ChREBP-Knockout Mice Show Sucrose Intolerance and Fructose Malabsorption

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Abstract: We have previously reported that 60% sucrose diet-fed ChREBP knockout mice (KO) showed body weight loss resulting in lethality. We aimed to elucidate whether sucrose and fructose metabolism are impaired in KO. Wild type mice (WT) and KO were fed a diet containing 30% sucrose with/without 0.08% miglitol, an α-glucosidase inhibitor, and these effects on phenotypes were tested. Furthermore, we compared metabolic changes of oral and peritoneal fructose injection. Thirty percent sucrose diet feeding did not affect phenotypes in KO. However, miglitol induced lethality in 30% sucrose-fed KO. Thirty percent sucrose plus miglitol diet-fed KO showed increased cecal contents, increased fecal lactate contents, increased growth of lactobacillales and Bifidobacterium and decreased growth of clostridium cluster XIVa. ChREBP gene deletion suppressed the mRNA levels of sucrose and fructose related genes. Next, oral fructose injection did not affect plasma glucose levels and liver fructose contents; however, intestinal sucrose and fructose related mRNA levels were increased only in WT. In contrast, peritoneal fructose injection increased plasma glucose levels in both mice; however, the hepatic fructose content in KO was much higher owing to decreased hepatic Khk mRNA expression. Taken together, KO showed sucrose intolerance and fructose malabsorption owing to decreased gene expression.

Keywords: carbohydrate-responsive element-binding protein; ketohexokinase; fructose; glucose transporter 5; glucose transporter 2

1. Introduction

Excess intake of high sucrose and fructose diet were thought to be associated with the development of obesity, metabolic syndrome and diabetes [1,2]. Many experimental animal studies, for example, experiments feeding 70% fructose-containing water, supported this hypothesis [2]. However, recent human epidemic data suggest that there is little association between metabolic syndrome and consumption of sucrose and fructose [3,4].

Moreover, the mechanism of sucrose and fructose metabolism remains unclear. Sucrose is a disaccharide composed of glucose and fructose, and is digested by intestinal sucrase-isomaltase (SI), which is inhibited by miglitol, an α-glucosidase inhibitor [5]. Fructose is more potent and has higher capacity of protein glycation than glucose, and thus is more harmful than glucose [6]. Fructose is metabolized in the intestine and liver. Previously, it has been considered that large amounts of fructose are metabolized mainly in the liver [7]. However, portal fructose levels are 10
times lower and plasma fructose levels are 100 times lower than plasma glucose levels [8,9].
Moreover, excess intake of fructose can cause dietary fructose malabsorption and thereby irritable bowel syndrome [10]. Taken together, we hypothesized that intestinal, but not hepatic, fructose absorption regulates portal and plasma fructose levels [11].

To clarify the intestinal sucrose and fructose metabolism, we focused on the phenotypes of high-sucrose diet-fed carbohydrate-responsive element-binding protein (ChREBP)-knockout (KO) mice [12]. ChREBP is a glucose-activated transcription factor that regulates glucose and lipid metabolism. We have formerly reported that high-sucrose diet-fed KO mice showed body weight loss and eventually lethality, although high-glucose diet- and high-starch diet-fed KO mice did not [12]. As SI is induced by sucrose, we wondered whether SI expression is decreased in KO mice [13]. Moreover, high-fructose diet-fed KO mice showed similar phenotypes (body weight loss and appetite loss) [14,15,16]. ChREBP regulates the gene expression of glucose transporter 5 (Glut5) and ketohexokinase (Khk), which regulate fructolysis [12,17,18]. Taken together, we speculated that altered sucrose and fructose metabolism may contribute to the pathology of sucrose intolerance and fructose malabsorption seen in KO mice.

In this study, we focused on the effect of ChREBP on sucrose and fructose metabolism in the liver and intestine. We tested whether 30% sucrose plus miglitol (S+M) diet-fed KO mice show phenotypes similar to sucrose intolerance. Furthermore, by comparing the results of oral and peritoneal fructose injection, we tried to clarify the role of hepatic and intestinal ChREBP in fructose metabolism. This study will be beneficial for understanding the mechanism of sucrose and fructose metabolism.

