High-fructose diet-induced hepatic expression of the Scd1 gene is associated with increased acetylation of histones H3 and H4 and the binding of ChREBP at the Scd1 promoter in rats

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ABSTRACT
Stearoyl-CoA desaturase-1 (SCD1) is a key enzyme in the biosynthesis of monounsaturated fatty acids, and the expression of the Scd1 gene is induced by the intake of the lipogenic sugar fructose. We examined the effects of a high-fructose diet on hepatic acetylation of histones H3 and H4 and the binding of carbohydrate response element-binding protein (ChREBP) on the Scd1 gene promoter in rats. Rats were fed a control diet or a high-fructose diet for 10 days. The intake of a high-fructose diet significantly increased histone H3 and H4 acetylation and ChREBP binding to the Scd1 gene promoter as well as the amount of triglyceride and the expression of the Scd1 gene.

These results suggest that short-term intake of high fructose upregulates expression of Scd1 by enhancing acetylation of histones H3 and H4 and binding of ChREBP at the Scd1 promoter.

Stearoyl-CoA desaturase-1 (SCD1) is a rate-limiting enzyme that catalyzes the conversion of saturated fatty acids into monounsaturated fatty acids, which are highly expressed in lipogenic tissues, such as liver and adipose tissues (Ntambi and Miyazaki 2003). Several studies, including ours, have reported that expression of Scd1 as well as the major fatty acid synthesis genes, such as those encoding fatty acid synthase (Fasn), are upregulated in rodents with fatty liver maintained on a diet rich in the major lipogenic sugar, fructose, even for short durations (1–2 weeks) (Miyazaki et al. 2004; Shimada et al. 2017). In addition, Miyazaki et al. have demonstrated that Scd1-knockout mice prevent fatty liver induced by high-fructose (Miyazaki et al. 2004). Therefore, it is most likely that high-fructose-induced fatty liver is closely related to enhanced hepatic expression of the Scd1 gene. Because excessive intake of fructose promotes the development of fatty liver disease and subsequently leads to the onset and development of insulin resistance, which is closely associated with metabolic diseases, such as diabetes and obesity (Herman and Samuel 2016), elucidation of the regulatory mechanisms of hepatic Scd1 expression induced by high-fructose may help in shaping key strategies for metabolic diseases, including fatty liver.

Possible mediators of regulatory mechanisms underlying expression of Scd1 include histone modifications, such as acetylation and methylation, as well as transcription factors. In particular, hyperacetylation of histones H3 and H4 is associated with the euchromatin region of the genome (Schübeler et al. 2004) and induces transcription by recruiting transcriptional complexes to target genes (Roh et al. 2005). Several studies have shown that rat pups during the suckling-weaning transition (Morishita et al. 2014) and SHR/NDmc-cp rats, a metabolic syndrome model (Suzuki et al. 2015), exhibit not only...
enhanced expression of *Fasn*, but also hyperacetylated histones H3 and H4 on the gene in the liver. Considering the above-mentioned reports that high-fructose induces hepatic expression of *Scd1* as well as *Fasn*, we hypothesized that the intake of high-fructose may affect the acetylation of histones on the *Scd1* gene. In this study, we examined whether hepatic expression of the *Scd1* gene and the acetylation of histones H3 and H4 on the *Scd1* promoter in rats fed a high-fructose diet differed from those in rats fed a starch-diet (control). In addition, we further examined the binding of a key lipogenic transcriptional factor, carbohydrate-responsive element-binding protein (ChREBP), because we recently showed that feeding of a high-fructose diet to rats increased the binding of ChREBP to the *Fasn* promoter (Shimada *et al.* 2019).

