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Omija fruit ethanol extract improves adiposity and related metabolic disturbances in mice fed a high-fat diet☆

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Abstract

This study investigated the biological and molecular mechanisms underlying the antiobesity effect of omija fruit ethanol extract (OFE) in mice fed a high-fat diet (HFD). C57BL/6J mice were fed an HFD (20% fat, w/w) with or without OFE (500 mg/kg body weight) for 16 weeks. Dietary OFE significantly increased brown adipose tissue weight and energy expenditure while concomitantly decreasing white adipose tissue (WAT) weight and adipocyte size by up-regulating the expression of brown fat-selective genes in WAT. OFE also improved hepatic steatosis and dyslipidemia by enhancing hepatic fatty acid oxidation-related enzymes activity and fecal lipid excretion. In addition to steatosis, OFE decreased the expression of pro-inflammatory genes in the liver. Moreover, OFE improved glucose tolerance and lowered plasma glucose, insulin and homeostasis model assessment of insulin resistance, which may be linked to decreases in the activity of hepatic gluconeogenic enzymes and the circulating level of gastric inhibitory polypeptide. These findings suggest that OFE may protect against diet-induced adiposity and related metabolic disturbances by controlling brown-like transformation of WAT, fatty acid oxidation, inflammation in the liver and fecal lipid excretion. Improved insulin resistance may be also associated with its antiobesity effects.

Keywords: Brown fat-selective genes; Fatty acid oxidation; Fecal lipid excretion; Insulin resistance; Omija fruit ethanol extract

1. Introduction

Excessive fat accumulation is a hallmark of obesity and is associated with a variety of metabolic disturbances, including insulin resistance, dyslipidemia and hepatic steatosis [1]. Adipose tissue is a critical component of metabolic control; mammals have two main types of adipose tissue: white adipose tissue (WAT), which plays a role in the storage of excess energy as triglycerides, and brown adipose tissue (BAT), which is specialized to dissipate chemical energy as heat [2]. Currently, browning of WAT is considered a promising approach in protecting against obesity and obesity-related complications.

Omija (*Schisandra chinensis*), a berry that possesses 5 flavors (salty, sweet, sour, pungent and bitter), contains a variety of phenolic compounds that have beneficial effects on health, including antioxidant and anti-inflammatory effects [3,4]. It has been used for medicinal purposes in Asia [5]. Recent studies have demonstrated that Omija alone or in combination with Grape Pomace has favorable effects on obesity and diabetes and improves energy metabolism by up-regulating the PGC-1α expression in the skeletal muscle during endurance exercise training [6–11]. Omija ethanol extract suppressed adipocyte differentiation and adipogenesis in 3T3-L1 preadipocytes and rats fed high-fat diet (HFD) containing omija ethanol extract (50 or 200 mg/kg body weight) had lower body weight and WAT weight compared to HFD control group [6]. Moreover, omija fruit water extract was shown to have high α-amylase and α-glucosidase inhibitory activity *in vitro* and postprandial blood glucose-lowering effect of omija extract (500 mg/kg body weight) was observed in rats [7]. Our previous studies also demonstrated the antiobesity and/or antidiabetic effects of a mixture of omija fruit extract (50 mg/kg body weight) and grape pomace extract (500 mg/kg body weight) in HFD-induced obese mice and type 2 diabetic *db*/*db* mice [8,9]. However, unlike a mixture of omija fruit extract and grape pomace extract, omija fruit extract alone (50 mg/kg body weight) did not alter body weight and WAT weight in HFD-fed obese mice [8]. Moreover, in overweight or obese subjects, a mixture of omija fruit extract (115 mg/day) and grape

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pomace (685 mg/day) did not alter body weight and body fat mass [10]. The effects of omija on obesity and related metabolic disturbances still remain unclear. In particular, there is lack of study about the effect of omija on energy expenditure, BAT weight and expression of BAT-selective genes in WAT.

Thus, this study sought to clarify the metabolic actions of long-term supplementation with omija fruit ethanol extract (OFE, 500 mg/kg body weight) in HFD-induced obese mice, in particular by focusing on its role in controlling brown-like transformation of WAT and ameliorating obesity-associated hepatic steatosis and insulin resistance.

2. Materials and methods

2.1. OFE preparation

Omija fruit (*S. chinensis* Baillon) was obtained from Gyeongsangbuk-do, Korea. Samples were prepared by adding 1 L of 50% ethanol to 100 g of dried omija fruit, followed by extraction at 80 °C for 2 h and cooling. The solution was filtered (Whatman paper No. 2), concentrated with a rotary vacuum evaporator, and stored at −70 °C. The final weight of the lyophilized powder of OFE was 39.7 g and it contained 8 mg/g schizandrin, 7 mg/g total flavonoid and 32 mg/g total polyphenol.

