ChREBP deficiency prevents high sucrose diet-induced obesity through reducing sucrase expression

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(Received 4 February, 2022; Accepted 7 June, 2022; Released online in J-STAGE as advance publication 19 August, 2022)

Obesity appears to be a major contributing factor for many health problems. Effective treatments for reducing weight gain, other than caloric restriction and exercise, are limited. The consumption of sugars is a major factor in the development of obesity in part by stimulating the transcription factor, carbohydrate response element binding protein (ChREBP), a process that is driven by de novo lipogenesis. Therefore, we hypothesized that inhibiting the action of ChREBP would be a promising strategy for alleviating these diseases. Using ChREBP deficient mice, the effect of a high intake of sucrose on body weight and blood glucose levels were investigated. Unlike wild type mice, ChREBP deficient mice did not gain much weight and their blood glucose and cholesterol levels remained relatively constant. In tracing it's cause, we found that the levels of expression of sucrase, an enzyme that digests sucrose, and both Glut2 and Glut5, a transporter of glucose and fructose, were not induced by feeding a high sucrose diet in the small intestine of ChREBP deficient mice. Our findings suggest that the inhibition of ChREBP could suppress weight gain even on a high sucrose diet.

Key Words: ChREBP, sucrose diet, sucrase, small intestine, obesity

Excessive food intake may be the single most significant cause of the current epidemic of obesity and obesity-associated diseases that are occurring in many countries. It is particularly noteworthy that type 2 diabetes mellitus is caused by several factors including lifestyle and obesity, and as a result, risks such as heart disease, non-alcoholic fatty liver disease (NAFLD), and other obesity-associated disorders are correspondingly increased. There are still no fundamental preventive or therapeutic methods for addressing this problem and effective strategies for combating the pathological consequences of caloric excess. Drugs that suppress the rapid rise in blood sugar level by delaying the absorption of sugars from the small intestine through blocking the decomposition of sugars are currently on the market. However, the side effects of this drug include symptoms such as abdominal bloating and diarrhea, and the deterioration of the patient’s quality of life (QOL) is a problem. Therefore, new medications with less side effects are awaited.

Once carbohydrate intake exceeds the short-term energy demand or glycogen storage capacity, sugars are converted into fatty acids for long-term storage by glycolytic and lipogenic pathways in the liver. A high carbohydrate diet induces the gene transcription of enzymes involved in the metabolic conversion of carbohydrate to stored fat. The transcription factor carbohydrate response element binding protein (ChREBP) mediates insulin independently from the gene expression of multiple liver enzymes responsible for converting excess carbohydrate to fatty acids for long-term storage. When the availability of glucose increases, ChREBP is translocated to the nucleus and binds to the carbohydrate response elements (ChoRE) in the promoter region of lipogenic enzyme genes and the gene encoding the glycolytic enzyme, liver pyruvate kinase (L-PK), to coordinately activate their transcriptions. In addition, ChREBP also regulates the expression of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), enzymes that are involved in fatty acid synthesis.

In ChREBP deficient (ChREBP KO) mice, de novo fatty acid synthesis in the liver and overall adiposity are decreased compared with wild type (WT) mice. ChREBP KO mice also exhibit sucrase intolerance and fructose malabsorption. For these reasons, when such mice are fed a 60% sucrose diet, they show body weight loss, ultimately resulting in lethality. More recently, we reported that mitochondrial cristae are hypoplasic due to decreased cardiolipin synthesis in the brown adipose tissue (BAT) of ChREBP KO mice. At the same time, in the BAT of ChREBP KO mice, the expression level of UCPI, an uncoupling protein involved metabolic and energy balance, was elevated, suggesting that de novo synthesized fat is basically decomposed in this mouse model.

