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Cell-Type–Specific, Ketohexokinase-Dependent Induction by Fructose of Lipogenic Gene Expression in Mouse Small Intestine

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ABSTRACT

Background: High intakes of fructose are associated with metabolic diseases, including hypertriglyceridemia and intestinal tumor growth. Although small intestinal epithelia consist of many different cell types, express lipogenic genes, and convert dietary fructose to fatty acids, there is no information on the identity of the cell type(s) mediating this conversion and on the effects of fructose on lipogenic gene expression.

Objectives: We hypothesized that fructose regulates the intestinal expression of genes involved in lipid and apolipoprotein synthesis, that regulation depends on the fructose transporter solute carrier family 2 member a5 [*Slc2a5* (glucose transporter 5)] and on ketohexokinase (*Khk*), and that regulation occurs only in enterocytes.

Methods: We compared lipogenic gene expression among different organs from wild-type adult male C57BL mice consuming a standard vivarium nonpurified diet. We then gavaged twice daily for 2.5 d fructose or glucose solutions (15%, 0.3 mL per mouse) into wild-type, *Slc2a5*-knockout (KO), and *Khk*-KO mice with free access to the nonpurified diet and determined expression of representative lipogenic genes. Finally, from mice fed the nonpurified diet, we made organoids highly enriched in enterocyte, goblet, Paneth, or stem cells and then incubated them overnight in 10 mM fructose or glucose.

Results: Most lipogenic genes were significantly expressed in the intestine relative to the kidney, liver, lung, and skeletal muscle. In vivo expression of *Srebf1*, *Acaca*, *Fasn*, *Scd1*, *Dgat1*, *Gk*, *Apoa4*, and *Apob* mRNA and of *Scd1* protein increased ($P < 0.05$) by 3- to 20-fold in wild-type, but not in *Slc2a5*-KO and *Khk*-KO, mice gavaged with fructose. In vitro, *Slc2a5*- and *Khk*-dependent, fructose-induced increases, which ranged from 1.5- to 4-fold ($P < 0.05$), in mRNA concentrations of all these genes were observed only in organoids enriched in enterocytes.

Conclusions: Fructose specifically stimulates expression of mouse small intestinal genes for lipid and apolipoprotein synthesis. Secretory and stem cells seem incapable of transport- and metabolism-dependent lipogenesis, occurring only in absorptive enterocytes. *J Nutr* 2020;00:1–9.

Keywords: chylomicron, epithelia, lipogenesis, lipids, liver, organoids, stem cells, sugars

Introduction

Increased fructose intake leads to portal vein and systemic fructosemia associated with nonalcoholic fatty liver and other metabolic diseases now at epidemic levels in the United States (1, 2). The small intestine plays a vital role in protecting the liver from fructose toxicity, as luminal fructose in modest concentrations can be metabolized intracellularly by intestinal cells into glucose and other metabolites, reducing the amount of fructose reaching the portal vein and liver (3, 4). After absorption, some fructose is converted by the small intestine to lipids, a process that then increases intestinal lipid secretion into the blood. In humans, labeled fructose in a mixed meal resulted in labeled palmitate incorporated in circulating chylomicrons, indicating small intestinal de novo lipogenesis from fructose (5–7). Dietary fructose also stimulated production of human intestinal triglycerides as well as apolipoprotein B48 (the structural protein for chylomicrons, APOB48, an RNA-edited product of the *Apob* gene) involved in the synthesis of chylomicrons (5, 8). Fructose feeding of hamsters for 3 wk increased not only enterocyte (ENT) incorporation of [^{3}H]acetate into fatty acids but also secretion of intestinal APOB48 and triglycerides (9). Fructose-fed, nonobese, adenomatous polyposis coli knockout (KO) mice (a model of intestinal tumorigenesis) not only converted fructose to but also increased synthesis of fatty acids that supported tumor growth (10). Because fructose increases the amounts of some inflammatory cytokines that alter the Wnt pathway, it can disrupt epithelial differentiation, leading to precancerous ectopic formation in the

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villi of ENTs inappropriately expressing stem cell markers (11). Thus, fructose-induced intestinal lipogenesis may contribute to metabolic diseases and also to the development of intestinal and colon cancer.

Because absorptive ENTs constitute ~70–80% of all small intestinal cells, studies attribute these fructose-induced responses to be solely due to this cell type (12). The adult small intestinal epithelium, however, is composed of intestinal stem cells (ISCs) that continuously proliferate and differentiate into absorptive ENTs as well as secretory enteroendocrine, Paneth (PAN), goblet (GOB), and other cells with unknown capacities for lipid synthesis and secretion.

