Slc2a5 (Glut5) Is Essential for the Absorption of Fructose in the Intestine and Generation of Fructose-induced Hypertension*

The identity of the transporter responsible for fructose absorption in the intestine in vivo and its potential role in fructose-induced hypertension remain speculative. Here we demonstrate that Glut5 (Slc2a5) deletion reduced fructose absorption by ~75% in the jejuna and decreased the concentration of serum fructose by ~90% relative to wild-type mice on increased dietary fructose. When fed a control (60% starch) diet, Glut5−/− mice had normal blood pressure and displayed normal weight gain. However, whereas Glut5+/+ mice showed enhanced salt absorption in their jejunum in response to luminal fructose and developed systemic hypertension when fed a high fructose (60% fructose) diet for 14 weeks, Glut5−/− mice did not display fructose-stimulated salt absorption in their jejunum, and they experienced a significant impairment of nutrient absorption in their intestine with accompanying hypotension as early as 3–5 days after the start of a high fructose diet. Examination of the intestinal tract of Glut5+/− mice fed a high fructose diet revealed massive dilatation of the caecum and colon, consistent with severe malabsorption, along with a unique adaptive up-regulation of ion transporters. In contrast to the malabsorption of fructose, Glut5+/− mice did not exhibit an absorption defect when fed a high glucose (60% glucose) diet. We conclude that Glut5 is essential for the absorption of fructose in the intestine and plays a fundamental role in the generation of fructose-induced hypertension. Deletion of Glut5 results in a serious nutrient-absorptive defect and volume depletion only when the animals are fed a high fructose diet and is associated with compensatory adaptive up-regulation of ion-absorbing transporters in the colon.

Fructose is a monosaccharide and is one of the three most important blood sugars along with glucose and galactose (1–3). It plays an essential role in vital metabolic functions in the body, including glycolysis and gluconeogenesis (4–6). Fructose is predominantly metabolized in the liver. A high flux of fructose to the liver perturbs glucose metabolism and leads to a significantly enhanced rate of triglyceride synthesis. In addition, fructose can be metabolized in the liver to uric acid, a potent antioxidant (7, 8).

The classic model of sugar absorption indicates that sodium glucose cotransporter 1 (Sglt1) and Glut5 absorb glucose and fructose, respectively, from intestinal lumen to cytosol, and Glut2 transports both glucose and fructose from the cytosol to the blood (9–19). Glut2 has high affinity for glucose and a moderate affinity for fructose, whereas Glut5 predominantly transports fructose with very low affinity for glucose (9–19; reviews in Refs. 14, 17–19). The expression of Glut5 or Glut2 in the small intestine increases in rats or mice fed a diet high in fructose or perfused with increased fructose concentration (11–14, 18, 19).

Glut2 is predominantly found on the basolateral membrane and in the cytoplasm of enterocytes at basal state but is thought to be recruited to the apical membrane in the presence of increased glucose or fructose in the intestinal lumen (11, 19). Given the fact that both Glut5 and Glut2 can transport fructose in vitro and given the ability of Glut2 to traffic to the apical membrane, the contribution of Glut5 to the absorption of fructose in vivo and systemic fructose homeostasis remains speculative.

The marked increase in dietary fructose consumption in the form of high fructose corn syrup, a common sweetener used in the food industry, table sugar, and fruits correlates with the increased incidence of metabolic syndrome, which is reaching an epidemic proportion in developed countries and is a major contributor to premature morbidity and mortality in our society (20–22). Increased dietary fructose intake recapitulates many aspects of metabolic syndrome, including dyslipidemia, insulin resistance, and hypertension in rat and mouse (23–26). Recent studies demonstrate that fructose-induced hyperten-

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The abbreviations used are: Sglt1, sodium glucose cotransporter 1; RT, reverse transcription.
sion is initiated by increased absorption of salt and fructose in the intestine (27); however, the one or more molecules (Glut2, Glut5, Glut7, or Sglt1) that are responsible for the absorption of fructose in the intestine remain speculative. Further, although Glut7, Glut5, and Glut2 can transport fructose in vitro, the role of Glut5 in in vivo fructose absorption remains unknown. To ascertain the role of Glut5 in fructose absorption in the intestine in vivo and fructose-induced hypertension, mice lacking the Glut5 gene (Glut5−/−) were placed on either high fructose or normal diet and compared with their wild-type littermates (Glut5+/+).

