Fructose-induced increases in expression of intestinal fructolytic and gluconeogenic genes are regulated by GLUT5 and KHK

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THE FRUCTOSE CONCENTRATION in human diets has increased almost 100-fold in the last 200 years (16, 33), and the current high rates of fructose intake are now linked to the development of the metabolic syndrome of insulin resistance, dyslipidemia, hypertension, and obesity (32, 47). Chronic consumption of high-fructose diets increases portal blood fructose concentrations, leading to fatty liver and perturbed liver function, as well as systemic blood fructose levels, leading to pathologies in peripheral organ systems (16). The rate of increase in portal blood fructose is directly dependent on intestinal processing of dietary fructose.

In the small intestine, glucose transporter protein, member 5 (GLUT5) is the primary fructose transporter responsible for the absorption of fructose into the cytosol. Unlike the intestinal glucose transporter sodium-dependent glucose transporter protein 1 (SGLT1), which is sodium dependent, GLUT5 transports fructose (Km = 6–14 mM) across the apical membrane down a chemical gradient (5). Fructose transport in the blood from the cytosol is mediated by GLUT2 (7). Although the liver is the primary organ that metabolizes most of the ingested fructose, the small intestine strongly expresses all fructose-metabolizing enzymes and is responsible for the catabolism of 10–30% of ingested fructose (3, 19, 34). Fructolysis is initiated by ketohexokinase (KHK, fructokinase), which converts fructose and ATP into fructose 1-phosphate and ADP, respectively. KHK has two isoforms, KHK-A and KHK-C. KHK-C has a 10-fold higher affinity for fructose (Km = 0.8 mM) and is mainly responsible for its metabolism (1). Aldolase-B cleaves fructose 1-phosphate into three-carbon intermediates, dihydroxyacetone phosphate and glyceraldehyde, and the latter is then converted by triokinase into glyceraldehyde 3-phosphate, which then joins the glycolysis pathway. Because fructolysis bypasses feedback regulation controlling glycolysis upstream of glyceraldehyde 3-phosphate, fructose catabolism results in the rapid accumulation of several metabolic intermediates (49) that are thought to play a crucial role in the development of metabolic disease. KHK, aldolase-B, and triokinase mediate most of fructolysis (20). Other enzymes do not contribute significantly to fructose metabolism due to their relatively much higher Km for fructose: hexokinase IV or glucokinase (>100 mM), fructose dehydrogenase (5 mM), fructose-3-phosphokinase (30 mM), and sorbitol dehydrogenase (100 mM) (15). The constitutive glycolytic hexokinase I has a Km (∼2 mM) for fructose that is ∼50-fold or more greater than for glucose (20). Thus, a deficiency of KHK and aldolase-B is responsible for major genetic disorders like essential fructosuria and fructose intolerance, respectively (18).

KHK-C is strongly expressed in mouse and rat enterocytes, hepatocytes, and renal proximal tubule cells (15, 50), whereas KHK-A is expressed in many other tissues, but levels are vanishingly low. Like those of GLUT5 and GLUT2 (16), the intestinal- and hepatic-specific activities of KHK, aldolase-B, and triokinase increase with dietary fructose (29). Expression of GLUT5 and other fructose-responsive genes is typically regulated by luminal and not endocrine signals (45).

GLUT5 plays a vital role in regulating the entry of fructose in our body. Deletion of GLUT5 reduces by >80% in vivo intestinal fructose absorption as well as serum fructose con-
Mice (GLUT5 and KHK) were mated in the CMR. Rab11a nonpurified diet (Purina Mills, Richmond, IN). Male and female KO facility of NJMS, under a temperature-controlled room with a temperature (2). Diet-induced specific increases in GLUT5 expression enhance the rate of fructose absorption and involve de novo mRNA and protein synthesis (25, 44). Once GLUT5 synthesis has been upregulated by dietary fructose, trafficking of GLUT5 to the apical membrane is important so transport activity matches the increased luminal fructose concentration. Ras-related protein in brain 11a (Rab11a) is an important GTPase associated with recycling endosomes critical for both endocytic and exocytic protein pathways (57). It is mainly associated with the apical recycling endosome in polarized epithelia (21), and regulates the movement of known brush-border biomarkers dipeptidyl peptidase and alkaline phosphatase to the apical membrane (46). The roles of Rab11a in regulating traffic of sugar transporters to the apical membrane and in trafficking of a transporter to the apical membrane on the regulation of enzymes mediating the catabolism of its substrate have not yet been investigated.