Fructose is transported across the apical membrane and down its chemical gradient into the cytosol via the intestinal fructose transporter glucose transporter 5 [GLUT5, encoded by solute carrier family 2 member a5 (*Slc2a5*)] (13, 14). Fructose is then rapidly metabolized by ketohexokinase (KHK, encoded by *Khk*) and aldolase into 3-carbon intermediates that can be converted to acetyl-CoA acting as precursors for the synthesis of triglycerides (15) in the intestinal cell. Some fructose is transported out of the intestine by GLUT2 (encoded by *Slc2a2*) into the hepatic portal vein and to the liver, where fructose is more lipogenic than glucose because it regulates the expression levels of sterol regulatory element-binding transcription factor 1 (*Srebf1*) that modulates expression of downstream lipogenic genes (16, 17) and because it is catabolized faster than glucose (18), thereby rapidly providing precursor carbons for fatty acid synthesis (19). Hepatic and intestinal lipogenesis begins with acetyl-CoA carboxylase 1 (encoded by *Acaca*) converting acetyl-CoA into malonyl-CoA, which is utilized by fatty acid synthase (encoded by *Fasn*) to produce palmitic acid. Palmitic and other longer chain SFAs can be desaturated by stearoyl-CoA desaturase 1 (SCD1, encoded by *Scd1*). During triglyceride synthesis, fatty acids are attached to monoacylglycerol, via monoacylglycerol acyltransferases, to make diacylglycerol and then, via diacylglycerol acyltransferases (DGAT1, encoded by *Dgat1*), to triglycerides. In the small intestine, DGAT1 is the isoform that synthesizes postprandial triglycerides (20), which are then packaged into chylomicrons coated with intestine-specific apolipoproteins APOB48 (encoded by *Apob*) and APOA4 (encoded by *Apoa4*) and other apolipoproteins. Chylomicrons, which are made only in intestinal cells, are then exocytosed to the lymph circulation. The proportion of chylomicrons from fructose-induced lipogenesis relative to total chylomicrons is likely related to dietary fructose concentrations.

Although many previous studies have demonstrated intestinal lipogenesis from fructose, no studies have examined the fructose-induced expression of genes involved in de novo lipogenesis and chylomicron formation (1, 17). Thus, we focused on documenting gene expression complementing dynamic metabolic data and hypothesized that fructose regulates in vivo the small intestinal expression of representative genes involved in lipogenesis as well as in apolipoprotein synthesis and also that regulation is dependent on GLUT5-mediated absorption and on KHK-mediated metabolism. We also noted that the identity of the cell type(s) capable of fructose-induced lipogenesis is unknown. Hence, we hypothesized that fructose regulation of lipogenic genes occurs specifically in absorptive ENT and not in secretory and stem cells. We tested this hypothesis by exposing intestinal organoids enriched in specific cell types to fructose at concentrations previously shown to upregulate fructolytic genes in vitro (12, 21, 22).

Materials and Methods

Animals

All procedures conducted in this study were approved by the Institutional Animal Care and Use Committee, New Jersey Medical School, Rutgers University. Tissues were harvested from adult, wild-type (WT; C57BL/6; Taconic Laboratories), *Slc2a5*-KO, or *Khk*-KO [both *Khk* isoforms A and B were ablated globally (1)] male mice. Mice were maintained under controlled temperatures (24°C) with a 12-h light–dark cycle and had free access to water and food (LabDiet Rodent 5001; Purina Mills).

Experimental design

Expression of lipogenic genes in different tissues.

Samples from the liver, lung, skeletal muscle, small intestine (mucosal scrapes), and kidney were harvested from 8-wk-old WT male mice [$n = 5$, except kidney ($n = 4$)] and then immediately frozen and stored at −80°C for future analyses of gene expression.

Effect of fructose metabolism and absorption on in vivo expression of lipogenic genes in the gut.

The experimental design and groups used in the in vivo study were previously described (23). Briefly, WT, *Slc2a5*-KO, and *Khk*-KO male, 5-wk-old mice were each randomly divided into 2 groups with access to food and water. They were then lightly anesthetized prior to gavage with 15% fructose (2 mL/100 g body weight, which is ~0.3 mL per mouse) or glucose (control) solutions; a "naïve" control was not evaluated because expression of all fructolytic enzymes required for synthesis of precursors of fructose-derived lipids was similar between glucose- and lysine-perfused intestines (1). Gavage feeding occurred twice a day for 2.5 d ($n = 4$–5), and mice were killed after a single gavage on the third day. The dose is roughly similar to humans drinking 3–4 cans of regular soda each day for 2.5 d. After gavage feeding, mice were returned to their cages each time, and they continued to have access to water and food (LabDiet Rodent 5001: 23.9% crude protein, 5.0% fat, 5.1% crude fiber, 48.7% nitrogen-free extract, and 7.0% ash; Purina Mills). During the 2.5 d, mice exhibited normal growth and body weight for their age. Initial body weights, final body weights, and feeding rates were not affected by diet or genotype

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Supplemental Tables 1–3 and Supplemental Figures 1–6 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://academic.oup.com/jn/.
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Abbreviations used: CCM, crypt culture medium; Cq, quantification cycle; DGAT1, diacylglycerol acyltransferase 1; ENT, enterocyte; *Gk*, glycerol kinase; GLUT, glucose transporter; GOB, goblet cell; *G6pc*, gene encoding for glucose-6-phosphatase catalytic subunit; ISC, intestinal stem cell; KHK, ketohexokinase; KO, knockout; PAN, Paneth cell; SCD1, stearoyl-CoA desaturase 1; *Si*, gene encoding for sucrase isomaltase; *Slc*, solute carrier family; TYP, typical; WT, wild type.

(initial body weight for all mice: 20.3 ± 0.1 g; final body weight: 21.0 ± 0.3 g; feeding rate: 4.8 ± 0.1 g mouse^{-1} d^{-1}). After the mice were killed, 6–8 cm of the proximal small intestine was harvested, the mucosa was scraped ice-cold, and scrapes were stored at −80°C.

Effect of fructose on expression of lipogenic genes in organoids enriched in different cell types.

Generation of intestinal organoids. Intestinal crypts were isolated from the proximal intestine of WT, *Slc2a5*-KO, and *Khk*-KO male, 5- to 8-wk-old mice fed a commercial, nonpurified rodent diet. Crypts were then cultured as previously described (12, 21) using a crypt culture medium (CCM) described in **Supplemental Table 1**.

