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The intake of red raspberry fruit is inversely related to cardiac risk factors associated with metabolic syndrome

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ABSTRACT

The effect of red raspberry fruit (RSP) was assessed in obesity-prone, Zucker Fatty rats as a model of cardiometabolic risk. RSP reduced fasting triglycerides and fasting glucose but did not appear to affect fasting insulin, low-density lipoprotein, or body weight gain. RSP did significantly reduce heart rate relative to time-matched CON rats. Noteworthy, RSP reduced left ventricular enlargement and wall thickening as measured by echocardiography, without impacting ejection fraction. Cardiac tissue was also evaluated for relative gene expression of key genes impacted in non-insulin-dependent diabetes mellitus (NIDDM). RSP upregulated the expression of myocardial adiponectin receptor 1 and apolipoprotein E, which may impact plasma cholesterol and triglyceride homeostasis. The lipoprotein lipase (Lpl) gene was down-regulated. On the contrary, RSP did not alter PPAR and NF-kB-related mRNA in heart tissue, but did alter nicotinamide phosphoribosyltransferase (Nampt) mRNA. RSP intake impacted cardiometabolic pathophysiology in this model, and molecular mechanisms deserve further study.

1. Introduction

One in four U.S. adults has metabolic syndrome and is therefore at-risk for the eventual development of diabetes and heart disease (Sun et al., 2012; Melanson et al., 2012). Metabolic syndrome includes phenotypes such as obesity, systemic inflammation, hyperglycemia/insulin resistance, and hyperlipidemia. These phenotypes dramatically increase the risk of developing type 2 diabetes mellitus and cardiovascular disease. Literature data suggests that regular intake of edible berries may prevent and treat many risk factors associated with metabolic syndrome and its cardiovascular complications (Vendrame, Del Bo', Ciappellano, Riso, & Klimis-Zacas, 2016; Castro-Acosta, Lenihan-Geels, Corpe, & Hall, 2016). Optimal dietary approaches can be a wise strategy to prevent or manage of the most common non-communicable diseases - type 2 diabetes mellitus.

Red Raspberries (*Rubus strigosus* syn. *R. idaeus* var. *strigosus*) are receiving increasing interest as a healthy functional food, because their regular consumption has been reported to decrease the risk of chronic diseases (Noratto, Chew, & Ivanov, 2016; Burton-Freeman, Sandhu, & Edirisinghe, 2016). The nutritional and health benefits of red raspberries are usually attributed to their chemical composition. Fruits contain a wide range of phytochemicals including anthocyanins and other flavonoids, flavonols, tannins and phenolic acids.

Red raspberry extracts affect cardiac transcription factors and genes/proteins related to antioxidant defense and inflammation (Jia et al., 2011; Jean-Gilles et al., 2012). As reported, the fraction of red raspberry phenolics demonstrates dose-dependent, antihypertensive effects in spontaneously hypertensive rats. It was suggested that this may be related to increased nitrous oxide activation and improved vascular endothelial dysfunction (Jia et al., 2011). In another study, polyphenol-enriched red raspberry extracts show cartilage-protecting and anti-inflammatory effects in an *in vivo* adjuvant-induced arthritis rat model (Jean-Gilles et al., 2012). Recently, the effect of red raspberry juice on early atherosclerosis in Syrian hamsters was reported. Findings suggest that moderate consumption of red raspberry juice can help prevent the development of early atherosclerosis, with the underlying mechanisms related to improved antioxidant status and serum lipid profiles (Suh et al., 2011). Taken together, these data prove that polyphenol-rich red raspberry fruits have potential cardio-protective effects.

To gain mechanistic insight into potential cardioprotection from red raspberry consumption, we employed Zucker Fatty rat model of metabolic syndrome. We studied the regulation of several genes involved in the pathogenesis of non-insulin-dependent diabetes mellitus (NIDDM).

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These insulin-resistant rats are vulnerable to multiple pathophysiologies as a result of prolonged hyperglycemia, including development of NIDDM, or type 2 diabetes. Hence, the aim of current study was to determine the effects of whole red raspberries on cardiovascular risk phenotypes (hypertension, cardiac fibrosis and cardiac function) and cardiac molecular mechanisms related to metabolic syndrome. In addition, we aimed to determine the inhibitory potential of red raspberry powder extract on glycemia-regulating enzymes *in vitro* which are linked to hypertension.

2. Materials and methods

2.1. Anthocyanin and total phenolic analysis of red raspberry freeze-dried powder

The red raspberry product was prepared by VanDrunen Farms (Momence, IL). The powder's nutrient analysis was conducted by VanDrunen Farms and its subsidiary, Futureceuticals. The quantification of anthocyanins and total phenolics of red raspberry powder have been evaluated by our group using the same method as we reported for tart cherries (Kirakosyan, Seymour, Urcuyo Llanes, Kaufman, & Bolling, 2009). Obtained results have been validated by liquid chromatography-mass spectrometry analysis (Kirakosyan et al., 2010). Anthocyanins content of the raspberry powder is described in Table 1.