Since ChREBP is a central regulator of glucose and lipid metabolism, it would be expected that inhibiting its activity would be a therapeutic target to diseases such as obesity and diabetes. Therefore, examining the effect of this on body weight and blood sugar level by feeding a high sucrose diet that does not cause death by slightly reducing the content of sucrose would be worthwhile experiment. In addition, sucrose and fructose malabsorption are caused by defects in fructose transporters, such as Glut5 and Glut2. ChREBP regulates both intestinal and hepatic fructose metabolism through controlling these transporters. Excessive fructose consumption is closely linked to the pathogenesis of a number of metabolic diseases. Shi et al. found that liver ChREBP protects mice against fructose-induced hepatotoxicity by regulating L-PK. Kato et al. reported that ChREBP KO mice showed sucrase intolerance and fructose malabsorption. In their article, the production of the mRNA of sucrase was suppressed in the upper small intestine of ChREBP KO mice by feeding sucrose (30%). ChREBP coordinately regulates sucrase by modulating the mRNA expression of sucrase and Glut5. However, measurements of blood glucose levels of

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KO mice that were fed a high sucrose diet over a medium to long term are not available.

In this study, WT and ChREBP KO mice were fed a high sucrose (50%) diet for 15 weeks and their body weights and blood glucose levels were evaluated. The findings indicate that, unlike WT mice, the body weight and blood glucose levels in ChREBP KO mice did not increase even when they were fed a high sucrose diet due to the low expression of sucrose in the small intestine. Based on these results, it would appear that inhibitors targeting ChREBP may be useful for addressing and controlling obesity and blood glucose levels.

Methods and Materials

Animal models. Homozygous ChREBP KO mice were a generous gift from Dr. Nakayama and Dr. Uyeda. Male C57BL/6j mice were purchased from Oriental Yeast, Co., Ltd. (Nagahama, Shiga, Japan) at the age of 4 weeks. All mice were housed in pathogen-free facilities at 22°C with a 12:12 h dark–light cycle with free access to water. Mice were assigned to different experimental groups. For dietary analyses, mice were housed at 22°C and fed either a standard chow (composed of 60% carbohydrate, 13% fat, and 27% protein on a caloric basis; Oriental MF, Oriental Yeast, Co., Ltd.), or high sucrose (50% MF, 50% sucrose; Oriental F2HScD, Oriental Yeast, Co., Ltd.) for 15 weeks (from 6 weeks of age to 20 weeks of age) (n = 8 per group). The high sucrose diet used in this study was 50% sucrose unless otherwise specified. The intake of food by the mice was measured as the difference in weight between the food put into the cage and that remaining at the end of 24 h. All experiments were approved by the Hyogo College of Medicine Animal care and Use Committee.

RNA extraction and RT-PCR. Total RNA was isolated from small intestine and liver samples using the Sepazol (Nacalai Tesque, Kyoto, Japan) reagent. The expression of different mRNAs of interest was evaluated by the reverse transcriptional polymerase chain reaction (RT-PCR) using a High RNA-to-cDNA Kit (Applied Biosystems).

Quantitative real-time PCR (qPCR). Relative semi-quantitative analysis of mRNA was performed using PCR using the specific primer sets listed below: mouse SGLT1 Forward, 5'-CGGAAGAAGCGATCTGAGAA-3', mouse SGLT1 Reverse, 5'-AATCAGACAGGATGAAACA-3'; mouse GLUT2 Forward, 5'-TCTTCCAGGCTGTCTTG-3', mouse GLUT2 Reverse,
5'-AATCATCCCCGTTAGGAACA-3', mouse GLUT5 Forward, 5'-AGAGCAACGATGGAGAAATAA-3', mouse GLUT5 Reverse, 5'-CCAGAGGAAGGACCAGTTC-3', mouse Sucrase Forward, 5'-ATCATCCCTACCCGGAAACC-3', mouse Sucrase Reverse, 5'-GCTGGTCATTTTTACACCCT-3'. The relative amounts of mRNA were calculated using the comparative CT method. Beta actin was used as an internal control.

**Measurement of blood glucose and fructose.** Blood glucose levels were measured using a portable blood glucose meter (Labo Gluco, Toronto, Canada). The total concentration of fructose and glucose (i.e., total hexose) was measured with a kit (ab83880; Abcam, Cambridge, UK), the concentrations of fructose were calculated from the corresponding concentration of both hexoses and glucose.

