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Diet-Induced Fatty Hepatic Steatosis in Male Spontaneously Diabetic Torii (SDT) Fatty Rats, A Genetic Model for Obese Type 2 Diabetes

T. Ohta[1*], Y. Motohashi[1], K. Miyajima[1], Y. Kemmochi[1], Y. Ishii[1], M. Shinohara[2] and T. Yamada[3]

[1]Japan Tobacco Inc., Central Pharmaceutical Research Institute, Osaka, Japan.
[2]CLEA Japan Inc., Planning and Development Section, Tokyo, Japan.
[3]Laboratory of Animal Genetics, Graduate School of Science and Technology, Niigata University, Niigata, Japan.

Authors' contributions

This work was carried out in collaboration between all authors. Authors TO, MS and TY designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors TO, YM, KM, YK and YI managed the analyses of the study, and performed the statistical analyses. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The Spontaneously Diabetic Torii (SDT) fatty rat is a metabolic syndrome model, showing obesity, hyperglycemia, dyslipidemia, and hypertension. Moreover, female SDT fatty rats exhibit hepatic steatosis. In this study, metabolic abnormalities, particularly in the liver, were assessed in male SDT fatty rats fed a diet containing 40% fat and 2% cholesterol (HFC-diet).
Location and Duration of Study: Niigata University, CLEA Japan and JT Central Pharmaceutical Research Institute, between January and December 2014.
Methodology: Male SDT fatty rats in control and HFC groups were fed a standard or HFC-diet (40% fat and 2% cholesterol, based on percentage of total calories) from 5 to 17 weeks of age, respectively. Body weight and blood chemistry parameters were periodically measured and a pathological analysis of the liver was performed at 17 weeks of age.

**Corresponding author: Email: takeshi.ota@jt.com;*

Results: In biological analyses, the HFC group showed increases in body weight, blood insulin, and total cholesterol during the experimental period and an increase in aspartate aminotransferase (AST) at 13 weeks of age. Blood glucose levels in HFC group decreased after 13 weeks of age. In pathological examinations, an increase in liver weight and hepatic steatosis, fatty change and hypertrophy in hepatocyte, were observed in the HFC group. Hepatic steatosis was not observed in the standard-diet group.
Conclusion: Male SDT fatty rats fed an HFC-diet may serve as a new nonalcoholic fatty liver disease (NAFLD) model.

Keywords: Hepatic steatosis; NAFLD; NASH; SDT fatty rat.

1. INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a common cause of liver diseases and is a hepatic manifestation of metabolic syndrome [1,2]. NAFLD consists of two pathological features: simple steatosis, which follows a benign non-progressive clinical course, and nonalcoholic steatohepatitis (NASH). NASH is a severe form of NAFLD and is considered to progress to liver cirrhosis and hepatocellular carcinoma [3,4]. With an increase in the number of patients showing metabolic diseases, including obesity, diabetes, and dyslipidemia, there is a concern regarding the prevalence of NAFLD and NASH [5]. Currently, however, there are no drugs specifically approved for the treatment of NAFLD/NASH. Animal models to recapitulate essential features of human diseases are required to develop novel therapies, including the development of new drugs for NAFLD/NASH.

The Spontaneously Diabetic Torii (SDT) fatty rat is an obese type 2 diabetic model that shows diabetes and its complications, such as retinopathy, nephropathy, and neuropathy [6-9]. Female SDT fatty rats exhibit hepatic steatosis; however, male SDT fatty rats do not exhibit fatty liver changes under the condition of standard diet feeding [10]. Progression of diabetes, including a decrease in blood insulin and an increase in blood glucose, was observed earlier in male SDT fatty rats than in the female rats, and diabetic grade was more critical in the male rats [11]. Male SDT fatty rats are expected to show fatty liver changes by inducing insulin resistance under the condition of high fat-diet feeding.

In this study, we intended to induce fatty liver diseases in male SDT fatty rats using a diet containing 40% fat and 2% cholesterol (HFC-diet).

2. MATERIALS AND METHODS

2.1 Animals and Diets

Male SDT fatty rats (CLEA Japan, Tokyo, Japan) were used in the study. Rats were divided into two groups at 5 weeks of age, a control group (CRF-1 group) and an HFC group (n=5). Rats in the control group were fed a standard diet (CRF-1, Charles River Japan, Yokohama, Japan), and rats in the HFC group were fed a high-fat/cholesterol diet (40% fat and 2% cholesterol, based on percentage of total calories, D09100301, Research Diets Inc., New Brunswick, NJ), from 5 to 17 weeks of age. The energy content of standard and high-fat/cholesterol diets was 3.59 and 4.06 kcal/g, respectively. Table 1 presents the composition of these diets. Animals were housed in a controlled room (with a 12 h lighting cycle) and allowed free access to water.

