antibody (BA-1000, Vector Labs, 1:1,000), 3,3′-Diaminobenzidine tetrachloride dihydrate was applied for 35 s. A positive reaction was identified as a dark brown stain. Pictures were taken with a Photometrics Cool Snap camera (Scanalytics, Fairfax, VA) mounted to a Nikon Eclipse E600 microscope. Pixel densities (relative to background, i.e., luminal pixel density) of the apical and basolateral regions of 10 sampled proximal tubules (PTs) per mouse were evaluated and averaged using Adobe Photoshop CS5 software. Values were obtained from tubule cross-sections by outlining areas approximating the inner one-third (“apical”) versus outer two-third (“basolateral”) regions of the tubule cross-section. The average density of each region and ratio of apical to basolateral staining were calculated for each tubule sampled.

Plasma and urine analyses. On 24-h urine, urine osmolality was measured using a freezing point osmometer (model 3900, Advanced Instruments, Norwood, MA). Urine fructose levels were measured by an enzyme-based chromogenic assay (EFP1-100, BioAssay Systems). Plasma creatinine was determined by an enzymatic assay kit (Mouse Creatinine Assay Kit no. 80350, Crystal Chem, Downers Grove, IL). Urine creatinine was determined by a Jaffe reaction assay kit (no. 500701, Cayman Chemical). Plasma and urine electrolytes (Na+, K+, and Cl−) were measured using the Medica EasyLyte Analyzer. Plasma and urine uric acid were measured by a colorimetric kit (no. 700320, Cayman Chemical). Plasma insulin and aldosterone were determined by ELISAs (no. 10-1247-01, Mercodia, Uppsala, Sweden; and no. 10004377, Cayman Chemical, respectively).

Western blot analysis of proteins. The kidney cortex (dissected free from the medulla) was prepared for Western blot analysis. Briefly, samples were homogenized in isolation buffer containing protease inhibitors (22). Protein concentration was determined by a bichromonic acid assay kit (Pierce), and protein was then solubilized in Lammeli buffer. Protein estimations were confirmed by Coomassie dye-stained loading gels. For immunoblot analysis, 10–25 μg of total protein were loaded from each sample onto individual gels of 10% or 12% polyacrylamide (Precast, Bio-Rad). Blots were probed with the following primary antibodies at the respective dilutions: 1) GLUT5 (H200, sc-30109, Santa Cruz Biotechnology, Santa Cruz, CA, 1:1,000); 2) insulin receptor β-subunit (MAB1139, Bioscience Research Reagents, Temple, CA, 1 μg/μl); 3) HNK-1 (sc-66024, Santa Cruz Biotechnology, 1:1,000); 4) Na+/K+-ATPase (AQP)2 [polyclonal rabbit from our laboratory (48, 58)]; 5) aquaporin (AQP)2 [polyclonal rabbit from our laboratory (48, 58)]; 6) β-actin (A5441, Sigma-Aldrich, 1:5,000); and 7) GADPH (SAB4105848, Sigma-Aldrich, 1:5,000). The blotting and detection approach has been previously described (22). Western blot band density on film was analyzed by Image J software (National Institutes of Health, Bethesda, MD) and normalized to β-actin or GADPH.

Statistical analysis. In general, data were analyzed using SigmaPlot (Systat Software, version 10, Evanston, IL). Two-way ANOVA was used to determine differences due to the main factors, sex, diet, and their interactions for all variables. One-way ANOVA followed by a multiple-comparisons test or unpaired t-tests were used to determine differences between individual pairs of means. P values of <0.05 were considered to be significant.

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**RESULTS**

**Metabolic effects of fructose feeding.** Body weight and weight gain (Table 1) were not affected by fructose feeding; however, male mice of both treatments were ~35% heavier and gained twice as much weight. Male mice had a significantly reduced ability to rapidly clear a bolus of intraperitoneal glucose (glucose tolerance) compared with female mice. Surprisingly, fructose feeding improved this ability in male mice. Male mice also had significantly increased plasma insulin, and there was a significant interactive term in that female mice showed a slight increase in plasma insulin with fructose and male mice showed a decrease. Plasma aldosterone was not significantly affected by sex or treatment.