2.2. Experimental animals and diets

C57BL/6J mice (4 weeks old, male, n=20) were purchased from Jackson Laboratory (Bar Harbor, ME, USA). All mice were individually housed at controlled temperature (22±2 °C) and humidity (40%±10%) under a 12-h light–dark cycle and fed a pelletized commercially nonpurified diet for acclimation for 1 week after delivery. Body weight-matched mice were randomly assigned (n=10/group) to one of two experimental diets for 16 weeks: HFD (20% fat based on AIN-76 diet plus 1% cholesterol, w/w), or HFD plus OFE (0.77%, w/w, approximately 500 mg/kg body weight/day). The HFD contained 40 kcal% fat, 17 kcal% protein, and 43 kcal% carbohydrate. In addition, 85% (w/w) of total fat was from lard, which contains high amounts of saturated fat, and 15% (w/w) of total fat was from soybean oil, an unsaturated fat source. The mice had free access to their assigned diet and the food consumption and body weight were measured daily and weekly, respectively. At the end of the experimental period, the mice were anesthetized with isoflurane (5 mg/kg body weight; Baxter, Deerfield, IL, USA) following a 12-h fast and blood was taken from the inferior vena cava to measure plasma biomarkers. After blood correction, the liver, WATs (epididymal, perirenal, retroperitoneal, mesenteric, subcutaneous, interscapular WAT), and BAT (interscapular BAT) were removed, rinsed with physiological saline and weighed. Among them, the liver and epididymal WAT were snap-frozen in liquid nitrogen and stored at −70 °C until enzyme activity and/or RNA analysis. All experimental procedures were performed in accordance with the protocols for animal studies approved by the Kyungpook National University Ethics Committee (Approval No. KNU 2014-133).

2.3. Energy expenditure

Energy expenditure was assessed using an indirect calorimeter (Oxylet; Panlab, Cornella, Spain). The mice were placed into individual metabolic chambers at 22±2 °C, with free access to food and water. Energy expenditure was calculated using the volume of CO_2 *vs.* volume of oxygen (VCO_2/VO_2) ratio.

2.4. Blood analysis

Plasma triglyceride, total cholesterol and glucose concentrations were determined using commercial kits (Asan Pharm, Seoul, Korea), and the concentration of plasma free fatty acid was determined using a commercial kit from Wako (Tokyo, Japan). The levels of plasma insulin and gastric inhibitory peptide (GIP) were determined using a multiplex detection kit (Bio-Rad, Hercules, CA, USA). All of the assays were performed according to the manufacturer's instructions. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as [fasting insulin concentration (mU/L)×fasting glucose concentration (mg/dl)×0.05551]/22.5. For the glucose tolerance test, at the 15th week after beginning the diet experiments, the mice were fasted for 12 h, and then injected intraperitoneally with glucose (0.5 g/kg body weight). Blood glucose levels were measured from the tail vein with a glucose analyzer (GlucoDr SuperSensor, Allmedicus, Korea) at 0, 30, 60 and 120 min after glucose injection.

2.5. Analysis of hepatic and fecal lipids

Hepatic lipids were extracted using the method of Folch et al. [12]. The dried lipid residues were dissolved in 1 ml of ethanol for FFA, triglyceride and cholesterol assays. Triton X-100 and a sodium cholate solution in distilled water were added to 200 μL of the dissolved lipid solution for emulsification. Hepatic FFA, triglyceride and cholesterol contents were analyzed with the same enzymatic kit used for the plasma analysis.

The feces from each group were collected daily for 1 week and extracted using the method of Folch et al. [12], with a slight modification. The feces were dried and extracted in ice-cold chloroform and methanol (2:1, v/v) for 24 h at 4 °C. After centrifugation at 900*g* for 10 min, the supernatant was collected, dried at 50 °C and dissolved in ethanol. Fecal lipid levels were estimated using the same method as used for the liver.

