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Purple sweet potato color attenuates hepatic insulin resistance via blocking oxidative stress and endoplasmic reticulum stress in high-fat-diet-treated mice

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Abstract

Purple sweet potato color (PSPC), a class of naturally occurring anthocyanins, has been reported to possess a variety of health-promoting properties. Emerging evidence indicates that PSPC can suppress postprandial hyperglycemia via inhibition of α-glucosidases. However, the protective effects of PSPC on hepatic insulin resistance and the precise mechanisms underlying these protective effects have never been investigated. In this study, our data showed that PSPC effectively improved the fasting blood glucose level, glucose and insulin tolerance by suppressing reactive oxygen species (ROS) production and by restoring glutathione (GSH) content and antioxidant enzymes' activities. PSPC further prevented the oxidative-stress-mediated endoplasmic reticulum (ER) stress in the livers of high-fat-diet (HFD)-treated mice. Moreover, PSPC dramatically suppressed the c-Jun-N-terminal kinase 1 and I kappa B kinase β activation and nuclear factor-kappa B p65 nuclear translocation caused by oxidative and ER stress in the livers of HFD-treated mice. Ultimately, PSPC notably restored the impairment of the insulin receptor substrate-1/phosphoinositide 3 kinase/protein kinase B (Akt) insulin signaling in the livers of HFD-treated mice. In conclusion, our findings indicate that PSPC protected against HFD-induced hepatic insulin resistance *via* decreasing ROS level and blocking ROS-mediated ER stress.

Keywords: PSPC; HFD; Insulin resistance; Oxidative stress; ER stress; NAFLD

1. Introduction

In the last 20–30 years, the nonalcoholic fatty liver disease (NAFLD) caused by excessive dietary fat has become increasingly prevalent in many countries worldwide. The chronic intake of a high-fat diet (HFD) can cause an abnormal accumulation of fat in liver and trigger toxic effects, which contribute to the pathogenesis of type 2 diabetes (T2D)and metabolic syndrome [1,2]. It is a general consensus that insulin resistance is a key pathophysiological factor in T2D and metabolic syndrome. The emerging evidence now suggests that insulin resistance plays an important role in the development of HFD-induced NAFLD [3-5].

Many contributing factors, such as inflammation, oxidative stress, endoplasmic reticulum (ER) stress, activated c-Jun-N-terminal kinase (JNK) and IkB kinase (IKK) are implicated in the pathology of insulin resistance [6-8]. Chronic oxidative stress that is mainly caused by mitochondrial dysfunction can develop NAFLD and metabolic syndrome [6,9]. In the context of an HFD, increased oxidative stress has been found in the liver, and the resulting oxidative damage can contribute to the pathogenesis of insulin resistance and metabolic syndrome [6]. It has been recently established that ER serves as a cellular stress sensor and can activate an adaptive program known as ER stresses under the conditions of accumulation of misfolded proteins and other stresses including lipid-overloading-mediated oxidative stress [10]. Recently, hepatic ER stress associated with oxidative stress has been described in HFD-treated animals [11,12]. Both prolonged oxidative and ER stress can impair insulin signaling mainly through the activation of JNK and IKK which can uncontrollably cause the inhibitory serine phosphorylation of insulin receptor substrates (IRS) and result in the insulin resistance [8].

Purple sweet potato color (PSPC), a class of naturally occurring anthocyanins derived from purple sweet potato storage roots, has been reported to possess unique color, nutrition and health-promoting benefits [13,14]. PSPC, which is more stable than many other plant

Abbreviations: PSPC, purple sweet potato color; HFD, high-fat diet; NAFLD, nonalcoholic fatty liver disease; T2D, type 2 diabetes; ER stress, endoplasmic reticulum stress; TG, triglyceride; TC, total cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ROS, reactive oxygen species; SOD, superoxide dismutases; GPx, glutathione peroxidase; PERK, pancreatic endoplasmic reticulum-resident kinase; eIF2α, eukaryotic translation initiation factor 2α; JNK, c-Jun-N-terminal kinase; IKK, IkB kinase; NF-κB, nuclear factor-κB; IRS, insulin receptor substrates; PI3K, Phosphoinositide 3 kinase; PKB (Akt), protein kinase B; GSK-3β, Glycogen synthase kinase-3β.

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pigments, can be recognized as a suitable candidate for the physiologically functional food factor [15,16]. PSPC exhibits a variety of pharmacological properties, including strong antioxidant, anti-inflammatory and neuroprotective effects [17-22]. Previous work by our group has demonstrated that PSPC exerted hepatoprotective effects by attenuating oxidative stress, inflammation and hepatocyte apoptosis in D-galactose-treated mice [23,24]. Recent studies indicate that anthocyanins from purple sweet potato can suppress postprandial hyperglycemia via inhibition of α-glucosidases [25]. Therefore, we hypothesize that PSPC plays a role in the blockage of hepatic insulin resistance.