**EXPERIMENTAL PROCEDURES**

**Animal Models and Experimental Diets**—Glut5−/− mice were generated as described (34). Briefly, a Glut5-floxed construct was generated by inserting one loxP site upstream of the predicted promoter and exon 1 and another loxP site downstream of exon 4 of the Glut5 gene, which has 14 exons. After establishing germ line transmission, intercrosses between Glut5floxed+/+ and a ubiquitous Cre-expressing line EIIA-Cre (35) resulted in offspring with the expected global deletion of the floxed exons 1–4 of Glut5 (34). Glut5−/− mice have normal growth and are fertile.

For the experiments, wild-type (Glut5+/+) and Glut5 knockout (Glut5−/−) mice were divided into two groups. One group was fed a diet high in fructose (60% fructose), and the other group received a control diet (60% starch). Diets were custom made at Harlan Teklad (Madison, WI). In separate studies, animals were fed a diet high in glucose (60% glucose).

All mice had free access to food and water. Mice were housed three to four mice/cage, in a temperature-controlled room (22.2 ± 0.5 °C) that maintained a cycle of 12-h dark and 12-h light. All experimental maneuvers were performed according to protocols approved by the Animal Care Committee at The University of Cincinnati or University of Hanover. In separate studies, Glut5+/+ mice were pair-fed at 1.5 g/day of control or high fructose diet for 5 days. Animals had free access to water.

**RNA Isolation and Northern Blot Hybridization**—Total cellular RNA was extracted from various mouse tissues, including duodenum, jejenum, ileum, caecum, proximal and distal colon, and kidney, according to established methods, quantitated spectrophotometrically, and stored at −80 °C. Hybridization was performed according to established protocols (36). The following DNA fragments were used as specific probes for Northern hybridization: for NHE3, a fragment encoding nucleotides 1883–2217; for DRA (Slc26a3), a 400-bp cDNA from the mouse DRA cDNA (EcoRI-EcoRI fragment); for Glut2 a fragment encoding nucleotides 64–501 (accession number NM_031197); for colonic H,K-ATPase, three pooled PCR products from rat (nucleotides 135–515, 2369–2998, and 3098–3678); and for Glut7, a fragment encoding nucleotides 1–1578 (accession number NM_001088382). Each Northern hybridization was performed on four separate samples from four different animals.

**Expression of Glut5 and Glut2 in COS7 Cells by RT-PCR**—The expression of Glut5 on RNA isolated from COS7 cells was determined by RT-PCR using the following primers: ACT CTC CAG CCC TGC TCA TG (sense) and CAG CTT CAC CAC CGA GAT G (antisense), which encodes the nucleotides 132–817 (GenBank™ accession number DC642055). The expression of Glut2 was examined using the following primers: CAC TAT GCT CGT CCT GTG (sense) and TCG GTA GCT GGA AGT GGT G (antisense), which encode nucleotides 591–1215 (GenBank™ accession number XM_001088382). The cycling parameters were as follows: 94 °C 1 min, followed by 94 °C 30 s, then 62 °C 30 s, and 68 °C 1 min 35 cycles.

**Luminal Membrane Vesicle Preparation**—Membrane vesicles were prepared from jejunal or proximal colon mucosa by the Mg2+ aggregation/differential centrifugation technique (37). Mucosa from three mice was pooled for each membrane preparation. Protein content was measured using bovine serum albumin as the protein standard.

**Immunofluorescence Labeling and Immunoblot Analysis**—Immunofluorescence labeling on sections from jejunum was performed as described (38, 39) using Glut5 or Glut2 antibodies in jejunum. For immunoblot analysis, microsomal membranes from the proximal colon were resolved by SDS-PAGE, membranes were blotted with anti-DRA or anti-NHE3 antibodies, and the reactions were visualized using chemiluminescence and captured on light sensitive imaging film (Kodak). Primary antibodies were polyclonal Glut5 antibodies (40), DRA antibodies (28), NHE3 antibodies, Glut2 antibodies (Chemicon International Inc, Temecula, CA) and monoclonal Glut5 antibodies (Alpha Diagnostic, San Antonio, TX).

