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Characterization of fat metabolism in the fatty liver caused by a high-fat, low-carbohydrate diet: A study under equal energy conditions

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A B S T R A C T

The pathology of fatty liver due to increased percentage of calories derived from fat without increased overall caloric intake is largely unclear. In this study, we aimed to characterize fat metabolism in rats with fatty liver resulting from consumption of a high-fat, low-carbohydrate (HFLC) diet without increased caloric intake. Four-week-old male Sprague-Dawley rats were randomly assigned to the control (Con) and HFLC groups, and rats were fed the corresponding diets ad libitum. Significant decreases in food intake per gram body weight were observed in the HFLC group compared with that in the Con group. Thus, there were no significant differences in body weights or caloric intake per gram body weight between the two groups. Marked progressive fat accumulation was observed in the livers of rats in the HFLC group, accompanied by suppression of de novo lipogenesis (DNL)-related proteins in the liver and increased leptin concentrations in the blood. In addition, electron microscopic observations revealed that many lipid droplets had accumulated within the hepatocytes, and mitochondrial numbers were reduced in the hepatocytes of rats in the HFLC group. Our findings confirmed that consumption of the HFLC diet induced fatty liver, even without increased caloric intake. Furthermore, DNL was not likely to be a crucial factor inducing fatty liver with standard energy intake. Instead, ultrastructural abnormalities found in mitochondria, which may cause a decline in β-oxidation, could contribute to the development of fatty liver.

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1. Introduction

Fatty liver has various causes, and the specific mechanisms underlying fatty liver remain unknown. The causal factors of diet-related fatty liver include excessive eating, excessive intake of fats, excessive intake of carbohydrates, deficiencies in vitamins and minerals, and malnutrition [1]. Although fatty liver is associated with obesity, recent studies have reported that fatty liver is not unusual in individuals who are not obese, not diabetic, and do not have metabolic syndrome [2,3]. Increases in the percentage of calories derived from fat have been identified as a problem in the modern Japanese diet [4]; however, this is not accompanied by changes in total caloric intake or body weight. Therefore, elucidation of the pathogenic mechanisms of fatty liver related to the percentage of calories derived from fat is needed.

Many studies have examined the occurrence of fatty liver due to excessive intake of fat, and the consumption of a high-fat diet

Abbreviations: HFLC, high-fat, low-carbohydrate; DNL, de novo lipogenesis; SD, Sprague-Dawley; GOT, glutamic-oxaloacetic transaminase; GPT, glutamic-pyruvic transaminase; TG, triglyceride; H&E, hematoxylin and eosin; DTT, dithiothreitol; PMSF, phenylmethylsulfonylfluoride; CST, Cell Signaling Technology; ATP-CL, ATP-citrate lyase; FAS, fatty acid synthase; DGAT2, diacylglycerol O-acyltransferase 2; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; PVDF, polyvinylidene difluoride; HRP, horseradish peroxidase; SEM, standard error of the mean.

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has been often used for induction of fatty liver in experimental animal models [5]. However, in these models, body and fat mass increase primarily because of the increased total caloric intake in mice and rats [6–8]. Therefore, it is unclear whether total caloric intake or fat intake is more important for the development of fatty liver. Accordingly, studies that can enhance fat-derived caloric intake without increasing overall caloric intake are needed; examination of the features of fatty liver under such a design would allow for elucidation of the pathogenic mechanisms of fatty liver and may facilitate the development of strategies for prevention of fatty liver.

In a previous study, obesity was not observed in young Sprague-Dawley (SD) rats consuming a high-fat diet owing to reduced food intake [9]. Therefore, in this study, we examined fat metabolism in fatty livers from nonobese SD rats consuming a high-fat, low-carbohydrate (HFLC) diet without increased caloric intake.

2. Materials and methods

2.1. Experimental design

Our study was approved by the Biological and Epidemiologic Research Committee for Animal Use of Wayo Women's University (Ichikawa, Japan). All procedures and protocols followed the standard guidelines for the care and use of laboratory animals. Male SD rats [Crl: CD (SD)] were purchased at 3 weeks of age (47.0–55.4 g body weight) from Charles River Laboratories Japan, Inc. (Yokohama, Japan). All animals were housed in individual cages in an animal room with controlled temperature (20.9 ± 0.4 °C) and humidity (44.6% ± 2.0%) and with a 12-h light (08:00–20:00)/dark (20:00–08:00) cycle. After a 1-week acclimation period, SD rats (4 weeks of age) were allocated to the control (Con) or HFLC groups using computer-generated randomization. The Con group received a standard rodent diet (10% fat, 70% carbohydrates, and 20% protein in kcal%; 3.84 kcal/g; #D12450B; Research Diets, Inc., New Brunswick, NJ, USA), and the HFLC group received a high-fat diet (60% fat, 20% carbohydrates, and 20% protein in kcal%; 5.24 kcal/g; #D12492; Research Diets, Inc.). Animals were fed the appropriate diet ad libitum and had free access to water. Body weight and food consumption were monitored daily throughout the study period.

