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Serine prevented high-fat diet-induced oxidative stress by activating AMPK and epigenetically modulating the expression of glutathione synthesis-related genes

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ABSTRACT

Serine deficiency has been observed in patients with nonalcoholic fatty liver disease (NAFLD). Whether serine supplementation has any beneficial effects on the prevention of NAFLD remains unknown. The present study was conducted to investigate the effects of serine supplementation on hepatic oxidative stress and steatosis and its related mechanisms. Forty male C57BL/6J mice (9 week-old) were randomly assigned into four groups (n = 10) and fed: i) a low-fat diet; ii) a low-fat diet supplemented with 1% (wt:vol) serine; iii) a high-fat (HF) diet; and iv) a HF diet supplemented with 1% serine, respectively. Palmitic acid (PA)-treated primary hepatocytes separated from adult mice were also used to study the effects of serine on oxidative stress. The results showed that serine supplementation increased glucose tolerance and insulin sensitivity, and protected mice from hepatic lipid accumulation, but did not significantly decreased HF diet-induced weight gain. In addition, serine supplementation protected glutathione (GSH) antioxidant system and prevented hypermethylation in the promoters of glutathione synthesis-related genes, while decreasing reactive oxygen species (ROS) in mice fed a HF diet. Moreover, we found that serine supplementation increased phosphorylation and *S*-glutathionylation of AMP-activated protein kinase α subunit (AMPKα), and decreased ROS, malondialdehyde and triglyceride contents in PA-treated primary hepatocytes. However, while AMPK activity or GSH synthesis was inhibited, the above-mentioned effects of serine on PA-treated primary hepatocytes were not observed. Our results suggest that serine supplementation could prevent HF diet-induced oxidative stress and steatosis by epigenetically modulating the expression of glutathione synthesis-related genes and through AMPK activation.

1. Introduction

Oxidative stress has been implicated in the physiopathology of nonalcoholic fatty liver disease (NAFLD), which is considered as the most common chronic liver disease worldwide. As important NAFLD pathogenic factors, high-fat diet (HFD) consumption or overnutrition results in lipid metabolism disorders and over-production of reactive oxygen species (ROS) [1]. The liver has been proven to be especially vulnerable to ROS damage as it is the central organ for lipid metabolism and plays a key role in the regulation of lipid homeostasis [2]. Excessive ROS impairs proteins, lipids and DNA, and further leads to proinflammatory cytokine secretion, hepatocyte degeneration, cell death, and fibrogenesis [3].

AMP-activated protein kinase (AMPK), as the cellular sensor of

Abbreviations: Acadm, medium-chain acyl-CoA dehydrogenase; AMPKα, AMP-activated protein kinase, α subunit; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BSO, buthionine sulfoximine; CBS, cystathionine-β-synthetase; CPT1a, carnitine palmitoyltransferase 1a; *DGAT*, diacylglycerol O-acyltransferase; Gclc, glutamate-cysteine ligase, catalytic subunit; Gclm, glutamate-cysteine ligase, modifier subunit; GSH, glutathione; GSH-Px, glutathione peroxidase; GSR, glutathione reductase; GSSG, oxidized glutathione; GST, glutathione *S*-transferase; Hcy, homocysteine; HFD, high-fat diet; LDH, lactate dehydrogenase; MDA, malondialdehyde; NAFLD, nonalcoholic fatty liver disease; nrf2, nuclear factor-erythroid 2-related factor-2; pAMPKα, phosphor-Thr172-AMPKα; ROS, reactive oxygen species; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; SOD, superoxide dismutase

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energy and redox status, plays an important role in mediating the adaptive response to dysfunctional lipid metabolism and oxidative stress [4,5]. AMPK activation promotes cell survival *via* regulation of mitochondrial biogenesis and expression of genes involved in antioxidant defense under oxidative conditions [6]. In addition, expression levels of superoxide dismutase (SOD), catalase and γ-glutamylcysteine synthetase, which are critical to the antioxidant defense system, were attenuated following knockdown of the AMPK α1 subunit [7]. Moreover, through decreased phosphorylation of AMPK, previous studies established that AMPK activation mediated the beneficial effects of several natural components (polysaccharide, gastrodin, demethyleneberberine, etc.) on high-fat diet-induced NAFLD by alleviating oxidative stress [8–10]. These results suggested activation of the AMPK signaling pathway might play an important role in the prevention of NAFLD.

Serine plays a critical role in the central metabolism, where it is involved in the synthesis of glutathione (GSH), protein (glycine and cysteine), sphingosine, phosphatidylserine and nucleotides [11,12]. According to the results of genome-scale metabolic modelling of hepatocytes, serine deficiency is concomitant with changes in metabolites involved in the interconversion of serine in patients with NAFLD [12]. These results raised the possibility that serine supplementation might have beneficial effects in the protection of NAFLD. Considering serine is a precursor of GSH synthesis [13] and involved in one-carbon metabolism [11], we speculated that serine might help alleviate oxidative stress and improve DNA methylation which are both closely related to the development of NAFLD [14]. Consequently, the present study aimed to investigate the effects of serine supplementation on GSH synthesis, oxidative stress, DNA methylation, and the AMPK pathway in HFD-induced mice.

2. Materials and methods

2.1. Animal care and experimental design

Forty male C57BL/6J mice (9 week-old) were purchased from the SLAC Laboratory Animal Central (Changsha, China). 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.

