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Chronic intermittent hypobaric hypoxia ameliorates endoplasmic reticulum stress mediated liver damage induced by fructose in rats

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ABSTRACT

Aim: High-fructose intake induces nonalcoholic fatty liver disease (NAFLD) and chronic intermittent hypobaric hypoxia (CIHH) has beneficial effects on the body. We hypothesized that CIHH has protective effects on the impaired hepar in fructose-fed rats.
Main methods: Sprague–Dawley rats (male, 160–180 g) were randomly divided into 4 groups: control group (CON), fructose group (FRUC, 10% fructose in drinking water for 6 weeks), CIHH group (simulated 5000 m altitude, 6 h per day for 6 weeks), and CIHH plus fructose groups (CIHH-F). Histopathology of liver, arterial blood pressure, blood biochemicals, hepatocyte apoptosis, and marker proteins of endoplasmic reticulum stress (ERS) were measured.
Key findings: The arterial blood pressure, body mass index, abdominal fat weight and liver weight were increased in FRUC rats but not in CIHH-F rats. Likewise, the serum glucose, insulin, insulin C peptide, triglyceride (TG) and total cholesterol (TC) were elevated in FRUC rats but not in CIHH-F rats after fasting 12 h. Meanwhile, the hepatic steatosis and hepatocyte apoptosis occurred in FRUC rats but not in CIHH-F rats. Finally the expression of ERS markers including GRP78 (glucose-regulated protein78), CHOP (C/EBP Homologous Protein), and caspase-12 in hepatic tissue were up-regulated in FRUC rats, but such up-regulation was not observed in CIHH-F rats.
Significance: Our results suggest that CIHH protect hepar against hepatic damage through inhibition of ERS in fructose-fed rats. CIHH might be the new therapy for NAFLD.

Introduction

Nonalcoholic fatty liver disease (NAFLD), the main expression of the metabolic syndrome (MS) in liver, is a common liver disease and the prevalence is likely to increase in the coming decades. NAFLD includes simple steatosis and nonalcoholic steatohepatitis (NASH). Simple steatosis is a benign pathological change without chronic alcohol consumption. NASH displays necro-inflammation and fibrosis, eventually leading to cirrhosis, hepatocellular carcinoma and end-stage liver disease. NASH is the most general pathogeny of cryptogenic cirrhosis [3]. The collecting evidence suggests that diabetes, obesity, and insulin resistance are tightly associated with ectopic fat accumulation especially in the liver. Hepatic steatosis might be the cause of insulin resistance [28]. However, the mechanism underlying NAFLD remains unknown, and an effective therapy for NAFLD is needed.

Recently, it has been shown that the endoplasmic reticulum (ER) is implicated in both the development of simple steatosis and the progression to NASH [27]. Disruption of ER homeostasis, often termed ER stress, has been demonstrated in liver and adipose tissues of patients with NAFLD [18]. The high fat diet-fed C57BL/6 mice featured enhanced lipogenesis and ER stress [17]. In human normal hepatocytes, L02 and HepG2 cell lines, ER stress induced by saturated fatty acid promoted apoptosis through the PERK (PKR-like Endoplasmic Reticulum Kinase)/ATF4 (Activating Transcription Factor 4)/CHOP (C/EBP Homologous Protein) signaling pathway [5,14]. Injection of ER stress inducer tunicamycin (TM) could result in NASH in mice demonstrating hepatic steatosis and inflammation. In mice treated with TM, hepatic triglycerides (TG) were increased but plasma lipids, such as TG, total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL), were decreased [16]. ER stress was also induced in liver of fructose-fed mice. 4-Phenylbutyric acid (PBA), an ERS inhibitor, was proved to attenuate both ER stress and hepatic lipid accumulation [34].

Chronic intermittent hypobaric hypoxia (CIHH) is also termed as exposure to hypoxia interrupted by normoxia. A lot of researches showed that CIHH had beneficial effects including cardioprotection [10,32], regulation of arterial blood pressure [25,33], prevention of arthritis [23], and facilitation of carotid sinus baroreflex [7]. Recently,

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our study demonstrated that CIHH can prevent cardiac dysfunction and normalize abnormality of plasma glucose, TC, TG, insulin, and insulin C peptide after fasting 12 h [35]. We also found that CIHH suppressed ERS induced by ischemia/reperfusion in myocardium [data unpublished]. So we hypothesized that CIHH might ameliorate hepatic damage of NAFLD through inhibiting ERS in fructose-fed rats.

Materials and methods

Chemicals

Fructose was purchased from Sigma (St. Louis, MO). The kit for terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) and hematoxylin–eosin (HE) was purchased from Roche Applied Science (Indianapolis, IN). Antibodies against GRP78 (glucose-regulated protein78), caspase-12 and CHOP were purchased from Abcam (Cambridge, UK), and antibody against GAPDH and all secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Nitrocellulose (NC) membrane was obtained from Hybond-C (Amersham Life Science, UK) and an enhanced chemiluminescence (ECL) kit was obtained from Beijing Applygen Technologies (Beijing, CN). Other chemicals and reagents were all of analytical grade.