Because intestinal processing of dietary fructose determines portal blood fructose concentrations that, under high fructose conditions perturb liver function as well as fructose and, eventually glucose, homeostasis, we investigated the mechanisms by which dietary fructose regulates the expression of intestinal enzyme systems that metabolize this sugar. Using genetically modified mice, we tested the hypothesis that GLUT5-mediated fructose transport, KHK-mediated fructolysis, and Rab11a-mediated GLUT5 trafficking are each required for fructose to induce expression of enzymes involved in fructolysis and gluconeogenesis. Because sweet taste receptors able to sense luminal sugars and artificial sweeteners have been localized in the small intestine (36), fructose may need not enter the enterocytes for induction to occur. The role of fructose transport in fructolytic and gluconeogenic enzyme regulation was evaluated using GLUT5 knockout (KO) mice. Intracellular fructose by itself and/or its metabolites may induce expression; thus, the role of metabolism was tested using KHK-KO mice. The intracellular distribution of KHK is known for cells in the kidney and liver (15), but not for those in the small intestine; hence, we immunolocalized KHK in enterocytes. The requirement of GLUT5 trafficking to the apical membrane was investigated using enterocyte-specific, Rab11a-KO (Rab11a<sup>Δ</sup>) mice.

MATERIALS AND METHODS

**Mice**

All procedures conducted in this study were approved by the Institutional Animal Care and Use Committee, New Jersey Medical School (NJMS), Rutgers University. Tissues collected from wild-type (WT) and genetically modified [GLUT5<sup>HZ</sup>/H11001, KHK<sup>H</sup> from Richard Johnson, University of Colorado], and WT GLUT5-KO (from Jian Zuo, St. Jude’s Children’s Research Hospital), lial cells, since global Rab11a deletion is fatal in utero (57).

Intracellular fructose by itself and/or its metabolites may induce expression; thus, the role of metabolism was tested using KHK-KO mice. The intracellular distribution of KHK is known for cells in the kidney and liver (15), but not for those in the small intestine; hence, we immunolocalized KHK in enterocytes. The requirement of GLUT5 trafficking to the apical membrane was investigated using enterocyte-specific, Rab11a-KO (Rab11a<sup>Δ</sup>) mice.

**Role of metabolism and intracellular trafficking in the regulation of fructolytic and gluconeogenic genes**

In study I, 4- to 5-week-old WT, GLUT5-HZ, and GLUT5-KO mice were randomly divided in three groups and then gavaged, under light anesthesia, with 30% lysine (nonsugar control), glucose (sugar control), or fructose (2 ml/100 g body wt, ~0.3 ml/mouse) solution two times a day for 2.5 days (n = 6/group). After gavage feeding, mice were observed for a few minutes for any abnormal behavior and then immediately returned to the cages where they resumed normal activity and ad libitum feeding of chow. Body weight and food intake were measured daily. Mice were killed 4 h after the last (5th) gavage in the morning of the 3rd day. The proximal part (6–8 cm) of the small intestine was scraped and snapped frozen in liquid nitrogen for future analyses by real-time PCR and Western blots.