**Hypertrophy of the kidney and osmotic diuresis.** Fructose feeding resulted in significantly increased kidney wet weight (Fig. 1A). The increase was ~19% and 10% in male and female mice relative to sex-respective control mice. Absolute weights of the kidneys were heavier in male mice (not shown), but no sex differences were observed when kidney weight was normalized to body weight. Urine volume (Fig. 1B) was also significantly higher in fructose-fed mice of both sexes (increases of 77% and 327% in male and female mice, respectively). Urine osmolality, however, was not significantly lower in fructose-fed mice (Table 2). This resulted in a large and significant increase in total osmoles excreted in fructose-fed mice (Fig. 1C). Moreover, there was no sex difference in this parameter when normalized to body weight. Dietary fructose led to a large significant increase in urinary fructose (Table 2). Urinary fructose was increased >100-fold in male mice and 40-fold in female mice. Interestingly, male control mice had significantly lower (less than half) urine fructose compared with female control mice. Gross differences in collagen deposition in the cortex were evaluated by Masson’s trichrome staining (Fig. 2). In general, no obvious differences were observed as a result of diet or sex.

**Uric acid metabolism.** Uric acid metabolism was evaluated as a determinant of ATP depletion. Urine uric acid was significantly increased by fructose feeding (>100%) in both male and female mice, with no sex differences (Fig. 3A). Surprisingly, uric acid concentrations in plasma were highest in male control mice and reduced significantly in both sexes by fructose (Fig. 3B). Uric acid clearance was highest in female mice fed fructose, with an increase of >500% compared with female control mice (Fig. 3C).

**Urine electrolytes.** Dietary fructose led to a significant increase in the excretion of Na+, K+, and Cl− in urine (Table 2).

There were no sex differences in this excretion (when normalized to body weight). The ratio of electrolytes in urine was also evaluated as an index of distal tubular electrolyte homeostasis. Fructose did not significantly affect any of these ratios (as assessed by two-way ANOVA); however, female mice had a lower ratio of K+ to Cl− in urine, and this difference was attenuated in fructose-fed mice. The fractional excretion of Na+, K+, and Cl− is shown in Fig. 4; mice are plotted individually to show increased variability in fructose-fed animals. Fructose increased the fraction excretion of all three electrolytes. This response was enhanced in male mice (highly significant interactive term). Differences in the fraction excretion of electrolytes between sexes were primarily driven by a fall in urine creatinine in male fructose-fed mice. Urine creatinine levels were 3.5 ± 0.5 μmol·day−1·25 g body wt−1 in male control mice, 1.6 ± 0.6 μmol·day−1·25 g body wt−1 in male fructose-fed mice, 2.9 ± 0.5 μmol·day−1·25 g body wt−1 in female control mice, and 5.4 ± 1.9 μmol·day−1·25 g body wt−1 in female fructose-fed mice (P = 0.032 between male control and male fructose-fed mice by unpaired t-test). In contrast, plasma creatinine was not significantly different between groups (not shown).

**Plasma electrolytes.** Plasma Na+, K+, and Cl− were all significantly affected by fructose (Fig. 5). Plasma Na+ (Fig. 5A) and Cl− (Fig. 5C) were reduced by fructose, but only in female mice (significant interactive term). Plasma K+, on the other hand, was increased by fructose in both sexes. The increase was larger in female mice, as female control mice had the lowest plasma K+. The ratio of Na+ to Cl− was not different between groups, which indicates that these two electrolytes were being regulated in parallel. In contrast, ratios of Na+ to K+ and K+ to Cl− were highly affected by fructose in that fructose reduced the former and increased the latter. There was also a significant interaction for both of these ratios in that the effects were strongest in female mice.

