Ingestion of a moderate high-sucrose diet results in glucose intolerance with reduced liver glucokinase activity and impaired glucagon-like peptide-1 secretion

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ABSTRACT

Aims/Introduction: Excessive intake of sucrose can cause severe health issues, such as diabetes mellitus. In animal studies, consumption of a high-sucrose diet (SUC) has been shown to cause obesity, insulin resistance and glucose intolerance. However, several in vivo experiments have been carried out using diets with much higher sucrose contents (50–70% of the total calories) than are typically ingested by humans. In the present study, we examined the effects of a moderate SUC on glucose metabolism and the underlying mechanism.

Materials and Methods: C57BL/6J mice received a SUC (38.5% sucrose), a high-starch diet (ST) or a control diet for 5 weeks. We assessed glucose tolerance, incretin secretion and liver glucose metabolism.

Results: An oral glucose tolerance test (OGTT) showed that plasma glucose levels in the early phase were significantly higher in SUC-fed mice than in ST-fed or control mice, with no change in plasma insulin levels at any stage. SUC-fed mice showed a significant improvement in insulin sensitivity. Glucagon-like peptide-1 (GLP-1) secretion 15 min after oral glucose administration was significantly lower in SUC-fed mice than in ST-fed or control mice. Hepatic glucokinase (GCK) activity was significantly reduced in SUC-fed mice. During the OGTT, the accumulation of glycogen in the liver was suppressed in SUC-fed mice in a time-dependent manner.

Conclusions: These results indicate that mice that consume a moderate SUC show glucose intolerance with a reduction in hepatic GCK activity and impairment in GLP-1 secretion. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2012.00208.x, 2012)

KEY WORDS: Glucagon-like peptide-1, Glucokinase, High-sucrose diet

INTRODUCTION

The term ‘sugars’ is commonly used to describe mono- and disaccharides, which represent an important part of total caloric intake1. Glucose, fructose and sucrose are the most commonly consumed sugars. Approximately 10% of daily caloric intake can be attributed to fructose, and fructose consumption has dramatically increased2,3. Excessive ingestion of sucrose promotes the development of type 2 diabetes mellitus, which is associated with obesity and insulin resistance1. Animal studies have shown that diets extremely high in sucrose cause numerous metabolic abnormalities, such as obesity, insulin resistance, glucose intolerance and dyslipidemia4. The impact of a high-sucrose diet (SUC) on insulin sensitivity remains controversial. Administration of a SUC (50–70% of the total calories) for 4–8 weeks induced insulin resistance in the liver and skeletal muscle5,6. However, in some experiments, mice fed a SUC (35–56% of the total calories) for 15–40 weeks developed glucose intolerance with enhanced insulin sensitivity7,8.

It is well recognized that the liver plays an important role in glucose homeostasis, in the rapid clearance of glucose in the postprandial state, and in the controlled production of glucose in the postabsorptive state9. The conversion of glucose into glycogen is a key pathway by which the liver removes glucose from the portal vein after a meal10. Hepatic glucokinase (GCK) plays a key role in glucose metabolism, as highlighted by the anomalies associated with Gck mutations10 and by the consequences of tissue-specific knock-out experiments11. Hepatic GCK, by which phosphorylation of glucose is a rate-determining step in glucose uptake and glycogen synthesis12,13, is responsible for postprandial
glucose disposal. Insulin positively regulates Gck gene expression in the liver, and thereby stimulates hepatic glucose uptake and glycogen synthesis. Expression of the hepatic Gck gene is reduced in diabetic animals with insulin deficiency and insulin resistance.

The incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinoctropic polypeptide (GIP) play a major role in glucose homeostasis through stimulation of insulin secretion and suppressing glucagon secretion, thereby contributing to limiting postprandial glucose excursions. Several nutrients, including triglycerides, fatty acids, proteins and carbohydrates, stimulate incretin secretion. Among these nutrients, glucose is one of the most potent stimulators of incretin secretion in rodents and humans. The two incretin hormones are responsible for approximately 50–70% of the postprandial insulin responses in healthy individuals. Several studies have shown a significant reduction in GLP-1 levels after mixed-meal ingestion in type 2 diabetes patients. However, whether chronic high-carbohydrate ingestion can change incretin secretion remains unknown.

The aim of the present study was to examine the effects of a moderate SUC (38.5% of the total calories) on glucose metabolism and the effects of chronic high-carbohydrate (corn starch or sucrose) ingestion on incretin secretion. The present findings show that consumption of a moderate SUC for 5 weeks results in glucose intolerance with a reduction in hepatic GCK expression and activity and impairment in GLP-1 secretion.