**Study II** was comprised of three experiments. In the first, the same protocol as in the first study was followed, except that WT, KHK-HZ, and KHK-KO mice were used. In the second experiment, 4- to 5-week-old WT mice (n = 6/group) were anesthetized and then gavaged with 15% glucose, fructose, or glyceraldehyde solution (2 ml/100 g) as previously described. A concentration of 15% was selected, since preliminary work showed that 30% glyceraldehyde was not tolerated well. The third experiment was the same as the second, except that KHK-KO mice were used because these are expected to have less endogenous glyceraldehyde and therefore are potentially more sensitive to glyceraldehyde feeding.

In study III, Rab11a<sup>Δ</sup> and WT (18-day-old) littermates were each randomly separated into two groups (n = 4/group) and gavaged with either 30% glucose or fructose solutions as previously described.

**Real-Time PCR**

Total RNA was extracted from the small intestinal mucosa of mouse using TRIzol reagent (Invitrogen, Carlsbad, CA). An RNasey mini kit (Qiagen, Valencia, CA) was used for purification of the RNA. The cDNA was generated using 5 μg of purified RNA by RT-PCR using a thermal cycler (Cycler, Bio-Rad, Hercules, CA) with a Super Script III Reverse Transcriptase kit (Invitrogen). Real-time PCR using Mx3000P (Stratagene, La Jolla, CA) was used to analyze cDNA as described in our earlier work (50). The Roche primer design software (http://www.roche-applied-science.com) was used to design various primers that were purchased from Integrated DNA Technologies (IDT, Coralville, IA). Elongation factor 1α (EFlα) was used as a housekeeping gene to correct for potential variability in loading. Previously published primer sequences for EFlα and KHK are listed in Tharabenjasin et al. (50). Primers for the following genes are as follows (forward, reverse, annealing temperature): aldolase-B: 5′-ATAGGAGACCCCCCATTTCT-3′, 5′-CTTCCAGGCTGTTATCAG-3′, 56°C; fructose-1,6-bisphosphatase (F-1,6-P<sub>2</sub>): 5′-TATACCCTGCCCAACAAACAA-3′, 5′-AACTATGGGTGTTGACTA-3′, 56°C; glucose-6-phosphatase (G-6-Pase): 5′-GGTACTATTTCCCATAGG-3′, 5′-ATCCGGAAGAACCAAACAG-3′, 54°C; hepxokinase I: 5′-ATGGTTGTGCCACTCCAGAC-3′, 5′-GACCCCGAAGTTGTTG-3′, 54°C.
RESULTS

Food Consumption and Body Weight

All mice in our experiments had ad libitum access to normal diet during the 2.5 days of gavage feeding of various substrates. Food intake and body weights were thus normal and virtually the same in all mice, except for those in study III (Table 1). Growth of adult mice was minimal over 3 days. Deletion of Rab11a from the intestinal cells had profound effects on growth. Mean initial and final body weights were different (P < 0.04). This was expected since the phenotype of Rab11aKO mice is characterized by running and slower growth compared with WT mice (57). Feeding rate was not measured in study III, since mice were returned to their dams after gavage feeding.

Effect of GLUT5 Deletion on Fructose-Induced Expression of Fructolytic and Gluconeogenic Genes

Deletion of GLUT5 abolished (0.02 ± 0.20) facilitated fructose transport compared (P < 0.0001) with that in WT mice (1.9 ± 0.2 nmol·mg⁻¹·min⁻¹). Mean relative GLUT5

