**Title**
Female spontaneously diabetic Torii fatty rats develop nonalcoholic steatohepatitis-like hepatic lesions

**Author(s)**
Ishii, Yukihito; Motohashi, Yu; Muramatsu, Makoto; Katsuda, Yoshiaki; Miyajima, Katsuhiro; Sasase, Tomohiko; Yamada, Takahisa; Matsui, Tohru; Kume, Shinichi; Ohta, Takeshi

**Citation**

**Issue Date**
2015-08-14

**URL**
http://hdl.handle.net/2433/217350

**Right**
© The Author(s) 2015. Published by Baishideng Publishing Group Inc. All rights reserved. This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See:
http://creativecommons.org/licenses/by-nc/4.0/

**Type**
Journal Article

**Textversion**
publisher

Kyoto University
Yukihito Ishii, Yu Motohashi, Makoto Muramatsu, Yoshiaki Katsuda, Katsuhiro Miyajima, Tomohiko Sasase, Takahisa Yamada, Tohru Matsui, Shinichi Kume, Takeshi Ohta

Author contributions: Ishii Y, Motohashi Y and Ohta T designed the research; Muramatsu M, Sasase T, Katsuda Y and Sasase T performed the research and analyzed the data; Ohta T, Yamada T, Matsui T and Kume S edited the manuscript; Ishii Y and Motohashi Y wrote the paper.

Institutional review board statement: The study was reviewed and approved by Japan Tobacco Biological/Pharmacological Research Institutional Review Board.

Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of Japan Tobacco Biological/Pharmacological Research Laboratories (IACUC protocol number: 00745).

Conflict-of-interest statement: We declare no conflicts of interest regarding this study.

Data sharing statement: No additional data are available.

Abstract

AIM: To investigate the histological features of the liver in spontaneously diabetic Torii (SDT) fatty rats compared with age-matched Sprague-Dawley (SD) rats.

METHODS: Female SDT Lepr<sup>a</sup> (SDT fatty) rats and age-matched SD rats were fed ad libitum. Body weight and biochemical parameters, such as serum glucose, triglyceride (TG), total cholesterol (TC), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels as well as fatty acid and TG accumulation in the liver were evaluated at 8 wk of age in the non-fasting state and at 8-wk intervals from 8 to 40 wk of age. Histopathological examinations of the liver were performed using hematoxylin and eosin and Sirius Red staining as well as double staining for ED-1 and toluidine blue. The expression of genes involved in TG synthesis, inflammation, and fibrosis was examined in the liver.

Basic Study

Female spontaneously diabetic Torii fatty rats develop nonalcoholic steatohepatitis-like hepatic lesions

Yukihito Ishii, Makoto Muramatsu, Yoshiaki Katsuda, Tomohiko Sasase, Takeshi Ohta, Japan Tobacco Inc., Central Pharmaceutical Research Institute, Osaka 569-1125, Japan

Katsuhiro Miyajima, Japan Tobacco Inc., Central Pharmaceutical Research Institute, Toxicology Research Laboratories, Hadano, Kanagawa 257-0024, Japan

Takahisa Yamada, Niigata University, Graduate School of Science and Technology, Nishi-ku, Niigata 950-2181, Japan

Tohru Matsui, Shinichi Kume, Kyoto University, Graduate School of Agriculture, Kitashirakawa, Sakyo-ku, Kyoto 606-8502, Japan

Received: December 25, 2014
Peer-review started: December 26, 2014
First decision: April 23, 2015
Revised: May 14, 2015
Accepted: June 9, 2015
Article in press: June 10, 2015
Published online: August 14, 2015
Nonalcoholic steatohepatitis (NASH) is presently well recognized as the most common chronic liver disease in the Western world[1]. NAFLD is strongly associated with central obesity, type 2 diabetes, dyslipidemia, hypertension, and insulin resistance[1]. NAFLD represents the pathology of fatty liver, including simple steatosis, nonalcoholic steatohepatitis (NASH), and cirrhosis[2]. In Western countries, 4%-22% of NAFLD cases lead to hepatocellular carcinoma[3-6]. The pathogenesis of NASH remains poorly defined, and effective pharmacological therapies for NASH have not been approved.