2.2 Biological Parameters

Food intake and body weight of the rats from 5 to 17 weeks of age were measured every four weeks. Blood chemistry parameters, including glucose, insulin, triglyceride (TG), total cholesterol (TC), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), were also evaluated every four weeks. Blood samples were collected from the tail vein of non-fasted rats. Serum glucose, TG, TC, ALT and AST were measured using commercial kits (Roche Diagnostics, Basel, Switzerland) and an automatic analyzer (Hitachi, Tokyo, Japan). Serum insulin was measured using a rat-insulin enzyme-linked immunosorbent assay (ELISA) kit (Morinaga Institute of Biological Science, Yokohama, Japan). Since there were multiple rats in each cage, calorie intake was calculated by dividing the total calorie intake of all animals in a cage by the number of animals per cage.

Table 1. Composition of experimental diets

Standard diet (CRF-1)		High-fat/cholesterol diet (D09100301)	
Ingredient	**g/100g**	**Ingredient**	**g/100g**
Water	8.2	Casein	22.1
Crude Protein	21.9	L-Cystine	0.3
Crude Fat	5.4	Maltodextrin 10	11.1
Crude Ash	6.3	Fructose	22.1
Crude Fiber	2.9	Sucrose	10.6
Nitrogen free extract	55.3	Soybean Oil	2.8
		Lard	2.2
Total	100.0	Primex shortening	14.9
		Mineral Mix	1.1
		Dicalcium phosphate	1.4
		Calcium carbonate	0.6
		Potassium citrate	1.8
		Vitamin mix	1.1
		Choline bitartrate	0.2
		Cholesterol	2.0
		FD&C yellow dye	0.1
		Total	99.9

CRF-1 diet was supplied by Charles River Japan and D09100301 was procured from Research Diets Inc

2.3 Tissue Sampling and Histopathology

Necropsy was performed when animals were 17 weeks of age, and liver weights were measured. These organs were fixed in 10% neutral buffered formalin. After resection, tissues were paraffin-embedded using standard techniques and subsequently thin-sectioned (3 to 5 μm). Sections were stained with hematoxylin and eosin (HE).

2.4 Statistical Analysis

Results of biological parameters and liver weight, with the exception of calorie intake, were expressed as the mean ± standard deviation (SD). Statistical analyses of differences between mean values were performed using an F-test, followed by a Student's t-test or Aspin-Welch's t-test. Differences were defined as significant at $p<0.05$.

3. RESULTS

3.1 Biological Parameters

Body weight in the HFC group increased after animals reached 9 weeks of age compared with weight in the CRF-1 group (Fig. 1A). Calorie intake in the HFC group was higher than intake in the CRF-1 group when animals were 13 and 17 weeks of age (HFC group, 248 kcal/body vs. CRF-1 group, 192 kcal/body, at 13 weeks of age). Serum glucose levels in the HFC group remained high (>700 mg/dl) during the experimental period; however, these levels decreased after rats reached 13 weeks of age compared with results in the CRF-1 group (Fig. 1B). Serum insulin levels in the HFC group significantly increased when animals were 9 weeks of age compared with results in the CRF-1 group (HFC group, 67.9±18.6 ng/ml vs. CRF-1 group, 40.8±14.0 ng/ml), and hyperinsulinemia persisted until the animals were 17 weeks of age (Fig. 1C). Serum TG levels in the HFC group were comparable with results in the CRF-1 group during the experimental period; however, serum TC levels in the HFC group were significantly elevated when animals were 9 weeks of age (HFC group, 314.7±40.8 mg/dl vs. CRF-1 group, 127.2±8.4 mg/dl), and a sustained increase in TC levels was observed (Figs. 2A and 2B). Serum ALT levels in the HFC group were comparable with results in the CRF-1 group, and serum AST levels in the HFC group significantly increased when animals were 13 weeks of age (Figs. 2C and 2D).

3.2 Pathology

Liver weight in the HFC group significantly increased compared with that in the CRF-1 group (Table 2). In histopathological analysis, severe fatty liver changes in hepatocytes were observed in all rats in the HFC group. However, fatty changes were not observed in the CRF-1 group (Table 3, Fig. 3). Moderate or slight hypertrophy of hepatocytes was also observed in the HFC group. Furthermore, an infiltration of inflammatory cells was observed in two rats in the HFC group (Table 3).