Table 1. Body weights and food consumption rates of mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Gavage Treatment</th>
<th>Initial Body Wt, g</th>
<th>Final Body Wt, g</th>
<th>Food Consumed, g/day</th>
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<tr>
<td>WT</td>
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<td>4.9 ± 0.3</td>
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<td>21.6 ± 0.9</td>
<td>4.9 ± 0.1</td>
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<td>5.1 ± 0.2</td>
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<tr>
<td>GLUT5-KO</td>
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<td>21.4 ± 0.2</td>
<td>4.9 ± 0.3</td>
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<td></td>
<td>Glucose</td>
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<td></td>
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<tr>
<td></td>
<td>Fructose</td>
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<td></td>
<td>5.0 ± 0.1</td>
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<tr>
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<tr>
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<td>4.7 ± 0.1</td>
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<td></td>
<td>Glyceraldehyde</td>
<td></td>
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<td>5.0 ± 0.4</td>
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<tr>
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<td>11 ± 0.3</td>
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<td>11 ± 0.2</td>
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<td>6.4 ± 0.2</td>
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<tr>
<td></td>
<td>Fructose</td>
<td></td>
<td>6.5 ± 0.4</td>
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Values are means ± SE. WT, wild type; GLUT5, glucose transporter protein, member 5; HET, heterozygous; KO, knockout; KHK, ketokexokinase.
mRNA level in WT was 1.0 ± 0.2, and there was none in the KO mice. The abrogation of fructose transport affected the response of fructolytic and gluconeogenic genes. By two-way ANOVA, the mRNA expression of KHK, aldolase-B, and triokinase varied with fructose feeding (P ≤ 0.04 for all three genes) and GLUT5 deletion (P ≤ 0.03) (Fig. 1, A, B, and C). There was also a significant interaction between fructose feeding and GLUT5 deletion (P ≤ 0.04), implying that the fructose effect depended on the genotype. In WT mice, fructose feeding increased KHK expression by 3.5-fold and aldolase-B as well as triokinase by two-fold (P ≤ 0.005 for all three enzymes). This fructose-induced increase in mRNA expression of genes specific to fructose metabolism was clearly prevented in GLUT5-HZ and GLUT5-KO mice. The mRNA expression of KHK, aldolase-B, and triokinase was similar in the small intestine of WT, GLUT5-HZ, and GLUT5-KO mice when gavaged with either lysine or glucose (P ≥ 0.2 in all cases), suggesting that baseline mRNA levels of these genes were independent of genotype, and did not compensate for GLUT5 ablation. Changes in the protein expression of KHK correlated with corresponding mRNA levels (Fig. 1A).

Fructose feeding (P ≤ 0.001) and GLUT5 deletion (P ≤ 0.02) affected the mRNA expression of the gluconeogenic genes F-1,6-P2 and G-6-Pase (Pinteraction < 0.01). Fructose increased in WT, but not in GLUT5-HZ and GLUT5-KO, mice the mRNA expression of F-1,6-P2 and G-6-Pase by more than threefold (Fig. 2, A and B). There was no significant difference in levels of these enzymes in GLUT5-KO and WT mice fed lysine and glucose (P ≥ 0.6 for both enzymes), suggesting that GLUT5 deletion did not affect constitutive mRNA expression of gluconeogenic genes. The finding that GLUT5 deletion abolishes the ability of dietary fructose to regulate fructolytic and representative gluconeogenic genes suggests that fructose transport is likely required for fructose-induced regulation. The effect of fructose on these genes is specific, since it has no effect on intestinal hexokinase I expression. By two-way ANOVA, hexokinase expression did not change with genotype (P = 0.30) and with diet (P = 0.50), and there was no interaction (P = 0.90). Mean hexokinase mRNA expression for the GLUT5-KO experiment was as follows: WT mice

Fig. 1. Effect of glucose transporter protein, member 5 (GLUT5) deletion on fructose-induced expression of fructolytic enzymes. The effect of fructose feeding and GLUT5 deletion on expression of various genes involved in fructolysis was evaluated by determining levels of mRNA from the jejunum of wild-type (WT), GLUT5 heterozygous (HZ), or GLUT5 knockout (KO) mice gavaged with 30% lysine, glucose, or fructose, two times a day at 0.3 ml/mouse. Otherwise, all mice had ad libitum access to chow. Jejunal mRNA expression of ketohexokinase (KHK) (A), aldolase-B (B), and triokinase (C). KHK protein expression was analyzed by Western blot (n = 2, A shows a representative blot) with β-actin as reference. Results were normalized to those in WT mice gavaged with lysine. Bars are means ± SE (n = 4–6), and those with different superscript letters are statistically different (P < 0.05) from each other as analyzed by 1-way ANOVA followed by least-significant difference (LSD) analysis. Bars that share the same superscript letter are the same. Detailed statistical analyses are described in the text. Upregulation of fructolytic enzymes requires transapical fructose transport mediated by GLUT5 in WT mice.