**Blood lipid contents.** Blood triglyceride levels were measured using the GK-GPO method according to the manufacturer's protocol by SRL (Tokyo, Japan). Blood cholesterol levels were measured using the COD-POD method according to the manufacturer’s protocol by SRL.

**Western blotting.** Protein samples were size-fractionated on a 10% SDS-PAGE gel and transferred to a PVDF membrane (Millipore, MA) by semi-dry blotting. The membranes were briefly rinsed with Tris-buffled saline containing 0.1% Tween 20 (TBS-T) and then incubated for 1 h at room temperature in TBS-T containing 5% skim milk (Difco, Detroit, MI). The blocked membrane was incubated with the primary antibody (Anti sucrase; Santa Cruz Biotechnology Inc., Santa Cruz, CA, 1:2,000) for 1 h at room temperature. Horseradish peroxidase-conjugated anti-mouse IgG (Zymed Laboratories Inc., South San Francisco, CA) was used as the secondary antibody, and proteins were visualized with ECL plus Western Blotting Detection Reagents (GE Healthcare, Piscataway, NJ) using LAS3000mini system (Fujifilm, Tokyo, Japan).

**Histological and IHC analysis.** Small intestine samples were fixed in 4% buffered formaldehyde (Wako, Osaka, Japan), paraffin-embedded, cut into 5-mm-thick sections and stained with standard H&E. Tissue sections were observed under a microscope at 20× magnification. Digital images were captured with a Leica DFC450 digital camera using LAS Software (Leica Microsystems Inc., Deerfield, IL). The sections were pretreated using heat-mediated antigen retrieval with a sodium citrate buffer (pH 6) for 30 min, then incubated with anti Sucrase-Isomaltase antibody (1:250 dilution; Santa Cruz Biocrude Laboratories, South San Francisco, CA) and analyzed by a fluorescence microscope (Leica DFC450, Wetzlar, Germany).

**Fig. 2.** Blood glucose levels in WT and ChREBP KO mice. (A) Blood glucose levels in the mice were measured at each age. Values are presented as the mean ± SE for 8–10 mice. Statistical significance: *p<0.01 vs high sucrose diet WT mice. (B) Concentration of cholesterol (total cholesterol (T-CHO), free cholesterol (F-CHO), and ester cholesterol (E-CHO)) in mice was measured at 20 weeks of age for both normal and high sucrose diets. Values are presented as the mean for 8–10 mice. Statistical significance: *p<0.05 vs WT mice, **p<0.01 vs WT mice.
Biotechnology Inc.) for 15 min at room temperature, with detection by a Dako-labeled streptavidin biotin-horseradish peroxidase kit.

**Sucrase activity.** Sucrase activity assays were performed using the small intestine according to the modified Dahlqvist’s method with minor modifications.20–22 Briefly, 20 mg of intestine was homogenized in 0.9% NaCl. A 20 μl aliquot of homogenates was kept at 37°C in the presence or absence of 5 μl of an inulin extract solution (10%, 5%, or 1%) or 5 μl of an acarbose solution (1%); 20 μl of substrate solution (0.056 M of sucrose) was added. After 1 h of incubation at 37°C, 25 μl of Tris buffer (3 M, pH 7.0) was added to stop the reaction. The fructose that was produced by the hydrolysis of sucrose was measured using a kit in which enzymatic reaction was coupled with the spectrophotometric detection of reaction end products (Diasys Diagnostic Systems, Holzheim, Germany). The amount of protein in the intestinal samples was determined by Lowry’s method.23

Sucrase activity was expressed as μmol of sucrose hydrolyzed into fructose/mg of protein per-minute. The inhibition was evaluated in vitro with increasing concentrations of sucrose in the presence of different concentrations of inulin extract. The type of inhibition was determined by a Lineweaver–Burk plot analysis of the data, from which the kinetics were calculated from the Michaelis–Menten equation.

**Data analysis.** Numerical values are expressed as the mean ± SEM. Student’s t test was used for evaluation of statistical significance. Data are presented as the mean ± SE and significance was set at p<0.05. In case of RT-PCR and Western blotting, one representative set of data is shown.