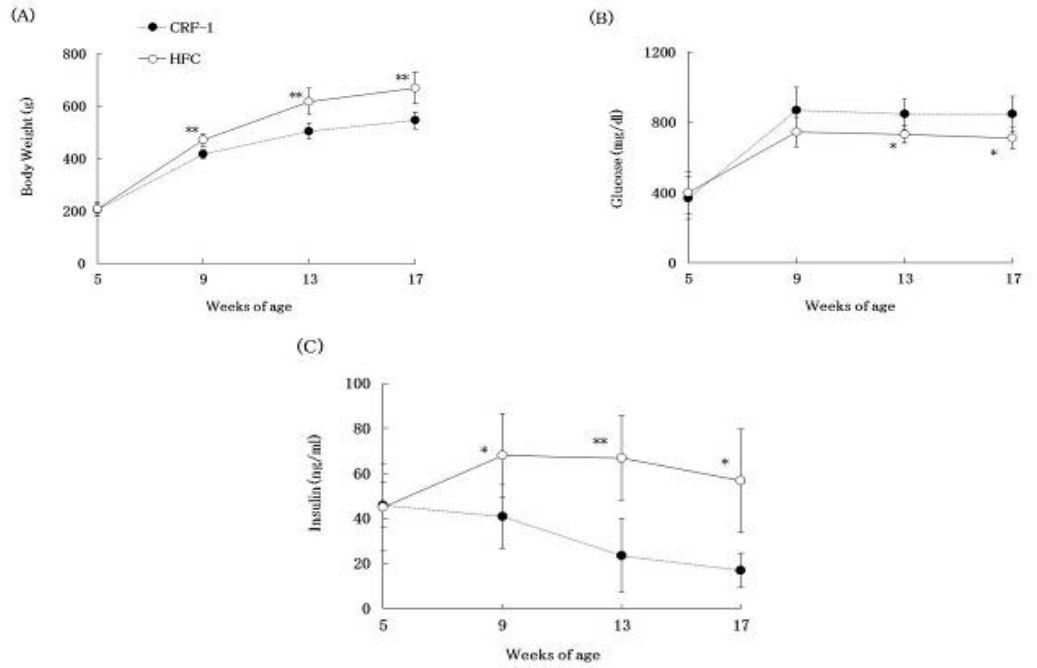


Fig. 1. Changes in body weight (A), and serum glucose (B) and insulin (C) levels in SDT fatty rats fed a standard diet (CRF-1 group) or a high-fat/cholesterol diet (HFC group). Data are represented as means ± SD (n=5)