MATERIALS AND METHODS

Animals and Diets

Twelve-week-old male C57BL/6J mice were obtained from Japan SLC (Shizuoka, Japan) and housed in a temperature-controlled room under a standard 12-h light/dark cycle. All procedures were carried out according to a protocol approved by the Nagoya University Institutional Animal Care and Use Committee. Mice were fed a normal chow diet of CE-2 (CLEA Japan, Osaka, Japan), containing 58.2% carbohydrates, 29.2% protein and 12.6% fat as energy content. After adaptation for 2 weeks, they were divided into three groups and fed a normal chow diet (NC), a high-starch diet (ST) supplemented with 38.5% corn starch or a high-sucrose diet (SUC containing 38.5% sucrose). The latter two diets were prepared by the addition of corn starch or sucrose, respectively, to CE-2 (Table 1). Mice were fasted for 16 h or were re-fed for 12 h after 24 h of starvation.

Plasma Biochemical Analyses

Blood glucose levels were measured with ANTSENSE II (Bayer Medical, Leverkusen, Germany). Plasma levels of insulin were determined by ELISA kit (Morinaga, Tokyo, Japan). Plasma triglycerides and free fatty acid levels were determined using the Triglyceride E test and NEFA C test (Wako Pure Chemical, Osaka, Japan), respectively. Plasma levels of total GIP and GLP-1 were determined using the GIP (TOTAL) ELISA kit (Linco Research, St. Charles, MO, USA) and an electro-

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Table 1 | Composition of experimental diets

| Protein  | NC  | ST   | SUC  |
|----------|-----|------|------|
| 292      | 180 | 180  |
| Fat      | 126 | 7.7  | 7.7  |
| Carbohydrates | 582 | 74.3 | 74.3 |
| (Sucrose) | –   | –    | (38.5)|

Data are expressed as % of total energy. NC, a normal chow diet; ST, a high-starch diet; SUC, a high-sucrose diet.

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Glucose Tolerance Test, Insulin Tolerance Test and Pyruvate Tolerance Test

Oral and intravenous glucose tolerance tests (OGTT and IVGTT, respectively) were carried out after 5 weeks of feeding with the NC, SUC or ST. After 16 h of food deprivation, glucose was given either orally at a dose of 2 g/kg (OGTT) or intravenously at a dose of 2 g/kg (IVGTT). After administration, blood was collected at 0, 10, 15 and 60 min for the measurement of glucose and insulin. For the insulin tolerance test (ITT), mice were deprived of food for 6 h before the test. Insulin was injected intraperitoneally at a dose of 0.6 U/kg. Blood was collected 0, 30, 60, 90 and 120 min after insulin injection. For the pyruvate tolerance test, the mice were deprived of food for 16 h and then injected intraperitoneally with pyruvate dissolved in saline (2 g/kg). Blood was collected 0, 15, 30, 60 and 90 min after the injection of pyruvate.

Isolation of Tissue Ribonucleic Acid and Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction

Extraction of total ribonucleic acid (RNA), synthesis of complementary deoxyribonucleic acid (DNA) and quantitative real-time reverse transcription-polymerase chain reaction (RT–PCR) were carried out as previously described. The primer sequences are shown in Table S1. The messenger RNA (mRNA) levels were normalized with respect to those of 36B4.

Measurement of Hepatic Glycogen and Triglyceride Content

For the determination of hepatic glycogen content, 100 mg of liver sample was digested in 0.5 mL of 1 mmol/L potassium hydroxide for 30 min in a 70°C water bath. The samples were briefly centrifuged, and 100 μL of the supernatant was removed and neutralized with 17 μL of 17.4 mol/L acetic acid. Glycogen was enzymatically cleaved to glucose by addition of 500 μL of 0.3 mol/L acetic buffer with 0.5% amyloglucosidase at 37°C. Glucose concentration was then measured using a Glucose C II test (Wako Pure Chemical). For the determination of hepatic triglyceride content, 100–200 mg of liver sample was homogenized for 10 min in 4 mL isopropanol with a Polytron disrupter. Triglyceride content was measured using a Triglyceride E test (Wako Pure Chemical).
Measurement of GCK Activity
GCK activity was measured as previously described. Briefly, liver was homogenized with the sample buffer, and a part of the homogenate was centrifuged for 90 min at 40,000 g at 4°C. Thereafter, the supernatant was harvested. The final glucose concentration in the assay mixture (200 mmol/L HEPES, 50 mmol/L KCl, 5 mmol/L MgCl₂, 1 mmol/L NAD⁺, 5 mmol/L adenosine triphosphate, 100 μg/mL bovine serum albumin and 1 unit/mL glucose 6-phosphate dehydrogenase) was adjusted to 0.5 or 50 mmol/L. The assay mixture (990 μL) was incubated for 5 min at 37°C, and the reaction was then started by the addition of 10 μL of the supernatant. The reaction velocity was measured as the rate of increase in absorbance monitored at 340 nm 2–3 min after initiation of the reaction. One unit was the amount of enzyme that catalyzed the phosphorylation of 1 μmol of glucose per minute. GCK activity was calculated using subtraction.