All animals were randomly assigned into four groups (n = 10): i) mice were fed on a low-fat diet (Control); ii) mice were fed on a low-fat diet supplemented with 1% (wt:vol) serine (Serine); iii) mice were fed on a high-fat diet (HF); and iv) mice were fed on a high-fat diet supplemented with 1% serine (HF + Serine). L-serine (Sigma-Aldrich, Shanghai, China) was supplemented in the drinking water at a concentration of 1% (wt/vol). The duration of experiment was 8 weeks. The low-fat diet consisted of 10% (kcal%) fat, 20% protein and 70% carbohydrate, while the high-fat diet consisted of 45% fat, 20% protein and 35% carbohydrate (Research Diets, Inc. New Brunswick, NJ, USA). Upon completion of the experiment, blood was taken from the retro-orbital sinus of each mouse after 4 h of fasting. Thereafter, following cervical dislocation, liver and white adipose tissue (WAT), including major subcutaneous WAT (inguinal WAT, ingWAT), and two representative visceral WATs (mesenteric WAT, mWAT and epididymal WAT, eWAT) were separated and weighed. Liver samples were either immediately fixed in formaldehyde solution for morphology observation or snap-frozen in liquid nitrogen and stored at − 80 °C until analysis. This study was approved by the animal welfare committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences.

2.2. Primary hepatocyte isolation and treatment

Primary hepatocytes from adult mice were isolated according to methods described in a previous study [15]. Briefly, fresh liver was minced and incubated with 0.2% collagenase IV at 37 °C for 15 min. The homogenized liver was then centrifuged at 1000 rpm for 5 min, and the resulting pellet was resuspended in DMEM-F12 medium. Isolated cells were subsequently obtained following filtration through a nylon mesh (61 μM pore size). Finally, cells were plated in individual 60-mm diameter culture dishes coated with 0.03% rat tail collagen and cultured in a 5% CO_2 atmosphere at 37 °C for further use. To induce oxidative stress, 200 μM palmitic acid (PA) (Sigma-Aldrich) conjugated to fatty acid-free bovine serum albumin (Sigma-Aldrich) was added to hepatocytes (~70% confluence) seeded in 6-well plates for 24 h. In addition, 2 μM Compound C (Aladdin, Shanghai, China), an inhibitor of AMPK, or 0.25 mM buthionine sulfoximine (BSO, Sigma-Aldrich), a specific inhibitor of glutamine-cysteine ligase were also added into hepatocytes for 24 h.

2.3. Biochemical assays

Biochemical assays for the following indexes were performed with commercially available kits: aspartate aminotransferase (AST), alanine aminotransferase (ALT), malondialdehyde (MDA), lactate dehydrogenase (LDH) and glucose (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), insulin, adiponectin, leptin (Cusabio Biotech Co., Ltd. Wuhan, China) and triglyceride (TG) (Beijing Strong Biotechnologies, Inc. Beijing, China).

2.4. Intraperitoneal glucose and insulin tolerance test

Intraperitoneal glucose test (IGTT) was conducted nine days before completion of the experiment and insulin tolerance test (ITT) were conducted 1 week before completion of the experiment. After a 6 h fasting period, mice were intraperitoneally injected with a dose of 1.0 g glucose or 0.65 U insulin per kg body weight. Blood was collected from the tail vein and glucose concentration was measured at 0, 30, 60 and 120 min after glucose injection using a One Touch Ultra Easy glucometer.

2.5. Hematoxylin-erosin and Oil Red O staining, and hepatic lipid determination

Liver samples were fixed with 4% formaldehyde and were paraffin embedded. Sections of 8-μm thickness were stained with hematoxylin–eosin (H&E). Liver samples were also placed in an optimum cutting temperature (tissue freezing medium) compound (Sakura, Tokyo, Japan), flash-frozen in a methylbutane-chilled bath at − 81 ± 2 °C, and then stored in liquid nitrogen. Sections of 10-μm were stained with Oil Red O. Liver samples (100 mg) were homogenized in 1 mL solution of 5% Nonidet P 40 Substitute (Sigma-Aldrich) and water. Slowly heat the samples to 90 °C in a water bath for 5 min, and then cool to room temperature. Subsequently, repeat the heating one more time and centrifuge at 13,000 rpm for 2 min to remove insoluble material. The supernatants were used for cholesterol and TG assays according to the manufacturer's instruction (Sigma-Aldrich). Protein was assayed using the Protein Assay Kit (Pierce Biotechnology, Rockford, IL, USA).

2.6. Determination of ROS and malondialdehyde

Hepatic ROS content was determined as previously described [16]. As with Oil Red O staining, 10-μm sections were sliced and stained with a 1 μM solution of dihydroethidium (Sigma-Aldrich) for 20 min at 37 °C in a humidified 5% CO_2 incubator. Samples were analyzed by fluorescence microscopy and Image Browser software (Leica, Wetzlar, Germany). ROS levels in primary hepatocytes were determined according to methods used in a previous study [17]. In brief, cells were cultured with 10 μM of 2′,7′-dichlorodihydrofluoresein diacetate (H_2DCFDA; Invitrogen Corporation, Carlsbad, CA) for 30 min and then washed twice with PBS. Cells were subsequently lysed and fluorescence intensity measured using a fluorescence spectrophotometer (Hitachi 4500, Japan) at excitation and emission wavelengths of 480 nm and