**p<0.05, ** p<0.01; significant difference compared with the CRF-1 group*

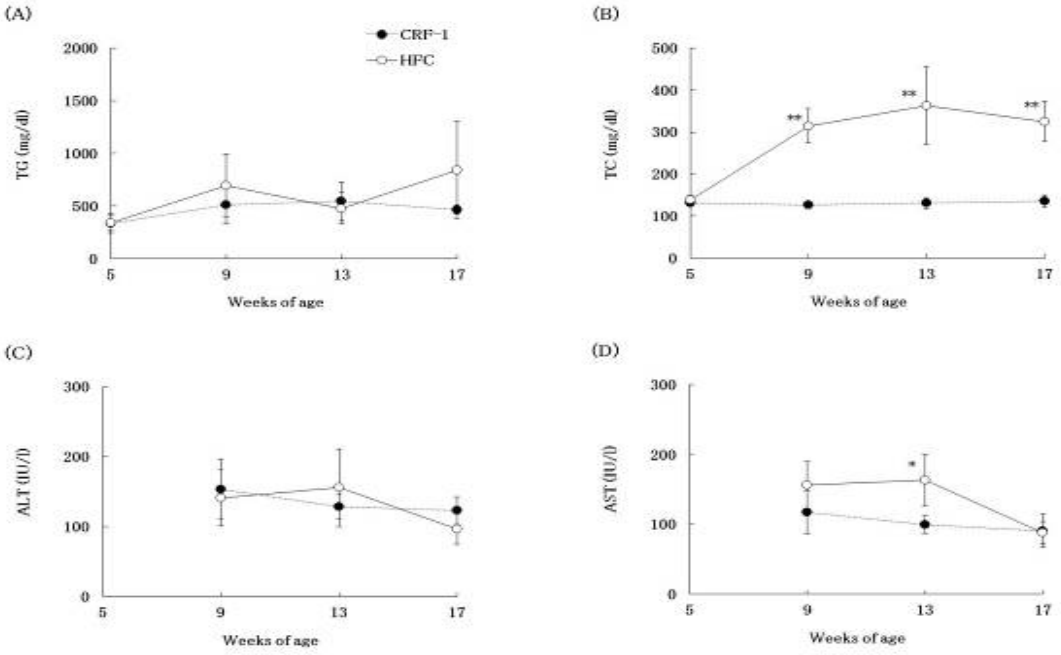


Fig. 2. Changes in serum triglyceride (TG) (A), total cholesterol (TC) (B), alanine aminotransferase (ALT) (C), and aspartate aminotransferase (AST) (D) levels in SDT fatty rats fed a standard diet (CRF-1 group) or a high-fat/cholesterol diet (HFC group). Data are represented as means ± SD (n=5)

**p<0.05, ** p<0.01; significant difference compared with the CRF-1 group*

Table 2. Liver weights in CRF-1 and HFC groups

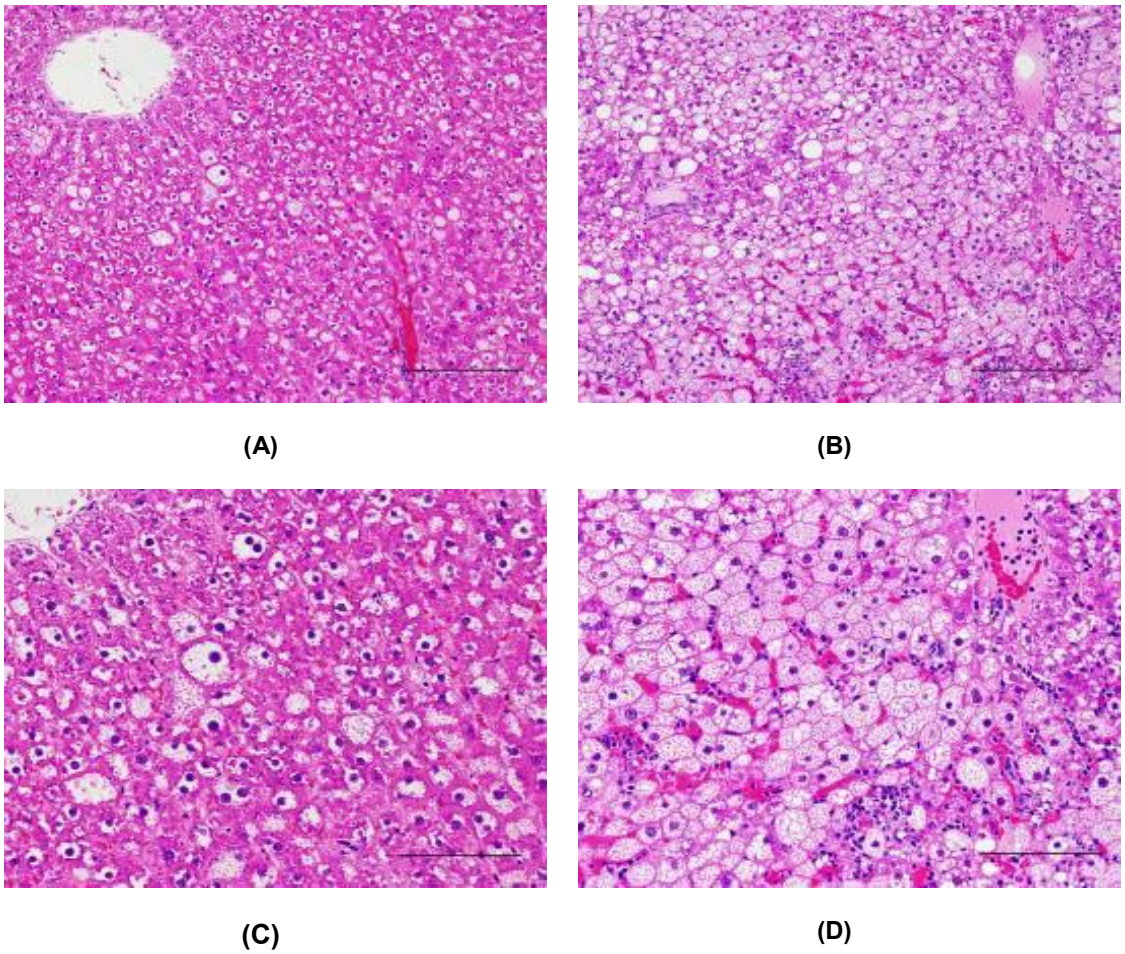
Group	Body weight (g)	Absolute weight (g)	Relative weight (g/g)
CRF-1	544.0±31.2	26.1±2.5	0.0478±0.02
HFC	667.9±59.5**	48.4±6.8**	0.0724±0.01**