**Expression of proteins involved in water balance.** Due to evidence of sex differences in plasma electrolytes with fructose feeding (modest hyponatremia in female mice), we tested the cortical expression of two major vasopressin-regulated proteins involved in urine concentrating, i.e., AQ2 in the collecting duct (CD) and NKCC2 in the thick ascending limb (TAL; Fig. 6). Both AQ2 and NKCC2 were more highly expressed in female control mice. Fructose feeding reduced both proteins, and the degree of reduction was greater in female mice.

**Expression of proteins involved in fructose metabolism/transport.** In contrast, the expression of proteins involved in fructose transport/metabolism or energy homeostasis (Fig. 7) due to evidence of sex differences in plasma electrolytes with fructose feeding (modest hyponatremia in female mice), we tested the cortical expression of two major vasopressin-regulated proteins involved in urine concentrating, i.e., AQ2 in the collecting duct (CD) and NKCC2 in the thick ascending limb (TAL; Fig. 6). Both AQ2 and NKCC2 were more highly expressed in female control mice. Fructose feeding reduced both proteins, and the degree of reduction was greater in female mice.

**Table 1. Metabolic and plasma parameters**

<table>
<thead>
<tr>
<th></th>
<th>Body Weight, g</th>
<th>Weight Gain, g/3 mo</th>
<th>Glucose Tolerance (Area Under the Curve), mg·h−1·dl−1</th>
<th>Plasma Insulin, μM</th>
<th>Plasma Aldosterone, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC group</td>
<td>32.0 ± 2.0A</td>
<td>9.1 ± 1.5A</td>
<td>504 ± 33A</td>
<td>1.14 ± 0.15</td>
<td>5.4 ± 2.1</td>
</tr>
<tr>
<td>MFr group</td>
<td>30.7 ± 1.9AB</td>
<td>8.1 ± 1.9A</td>
<td>367 ± 74B</td>
<td>0.58 ± 0.13</td>
<td>6.9 ± 2.7</td>
</tr>
<tr>
<td>FC group</td>
<td>22.0 ± 0.4R</td>
<td>3.4 ± 1.0B</td>
<td>286 ± 36B</td>
<td>0.40 ± 0.14</td>
<td>21.3 ± 13.8</td>
</tr>
<tr>
<td>FFr group</td>
<td>23.8 ± 0.4BC</td>
<td>4.0 ± 1.0B</td>
<td>317 ± 23R</td>
<td>0.54 ± 0.13</td>
<td>18.0 ± 6.9</td>
</tr>
</tbody>
</table>

Results of two-way ANOVA (diet × sex) (P values)

| Diet  | 0.87 | 0.90 | 0.26 | 0.15 | 0.92 |
| Sex   | <0.001* | 0.002* | 0.008* | 0.012* | 0.12 |
| Inter | 0.28 | 0.56 | 0.083 | 0.02* | 0.77 |

Values are means ± SE; n = 6 animals/group. Mice were divided into the following groups: male mice fed control diet (MC group), male mice fed fructose (MFr group), female mice fed control diet (FC group), and female mice fed fructose (FFr group). A,B,C Letters indicate the results of a multiple-comparisons test after a significant (P < 0.05) one-way ANOVA; “A” is equal to “AB” but not “B,” etc. *Significant values (P < 0.05) for two-way ANOVA results.
To determine whether sex or dietary fructose affect the order for luminal-to-interstitial directional transport to occur, the PT requires apical brush-border localization in the first enzyme in fructose metabolism, and the insulin receptor subunit were increased by fructose only in male mice. We will discuss these findings and interpretation in greater detail below.

Glucose reabsorption in the PT (63), it does not transport Na+/K+-ATPase expression in regions of the kidney. To determine whether electrolyte differences might result from altered driving forces, we evaluated protein levels (Fig. 9) of \( \alpha_1 \)-subunit of Na\(^+\)/K\(^+\)-ATPase pump in homogenates from the cortex, inner stripe of the outer medulla, and inner medulla. Female mice had modest but significantly higher levels of the \( \alpha_1 \)-subunit in homogenates from the cortex (primarily PT associated) and inner medulla (CD associated) compared with male mice (two-way ANOVA). In addition, fructose had a tendency to increase expression in female mice only in all three regions, leading to a significant interactive term for homogenates from the inner stripe of the outer medulla (TAL associated) and inner medulla with a strong trend (\( P = 0.061 \)) in cortex homogenates.