2. Materials and Methods

2.1. Materials
Sucrose, fructose and glucose measurement kits were purchased from Wako Pure Chemicals (Osaka, Japan). Lactate measurement kits were purchased from Kyowa Medex Co. (Tokyo, Japan). Triglyceride and cholesterol measurement kits were purchased from Wako Pure Chemicals. Glucose-6-phosphate dehydrogenase (G6PDH), phosphoglucone isomerase, hexokinase and NADP were purchased from Roche Custom Biotech Inc. (Mannheim, Germany).

2.2. Animals, and sucrose and sucrose+miglitol diets

Animal experiments were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). All animal care was approved by the Animal Care Committee of the University of Gifu. Mice were housed at 23°C on a 12-h light/dark cycle. KO mice were backcrossed for at least 10 generations onto the C57BL/6J background [19].

Mice had free access to water and were fed an autoclaved CE-2 diet (CLEA Japan, Tokyo, Japan). Wild type (WT) and KO mice were housed separately with a total of three mice per cage. To examine mortality and body weight changes, 12 weeks old male WT and KO mice were fed a 30% sucrose diet (S; protein 17% kcal, carbohydrate 73% kcal, fat 10% kcal) or a 30% sucrose + 0.08% miglitol diet (S+M; protein 17% kcal, carbohydrate 73% kcal, fat 10% kcal, miglitol 0.08%) for 8 weeks [20]. To examine phenotypes (tissue weight, tissue metabolites, plasma profile, mRNA levels), 18 weeks old male WT and KO mice were fed S or S+M diets for 7 days. The diets were purchased from Research Diets Inc. (New Brunswick, NJ, USA). Miglitol was gifted by Sanwa Kagaku Kenkyuusho Co. (Nagoya, Aichi, Japan).

2.2. Liver glycogen, triglyceride, cholesterol and fructose contents, and plasma profile measurements

The liver glycogen content was measured as previously reported [12,19]. Liver lipids were extracted using the Bligh and Dyer method [21], and measured using triglyceride (Wako Pure Chemicals) and cholesterol E-tests (Wako). Liver fructose contents were measured by enzymatic methods [22]. Briefly, freeze-clamped tissues (100 mg) were homogenized in 2 ml of cold 6%
perchloric acid, neutralized and centrifuged. The assay is based on the oxidation of glucose as glucose-6-phosphate (G6P) using G6PDH. Fructose-6-phosphate is converted to G6P by the phosphoglucone isomerase enzyme, and subsequently oxidized by the G6PDH in the assay mixture. The fructose concentration is determined as the difference in G6P concentration before and after phosphoglucone isomerase treatment. All enzymes were purchased from Roche Custom Biotech Inc. Blood plasma was collected from the retro-orbital venous plexus following ad libitum feeding or after a 6-h fast. Blood glucose levels were measured using a FreeStyle Freedom monitoring system (Nipro, Osaka, Japan). Plasma triglycerides and total cholesterol levels were determined using the commercial kits, triglyceride E-test (Wako) and cholesterol E-test (Wako), respectively.

2.3. Cecal contests weight, cecal lactate contents and intestinal bacterial flora

Mice fed with S or S+M were sacrificed at 19 weeks of age by cervical dislocation. After tissue weight, length of intestine and cecal contents were measured, the intestine and liver were immediately snap-frozen in liquid nitrogen and stored at −80°C until further analysis of hepatic triacylglycerol and cholesterol contents, and quantitative PCR. For measurement of cecal lactate contents, frozen cecal content (20 mg) was homogenized in 80 μl of cold 6% perchloric acid, neutralized and centrifuged. Supernatants were collected and measured by a lactate measurement kit (Kyowa Medex). Terminal restriction fragment length polymorphism (T-RLFP) flora analysis of cecal contents was performed by Techo Suruga Labo Inc. (Shizuoka, Shizuoka, Japan) [23].

2.4. Oral and intraperitoneal fructose-loading test

Fructose (3 g/kg BW) was orally or intraperitoneally injected into 14 weeks old male WT and KO mice. Plasma glucose was measured at the indicated times. For liver fructose contents and mRNA expression analyses, mice were sacrificed at 0, 1 or 4 h, and the liver and intestine were removed and stored at −80°C until further analysis.