An increasing amount of studies have indicated that multiple contributing factors facilitate the development of insulin resistance in animal models of lipid overload [7,8,10,11]. However, the exact cellular and molecular mechanisms underlying HFD-induced insulin resistance and the relationships among these contributing factors have not been well established. Moreover, the protective effects of PSPC on hepatic insulin resistance induced by HFD and the precise mechanisms underlying these protective effects have never been investigated. This study is designed to address these issues.

2. Materials and methods

2.1. Animals and administration

All experimental protocols and euthanasia procedures were approved by the Institutional Animal Care and Use Committee of Xuzhou Normal University. Eight-week-old male ICR mice were purchased from the Branch of National Breeder Center of Rodents (Shanghai, China). Before the experimental stage, the mice were maintained under constant conditions [temperature (23°C±1°C) and humidity (60%)] and had free access to rodent food and tap water and kept under a 12-h light/dark schedule (lights on 08:30–20:30). After acclimatization to the laboratory conditions for 1 week, mice were randomly subdivided into four groups (10 animals each). Each group received one of the following treatments for 20 weeks: vehicle control group: normal diet (11.4% fat), daily oral administration of sterile distilled water containing the PSPC solvent [0.1% Tween-80 (Sigma-Aldrich, St. Louis, MO, USA)]; HFD group: HFD (60% fat), daily oral administration of sterile distilled water containing the PSPC solvent; HFD+PSPC group: HFD, daily oral administration of PSPC [Qingdao Pengyuan Natural Pigment Research Institute, China; the major components of PSPC by high-performance liquid chromatography analysis are cyanidin acyl glucosides and peonidin acyl glucosides (>90%), and the others are other flavonoids] at doses of 700 mg/kg/day; PSPC group: normal diet, daily oral administration of PSPC at doses of 700 mg/kg/day.

Body weight of mice was measured every week after 6-h fasting. After 20 weeks of treatment, mice were deeply anesthetized and sacrificed. The livers were immediately collected for experiments or stored at −70°C for later use. Concurrently, the whole blood of mice was collected into heparinized test tubes and centrifuged at 2000*g* for 15 min at 4°C to separate serum, and the serum was stored at −70°C for biochemical analysis.

2.2. Glucose and insulin tolerance tests

After 17 and 19 weeks on an HFD, respectively, glucose and insulin tolerance tests were performed in fasted mice (6 h) with oral administration of glucose (2 g of glucose per kg of body weight) or intraperitoneal injection of insulin (0.75 U of insulin per kg of body weight), respectively. For the glucose tolerance test, blood glucose values were measured immediately before and 15, 30, 60, 90 and 120 min after oral administration of glucose. For the insulin tolerance test, blood glucose levels were measured immediately before and 15, 30, 60, 90 and 120 min after insulin injection. Blood samples were taken by tail venipuncture, and blood glucose levels were measured with an Ascensia Elite glucose meter (Bayer Corporation, Mishawaka, IN, USA).

2.3. Tissue homogenates

For biochemical studies, the liver was promptly dissected and homogenized in 1:10 (w/v) 50 mM (pH 7.4) ice-cold Tris-buffered saline containing a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA) with 10 strokes at 1200 rpm in a Teflon-glass Potter–Elvehjem homogenizer (Kontes, Vineland, NJ, USA). Homogenates were centrifuged at 12,000*g* for 10 min, and the supernatant was used to determine reactive oxygen species (ROS) and glutathione (GSH) levels and antioxidant enzymes activities.

For Western blot analysis, the liver was homogenized in 1:5 (w/v) ice-cold radioimmunoprecipitation assay (RIPA) lysis buffer (1× Tris-buffered saline, 1% Nonidet P40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 0.004% sodium azide) supplemented with 30 μl of 10 mg/ml phenylmethanesulfonyl fluoride solution, 30 μl of sodium orthovanadate (Na_3VO_4) and 30 μl of protease inhibitors cocktail per gram of tissue. The homogenates were sonicated four times for 30 s with 20-s intervals using a sonicator and centrifuged at 15,000*g* for 10 min at 4°C, and then the supernatant was collected and stored at −70°C for Western blot studies. Nuclear factor (NF)-κB p65 expression was determined by Western blot assessment in cytoplasmic and nuclear extracts of liver tissues that were obtained using a nuclear/cytoplasmic isolation kit (Pierce Biotechnology, Inc., Rockford, IL, USA). Protein levels in the supernatants were determined using the bicinchoninic acid assay kit (Pierce Biotechnology, Inc., Rockford, IL, USA).