To understand the complicated features of NAFLD/ NASH, animal models offer a source of important information. It has been reported that ob/ob mice, db/db mice, and KK-A' mice, as NAFLD animal models, exhibit spontaneous hepatic steatosis based on insulin resistance and obesity, but these mice do not progress to liver fibrosis when fed a normal diet[7-9]. In contrast, methionine and choline-deficient (MCD) diet-induced NASH is accompanied by hepatic inflammation and fibrosis in ob/ob mice and db/db mice[10,11].

Spontaneously diabetic Torii Lepr-rd (SDT fatty) rats, which are generated by introducing the fa allele of the Zucker fatty rat into the SDT rat genome, represent a new model of obese type 2 diabetes. SDT fatty rats exhibit hyperglycemia that leads to obesity associated with hyperglycemia, hyperinsulinemia, and hyperlipidemia at a young age compared with SDT rats[12,13]. Compared with SDT rats, this early onset of diabetes in SDT fatty rats is considered to be caused by obesity resulting from hyperphagia. Furthermore, female SDT rats also exhibit hyperglycemia at the same young age as male SDT fatty rats[14,15].

SDT fatty rats have been used to investigate the effects of a high-fat diet in a previous study. In this prior study, the rats fed a high-fat diet had improved hyperglycemia and increased serum glucagon-like peptide-1 (GLP-1) levels after refeeding[16]. Moreover, histopathological observations showed improvement in the fatty liver and pancreatic abnormalities. This previous report showed beneficial effects on diabetes in SDT fatty rats fed a high-fat diet.

With regard to the metabolic responses to a high-fat diet in Sprague-Dawley (SD) rats, steatohepatitis associated with hepatic fatty changes, inflammation, and mitochondrial lesions has been confirmed[17]. C57BL/6 mice fed a high-fat diet also develop steatohepatitis[18]. However, the degree of hepatic fibrosis is less severe in animals fed a high-fat diet compared with choline-deficient diet models.

In the present study, we investigated the histological features of the liver in SDT fatty rats without manipulating the diet in comparison with age-matched SD rats. In addition to the pathophysiology, the biochemical parameters, hepatic lipid content, and expression of genes were examined.

MATERIALS AND METHODS

Animals
This experiment was conducted in compliance with the Guidelines for Animal Experimentation of Japan Tobacco Biological/Pharmacological Research Laboratories. The animal protocol was designed to...
minimize pain or discomfort to the animals. Female SDT fatty rats from our colony were used in the study. At 8 wk of age, SDT fatty rats were divided into 5 groups for necropsy at 8, 16, 24, 32, and 40 wk of age. SD rats [Crj: CD (SD); Charles River Japan, Yokohama, Japan], as control animals, at 8 wk of age were also divided into the same 5 groups as the SDT fatty rats. In the experimental period, one SDT fatty rat in the 24-wk necropsy group died due to unidentified causes, leaving 4 animals in this group. The rats were housed individually in suspended bracket cages in a climate-controlled room with a temperature of 23 ± 3 °C, humidity 55% ± 15%, and a 12-h dark-light cycle, and the rats had free access to a commercial diet (CRF-1, Charles River Japan, Yokohama, Japan) and water. The rats were anesthetized with isoflurane inhalation before the procedures.

Biological parameters

Body weight and biochemical parameters, such as serum glucose, triglyceride (TG), total cholesterol (TC), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels, were measured at 8 wk of age and thereafter at 8-wk intervals until 40 wk of age. Blood samples were collected from the tail vein of the rats. The samples were stored in ice water until measurement. Serum glucose, TG, TC, ALT, and AST levels were measured as biochemical parameters using commercial kits (Roche Diagnostics, Basel, Switzerland) in an automatic analyzer (Hitachi 7170S; Hitachi, Tokyo, Japan).