Fig. 2. Effect of GLUT5 deletion on fructose-induced expression of representative genes in gluconeogenesis. Jejunal mucosa were obtained from WT, GLUT5-HZ, or GLUT5-KO mice gavaged with lysine, glucose, or fructose. Levels of mRNA of fructose-1,6-bisphosphatase (F-1,6-P2, A) and glucose-6-phosphatase (G-6-Pase, B). Bars, normalization, and analysis are described in Fig. 1. Upregulation of genes in gluconeogenesis requires GLUT5-mediated fructose uptake.
Expression of KHK, aldolase-B, and triokinase (Fig. 3, tose-KO/H11005) markedly affected the mRNA expression of F-1,6-P2 (Fig. 4A). These treatments also affected G-6-Pase expression (P < 0.001 and 0.02, respectively, Fig. 4B). The effect of fructose feeding on F-1,6-P2, but not G-6-Pase, mRNA expression was genotype dependent (Pinteraction < 0.005). In WT but not in KHK-KO mice, fructose feeding increased by four- to fivefold the mRNA expression of F-1,6-P2 and G-6-Pase; thus, upregulation of these gluconeogenic genes is dependent on KHK-mediated metabolism. There was no significant difference in mRNA expression of F-1,6-P2 and G-6-Pase in WT, KHK-HZ, and KHK-KO mice gavaged with either lysine or glucose (P ≥ 0.4 in all cases for both enzymes), suggesting that deletion of KHK did not affect their basal mRNA expression.

These fructose-induced increases in expression are specific to fructolytic and gluconeogenic genes because levels of hexokinase I did not change with genotype (P = 0.20) and with diet (P = 0.09), with no interaction (P = 0.30). Mean hexokinase mRNA for the KHK-KO experiment was as follows: lysine-WT = 1.0 ± 0.2, lysine-HZ = 1.1 ± 0.3, lysine-KO = 1.3 ± 0.4, glucose-WT = 0.8 ± 0.3, glucose-HZ = 0.6 ± 0.3, glucose-KO = 1.1 ± 0.2, fructose-WT = 0.5 ± 0.1, fructose-HZ = 0.8 ± 0.3, and fructose-KO = 0.6 ± 0.2.

Effect of Glyceraldehyde Feeding on Fructose-Induced Gene Expression

WT mice. Fructose feeding (P ≤ 0.04 for all three genes, by one-way ANOVA) increased the mRNA expression of KHK, aldolase-B, and triokinase, but glucose or glyceraldehyde gavaged with lysine (lysine-WT) = 1.0 ± 0.1, lysine-HZ = 1.3 ± 0.4, lysine-KO = 1.0 ± 0.1, glucose-WT = 0.7 ± 0.2, glucose-HZ = 1.0 ± 0.2, glucose-KO = 1.0 ± 0.1, fructose-WT = 0.7 ± 0.1, fructose-HZ = 1.0 ± 0.2, and fructose-KO = 0.7 ± 0.2.

