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Effects of maternal serine supplementation on high-fat diet-induced oxidative stress and epigenetic changes in promoters of glutathione synthesis-related genes in offspring

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ABSTRACT

To elucidate the effects of maternal serine supplementation during pregnancy on high-fat (HF) diet-induced oxidative stress in the offspring and the epigenetic mechanisms involved, a control diet, HF diet, or HF diet supplemented with different concentrations of serine dissolved in water was fed to C57BL/6J mouse dams during gestation. Eight male offspring from each dietary group were sacrificed at weaning, Consequently, the serum and hepatic oxidative stress markers and lipid levels in weanlings from dams fed the HF diet supplemented with 1% serine were lower than those of offspring from dams fed the HF diet. Maternal serine supplementation prevented promoter DNA hypermethylation of HF diet-induced glutathione synthesis-related genes, and increased the gene expression and resultant protein contents in the weanlings. Our results therefore suggest that maternal serine supplementation during pregnancy alleviates HF diet-induced oxidative stress by epigenetically regulating glutathione synthesis in the weanling.

1. Introduction

Offspring are sensitive to changes in maternal nutrition during pregnancy and lactation. Maternal overnutrition in these periods results in an increased risk of the metabolic syndrome in the offspring and even altered pathophysiology throughout their whole life (Ohta et al., 2017; Simmons, 2005). Variations in early-life environmental factors, including nutrition, can induce different phenotypes in an organism, a phenomenon known as programming (Burdge, Hanson, Slater-Jefferies, & Lillycrop, 2007). It has been suggested that epigenetic regulatory mechanisms, such as DNA methylation, histone modifications, and microRNA-mediated post-transcriptional regulation, are involved in the nutritional programming of offspring. For example, maternal nutrition can affect the regulation of metabolism in offspring (e.g., cholesterol homeostasis and gluconeogenesis) by regulating the methylation status of CpGs on the promoters of genes related to gluconeogenesis and cholesterol metabolism (Cai, Jia, Lu et al., 2014; Cai, Jia, Song et al., 2014).

Oxidative stress has been suggested to play a critical role during the development of the pathophysiological process in offspring. Accumulated triglycerides (TGs) and decreased gene expression of the antioxidative enzymes glutathione peroxidase 1 and superoxide dismutase 1 were observed in the liver of offspring from rat dams fed a high-fat (HF) diet (Zhang, Strakovsky, Zhou, Zhang, & Pan, 2011). Furthermore, HF diet feeding in mouse dams induced a high hepatic content of phosphatidylcholine hydroperoxide (an oxidative stress marker) in the offspring at 11 weeks of age (Ito et al., 2016).

Methyl donors, including methionine, betaine, and folic acid, are able to improve upon the epigenetic modifications and thereby rectify the metabolic disorders in offspring caused by maternal factors (Lillycrop et al., 2010; Weaver et al., 2005). A maternal diet supplemented with betaine modified the hepatic expression of gluconeogenic and cholesterol metabolic genes by improving promoter CpG hypermethylation of related genes (Cai, Jia, Lu et al., 2014; Cai, Jia, Song

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; CBS, cystathionine β-synthase; DNMT, DNA methyltransferase; GCLC, glutamate-cysteine ligase catalytic subunit; GCLM, glutamate-cysteine ligase modifier subunit; GSH, glutathione; LPO, lipid hydroperoxide; MDA, malondialdehyde; ROS, relative oxygen species; TC, total cholesterol; TG, triglyceride

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et al., 2014). In addition, a HF diet supplemented with a complex of methyl donors (choline, vitamin B12, betaine, folic acid) in lactating mothers prevented hepatic lipid accumulation in the adult offspring (Cordero, Milagro, Campion, & Martinez, 2014). However, whether maternal dietary methyl donors can alleviate HF-induced oxidative stress in the offspring remains to be elucidated.

Recently, the nutritionally nonessential amino acid serine was suggested to be involved in one-carbon metabolism by supplying one-carbon units to the methionine cycle (Parker & Metallo, 2016). Moreover, dietary serine could help to alleviate hepatic oxidative stress and prevent lipopolysaccharide-induced intestinal oxidative stress by improving glutathione (GSH) synthesis (Zhou, 2017; Zhou et al., 2017). Furthermore, serine also epigenetically modulated the expression of GSH synthesis-related genes in HF diet-fed mice (Zhou, 2018). In addition, a recent study using mouse model reported a maternal effect of serine on offspring body weight (Nagamachi et al., 2018). Consequently, the present study was conducted with mouse model to elucidate the effects of maternal serine supplementation during pregnancy on HF diet-induced oxidative stress in the offspring and on the epigenetic mechanisms involved.