Tissue sampling and histopathology

Necropsy was performed at 8, 16, 24, 32, and 40 wk of age (n = 5 for each age, except 24 wk of age with n = 4). All animals were sacrificed by exsanguination under isoflurane anesthesia. The livers were sampled for measurement of gene expression, hepatic lipid content, and histopathology. Samples for determination of gene expression and hepatic lipid content were stored at -80 °C until analysis. For histopathology, the livers were immediately fixed in 10% neutral-buffered formalin. After resection, the tissue was paraffin-embedded by standard techniques and sectioned (3 to 5 µm). The sections were stained with hematoxylin and eosin (HE). The samples were sampled for measurement of gene expression, hepatic lipid content, and histopathology. Samples were also divided into the same 5 groups as the SDT fatty rats. In the experimental period, one SDT fatty rat in the 24-wk necropsy group died due to unidentified causes, leaving 4 animals in this group. The rats were housed individually in suspended bracket cages in a climate-controlled room with a temperature of 23 ± 3 °C, humidity 55% ± 15%, and a 12-h dark-light cycle, and the rats had free access to a commercial diet (CRF-1, Charles River Japan, Yokohama, Japan) and water. The rats were anesthetized with isoflurane inhalation before the procedures.

Immunostaining

The paraffin-embedded tissue sections were also stained with Sirius Red and toluidine blue (TB), and immunohistochemistry was performed for ED-1 (CD68: 1:100, BMA BIOMEDICALS, Switzerland) and alpha-smooth muscle actin (α-SMA; 1A4, 1:50, Dako, Denmark).

Hepatic TG and FFA content

An approximate 100 µg portion of the liver, 0.5 mL of methanol, and zirconia beads were added to tubes. The liver portion was homogenized using a mixer mill (MM300 Retch) (25 Hz for 10 min). To the homogenized solution, 1 mL of chloroform was added and mixed thoroughly. The mixture was then centrifuged (10000 g for 5 min at 4 °C), and the resultant supernatant was collected. Solvents contained in 0.5 mL of the supernatant were dried under a stream of nitrogen gas. To the residue, 0.5 mL of 2-propanol was added, and the residue was then dissolved again. The TG concentration of the 2-propanol solution was determined using the biochemistry automatic analyzer (Hitachi 7170S; Hitachi, Tokyo, Japan). The free fatty acid (FFA) concentration was analyzed using a NEFA-C kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

