Available online at www.sciencedirect.com

ScienceDirect

Journal of Nutritional Biochemistry 28 (2016) 51–60

Journal of Nutritional Biochemistry

A nutritional nonalcoholic steatohepatitis minipig model☆,☆☆

Sin-Jin Li[a], Shih-Torng Ding[a], Harry J. Mersmann[a], Chun-Han Chu[a], Chia-Da Hsu[b], Ching-Yi Chen[a,*]

[a]Department of Animal Science and Technology, National Taiwan University, No. 50, Lane 155, Sec 3, Keelung Rd, Taipei 10672, Taiwan
[b]School of Veterinary Medicine, National Taiwan University, No. 1, Sec 4, Roosevelt Rd, Taipei 106, Taiwan

Received 9 July 2015; received in revised form 26 September 2015; accepted 30 September 2015

Abstract

Background and Aims: The objective of this study was to elucidate whether a Western diet was associated with nonalcoholic steatohepatitis (NASH), and the relationship between NASH, autophagy and endoplasmic reticulum (ER) stress.
Methods: Four-month-old Lee–Sung minipigs were randomly assigned to two groups: control diet (C) and Western diet (W), for a 5-month experimental period.
Results: Feeding a Western diet produced a body composition with more fat, less lean and a greater liver weight. Compared with C pigs, W pigs also exhibited an elevated level of plasma insulin and free fatty acid. The W pigs displayed glucose intolerance, lower circulation antioxidant capacity and greater hepatic oxidative stress. Furthermore, pig fed the W diets had increased collagen accumulation in the liver and elevated systemic inflammation [tumor necrosis factor α and interleukin (IL)-6]. Compared with C pigs, W pigs had higher hepatic ER stress-related protein expression of GRP94, CHOP and caspase-12. The W pigs also had greater hepatic autophagy-related protein expression of p62 and LC3II. In an obesity antibody array analysis, W pigs had higher type 2 diabetes mellitus- (insulin-like growth factor 1, osteoprotegerin and resistin), atherosclerosis- (vascular endothelial growth factor, platelet-derived growth factor-AA and plasminogen activator inhibitor-I) and inflammation- [IL-1, macrophage-stimulating protein alpha, X-linked ectodermal dysplasia receptor and serum amyloid A (SAA)] related protein expressions. In addition, W pigs had greater plasma SAA concentration than C pigs and plasma SAA level was highly associated with IL-6.
Conclusions: We successfully established a NASH pig model, and our findings suggested an association of NASH with ER stress and autophagy. The SAA has potential as a novel plasma biomarker for nonalcoholic fatty liver disease pigs.

Keywords: ER stress; Autophagy; NAFLD; SAA; IL-6; Lee–Sung minipigs

1. Introduction

When nutrient uptake and energy expenditure are in imbalance, ectopic deposition of lipid in nonadipose tissues such as the liver may result [1]. A nonalcoholic fatty liver disease (NAFLD) patient has chronic lipid metabolic imbalance, with excessive lipid supply to the liver, either from *de novo* lipogenesis or from excess dietary fat, to cause lipotoxic injury of hepatocytes. Nonalcoholic steatohepatitis (NASH) is a severe type of NAFLD worldwide [2]. Inflammation and oxidative stress can activate fibrogenesis in hepatic stellate cells (HSCs) and fibrosis is linked to NASH. Hyperinsulinemia in NASH can progress to cirrhosis with a high risk of liver failure and hepatocellular carcinoma [3].

Both the endoplasmic reticulum (ER) and autophagy have important functions in protein sorting, cellular homeostasis and energy balance. The ER is an important cellular organelle in which protein folding, calcium homeostasis and lipid biosynthesis occur. Abnormal lipid metabolism and excessive hepatic lipid accumulation disrupt ER homeostasis and are termed ER stress [4]. Under ER stress, the unfolded protein response (UPR) is activated. However, prolonged ER stress triggers apoptosis signaling and leads to cell death. Hepatic steatosis and insulin resistance trigger ER stress and decrease normal ER function to increase the risk of developing NAFLD [5]. Autophagy is a mechanism to maintain cellular homeostasis by degradation of proteins and cytoplasmic organelles. Autophagy dysfunction is observed during the development of NAFLD [6]. A hepatocyte model proves that an increase in autophagy function improves cell survival and protects against NAFLD-induced lipotoxic cell death [7]. Impaired autophagy flux promotes the prevalence of NAFLD and NASH, and contributes to insulin resistance and progression of hepatic steatosis to liver injury [8].