Effect of KHK Deletion on Fructose-Induced Gene Expression

Fructose feeding (P < 0.0001–0.01) and KHK deletion (P < 0.0001–0.007, by 2-way ANOVA) significantly affected the mRNA expression of KHK, aldolase-B, and triokinase (Fig. 3, A, B, and C). Significant interactions suggested that genotype modulated the effect of fructose on expression of these three genes (Pinteraction < 0.003–0.010). In WT mice, fructose feeding increased by four- to sevenfold mRNA expression of KHK, aldolase-B, and triokinase. Protein expression of KHK corresponded with its mRNA levels. As expected, no KHK transcript was detected in KHK-KO mice while KHK-HZ mice had ~35% less mRNA than WT. Fructose-induced increases in aldolase-B and triokinase were clearly prevented by KHK ablation, which did not affect the basal mRNA expression of these genes (P ≥ 0.9 in all cases) in lysine- and glucose-fed mice. There were no compen-
feeding did not (Fig. 5, A, B, and C). The reason for the modest effect of fructose feeding in this experiment relative to those in previous experiments was probably the reduced fructose dose (15% compared with the previously used 30%). Fructose also increased the protein expression of KHK. The mRNA expression of gluconeogenic genes F-1,6-P2 and G-6-Pase in mice fed glucose and glyceraldehyde was also less compared with those fed fructose (P < 0.05 in all cases, Fig. 6, A and B). These results clearly suggest that glyceraldehyde, despite being a fructose-specific metabolite, failed to induce the expression of fructolytic and gluconeogenic genes in WT mice. The mRNA expression of aldolase-B and triokinase as well as F-1,6-P2 and G-6-Pase (data not shown) was each independent of diet (P > 0.05 in all cases), indicating that glyceraldehyde could not induce fructose-responsive genes in the KHK-KO mice. These results also confirmed previous findings that fructose metabolism is required for fructose to induce various genes involved in fructolysis as well as in gluconeogenesis.

**Intracellular Location of KHK**

We have previously shown that KHK distribution tended to be homogeneous among enterocytes along the crypt-villus axis (50). In renal proximal tubules, KHK expression was greater in the medulla than in the cortex (38). Although villus location had no effect on its expression, KHK seems to be concentrated...
in the basolateral region of the cytosol, below the basal, elongated nuclei of enterocytes (Fig. 10, A–C) facing the basolateral membrane. At higher magnification, the greater concentration of KHK in the basal region is observed in cells near the villus tip as well as in the mid and lower villus regions (Fig. 10, D–F).

DISCUSSION

GLUT5 Is Required for Induction of Fructolytic and Gluconeogenic Genes

Signals from T1R2/T1R3 receptors in the apical mucosal membrane that sense but do not absorb luminal sugars in mammalian intestine (36, 56) upregulate SGLT1 and may also directly stimulate expression of fructolytic and gluconeogenic genes as part of an adaptation to a sweeter diet. Because fructose-induced increases in the expression of these genes are abrogated in GLUT5-KO mice, potential signaling from these sweet receptors is either insufficient or unrelated to fructose metabolism. In contrast, transport via GLUT5 is essential for fructose-induced upregulation of intestinal fructolysis and gluconeogenesis, indicating that contributions of other intestinal GLUTs that can potentially transport fructose [GLUTs 2, 7, 8, and 12 (6, 14, 27)] are also insufficient. In the rat brain, fructose dose dependently upregulated the expression of GLUT2 and of GLUT5 transporters, leading to increases in KHK levels and in pyruvate as well as ATP production (54).