mRNA quantification with real-time quantitative PCR

Total RNA was extracted from the liver with the GenElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich, St. Louis, MO, United States) at 8, 16, 24, 32, and 40 wk of age. RNA was transcribed into cDNA using M-MLV reverse transcriptase and random primers (Invitrogen, Carlsbad, CA, United States). The reaction mixture was incubated for 10 min at 25 °C, 1 h at 37 °C, and 5 min at 95 °C. Real-time PCR quantification was performed in a 50 µL reaction mixture with an automated sequence detector combined with ABI Prism 7700 Sequence Detection System software (Applied Biosystems, Foster City, CA, United States). The reaction mixture contained 50 ng of synthesized cDNA, 3.5 mM MgCl₂, 0.3 µM/L primers, 0.1 µM/L probes, and 1.25 units of AmpliTaq Gold®. The cycle parameters included 10 min at 95 °C followed by 40 cycles of 15 s at 95 °C and 60 s at 60 °C. The following primers and FAM-conjugated probes were designed using Primer Express software (Applied Biosystems): SREBP-1c (forward, CGACTACATCCCGTTCTCTACAGC; reverse, TTTTGATGACTTGCAGG; probe, CAGCAACCAGAAACTCAACGGAGAAGAC), DGAT2 (forward, GCTGATAGCTGCTCTCTACTTCACC; reverse, GTGTCCCATGTTGGATTTGGTG; probe, ACATTGACAGCCGTTATCGTGA; probe, GGACGTTGTGTTACTCCGTAGCC), MTP (forward, GGACGTTGTGTTACTCCGTAGCC; reverse, GTGTCCCATGTTGGATTTGGTG; probe, ACATTGACAGCCGTTATCGTGA; probe, GGACGTTGTGTTACTCCGTAGCC), FAS (forward, ACTGAAAGGCATTACTCGGTCC; reverse, GTGTCCTCAGTTGGATTTGGTG; probe, TTCCGCCAGAGCCCTTTGATAGTGG), MTP (forward, GGACGTTGTGTTACTCCGTAGCC; reverse, ACATTGACAGCCGTTATCGTGA; probe, GATCCCCAGCGAGCTGATCCAC), and β-actin (purchased from Applied Biosystems). The expression of the following genes were confirmed using Taqman Gene Expression Assays on Demand and Universal Maser Mix: ACC (Rn00573474_m1), α-SMA (Rn01759928_g1), MCP-1 (Rn00580555_m1), TGF-β (Rn99999016_m1), TNF-α (Rn99999017_m1), and
collagen type 1 (Rn01463848_m1).

Statistical analysis
The results of the biological parameters are expressed as the mean ± SD. Statistical analysis of differences between mean values was performed using an F-test followed by the Student t-test or the Aspin-Welch t-test. Differences were considered significant at P < 0.05. The statistical methods of this study were reviewed by Dr. Hisayo Morinaga from Biological/Pharmacological research laboratories, Central Pharmaceutical Research Institute, Japan Tobacco Inc.

RESULTS

Body weight and biochemical parameters
SDT fatty rats showed obesity at 8 wk of age. The mean bodyweight at 8 wk was significantly higher in SDT fatty rats compared with SD rats (328 ± 40 g vs 216 ± 8 g; P < 0.01). Obesity was sustained in SDT fatty rats until 24 wk of age (Figure 1A). The serum glucose levels in SDT fatty rats were higher than those in SD rats at 8 wk of age, and the increase was sustained until 40 wk of age (Figure 1B). The serum TG levels in SDT fatty rats increased until 24 wk of age, and they slightly decreased after 32 wk (Figure 1C). The serum TC levels in SDT fatty rats increased throughout the observation period (Figure 1D). The serum TG and TC levels in SDT fatty rats were higher than those in SD rats until 40 wk of age. Furthermore, the serum AST and ALT levels were significantly elevated at 8 wk, and these high levels were sustained until 40 wk of age (Figure 1E and F).

Liver weight and hepatic TG and FFA content
The liver weights were increased in SDT fatty rats until 24 wk of age and slightly decreased thereafter, but they were greater than those in SD rats throughout the experiment, except at 24 wk of age (Figure 2A). The hepatic TG content in SDT fatty rats was higher than that in SD rats at 8 wk of age, (129 ± 77 mg/g tissue vs 10 ± 2 mg/g tissue; P < 0.01). The hepatic TG content was markedly elevated until 16 wk of age in SDT fatty rats, and gradually decreased to the level in SD rats (Figure 2B). The hepatic FFA content showed a similar change to hepatic TG content in SDT fatty rats because it increased until 16 wk and gradually decreased after 32 wk of age (Figure 2C).

Histopathological analyses
The liver histopathology was examined by HE staining. Histopathologically, severe changes (3+) of the liver, including fatty and vacuolar changes, were observed in SDT fatty rats at 8 wk of age. Moderate changes (1+–2+) of hypertrophy and very slight or slight changes in inflammation were also observed in SDT fatty rats at 8 wk of age (Table 1). Moreover, moderate changes (2+) indicating fibrosis were observed in SDT rats at 32 wk of age.