Immunoblotting Analysis
After 16 h of food deprivation, 5 units of human regular insulin (Eli Lilly, Indianapolis, IN, USA) were injected intravenously. The liver and skeletal muscle tissue samples were rapidly extracted 3 min after the injection. Immunoblotting analysis was then carried out as previously described, by using an antitotal AKT antibody (at a dilution of 1:1000; Cell Signaling Technology, Beverly, MA, USA), an antiphospho-AKT (Ser 473) antibody (at a dilution of 1:1000; Cell Signaling Technology), an antityrosine kinase-3β (GSK-3β) antibody (at a dilution of 1:1000; BD Biosciences, Sparks, MD, USA), or an antiphospho-GSK-3β (Ser 9) antibody (at a dilution of 1:1000; Cell Signaling Technology). The intensity of the signals was quantified using NIH image software (National Institutes of Health, Bethesda, MD, USA). Phosphorylation was expressed as a percentage of the amount of phospho-AKT or phospho-GSK-3β relative to the total amount of AKT or GSK-3β, respectively.

Statistical Analysis
Results are expressed as mean ± SEM of values obtained from several experiments, and statistical significance was evaluated using analysis of variance (ANOVA) with Bonferroni post-hoc tests. *P < 0.05 was considered statistically significant.

Table 2: Bodyweight, insulin and metabolic parameters in fasted and fed state

|                  | Fasted   |        | Fed       |        |
|------------------|----------|--------|-----------|--------|
|                  | NC       | ST     | SUC       |        |
|                  |          |        |           |        |
| Bodyweight (g)   |          |        |           |        |
| Energy intake (kJ/day) |          |        |           |        |
| Glucose (mmol/L) | 5.76 ± 0.21 | 5.69 ± 0.05 | 5.74 ± 0.29 |        |
| Insulin (pmol/L) | 706 ± 148 | 634 ± 140 | 436 ± 78  |        |
| Triglycerides (mmol/L) | 1.05 ± 0.08 | 1.23 ± 0.13 | 1.21 ± 0.07 |        |
| Free fatty acids (mmol/L) | 1.54 ± 0.09 | 1.59 ± 0.11 | 1.45 ± 0.13 |        |
|                  | 267 ± 0.3 | 276 ± 0.8 | 275 ± 0.3 |        |
|                  | 480 ± 1.6 | 466 ± 0.4 | 475 ± 0.8 |        |
|                  | 114 ± 0.8 | 118 ± 0.5 | 125 ± 0.6 |        |
|                  | 1456 ± 13.8 | 2044 ± 11.1 | 1389 ± 15.7 |        |
|                  | 1.28 ± 0.15 | 1.13 ± 0.09 | 1.17 ± 0.17 |        |
|                  | 0.63 ± 0.09 | 0.61 ± 0.07 | 0.56 ± 0.08 |        |

Data are expressed as means ± SEM of values. *P < 0.05 compared with normal chow diet (NC)-fed mice and high-sucrose diet (SUC)-fed mice; n = 4 or more per group. ST, a high-starch diet.

RESULTS
Effects of Carbohydrates on Metabolic Parameters
To examine the effects of a diet containing high levels of not only sucrose, but also carbohydrates, we divided mice into three groups: NC, ST and SUC. After 5 weeks on the respective diets, no differences in bodyweight or energy intake were observed among the groups. Furthermore, no significant differences in the levels of plasma glucose, triglycerides or free fatty acids in either a fasted or a fed state were observed, although insulin levels in the fed state were higher in ST-fed mice than in the other groups (Table 2).

