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Original Research

Resveratrol prevents hepatic steatosis and endoplasmic reticulum stress and regulates the expression of genes involved in lipid metabolism, insulin resistance, and inflammation in rats

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ABSTRACT

Previous research demonstrated that resveratrol possesses promising properties for preventing obesity. Endoplasmic reticulum (ER) stress was proposed to be involved in the pathophysiology of both obesity and hepatic steatosis. In the current study, we hypothesized that resveratrol could protect against high-fat diet (HFD)-induced hepatic steatosis and ER stress and regulate the expression of genes related to hepatic steatosis. Rats were fed either a control diet or a HFD for 12 weeks. After 4 weeks, HFD-fed rats were treated with either resveratrol or vehicle for 8 weeks. Body weight, serum metabolic parameters, hepatic histopathology, and hepatic ER stress markers were evaluated. Moreover, an RT^2 Profiler Fatty Liver PCR Array was performed to investigate the mRNA expressions of 84 genes related to hepatic steatosis. Our work showed that resveratrol prevented dyslipidemia and hepatic steatosis induced by HFD. Resveratrol significantly decreased activating transcription factor 4, C/EBP-homologous protein and immunoglobulin binding protein levels, which were elevated by the HFD. Resveratrol also decreased PKR-like ER kinase phosphorylation, although it was not affected by the HFD. Furthermore, resveratrol increased the expression of peroxisome proliferator-activated receptor δ, while decreasing the expression of ATP citrate lyase, suppressor of cytokine signaling-3, and interleukin-1β. Our data suggest that resveratrol can prevent hepatic ER stress and regulate the expression of peroxisome proliferator-activated receptor δ, ATP citrate lyase, suppressor of cytokine signaling-3, tumor necrosis factor α, and

Abbreviations: ER, Endoplasmic reticulum; HFD, high fat diet; ATF4, activating transcription factor 4; CHOP, C/EBP-homologous protein; BiP, immunoglobulin binding protein; PERK, PKR-like ER kinase; PPARδ, peroxisome proliferator-activated receptor δ; ACLY, ATP citrate lyase; SOCS-3, suppressor of cytokine signaling-3; TNFα, tumor necrosis factor α; IL-1β, interleukin-1β; SIRT1, Sirtuin 1; IRE-1α, inositol-requiring enzyme-1α; TC, total cholesterol; TG, triacylglcerol; HOMA-IR, Homeostatic model assessment-insulin resistance; NAFLD, nonalcoholic fatty liver disease; UPR, unfolded protein response; SREBP-1, sterol regulatory element-binding protein-1.

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interleukin-1β in diet-induced obese rats, and these effects likely contribute to resveratrol's protective function against excessive accumulation of fat in the liver.

1. Introduction

Obesity is often associated with hepatic steatosis and hyperlipidemia and is indicated by an excessive accumulation of fat in the liver and excess fat in the circulation, respectively [1]. Resveratrol (trans-3,5,4'-trihydroxystilbene) is one of the natural polyphenolic compounds that are mainly found in grape skin, peanut roots and other various plants. It is known for its anti-inflammatory, antioxidant, anti-ageing, and cardioprotective properties [2–6]. Recently, resveratrol has been suggested to be effective in preventing the development of obesity and diabetes [7–9]. Resveratrol has also been reported to have a promising effect for preventing hepatic steatosis in obese animals [10–12]. In a randomized double-blind study in obese men, resveratrol decreased the intrahepatic lipid content, serum triglycerides, and alanine-aminotransferase [13]. Faghihzadeh et al reported that resveratrol supplementation was associated with a significant reduction in the liver enzyme alanine aminotransferase and the hepatic steatosis grade compared with the placebo in patients with nonalcoholic fatty liver disease [14]. However, the mechanisms underlying the hepatoprotective effects of resveratrol in vivo still remain to be investigated.