Directed differentiation of intestinal stem cells. After 2–3 d in CCM, intestinal crypts grew into typical (TYP) organoids. Organoids cultured throughout in CCM (without Wnt or Notch inhibitors or activators) are referred to as TYP organoids because these have all cell types represented in roughly the same proportion as that found in vivo. On the third day, organoids were directed either to remain mostly undifferentiated and become enriched in stem cells or to differentiate and become enriched mostly in ENT, GOB, or PAN cells. Directed differentiation was achieved by activating and/or inhibiting Wnt and Notch signaling pathways (12, 21) (**Supplemental Table 2**). Our previous studies showed that directed differentiation resulted in organoids composed mostly of ENT (~90% enrichment), GOB (80%), PAN (65%), or ISC (90%) cells.

Identifying the intestinal cell type involved in lipogenesis. After 2–3 d in differentiation media when enrichment in specific cell types was confirmed by biomarkers, organoids were treated with 10 mM fructose or glucose (control) for 24 h (12). We previously showed that rats fed high-fructose (65%) pellets had luminal concentrations of 26 mM (24); hence, 10 mM is physiological when mice are fed fructose at lower concentrations. Organoids were then harvested and stored at −80°C for subsequent analysis of mRNA expression.

Real-Time qPCR

Total RNA was extracted from tissues or organoids using TRIzol (Invitrogen) and then purified with an RNeasy micro kit (Qiagen) as previously described (12). After analyses of several typical housekeeping genes (**Supplemental Figure 1**), 18S rRNA was selected to normalize expression among liver, lung, intestine, skeletal muscle, and kidney, and β-actin was used for intestinal cell types. Primer sequences (Integrated DNA Technologies) are listed in **Supplemental Table 3**.

Western blot analysis

Briefly, after perfusion, the intestinal mucosa was immediately scraped from 10 cm of jejunum, snap-frozen in liquid nitrogen, and then stored at −80°C [for details, see Cui et al. (25)]. The mucosa was subsequently placed in 20 mL of ice-cold mannitol/Tris buffer (300 mM mannitol, 1 mM Tris·HCl, pH 7.4) and homogenized with a Polytron homogenizer (setting 9) for 30 s. Total protein [40 μg (Bio-Rad Laboratories)] from homogenate samples was mixed with 2 × Laemmli sample buffer (Sigma-Aldrich) and run on a 10% SDS-PAGE using a Mini-PROTEAN II cell (Bio-Rad Laboratories) for 2 h. The proteins were transferred onto a nitrocellulose membrane (Amersham Biosciences) for 1 h at room temperature using the model 6000 Electroblotter (E&K Scientific Products). Membranes were probed with primary antibody against SCD1 (1:1000; Sigma Aldrich), and all membranes were subsequently stripped and reprobed with primary antibody against the housekeeping protein β-actin (1:1000; Millipore).

Statistical analyses

Data are presented as means ± SEs. Differences of expression among different tissues and among different organoid types were analyzed with 1-way ANOVA. A multiple post hoc test (Tukey's) was done to determine significant differences between means. The effect of fructose on expression for each tissue or organoid was also analyzed by 1-way ANOVA. Differences were considered significant at $P \leq 0.05$ (GraphPad Prism 8; GraphPad Software).

Results

Expression of lipogenic genes in the gut relative to those in other organ systems

Relative expression levels of *Srebf1*, normalized to those in the small intestine, were generally similar among the various tissues except for skeletal muscle, which expressed approximately half of those in other organs (**Figure 1**). *Acaca* was also relatively similarly expressed among different organ systems, with renal, lung, and liver expression being 3- to 5-fold greater relative to the intestine and muscle. Both *Fasn* and *Scd1* were expressed in the liver 2- to 500-fold greater than in all other tissues. Although modest relative to that in the liver, absolute expression in the gut of *Fasn* and *Scd1* was still plentiful because an estimate of RNA quantity, the quantification cycle (Cq), was ~24 for both genes, whereas housekeeping gene Cqs were ~12–18 (Supplemental Figure 1), and those of very-low-abundance genes were ~32–35. Expression of *Dgat1* and *Apoa4* was at least 10-fold greater in the small intestine compared with all other tissues. *Apob* expression in the liver and small intestine was 4- to 20-fold greater than that in lung, skeletal muscle, and kidney. Expression levels of *Gk* were >5-fold in the kidney compared with other tissues, although modest amounts were expressed in the small intestine. As expected for gluconeogenic tissues, *G6pc* expression was >20-fold in the liver and kidney compared with other tissues (Figure 1). *Mlxipl* expression was 3- to 30-fold greater in the liver compared with other tissues. Thus, in the small intestine, genes involved in fatty acid synthesis were expressed modestly, whereas those involved in triglyceride synthesis and chylomicron packaging were expressed abundantly. For comparison, expression of genes (*Slc5a1* and *Slc2a5*) coding for sugar transporters was generally abundant in the small intestine compared with all other tissues (**Supplemental Figure 2**A1, A2).

