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Original Research

Sesamin ameliorates hepatic steatosis and inflammation in rats on a high-fat diet via LXRα and PPARα

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ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) is defined by a nonalcohol relevant pathological accumulation of fat in the liver. Previous studies have shown that sesamin exerts antioxidant effects and improves lipid metabolism of the fatty liver. In this study, we hypothesized that sesamin improves lipid homeostasis of Sprague-Dawley rats fed a high-fat diet (HFD) by regulating the expression of genes related to de novo lipogenesis and β-oxidation. We induced NAFLD in rats with HFD and examined the effect of sesamin in vivo. The results showed that HFD rats accumulated total cholesterol and triacylglycerols in the liver and developed inflammation, as evidenced by the elevation of interleukin-6 and tumor necrosis factor–α in the liver and serum. Sesamin attenuated the disease progression by improving the blood lipid profile in a dose-dependent manner. Sesamin reduced the serum levels of total cholesterol, triacylglycerols, low-density lipoprotein cholesterol, and free fatty acid, whereas it increased the level of high-density lipoprotein cholesterol. Meanwhile, sesamin increased the activities of hepatic glutathione peroxidase and superoxide dismutase while reducing the level of malonaldehyde and cytochrome P450 2E1. Furthermore, higher doses of sesamin reduced the expression of liver X receptor α and its downstream target genes, whereas it upregulated the peroxisome proliferator-activated receptor α–mediated signaling. These findings suggest that sesamin attenuates diet-induced dyslipidemia and inflammation of NAFLD in rats via mechanisms regulated by liver X receptor α and peroxisome proliferator-activated receptor α.

Abbreviations: ACOX1, acyl CoA-oxidase 1; ANOVA, analysis of variance; ApoB100, apolipoprotein B100; CAT-1, cationic amino acid transporter 1; cDNA, complementary DNA; CYP2E1, cytochrome P450 2E1; FAS, fatty acid synthase; FFA, free fatty acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GSH-Px, glutathione peroxidase; HDL-C, high-density lipoprotein cholesterol; HFD, high-fat diet; IL6, interleukin-6; LDL-C, low-density lipoprotein cholesterol; LXRα, liver X receptor α; MDA, malonaldehyde; MTTP, microsomal triglyceride transfer protein; NAFLD, nonalcoholic fatty liver disease; PPARα, peroxisome proliferator-activated receptor α; qPCR, quantitative polymerase chain reaction; SOD, superoxide dimutase; SREBP1c, sterol regulatory element-binding protein 1c; TAG, triacylglycerols; TC, total cholesterol; TNFα, tumor necrosis factor–α.

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1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is defined by the pathological accumulation of fat in the liver without alcohol, which is characterized by hepatic steatosis, liver cell injury, and lobular hepatitis [1]. Although the pathogenesis of NAFLD is not completely clear, multiple mechanisms, such as aberrant lipid metabolism, insulin resistance, dysregulated cytokine production, oxidative stress, and inflammation in hepatocytes, are believed to be involved [2-5]. The pathogenesis of hepatic steatosis is widely considered to result from an imbalance between lipid availability and consumption, which can be caused by defects in many biological processes, such as enhanced de novo lipogenesis, decreased mitochondrial fatty acid β-oxidation, and/or lipid export from the liver [6-8]. At a molecular level, many nuclear receptors are involved in lipid homeostasis. Liver X receptor α (LXRα) and peroxisome proliferator-activated receptor α (PPARα) play crucial roles in the regulation of fatty acid metabolism [9]. LXRα antagonists reduce the expression of sterol regulatory element-binding protein 1C (SREBP1c) and fatty acid synthase (FAS) in the liver, suggesting that the reduction of triacylglycerols (TAG) caused by LXRα inhibition might be associated with decreases in the expression of proteins mediating hepatic fatty acid synthesis [10,11]. PPARα attenuates hepatic fat accumulation by upregulating the levels of acyl CoA-oxidase 1 (ACOX1) and cationic amino acid transporter 1 (CAT-1), thereby enhancing fatty acid oxidation [12,13].