2. Materials and methods

2.1. Animal care and experimental design

Male and female virgin C57BL/6J mice (9 weeks old) were purchased from the SLAC Laboratory Animal Central (Changsha, China). After an adaptation period of 1 week, the mice were fed a control diet and mated, and the females were then checked every morning for postcopulatory plugs. Pregnant mice were housed individually and randomly separated into five dietary groups: control (CON) diet (10% as fat, 70% as carbohydrate, and 20% as protein; Research Diets, New Brunswick, NJ, USA); HF diet (45% as fat, 35% as carbohydrate, and 20% as protein; Research Diets); HF diet with 0.1% (wt:vol) serine dissolved in water (low serine, LS); HF diet with 0.5% (wt:vol) serine dissolved in water (medium serine, MS); and HF diet with 1% (wt:vol) serine dissolved in water (high serine, HS). After birth, all dams did not receive further serine supplementation. Dams from each dietary group were randomly assigned 8 pups each (4 males and 4 females) until weaning at 3 weeks of age. Half of the male weanlings from each group were then sacrificed by cervical dislocation, and their plasma and liver samples were collected. The remaining male offspring from dams fed CON, HF, and HS diets were further fed either the CON diet or the HF diet for another 12 weeks without serine supplementation. Then, they were sacrificed by cervical dislocation at 15 weeks of age, and their blood and liver samples were collected. Food intake was recorded every day and the body weight was measured every week. The breeding schedule was showed in Fig. 1. All mice were housed under standard conditions, in pathogen-free colonies (temperature, 22 ± 2 °C; relative humidity, 50 ± 5%; lighting cycle, 12 h/d), with free access to food and water. This study was approved by the animal welfare committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences, and all the procedures were carried out according to the rules established by the committee.

2.2. Determination of aspartate aminotransferase, alanine aminotransferase, glucose and glutathione levels

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and glucose levels were determined by the colorimetric method using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The reduced GSH content was measured using a commercial colorimetric assay kit (Cayman Chemical Company, Ann Arbor, MI, USA) according to the manufacturer's instructions.

2.3. Determination of triglyceride and cholesterol levels

Serum TG was determined using a commercial kit according to the manufacturer's instructions (Beijing Strong Biotechnologies, Inc., Beijing, China). Liver samples were pretreated according to the method described in a previous study (Zhou, 2017), and the cholesterol and TG contents were assayed using commercial kits according to the manufacturer's instructions (Sigma-Aldrich, Shanghai, China).

2.4. Determination of reactive oxygen species and lipid oxidation biomarkers

The hepatic reactive oxygen species (ROS) content was analyzed as previously described (Zhou, 2017). Liver samples were embedded in an optimum cutting temperature (tissue-freezing medium) compound (Sakura, Tokyo, Japan), immediately frozen in a methylbutane-chilled bath at –80 ± 2 °C, and then stored in liquid nitrogen. For imaging, 10-μm sections were sliced and stained with dihydroethidium (Sigma-Aldrich) at a concentration of 1 μM for 20 min at 37 °C. Representative fluorescence microscopy images were taken and analyzed by Image Browser software (Leica, Wetzlar, Germany). Liver samples were prepared and malondialdehyde (MDA) and lipid hydroperoxide (LPO) contents were determined using a commercial kit according to the manufacturer's instructions (Northwest Life Science Specialties, Vancouver, WA, USA).

2.5. Hematoxylin–eosin and Oil Red O staining

Liver samples were fixed with 4% formaldehyde and embedded in paraffin, and the paraffin blocks were then sliced into 8-μm-thick sections and stained with hematoxylin–eosin (H&E). Samples were also prepared in the same way as was done for ROS detection, and 10-μm sections were stained with Oil Red O.

2.6. Determination of cystathionine beta-synthase, glutamate-cysteine ligase, and DNA methyltransferase, serine and homocysteine concentrations

Cystathionine beta-synthase (Cbs), glutamate-cysteine ligase catalytic subunit (Gclc), glutamate-cysteine ligase modifier subunit (Gclm), DNA methyltransferase 1 (Dnmt1), and Dnmt3b concentrations were determined by the colorimetric method with commercially available kits (USCN, Wuhan, China). Liver samples were pretreated according to previous study (Zhou, 2018) and the supernatant was analyzed by an ion-exchange amino acid analyzer (L8800, Hitachi, Tokyo, Japan) to determine serine content. Homocysteine (Hcy) content was measured by ELISA kits (Cell Biolabs, Inc. San Diego, CA, USA) according to the manufacturer's instructions.

2.7. RT-qPCR analysis

Total RNA was isolated using the TRIzol reagent (Invitrogen Corporation, Carlsbad, CA, USA) (Jin et al., 2017). RT-qPCR was performed with a Roche LightCycler 480 II system, in a 10-μL assay solution containing 1 μL of cDNA template, 5 μL of SYBR Green mix (Takara, Dalian, China), 0.2 μL of ROX, 3 μL of diethylpyrocarbonate-treated deionized H_2O, and 0.4 μL each of the forward and reverse primers (primers information shown in the Supplementary Materials). All samples were run in triplicate and the average values were calculated.

2.8. Pyrosequencing

Genomic DNA was extracted from liver samples and then modified by sodium bisulfate, using the EZ DNA Methylation-Gold Kit D5005 (ZYMO Research, Beijing, China). A specific region in the promoters of *Cbs*, *Gclc*, and *Gclm* was PCR-amplified respectively using a PCR kit