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Basic nutritional investigation

Anthocyanins in chokeberry and purple maize attenuate diet-induced metabolic syndrome in rats

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ABSTRACT

Objective: Increased consumption of fruits and vegetables as functional foods leads to the reduction of signs of metabolic syndrome. The aim of this study was to measure and compare cardiovascular, liver, and metabolic parameters following chronic administration of the same dose of anthocyanins either from chokeberry (CB) or purple maize (PM) in rats with diet-induced metabolic syndrome.
Methods: Male Wistar rats were fed a maize starch (C) or high-carbohydrate, high-fat diet (H) and divided into six groups for 16 wk. The rats were fed C, C with CB or PM for the last 8 wk (CCB or CPM), H, H with CB or PM for the last 8 wk (HCB or HPM); CB and PM rats received ~8 mg anthocyanins/kg daily. The rats were monitored for changes in blood pressure, cardiovascular and hepatic structure and function, glucose tolerance, and adipose tissue mass.
Results: HCB and HPM rats showed reduced visceral adiposity index, total body fat mass, and systolic blood pressure; improved glucose tolerance, liver, and cardiovascular structure and function; decreased plasma triacylglycerols and total cholesterol compared with H rats. Inflammatory cell infiltration was reduced in heart and liver.
Conclusion: CB and PM interventions gave similar responses, suggesting that anthocyanins are the bioactive molecules in the attenuation or reversal of metabolic syndrome by prevention of inflammation-induced damage.

Introduction

Fruits and vegetables rich in antioxidant phytochemicals such as flavonoids and polyphenols are effective in attenuating the signs of metabolic syndrome [1,2]. An increased intake of fruits and vegetables has been associated with a decrease in cardiovascular diseases [3], and better control of type 2 diabetes [4] and nonalcoholic fatty liver disease (NAFLD) [5]. Anthocyanins are bioactive flavonoids that give red to purple colors to a wide range of fruits and vegetables including rhubarb, cabbage, berries, cherries, and red grapes [6]. Regular consumption of anthocyanins has been credited with reducing risk for chronic diseases such as obesity, NAFLD, diabetes and CVDs [7–9]. It is estimated that the average US adult consumes ~1000 mg of polyphenols, including up to 215 mg of anthocyanins, daily [9,10].

This study focuses on black chokeberry (CB) and purple maize (PM) as two rich dietary sources of anthocyanins similar to purple carrots and Queen Garnet plums [11,12]. Black CB (*Aronia melanocarpa*) is described as an attractive garden plant, native to eastern North America but now grown widely in northern Europe, primarily Poland, where the sour fruit is eaten raw or processed for incorporation into foods. They are rich in cyanidin anthocyanins, chlorogenic acids, and proanthocyanidins, and also contain quercetin flavonols [13]. Although these components indicate that black CBs may be an effective functional food, more rigorous studies are needed to support popular indications

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for heart disease, hypertension, hyperlipidemia, and urinary tract infections, as well as actions against bacteria and viruses, and to strengthen memory and digestion [14,15]. In individuals with metabolic syndrome, CB extract decreased blood pressure and plasma lipid concentrations with no change in body weight [16]. CB attenuated body weight gain and insulin resistance in rats fed a fructose-rich diet [17].

PM has been cultivated in the Andean region, especially Peru and Bolivia, for centuries where it is used as a food and a colorant in a drink believed to improve health [18,19]. Treatment with PM (*Zea mays*) decreased abdominal adiposity [20], improved glucose metabolism [21], and decreased blood pressure in healthy humans [22]. However, there is no clear evidence that intervention with these anthocyanin-containing traditional functional foods will improve the widespread organ dysfunction observed in patients with metabolic syndrome, despite improvements in individual signs.

This study has compared the cardiovascular, liver, and metabolic responses of two dietary sources of anthocyanins, CB and PM, at the same daily anthocyanin dose as in rats fed cyanidin 3-glucoside or Queen Garnet plums [12] using the same high-carbohydrate, high-fat diet as a model of human metabolic syndrome [23]. These measurements included systolic blood pressure, echocardiography, vascular reactivity, collagen deposition and stiffness of heart, plasma biochemistry, and histology for structural changes on heart and liver. The results suggested that an adequate intake of foods containing cyanidin-type anthocyanins can normalize the metabolic, cardiovascular, and liver changes induced by a high-carbohydrate, high-fat diet by decreasing infiltration of inflammatory cells in the organs.

Materials and methods

Analysis of chokeberry juice and purple maize flour

CB juice was supplied by Fasbay Pty Ltd, Sydney, Australia and PM flour (PM) was supplied by Spectrum Ingredients Pte Ltd, Singapore. The anthocyanin contents were determined by high-performance liquid chromatography (HPLC) based on the method outlined in the British Pharmacopoeia 2014 (Eur. Pharm 2394) using an Agilent 100 series HPLC system. Briefly, samples were prepared by extraction by 2% v/v HCl in methanol, using sonication for 15 min in volumetric flasks, then made up to volume and diluted as required to be within the standard calibration. Analysis was performed using a gradient of mobile phases A (water and formic acid, 91.5:8.5) and B (acetonitrile, methanol, water and formic acid, 22.5:22.5:41.5:8.5) over 56 min. The gradient ran from 7 to 25% B in 35 min, to 65% at 45 min followed by 100% B to 50 min and return to 7%. The column used was a Phenomenex 250 mm C18 5 μm column with a flow rate of 1 mL/min and temperature 30°C. Detection and quantification were performed using a diode array detector (DAD) at 535 nm with cyanidin chloride (PhytoLab, CAS No. 528-58-5, B# 80022 5368) as the calibrating standard. Total anthocyanins were calculated as cyanidin chloride and cyanidin 3-glucoside by mass correction.