2.5. RNA isolation and quantitative real-time PCR

Total RNA isolation, cDNA synthesis and real-time PCR analysis were performed as previously described [12,19]. Real-time PCR primers for mouse/rat Chrebp, liver type pyruvate kinase (Pklr), glucose transporter 2 (Glut2), fibroblast growth factor-21 (Fgf-21) and RNA polymerase II (Pol2) have been previously reported [19]. Primers used for Glut5, Khk and Si were as follows: Glut5 Forward, 5′-CGGCTTCTCCACCTGCTC-3′, Glut5 Reverse, 5′-CGTGTCCTATGACGTAGACAATGA-3′; Khk-C Forward, 5′-GCTGACTTCAGGCAGAGG-3′, Khk-C Reverse, 5′-CCTTCTCAAAGTCCTTAGCAG-3′; Si Forward, 5′-CTTGCATCCAGTTCAGTGT-3′, Si Reverse, 5′-CAGGTGACATCCAGTGTCCAT-3′. All amplifications were performed in triplicate. The relative amounts of mRNA were calculated using the comparative CT method. Pol2 expression was used as an internal control.

2.6. Statistical analysis

All values are presented as means ± SD. Data were analyzed using Tukey’s test. A value of p < 0.05 was considered statistically significant.

3. Results

3.1. ChREBP shows intolerance to modest amounts of sucrose and miglitol diet

We have reported that a high-sucrose diet (60% sucrose) caused decreased appetite and eventually lethality in KO mice [12]. First, we investigated whether KO mice have any problems with sucrose digestion. We tested whether a medium amount of sucrose (30%) feeding causes body weight loss. A 30% sucrose diet was not lethal, although the body weight gain of 30% sucrose-diet-fed KO (KO S) mice was much lower than that of 30% sucrose-fed WT (WT S) mice (Figure 1A and B).
Figure 1. 30% sucrose + 0.08% miglitol diet causes body weight loss and high lethality.

Twelve weeks old male wild type (WT) mice and ChREBP knockout (KO) mice were fed a 30% sucrose (S) or 30% sucrose plus 0.08% miglitol (S+M)-containing diet for 8 weeks. (A) Survival rate. (B) Body weight change. Data represented as mean±S.D. (n=12 per group). 1WT S vs WT S+M, p<0.05, 2KO S vs KO S+M, p<0.05, 3WT S vs KO S, p<0.05, and 4WT S+M vs KO S+M, p<0.05.

Interestingly, addition of miglitol, which inhibits sucrose digestion in the upper intestine, caused decreased body weight and increased mortality (50% and 75% 4 and 8 weeks after feeding the specific diet, respectively; Figure 1A and 1B). Next, we examined the following parameters 1 week after feeding the specific diet. The body weight changes and food intake of KO S mice were similar to those of WT S mice (Table 1). However, the body weight and food intake of sucrose plus miglitol (S+M) diet-fed KO (KO S+M) mice were significantly decreased compared with WT S+M mice. Consistently, the liver, epidydimal fat tissue and brown adipose tissue weight was decreased in KO S+M mice compared with WT S+M mice. (Table 1). In contrast, the locomotor activity was similar among the groups (Table 1).
Table 1. The effect of 30% sucrose and 0.08% miglitol diet on wild type mice and ChREBP knockout mice.