**Blood Pressure Measurement**—Systolic blood pressure in conscious mice was measured by tail cuff using a BP-2000 (Visitech Systems). Measurements for each mouse represent mean value of three consecutive recordings performed in the last week of experiments.

**Histopathologic Analysis of Intestine**—Microscopic analysis of hematoxylin and eosin staining of sections from duodenum, jejunum, caecum, and proximal and distal colon was performed according to established methods.

**Measurement of Fluid (Salt) Absorption in Jejunum**—Glut5+/+ and Glut5−/− mice were anesthetized by the administration of 10 μl/g intraperitoneal haloperidol/midazolam/fentanyl mixture (haloperidol, 12.5 mg/kg, fentanyl, 0.325 mg/kg, and midazolam, 5 mg/kg body weight). Abdomen was opened, and a 10- to 15-cm segment from jejunum with an intact blood supply was isolated, gently flushed, and then perfused (Perfusor compact, Braun) in vivo, as described from our laboratories (27). The perfusate was collected in a pre-weighed 4.5-ml collecting tube. After a 30-min period, the tube was weighed again, and the difference of the two was taken as the amount of fluid recovered. To examine the effect of luminal fructose, the jejunal segment was perfused with isotonic solution NaCl plus fructose (130 mM NaCl plus 40 mM fructose) or NaCl alone (150 mM NaCl) as control. At the end of the experiments, mice were sacrificed by cervical dislocation, and the length of jejunum was measured.

**Fructose Assay**—Blood fructose concentration was measured according to established methods. Blood samples were immediately frozen in a −20 °C freezer after euthanasia. To eliminate interference from glucose in the samples, 100 μl of sample (plasma or hemolyzed blood) was mixed with 100 μl of saline, and 0.5 unit of glucose oxidase was added in 2 μl (0.86 mg/ml 25
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mm Tris Buffer, pH 8.1). 200 μl of the sample mixture was then transferred to an Eppendorf tube, and 31 μl of 3 N HClO₄ was added, and the tubes were placed in an ice bath for 15–20 min to precipitate proteins. After centrifugation (19,600 × g, 30 min, 4°C), 140 μl of the supernatant was neutralized by adding 27 μl of 2.5 N KHCO₃. Fructose was measured using an enzymatic-fluorometric procedure assaying the last step of fructose metabolism, which involves the conversion of glucose 6-phosphate and NADP to 6-phosphate gluconolactone and NADPH by glucose-6-phosphate dehydrogenase (41). Fructose concentration is determined by the subtraction of glucose prior to isomerization from total glucose at the end of the assay. All enzymes (hexokinase, glucose-6-phosphate dehydrogenase, and phosphoglucone isomerase) were purchased from Roche Applied Science (Indianapolis, IN). Fluorescence was detected using a FL600 fluorometric plate reader from BioTek (Winooski, VT). The assay was based on the reduction of NADP to NADPH, which was detected fluorometrically (excitation: 460 nm, emission: 530 nm). All assays were against standard series of analytical grade fructose.

Histopathological Examination of Intestine—Mice were euthanized, and tissues were harvested within 10 min. Tissues were fixed in Amesterdam solution and then processed and embedded in paraffin blocks using standard methods. Sections were cut and stained using hematoxylin/eosin.

Real-time Quantitative PCR—The real-time PCR was performed in a 7300 Sequence Detector from Applied Biosystems. Primers and probe sets were purchased from Applied Biosystem TaqMan Gene Expression Master Mix (P/N 4369016), and include Glut 2 (Assay ID Mm00446224_m1), Glut 5 (Assay ID Mm00600311_m1), Sglt-1 (Assay ID Mm00451203_m1), and glyceraldehyde-3-phosphate dehydrogenase (Assay ID Mm_99999915_g1) TaqMan Gene Expression Assays. The real-time PCR was carried out in triplet in a final volume of 20 μl containing TaqMan® Universal PCR Master Mix at 10 μl, 20× TaqMan Gene Expression Master Mix at 1 μl, and cDNA diluted in RNase-free water at 9 μl. Samples were heated for 50 °C 2 min, 95 °C 10 min, and then for 40 cycle of 50 s at 95 °C and 60 °C 1 min. Results were presented as cycle threshold. The cycle threshold values of each transporter were individually calibrated to glyceraldehyde-3-phosphate dehydrogenase. The changes in mRNA expression for Glut5, Glut2, and Sglt1 in high fructose or high glucose diet were determined relative to the respective control groups of mice on regular (control) diet after changes in mRNA expression for Glut5, Glut2, and Sglt1 in high fructose diet over the same period relative to the control diet. However, food intake in Glut5 null mice remained stable with constant body weight after 5–7 days on ad libitum high fructose diet relative to control diet. However, Glut5−/− mice experienced significant weight loss on the high fructose diet over the same period relative to the control diet.