Sesamin, a major lignan present in sesame seeds and sesame seed oil, is known to have many biological activities [14]. Previous in vivo studies have demonstrated the antioxidant activity of sesamin and have shown that sesamin protects the liver from the oxidative stress induced by carbon tetrachloride or acetaminophen [15,16]. Sesamin has also been found to regulate lipid metabolism and reduce cholesterol levels [17]. In addition, some studies have suggested that sesamin can ameliorate abnormal lipid metabolism of the fatty liver [18,19]. However, the full-range effects of sesamin on NAFLD in vivo and the underlying mechanisms remain uncharacterized.

Therefore, the aim of the present study was to investigate the effects of sesamin on NAFLD induced by a high-fat diet (HFD) in rats and to explore the possible molecular mechanisms of those effects. We hypothesized that sesamin supplementation would improve hepatic steatosis by regulating the LXRα and PPARα signaling pathways. To test this hypothesis, we treated NAFLD rats induced by HFD with different doses of sesamin and examined whether sesamin could ameliorate hepatic steatosis and inflammation. In parallel, we determined the effects of sesamin on lipid accumulation and inflammation levels, serum lipid profile and gene expression of oxidative stress markers, and steatohepatitis-related indicators.

2. Methods and materials

2.1. Materials

Sesamin was purchased from Sigma-Aldrich (St Louis, MO, USA). The stock solutions at the concentrations of 8, 16, and 32 mg/mL were prepared freshly in plant oil every week and stored at 4°C. The plant oil is composed of rapeseed oil and soybean oil at a ratio of 7:3 as reported in a previous study [20]. Considering the high content of 22:1n9 cis (erucic acid; 30%-50%) in crude rapeseed oil, we chose refined rapeseed oil, which is low in erucic acid (<5%), and added 30% soybean oil to improve the essential fatty acid composition of the lipid. The HFD and the control diet were obtained from Research Diets (Research Diets, New Brunswick, NJ). All reagents were of analytical grade, unless specified otherwise.

2.2. Animals and treatments

A total of 45 male 8-week-old Sprague-Dawley rats were purchased from the Animal Experiments Research Centre in Xi'an Jiaotong University and randomly assigned to 5 groups (9 rats/group) with similar mean body weights. Two groups of rats were used as controls, with one group (the Ctrl group) maintained on a control diet (15.8 kcal% [15.8 kJ%] fat; AIN 93G, D10012G, Research Diets) and the other (the HFD group) on an HFD (45 kcal% [45 kJ%] fat; D12451, Research Diets). The other 3 groups of rats were also fed with HFD but were supplemented with sesamin 5 weeks after switching to HFD via daily intragastric administration of 40, 80, or 160 mg/kg body weight (bw) of sesamin (corresponding to the low-, mid-, or high-dose groups, respectively) (Table 1). The rationale for the doses of sesamin in our study is that the doses were determined according to some previous studies both in human and in animals [21-23] and our preliminary data. According to our previous study [24], the doses of sesamin used in this study were safe for rats. At the time of sesamin administration, the Ctrl and HFD groups of rats were given saline and plant oil, respectively. The animals were maintained individually in an air-conditioned room at 24°C with a 12:12 light-dark cycle and ad libitum access to food and autoclaved water. The animals were weighed weekly, and the consumption of water and food was calculated.

The sesamin treatment lasted for 7 weeks. At the end of the feeding study, all rats were anesthetized with 3% pentobarbital after 12 hours of fasting. Loss of reflex was confirmed by pricking the animals' feet and legs with forceps. Blood was collected from the tail and centrifuged at 3000 rpm for 15 minutes at 4°C, and the supernatants were collected and stored at –70°C. All rats were euthanized by cervical dislocation. Then, 0.2 g of liver tissue was excised and homogenized using a polytron homogenizer in 10 vol of saline solution at 4°C for 30 seconds, and the homogenate was centrifuged at 12,000 rpm for 10 minutes. The protein concentration in the resulting supernatant was determined by a Bradford assay [25]. The serum and hepatic levels of interleukin-6 (IL6) and tumor necrosis factor–α (TNFα) were measured by enzyme-linked immunosorbent assays according to the manufacturer's instructions (Thermo Scientific, Leicestershire, UK) and expressed as pg/mL of serum and pg/mg liver protein, respectively. Meanwhile, liver slices were also collected, fixed with 10% buffered formalin, and embedded in paraffin. All tissue slides were cut at a thickness of 5 μm and stained with hematoxylin and eosin. Images were captured with an Olympus BX60 camera, and data were collected from 5 random fields per rat at 400× magnification, as previously reported [26]. The study and all of the procedures