**DISCUSSION**

The consumption of fructose, around the world, has increased substantially in the last three to four decades (8, 9, 53, 57). However, whether or not this represents an inherent health risk and whether certain populations may be more susceptible are still uncertain. Our experiments were aimed to determine whether there were sex differences primarily in the renal-specific responses to fructose feeding in the mouse. We used mixed genetic background (~80% 129/SV and 20% C57Bl6/J). It is important to note that background strain may affect overall results. Our mixed background would be predicted to increase overall variability, and therefore any sex differences found might be considered more robust. Overall, we found a pattern of differences that we interpret as supportive of greater PT metabolism of fructose by male mice and a greater impact, i.e., sensitivity of the distal tubule (TAL and CD) in female mice. We will discuss these findings and interpretation in greater detail below.

Like glucose, fructose has the capacity to be reabsorbed from the filtrate in the PT. Whereas Slc5a2, i.e., Na\(^+\)-glucose cotransporter 2 (SGLT2), is responsible for the majority of glucose reabsorption in the PT (63), it does not transport fructose (63). In the Slc2 family, Slc2a5 (GLUT5) appears to be the major facilitator of fructose reabsorption across the apical membrane of the PT (2). Our Western blot analysis results support our conclusion that male mice have higher total PT cellular levels of GLUT5 protein under control and dietary fructose feeding (Fig. 6). Furthermore, whereas the expression of GLUT5 increased twofold in male fructose-fed mice, it was unresponsive in female mice. The increase in renal GLUT5 with fructose feeding in male mice confirmed the observation of other laboratories (1, 2).

Nevertheless, our immunohistochemical analysis (although clearly not as quantitative) did not show increased density in male animals or due to fructose in either sex. In fact, female

*Fig. 1. Fructose increased kidney weight and urine volume. A: kidney weight (average of both kidneys). B: urine volume (24 h). C: urine osmoles excreted (24 h). Mice were divided into the following groups: male mice fed control diet (MC group), male mice fed fructose (MFr group), female mice fed control diet (FC group), and female mice fed fructose (FFr group). All data were normalized to 25 g of body weight (bw). “A” is significantly greater than “B” but not “AB” [results of a multiple-comparisons test after a significant (\( P < 0.05 \)) one-way ANOVA]; two-way ANOVA (sex \( \times \) diet) results are provided in the graphs.*
mice (on both diets) had a higher ratio of apical to basolateral staining, potentially indicative of transport potential. The absence of finding an increase in GLUT5 due to fructose feeding with immunohistochemistry in either sex might be explained by hypertrophy of the PT (10% in female mice and 19% in male mice), which may have diluted the signal with immunohistochemistry but would not confound Western blot analysis. Nonetheless, in female mice fed fructose, both apical and basolateral staining, as quantified, was significantly reduced (relative to same-sex control mice). Thus, it appears that female mice either downregulate or at least have attenuated upregulation of GLUT5 expression (relative to male mice) in response to dietary fructose, in agreement with the Western blot analysis results and directional changes in KHK.

Based on the fairly high increase in fructose in urine in fructose-fed mice, it is likely that fructose uptake into PT cells is not nearly as efficient as that of glucose. In support of this, the $K_m$ of GLUT5 in rat has been reported to be 12.6 mM, similar to the small intestine, and rather high relative to blood levels (0.1–0.3 mM in rats that consumed fructose) (11). A more recent study (15) examining mouse GLUT5 in transfected oocytes found similar kinetics to those of the rat. In comparison, SGLT2 has a $K_m$ of 1.6 mM for glucose, but blood levels are near 5.5 mM (35). However, the fact that male mice had more GLUT5 and this level was increased by fructose suggests that they had the capacity to adapt in this manner. This may be due to the fact that GLUT5 is an “Sry-regulated” gene (15). Sry is a transcription factor produced from the Y chromosome.

