Food and Chemical Toxicology 118 (2018) 190–197

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Effects of high fructose intake on liver injury progression in high fat diet induced fatty liver disease in ovariectomized female mice

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ARTICLE INFO

Keywords:
Fructose
Estrogen
Hepatic steatosis
Menopause
Steatohepatitis

ABSTRACT

Epidemiology shows that the morbidity of nonalcoholic fatty liver disease (NAFLD) is increased in postmenopausal women and chronic high fructose intake induces NAFLD progression. To analyze the effects of high fructose intake on estrogen deficiency, we evaluated liver disease progression using ovariectomized mice fed with a high fat diet (HFD) for 12 weeks. Hepatic steatosis developed in all HFD groups. Fructose intake significantly increased the liver weight and serum alanine aminotransferase, which was not exacerbated by ovariectomy alone. Ovariectomy enhanced the hepatic inflammatory activity shown by tumor necrosis factor α upregulation in the groups with or without fructose intake. Both fructose intake and ovariectomy increased the hepatocytes with ballooning degeneration and hepatic macrophage infiltration and activated hepatic stellate cells. Coexistence of fructose intake and ovariectomy markedly enhanced liver cell destruction, macrophage accumulation, and progression of fibrosis. Liver damage was ameliorated by 17β-estradiol supplementation. These findings suggest that high fructose intake enhanced the progression of NAFLD in ovariectomized female mice.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) has been increasing worldwide and becoming the most frequent disease of the liver. Nonalcoholic steatohepatitis (NASH) is a severe form of NAFLD characterized with sustained inflammation and fibrosis, eventually developing into liver cirrhosis and hepatocellular carcinoma (Sugimoto and Takei, 2011). To prevent liver related death in NASH patients, detailed understanding of factors associated with the progression of liver disease is necessary.

Insulin resistance and obesity are recognized as major factors that exacerbate NAFLD. In addition, menopause and unbalanced dietary carbohydrate contents are associated with NAFLD development (Suzuki and Abdelmalek, 2009). The prevalence of NAFLD is generally higher in men than in women, but epidemiological studies show that this sex difference disappears after menopause with increasing prevalence of NAFLD in women (Luo and Ishigami, 2015). In postmenopausal women, the presence of central obesity is significantly associated the risk of NAFLD (Chung et al., 2015). Because hepatic fatty acid oxidation is impaired in estrogen deficiency, reduced fat oxidation in postmenopausal women likely accelerates visceral fat, leading to obesity and NAFLD (Lovejoy et al., 2008; Nemoto et al., 2000). In addition to the effects on metabolism, estrogen modulates immune-inflammatory activity. Thus estrogen withdrawal may affect intrahepatic proinflammatory activity, leading to increased liver damage (Monteiro et al., 2014). These observations suggest that both the metabolic and immune-inflammatory changes in estrogen deficiency may exacerbate NAFLD development in postmenopausal woman.

Chronic high fructose intake induces hepatic steatosis (Lim et al., 2010; Castro et al., 2011). Fructose is principally metabolized in the liver, where it can be converted into fatty acids without physiological regulation by phosphofructokinase, stored in the form of triglycerides (TGs) (Softic et al., 2016). Fructose is believed to increase the generation of advanced glycation endproducts and reactive oxygen species

Abbreviations: αSMA, *alpha* smooth muscle actin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; hCLS, hepatic crown-like structure; HFD, high fat diet; MCP-1, monocyte chemoattractant protein-1; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; OVX, ovariectomy; PCR, polymerase chain reaction; ROS, reactive oxygen species; SD, standard diet; SO, sham operation; TG, triglyceride; TGFβ, transforming growth factor-β; TNFα, tumor necrosis factor-α

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https://doi.org/10.1016/j.fct.2018.05.006
Received 7 September 2017; Received in revised form 21 April 2018; Accepted 4 May 2018
Available online 09 May 2018
0278-6915/

(ROS), thereby promoting the hepatocyte damage (Takeuchi et al., 2015; Kohli et al., 2010). The association of fructose and NAFLD is well known, however, the effects of chronic consumption of fructose on postmenopausal woman are not well understood. Because a nationwide survey in the US reported the increased dietary fructose intake in all sex and age groups from 1977 to 2004 (Marriott et al., 2009), high fructose intake may have contributed to the increased number of postmenopausal women becoming NAFLD patients. Thus, understanding the effects of high fructose intake during the postmenopausal stage may be helpful when considering fructose restriction to prevent NAFLD progression.

In this study, we evaluated the effects of high fructose intake on the progression of liver disease in ovariectomized female mice. To simulate NAFLD patients, mice were fed with a high fat diet (HFD) to induce obesity. We observed that either the fructose intake or ovariectomy enhanced the fatty liver disease; however, coexistence of these two insults further exacerbated liver damages and augmented hepatic fibrosis. Our observations suggest that high fructose intake accelerates the progression of NAFLD in estrogen deficiency.

