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Diet high in fructose promotes liver steatosis and hepatocyte apoptosis in C57BL/6J female mice: Role of disturbed lipid homeostasis and increased oxidative stress

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ABSTRACT

The effects of high (H)-fructose (FR) diet (D) (HFRD) on hepatic lipid homeostasis, oxidative stress, inflammation and hepatocyte apoptosis were investigated in 6-week old female C57BL/6J mice fed a regular chow (ContD) or HFRD (35% fructose-derived calories) for 3 weeks. HFRD-fed mice exhibited increased levels of hepatic steatosis with a significant elevation of serum levels of triglyceride, cholesterol and TNFα compared to ContD-fed mice ($P<0.05$). HFRD-fed mice exhibited ~2.7- fold higher levels FAS along with significantly decreased protein levels of adiponection-R2 (~30%), P-AMPK (~60%), P-ACC (~70%) and RXR-α (~55%), suggesting decreased hepatic fat oxidation compared to controls. Interestingly, hepatic fatty acid uptake into hepatocytes and lipolysis were significantly increased in HFRD-fed mice, as shown by decreased CD36 and fatty acid transporter protein-2, and increased adipose triglyceride lipase, respectively ($P<0.05$). Increased hepatic levels of iNOS and GSSG/GSH suggest elevated oxidative stress with a higher number of macrophages in the adipose tissue in HFRD-fed mice ($P<0.05$). Significantly elevated rates of hepatocyte apoptosis (~2.4-fold), as determined by TUNEL analysis with increased Bax/Bcl2 ratio and PARP-1 levels (~2- and 1.5-fold, respectively), were observed in HFRD-fed mice. Thus, HFRD exposure increased hepatic steatosis accompanied by oxidative stress and inflammation, leading to hepatocyte apoptosis.

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD) represents the most prominent form of chronic liver diseases, affecting numerous people at different ages (Wieckowska et al., 2007). Despite advances in this field, knowledge on the pathogenesis of NAFLD is still incomplete and the understanding of the mechanisms underlying the development of NAFLD is of extreme importance. According to the most widespread and prevailing model of "two-hit hypothesis", the "first hit" involves lipid accumulation in the hepatocytes (Duvnjak et al., 2007). The first 'hit' is the accumulation of intrahepatic lipid (hepatic steatosis) owing to an imbalance of normal hepatic lipid metabolism of fat synthesis and oxidation, which results in either excessive lipid influx, decreased lipid clearance, or both (Duvnjak et al., 2007). The "first hit" increases the vulnerability of the liver to many additional factors that constitute the "second hit" and promote hepatic injury such as apoptosis, inflammation and fibrosis/cirrhosis. Increased oxidative stress, pro-inflammatory cytokines/chemokines, endotoxemia, insulin

Abbreviations: ACC, acetyl-CoA carboxylase; Adiponectin R2, adiponectin receptor 2; AFLD, alcoholic fatty liver disease; ALT, alanine aminotransferase; AMPK, AMP-activated protein kinase; ATGL, adipose triglyceride lipase; Bax, Bcl-2-associated X protein; Bcl2, B-cell lymphoma 2; CAT, catalase; CD36, cluster of differentiation 36; ContD, regular chow control diet; FAS, fatty acid synthase; FATP2, fatty acid transport protein 2; GSH, reduced glutathione; GSSG, oxidized glutathione disulfide; HFD, high fat diet; HFRD, high (H)-fructose (FR) diet (D); HO-1, heme oxygenase-1; HSL, hormone-sensitive lipase; iNOS, inducible nitric oxide synthase; NAFLD, Non-alcoholic fatty liver disease; P-ACC, phospho-acetyl-CoA carboxylase; P-AMPK, phospho-AMP-activated protein kinase; PARP-1, poly-ADP-ribosyl polymerase-1; PPARα, peroxisome proliferator-activated receptor α; RNS, reactive nitrogen species; ROS, reactive oxygen species; RXRα, Retinoid X receptor α; SCD1, stearoyl-CoA desaturase-1; SOD, superoxide dismutase; TG, triglyceride; TNFα, tumor necrosis factor α; VLDL, very low density lipoprotein.

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resistance and adipokines are considered “second hit” (Buzzetti et al., 2016). The resulting lobular inflammation leads to ballooning degeneration and perisinusoidal fibrosis, which are preceded by hepatic apoptosis and inflammation, with resulting scarring and progression to non-alcoholic steatohepatitis (NASH) (Syn et al., 2009). Understanding of the etiology and different dietary factors that induce the “two hits” is essential in the prevention and treatment of NAFLD.