Table 2. Urine analysis

<table>
<thead>
<tr>
<th>Group</th>
<th>Urine Osmolality, osm/kg H2O</th>
<th>Urine Fructose, μmol/day</th>
<th>Urine Na⁺, μmol/day</th>
<th>Urine K⁺, μmol/day</th>
<th>Urine Cl⁻, μmol/day</th>
<th>Ratio of Na⁺ to Cl⁻</th>
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<th>Ratio of K⁺ to Cl⁻</th>
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<tbody>
<tr>
<td>MC group</td>
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<td>105 ± 18AB</td>
<td>71 ± 15B</td>
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<td>1.72 ± 0.25AB</td>
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<tr>
<td>MFr group</td>
<td>2.54 ± 0.36</td>
<td>430 ± 132</td>
<td>105 ± 19A</td>
<td>240 ± 45A</td>
<td>145 ± 27A</td>
<td>0.73 ± 0.03</td>
<td>0.44 ± 0.03</td>
<td>1.68 ± 0.10A</td>
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<tr>
<td>FC group</td>
<td>2.85 ± 0.18</td>
<td>8.19 ± 1.56*</td>
<td>47 ± 7B</td>
<td>68 ± 15B</td>
<td>62 ± 9B</td>
<td>0.77 ± 0.12</td>
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<td>FFr group</td>
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<td>334 ± 74</td>
<td>91 ± 12AB</td>
<td>174 ± 23AB</td>
<td>115 ± 14AB</td>
<td>0.79 ± 0.05</td>
<td>0.53 ± 0.05</td>
<td>1.52 ± 0.10AB</td>
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Results of two-way ANOVA (diet × sex) (P values)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Sex</th>
<th>Interaction</th>
<th>P</th>
<th>P</th>
<th>P</th>
<th>P</th>
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<tbody>
<tr>
<td>Diet</td>
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<td>&lt;0.001†</td>
<td>0.002†</td>
<td>0.46</td>
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<td>Diet</td>
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<td>0.29</td>
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<td>0.28</td>
<td>0.66</td>
<td>0.086</td>
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<td>Diet</td>
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<td>0.61</td>
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<td>0.36</td>
<td>0.96</td>
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<tr>
<td>Diet</td>
<td>0.27</td>
<td>0.44</td>
<td>0.76</td>
<td>0.61</td>
<td>0.55</td>
<td>0.36</td>
<td>0.96</td>
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<tr>
<td>Sex</td>
<td>0.12</td>
<td>0.29</td>
<td>0.44</td>
<td>0.081</td>
<td>0.28</td>
<td>0.66</td>
<td>0.086</td>
</tr>
<tr>
<td>Interaction</td>
<td>0.48</td>
<td>0.27</td>
<td>0.76</td>
<td>0.61</td>
<td>0.55</td>
<td>0.36</td>
<td>0.96</td>
</tr>
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</table>

Values are means ± SE; n = 6 mice/group. Daily excretion of urine fructose, Na⁺, K⁺, and Cl⁻ were normalized to 25 g of body weight. Letters indicate the results of a multiple-comparisons testing after a significant (P < 0.05) one-way ANOVA. “A” is equal to “AB” but not “B”. *Significantly different from the MC group by an unpaired t-test. †Significant values (P < 0.05) for two-way ANOVA results.
A mosome that increases the expression of this transporter, similar to other proteins involved in differentiation of the male sex (55). In a recent study by Aoyama et al. (1), increased outer cortical GLUT5 expression in response to dietary fructose corresponded temporally to increased tubulointerstitial fibrosis in the DBA/2N mouse strain, suggesting that cellular uptake of the sugar was necessary to initiate some modes of renal pathology.