RESULTS

Expression of Glut5 in the Intestine and Kidney in Glut5+/+ and Glut5−/− Mice—As shown in Fig. 1a, Glut5 mRNA expression is highly abundant in the small intestine, with lower levels in the kidney in wild-type animals. However, the expression of Glut5 was completely absent in the intestine and kidney of Glut5 null mice. Immunofluorescence labeling with Glut5 antibody demonstrated apical labeling of jejunal villi in wild-type animals but did not detect any labeling in Glut5 null mice (Fig. 1b).

[^14]C]Fructose Uptake in Cultured Cells and in Luminal Membrane Vesicles from Jejunum—The 10-min influx of [^14]C]fructose into transiently transfected COS-7 cells was measured. As shown in Fig. 1c (left panel), Glut5-expressing cells demonstrated a robust [^14]C]fructose uptake relative to sham-transfected cells. Glut5-expressing cells did not transport [^14]C]glucose (Fig. 1c, middle panel). The residual [^14]C]fructose uptake or elevated baseline [^14]C]glucose uptake in sham-transfected COS7 cells could be mediated via endogenous Glut2 but not Glut5, because RT-PCR experiments detected the expression of Glut2 but not Glut5 (data not shown).

To ascertain the role of Glut5 in fructose uptake in the intestine, [^14]C]fructose influx was assayed in luminal membrane vesicles isolated from jejunum. [^14]C]Fructose uptake at 30 s decreased by ~75% in Glut5−/− mice (p < 0.05, n = 4) (Fig. 1d, left panel). The [^14]C]glucose uptake was not significantly altered in luminal membrane vesicles isolated from small intestine of Glut5−/− ko mice (Fig. 1d, right panel).

Role of Glut5 in Dietary Fructose Absorption—To examine the role of Glut5 in fructose absorption in the intestine, Glut5+/+ and Glut5−/− mice were fed either a high fructose (60% fructose) or a normal (60% starch) diet for 5–7 days.

Body Weight—Food intake in Glut5+/+ and Glut5−/− mice on control diet was similar (3.11 ± 0.4 and 3.23 ± 0.6 g/day in Glut5+/+ and Glut5−/− mice, respectively; n = 5, p > 0.05), however, food intake in Glut5−/− mice on the high fructose diet was significantly decreased relative to Glut5+/+ mice (3.43 ± 0.5 g/day in Glut5+/+ mice and 1.56 ± 0.20 g/day in Glut5−/− mice, n = 5, p < 0.01). These measurements represent average daily intake for the first 3 days of the experiments. For accurate comparison, a separate Glut5+/+ mice group was fed at a reduced rate of 1.4 ± 0.15 g of food per day/mouse, which was comparable to the food intake in Glut5−/− mice fed a high fructose diet.

As noted in Fig. 2a (left and right panels), Glut5+/+ mice remained stable with constant body weight after 5–7 days on ad libitum high fructose diet relative to control diet. However, Glut5−/− mice experienced significant weight loss on the high fructose diet over the same period relative to the control diet.
Glut5/H11002/H11002 mice showed 28% reduction in body weight on the high fructose diet (Fig. 2a, left panel). The Glut5/H11001/H11001 mice on restricted control or high fructose diet showed 13 and 15% reduction in their body weight, respectively, over the duration of experiment (Fig. 2a, right panel). These results indicate that Glut5/H11002/H11002 mice on the high fructose diet demonstrate significant weight loss, far and above what can be explained on the basis of reduced food intake.

