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Red raspberries suppress NLRP3 inflammasome and attenuate metabolic abnormalities in diet-induced obese mice☆

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Abstract

The NLR family pyrin domain containing 3 (NLRP3) inflammasome plays a critical role in insulin resistance and the pathogenesis of type 2 diabetes. Red raspberry (RB) contains high amounts of dietary fibers and polyphenolic compounds, which are known for their anti-oxidative and anti-inflammatory effects. This study evaluated the preventive effects of RB supplementation on the NLRP3 inflammasome activation and associated metabolic abnormalities induced by high fat diet (HFD). Wild-type male mice (six weeks old) were randomized into 4 groups receiving a control or typical western HFD supplemented with or without 5% freeze-dried RB for 12 weeks, when mice were sacrificed for tissue collection. HFD feeding substantially increased body weight, which was alleviated by RB supplementation towards the end of the feeding trial. Dietary RB restored the baseline blood glucose level, ameliorating glucose intolerance and insulin resistance, which were aggravated by HFD. Additionally, HFD reduced O_2 expenditure and CO_2 production, which were ameliorated by RB consumption. The liver is the key site for energy metabolism and a key peripheral tissue responsive to insulin. RB supplementation reduced hepatic lipid accumulation in HFD mice. In agreement, RB consumption suppressed hepatic NLRP3 inflammasome activation and reduced interleukin (IL)-1β and IL-18 production in HFD mice, accompanied with normalized mitochondriogenesis. These results suggest that RB consumption improves insulin resistance and metabolic dysfunction in diet-induced obesity, which is concomitant with suppression of NLRP3 inflammasome elicited by HFD. Thus, dietary RB intake is a promising strategy for ameliorating diet-induced metabolic abnormalities.

Keywords: Red raspberry; Obesity; Insulin resistance; Liver; Inflammasome; Mitochondriogenesis; High-fat diet

1. Introduction

Obesity is a worsening problem worldwide, and is closely linked to type II diabetes (T2D), cardiovascular diseases and a number of cancers [1]. In the United States, more than one third of the population are obese, and another one third are over-weight, which incur huge medical costs and loss of human capital [2–4]. The typical "Western diet" containing high energy and low dietary fiber is mainly blamed for the current obesity epidemics. Excessive dietary energy due to Western diet consumption leads to chronic hyperlipidemia and ectopic deposition of lipids in peripheral tissues resulting in insulin resistance [5]. Insulin insensitivity, in return, reduces the capacity of peripheral tissues to utilize glucose and fatty acids and promotes hepatic glucose production, further worsening these adverse changes and causing serious metabolic disorders [6].

Liver and skeletal muscle are primarily peripheral tissues responsive to insulin; muscle and hepatic insulin resistance is a precondition for the development of T2D [7]. Hepatic lipid accumulation, inflammation, and insulin resistance are highly correlated [8]. Under obese condition, excessive hepatic lipid accumulation induces inflammation and other pathological changes [9]. Inflammation activates pro-inflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and c-Jun N-terminal kinase (JNK) signaling, which induces insulin receptor substrate-1 (IRS-1) serine phosphorylation and insulin resistance [10,11]. The NLR family pyrin domain containing 3 (NLRP3) inflammasome is an important "inflammation processer", which can sense and be activated by a vast array of metabolic molecules such as ATP [12], glucose [13], fatty acid [14] and uric acid crystals [15], further leading to maturation and secretion of proinflammatory cytokines including IL-1β and IL-18. Consistently, the NLRP3 inflammasome has been implicated in the progression of T2D and insulin resistance [14,16]. Hepatic lipid accumulation activates NLRP3 inflammasome, inducing insulin resistance [14,17].