Rats and diets

The experimental groups consisted of 72 male Wistar rats (8- to 9-wk old; weighing 335 ± 3 g) purchased from Animal Resource Centre, Murdoch, WA, Australia and individually housed in a temperature-controlled (20 ± 2°C), 12-h light/dark cycle environment with ad libitum access to water and rat diet at the University of Southern Queensland Animal House. All experimentation was preapproved by the Animal Ethics Committee of the University of Southern Queensland under the guidelines of the National Health and Medical Research Council of Australia. The rats were randomly divided into six separate groups (n = 12 each) and fed with maize starch (C), maize starch + chokeberry juice (CCB), maize starch + purple maize flour (CPM), high-carbohydrate, high-fat (H), high-carbohydrate, high-fat + chokeberry juice (HCB), and high-carbohydrate, high-fat + purple maize flour (HPM).

The preparation and macronutrient composition of basal diets, including the dietary fatty acid profiles, have been described previously [23–25]. C and H rats received their diets for 16 wk and CCB, CPM, HCB, and HPM rats received C or H diets for the first 8 wk and both diets were supplemented with CB juice 50 mL/kg or PM flour 50 g/kg by replacing equivalent amounts of water for an additional 8 wk. The drinking water in all H diet-fed groups was augmented with 25% fructose for the duration of the study. Body weight and food and water intakes were measured daily and feed efficiency (%) was calculated [24] using the following equation:

$$\text{feed conversion efficiency (\%)} = \frac{\text{increase in body weight (\%)}}{\text{daily energy intake (kJ)}} \times 100$$

Increase in body weight (BW; %): BW difference between day 56 (week 8) and day 112 (week 16); daily energy intake: average of daily energy intake from week 8 to week 16.

Oral glucose tolerance test

For the oral glucose tolerance test, glucose concentrations in blood collected by tail prick on tail vein of overnight food-deprived rats were measured using Medisense Precision Q.I.D glucose meter (Abbott Laboratories, Bedford, MA, USA). Fructose-supplemented drinking water in H, HCB, and HPM rats was replaced with normal water for the overnight food-deprivation period. The rats were given 2 g/kg BW of glucose as a 40% aqueous solution via oral gavage. Tail vein blood samples were taken at 30, 60, 90, and 120 min after glucose administration.

Body composition measurements

Dual energy x-ray absorptiometric (DXA) measurements were performed on rats after 16 wk of feeding, 2 d before rats were sacrificed for pathophysiological assessments, using a Norland XR36 DXA instrument (Norland Corp., Fort Atkinson, WI, USA). DXA scans were analyzed using the manufacturer's recommended software for use in laboratory animals (Small Subject Analysis Software, version 2.5.3/1.3.1, Norland Corp.) [24]. The precision error of lean mass for replicate measurements, with repositioning, was 3.2%. Visceral adiposity index (%) was calculated [23].

Cardiovascular measurements

Systolic blood pressure was measured under light sedation following intraperitoneal injection of Zoletil (tiletamine 10 mg/kg, zolazepam 10 mg/kg; Virbac, Peakhurst, NSW, Australia) using a MLT1010 Piezo-Electric Pulse Transducer and inflatable tail-cuff connected to a MLT844 Physiological Pressure Transducer using PowerLab data acquisition unit (ADInstruments, Sydney, Australia) [24].

Anesthesia using Zoletil (tiletamine 10 mg/kg and intraperitoneal zolazepam 10 mg/kg) and Ileum xylazil (xylazine 6 mg/kg; Troy Laboratories, Smithfield, NSW, Australia) was used for echocardiographic examination (Hewlett Packard Sonos 5500, 12 MHz transducer) performed at 16 wk [23–25], in accordance with the guidelines of the American Society of Echocardiography using the leading-edge method [26].

The left ventricular (LV) function of the rats in all groups was assessed using the Langendorff heart preparation [23–25]. Terminal anesthesia was induced via intraperitoneal injection of pentobarbitone sodium (Lethabarb, 100 mg/kg). After heparin (200 IU; Sigma-Aldrich Australia, Sydney, Australia) administration through the right femoral vein, blood (~5 mL) was taken from the abdominal aorta. Isovolumetric ventricular function was measured by inserting a latex balloon catheter into the left ventricle of the isolated heart connected to a Capto SP844 MLT844 physiological pressure transducer and Chart software on a MacLab system (ADInstruments Australia and Pacific Islands, Bella Vista, NSW, Australia). All LV end-diastolic pressure values were measured during pacing of the heart at 250 beats per minute using an electrical stimulator. End-diastolic pressures were obtained starting from 0 to 30 mm Hg.

Thoracic aortic rings (~4 mm in length) were suspended in an organ bath chamber with a resting tension of ~10 mN. Cumulative concentration–response (contraction) curves were measured for noradrenaline (Sigma-Aldrich Australia, Sydney, Australia); concentration–response (relaxation) curves were measured for acetylcholine (Sigma-Aldrich Australia) and sodium nitroprusside (Sigma-Aldrich Australia) in the presence of a submaximal (70%) contraction to noradrenaline [25].

Organ weights

The right and left ventricles were separated after perfusion experiments and weighed. Liver and retroperitoneal, epididymal, and omental fat pads were collected after heart removal and blotted dry for weighing. Organ weights were normalized relative to the tibial length at the time of their removal (in mg/mm).

Histology

Two rats per group were taken exclusively for histological analysis. Two slides were prepared per tissue specimen and two random, nonoverlapping fields