Fructose feeding and CIHH treatment

All animal procedures were complied with according to the Animal Management Rule of the Ministry of Health, People's Republic of China (Documentation No. 55, 2001) and EU Directive 2010/63/EU for animal experiments. Sprague–Dawley rats (male, 160–180 g, Hebei Medical University Animal Center) were randomly divided into 4 groups: control (CON), fructose-fed (FRUC), CIHH, and CIHH plus fructose-fed (CIHH-F).The model of MS induced by fructose in rats was the same as our previous studies [35]. Briefly, the rats in the FRUC and CIHH-F groups were fed with 10% fructose in water for 6 weeks. The methods of CIHH were described in our previous studies [10]. Simplified, the rats in the CIHH and CIHH-F groups were in a hypobaric chamber, and were exposed to hypobaric hypoxia (simulated 5000 m altitude for 6 weeks, 6 h per day, PB = 404 mm Hg, PO_2 = 84 mm Hg). All rats drank water freely, fed a standard laboratory diet, and were housed in a temperature-controlled room (22 ± 1 °C) with a 12 h/12 h light/dark cycle (lights on at 06:00 am). The volume of water intake was counted each day and body weight was measured once a week.

Measurement of blood pressure

The measurement of systolic arterial blood pressure (SAP) of tail artery was as described previously [6]. Briefly, SAP was determined in conscious rats by a tail-cuff pressure meter (LE5001, Pressure Meter, Powerlab, ADInstruments Company, AUS). SAP was determined 3 times in each rat and the mean was counted.

Measurement of biochemicals in blood and tissue

6 weeks later, 2.5 ml blood for each rat was collected from the angular vein after a 12 h fasting. The blood sample was centrifuged at 3000 rpm for 15 min and plasma was collected. Plasma glucose was determined by glucose oxidation method (Beijing Kemeidongya Company, CN), plasma TC was determined by cholesterase method (Shangdong 3V Biology Company, CN), plasma TG was determined by hydrolase method (Shangdong 3V biology company, CN), and the concentration of plasma insulin and insulin C peptide was determined by enzyme linked immunosorbent assay (ELISA) (Beijing North Biology Technique Institute, CN).

HE and TUNEL staining

A small segment of liver was fixed in 4% paraformaldehyde for 12 h, embedded in paraffin, cut into 6-μm-thick sections, stained with HE, and then observed under an optical microscope (BX 50, Olympus Optical, Japan). Apoptosis of hepatocytes was determined by TUNEL staining according to the manufacturer's instructions. Simplified, after deparaffinization and fixation, the sections were immersed in 20 mg/ml proteinase K for 20 min. After refixation and equilibration, sections were incubated at 37 °C for 60 min with biotinylated nucleotide and the terminal deoxynucleotidyl transferase recombinant enzyme, blocked in 0.3% H_2O_2, then incubated with streptavidin horseradish peroxidase (HRP) solution, and then detected with 0.05% diaminobenzidine in PBS containing 0.03% H_2O_2. Quantitative analysis was operated by determining the ratio of TUNEL-positive/total hepatocytes in 10 random high-power fields for each section.

Caspase-3 activity

Liver samples were collected and rinsed with PBS, homogenized and lysed in lysis buffer for 15 min on ice, then centrifuged at 12,000 × *g* for 10 min at 4 °C. The caspase-3 activity was detected by a commercial kit (Applygen Technologies Inc., Beijing, CN). The operation was made in accordance with the manufacturer's instruction. The quantification of caspase-3 activity was normalized by protein content.

Western blot analysis

100 mg of hepatic samples were frozen by liquid nitrogen, homogenized in 1 ml lysis buffer, and then centrifuged at 10,000 rpm for 10 min at 4 °C. Protein extracts were resuspended in a 6× sample buffer, and boiled in water for 15 min at 100 °C. 100 μg of samples were electrophoresed on 10% SDS-PAGE and transferred to NC membranes. The blots were then incubated with primary antibodies anti-GRP78 (1:1000), anti-caspase-12 (1:300), and anti-CHOP (1:1000) respectively at 4 °C overnight, and then with secondary antibody for 1 h at room temperature. The reaction was visualized by the ECL method, and the bands were analyzed by NIH image software three times. The protein contents were normalized to that of GADPH.

Statistical analysis

Data are expressed as mean ± SD. One-way analysis of variance was used to compare more than two groups, and Tukey's honestly significant difference test was used to test for differences between-individual groups. A $P < 0.05$ was considered statistically significant.

Results

Body mass index (BMI), intra-abdominal fat, and liver weights

Compared with CON rats, the BMI, intra-abdominal fat weight, and liver weight of FRUC rats were all significantly increased (all $P < 0.05$, Table 1), and CIHH could inhibit completely the increase induced by fructose (all $P < 0.05$, Table 1). However, there were no differences in BMI, intra-abdominal fat, and liver weights among CON, CIHH, and CIHH-F rats (all $P > 0.05$, Table 1).

SAP and blood biochemicals

Compared with CON rats, SAP in FRUC rats was significantly increased, and CIHH completely inhibited the increase of SAP induced by fructose ($P < 0.05$, Table 2). However, there were no differences in SAP among CON, CIHH, and CIHH-F rats ($P > 0.05$, Table 2). Similarly, the levels of plasma glucose, TC, TG, insulin and insulin C peptide after the 12 h fasting were increased in FRUC rats compared with CON rats,