**Results**

**ChREBP KO mice gain less weight even on a high sucrose diet.** To examine how a high sucrose diet affects body weight in ChREBP KO mice, the mice were fed a normal or a high sucrose diet (50% sucrose) for 15 weeks. Body weights were measured once per week, and food intake per 24 h was measured at 12 and 20 weeks. As shown in the photograph in Fig. 1A, the ChREBP KO mouse was clearly thinner than the WT mouse that was given a high sucrose diet. WT mice showed dramatic increase in body weight at all points on the high sucrose diet, whereas ChREBP KO mice, on the contrary, did not gain weight on the high sucrose diet compared to the normal diet (Fig. 1A). On the other hand, the WT and ChREBP KO mice both showed similar weight gain curves when fed a normal diet. In this experiment, the amount of food consumption was the same between WT and ChREBP KO mice in the case of feeding a high sucrose diet (Fig. 1B).

**Blood glucose levels of ChREBP KO mice do not increase even on a high sucrose diet.** A high sucrose diet usually results in increased blood glucose levels. In fact, the high-sucrose diet increased the blood glucose levels of WT mice to over 200 mg/dl at all ages (Fig. 2A). In contrast, the blood glucose levels of the ChREBP KO mice were not increased despite being fed the high sucrose diet, and were somewhat lower than that for a normal diet (Fig. 2A). Namely, when ChREBP KO mice were fed a high sucrose diet, blood glucose levels were decreased somewhat.

**A ChREBP deficiency protects against sucrose induced lipid synthesis.** Blood cholesterol levels were next measured. As shown in Fig. 2B, in the case of WT mice, the concentration of total cholesterol increased by 1.8-fold in the case of the high sucrose diet [total cholesterol (T-CHO) = free cholesterol (F-CHO) + ester cholesterol (E-CHO)]. In contrast, there was no change in cholesterol content in the ChREBP KO mice.

**Blood fructose concentrations were decreased in ChREBP KO mice.** Blood fructose levels were measured for the ChREBP KO mice that were on high sucrose diets in an attempt to better understand the nature of the metabolism of glucose and fructose in these mice. Under the normal diet at 10 weeks of age, fructose levels were 0.95 ± 0.38 mg/dl in WT mice and 0.43 ± 0.26 mg/dl in the ChREBP KO mice (Fig. 3A). When a high sucrose diet was fed for 15 weeks (20 weeks of age), the fructose levels were measured once per week, and food intake per 24 h was measured.

![Fig. 3](image-url) **Blood fructose levels in WT and ChREBP KO mice.** (A) Concentration of blood fructose in mice was measured for mice at 10 weeks of age under a normal diet. Values are presented as the mean ± SE for 8–10 mice. (B) Concentration of blood fructose in mice was measured at 20 weeks of age under both normal and high sucrose diets. Values are presented as the mean ± SE of 8–10 mice.
levels in the WT mice were doubled on the normal diet. On the contrary, in the ChREBP KO mice, the fructose concentrations for the high sucrose diet were reduced to one-fourth that of the normal diet (Fig. 3B).

**Glut2 and Glut5 expression were decreased in the small intestines of ChREBP KO mice under a high sucrose diet.** In order to ascertain whether the decrease in blood fructose concentration was due to the inhibition of absorption from the small intestine, the level of expression of the glucose transporter in the small intestine was examined. The mRNA expression of Glut2 in the small intestine was reduced by half in the ChREBP KO mice, but there was no significant difference in the expression levels of Glut5 and SGLT1 in the case of the normal diet (Fig. 4). In addition to these results, when a high sucrose diet was continuously fed, the induction of Glut2 and Glut5 expression was observed in WT mice at 20 weeks of age, but neither of these proteins was induced in the ChREBP KO mice (Fig. 4). These data show that the impaired absorption of glucose and fructose is caused by the reduced expression of Glut2 and Glut5 in the small intestine in ChREBP KO mice, even when they are fed high sucrose diet.