Fructose-induced increases in lipogenic gene expression depend on fructose transport and metabolism

In WT mice, fructose increased by 3- to 20-fold the small intestinal expression of all representative genes (*Srebf1*, *Acaca*, *Fasn*, *Scd1*, and *Dgat1*) involved in lipogenesis and triglyceride synthesis (**Figure 2**A–E). Deletion of *Slc2a5* and of *Khk* each abrogated the fructose effect in all these genes. Compared with WT mice, deletion of *Khk* reduced by >10-fold the expression of *Acaca* and *Fasn*. The pattern of expression of *Apoa4*, *Apob*, and *Gk* (Figure 2F and G) was similar in that fructose

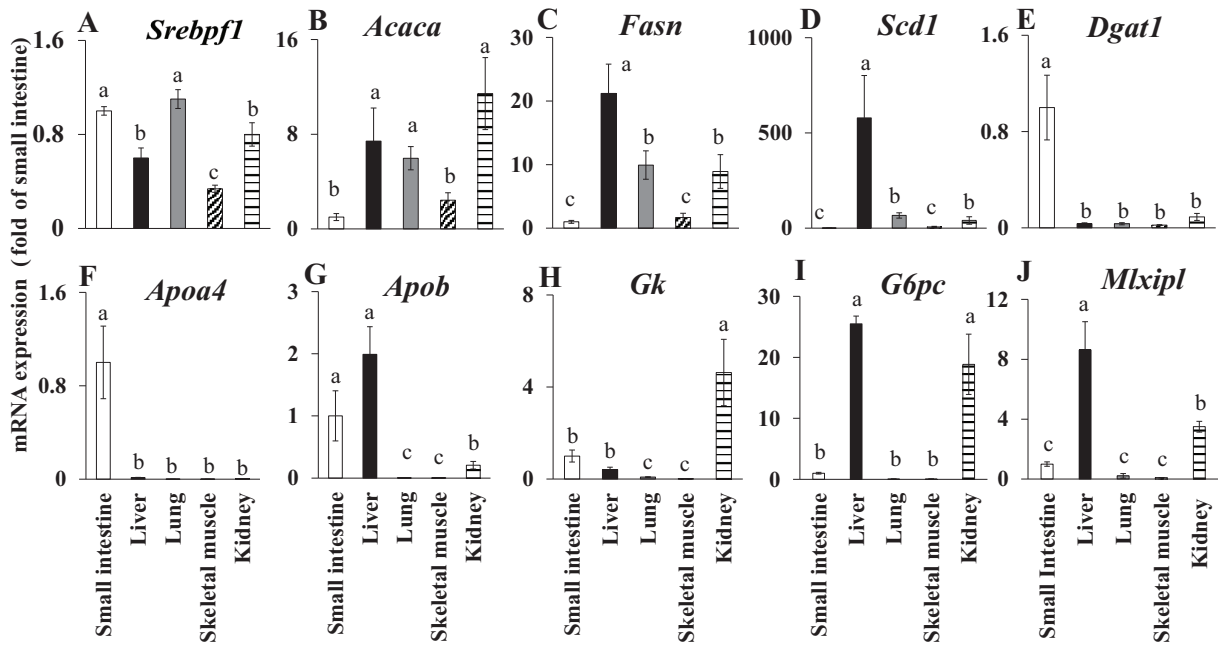

FIGURE 1 Expression of representative lipogenic genes in different organ systems. Tissues from the small intestine, liver, lung, skeletal muscle, and kidney were obtained from WT mice fed a nonpurified diet. mRNA concentrations of genes involved in lipogenesis and apolipoprotein synthesis as well as of *G6pc*, *Slc5a1*, *Mlxipl*, and *Slc2a5* mRNA were normalized to those in the small intestine (= 1.0). Results are means ± SEMs, $n = 4$–5. Different superscripts indicate significantly different means, where superscripts a > b > c. $P \leq 0.05$, as determined by 1-way ANOVA. *Rn18s* was used as housekeeping reference gene (Supplemental Figure 1). *Acaca*, acetyl-CoA carboxylase 1; *Apoa4*, apolipoprotein A-4; *Apob*, apolipoprotein B; *Dgat1*, diacylglycerol acyltransferases; *Fasn*, fatty acid synthase; *Gk*, glycerol kinase; *G6pc*, glucose-6-phosphatase catalytic subunit; *Mlxipl*, MLX-interacting protein-like (also known as carbohydrate response element-binding protein); *Scd1*, stearoyl-CoA desaturase 1; *Srebf1*, sterol regulatory element-binding transcription factor 1; WT, wild type.

upregulated by 3- to 15-fold their mRNA concentrations in WT, but there was no fructose upregulation in *Slc2a5*-KO and *Khk*-KO mice. In fact, *Gk* expression increased almost 3-fold with glucose in *Slc2a5*-KO mice compared with glucose in WT.

As in previous work (1), fructose increased by 3.5-fold *G6pc* expression in WT but not in *Slc2a5*-KO and *Khk*-KO mice (Figure 2I). Expression of *Mlxipl*, which regulates glycolysis, did not increase with fructose feeding and was 2- to 5-fold greater in the glucose-fed *Slc2a5*-KO and *Khk*-KO

