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# Fat and carbohydrate in western diet contribute differently to hepatic lipid accumulation

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ABSTRACT

We investigated the contributions of dietary fat and dietary carbohydrate to the development of fatty liver induced by western diet (WD). Compared with WD-fed wild type (WT) mice, livers of WD-fed ChREBP$^{-/-}$ mice showed lipid droplets of varying sizes around the hepatic lobules, while hepatic triglyceride and cholesterol contents were only modestly decreased. Inflammation and fibrosis were suppressed in ChREBP$^{-/-}$ mice. In addition, compared with WD-fed WT mice, ChREBP$^{-/-}$ mice showed decreased β-oxidation, ketogenesis and FGF21 production, increased intestinal lipid absorption, and decreased VLDL secretion. These findings suggest that dietary fat and carbohydrate contribute differently to the development of fatty liver.



## 1. Introduction

High fat/high carbohydrate diets, i.e., a "western diet," are associated with non-alcoholic fatty liver disease (NAFLD), which is characterized by hepatic lipid accumulation not due to excess alcohol intake [1–4]. The pathophysiology of NAFLD involves (1) increased *de novo* synthesis of fatty acids in hepatocytes, (2) retention of lipids due to impaired hepatocyte apolipoprotein secretion, (3) β-oxidation of fatty acids and (4) excess dietary fat and carbohydrate intake [1–4]. Excess dietary glucose is primarily converted into triglyceride (TAG). *De novo* lipogenesis is regulated by insulin and glucose through activation of SREBP1c and carbohydrate response element binding protein (ChREBP), respectively [3,5–7]. ChREBP contributes in about half of the hepatic *de novo* lipogenesis [3,5–7]. Moreover, we and other groups have previously reported that ChREBP deletion in *ob/ob* mice improves weight gain, glucose intolerance, and fatty liver [8,9]. Considered together with the finding that fatty acid suppresses ChREBP transactivity through AMPK activation in hepatocytes [10,11], ChREBP may well act as an important mediator of dietary carbohydrate action in the development of fatty liver.

Here we examine the roles of dietary fat and dietary glucose on hepatic steatosis using ChREBP knockout mice. Some groups have compared the effects of feeding a high fat diet or a high carbohydrate diet on the development of NAFLD. However, an entirely carbohydrate diet or high fat diet is rarely taken, while a high fat/high carbohydrate diet, i.e. a western diet (WD), is often chosen. To better understand the mechanism of fatty liver development induced by WD, we investigated the roles of dietary fat and dietary carbohydrate in WD feeding with particular attention to ChREBP action.

## 2. Materials and methods

### 2.1. Animals, western diet feeding and tissue preparation

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. ChREBP$^{-/-}$ mice were backcrossed for at least 10 generations into the C57BL/6J background [12]. Male mice were used for all studies, and all experiments were performed using littermates. Mice had free access to water and were fed an autoclaved CE-2 diet (CLEA Japan, Tokyo, Japan) as the normal diet (ND). A high fat/high carbohydrate/high cholesterol diet called WD (western

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diet) was purchased from Research Diets (New Brunswick, NJ, USA). The proportions of calories derived from nutrients were as follows: 28.9 kcal% protein, 12.0 kcal% fat, 59.1 kcal% carbohydrate with 3.4 kcal/g energy density for ND and 17 kcal% protein, 41 kcal% fat, 42 kcal% carbohydrate with 4.7 kcal/g energy density for WD. WD also contained 34 g% sucrose and 1.0 g% cholesterol. Wild type (WT) and ChREBP$^{-/-}$ mice were separated and housed three mice per cage. Body weight was measured weekly between the ages of 7 and 21 weeks. Beginning at 8 weeks of age, the normal chow diet was discontinued and the mice were fed WD. Mice were killed at 21 weeks of age by cervical dislocation. All tissue samples were immediately placed into liquid nitrogen and stored at −80 °C until further analysis for hepatic TAG and cholesterol content and for quantitative PCR.

### 2.2. Measurement of liver triglyceride and cholesterol content and plasma profile

Liver lipids were extracted using the method of Bligh and Dyer [13] and measured using Triglyceride E-test (Wako Pure Chemicals, Osaka, Japan) and Cholesterol E-test (Wako). Blood plasma was collected from the retro-orbital venous plexus *ad libitum* or after a 6-h fast. Blood glucose and beta-hydroxybutyrate (β-OHB) were measured using a FreeStyle Freedom monitoring system (Nipro, Osaka, Japan). Plasma insulin, free fatty acid, fibroblast growth factor 21 (FGF21), triglyceride, and total cholesterol levels were determined using commercial assay kits as follows: mouse insulin ELISA kit (H type) (Shibayagi, Gunma, Japan), NEFA C-test (Wako Pure Chemicals, Tokyo, Japan), mouse/rat FGF21 ELISA kit (R&D Systems, Minneapolis, MN), Triglyceride E-test (Wako Pure Chemicals, Osaka, Japan), and Cholesterol E-test (Wako Pure Chemicals, Osaka, Japan).

### 2.3. RNA isolation and quantitative real-time PCR

Total RNA isolation, cDNA synthesis and real time PCR analysis were performed as previously described [14]. Real time PCR primers for mouse *Chrebp*, fatty acid synthase (*Fas*), liver type pyruvate kinase (*Pklr*), acyl CoA oxidase (*Acox*), *Fgf21*, peroxisome proliferator-activated receptor alpha (*Ppara*), microsomal triglyceride transfer protein (*Mttp*), sterol regulatory element-binding protein 1 c (*Srebp1c*), sterol regulatory element-binding protein 2 (*Srebp2*), 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*Hmgcr*), Niemann-Pick C1 Like 1 (*Npc1l1*), Cd36, diacylglycerol acyl transferase 2 (*Dgat2*), tumor necrosis factor alpha (*Tnfa*), monocyte chemotactic protein 1 (*Mcp1*), Cd68, tissue inhibitor of metalloproteinase (*Timp*), transforming growth factor beta 1 (*Tgfb1*), collagen 1 (*Col1*) and RNA polymerase II (*Pol2*) were previously reported [14–21]. All amplifications were performed in triplicate. The relative amounts of mRNA were calculated using the comparative CT method. Expression of Pol2 was used as an internal control.