Fructose Absorption—The concentration of fructose in the blood significantly increased in Glut5/H11001/H11001 mice when fed an ad libitum high fructose diet relative to control diet, with fructose concentration of 44 ± 8 μM/dl in mice on control diet and 175 ± 19 μM/dl in mice on the high fructose diet (p < 0.05, n = 4) (Fig. 2b, left two bars). However, fructose concentration did not increase in Glut5/H11002/H11002 mice on the high fructose diet relative to control diet, with concentrations of 26 ± 5 μM/dl in mice on control diet and 37 ± 6 on the high fructose diet (p > 0.05, n = 4) (Fig. 2b, right two bars). The blood fructose concentration in Glut5/H11001/H11001 mice on restricted control or high fructose intake was 29 ± 4 and 126 ± 10 μM/dl, respectively. These results demonstrate that Glut5/H11002/H11002 is essential for the absorption of fructose in the intestine.
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Glut5 Deletion Causes Malabsorption in Mice Only When Fed a Diet High in Fructose—We next examined the gastrointestinal tract of Glut5−/− mice to determine the nature and mechanism of the malabsorption relative to Glut5+/+ mice.

Gross Morphology of Intestinal Tract—Autopsy revealed that Glut5+/+ and Glut5−/− mice on control diet and Glut5+/+ mice on the high fructose diet had normal looking intestines (Fig. 3a, bottom panels and left top panel). However, to our surprise, Glut5−/− mice on the high fructose diet had extremely distended colon, and its contents appeared to contain both fluid and gas (Fig. 3a, top right panel). All segments of the intestinal tract of Glut5−/− mice on the high fructose diet were enlarged compared with those of Glut5+/+ mice (Fig. 3b). The caecum and proximal colon showed the highest degree of dilatation (Fig. 3, a and b). The cecal contents in Glut5−/− mice were significantly increased (≈10-fold) as compared with Glut5+/+ mice on the high fructose diet (Fig. 3c). The length of small intestine was comparable in Glut5−/− compared with those of Glut5+/+ and Glut5−/− mice (Fig. 3d). Similarly, the area of caecum and length of colon were significantly increased in Glut5−/− mice on the high fructose diet relative to Glut5+/+ mice (Fig. 3d). The length of small intestine and colon and area of caecum were not different in Glut5+/+ mice on ad libitum or restricted high fructose diet (Fig. 3d). The stool in Glut5−/− mice on the high fructose diet was not watery. It is worth mentioning that these dramatic changes occurred in only 5–7 days after the start of high fructose diet.

Regulation of Glut2 and Sglt1 in the Small Intestine by High Fructose or High Glucose Diet in Glut5+/+ and Glut5−/− Mice—Glut5 mRNA expression in the jejunum increased in Glut5+/+ mice fed a diet high in fructose relative to control but remained the same in Glut5−/− mice on control or high fructose diet (Fig. 4a, left panel). The mRNA expression of Glut7 remained low in Glut5−/− mice and was comparable to Glut5+/+ animals on control diet and did not change in Glut5−/− or Glut5+/+ mice on the high fructose diet (data not shown). The expression of Sglt1 increased significantly in the small intestine of Glut5−/− mice on the high fructose diet (Fig. 4a, right panel) but increased mildly in Glut5−/− mice on the high fructose diet relative to their littermates on control diet (Fig. 4a, right panel). Quantitative RT-PCR on RNA samples from experimental groups showed a ~70% and ~140% increase in Glut2 and Sglt1, respectively, in Glut5+/+ mice on the high fructose diet relative to control diet (Fig. 4b, left and right panels). In contrast the expression of Glut2 and Sglt1 increased by only ~10 and 30%, respectively, in Glut5−/− mice on the high fructose diet relative to control diet (Fig. 4b, left and right panels).

To examine the distribution and regulation of Glut2, immunofluorescence labeling was performed in jejunum of Glut5+/+ and Glut5−/− mice on control or high fructose diet. The results are shown in Fig. 4c and demonstrate the absence of Glut2 label-
ing on the apical membrane of jejunal villi in Glut5+/+ mice on control diet (left top panel). Glut2 was only detected on the basolateral membrane in control animals (left top panel). In addition to the basolateral membrane, Glut2 was also detected on the apical membrane of jejunum in Glut5−/− mice fed a diet high in fructose (Fig. 4c, right top panel). In contrast, Glut2 labeling was detected on both the apical and basolateral membrane of jejunum in Glut5−−−/− mice fed a control diet (Fig. 4c, left bottom panel). The apical localization of Glut2 as also evident in Glut5−−−/− mice fed a diet high in fructose (Fig. 4c, right bottom panel).