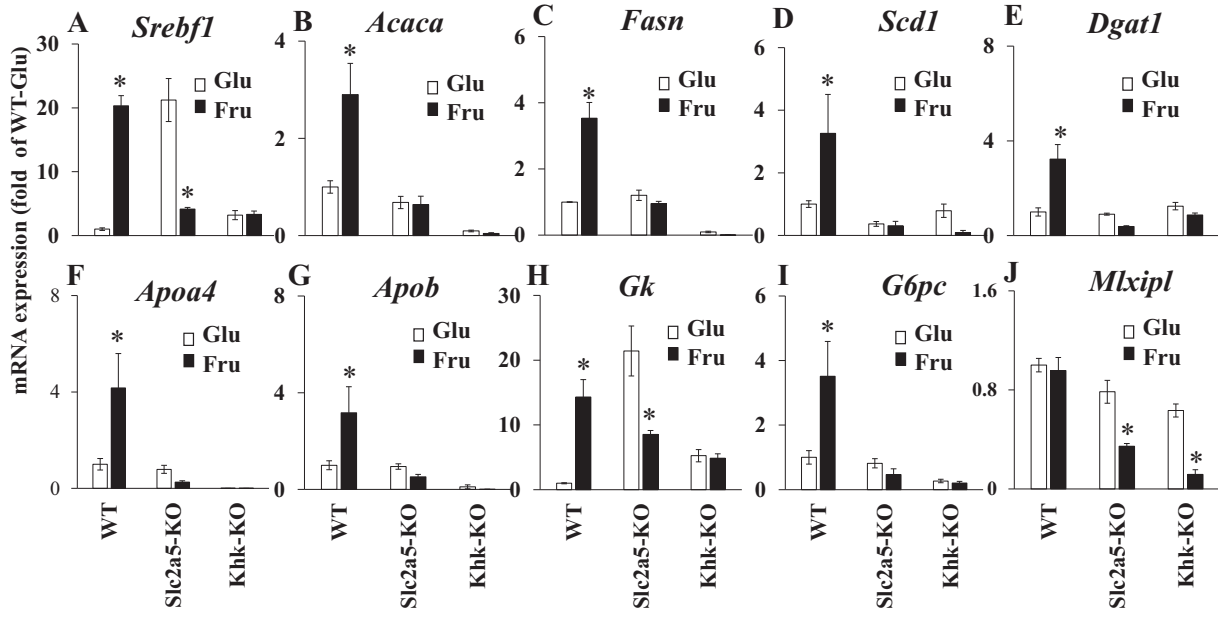

FIGURE 2 Fructose increased expression of lipogenic genes in gavage-fed WT mice. Jejunal mucosa were obtained from WT, *Slc2a5*-KO, and *Khk*-KO mice gavaged with Fru or Glu (control) solutions twice a day for 2.5 d. Expression was normalized to those of Glu-fed WT mice (= 1.0). Results are means ± SEMs, $n = 4$–5. ∗Different from Glu, $P < 0.05$. Fru, fructose; Glu, glucose; KO, knockout; WT, wild type.

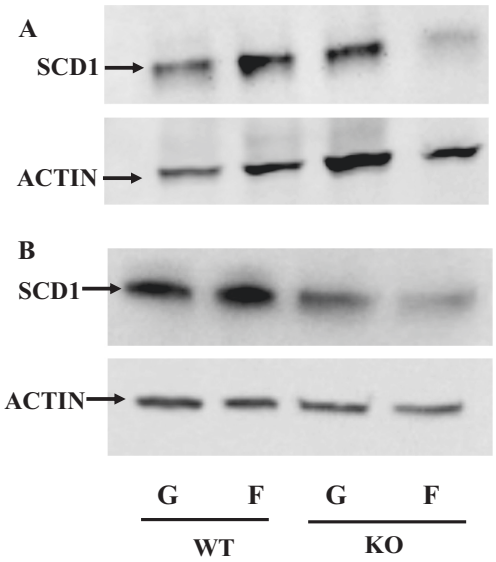

FIGURE 3 Fructose increased SCD1 protein concentrations in gavage-fed WT but not in *Slc2a5*-KO and *Khk*-KO mice. (A) Protein expression levels by Western blot of SCD1 in intestinal mucosa from WT and *Slc2a5*-KO mice gavage fed with fructose or glucose (control) solutions twice a day for 2.5 d. (B) Protein expression levels of SCD1 in WT and *Khk*-KO mice. A duplicate Western blot depicted similar results. F, fructose; G, glucose; KO, knockout; WT, wild type.

mice (Figure 2J). Expression of the nonlipogenic, nonfructolytic gene *Slc5a1* was not affected by fructose feeding, suggesting that the fructose effect on lipogenic and apolipoprotein genes is specific (Supplemental Figure 2B1, B2). *Slc2a5* expression increased with fructose in WT but not in *Khk*-KO mice, as previously shown (23).

Compared with those gavaged with glucose, protein concentrations of SCD1 increased in WT mice gavaged with fructose (**Figure 3**), a finding similar to that found for mRNA concentrations. On the other hand, deletion of *Slc2a5* (Figure 3A) and *Khk* (Figure 3B) abolished the effects of fructose on SCD1, suggesting that the fructose-induced increase in expression required both GLUT5-mediated fructose transport and KHK-mediated metabolism.

Directed differentiation of organoids enriched in specific cell types

The mRNA concentrations of distinct biomarkers confirmed the differentiation of stem cells into specific cell types and were compared with those of TYP (control, contains all cell types) organoids (**Figure 4**A–D). Expression of leucine-rich repeat-containing G protein-coupled receptor-5 (*Lgr5*, a stem cell biomarker) was 4- to 50-fold greater in organoids directed to consist mostly of stem cells. Sucrase isomaltase (*Si*, a biomarker of ENTs) expression confirmed the enrichment of ENTs in organoids directed to differentiate primarily to this cell type. *Si* expression was also high in TYP organoids (~0.3-fold compared with ENT), which also contained many ENTs. Expression of mucin 2 (*Muc2*, biomarker of GOB) was almost 300-fold greater in organoids directed to differentiate into GOB compared with TYP and to other organoids. Expression of the Paneth cell biomarker lysozyme (*Lyz*) confirmed the enrichment of PAN in organoids directed to differentiate to this cell type (~10-fold compared with that in TYP). The distinct morphology of each organoid enriched in different cell types (Figure 4E) was similar to that previously shown (22). Thus, the organoids were each enriched in a specific cell type as directed.