Glucose Tolerance Test and Insulin Tolerance Test
The OGTT showed no differences in blood glucose levels at baseline or 60 min among the groups (Figure 1a). Plasma glucose levels in SUC-fed mice were significantly higher 10 and 15 min after oral glucose administration than those in NC- and ST-fed mice (Figure 1a), whereas insulin levels were similar among the groups at all times (Figure 1b). To investigate the ability of pancreatic islets to secrete insulin, we carried out an IVGTT. An IVGTT shows glucose-stimulated insulin secretion in vivo, because glucose does not pass through the portal vein after an intravenous glucose injection. The IVGTT showed no differences among the groups in glucose concentrations after intravenous administration of glucose at the indicated periods (Figure 1c). Insulin levels did not differ among the groups at any time (Figure 1d). The ITT showed that blood glucose levels after insulin injection were significantly lower in the SUC-fed mice than in the NC- and ST-fed mice (Figure 1e). Insulin-induced phosphorylation of AKT in the skeletal muscle was enhanced in SUC-fed mice (Figure 1f). These results show that the ability to secrete insulin is conserved, and that peripheral insulin sensitivity is enhanced in SUC-fed mice.

Effects of Carbohydrates on Incretin Secretion
No differences in plasma GLP-1 levels in the fasting state were observed among the groups (Figure 2b). Oral glucose administration induced GLP-1 secretion, and peak plasma GLP-1 levels were observed at 15 min (Figure 2a). An unexpected finding is that plasma GLP-1 levels 15 min after oral glucose
administration were significantly lower in the SUC-fed mice than in the NC- and ST-fed mice (Figure 2b). Plasma GIP levels at baseline were similar among the groups (Figure 2d). Peak GIP levels were observed 15 min after oral glucose administration (Figure 2c), and GIP levels at 15 min were not suppressed in the SUC-fed mice (Figure 2d).

**Hepatic Glucose Metabolism in SUC-Fed Mice**

To investigate the effects of carbohydrates on hepatic glucose metabolism, we carried out a pyruvate tolerance test. As shown in Figure 3a, glucose levels throughout the pyruvate tolerance test were significantly lower in the SUC-fed mice than in the NC- and ST-fed mice. Glucose-6-phosphatase (G6pc) mRNA expression in the fasted state was comparable among the groups, whereas phosphoenolpyruvate carboxykinase (Pepck) mRNA expression increased in the SUC-fed mice (Figure 3b). The expression of G6pc and Pepck mRNA in the re-fed state was comparable among the groups (Figure 3b). Thereafter, we assessed glycogen content in the liver. Storage of glycogen in the liver did not differ among the groups in the fed state (NC 60.7 ± 2.6 mg/g liver; ST 55.3 ± 3.2 mg/g liver; SUC 61.3 ± 2.5 mg/g liver), and after 16 h of fasting, glycogen content was undetectable in all groups. However, accumulation of glycogen throughout the OGTT was significantly suppressed in the SUC-fed mice 60 and 120 min after oral glucose administration (Figure 3c). The expression levels of Gck mRNA were lower in the fasting SUC-fed mice than in the NC-fed mice (Figure 3d). Total GCK activity during fasting was reduced by approximately 50% in the SUC group compared with the other groups, showing decreased GCK expression in the SUC-fed mice (Figure 3e).

**Lipid Metabolism in the Liver**

Next we examined lipid metabolism. Hematoxylin–eosin staining showed no vacuolization in the liver in the SUC-fed mice (data not shown). However, hepatic triglyceride content in the fed state was significantly higher in the SUC-fed mice than in the NC- and ST-fed mice (Figure 4a). In the fed state, gene expression of lipogenic enzymes, including fatty acid synthase, acetyl-CoA carboxylase α (Acaca) and stearoyl-CoA desaturase 1 (Scd1), was significantly higher in the livers of the SUC-fed mice than in the livers of the NC-fed mice; furthermore, the expression of Acaca and Scd1 mRNA was higher than in the NC- and ST-fed mice (Figure 4b). Gene expression of lipolytic
enzymes, including acyl-CoA oxidase, medium-chain acyl-CoA dehydrogenase and carnitine palmitoyltransferase 1a, in the liver was comparable among the groups (Figure 4c). However, the ratio of insulin-induced phosphorylation of AKT and GSK-3β in the liver was similar among the groups (Figure 4d,e), showing that the insulin signal was conserved in the liver of the SUC-fed mice.

**DISCUSSION**

In the present study, we observed hyperglycemia in the early phase after oral glucose administration. The liver is known to play an important role in the disposal of an oral glucose load in the early phase, whereas peripheral tissues play a role in the late phase after oral glucose loading. These findings suggest that abnormal glucose metabolism in the liver caused glucose intolerance in the SUC-fed mice.