Endoplasmic reticulum (ER) stress has recently been proposed to play a crucial role in lipotoxicity, insulin resistance, inflammation, and oxidative stress; all of these are linked to both obesity and hepatic steatosis [15]. Sirtuin 1 (SIRT1) is an NAD+ dependent deacetylase that functions as a key metabolic/energy sensor and mediates homeostatic responses to nutrient availability [16]. It has been demonstrated that hepatic overexpression of SIRT1 in mice attenuates endoplasmic reticulum stress and hepatic steatosis [17]. Although resveratrol is a well-known SIRT1 activator [18], it remains unclear whether resveratrol prevents hepatic ER stress in vivo.

In this study, we hypothesized that resveratrol could protect against high-fat diet (HFD)–induced hepatic ER stress and regulate the expression of genes related to hepatic steatosis. We examined the effects of resveratrol on hepatic steatosis and ER stress markers (activating transcription factor 4 [ATF4], C/EBP-homologous protein [CHOP], immunoglobulin binding protein [BiP], PKR-like ER kinase [PERK], and inositol-requiring enzyme-1α [IRE-1α]) in Sprague–Dawley rats fed a HFD. We also investigated the effects of resveratrol on the expression of 84 genes related to hepatic steatosis, using an RT^2 profiler Rat Fatty Liver PCR Array. Subsequently, a Western blot was used to examine the protein levels to validate the result of the polymerase chain reaction (PCR) array.

2. Methods and materials

2.1. Animals

Six-week-old male Sprague-Dawley rats weighing approximately 190 g were purchased from the SPF animal center (Beijing, China). The rats were kept in a room with controlled temperature (20–23 °C) and lighting (alternating 12-hour periods of light and dark). The animals had ad libitum access to water and were fed throughout the trial period. All of the procedures were approved by the Ethics Committee for Animal Studies of Capital Medical University, China.

2.2. Research design, diet and resveratrol treatment

Initially, the animals were fed either a control diet (n = 10) or a HFD (n = 20) for 4 weeks. The control diet was a normal chow diet (Beijing HFK Bioscience, Beijing, China) composed of balanced levels of protein, carbohydrate, and fat in a proportion of 20%/70%/10% of the total calories, respectively. The composition of the HFD (Research Diet, New Brunswick, NJ, USA) was changed to a proportion of 20%/35%/45% of the total calories. The normal chow diet contained 3.85 kcal/g and the HFD contained 4.73 kcal/g. The ingredient composition of the diets fed to rats is shown in Table 1. After 4 weeks, the rats fed the HFD were randomly distributed into 2 groups of 10 rats each: the HFD group and the HFD plus resveratrol (HFD + Res) group. The rats that were fed the control diet were classified as the control (C) group. The rats in the HFD + Res group were orally administered resveratrol (100 mg/kg per day, Sigma, St. Louis, MO, USA) using gastric gavage. The rats in the C and HFD groups were given the same volume of normal saline. The body weights of the rats of all three groups were measured weekly. The rats were fed and treated as such for the next 8 weeks. At the end of the experiment, after an overnight fasting, the animals were killed by using an intraperitoneal injection of chloral hydrate. Blood was collected and the liver was dissected,

Table 1 – Ingredient composition of the experimental diets

Ingredient(g)	C	HFD	HFD+Res
Casein, 80 Mesh	200	200	200
L-Cystine	3	3	3
Corn starch	315	72.8	72.8
Maltodextrin 10	35	100	100
Sucrose	350	172.8	172.8
Cellulose, BW200	50	50	50
Soybean oil	10	25	25
Lard	30	177.5	177.5
Mineral mix S10026	10	10	10
DiCalcium phosphate	13	13	13
Calcium carbonate	5.5	5.5	5.5
Potassium citrate, 1 H2O	16.5	16.5	16.5
Vitamin mix V10001	10	10	10
Choline bitartrate	2	2	2

C, control; HFD+Res, HFD plus resveratrol (100 mg/kg per day). C is a normal diet with 10% fat, provided by Beijing HFK Bioscience (catalogue number: 1022), China. HFD and HFD+Res, diet with 45% fat, were purchased from Research Diet (catalogue number: D12451), USA.