This difference in GLUT5 expression may have also precipitated the ~100% increase in the expression of KHK in male fructose-fed mice. KHK and GLUT5 expression are thought to be limited exclusively to the PT in kidney (18, 19). Thus, while other renal tubule cells may reabsorb filtered fructose or transport it from the basolateral side, it is unclear whether they can metabolize it. PT cells have similarities to hepatic cells, the major metabolic site for absorbed fructose. In that regard, our results do contrast to what has been reported in rats fed fructose, where female rats were found to have a greater upregulation of fructokinase (KHK) in the liver compared with male rats (61). In addition, female rats in that study (6) suffered additional consequences related to diet (impaired glucose tolerance test and elevated plasma insulin) to a greater extent than male rats (61). This did agree with our findings in mice on the whole body impact of dietary fructose, which, we found, were somewhat more apparent in female mice, e.g., increased plasma insulin and a trend for reduced glucose tolerance test.

We also found that male mice had an approximately fourfold increase in the expression of renal cortical insulin receptor (β-subunit) with fructose feeding, whereas female mice started out with higher levels of expression, which did not change with fructose. Whether this increase in receptor number led to greater signaling capacity in these cells or was in response to insulin receptor “resistance” is not known. We have found a...
number of factors that influence expression of the renal insulin receptor, including obesity and high-fat diets (decrease expression) as well as insulin infusion (increase its expression) (59). Impaired insulin signaling in the PT may be associated with enhanced gluconeogenesis at this site (28).

Nonreabsorbed fructose travels down the tubule lumen, where it can act as an osmolyte, drawing in fluid, leading to osmotic diuresis. We did not find any sex differences in the absolute or body weight-normalized amount of fructose in urine, which was substantial in both sexes (430 μmol or ~77 mg/day for male mice) and represented ~0.3 kcal (4 kcal/g × 0.077 g fructose). Nonetheless, maintenance energy requirements for a lean mouse have been estimated at 124 kcal·kg⁻¹·day⁻¹ (39) or 10 kcal/day for a 30-g mouse. Thus, urinary fructose would represent only ~3% of daily energy requirements and should not have substantially affected nutritional status.

Although we did not measure food intake as it is difficult with pelleted diet in chronic studies, we did monitor urine electrolytes as an index of food intake in the chronic state. The diets were formulated to contain the same percentage of these electrolytes. While there were no significant differences in body weight gain between fructose- and control diet-fed mice, urine electrolytes were increased by 94%, 129%, and 104% for Na⁺, K⁺, and Cl⁻, respectively, in male mice and by 94%, 156%, and 85% in female mice for fructose-fed animals. It is not entirely clear what is driving this increase in urinary electrolytes. The possibilities include 1) increased food consumption due to lower energy bioavailability of fructose and/or increased basal metabolic rate or 2) losses from other tissues beds, such as bone, muscle, and extracellular fluid. Calculation of the fractional excretion of electrolytes revealed a greater response in male mice; however, this sex effect was primarily driven by a fall in urine creatinine in male fructose-fed mice rather than increased urine electrolytes. Why male and not female mice experienced this reduction in urine creatinine with fructose feeding is unclear. Plasma creatinine levels were not significantly different.

The osmotic diuresis produced by fructose may have been the cause of the slight, but significant, disruption in plasma...
electrolyte homeostasis. Overall, fructose increased the ratio of $\text{K}^+$ to both $\text{Cl}^-$ and $\text{Na}^+$ in the plasma, and this effect was significantly greater in female mice. Female mice fed fructose had significantly lower plasma $\text{Na}^+$ and $\text{Cl}^-$ than all other groups. Both sexes experienced some rise in serum $\text{K}^+$. Although the fall in serum $\text{Na}^+$ and $\text{Cl}^-$ in female mice was modest, it might be exacerbated with aging or comorbidities.

Fig. 6. Aquaporin (AQP2) and $\text{Na}^+$-$\text{K}^+$-$2\text{Cl}^-$ cotransporter (NKCC2) abundance showed greater sensitivity to fructose in female mice. A: Western blots of cortex homogenates (each lane shows a different mouse sample) loaded with equal amounts of protein and probed with antibodies against AQP2, NKCC2, and GAPDH. GAPDH (reprobe) was used to normalize loading. “A” is significantly greater than “B” but not “AB” [results of a multiple-comparisons test after a significant ($P < 0.05$) one-way ANOVA]; two-way ANOVA (sex $\times$ diet) results are provided in the graph (B).