Contrary to fructose malabsorption, Glut5−−−/− mice displayed normal growth and weight gain relative to Glut5+/+ mice when fed a high glucose (60% glucose) diet for 7 days. Further, there was no intestinal enlargement or caecal dilatation (data not shown). Northern hybridizations demonstrated that the expression of Glut2 and Sglt1 increased significantly and comparably in Glut5+/+ and Glut5−−−/− mice on high glucose diet for 7 days (Fig. 4d, left and right panels).

Quantitative RT-PCR on RNA samples showed ~130 and ~240% increase in Glut2 and Sglt1, respectively, in Glut5+/+.
mice on high glucose diet relative to control diet (Fig. 4e). The expression levels of Glut2 and Sglt1 increased by ~110 and ~190%, respectively, in Glut5−/− mice on high glucose diet relative to control diet (Fig. 4e).

Effect of Luminal Fructose on Salt Absorption and Glut5 Expression in Jejunum and Fructose-induced Hypertension—We next examined the effect of luminal fructose on salt absorption in jejunum. As shown in Fig. 5a, perfusion of
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jejunum with 40 mm fructose in vivo resulted in a significant increase in fluid (salt) absorption in Glut5+/+ mice (p < 0.05, n = 4). However, fructose-stimulated salt absorption was completely absent in Glut5−/− mice (p > 0.05, n = 4). Interestingly, basal fluid absorption seems to be increased in jejunum of Glut5−/− mice.

Glut5+/+ mice fed a diet high in fructose for 14 weeks demonstrated significant up-regulation of Glut5 expression in their jejunum, consistent with increased capacity to absorb fructose (Fig. 5b). Lastly, we show that Glut5+/+ survived and developed systemic hypertension on 14 weeks on the high fructose diet (Fig. 5c). Interestingly and contrary to Glut5+/+ mice, the blood pressure in Glut5−/− mice on the high fructose diet decreased significantly several days after the start of the experiment. This occurred as early as 5 days into the course of fructose feeding (Fig. 5d). Systolic blood pressure in Glut5−/− mice on control diet was 103 ± 2 mm Hg and in Glut5+/+ mice on control or high fructose diet for 5 days was 101 ± 3 or 103 ± 3 mm Hg, respectively (p > 0.05, n = 5 for each group). Glut5−/− mice developed severe hypovolemic shock and died after 7–10 days of increased dietary fructose intake. Blood pressure in other experimental groups was normal (Fig. 5d).

Histological Analysis of Intestine—H and E staining revealed that epithelial cells in the caecum and proximal colon in Glut5−/− mice on the high fructose diet displayed significantly distended mucosal goblet cells in their caecum and proximal colon. The histology in Glut5+/+ mice on the high fructose diet appeared normal. Whether the changes in Glut5−/− mice on the high fructose diet are secondary to excessive bowel fluid transport associated with hyperosmolar loading or unique to Glut5−/− mice on increased dietary fructose intake remain to be determined.

Expression of Ion Transporter in Intestine of Glut5−/− Mice on High Fructose Diet: Adaptive Regulation of Ion Transporters in the Colon in Glut5−/− Mice on Increased Dietary Fructose Intake—Despite the dilated colon and watery luminal content in the caecum and proximal colon in Glut5−/− mice fed a high fructose diet (Fig. 3), stool was reasonably formed, strongly suggesting adaptive regulation of electrolyte-absorbing transporters in the colon. This issue was investigated. Northern hybridization demonstrated that the expression of the apical Na+/H+ exchanger NHE3 (Slc9a3) and the Cl−/HCO3− exchanger DRA (Slc26a3) was significantly increased in the proximal colon of Glut5−/− mice when fed a high fructose diet (Fig. 6a). The expression of NHE3 and DRA in the colon remained unchanged in Glut5+/+ mice on the high fructose diet relative to control diet and was comparable to Glut5−/− mice on control diet (Fig. 6a).