Fructose Metabolism Is Required for Induction

Although fructose is now a significant constituent of diets worldwide, regulation of intestinal fructolytic enzymes by its substrate has only been studied by Crouzoulon and Korieh (9) who showed that adaptive increases in KHK, aldolase-B, and triokinase activities last for several days even after fructose is removed from the diet. Chronic consumption of high-fructose diets increases KHK expression in rat kidney (8) as well as in the liver of female rats (51). In Caco-2 cells, a glucose-dependent G-6-Pase mRNA increase is strictly regulated by glucose metabolism (30). Here we show that fructose must be metabolized by KHK for upregulation to occur. It is not clear whether further catabolism of the KHK product fructose 1-phosphate by aldolase-B is required, because one of the products of aldolase-B is glyceraldehyde, which failed to upregulate expression. It is possible that this failure is due to exogenous glyceraldehyde being gavaged in insufficient quantities or not being taken up by intestinal cells, although others
have shown it can be transported by a saturable, competitively inhibited electrogenic and Na\(^+\)/H\(^+\)-dependent transporter in the pancreas (13). The second aldolase-B product, dihydroxyacetone phosphate, and the triokinase product, glyceraldehyde 3-phosphate, cannot be the signals because both are glycolytic intermediates and thus cannot mediate a fructose-specific response. If indeed glyceraldehyde is not the inducer, then a genetic model with aldolase-B deletion will provide a clearer conclusion and indicate that, if aldolase-B is not required, then the signal is likely a reduction in ATP levels or an increase in ADP as well as reduce ATP-to-ADP ratios (19, 50). The intracellular location of KHK suggests that fructose may be metabolized mainly near the basolateral pole.

AMP-activated protein kinase (AMPK) kinase and AMPK are highly expressed in the small intestine (23) along with KHK and may mediate the effect of fructose, since this sugar reduces ATP-to-AMP ratios in a KHK-dependent manner (50), and AMPK is activated by reduced ATP-to-AMP ratios. Intestinal AMPK activation by 5-aminoimidazole-4-carboxamide ribonucleotide increases GLUT5 expression in the small intestine of perfused rats (Patel and Ferraris, unpublished observations). Moreover, fructose-induced activation of hepatic AMPK requires KHK (52). It is interesting to note that fructose modulation of AMPK may be confounded by gender. The expression of KHK and the activity of G-6-Pase were significantly enhanced via AMPK phosphorylation in the liver of female adult rats (52) and in the female offspring from fructose-fed dams (37). However, AMPK has also been shown to repress hepatic gluconeogenesis (31).

Because its catabolism, unlike that of glucose, to glycolytic intermediates is not subject to feedback inhibition, fructose is highly lipogenic and uricemic (4, 26). Our findings support the hypothesis that the biochemical basis of its pathological effects is the absence of feedback inhibition because excessive fructose feeding does not inhibit, but rather stimulates, fructolytic gene expression. Fructose feeding is known to increase cytosolic levels of three-carbon glycolytic intermediates (49) that can then be used for gluconeogenesis and acetyl-CoA, with entry in the tricarboxylic acid cycle or use in lipid synthesis. It

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**Fig. 8.** Expression of fructolytic genes in Rab11\textsubscript{aIEC} mice. Expression of KHK (A), aldolase-B (B), and triokinase (C) was analyzed by real-time PCR in weaning WT and Rab11\textsubscript{aIEC} mice gavaged with 30% glucose or fructose two times a day (0.1 ml/mouse) for 2.5 days. Mice were returned to dams after feeding. Results were normalized to WT mice gavaged with glucose. Bars are means ± SE (n = 4), and those with different superscript letters are statistically different (P < 0.05) from one another as analyzed by 1-way ANOVA. Rab11\textsubscript{a}-mediated trafficking of GLUT5 to the apical membrane is required for fructose-induced regulation of genes involved in fructose metabolism.

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**Fig. 9.** Expression of gluconeogenic genes in Rab11\textsubscript{aIEC} mice. Expression of F-1,6-P\(_2\) (A) and G-6-Pase (B) in the intestinal mucosa. Bars, normalization, and analysis are described in Fig. 8. Rab11\textsubscript{a}-mediated trafficking of GLUT5 to the apical membrane is required for fructose-induced regulation of genes involved in gluconeogenesis.
is likely that increased levels of these intermediates stimulate the gluconeogenic pathway because expression of the critical enzymes F-1,6-P₂ and G-6-Pase are also dramatically stimulated by dietary fructose. Fructose feeding increases blood concentrations of the triglyceride precursor glycerol (22). We did not assess intestinal expression of genes in lipogenesis, which occurs mainly in hepatic and adipose tissues.