Four-week-old male Wistar rats (Japan SLC, Inc., Shizuoka, Japan) received a control diet (*n* = 6) or a high-fructose diet (*n* = 8) for 10 days. The diets were AIN93G-based and contained 64.9% (w/w) carbohydrate (64.9% α-cornstarch in the control diet for a short duration (10 days) significantly increased hepatic triglyceride levels and the expression of *Scd1* in rats (Fig. 1A and 1B). In addition, we showed that high-fructose induced acetylation of histones H3 and H4 on the *Scd1* promoter (Fig. 2A and 2B). Several studies have reported that the perfusion and acute administration of fructose solution, compared with those of glucose, increased acetylation of histones H3 and H4 on a key fructose transporter gene, solute carrier family 2, member 5 (*Slc2a5*), known as Glut5, and the expression of the gene in rat and mouse jejunum (Suzuki *et al.* 2011; Yoshinaga *et al.* 2012; Honma *et al.* 2013). Based on the fact that fructose is absorbed via GLUT5 in the small intestine and is rapidly used for *de novo* fatty acid synthesis in the liver, it is likely that a high-fructose signal effectively induces histone acetylation on fatty acid synthesis genes, including *Scd1* in the liver, as well as *Slc2a5* in the small intestine.

ChREBP is activated in response to glucose efflux and binds to carbohydrate response elements (ChoREs) in the promoters of fatty acid synthesis genes to induce their expression (Rufo *et al.* 2001; Yamashita *et al.* 2001). In the present study, we found that ChREBP bound to the promoter regions (−740, −480, and −90 bp), around which each ChoRE-like sequence was located, in rat liver, and that rats fed a high-fructose diet showed remarkably increased binding of ChREBP to the *Scd1* promoter regions (−480 and −90 bp) (Fig. 2C). To the best of our knowledge, this is the first *in vivo* demonstration of histone acetylation in response to carbohydrate metabolism.
Scd1 regulation by fructose

Moreover, Erion et al. demonstrated that ChREBP-knockdown reduced the hepatic expression of Scd1 and Fasn in rats fed a high-fructose diet (Erion et al. 2013). Thus, considering the above-mentioned reports and our data, it is likely that high-fructose-induced ChREBP also regulates Scd1 in the liver. It should be examined further by a DNAase footprinting assay whether a high-fructose diet enhancing the hepatic binding of ChREBP as well as the acetylation of histones H3 and H4 to the Scd1 promoter in rats. Janevski et al. showed that intake of a high-fructose diet compared with a high-glucose diet promoted ChREBP nuclear translocation in rat liver (Janevski et al. 2012). In addition, we previously reported that feeding of a high-fructose diet, compared with that of a high-glucose diet, increased the hepatic binding of ChREBP to the ChoRE of the Fasn promoter region in rats (Shimada et al. 2019). Moreover, Erion et al. demonstrated that ChREBP-knockdown reduced the hepatic expression of Scd1 and Fasn in rats fed a high-fructose diet (Erion et al. 2013). Thus, considering the above-mentioned reports and our data, it is likely that high-fructose-induced ChREBP also regulates Scd1 in the liver. It should be examined further by a DNAase footprinting assay whether...
er high-fructose-induced ChREBP binds to the ChoRE-like sequences around −480 and −90 bp of the Scd1 gene. However, the relationship between histone acetylation and ChREBP on the Scd1 gene in the liver of rats fed a high-fructose diet remains unclear. Burke et al. (2009) showed that glucose signaling formed the complex composed of ChREBP and CREB-binding protein (CBP), a histone acetyltransferase, on a carbohydrate-responsive gene, pyruvate kinase in insulinoma cells. In addition, we previously reported that intake of a high-starch diet, compared with that of a low-starch diet, enhanced not only the acetylation of histones H3 and H4, but also the binding of CBP at the carbohydrate-responsive gene, maltase-glucoamylase in mouse jejunum (Mochizuki et al. 2010). Therefore, lipogenic signals via intake of high-carbohydrate, especially that of high-fructose, may induce the formation of ChREBP-CBP complex and acetylate histones in the Scd1 gene in the liver. This hypothesis requires further investigation.

In conclusion, we demonstrated in the present study that short-term intake of high-fructose diet increases expression of Scd1 gene, and acetylation of histones H3 and H4 and binding of ChREBP at the Scd1 promoter in rat fatty liver. The results suggest that short-term intake of high fructose upregulates expression of Scd1 gene by enhancing acetylation of histones H3 and H4 and binding of ChREBP at the Scd1 promoter. Therefore, the regulation of hepatic expression of Scd1 gene may be useful for the prevention of high-fructose-induced fatty liver and the subsequent insulin resistance.

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CONFLICT OF INTERESTS

There are no conflicts of interest to declare.

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