Immunoblot analysis with NHE3 and DRA antibodies on membrane proteins isolated from proximal colon of Glut5−/− and Glut5+/+ mice indicated that the abundance of NHE3 and DRA significantly increased in the proximal colon of Glut5−/− mice fed a high fructose diet relative to Glut5+/+ mice on the same diet (Fig. 6b). The abundance of NHE3- or DRA-specific band, when adjusted for β-actin intensity as loading control, was increased by ~320 and ~210%, respectively, in Glut5−/− mice fed a diet high in fructose (p < 0.05 versus Glut5+/+ on high fructose or control diet and Glut5−/− mice on control diet, n = 4 in each group).

The expression of NHE3 and DRA increased in the distal colon in Glut5−/− mice fed a high fructose diet as compared with Glut5+/+ mice (data not shown). Surprisingly, colonic H-K-ATPase expression did not show any up-regulation in the distal colon of Glut5−/− mice on the high fructose diet relative to control diet (Fig. 6c, left panel). Further, the expression of H-K-ATPase was actually decreased by 42% in Glut5−/− mice relative to Glut5+/+ mice on the normal diet (Fig. 6c, left panel). This is in distinct contrast to the significant up-regulation of colonic H-K-ATPase in the distal colon of DRA (Slc26a3)
knockout mice, which have diarrhea and dilatation of the colon (28) (Fig. 6c, right panel). These results indicate that Glut5−/− mice display distinct adaptive regulation of ion transporters in their colon that is unique to this animal model and different from other reported models of diarrhea or increased delivery of fluid to the large intestine.

**Luminal pH in Caecum and the Kidney Function in Glut5−/− Mice on High Fructose Diet**—The luminal pH in the caecum was similar in both groups, with the pH of 6.4 ± 0.3 in Glut5+/+ and 6.2 ± 0.4 in Glut5−/− mice (p > 0.05), suggesting that the non-absorbed fructose in the small intestine was not metabolized to short-chain fatty acids in the time period examined in the experiments. Glut5−/− mice on the high fructose diet showed signs of volume depletion as evident by a 2.5-fold increase in the concentration of blood urea nitrogen relative to Glut5+/+ mice on the control diet or Glut5+/+ mice on the high fructose or control diet.

**DISCUSSION**

The present studies provide convincing evidence that Glut5 is essential for the absorption of fructose in the intestine (Figs. 1–3). Glut5 null mice displayed ~75% reduction in radiolabeled fructose uptake in luminal membranes from jejunum and showed no increase in blood fructose concentration when fed a diet high in fructose (Fig. 1). Glut5 null mice developed severe malabsorption when fed a high fructose diet, which presented as decreased nutrient absorption in the small intestine, distended caecum, and colon resulting from increased delivery of nutrients to the colon, decreased vascular volume (hypovolemia), hypotension, and eventually shock (Figs. 3 and 5), all hallmarks of profound fructose malabsorption. To the best of our knowledge, this is the first report of nutrient malabsorption in a genetically engineered mouse model.

Examination of the gastrointestinal tract revealed that all seg-
ments of the intestinal tract in Glut5<sup>-/-</sup> mice on the high fructose diet were enlarged compared with those of Glut5<sup>+/+</sup> mice (Fig. 3). The histological examination of the colon supported this conclusion ("Results"). The caecum was massively enlarged in Glut5<sup>-/-</sup> mice on the high fructose diet as shown in Fig. 3, due to increased delivery of unabsorbed fluid from the small intestine. In addition to malabsorption, another contributing factor to the weight loss in Glut5<sup>-/-</sup> mice on the high fructose diet is decreased food intake. However, Glut5<sup>+/+</sup> mice on the restricted high fructose diet did not develop bowel dilatation and did not display as much weight loss as Glut5<sup>-/-</sup> mice (Fig. 2), indicating that the intestinal phenotype in Glut5<sup>-/-</sup> mice on the high fructose diet is not due to reduced food intake. Glut5<sup>-/-</sup> mice exhibited relatively formed stool when fed a high fructose diet and Glut5<sup>+/+</sup> mice on control or high fructose diet and Glut5<sup>-/-</sup> mice on control diet all had formed, solid stool. Glut5<sup>-/-</sup> mice on the high fructose diet displayed vascular volume depletion and reduced kidney function, as measured by blood urea nitrogen concentration in animals on the high fructose diet for 7 days.