2.4. Determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels

The serum levels of ALT and AST were measured spectrophotometrically using kits according to manufacturer instructions (Jiancheng Institute of Biotechnology, Nanjing, China). The activities of ALT and AST were expressed as an international unit (U/L).

2.5. Determination of hepatic lipids

Lipids were extracted from approximately 200-mg frozen liver samples using chloroform:methanol (2:1 v/v) solution, as described by Folch and Lees [26]. The extraction solution of hepatic lipids was used to determine the levels of triglyceride, free fatty acid and total cholesterol using the LabAssay corresponding kit (Wako Chemicals, Richmond, VA, USA) according to the manufacturer's instructions.

2.6. Determination of redox status

2.6.1. ROS assay

ROS was measured as described based on the oxidation of 2',7'-dichlorodihydrofluorescein diacetate to 2',7'-dichlorofluorescein [19]. Briefly, the homogenate was diluted 1:20 (v/v) with ice-cold Locke's buffer [154 mM NaCl, 5.6 mM KCl, 3.6 mM NaHCO3, 2.0 mM CaCl2, 10 mM D-glucose and 5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4] to obtain a tissue concentration of 10 mg/ml. The reaction mixture (1 ml) containing Locke's buffer, 0.2 ml of homogenate and 10 μl of 5 mM 2',7'-dichlorodihydrofluorescein diacetate was incubated for 15 min at room temperature to allow the 2',7'-dichlorodihydrofluorescein diacetate to be incorporated into any membrane-bound vesicles and the diacetate group to be cleaved by esterases. After 30 min of further incubation, the conversion of 2',7'-dichlorodihydrofluorescein diacetate to the fluorescent product 2',7'-dichlorofluorescein was measured using a spectrofluorometer with excitation at 484 nm and emission at 530 nm. Background fluorescence (conversion of 2',7'-dichlorodihydrofluorescein diacetate in the absence of homogenate) was corrected by the inclusion of parallel blanks. ROS formation was quantified from a 2',7'-dichlorofluorescein standard curve, and the data are expressed as pmol 2',7'-dichlorofluorescein formed/min/mg protein.

2.6.2. GSH assays

The levels of GSH in hepatic supernatant were determined according to the protocols of a commercially available GSH assay kit (Cayman Chemical, Ann Arbor, MI, USA). After reaction with 5,5-dithiobis-(2-nitrobenzoic acid), the GSH levels were determined at 405 nm with a spectrophotometer (Shimadzu UV-2501PC). The results were expressed as the contents (μmol GSH) per mg protein.

2.6.3. Measurement of antioxidant enzymes activities

Cu, Zn-superoxide dismutases (SOD) activity was measured using the method of McCord and Fridowich [27]. Solution A was prepared by mixing 100 ml of 50 mM phosphate-buffered saline (pH 7.4) containing 0.1 mM EDTA and 2 μmol of cytochrome c with 10 ml of 0.001 N NaOH solution containing 5 μmol of xanthine. Solution B contained 0.2 U xanthine oxidase/ml and 0.1 mM EDTA. Fifty microliters of a tissue supernatant was mixed with 2.9 ml of solution A, and the reaction was started by adding 50 μl of solution B. Change in absorbance at 550 nm was monitored in a spectrophotometer (Shimadzu UV-2501PC, Shimadzu Corporation, Japan). A blank was run by replacing the supernatant with 50 μl of ultra pure water. Cu, Zn-SOD levels were expressed as units per mg protein with reference to the activity of a standard curve of bovine Cu, Zn-SOD under the same conditions.

The glutathione peroxidase (GPx) activity assay was based on the method of Paglia and Valentine [28]. *tert*-Butylhydroperoxide was used as substrate. The assay measures the enzymatic reduction of H_2O_2 by GPx through consumption of reduced GSH that is restored from oxidized glutathione (GSSG) in a coupled enzymatic reaction by glutathione reductase (GR). GR reduces GSSG to GSH using reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) as a reducing agent. The decrease in absorbance at 340 nm due to NADPH consumption was measured in a Molecular Devices M2 plate reader (Molecular Devices Corporation, Menlo. Park, CA, USA). GPx activity was computed using the molar extinction coefficient of 6.22 mM^{-1} cm^{-1}. One unit of GPx was defined as the amount of enzyme that catalyzed the oxidation of 1.0 μmol of NADPH to $NADP^+$ per minute at 25°C.

2.7. Western blot analysis

Samples (80 μg proteins) were separated by denaturing sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Roche Diagnostics Corporation, Indianapolis, IN, USA) by electrophoretic transfer. The membrane was blocked with 5% nonfat milk and 0.1% Tween-20 in tris-buffered saline (TBS) and incubated overnight with primary antibodies. These