Fructose-induced expression of lipogenic genes occurs only in enterocytes in WT mice

Because fructose stimulated the mRNA expression levels of lipogenic genes in vivo in WT mice, we next investigated whether fructose stimulation of lipogenic genes was specific to one or more cell types. Typical organoids contain cell types at proportions similar to those in vivo; thus, they contain mainly ENTs and respond similarly to ENT-enriched organoids (12, 22).

With the exception of *Acaca*, which increased in expression with fructose incubation in 3 cell types, the fructose-induced upregulation of mRNA expression of lipogenic genes was observed only in typical and in ENT organoids (**Figure 5**A–E). This suggested that the effect of fructose on gene expression depended on cell type. Fructose incubation increased expression of lipogenic genes by 1.5- to 5-fold in TYP and by 2- to 4-fold in ENT organoids. It is unclear why fructose increased *Acaca* expression in several cell types, but this gene, which is expressed in human intestinal epithelial cells, is regulated transcriptionally by multiple promoters that modulate *Acaca* mRNA abundance as a general response to nutritional status (17).

The pattern of expression of *Apoa4*, *Apob*, and *Gk* (Figure 5F–H) was similar to that of lipogenic genes in that fructose increased expression by 2- to 4-fold only in TYP and ENT organoids. Confirming previous work, fructose increased *G6pc* expression in virtually all cell types except ISC. Fructose had no effect on *Mlxipl* expression (Figure 5J). In summary, expression of most genes involved in lipogenesis and apolipoprotein synthesis increased with fructose incubation primarily in ENT and TYP organoids because ENT cells also comprised most of the cells that constituted TYP organoids. The fructose effect on lipogenic genes was specific for ENTs because expression of *Slc2a5* increased with fructose in all differentiated cell types and also because *Slc5a1* expression did not change with fructose in any cell type (Supplemental Figure 2C1, C2).

Slc2a5 deletion prevents the majority of fructose effects in enterocytes

Because fructose effects on expression of enzymes involved in lipogenesis and apolipoprotein synthesis were observed only in ENT, we evaluated the role of *Slc2a5* deletion on fructose effects only in ENT and ISC (as control) organoids. The enrichment of these organoids in ENT and ISC biomarkers is shown in **Supplemental Figure 3**. Fructose effects on expression of most, but not all, genes involved in lipogenesis and lipoprotein synthesis were prevented by the deletion of *Slc2a5* (**Supplemental Figure 4**). Fructose-induced increases in *Srebf1*, *Fasn*, *Scd1*, *Gk*, and *Apob* were abolished in ENTs of *Slc2a5*-KO mice. In contrast to these genes, mRNA concentrations of *Acaca*, *Dgat1*, and *Apoa4* increased modestly with fructose. Fructose increased *G6pc* expression in *Slc2a5*-KO mice—a finding similar to that of our previous study (12). *Mlxipl* (Supplemental Figure 4) and *Slc5a1* (not shown) expression was not significantly affected by fructose. *Slc5a1* is expressed mainly in differentiated cells and is sometimes used as a biomarker for differentiation (12).

Khk deletion abolishes fructose effects

The enrichment of these ENT and ISC organoids in ENT and ISC, respectively, biomarkers is shown in **Supplemental**