<table>
<thead>
<tr>
<th></th>
<th>WT S</th>
<th>WT S+M</th>
<th>KO S</th>
<th>KO S+M</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g) before</td>
<td>31.0 ± 1.77</td>
<td>29.6 ± 1.83</td>
<td>27.7 ± 1.62</td>
<td>26.6 ± 2.21</td>
</tr>
<tr>
<td>BW (g) after</td>
<td>29.3 ± 1.22</td>
<td>27.6 ± 0.91</td>
<td>25.2 ± 1.00</td>
<td>20.9 ± 1.00</td>
</tr>
<tr>
<td>BW (%BW) difference</td>
<td>-5.38 ± 2.47</td>
<td>-7.5 ± 0.88</td>
<td>-8.97 ± 3.21</td>
<td>-21.5 ± 2.14</td>
</tr>
<tr>
<td>Liver (%BW)</td>
<td>5.33 ± 0.30</td>
<td>5.23 ± 0.23</td>
<td>7.12 ± 1.79</td>
<td>4.97 ± 0.57</td>
</tr>
<tr>
<td>Epididymal fat weight</td>
<td>1.78 ± 0.55</td>
<td>1.69 ± 0.32</td>
<td>1.35 ± 0.30</td>
<td>0.47 ± 0.16</td>
</tr>
<tr>
<td>Brown adipose tissue</td>
<td>0.40 ± 0.09</td>
<td>0.38 ± 0.05</td>
<td>0.30 ± 0.07</td>
<td>0.26 ± 0.06</td>
</tr>
<tr>
<td>Locomotor activity</td>
<td>14550 ± 3788</td>
<td>12778 ± 2984</td>
<td>12875 ± 2303</td>
<td>10800 ± 2066</td>
</tr>
<tr>
<td>food intake (g/day)</td>
<td>2.51 ± 0.63</td>
<td>2.33 ± 0.26</td>
<td>2.53 ± 0.17</td>
<td>1.77 ± 0.30</td>
</tr>
<tr>
<td>Plasma glucose (mg/dL)</td>
<td>100.6 ± 9.6</td>
<td>96.3 ± 8.3</td>
<td>80.3 ± 10.8</td>
<td>57.6 ± 6.8</td>
</tr>
<tr>
<td>Plasma triglyceride (mg/dL)</td>
<td>137.2 ± 49.4</td>
<td>161.7 ± 54.2</td>
<td>72.7 ± 17.5</td>
<td>70.2 ± 14.2</td>
</tr>
<tr>
<td>Plasma T-Chol (mg/dL)</td>
<td>127.5 ± 15.3</td>
<td>130.6 ± 4.4</td>
<td>60.3 ± 7.8</td>
<td>65.4 ± 6.4</td>
</tr>
<tr>
<td>Liver Glycogen content</td>
<td>38.6 ± 14.3</td>
<td>50.4 ± 17.4</td>
<td>83.5 ± 36.2</td>
<td>56.9 ± 27.4</td>
</tr>
<tr>
<td>Liver Cholesterol content (mg/g liver)</td>
<td>6.60 ± 1.97</td>
<td>5.54 ± 1.50</td>
<td>2.72 ± 0.84</td>
<td>1.35 ± 0.45</td>
</tr>
<tr>
<td>Liver Cholesterol content (mg/g liver)</td>
<td>0.99 ± 0.32</td>
<td>1.54 ± 0.79</td>
<td>0.44 ± 0.14</td>
<td>0.56 ± 0.33</td>
</tr>
</tbody>
</table>

Regarding the plasma profile, the plasma glucose levels were lowest in KO S+M mice. Plasma triglyceride and total cholesterol levels in KO S and KO S+M mice were lower than those in WT S and WT S+M mice (Table 1). The liver triglyceride and cholsterol contents in KO S and KO S+M mice were also lower than those in WT S and WT S+M mice (Table 1). The liver glycogen content in KO S mice was increased; however, in KO S+M mice it was decreased owing to appetite loss (Table 1). Thus, KO S+M mice showed sucrose intolerance similar to high-sucrose diet-fed KO mice.

3.2. Sucrose plus miglitol diet-fed KO mice show cecum enlargement

Next, we checked intestinal changes in WT and KO mice. The length of the small intestine was comparable in WT S, WT S+M, KO S and KO S+M mice (Figure 2A). The cecal enlargement and cecal contents in KO S mice were higher than those in WT S mice (Figure 2B and C). Although the food-loading test was performed only for one week, the cecal content in KO S+M mice was about 3.5 times higher than that in WT S and WT S+M mice (Figure 2B and C). Moreover, analysis of the intestinal flora and cecal contents showed that the ratios of Bifidobacterium and lactobacillales, and the cecal lactate contents were the highest in KO S+M mice (Figure 2D–F). In contrast, the abundance of clostridium cluster XIVa was dramatically diminished in KO S+M mice (Figure 2D).
Figure 2. Sucrose plus miglitol diet-fed KO mice show cecal enlargement, higher lactate contents and altered intestinal flora.