Polyphenolic compounds are known for their beneficial effects in prevention of obesity and associated metabolic disorders. Resveratrol, a polyphenolic compound found in grape skin and other fruits, reduces

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body weight gain and fat mass in C57BL/6 mice [18] and CD1 mice [19] as well as patients with metabolic syndrome [20]. Raspberry seed flour extract which contains a high level of polyphenols improves high sucrose mediated dyslipidemia and hepatic oxidative stress in high-fat induced obese mice [21]. Consistently, red raspberry (RB) extract reduces inflammation and onset of clinical symptom of arthritis in a rat model of experimental induced arthritis [22,23]. Recent studies indicated that dietary RB juice, RB puree concentrate, and selected phytochemicals abundant or unique to raspberry such as ellagic acid and raspberry ketone mitigated body weight gain in mice fed a high-fat, high-calorie diet [24]. In addition, RB contains high dietary fibers (~6.5% of fresh fruit and 45.6% of dry matter, USDA National Nutrient Database), which are expected to further enhance its beneficial role in obesity prevention, either through inhibiting lipid absorption or enhancing the prebiotic effects by providing fermentation substrates [25,26]. The current study is aimed to evaluate preventive roles of RB in improving lipid homeostasis and insulin resistance using a diet-induced obesity mouse model, and further examine its ability to suppress hepatic inflammasome induced by high fat diet (HFD).

2. Materials and methods

2.1. Experimental design and animal diet

Six-week-old wild-type male mice (C57BL/6) were randomized into 4 groups (n= 10 per group) receiving a control rodent diet (10% energy from fat, D12450B, Research Diet, New Brunswick, NJ) or a typical western high energy diet (60% energy from fat, D12492, Research Diet) supplemented with or without 5% freeze dried RB powder (based on dry feed weight) for 12 weeks. This resulted in four treatments: control (CON), raspberry (RB), high energy diet (HFD), and high energy diet plus RB (HFDRB). All mice were housed in a temperature-controlled room with a 12 h light and 12 h dark cycle and had free access to diet and drinking water. At the 10th and 11th weeks of dietary supplementation, mice were subjected to glucose tolerance test and insulin sensitivity test, respectively. Mice were euthanized at the end of 12-week trial. All animal procedures were approved (BAF#04316–001) by the Washington State University Animal Care and Use Committee.

Organic frozen red raspberries were purchased from a local Safeway supermarket (Pullman, WA), originally distributed by Lucerne Foods, Inc. They were freeze dried in VirTis freeze drier (Vertis Comp., Gardiner, NY, USA) and ground into powder, then shipped to the Research Diets, Inc. for preparation of customized experimental diet containing 5% (W/W) of freeze-dried RB powder. The RB powder contains 1.09±0.05% gallic acid equivalent polyphenolics, 4.2±0.12% protein, 1.9±0.03% fat, 0.8±0.02% ash, and 16.1±0.45% moisture. The dose of RB supplementation at 5% dry weight is practical for human consumption. The average daily feed consumption was 2.40 g/mouse, which equals to 120 mg of RB per day for an adult mouse of 20 g (*i.e.*, 6 g RB/day/kg body mass). This is equivalent to about 29 g of freeze-dried RB intake for an adult with 60 kg body weight according to a published formula [27]. The similar supplemental level of RB has been used in a previous mice study [28].

2.2. Basal metabolic rate analysis

Before and after the dietary treatment, the basal metabolic rate (BMR) (the volume of oxygen consumption per minute (VO_2), the volume of CO_2 production (VCO_2) and respiratory exchange ratio (RER)) of mice were measured during 24 h period using a CLAMS indirect open circuit calorimetry system (Columbus Instruments, Columbus, Ohio) in a temperature-controlled room with a 12 h light and 12 h dark cycle. Mice in each chamber had free access to food and water.

2.3. Tissue collection and fixation

Mice were anesthetized with CO_2 inhalation and followed by cervical dislocation. The blood samples were collected *via* cardiac puncture. The resultant serum was stored at −80°C for serum triglyceride analysis. The liver was dissected, and the right lobe of the liver was rapidly frozen in liquid nitrogen for biochemical analyses, while a portion of the left lobe at a constant location was frozen in OCT medium for cryo-section preparation and histological examination.

2.4. Body mass index and adiposity calculation

Body mass index (BMI, kg/m^2) was calculated as body weight (kg) divided by body length squared (without tail, m^2). Adiposity percentage (%) was determined by the sum of fat tissues (subcutaneous, epididymal, and abdominal) divided by body weight times 100.