2.6. Hepatic enzyme analysis

Hepatic mitochondrial, cytosolic and microsomal fractions were prepared according to Hulcher and Oleson [13], with a slight modification, and protein concentrations were determined using the Bradford method [14]. Cytosolic fatty acid synthase activity was measured by monitoring the malonyl coenzyme A-dependent oxidation of NADPH at 340 nm [15]. Activity represented the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) in nmol/min/mg protein at 37 °C. Mitochondrial fatty acid β-oxidation was measured by monitoring the reduction of NAD+ to NADH at 340 nm [16]. Mitochondrial carnitine palmitoyltransferase (CPT) was assayed using previously published methods [17]. Microsomal phosphatidate phosphohydrolase (PAP) activity was determined using a spectrophotometric method [18]. Glucokinase activity was measured in the cytosol using a spectrophotometric assay as described by Davidson and Arion [19], whereby the formation of glucose-6-phosphate at 37 °C was coupled to its oxidation by glucose-6-phosphate dehydrogenase and NAD+. Glucose-6-phosphatase (G6Pase) activity in the microsome was determined with a spectrophotometric assay according to the method of Alegre et al. [20]. Phosphoenolpyruvate carboxykinase (PEPCK) activity was monitored in the direction of oxaloacetate synthesis using the spectrophotometric assay developed by Bentle and Lardy [21].

2.7. RNA isolation and real-time reverse transcription polymerase chain reaction (RT-PCR) analysis

Liver and epididymal WAT were homogenized in TRIzol reagent (Invitrogen Life Technologies, Grand Island, NY, USA) and total RNA was isolated according to the manufacturer's instructions. DNase digestion was used to remove any DNA contamination and RNA was re-precipitated in ethanol to ensure no phenol contamination. For quality control, RNA purity and integrity were evaluated using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Equal amounts of RNA from each of the study groups were pooled to normalize individual differences. Total RNA (1 μg) was reverse-transcribed into cDNA using the QuantiTect reverse transcription kit (Qiagen, Hilden, Germany). Expression of mRNA was quantified by real-time RT-PCR, using the SYBR green PCR kit (Qiagen) and the CFX96TM real-time system (Bio-Rad, Hercules, CA, USA). Cycle thresholds were determined based on SYBR green emission intensity during the exponential phase. Using the $2^{-\Delta\Delta Ct}$ method, the fold changes were calculated. Transcripts of GAPDH were also amplified from the samples in order to assure normalized real-time RT-PCR detection. The primer pairs were as follows: PPARα, 5′-GCTGGAGGGTTCGTGGAGTC-3′ and 5-CGGTGAGATACGCCCAAATGC-3; UCP1, 5′-AGATCTTCTCAGCCGGAGTTT-3′ and 5′-CTGTACAGTTTCGGCAATCCT-3′; CIDEA, 5′-TTTCAAACCATGACCGAAGTAGCC-3′ and 5′-CCTCCAGCACCAGCGTAACC-3′; COX8β, 5′-TGTGGGGATCTCAGCCATAGT-3′ and 5′-AGTGGGCTAAGACCCATCCTG-3′; PGC1α, 5′-AAGTGTGGAACTCTCTGGAACTG-3′ and 5′-GGGTTATCTTGGTTGGCTTTATG-3′; FAS, 5′-GCTGCGGAAACTTCAGGAAAT-3′ and 5′-AGAGACGTGTCACTCCTGGACTT-3′; TLR4, 5′-AAGAGCCGGAAGGTTATTGTG-3′ and 5′-CCCATTCCAGGTAGGTGTTTC-3′; IL6, 5′-GAGGATACCACT-CCCAACAGACC-3′ and 5′-AAGTGCATCATCGTTGTTCATACA-3′; GAPDH, 5′-ACAATGAATACGGCTACAGCAACAG-3′ and 5′-GGTGGTCCAGGGTTTCTTACTCC-3′

2.8. Morphological analysis of liver and epididymal fat

The liver and epididymal WAT were fixed in 10% (v/v) paraformaldehyde/phosphate-buffered saline and embedded in paraffin for staining with hematoxylin and eosin. Stained areas were viewed using an optical microscope (Zeiss Axioscope, Germany) with a magnifying power of ×200.

2.9. Statistical analysis

All data were expressed as mean±S.E.M. Statistical analysis was carried out using the statistical package for the social sciences software (SPSS) program. Significant differences between the two groups were determined using the Student's t test.

3. Results and discussion

In the present study, long-term supplementation of OFE did not alter body weight and food intake in HFD-fed obese mice (Fig. 1A and B). However, epididymal, visceral and total WAT weights and epididymal adipocyte size were decreased in mice whose diet was supplemented with OFE (Fig. 1C and D). This result was in accordance with a previous study, which demonstrated that supplementation with omija ethanol extract for 5 weeks protected against adiposity in HFD-fed rats [6].