There is strong evidence that the diet may affect the development of NAFLD (Le and Bortolotti, 2008). Dietary fructose is a major candidate for causing NAFLD. Fructose is a monosaccharide which is commonly used as a sweetener, e.g., in high fructose corn syrups. Industrially, it is frequently found in soft drinks and pre-packaged foods (Akar et al., 2012). Epidemiologic data suggest that there has been a significant rise in calories consumed from saturated fat and fructose rich foods (Bray et al., 2004). Fructose consumption accounts for approximately 10.2% of all calories in our average diet in the United States (Vos et al., 2008). Fructose intake is 2–3 fold higher in patients with NASH compared to BMI-matched controls and recently daily fructose ingestion has been associated with increased hepatic fibrosis (Ouyang et al., 2008). This has been paralleled by an increasing prevalence of obesity and its associated hepatic comorbidity, namely NAFLD (Cave et al., 2007). A correlation is observed between dietary fructose intake and the prevalence of metabolic syndrome and fatty liver (Bantle, 2009). Recent data suggest that increased fructose consumption elevates fat mass, *de novo* lipogenesis and inflammation while it promotes insulin resistance and post-prandial hypertriglyceridemia, particularly in overweight individuals (Cave et al., 2007). Further, studies have indicated that the development of NAFLD may be frequently associated with excessive dietary fructose consumption (Ouyang et al., 2008). Whether increased fructose consumption positively correlates with the development of NAFLD or promotes the transition from NAFLD to NASH and more advanced stages of liver damage remains unclear. The role of dietary saturated fat and fructose in triggering these mechanism(s) of fibrosis progression in NASH still need further investigations.

Even though growing evidence suggests that fructose contributes to the development and severity of NAFLD, the biological mechanism underlying fructose- caused NAFLD occurrence and progression to NASH is not clearly understood and is probably due to a number of other factors that are expressed in a context of genetic predisposition and sedentary life style. Increased oxidative stress is a major contributor in the pathogenesis of NAFLD (Browning and Horton, 2004). It is suggested that increased accumulation of liver triglycerides leads to increased oxidative stress in the hepatocytes of animals and humans (Browning and Horton, 2004). Data from animal models have shown increased oxidative stress due to increased fat influx into the liver (Hensley et al., 2000). In addition, pathological increases in cell death in the liver as well as in peripheral tissues have emerged as an important mechanism involved in the development and progression of NAFLD (Alkhouri et al., 2011). In fact, increased hepatocyte cell death mainly by apoptosis is frequently observed in patients with NAFLD (Alkhouri et al., 2011; Feldstein et al., 2003; Ribeiro et al., 2004). However, little has been reported about the fructose-induced oxidative stress and the development of hepatic apoptosis in NAFLD in the context of the “two-hit hypothesis.” Based on the current background, we aimed to evaluate the effects of dietary high fructose on hepatic lipid accumulation and increased oxidative stress with inflammation as the first and second hits, respectively, and then study the underlying mechanisms for the development of hepatocyte apoptosis and adipose inflammation in young female C57BL/6J mice.

2. Materials and methods

2.1. Animal model

Animal experiments were performed in accordance with the National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee. Age-matched (6 weeks) female C57BL/6 mice were obtained from Jackson Laboratories. After one week of acclimation, mice were randomly assigned to two groups ($n \geq 6$/group) fed either a regular chow as control diet (ContD, 7017 NIH-31 open formula mouse/rat sterilizable diet, http://www.envigo.com/resources/data-sheets/7017-datasheet-0915.pdf) or a high (H)-fructose (FR) diet (D) (HFRD, 35% fructose-derived calories, D15101101 specially formulated by Research Diets, New Brunswick, NJ, USA) for 3 weeks (Table 1). The NIH-31 rodent diet contains simple sugar content from natural ingredients at an estimated content of 2–5% by weight. There was also no additional mono- or di-saccharide such as glucose, fructose and sucrose or otherwise added. In addition, generally rodent chow contains <0.5% fructose whereas HFRD contains 35% fructose. The mice were housed in groups of three per cage at 22 °C with a 12 h light/dark cycle and given free access to diet and water. Body weight for each animal and their food intake were recorded weekly during the feeding period. After 3 weeks, the livers and adipose tissues were excised from the decapitated mice, which were fasted overnight (12 h), and immediately snap frozen, while individual trunk blood samples following decapitation of sedated mice were collected for serum preparation, as described (Choi et al., 2016a, 2016b). All samples were stored at −80 °C until analysis.

2.2. Histopathology analysis

Liver tissue sections from the largest lobe were fixed in neutralized formalin (10%) before being stained with hematoxylin and eosin (H&E). Following staining, hepatic histological examination was performed with the histological scoring system for NAFLD blindly, as described (Abdelmegeed et al., 2017; Kleiner et al., 2005).

2.3. Measurements of the hepatic contents of triglyceride (TG) as well as serum levels of metabolic parameters

Liver tissues (50 mg wet weight) were homogenized in 5% Triton

Table 1
Composition of standard chow control diet (ContD) and HFRD.

	ContD (3.0 kcal/g)	HFRD[a] (3.9 kcal/g)	
	% kcal from	% kcal from	
Protein	24	21	
Carbohydrate	62	68	
Fat	14	12	
Ingredient		g	*kcal*
Casein		200	800
DL-Methionine		3	12
Corn Starch		318	1272
Fructose		342	1368
Cellulose		50	0
Corn Oil		50	450
Mineral Mix		35	0
Vitamin Mix		1	4
Choline Bitartrate		2	0
Total		1001	3906

[a] HFRD: D15101101, a high-fructose rich diet specially formulated by Research Diets.