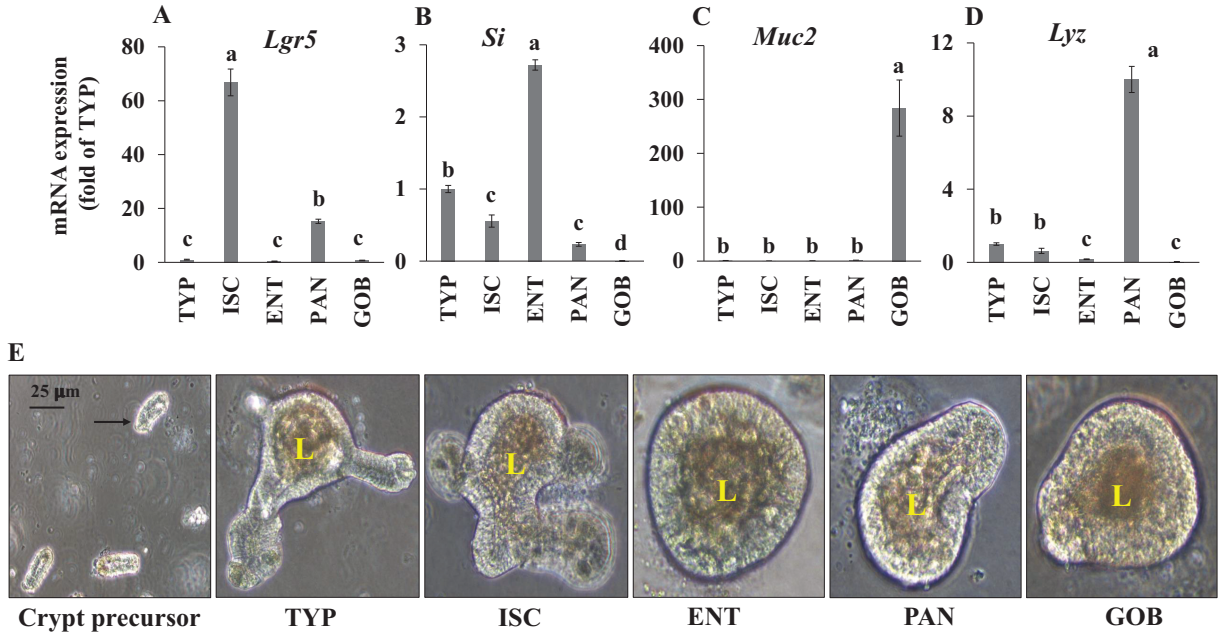

FIGURE 4 Expression levels of biomarkers of different cell types and brightfield images of organoids. Expression of biomarkers (A. *Lgr5*, B. *Si*, C. *Muc2*, D. *Lyz*) was normalized to that in TYP organoids (= 1.0). Results are means ± SEMs, $n = 3$–6. Different superscripts indicate significantly different means, where superscripts a > b > c. $P \leq 0.05$, as determined by 1-way ANOVA. (E) Representative brightfield images (left to right) of crypts (arrow) harvested from mucosa; TYP organoids; as well as organoids enriched in ISCs, ENTs, and PAN and GOB cells. Images are shown at 20× magnification. ENT, enterocyte; GOB, goblet; ISC, intestinal stem cell; L, lumen; PAN, Paneth; TYP, typical.

Figure 5. Whereas deletion of *Slc2a5* abolished fructose effects in most, but not all, lipogenic genes, deletion of *Khk* abolished fructose effects in all genes involved in lipogenesis and lipoprotein synthesis (**Supplemental Figure 6**). Expression of *G6pc*, *Mlxipl*, and *Slc5a1* genes not directly involved in lipogenesis was not significantly affected by fructose in these mice.

Discussion

The main findings in our study are that *1*) fructose induces the expression of almost all genes involved in lipid and apolipoprotein synthesis in the small intestine, *2*) these effects are entirely dependent on fructose metabolism, and *3*) fructose-induced expression is limited to differentiated ENTs.

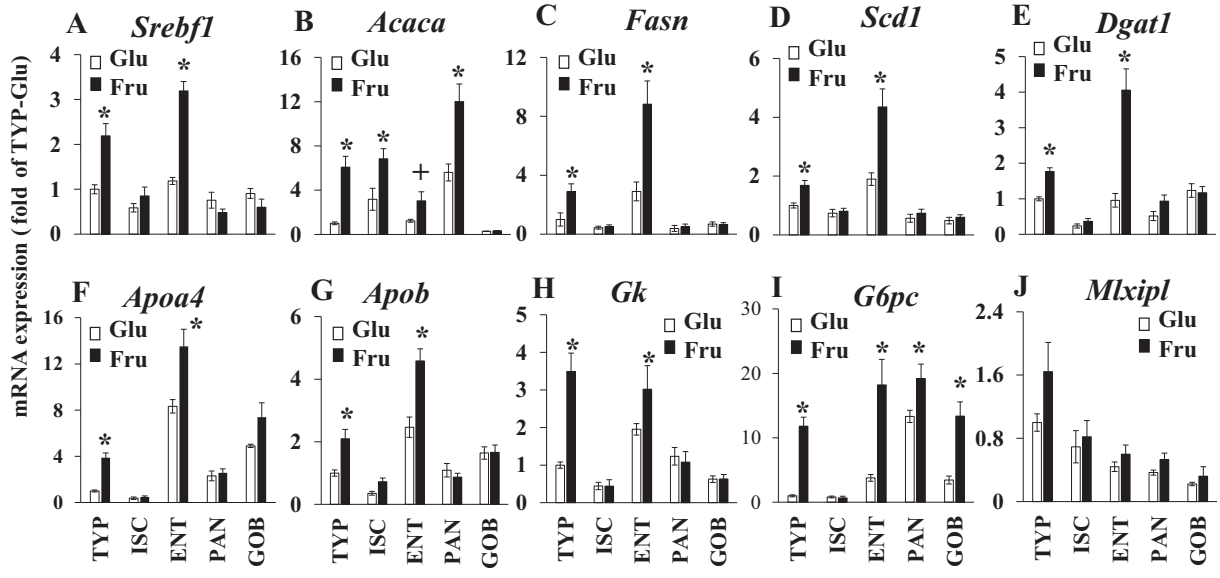

FIGURE 5 Fructose increased expression of lipogenic genes only in enterocytes. Each organoid was treated with 10 mM Glu (control) or 10 mM Fru for 24 h. Concentrations of mRNA in all organoids were normalized to those in TYP organoids treated with 10 mM Glu (= 1.0). Results are means ± SEMs, $n = 3$–6. ∗Different from Glu, $P < 0.05$; $^{+}P = 0.06$. ENT, enterocyte; Fru, fructose; Glu, glucose; GOB, goblet; ISC, intestinal stem cell; PAN, Paneth; TYP, typical.

Baseline expression of lipogenic and lipoprotein genes in the small intestine

Although the liver and adipose tissues are known sites of lipogenesis, no studies have directly compared expression of genes involved in lipid and apolipoprotein synthesis among other organ systems. These genes were found expressed in the rodent and human kidney (26, 27), lungs (28, 29), small intestine (30–32), and even skeletal muscle (33), but there were no comparisons within the same study. Our findings indicate that in mice fed a commercial rodent diet, the baseline, unstimulated expression of almost all genes involved in triglyceride and apolipoprotein synthesis is modest to abundant in the small intestine compared with other organ systems.