2. Materials and methods

2.1. Animal protocol

All female C57BL/6J mice (7 weeks old, 17 − 19g; Charles River Laboratories, Yokohama, Japan) were housed at 22–23 °C in a 12:12-h light: dark cycle. All studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health) and were approved by the Animal Care Committee of Kyushu University. The standard diet (SD) (Oriental Yeast, Tokyo, Japan) consisted of 58.2% carbohydrate, 4.8% fat, and 20.8% protein. The HFD (Oriental Yeast, Tokyo, Japan) consisted of 48.3% carbohydrate (no glucose, no sucrose), 30.1% fat (containing 4.3% cocoa butter, 3.0% lard, and 1.25% cholesterol), and 22.8% protein. The water containing 30% fructose was prepared using D- (−) - Fructose (Sigma, St. Louis, MO). At 1 week after arrival, mice (n = 40) underwent bilateral ovariectomy (OVX) (n = 16) or sham operation (SO) (n = 24). One week after the surgery, mice were divided into the following five groups (n = 8 per group): SO-SD group, SO mice with SD and tap water, SO-HFD group, SO mice with HFD and tap water, OVX-HFD group, OVX mice with HFD and tap water, SO-HFD-Fr group, SO mice with HFD and 30% fructose, OVX-HFD-Fr group, OVX mice with HFD and 30% fructose. All groups were fed for 12 weeks. The experimental design of the groups is shown in Fig. 1. In the estrogen replenishment study, mice (n = 15) were divided into three groups and underwent SO (n = 5), and OVX (n = 5), OVX with subcutaneous implantation of 17β-estradiol sustained release (0.5 mg/pellet, 90-day release, Innovative Research of America, Sarasota, FL) (n = 5). From 1 week after surgery, all mice were fed for 12 weeks with HFD and 30% fructose. At the end of the experimental period, the mice fasted overnight and then euthanized after being weighed. Blood was collected from the inferior vena cava, centrifuged (3000 rpm, 5 min, 4 °C), and serum was collected. The livers were harvested and fixed with 10% buffered formalin for histological examination or immediately frozen in liquid nitrogen for mRNA extraction and lipid analysis.

2.2. Measurement of serum biochemical markers and liver lipid content

Serum alanine aminotransferase (ALT) was measured using the transaminase CII test kit (Wako Pure Chemical Industries, Tokyo, Japan). Total lipids were extracted from the liver as described previously (Miura and Suzuki, 2014). Serum and liver TG were measured using TG E-test kit as manufacture's protocol (Wako Pure Chemical Industries, Tokyo, Japan).

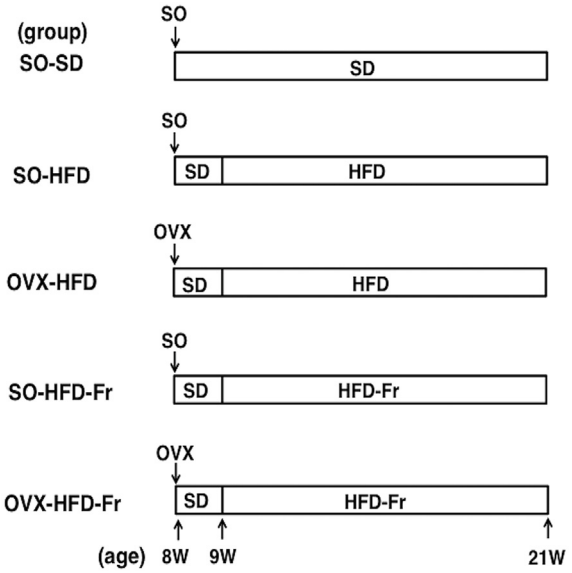

Fig. 1. Design of experimental groups. Female C57BL/6J mice (8 weeks old) underwent bilateral ovariectomy (OVX) or sham operation (SO). One week after the surgery, mice were divided into the following five groups (n = 8 per group); SO-SD group, SO mice with standard diet (SD) and tap water, SO-HFD group, SO mice with high fat diet (HFD) and tap water, OVX-HFD group, OVX mice with HFD and tap water, SO-HFD-Fr group, SO mice with HFD and 30% fructose, OVX-HFD-Fr group, OVX mice with HFD and 30% fructose. After a 12 week diet, histological and biochemical analyses were performed.

2.3. Histological and immunohistochemical analyses

The liver samples were fixed and embedded in paraffin. 5 μm thick sections were cut from each block and randomly assigned to 4 types of staining, i.e., hematoxylin and eosin (HE), Masson trichrome, and immunostaining for F4/80 and α-smooth muscle actin (αSMA). Anti-F4/80 antibody (1:100) (MCA497GA, Bio-Rad, Hercules, CA) and anti-αSMA antibody (1: 100) (Abcam, Cambridge, UK) were commercially purchased. The Histological scoring were assessed quantitatively by an expert hepatopathologist (H.S.) who was blinded to the sample's background. The observer evaluated in five fields of view at a magnification of 200× in each section, 5 sections for each animal. The numbers of ballooned hepatocytes and hepatic crown-like structure (hCLS) were quantified in HE stained sections (6 animals in each group) and F4/80 immunostained sections (6 animals in each group), respectively. The immune-positive area for F4/80 or αSMA was quantified in the same way as above in respective immunostaining (6 animals in each group) using Leica Application Suite Ver. 4.2 software (Leica Microsystems, Heerbrugg, Switzerland). The fibrosis score was determined by reference to the classification of Brunt et al. (Brunt et al., 1999; Kleiner et al., 2005). Magnifications of photographs shown in figures were 200×.

2.4. Quantitative real-time PCR

Total RNA was prepared from liver specimens using a TRIzol reagent (Invitrogen, Carlsbad, CA) and cDNA was synthesized from 1 μg of RNA by GeneAmp RNA polymerase chain reaction (PCR) (Applied Biosystems, Hammonton, NJ). Real-time PCR was performed using LightCycler FastStart DNA Master SYBR Green I (Roche, Basal, Switzerland). To control for variations in reactions, all PCR data were normalized against glyceraldehyde 3-phosphate dehydrogenase expression. The primer sequences used are listed in Table 1.