The impaired hepatic glucose metabolism in type 2 diabetes involves both the fasting state, in which gluconeogenesis appears to be increased, and the fed state, in which insulin suppression of glucose production is reduced. Experimental studies have shown that consumption of sucrose enhances gluconeogenesis with impaired insulin sensitivity, resulting in impaired glucose metabolism in the liver. We found that liver glycogen was completely depleted after a 16-h fast, indicating that glucose production through glycogenolysis was minimized. Furthermore, we showed that glucose production from pyruvate was suppressed, which conflicts with the expression of G6pc and Pepck mRNA in a fasting state. The pyruvate tolerance test is known to measure the capacity of the liver to convert pyruvate to glucose, which would normally be inhibited by insulin. In the present study, hepatic lipogenesis was enhanced in SUC-fed mice. Enhanced lipogenesis in the liver might increase fatty acid synthesis, and thereby reduce glucose production, from pyruvate. Therefore, the present results show that gluconeogenesis in SUC-fed mice is suppressed in a fasted state.

A previous study showed that hepatic glucose uptake was reduced, and that hepatic glucose excursion was increased in patients with type 2 diabetes, because inadequate activation of hepatic GCK presumably decreased the uptake of extracellular...
glucose Clamp studies in animals with euglycemic hyperinsulinemia showed that impaired insulin action in the liver reduced glucose disposal in SUC-fed rats, although a change in hepatic GCK activity was not examined. Thus, we investigated GCK activity and glycogen storage in the liver, which represents glucose uptake. We showed that hepatic GCK expression and activity were attenuated in the SUC-fed mice, and that glycogen storage was suppressed during the OGTT, suggesting that reduced glucose disposal through the reduction of GCK activity is responsible for diabetic glucose intolerance in mice fed a moderate SUC.

As mentioned previously, impaired insulin action is thought to modify GCK activity in the livers of SUC-fed mice. An unexpected finding from the present study was that insulin-induced phosphorylation of AKT and GSK-3β did not change in the SUC-fed mice, showing that the insulin signal is conserved in the liver. Thus, this finding suggests that a SUC reduces hepatic GCK expression and activity independent of insulin action. Further investigations are required to clarify the mechanism of reduced GCK expression in the liver of SUC-fed mice.

We found elevated triglyceride content and lipogenic enzyme expression with conserved hepatic insulin action of insulin in the SUC-fed mice. Liver fat accumulation is proposed to link obesity and insulin resistance. Whether liver fat accumulation is a result or a cause of peripheral insulin resistance and glucose intolerance remains controversial. Exposure of the liver to large quantities of fructose (and sucrose-containing fructose) leads to rapid stimulation of lipogenesis and accumulation of triglycerides, which in turn contributes to reduced insulin sensitivity and hepatic insulin resistance/glucose intolerance. Accordingly, our observations suggest that liver fat accumulation is not a result of insulin resistance.

Previous studies have shown that SUC feeding for a long period decreases insulin secretion. In our experiments, no
apparent decrease in insulin secretion was observed. However, we assumed that insulin secretion was relatively impaired in this model. In fact, we found that serum insulin levels were similar throughout the OGTT, although serum glucose levels in SUC-fed mice were higher in the early phase after oral glucose administration, but not after intravenous glucose administration.

We further showed impaired GLP-1 secretion in response to oral glucose loading in SUC-fed mice. These findings therefore suggest that insulin secretion is relatively impaired throughout the OGTT in SUC-fed mice.

An animal study showed lower basal plasma GLP-1 levels and diminished GLP-1 response to oral glucose administration in mice on a high-fat diet compared with those on a low-fat diet. High levels of non-esterified fatty acids during fasting and after meals, accompanied by insulin resistance, have been speculated to inhibit nutrient-mediated GLP-1 secretion in obese individuals. However, the effects of a high-carbohydrate diet on incretin secretion have not yet been reported. In the present study, we found impaired GLP-1 secretion, but not GIP secretion, in response to oral glucose loading in the SUC-fed mice, but not in the ST-fed mice; ours is the first study that determined the effects of chronic high-carbohydrate ingestion on incretin secretion. Our observations are consistent with those of previous studies in humans that postprandial GLP-1 levels were reduced in patients with type 2 diabetes compared with healthy subjects, but that GIP secretion remained relatively intact. This finding suggests that excess ingestion of sucrose contributes to impaired GLP-1 secretion in patients with type 2 diabetes. A reduction in insulin signaling in the peripheral tissues could not explain impaired GLP-1 secretion in this model. Therefore, further investigations of the effects of a SUC on the function of intestinal L-cells are required to clarify this mechanism.

In conclusion, we showed that a moderate SUC results in glucose intolerance with a reduction in hepatic GCK expression and activity, and impaired GLP-1 secretion.

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article:

**Table S1** | Primers used for quantitative real-time polymerase chain reaction

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