Dietary fructose upregulates expression of lipogenic genes in vivo

Increased intestinal mRNA expression of lipogenic and lipoprotein genes likely precedes the intestinal conversion, in humans and animal models, of fructose to lipids as well as to lipid precursors (3, 5–9). It also likely precedes the marked increases in blood concentrations of triglyceride-rich, intestine-specific APOB48 following fructose intake (8, 9), although APOB48 concentrations are also affected by clearance rates in vivo (34). It is possible that the already substantial fructose-induced increase in mRNA concentrations of lipogenic genes after 2.5 d may not have reached a final, stable point and could have increased further. Fructose also increases hepatic expression of genes involved in lipogenesis (4, 16, 35–39). In the liver, increased expression induced by fructose of genes involved in lipid and apolipoprotein synthesis has been shown to precede increased hepatic lipid production (16, 38–40). Fructose stimulates the expression of these lipogenic genes in rat liver via the transcription factor(s) sterol regulatory element-binding transcription factor 1c (SREBP-1c) and/or carbohydrate response element-binding protein (ChREBP) encoded by *Mlxipl* (16, 36, 40) and also by other transcription factors (41, 42). Hyperinsulinemia arising from chronic consumption of high-fructose diets may confound our findings on increases in intestinal *Srebf1* expression that is regulated by insulin (17). Acute fructose intakes in vivo, however, either have no effect on or decrease postprandial insulin concentrations (43); thus, our findings may not be compromised by perturbations in insulin concentrations.

GLUT5-mediated transport from the lumen and KHK-mediated metabolism have been shown to be essential in fructose-induced changes in fructolytic enzyme expression in the liver, small intestine, and kidney in vivo (1, 23, 35, 37). For dietary fructose to regulate gene expression in vivo, ingested fructose must be absorbed by its transporter GLUT5 and not merely "tasted" by intestinal sweet receptors that sense the nutrient composition of luminal contents (44). The fructose-induced increases in lipogenic and apolipoprotein gene expression require fructose to be metabolized to fructose-1-phosphate. Thus, deletion of *Khk* can prevent fructose-induced increases in intestinal lipogenesis. Likewise, *Khk*-KO mice are protected from fructose-induced hepatic steatosis and insulin resistance (39, 45).

The cell type mediating fructose-induced upregulation of lipogenic genes

Although dietary fructose upregulates the in vivo expression of lipogenic and apolipoprotein genes, an unresolved question in our understanding of gut adaptation is whether this fructose-induced upregulation is characteristic of all, or only of a subset of, epithelial cell types lining the intestinal crypts and villi. Because absorptive ENTs make up ~75–80% of small intestinal cells in vivo, ENTs are the likely drivers of fructose-regulated lipogenic genes; however, it remains unclear *1*) whether other cell types that make up the intestinal epithelium express lipogenic genes and *2*) whether the lipogenic genes in those cells are responsive to fructose. A similar question has been asked regarding sugar-processing genes such as *Si*, *Slc2a5*, and *Khk* that in vivo are upregulated by dietary fructose, resulting in increased absorption and portal blood concentrations of fructose (1, 46). Using a similar specialized organoid culture method, we then demonstrated that in all cell types, except stem cells, fructose upregulates expression of these genes. Although differentiation from stem cells is adequate for fructose-induced upregulation of fructolytic genes (12), further specialized differentiation into absorptive ENTs seems to be required for fructose-induced upregulation of genes involved in lipid and apolipoprotein synthesis. Because organoids from nondiabetic mice were incubated with fructose and were not exposed to changes in insulin concentrations, fructose effects on intestinal *Srebf1* and other lipogenic genes may be independent of insulin. The liver is also highly responsive to dietary fructose concentrations, but it is not clear which of the 4 major hepatic cell types (hepatocytes, hepatic stellate cells, Kupffer cells, or liver sinusoidal endothelial cells) is or are involved in adapting to fructose.

Although *Slc2a5* deletion resulted in a global in vivo inhibition of fructose-induced upregulation of lipogenic genes in gavage-fed mice, it did not inhibit fructose-induced in vitro upregulation of lipogenic genes *Acaca*, *Dgat1*, and *Apoa4* in organoids. This is because *Slc2a5* is apical and mediates the transport of dietary fructose from the lumen into the cell, so without *Slc2a5*, fructose cannot be absorbed in vivo. Because organoids develop with the lumen inside, organoid incubation with fructose exposes only the basolateral side, where the basolateral fructose transporter GLUT2 can mediate the transport of fructose from the medium into the cell. It is possible that fructose in the organoid media absorbed by basolateral GLUT2 was sufficient to upregulate *Acaca*, *Dgat1*, and *Apoa4* in ENT organoids. Expression in organoids of *Srebf1*, *Fasn*, *Scd1*, *Gk*, and *Apob* did not increase with fructose because these genes may require *Slc2a5* not as a transporter but, rather, as a transceptor sensing intracellular fructose and affecting intracellular signals regulating expression of these genes (13). In our previous organoid work, several fructolytic genes were also thought to depend on *Slc2a5* as a transceptor and not as a transporter (12).

These directed organoids contain other cell types that could potentially confound our findings. However, each organoid type is more highly enriched in a specific cell compared to in situ populations in the small intestine, providing us an experimental approach able to estimate mRNA expression in different cell types—an objective that would be difficult to pursue in vivo and even in vitro because isolated intestinal cells have extremely short life spans (~1 h) unless cultured in CCM and Matrigel into organoids (22).

Conclusions

Our study extends previous findings of small intestinal de novo lipogenesis from dietary fructose. Enhanced expression of genes involved in this process that takes place only in enterocytes requires fructose metabolism, suggesting that fructose metabolites are likely precursors of lipids incorporated in chylomicrons after fructose feeding (5–7).

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