2.2. Sampling

At 12 weeks of age, all animals were fasted overnight to exclude the influence of diet until the day before sampling. The rats were then anesthetized with isoflurane, and blood samples were collected into serum gel separator tubes (VENOJECT II plastic vacuum tubes; Terumo, Tokyo, Japan) and centrifuged to separate the serum for blood chemistry tests. The liver was rapidly resected from the anesthetized rats. The rats were then sacrificed by fatal exsanguination. A portion of the collected hepatic tissue was immersed in 10% formalin and 2.5% glutaraldehyde for histological analysis and electron microscopy, respectively. The remaining tissues were frozen in liquid nitrogen and stored at −80 °C until biochemical analysis.

2.3. Serum analysis

Serum levels of glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), and leptin were measured using commercially available kits according to the manufacturer's instructions (GOT and GPT: Dri-Chem 4000, Fujifilm Corp., Tokyo, Japan; leptin: YK050 Rat Leptin ELISA Kit, Yanaihara Institute, Shizuoka, Japan).

2.4. Liver tissue preparation for triglyceride (TG) analysis

To extract lipids, frozen liver samples were powdered and homogenized with a 2:1 (v/v) chloroform-methanol mixture [10]. Hepatic TG levels were determined using a kit (TG E-Test; Wako Pure Chemical Industries Ltd., Osaka, Japan).

2.5. Histological examination

Formalin-fixed liver samples were sent to SRL Inc. (Tokyo, Japan) for embedding with paraffin and staining with hematoxylin and eosin (H&E). Photomicrographs of the sections were made for digital storage using a microscope equipped with a CCD camera.

2.6. Electron microscopy

Hepatic tissues were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4), postfixed with 1% osmium tetroxide in the same buffer, dehydrated in ethanol, and embedded in Epon 812 (TAAB, England). Ultrathin sections were cut, stained with uranyl acetate followed by lead citrate, and examined with a JEOL 1220 electron microscope (JEOL Ltd., Japan).

2.7. Western blotting

The expression of ATP-citrate lyase (ATP-CL), fatty acid synthase (FAS), and diacylglycerol O-acyltransferase 2 (DGAT2) was examined by western blotting. Frozen hepatic tissues (approximate 60–70 mg) were homogenized in seven volumes of ice-cold homogenization buffer (Chaps buffer; Cell Signaling Technology Inc. [CST], MA, USA) containing phenylmethylsulfonylfluoride (PMSF; CST), dithiothreitol (DTT; Wako Pure Chemical Industries Ltd.), protease inhibitor (Roche Diagnostics GmbH, Mannheim, Germany), and phosphatase inhibitor (Roche Diagnostics GmbH). Homogenates were centrifuged at 10000 × *g* for 20 min at 4 °C to collect supernatants. Protein concentrations were determined using a BCA Protein Assay Kit (Bio-Rad Laboratories, CA, USA). Approximately 10 μg of total protein was applied to each lane on 7.5–15% polyacrylamide gels and separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were then electrotransferred onto polyvinylidene difluoride (PVDF) membranes. Membranes were blocked with 5% nonfat dry milk and then incubated for 1 h at room temperature with the following primary antibodies: *anti*-ATP-CL (1:1000; CST), *anti*-FAS (1:1000; CST), *anti*-DGAT2 (1:1000; Abcam plc, Cambridge, UK), and *anti*-β-actin (1:1000; CST). Membranes were then incubated with anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibodies (1:4000; CST) for 1 h at room temperature. Proteins were detected using ECL Prime reagent (GE Healthcare, Piscataway, NJ, USA). The signals were captured using an imaging system (GE Healthcare). Densitometry analysis was conducted using Image Quanr TL (Ver.8.1.) software (GE Healthcare).

2.8. Statistical methods

Student's *t* tests were used to analyze individual differences. All values are expressed as means ± standard errors of the means (SEMs). Differences with *P* values of less than 0.05 were considered statistically significant.

3. Results

3.1. Animal characteristics

The results of body weight and food intake analyses are shown

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