Fibrosis was also confirmed by Sirius Red staining (Figure 3A-D). Consistent with the HE staining, similar findings of fibrosis were observed in SDT fatty rats. However, these changes were not observed in SD rats (Table 1). Activated hepatic stellate cells (HSCs) in the liver were investigated by α-SMA immunostaining. The SD rats showed few HSCs at 32 wk of age. Consistent with the presence of hepatic fibrosis, there were increased numbers of α-SMA-positive, activated HSCs in SDT fatty rats (Figure 3E and F). To examine the macrophages and mast cells in the liver, liver sections were immunostained with ED-1 and TB. Increased macrophage and mast cell counts, as indicated by ED-1- and TB-positive stained cells, respectively, were found in SDT fatty rats at 32 wk of age, and these cells were scarcely observed in SD rats (Figure 3G and H).

Liver mRNA expression
The mRNA levels of lipogenic genes (SREBP-1c, FAS, ACC, DGAT2, and MTP), inflammatory genes (MCP-1 and TNF-α), and fibrotic genes (collagen Type 1, TGF-β1, and α-SMA) in the liver were measured by real-time PCR at 8-wk intervals. At 8 wk of age, the expression of lipogenic genes (such as SREBP-1c, FAS, and ACC) increased in SDT fatty rats compared with SD rats (Figure 4A-C). The DGAT2 mRNA level was not increased in SDT fatty rats (Figure 4D), and the MTP mRNA level, which is related to secretion of very low-density lipoprotein (VLDL), was decreased by 64% in SDT fatty rats compared with SD rats (Figure 4E). After 16 wk of age, SDT fatty rats showed decreased expression of lipogenic genes (such as SREBP-1c, FAS, and ACC). The expression of lipogenic genes was comparable between SDT fatty rats and SD rats, but the DGAT2 and MTP mRNA levels in SDT fatty rats were lower than those in SD rats. The TNF-α and MCP-1 inflammatory genes were increased by 16.1% and 38.9%, respectively, in SDT fatty rats at 16 wk of age (Figure 4F and G). The mRNA levels of fibrotic genes, namely collagen type 1 and α-SMA, tended to increase in SDT fatty rats compared with SD rats at 16 and 24 wk of age. The TGF-β1 mRNA levels in SDT fatty rats also tended to increase at 32 wk of age.

DISCUSSION
In a previous study, we investigated diabetes mellitus in female SD T fatty rats by comparing these rats with age-matched female SD rats. Female SDT fatty rats showed metabolic abnormalities such as hyperglycemia, hyperinsulinemia, and dyslipidemia at a young age, which were similar to the abnormalities observed in male SDT fatty rats[14]. Furthermore, diabetic complications such as renal lesion and cataracts were observed at 16 wk of age in female SDT fatty rats, and were comparable to the complications
observed in male SDT fatty rats\textsuperscript{[15]}. Sexual differences in SDT fatty rats did not exist in the progression of diabetes. Thus, female SDT fatty rats are an important animal model of metabolic syndrome with diabetes. In Zucker diabetic fatty rats, hepatic steatosis is observed without dietary manipulation, but hepatic fibrosis is not observed\textsuperscript{[19]}. Although \textit{ob/ob} and \textit{db/db} mice develop hepatic steatosis associated with obesity and insulin resistance, these mice do not spontaneously progress to significant steatohepatitis\textsuperscript{[8,9]}. \textit{Ob/ob} and \textit{db/db} mice show significant steatohepatitis development when fed a MCD diet, but only \textit{db/db} mice fed a MCD diet have increased fibrosis in the liver\textsuperscript{[10,11]}. \textit{Db/db} mice require other stimulation in addition to the background condition, such as obesity, diabetes, and dyslipidemia, to trigger progression to fibrosis\textsuperscript{[10]}

In the present study, we investigated the pathophysiological changes of the liver in female SDT fatty rats compared with SD rats. We used SD rats as the control, instead of SDT rats, because we wanted to focus on the investigation of pathophysiological changes.