Abbreviations: T2DM, type 2 diabetes mellitus; CVD, cardiovascular disease; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; HSCs, hepatic stellate cells; ER, endoplasmic reticulum; UPR, unfolded protein response; MCD, methionine- and choline-deficient; HFD, high-fat diet; GRP94, glucose-regulated protein of 94 kDa; CHOP, C/EBP homologous protein; LC3, microtubule-associated protein 1A/1B-light chain 3; BNIP3, BCL2/adenovirus E1B 19 kDa protein-interacting protein 3; PARP, poly(ADP-ribose) polymerase.

☆ Conflict of interest: None declared.

☆☆ Financial support: This work was supported by the Research Grant MOST 103-2313-B-002-035 (from the Ministry of Science and Technology), MOST 104-2313-B-002-038-MY3 (from the Ministry of Science and Technology) and NTU-CESRP-104R7615-3 (from National Taiwan University).

* Corresponding author at: Department of Animal Science and Technology, National Taiwan University, No. 50, Lane 155, Sec 3, Keelung Rd, Taipei 10672, Taiwan. Tel.: +886 2 33665283; fax: +886 2 27324070.
E-mail address: ronichen@ntu.edu.tw (C.-Y. Chen).

http://dx.doi.org/10.1016/j.jnutbio.2015.09.029
0955-2863/

Rodent models have been widely used in NAFLD-related research; however, there is no single rodent model that includes the full symptoms of human NAFLD disease progression [9]. A methionine- and choline-deficient (MCD) diet is the most frequently used dietary model for NAFLD, but this is an abnormal nutrient-deficient dietary model. Furthermore, the MCD rodent model does not have all of the symptoms of NAFLD, including insulin resistance and overweight [10]. Compared with the MCD rodent model, the high-fat diet (HFD) rodent model does not cause severe liver injury and it takes a long feeding time to achieve NAFLD [11,12]. In contrast to rodents, swine physiological characteristics, such as cardiac anatomy, body size, propensity for sedentary behavior, and metabolism of dietary carbohydrates and lipids, are more similar to humans [12]. The body size, feeding cost and space demands of miniature pigs have advantages compared with domestic pigs [13]. Long-term feeding of a Western diet to Lee–Sung miniature pigs not only increases body weight and fat accumulation but also induces the metabolic syndrome including hyperglycemia, hyperlipidemia, hypertension and fatty liver [14]. The present study showed that pigs fed the Weston diet had hepatic fibrosis, insulin resistance and inflammation demonstrating that the pig fed this diet could be used as a NASH model. The current study determined the relationship between autophagy, ER stress and Western diet-induced NASH in these pigs. Furthermore, we used an obesity antibody array to search for a suitable biomarker for NASH in pigs.

2. Materials and methods

2.1. Animals and experiment diets

All animal care procedures used in this study were approved by the Institutional Animal Care and Use Committee of the National Taiwan University. Four-month old Lee–Sung miniature pigs (LS) from National Taiwan University were used in this study. Pigs with an initial body weight of 19.4±1.0 kg were randomly divided into two groups (five barrows and five gilts per group) and fed the control diet (C) or a Western diet (W) for 5 months. The two dietary compositions and feeding condition were described previously [14]. The control diet consisted, on a caloric basis, of 58.0% carbohydrate, 13.5% fat and 28.5% protein (metabolic energy=2.4 kcal/kg). The Western diet consisted of 35.3% carbohydrate, 45.7% fat and 18.3% protein (metabolic energy=3.8 kcal/kg). Feed was provided twice daily at 9:00 am and 5:00 pm, and water was available *ad libitum*. Pigs of the same sex and treatment were housed together in a sheltered pen. Pigs were allowed to acclimate at the research facilities for 1 week before starting the experiment. After 5 months of feeding, animals were sacrificed by intravenous injection of an overdose of barbiturate. Liver were removed, weighed and the lower portion of the left lobe was frozen in liquid nitrogen and stored at −80°C until analyses.

2.2. Body composition analysis

During the fifth month of diet feeding, pigs were fasted (12–16 h) and then sedated with Zoletil-50 (10 mg/kg im). The whole body composition as lean (lean/body weight, %), fat (fat/body weight, %) and water was determined using a quantitative magnetic resonance system, the Eco-MRI (EchoMRI-D Whole Body Composition Analyzer, Echo-MRI, SN, USA).

2.3. Blood analysis

Blood samples were taken from the jugular vein after an overnight fast and collected in an EDTA vacutainer tube (BD Vacutainer, Plymouth, UK). Plasma samples were collected by centrifugation at 200×*g* at 4°C for 20 min and stored at −80°C until analyses. Blood variables were measured using commercial kits: plasma glucose (GL2603, Rando, Antrim, UK) and plasma free fatty acids (FFAs; 700310; Cayman Chemical Company, Ann Arbor, MI, USA). Plasma insulin was measured by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (80-INSPO-E01; ALPOCO Diagnostics, Windham, NH, USA). All procedures were according to the manufacturer's instructions. The absorbance or fluorescence was measured using microplate assays (BioTek, Synergy H1, Ashton Vale, BRS, USA). All analyses were in triplicate.