Fig. 7. Proteins involved in energy metabolism are upregulated by fructose in male mice. A: Western blots of cortex homogenates (each lane shows a different mouse sample) loaded with equal amounts of protein and probed with antibodies against glucose transporter (GLUT)5, ketohexokinase (KHK), insulin receptor $\beta$-subunit (IR-$\beta$), and GAPDH. GAPDH (reprobe) was used to normalize loading. “A” is significantly greater than “B” but not “AB” [results of a multiple-comparisons test after a significant ($P < 0.05$) one-way ANOVA]; two-way ANOVA (sex $\times$ diet) results are provided in the graph (B).

Fig. 8. GLUT5 immunohistochemistry. A: representative stained sections from the deep cortical region of the kidney from a MC mouse (top left), MFr mouse (top right), FC mouse (bottom left), and FFr mouse (bottom right). Magnification: $\times 400$. B: summary of density semiquantification of “apical” and “basolateral” aspects of tubules sampled (sampling 10 tubules/mouse, $n = 4$ mice/group). C: summary of average mean ratios of apical to basolateral staining calculated for each tubule. *Significant ($P < 0.05$) difference between control- and fructose-fed mice within the female sex (by unpaired $t$-test). “A” is significantly greater than “B” [results of a multiple-comparisons test after a significant ($P < 0.05$) one-way ANOVA]; two-way ANOVA (sex $\times$ diet) results are provided in the graphs.
Fig. 9. Regulation of Na⁺−K⁺-ATPase (α1-subunit) in kidney regions by fructose. A–C: Western blots of whole cell homogenates prepared from the cortex (CTXH; A), inner stripe of the outer medulla (OMH; B), and inner medulla (IMH; C). Each lane was loaded with the same amount of protein from a different mouse sample and probed with a commercial antibody against the Na⁺−K⁺-ATPase α1-subunit. The lower portion of each blot was probed with β-actin to control for loading. D: densitometric summary. “A” is significantly greater than “B” but not “AB” [results of a multiple-comparisons test after a significant (P < 0.05) one-way ANOVA]; two-way ANOVA (sex × diet) results are provided in the graph.

The relative hyponatremia and hypochloremia in female fructose-fed mice were accompanied by a fall in the expression of two major proteins involved in water balance, i.e., AQP2 and NKCC2. We also found a fall in the abundance of these two proteins in our study done in male rats in response to fructose (54). The cause-and-effect relationship between the fall in these proteins and the 237% increase in urine volume in female mice is uncertain. A reduction in the level of these proteins would clearly facilitate water excretion both by affecting urine concentrating and diluting capacity of the TAL and affecting permeability of the CD. The fall in these proteins and the 237% increase in urine volume in female mice may be strain specific (1). Nevertheless, it is not clear whether the changes in female mice represent adaptive, or neutral responses to fructose. Additional studies are warranted to address differences in vulnerabilities to dietary fructose between the sexes and the mechanisms underlying them.

In summary, fructose feeding resulted in differential responses at the level of the kidney in male versus female mice. Our findings are consistent with more proximal effects in male mice that may arise from increased cellular uptake and metabolism and a more distal phenotype in female mice, with effects on serum electrolytes and expression of transporters/channels that regulate these parameters. Nonetheless, it is not clear whether the changes in female mice represent adaptive, maladaptive, or neutral responses to fructose. Additional studies are warranted to address differences in vulnerabilities to dietary fructose between the sexes and the mechanisms underlying them.

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Sex Differences in Fructose Metabolism

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: N.S. and L.L. performed experiments; N.S., L.L., and C.M.E. analyzed data; N.S. and C.M.E. interpreted results of experiments; N.S. and C.M.E. prepared figures; N.S., L.L., and C.M.E. drafted manuscript; N.S., L.L., and C.M.E. edited and revised manuscript; N.S., L.L., and C.M.E. approved final version of manuscript; C.M.E. conception and design of research.

REFERENCES

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