The results of the present studies indicate that the role of Glut2 in fructose absorption in the intestine is minimal, at least under the current experimental protocols. This is supported by several lines of evidence: First, the uptake of [14C]fructose in luminal membrane vesicles from jejunum was almost abolished in Glut5<sup>-/-</sup> mice; second, the concentration of fructose in the blood did not increase in Glut5<sup>-/-</sup> mice fed a high fructose diet relative to control diet (Fig. 1). The generation of massive malabsorption in Glut5 null mice fed a diet high in fructose strongly suggests that Glut2 does not compensate for the lack of Glut5 in the intestine, specifically when it relates to the absorption of increased fructose load. This issue deserves further emphasis, because Glut2 was clearly detected on the apical membrane of the intestinal villi in Glut5<sup>-/-</sup> mice (Fig. 4c), therefore discounting any possibility of impaired Glut2<sup>-/-</sup> trafficking as the reason for fructose malabsorption. Our results further demonstrated that, contrary to its essential role in fructose absorption, Glut5 does not play a significant role in glucose absorption in the intestine. This is specifically highlighted by the normal weight gain, lack of caecum, and colon dilatation and appropriate up-regulation of Glut2 and Sglt1 in Glut5<sup>-/-</sup> mice fed a diet high in glucose ("Results").

The reduction in the food intake in Glut5<sup>-/-</sup> mice on the high fructose diet relative to Glut5<sup>+/+</sup> mice ("Results") was evident from the very first day. The reason for the decreased appetite, which was specific only to fructose-containing diet and not to glucose or starch-containing diet, remains speculative. RT-CR and Northern hybridization demonstrated abundant expression of Glut5 in the tongue (data not shown). Whether the ablation of Glut5 affects the taste perception of fructose or its sweetness in the diet remains speculative and warrants investigation.

The adaptive regulation of ion transporters in the colon in Glut5<sup>-/-</sup> mice fed a high fructose diet was distinct from other reported models of diarrhea or increased delivery of fluid to the large intestine. Decreased absorption of fluid in the small intestine and its increased delivery to the colon causes robust adaptive up-regulation of colonic H-K-ATPase and ENaC in NHE3 or DRA null mice (28, 29). However, the expression of colonic H-K-ATPase was actually decreased in the distal colon of Glut5<sup>-/-</sup> mice fed a high fructose diet (Fig. 6c), despite increased delivery of fluid to the colon (Figs. 1–3). The reasons for the differential regulation of H-K-ATPase in the colon of Glut5<sup>-/-</sup> mice versus DRA or NHE3 null mice warrant further investigation.

Metabolic syndrome, which is manifested by visceral obesity, hypertension, glucose intolerance, insulin resistance, and atherogenic dyslipidemia (20–22), is reaching epidemic proportions worldwide, with over 40 million in the United States alone. Its increased incidence correlates with marked increase in the amount of dietary fructose consumption from high fructose corn syrup (30–33). Recent studies demonstrated that fructose stimulates salt absorption in the small intestine and kidney and causes hypertension by up-regulating the expression of the chloride and sodium-transporting transporters PAT1 (Slc26a6) and NHE3 (27). The present studies demonstrate that fructose-stimulated salt absorption is abolished in Glut5<sup>-/-</sup> mice jejenum (Fig. 6), and Glut5<sup>-/-</sup> mice develop hypotension rather than hypertension when fed a diet high in fructose (Figs. 2 and 6). Taken together, these results indicate that Glut5 plays a fundamental role in the pathogenesis of fructose-induced hypertension.

The basis and impact of enhanced salt absorption at basal state and in the absence of fructose in Glut5<sup>-/-</sup> jejunum (Fig. 5) remains speculative, and additional studies are needed to ascertain the significance of this finding.

In conclusion, our studies strongly suggest that Glut5 is essential for the absorption of fructose in the intestine and the generation of fructose-induced hypertension. Our results further indicate that the role of Glut2 in the absorption of fructose in the intestine from fructose-rich diets is minimal. Inhibitors of Glut5 could play an important role in the prevention of hypertension and subsequent kidney damage in fructose-induced hypertension.

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