2.4. Oxygen radical absorbance capacity

Total antioxidant capacity in plasma was analyzed by the oxygen radical absorbance capacity (ORAC) assay as described by Hsieh et al. [15]. Briefly, 100 μL of 3.78 mg/L β-phycoerythrin (P1286; Sigma, St. Louis, MO, USA) was added to a 96-well microplate (black). Then, 30 μL of phosphate buffer (67.5 mM Na_2HPO_4 and 7.5 mM KH_2PO_4, pH 7.4) or 30 μL of 5 μmol/L trolox (93510; Fluka, St. Louis, MO, USA) or 30 μL of 10-fold diluted plasma was added as blank, control, and sample groups, respectively. Next, 30 μL of 2,2′-Azobis (2-methylpropionamidine) dihydrochloride (440914, Sigma) was added to each well, and then the fluorescence was immediately measured (excitation: 515 nm, emission: 577 nm) and then every 5 min with a fluorescent microplate reader (BioTek) until the fluorescent value was below 100. The area under the fluorescence curve (AUC) was used to calculate differences in decay curves between the blank and either the control or the sample. The ORAC values were estimated by linear regression of the area vs. sample concentration and expressed as μmol Trolox equivalents (TE) per g of plasma protein. Protein concentrations were determined using Bradford assay reagents (B6916; Sigma).

2.5. Thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances (TBARS) were measured as the marker of oxidative stress [14]. A liver sample was homogenized in RIPA lysis buffer (20-188; Millipore, Billerica, MA, USA) and centrifuged at 14,000×*g* for 10 min at 4°C. The supernatant fraction was used for analysis. One hundred eighty microliters of TBA solution was added to 120 μL of sample. Then, 1.02 ml of trichloroacetic acid–HCl reagent was added and the vials were boiled for 30 min. After cooling to room temperature, the vials were centrifuged at 5000×*g* for 3 min at 4°C. The absorbance was measured at 535 nm using microplate assays (BioTek).

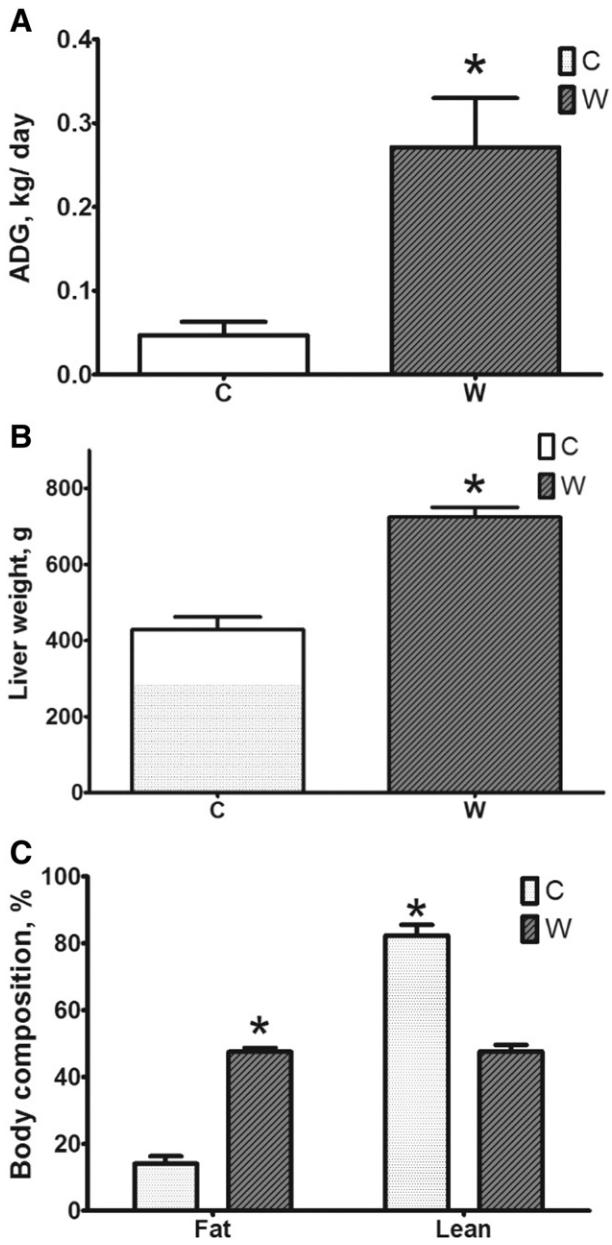

Fig. 1. Average daily gain (ADG) (A), liver weight (B) and body composition (C). C= control diet; W=Western diet. All results are expressed as mean±S.E.M. $n=10$. *$P<.05$ vs. control group.