*Data represented mean ± SD (n=5). **p<0.01 vs. CRF-1 group*

Table 3. Histopathological findings of liver in CRF-1 and HFC groups

	CRF-1 group					HFC group				
Animal no.	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Findings:										
Fatty change, hepatocyte	-	-	-	-	-	3+	3+	3+	3+	3+
Hypertrophy, hepatocyte	-	-	-	-	-	2+	+	2+	2+	+
Infiltration, inflammatory cell	-	-	-	-	-	±	-	-	-	±
Glycogen deposition, hepatocyte	+	±	±	+	+	-	-	-	-	-

±: Very slight, +: Slight, 2+: Moderate, 3+: Severe



(A) **(B)**

(C) **(D)**

Fig. 3. Histopathological analysis of liver. HE stain. (A) SDT fatty rat fed a standard diet (CRF-1 group). Bar=200 μm. (B) SDT fatty rat fed a high-fat/cholesterol diet (HFC group). Bar=200 μm. (C) CRF-1 group. Bar=100 μm. (D) HFC group. Bar=100 μm.Severe hepatic steatosis was observed in the HFC group (B). Inflammatory cell infiltration was also observed in the HFC group (D)

4. DISCUSSION

Various animal models showing NAFLD/NASH-like hepatic lesions were developed to gain a better understanding of the pathogenesis of these diseases. These animal models also play pivotal roles in developing new drugs for NAFLD/NASH. Two types of models, genetic and diet-induced models, are reported as NAFLD models [12-14], and have different mechanisms of fat accumulation. Genetic models such as ob/ob mice, db/db mice, and KK-Ay mice, develop fatty liver with obesity, insulin resistance, and diabetes mellitus, but do not exhibit hepatic inflammation and fibrosis. Other transgenic and knockout mouse models, sterol regulatory element-binding protein (SREBP)-1c over expressing mice or phosphatase and tensin homolog deleted on chromosome 10 (PTEN) deleted mice, develop a more severe hepatic phenotype, but do not exhibit the metabolic syndromes, such as obesity and insulin resistance that accompany this disease [15,16].

SDT fatty rats, made by introducing the allele of the Zucker fatty rat into the SDT rat genome, represent a new model for obese type 2 diabetes [17]. Female SDT fatty rats exhibit hepatic steatosis under the condition of standard diet feeding [10]. On the other hand, male SDT fatty rats do not exhibit hepatic steatosis with a standard diet. Since blood insulin levels in male rats decreased remarkably with aging [7], reducing the anabolic effect is not considered to induce fat accumulation. In this study, however, hepatic steatosis was induced in male rats fed a high fat/cholesterol diet, D09100301. In the diet, D09100301, the source of the trans-fats is hydrogenated vegetable oil shortening, and the cholesterol content is higher (2% by wt). The higher cholesterol content (2%) is considered to induce a significant hypercholesterolemia in HFC group compared with CRF-1 group (the cholesterol content, <0.1%). The HFC diet results in more frequent and severe NAFLD/NASH phenotypes [18,19]. Indeed, in this study, significant hepatic steatosis with partly inflammatory changes was observed in male SDT fatty rats fed the HFC diet, including obesity, hyperinsulinemia, and hypercholesterolemia (Table 2, Fig. 3). Since NASH like changes were induced in a normal mice fed the diet (D09100301) [17], it is considered to be important to compare the differences in pathophysiology between a normal rat and a diabetic rat by feeding the HFC diet. Moreover, quantitative analyses, such as hepatic lipid levels and the histological NAFLD/NASH scores, and the other biochemical analyses, such as high density lipoprotein (HDL) cholesterol, adiponectin and leptin levels, are essential to be the better understanding in pathophysiology in NAFLD/NASH and evaluate the pharmacological effects on new drugs in these diseases. The “two-hit” theory; the first hit causing fat accumulation in hepatocytes, and the second hit causing inflammation and fibrosis, is proposed as the pathogenesis for NAFLD/NASH [20,21]. In further studies, it will be necessary to elucidate whether longer treatment using this diet will lead to the development of hepatic steatosis with severe inflammation, cellular ballooning, and varying degrees of fibrosis in male SDT fatty rats. Also, examination in female SDT fatty rats fed the HFC diet will be an important study.

5. CONCLUSION

A high-fat/cholesterol diet in male SDT fatty rats induced hepatic steatosis. Male SDT fatty rats fed an HFC-diet may serve as a new NAFLD model.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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