Eighteen weeks old male wild type (WT) mice and ChREBP-knockout (KO) mice were fed a 30% sucrose (S) or 30% sucrose plus 0.08% miglitol (S+M)-containing diet for 7 days. (A) Lengths (cm) of small intestine. (B) Representative image of intestinal enlargement. (C) Weight of cecal contents (% BW). (D, E) Gut microbes in cecum contents of WT and KO mice are expressed as a percentage of total DNA sequences. (F) Cecal lactate contents (mg/g). Data represented as mean±SD (n = 6 per group). *p < 0.05, 1WT S vs WT S+M, p<0.05, 2KO S vs KO S+M, p<0.05, 3WT S vs KO S, p<0.05, and 4WT S+M vs KO S+M, p<0.05. BW: body weight.
3.3. Miglitol affects the expression of ChREBP target genes in the intestine

Next, we tested the sucrose and fructose metabolism in relation to gene expression. In WT S mice, the expression of sucrose metabolism (Si), fructose metabolism (Glut2, Glut5 and Khk), and Chrebp and its target genes was highest in the upper intestine (Figure 3). Upon addition of miglitol, the mRNA expression of these genes was highest in the middle and lower intestine. In the liver, the mRNA expression of these genes was not affected by the addition of miglitol. Interestingly, the expression of Glut5 mRNA in the liver was much lower than in the intestine (Figure 3E). By contrast, the mRNA levels of the abovementioned genes were lower in the KO mice than in the WT and the effect of miglitol on these mRNA levels was suppressed in KO S mice (Figure 3A–F). As compared with Glut5 expression, SGLT1 mRNA levels were not affected by ChREBP gene deletion (data not shown). Thus, we concluded that ChREBP regulates sucrose and fructose metabolism through gene expression.
Figure 3. Effect of miglitol and the ChREBP gene deletion on genes related to ChREBP, fructose and sucrose metabolism.

Eighteen weeks old male wild type (WT) mice and ChREBP-knockout (KO) mice were fed a 30% sucrose (S) or 30% sucrose plus 0.08% miglitol (S+M)-containing diet for 7 days. The intestine was divided into three parts (upper, middle and lower) and the mRNA levels were measured by real-time PCR. (A) Chrebp; (B) liver pyruvate kinase (Pklr); (C) sucrase isomerase (Si); (D) ketohexokinase (Kkh); (E) glucose transporter 5 (Glut5); (F) Glucose transporter 2 (Glut2). Data represented as mean ± SD (n = 3 per group). 1WT S vs WT S+M, p<0.05, 2KO S vs KO S+M, p<0.05, 3WT S vs KO S, p<0.05, and 4WT S+M vs KO S+M, p<0.05.

3.4. Fructose is difficult to metabolize in the intestine, but not in the liver

As KO mice showed disturbance not only in sucrose metabolism but also in fructose metabolism, we next tested the role of intestinal and hepatic ChREBP in fructose metabolism. After oral fructose injection, fructose is absorbed in the intestine (Figure 4A). After peritoneal injection, fructose is absorbed in the portal vein (figure 4B) [24]. In the oral fructose-loading test (3 g/kg BW), the plasma glucose levels in WT mice only modestly increased to 120 mg/dL at 30 min (Figure 4A). In KO mice, the plasma glucose levels at 30 min were slightly lower than those in WT mice (Figure 4A). By contrast, in peritoneal fructose loading, the plasma glucose levels in WT mice increased to 200 mg/dL at 30 min (Figure 4B). In KO mice, the plasma glucose levels were lower than those in WT mice, and the peak time shifted right (Figure 4B). Consistent with these results, the hepatic fructose content in the oral fructose-loading test (at 0 h and 1 h) was undetectable (Figure 4C). Therefore, we concluded that fructose is difficult to metabolize and absorb in the intestine. In contrast, the fructose content after the peritoneal fructose-loading test at 1 h was measurable. Moreover, in KO mice, the
hepatic fructose content at 1 h was about 3 times higher than that in WT mice (Figure 4D). These results suggest that hepatic fructose metabolism was inhibited at the level of KHK in the liver of KO mice.

**Figure 4. Oral and peritoneal fructose injection test.**

Oral (A) and peritoneal (B) injected fructose is absorbed in intestine and portal vein, respectively. Time course of glucose concentration after oral (C) or peritoneal (D) fructose injection. Liver fructose content at 0hr and 1hr after oral (E) or peritoneal (F) fructose injection. Data are presented as means ± SD (n = 6 per group). *WT vs KO, p<0.05.