Although the small intestine in adult mammals is thought to have a low capacity for gluconeogenic activity (41), recent studies (reviewed in Ref. 35) suggest that intestinal gluconeogenesis may contribute up to 25% of total endogenous glucose production during fasting, thereby, via the periportal neural system, modulating hunger sensations and whole body glucose homeostasis, especially between meals. Even though intestinal gluconeogenic activity is controversial, the expression levels of F-1,6-P₂ and G-6-Pase mRNA in our intestinal samples, while modest, are nevertheless significant (relatively lower than those of fructolytic genes but greater than that of hexokinase) and definitely regulated as indicated by sensitivity to luminal fructose and as we have consistently observed in previous work (11). This rapid response to luminal substrate concentration implies physiological significance. In fact, we found that chronic fructose feeding may cause KHK-dependent increases in portal glucose levels (40). Fructose also stimulates G-6-Pase and F-1,6-P₂ expression in the liver of rats (28, 53).

Similar links between substrate metabolism and enzyme regulation have not been found for the intestine but have been demonstrated in the liver, pancreas, and kidney. Regulation of hepatic and pancreatic GLUT2 expression by glucose depends on glucose metabolism (17, 42), whereas that of renal glutaminase and glutamate dehydrogenase depends on glutamine metabolism (12, 39). Thus, in several tissues and for several enzyme systems, upregulation requires metabolism of their substrates.

It is possible that the absence of KHK (as well as GLUT5) in nonintestinal tissues may result in changes in those tissues that can nonspecifically alter fructose-inducible expression of fructolytic and gluconeogenic genes in the small intestine. This possibility is remote, however, because KHK-KO and GLUT5-KO mice were also gavage fed the control solutions lysine and glucose, which would account for these nonspecific effects.

Role of Endosomal Trafficking

There has not been any study on the role of endosomal trafficking on the regulation of cytosolic enzymes. Rab8a, Rab11, and Rab13 regulate endosomal trafficking and mediate the insertion of GLUT4 to the surface membrane of skeletal muscle in an insulin-dependent manner (48, 58). These Rabs can also regulate the sorting of membrane proteins in polarized epithelia. Thus, Rab8 mediates the anterograde trafficking of the proton-dependent peptide transporter PEPT1 and of SGLT1 from the cytosol to the intestinal apical membrane (43) while Rab11a mediates trafficking of apical proteins to the intestinal membrane (46). With reduced Rab11a-mediated GLUT5 trafficking to the apical membrane and, consequently, decreased fructose transport and metabolism in the cytosol, expression of fructolytic and gluconeogenic genes does not increase even when dietary fructose levels increase. Thus, findings in this experiment support and supplement those observed using GLUT5- and KHK-KO mice. Rab11a ablation may also reduce expression of gluconeogenic genes in glucose-gavaged Rab11a-KOIEC relative to WT mice because Rab8 is mislocalized in Rab11a-KO mice (46), reducing SGLT1-mediated glucose uptake.

Perspectives and Significance

Fructose uptake and metabolism by enterocytes thus contribute significantly to intestinal processing of this sugar, and...
may influence portal fructose and glucose concentrations. After high levels of dietary fructose are consumed, fructose enters the enterocyte via GLUT5, and some are metabolized by KHK in the cytosol while the remainder exits to the blood via GLUT2. The KHK-mediated step likely represents the signal that increases fructose-induced expression of fructolytic and gluconeogenic enzymes. The signal is specific, since it does not affect hexokinase I expression, and is likely dose dependent because the magnitude of fructose-induced increases is greater with 30 than 15% fructose, and because a reduction in fructose perfusion of 75% reduces fructose-induced increases is greater with 30 than 15% fructose, and because a reduction in fructose perfusion of 75% reduces expression and/or for valuable discussion. We thank Drs. R. Buddington, R. Briant, and CA-178599 to N. Gao.

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REFERENCES


DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