**Both the level of expression and the activity of sucrose were suppressed in the small intestine of ChREBP KO mice.** Sucrose is degraded by sucrase in the small intestine, and it is then absorbed as glucose and fructose. It is well known that Glut5 expression is induced by fructose. To investigate the mechanism responsible for why the expressions of Glut2 and Glut5 are not induced in ChREBP KO mice on a high sucrose diet, the level of expression of sucrase itself was measured by quantitative real-time PCR and Western blotting. As shown in Fig. 5A and B, sucrase mRNA and the corresponding protein expression were induced by feeding a high sucrose diet in the WT mice. In contrast, sucrase mRNA was not induced in the ChREBP KO mice by feeding the high sucrose diet (Fig. 5A), and the sucrose protein levels were also much lower than those in WT mice under both the normal and high sucrose diets (Fig. 5B).

Similarly, also showed much lower levels of sucrose expression on the surface of the microvilli of the small intestine in ChREBP KO mice compared to WT mice, although H&E staining showed no significant difference between the mice (Fig. 5C). In addition, in the case of the WT mice, sucrase activity was increased in response to protein levels on a high sucrose diet, but remained low in the ChREBP KO mice (Fig. 5D). These results demonstrate that the digestion of sucrose is suppressed in the small intestine of ChREBP KO mice.

**Discussion**

The findings reported in this paper show that a ChREBP deficiency reduces the expression of sucrose, which leads to the digestion and absorption of sucrose being suppressed. Furthermore, as a result, the body weight, blood glucose and cholesterol levels in ChREBP KO mice did not increase even when a high sucrose diet was fed. A previous study of ChREBP KO mice on a normal diet exhibited phenotypic effects in which plasma glucose levels were slightly increased from 150 to 190 mg/dl, a 2-fold increase in liver glucose-6-P concentration and a 3-fold increase in liver glycogen compared with WT. In addition, the ChREBP KO mice also had markedly higher liver phosphoenolpyruvate and lower pyruvate concentrations, reflecting their lower LPK activity. As a result of the lower levels of most of the enzymes involved in the fatty acids synthesis pathway and also malic enzyme, the ChREBP KO mice had lower levels of epididymal fat, brown fat mass and serum free fatty acid levels. The hepatic triglyceride content of the ChREBP KO mice were essentially the same as that for WT mice, suggesting that the storage of dietary fat in these animals had not been altered. Moreover, attempts to feed ChREBP KO mice a 60% sucrose or a high fructose diet led to a further decrease in plasma free fatty acids and death by hypothermia and starvation, suggesting that the defect in fatty acids synthesis observed in ChREBP KO mice leads to a deficit in energy and heat producing substrates, i.e., free fatty acids.

Although ChREBP KO mice could not survive when the sucrose content of the diet was 60%, we found that they could survive when a 50% sucrose diet was fed. After feeding a 50% sucrose diet, the WT mice gained substantial amounts of body weight and this gain was age dependent. In contrast, no weight gain was observed in the ChREBP KO mice compared to a normal diet. We therefore conclude that if the expression of ChREBP is suppressed, then the increase in body weight and blood glucose levels can be inhibited. Based on these findings, two possibilities were considered in the case of the ChREBP KO
mice: sucrose is digested but the digested fructose and glucose are not absorbed or the possibility that the sucrose is not digested. Kato et al.\textsuperscript{15} previously reported that a 30% sucrose diet suppressed the mRNA level of sucrase in the small intestines of ChREBP KO mice. Consistent with their results, we also observed that the protein expression levels and enzymatic
activities, in addition to the mRNA levels of sucrase in the ChREBP KO mice, were much lower than those in the WT mice that were on a 50% sucrase diet (Fig. 5). In addition, the expression of both Glut2 and Glut5, which are related to the absorption of glucose and fructose, was lower in the ChREBP KO mice (Fig. 4). Namely, since ChREBP KO mice are not able to digest sucrase nor to absorb glucose and fructose (Fig. 4), they did not gain weight (Fig. 1), blood glucose and cholesterol (Fig. 2) on the high sucrose diet.