3.5. ChREBP regulates the expression of genes related to fructose metabolism in the intestine

Finally, we examined whether fructose induces the expression of intestinal and hepatic ChREBP target genes. After oral fructose injection, the expression of intestinal ChREBP target genes (Chrebp, Pklr) and fructose metabolism genes (Glut2, Glut5 and Khk) in WT mice increased in a time-dependent manner, while the mRNA expression of these genes was much lower in KO mice (Figure 5A–F). Consistent with the plasma glucose levels, the mRNA expression of the hepatic ChREBP target genes (Chrebp, Pklr and Fgf-21) and fructose metabolism genes (Glut2, Glut5 and Khk) was not affected by fructose (Figure 5A–F). After peritoneal fructose injection, the hepatic mRNA expression of Chrebp, Pklr, Glut2, Glut5 and Khk in WT mice increased in a time-dependent manner; however, this induction was diminished in KO mice. By contrast, the intestinal mRNA levels of these genes were not affected by fructose injection (Figure 5A–F). In the liver, Fgf-21 mRNA levels in KO mice were lower than those in WT mice. However, the hepatic Fgf-21 mRNA levels in WT mice were not induced by oral or peritoneal fructose injection (Figure 5C). Thus, we concluded that oral and peritoneal fructose injection mainly induced intestinal and hepatic fructose metabolism genes regulated by ChREBP, respectively.
Figure 5. Effect of oral and peritoneal fructose injection on genes related to Chrebp and fructose metabolism.
After oral or peritoneal fructose injection (3 kg/kg BW), the mRNA expression of Chrebp (A), liver type pyruvate kinase (Pklr) (B), fibroblast growth factor-21 (Fg21), (C), ketohexokinase (Khk), (D), glucose transporter 5 (Glut5), (E) and glucose transporter 2 (Glut2) (F) in the intestine and liver was measured by real-time PCR analysis. n = 3 per group. 1WT S vs WT S+M, p<0.05, 2KO S vs KO S+M, p<0.05, 3WT S vs KO S, p<0.05, and 4WT S+M vs KO S+M, p<0.05.

4. Discussion

In this study, we tried to identify the mechanism by which ChREBP-KO mice show sucrose intolerance. Thirty percent sucrose (30%) diet-fed KO mice did not present the body weight loss and lethality seen in 60% sucrose diet-fed KO mice; however, SI inhibition by miglitol successfully exhibited sucrose intolerance. Increased fecal lactate contents, and increased growth of lactobacillales and *Bifidobacterium*, consistent with increased lactate contents, was seen only in S+M fed KO mice. These findings were consistent with decreased expression of sucrose and fructose metabolism-related genes, which are regulated by ChREBP. Moreover, oral and peritoneal fructose injection mainly induced ChREBP-regulated intestinal and hepatic fructose metabolism genes, respectively. These results suggest that alternations in the expression of both sucrose and fructose-related genes contribute to sucrose intolerance and fructose malabsorption in KO mice (Figure 6).

Figure 6. The mechanism that ChREBP knockout mice showed sucrose intolerance and fructose malabsorption.

(A) In 30% sucrose plus 0.08% miglitol diet fed wild type mice (WT), sucrose was digested into glucose and fructose in upper intestine. Glucose was almost absorbed in upper intestine. In contrast, fructose was partly absorbed and unabsorbed fructose was used for intestinal bacterial growth.

(B) In 30% sucrose plus 0.08% miglitol diet fed ChREBP knockout mice (KO), owing to decreased sucrase-isomaltase (SI) expression or SI inhibition by miglitol, undigested sucrose was moving...
metformin and carbohydrates are consumed by patients with diabetes mellitus, the combination therapy of suppression of ChREBP, and thereby decreased

Considering metformin can inhibit ChREBP activity [36], abdominal side effects may be due to viewpoint, metformin sometimes causes abdominal discomfort (diarrhea and vomiting) [35].