**Figure 1** Changes in body weight and biological parameters in spontaneously diabetic Torii fatty rats and Sprague-Dawley rats. A: Body weight; B: Glucose; C: Triglyceride; D: Total cholesterol; E: Alanine aminotransferase; F: Aspartate aminotransferase. Data are shown as mean ± SD (n = 4-5). \textsuperscript{a}P < 0.05, \textsuperscript{b}P < 0.01, vs Sprague-Dawley rats. SDT: spontaneously diabetic Torii; SD: Sprague-Dawley.
Figure 2. Changes in liver weight, hepatic triglyceride, and fatty acid contents in Spontaneously Diabetic Torii fatty rats and Sprague-Dawley rats. A: Liver weight; B: Hepatic triglyceride content; C: Hepatic fatty acid content. Data are shown as the mean ± SD (n = 4-5). Data are shown as the mean ± SD (n = 4-5).

Table 1. Microscopy findings of the liver in female spontaneously diabetic Torii fatty rats and Sprague-Dawley rats

<table>
<thead>
<tr>
<th></th>
<th>8 wk</th>
<th>16 wk</th>
<th>24 wk</th>
<th>32 wk</th>
<th>40 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
<td>SDT fatty</td>
<td>SD</td>
<td>SDT fatty</td>
<td>SD</td>
</tr>
<tr>
<td>Hepatosteatosis (Vacuolar change/fatty change)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>±</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2+</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>3+</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Hypertrophy of hepatocytes (with vacuolation/fatty change)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>5</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>±</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2+</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>3+</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Fibrosis, focal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>±</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Infiltration, inflammatory cell, periportal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>±</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
changes of the liver in SDT fatty rats. In our previous study, we confirmed that female SDT rats only exhibit a fatty liver based on histopathology but not fibrosis at 40 wk of age. Furthermore, male SDT rats, which are a severe type 2 diabetic model, do not exhibit a fatty liver and liver fibrosis at 24 wk of age.

SDT fatty rats showed hyperglycemia associated with increased body weight at 6 wk of age, and they exhibited severe hyperglycemia after 6 wk of age, which is considered to be due to a decrease in insulin...
Ishii Y et al. New NASH-like animal model

![Graph A](chart1.png)

![Graph B](chart2.png)

![Graph C](chart3.png)

![Graph D](chart4.png)

![Graph E](chart5.png)

![Graph F](chart6.png)

![Graph G](chart7.png)

![Graph H](chart8.png)
levels. Indeed, our previous study has shown that insulin levels in SDT fatty rats increase until 6 wk of age and gradually decrease thereafter, but the levels are significantly higher than those in SD rats until 20 wk of age (8.89 ± 7.07 ng/mL vs 1.42 ± 0.49 ng/mL; P < 0.05). From 20 to 40 wk of age, the insulin levels in SDT fatty rats are slightly greater than those in SD rats, and it peaked at 16 wk of age. After 16 wk of age, it gradually decreased to the levels observed in the SD rats. The mRNA expression levels of MCP-1 and TNF-α at 16 wk of age were increased, suggesting that SDT fatty rats had accelerated liver injury and inflammation. The mRNA expression of collagen type 1 and α-SMA tended to increase in SDT fatty rats. With regard to the pathophysiology, hepatic fibrosis was observed in SDT fatty rats at 32 wk of age. These findings indicated that female SDT fatty rats spontaneously develop nonalcoholic steatohepatitis-like hepatic lesions without dietary manipulation.