### 2.4. Intestinal lipid absorption test and VLDL secretion test

Intestinal lipid absorption test and VLDL secretion test were performed according to previously reported papers [22,23]. Briefly, 200 μl olive oil was orally administered to 18 h-starved mice 30 min after administration of 500 mg/kg BW tyloxapol (Sigma). As with the VLDL secretion test, 500 mg/kg BW tyloxapol was administered intra-peritoneally to 5 h-starved mice. Blood sampling was performed at the indicated times. The intestinal lipid absorption rate (mg/dl/h) was calculated as TG at 3 h − TG at 2 h, because plasma TG and time (h) have a good linear correlation ($R^2 = 0.99$) over 1–4 h. Similarly, the VLDL secretion rate (mg/dl/h) was calculated as TG at 3 h − TG at 2 h, because of a good linear correlation ($R^2 = 0.98$) over 1.5–3.0 h.

### 2.5. Statistical analysis

All values are presented as mean ± standard deviation. Data were analyzed using Student's *t*-tests. A *p*-value <0.05 was considered statistically significant.

## 3. Results

### 3.1. ChREBP deletion fails to alleviate WD diet-induced fatty liver

WT and ChREBP$^{-/-}$ mice were fed with ND and WD. As anticipated, WD feeding induced obesity in WT mice. In contrast, the average body weight of WD-fed ChREBP$^{-/-}$ mice was lower than that in wild type and in ND-fed ChREBP$^{-/-}$ mice (ND-fed WT mice 30.35 ± 2.27 g, ND-fed ChREBP$^{-/-}$ mice 30.68 ± 2.42 g, WD-fed WT mice 35.91 ± 3.48 g, BW, WD-fed ChREBP$^{-/-}$ mice 29.71 ± 2.69 g (Supplementary Table 1). WD-fed ChREBP$^{-/-}$ mice exhibited increased hepatomegaly (ND-fed WT mice 4.52 ± 0.36% BW, ND-fed ChREBP$^{-/-}$ mice 5.2 ± 0.5% BW, WD-fed WT mice 5.69 ± 0.45% BW, WD-fed ChREBP$^{-/-}$ mice 8.24 ± 0.31% BW) and lower adiposity compared with WD-fed WT mice (Supplementary Table 1). Compatible with these findings, the food intake of WD-fed ChREBP$^{-/-}$ mice was much lower than that of WD-fed WT mice (ND-fed WT mice 3.42 ± 0.32 g, ND-fed ChREBP$^{-/-}$ mice 3.83 ± 0.35 g, WD-fed WT mice 3.24 ± 0.80 g, WD-fed ChREBP$^{-/-}$ mice 2.85 ± 0.32 g) (Supplementary Table 1). Livers of WT mice had histologically small fat droplets near the central vein and large fat droplets near the portal vein (Fig. 1A). In contrast, livers of ChREBP$^{-/-}$ mice had lipid droplets of various sizes around all of the hepatic lobules (Fig. 1A). In addition, liver triglyceride and cholesterol content in ChREBP$^{-/-}$ mice were lower than those in WT mice (Fig. 1B and C). These data suggest the development of hepatic steatosis in WD-fed ChREBP$^{-/-}$ mice.

### 3.2. Decreased β−oxidation and ketogenesis in WD-fed ChREBP$^{-/-}$ mice

We then examined the effects of ChREBP on β−oxidation and ketogenesis. After 24 h starvation, plasma glucose levels of WD-fed ChREBP$^{-/-}$ mice exhibited a tendency to be lower than those of WD-fed WT mice (Fig. 2A). Moreover, plasma insulin levels of WD-fed ChREBP$^{-/-}$ mice were significantly lower than those of WD-fed WT mice (Fig. 2B). In WD-fed ChREBP$^{-/-}$ mice, the HOMA-R insulin sensitivity index was significantly improved (ND-fed WT mice 0.90 ± 0.42, ND-fed ChREBP$^{-/-}$ mice 0.77 ± 0.40, WD-fed WT mice 3.87 ± 0.87, WD-fed ChREBP$^{-/-}$ mice 0.93 ± 0.77) [24]. In addition, in WD-fed ChREBP$^{-/-}$ mice, 24 h-fasted FFA levels tended to be lower than those in WT mice (Fig. 2C) and 24 h-fasted plasma β-OHB levels were lower than those in WT mice (Fig. 2D). Furthermore, plasma FGF21 levels were lower in WD-fed ChREBP$^{-/-}$ mice than those in WD-fed WT mice (Fig. 2E). Consistent with these findings with respect to plasma β-OHB and FGF21 levels, *Acox*, *Ppara*, and *Fgf21* mRNA tended to be decreased in WD-fed ChREBP$^{-/-}$ mice (Fig. 2F).

### 3.3. Increased intestinal lipid absorption and decreased VLDL formation in WD-fed ChREBP$^{-/-}$ mice

Plasma TG levels in ND and WD-fed ChREBP$^{-/-}$ mice were lower than those in ND and WD-fed WT mice. In contrast, plasma cholesterol levels in WT mice were similar to those of ChREBP$^{-/-}$ mice (Fig. 3A and B).