2.5. Histological evaluation of hepatic lipid accumulation

Frozen preserved liver cryo-tissues were sectioned at 5 μm thickness and subjected to Oil-Red-O staining for accumulation of lipids per a previous published method [29]. After counterstaining with hematoxylin, images were taken using a Lecia DM2000 LED light microscope (Chicago, IL, USA). The quantification of Oil-Red-O stained lipid droplets was performed using the Image J 1.30v software (split color channels) [30].

2.6. Immunoblotting

The content of the selected proteins was analyzed by immunoblotting as previously described [31]. Briefly, protein samples were extracted from liver tissues and separated by 4–20% SDS-PAGE followed with nitrocellulose membrane transferring. Primary antibodies against IRS-1, phospho-IRS-1, Akt, phospho-Akt, caspase 1 (CASP1) p20, and IL-1β were purchased from Cell Signaling Technology (Beverly, MA). Anti-β-tubulin and IL-18 antibodies were obtained from DSHB at the University of Iowa (Iowa City, IA). The band density was quantified using Imager Scanner II and ImageQuant TL software (Amersham Bioscience, Sunnyvale, CA, USA), and normalized to the β-tubulin content.

2.7. Intraperitoneal glucose tolerance test (IPGTT) and insulin tolerance test (ITT)

Mice were subjected to IPGTT as previously described [30]. Briefly, mice were fasted for 6 h before testing and administered intraperitoneally with 2.0 g D-glucose/kg body weight. Blood samples were collected from the tail veil at 0, 15, 30, 60, 90 and 120 min post injection. Glucose concentrations were measured by glucose oxidase method using a glucose meter (Bayer Contour, Tarrytown, NY, USA).

For ITT test, mice were fasted for 6 h before testing and administered intraperitoneally with 0.625 U insulin/kg body weight (Sigma, St. Louis, MO, USA). Blood samples were collected from the tail veil at 0, 15, 30, 60, and 120 min post injection. Glucose concentrations were measured by glucose oxidase method using a glucose meter (Bayer Contour).

2.8. Triglyceride content analysis

The serum and hepatic triglyceride contents were analyzed with a triglyceride colorimetric assay kit from Cayman (Ann Arbor, MI, USA) per manufacturer's instruction.

2.9. Quantitative reverse transcriptase PCR (qRT-PCR)

Total RNAs were extracted using Trizol (Life Technologies, Grand Island, NY, USA) and treated with DNase I (Qiagen, Valencia, CA, USA) followed by purification with RNeasy® Mini Kit (Qiagen). cDNA was synthesized using an iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) according to manufacturer's instruction. qRT-PCR reactions were performed with SYBR green master mix (Bio-Rad, Hercules, CA, USA) on a Bio-Rad CFX384 real-time PCR unit. β-Tubulin was used as a reference gene. All primer sequences are listed in the Supplementary Table 1.

2.10. Statistical analysis

Statistical analyses were conducted as previously described [31]. Data were analyzed as a complete randomized design using GLM (General Linear Model of Statistical Analysis System, SAS, 2000). Data were expressed as mean±standard error of mean (S.E.M.). A significant difference was considered as $P \le .05$.

3. Results

3.1. RB ameliorated insulin resistance in diet-induced obese mice

Mice of different dietary groups had a similar daily feed intake (Fig. 1A). HFD feeding substantially increased body weight, which was alleviated by RB supplementation towards the end of feeding trial (Fig. 1B). The BMI was higher in mice with HFD regardless of RB supplementation (Fig. 1C). Adiposity, based on the ratio of fat weight/body weight, was markedly increased in HFD-fed mice, which tended to be reduced by RB supplementation (Fig. 1C, P<.1). The fasting baseline blood glucose level in HFD-fed mice was elevated at 4, 8 and 12 weeks of dietary treatment, which was all restored by RB supplementation, while dietary RB had no effects on blood glucose level in CON-fed mice (Fig. 1D). Furthermore, RB consumption not only ameliorated glucose intolerance (Fig. 2A), but also improved insulin resistance (Fig. 2B) induced by HFD.

The basal metabolic rate analysis showed that VO_2 consumption (Fig. 3A) and VCO_2 excretion (Fig. 3B) were reduced in HFD mice,