Curiously, the ChREBP KO mice that were on the high sucrose diet showed significantly lower blood fructose concentrations compared to the normal diet (Fig. 3B), although the enzymatic activity of sucrase was nearly the same (Fig. 5D). This can be attributed to the lower expression of Glut5, which mainly controls the absorption of fructose, in ChREBP KO mice (Fig. 4).

Another possible reason is that fructose is converted into glucose to maintain blood glucose levels because of the lower digestion of sucrose (Fig. 5). Fructose in the blood is known to be partially converted into glucose. Indeed, the blood glucose levels the ChREBP KO mice on the high sucrose diet were lower than those on the normal diet but normal levels were maintained (Fig. 2A).

Sucrase plays an important role in the digestion and absorption of sucrose in the small intestine. Analysis of the promoter region involved in the regulating the expression of sucrase revealed the presence of a potential sequence to which ChREBP could bind, but no exact match was found (data not shown). A further analysis of the promoter region of sucrase will be needed. The mechanism of sucrase gene expression was extensively investigated, and it has been reported that glucose negatively regulates sucrase gene expression through the hepatocyte nuclear factor1 (HNF-1).25,27 Because the blood glucose levels in ChREBP KO mice are slightly higher than that of WT mice on a normal diet,26 this study’s expression of sucrase may be suppressed. In addition, sucrose is a highly glycosylated 215 kDa membrane glycoprotein.27-29 Human sucrase has numerous N-linked sugar chain binding sites, and the addition of complex-type, high-mannose-type N-linked sugar chains, and O-linked sugar chains has been confirmed.30-32 Therefore, the addition of sugar chains may be involved in the stability and expression level of the sucrase protein itself. Considering these collective findings, it is highly possible that ChREBP is not directly involved in the expression of sucrase.

Blood sugar levels increase as glucose is absorbed by blood vessels. A rapid rise in blood glucose level after a meal is a risk factor for later complications including myocardial infarction and strokes. When food in the form of starch is taken into the body, it is converted into disaccharides and then into glucose by an enzyme called α-glucosidase, and is then absorbed into blood vessels, leading to an increase in blood sugar level. Inhibiting the action of α-glucosidase can delay the absorption of sugar into blood vessels and slow down the rise in postprandial blood glucose. However, the problem here is that glucosidase enzymes cleave a variety of glycosidic bonds in saccharides and have little substrate specificity. Because of this, the glucosidase inhibitors that are currently in use may cause unexpected side effects. Decreasing sucrose due to ChREBP inhibition would be expected to be more beneficial than existing glucosidase inhibitors because no change in body weight or blood glucose level would be observed in a normal diet and it is highly possible that the effect is only due to a high sucrose diet. Further studies will need to be conducted to determine the expression level of other digestive enzymes in the small intestine in ChREBP KO mice.

In summary, the findings of this study indicate that a ChREBP deficiency does not result in weight gain or elevated blood glucose levels in mice that are on a high sucrose diet. The underlying mechanism responsible for this can be attributed to deficiency of ChREBP, leading to the inhibition of the digestion and absorption of sucrose in the small intestine. Therefore, compounds that inhibit the activation of ChREBP would be promising therapeutic drugs for the treatment of obesity and type 2 diabetes in terms of suppressing the absorption of sucrose.

Author Contributions

HS and NF conceived and wrote the manuscript. HS, LL, MI, and HE designed and carried out experiments. DY performed the statistical analysis. HS and KS analyzed the data. All authors read and approved the final version of the manuscript.

Acknowledgments

This work was supported in part by Grant-in-Aid for Scientific Research C grant numbers 17K01883 (HS), 20K11570 (HS), and 18K11147 (KS), and in part supported by “Hyogo College of Medicine Diversity Grant for Research Promotion” under MEXT Funds for the Development of Human Resources in Science and Technology: “Initiative for Realizing Diversity in the Research Environment (Characteristic-Compatible Type).”

We wish to thank Dr. Milton S Feather from Technical Editing Services for editing a draft of this manuscript.

Conflict of Interest

No potential conflicts of interest were disclosed.

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