2.2. Enzymes

2.2.1. In vitro α-glucosidase inhibition assay

Alpha-glucosidase Inhibition was assessed using the method described by Schmidt, Lauridsen, Dragsted, Nielsen, and Staerk (2012). In brief, 90 µL of 0.1 M phosphate buffer (pH 7.5), 10 µL test sample dissolved in DMSO (0.1, 1, 5, and 10 mg/mL, accordingly), and 80 µL of enzyme solution were added to each well. The mixture was incubated at 28 °C for 10 min before adding 4-Nitrophenyl-α-D-glucopyranoside (PNPG) to a final volume of 200 µL. The blank was carried out in a similar manner, with the test sample replaced by solvent. The hydrolysis rate of PNPG to release p-nitrophenolate was monitored at 405 nm every 30 s for 35 min. Incubation and absorbance measurements were performed with a BioTek Eon™ microplate reader with built-in incubator, controlled by Gene5 2.0 software. The α-glucosidase inhibitory activity was expressed as percentage inhibition and was calculated using the following formula:

$$\%\ inhibition = (Slope_{blank} - Slope_{sample})/Slope_{blank} \times 100.$$

2.2.2. In vitro α-amylase inhibition assay

The α-amylase inhibition assay was conducted according to the method described by (Trinh, Staerk, & Jäger, 2016). In brief, 80 µL of 0.1 M phosphate buffer (pH 6.0), 20 µL test sample dissolved in DMSO (0.1, 1, 5, and 10 mg/mL, accordingly), and 80 µL of the enzyme solution were added to each well. After incubation at 37 °C for 10 min, the reaction was started by adding 2-Chloro-4-nitrophenyl-α-D-maltotrioside (CNP-G3) to a final volume of 200 µL. The blank was carried out in a similar manner, with the test sample replaced by solvent. Absorbance was measured at 405 nm every third minute for 30 min on the same instrument as described above. The α-amylase inhibitory activity was expressed as percentage inhibition and was calculated using the following formula:

$$\%\ inhibition = (Slope_{blank} - Slope_{sample})/Slope_{blank} \times 100.$$

Table 1
Anthocyanins and total phenolics content in red raspberry powder.

Anthocyanins	Content (mg/g dry weight)
Cyanidin-3-sophoroside	0.629
Cyanidin-3-glucosylrutinoside	0.946
Cyanidin-3-glucoside	0.484
Cyanidin-3-sambubioside	0.028
Cyanidin-3-rutinoside	0.866
Pelargonidin-3-rutinoside	0.009
Unknown anthocyanins	~0.020
Total anthocyanins[a]	2.982 mg
Total Phenolics[b]	12.480 mg

[a] Total anthocyanins are expressed as mg/g dry weight of Cyanidin 3-glucoside equivalent.
[b] Total phenolics are expressed as mg/g dry weight of Gallic acid equivalent.

2.2.3. Angiotensin I-converting enzyme (ACE) inhibition assay

ACE inhibition was assayed by the slightly modified method as previously described (Kwon, Apostolidis, & Shetty, 2008). The substrate, hippuryl-histidyl-leucine (HHL), and angiotensin I-converting enzyme (ACE) from rabbit lung were purchased from Sigma (Sigma Chemical Co., St. Louis, MO). 40 µL of extract (10 mg/mL red raspberry powder dissolved in 80% MeOH) were incubated with 60 µL of 1.0 M NaCl-borate buffer (pH 8.3) containing 1.0 mU ACE solution at 37 °C for 10 min. After pre-incubation, 20 µL of 5.0 mM substrate (HHL) solution was added to reaction mixture. Test solutions were incubated with agitation at 37 °C for 30 min. The reaction was stopped with 250 µL of 0.5 M hydrogen chloride. The hippuric acid (HA) formed was detected and quantified by HPLC method (Shimadzu HPLC equipped with a photodiode array detector (SPD-10A system from Shimadzu, Japan). The HPLC conditions were as follows: a Phenomenex Luna™ column (Torrance, CA 90,501) (5 µm pore size, C-18, 150 mm × 4.60 mm), flow rate of 0.4 mL per min. The isocratic solution of 75% H_2O + 0.1% TFA and 25% of C_2H_3N was used. Detection set at 226 nm. Pure hippuric acid (Sigma Chemical Co., St. Louis, MO) was used to calibrate the standard curve and retention time. The % inhibition was calculated by formula:

$$\%\ inhibition = (A - B)/A \times 100$$

A is the peak area of HA without adding ACE inhibitors; B is the peak area of HA with adding ACE inhibitors.

2.2.4. Lipoxygenase activity inhibition assay

The inhibition of lipoxygenase was conducted using a Lipoxygenase Inhibitor Screening Assay Kit (Cayman Chemical, Ann Arbor, MI USA). This assay kit provides an accurate and convenient method for screening lipoxygenase inhibitors. This assay measures the hydroperoxides generated from the incubation of a lipoxygenase (15-LO) with arachidonic acid. Percent enzyme inhibition was calculated as $100 \times [(\Delta A1 - \Delta A2)/\Delta A1]$, where $\Delta A1$ and $\Delta A2$ are values for increase in absorbance at 500 nm for sample without test substance and with test substance, respectively. Quercetin was used as a positive control.

2.3. Animal care

Male Zucker Fatty rats (6 weeks old) were acquired from Harlan (Indianapolis, IN, USA) and were housed two per cage. Rats were housed on 12-h light:dark cycles. Twelve rats per group were used for feeding experiment. This sample size is based upon our extensive experience with this model and the inherent variability in required cardiac outcome measures. The study design and sample size allowed 80% power to detect a 20% difference from diet in a composite score of fasting glucose, fasting triglyceride, and fasting total cholesterol. Power was based on a 0.05 level two-tailed Wilcoxon Rank-Sum test assuming a lognormal distribution of the data. The sample size takes account one interim analysis.

Rats were feed (20 g of diet per day) with either our control, base diet or a raspberry-supplemented diet (2% red raspberry freeze-dried powder) for a 12-week period. Base diet was a modified "Western-styles