S+M-fed KO mice have not only sucrose intolerance, but also fructose malabsorption. From a clinical viewpoint, fructose was decreased by 90%, compared with WT mice [31]. Therefore, decreased "intestinal" absorption capacity of a facilitated transport system [33,34]. Moreover, in these GLUT5-KO mice, fructose absorption was decreased by 75% in the jejunum and the concentration of serum fructose was decreased by 90%, compared with WT mice [31]. Therefore, decreased “intestinal” Glut5 mRNA may contribute to the lower intestinal fructose absorption in KO mice, suggesting that S+M-fed KO mice have not only sucrose intolerance, but also fructose malabsorption. From a clinical viewpoint, metformin sometimes causes abdominal discomfort (diarrhea and vomiting) [35]. Considering metformin can inhibit ChREBP activity [36], abdominal side effects may be due to suppression of ChREBP, and thereby decreased Glut5 mRNA expression. If excess amounts of carbohydrates are consumed by patients with diabetes mellitus, the combination therapy of metformin and α-glucosidase inhibitor may increase abdominal side effects.
SI has important roles in the regulation of intestinal sucrose absorption [37]. SI is an enzyme that digests sucrose into glucose and fructose. SI mRNA is induced by sucrose and fructose [13,38]. Moreover, it has been reported that glucose “negatively” regulates human SI gene expression through two HNF binding sites in Caco-2 cells [39,40]. Therefore, it is reasonable that ChREBP does not directly regulate SI. However, we found that SI mRNA levels in the intestine of KO mice were lower than those in WT. We considered some potential pathways through which ChREBP indirectly regulates SI mRNA expression. First, the amount of sucrose intake by KO mice may be lower than the intake by WT because of appetite loss in KO mice. Second, intracellular metabolites derived from sucrose may be a signal for induction of SI genes. As ChREBP regulates glucose and fructose metabolism, intracellular metabolites may be decreased in KO mice. Interestingly, it has been reported that independently of ChREBP, fructose uniquely induces SREBP1c and fatty acid synthesis genes, resulting in impaired insulin signaling [41]. Although further investigation is still needed, decreased SI mRNA levels in KO mice also partly contribute to the pathogenesis of sucrose intolerance.

In addition to decreased sucrose metabolism, decreased fructose metabolism has a more important role in the pathogenesis of sucrose intolerance in KO mice. We and other groups have reported that ChREBP has an important role in regulating fructose metabolism [11,12,14-17]. Many of the fructose metabolism genes (Glut2, Glut5, Khk and aldolase B) are ChREBP-target genes [12, 17, 18]. The mRNA levels of Khk, Glut2 and Glut5 in intestine-specific ChREBP-KO mice were much lower than in WT mice after oral fructose injection [18]. Consistently, our data showed that the mRNA levels of Khk, Glut2 and Glut5 in KO mice were much lower than in WT mice. Moreover, oral fructose injection induced Khk, Glut2 and Glut5 mRNA levels in a time-dependent manner only in WT mice. These results reconfirmed that ChREBP coordinates with intestinal fructose metabolism by modulating Khk, Glut2 and Glut5 gene expression.

Hepatic Khk has important roles in liver fructose metabolism [42,43]. It has been reported that the plasma fructose levels in Khk−/− mice were 10 times higher than those in WT and Glut5−/− mice [42]. Consistently, the hepatic fructose content in KO mice was much higher after peritoneal fructose injection, which is consistent with decreased Khk mRNA levels in the liver of KO mice. As with hepatic fructose transport, hepatic Glut5 mRNA levels were much lower than in the intestine, which is consistent with a previous study [32]. Considering that the plasma fructose levels in Glut5−/− mice were much lower than in Khk−/− mice, other fructose transporters may regulate hepatic fructose uptake. Our data suggest that hepatic Khk rather than Glut5 regulates hepatic fructose metabolism.

5. Conclusions

In conclusion, both sucrose feeding and SI inhibitor caused sucrose intolerance and fructose malabsorption in ChREBP-KO mice. ChREBP coordinates with intestinal fructose metabolism by modulating the mRNA expression of intestinal SI and Glut5, and hepatic Khk. Considering intestinal absorption of fructose is more difficult than that of glucose, intestinal ChREBP rather than hepatic ChREBP has an important role in the pathology of sucrose intolerance and fructose malabsorption.

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Author Contributions: Katsumi Iizuka conceived and designed the experiments; Takehiro Kato and Ken Takao performed the experiments; Takehiro Kato and Katsumi Iizuka analyzed the data; Tadahiro Kitamura and Yukio Horikawa gave a support in the literature review; Katsumi Iizuka and Jun Takeda wrote and revised the paper. All the authors approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.
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