The hepatic steatosis in SDT fatty rats observed at 8 wk of age resulted from increased hepatic TG synthesis and decreased VLDL secretion, and histopathological findings, such as fatty changes in the hepatocytes, showed hepatic steatosis. Indeed, the hepatic FFA content, which is a component of TG, was also increased. The expression of genes related to lipogenesis, FAS, and ACC1 was increased, and the expression of MTP, which is a gene related to VLDL secretion, was decreased in SDT fatty rats at 8 wk of age. Furthermore, the FFA content in the liver was attributed to the degradation of serum lipoproteins and uptake of serum FFA. The hyperlipidemia in SDT fatty rats is considered to supply fatty acids to the liver.

In terms of the hepatic steatosis, SDT fatty rats are comparable to other rat strains at 8 wk of age. However, the hepatic steatosis in SDT fatty rats observed after 24 wk of age may be based on the decreased de novo lipogenesis. The mRNA expression of SREBP-1c, which regulates FAS and ACC1 mRNA expression, was decreased in SDT fatty rats after 16 wk of age. Some complex mechanisms involved in the decrease in SREBP-1c mRNA expression of liver should be considered. It is known that insulin activates the SREBP-1c promoter in liver, thereby increasing fatty acid and triglyceride synthesis. A previous study has shown that the serum insulin levels are decreased in SDT fatty rats after 6 wk of age. The reduction of SREBP-1c levels could contribute to the decrease in serum insulin levels. The reduction of hepatic steatosis has also been shown in Otsuka Long-Evans Tokushima fatty rats after 30 wk of age. The change in hepatic steatosis is considered to be associated with a decrease in SREBP-1c expression. The change in hepatic steatosis in SDT fatty rats may be partly due to decreased SREBP-1c expression based on the decreased serum insulin levels. Further study is required to confirm this mechanism.

The spontaneous development of steatohepatitis and hepatic fibrosis in SDT fatty rats could be explained by the increase in hepatic FFA. The excess FFA could serve as precursors for the production of lipid peroxidation, which can activate hepatic stellate cells and thereby result in the development of fibrosis. Indeed, an increase in α-SMA level, which is an indicator of activated hepatic stellate cells, was observed. In addition to lipid peroxidation, CYP2E1 may be involved in hepatic fibrosis development in SDT fatty rats. It has been reported that fatty acids increase both CYP2E1 mRNA and protein levels in the liver of high-fat diet fed animals. CYP2E1 produces high levels of reactive oxygen species, which play an important role in the development of NASH and fibrosis.

The difference in the pathophysiology of the liver between SDT fatty rats and other diabetic animal models remains unknown. However, the hepatic steatosis

---

**Figure 4** Expression of genes related to triglyceride synthesis, triglyceride secretion, inflammation, and fibrosis in spontaneously diabetic Torii fatty rats and SD rats. A: SREBP-1c; B: FAS; C: ACC; D: DGAT2; E: MTP; F: TNF-α; G: MCP-1; H: Collagen type 1; I: α-SMA; J: TGF-β. Data are shown as the mean ± SD (n = 4-5). *P < 0.05, †P < 0.1, vs SD rats. SDT: Spontaneously diabetic Torii; SD: Sprague-Dawley.
Acknowledgments

We wish to thank Mr. Nobuhiro Inaba and Mr. Naruhisa Ryamon (JT Creative Service Co., Ltd.) for long-term animal care.

References

2 Greenfield V, Cheung O, Sanyal AJ. Recent advances in nonalcoholic fatty liver disease. Curr Opin Gastroenterol 2008; 24: 320-327 [PMID: 18408460 DOI: 10.1097/MOG.0b013e3282fbccf2]


Zern MA, Leo MA, Giambra ME, Lieber CS. Increased type 1 procollagen mRNA levels and in vitro protein synthesis in the baboon model of chronic alcoholic liver disease. Gastroenterology 1985; 89: 1123-1131 [PMID: 4043669]


Ishii Y et al. New NASH-like animal model


P-Reviewer: Qiu LX, Sertoglu E, Shimizu Y  S-Editor: Ma YJ  L-Editor: Cant MR  E-Editor: Ma S