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The effects of anthocyanins on body weight and expression of adipocyte's hormones: Leptin and adiponectin

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ABSTRACT

The purpose of the study was to assess the effects of anthocyanin-rich Queen Garnet plum juice (QGPJ) on body weight and expression of leptin and adiponectin. Queen Garnet plum is a Japanese plum (*Prunus salicina*) cultivar developed through a Queensland Government breeding program with very high levels of anthocyanins (272 mg/100 g). Twenty healthy volunteers were supplemented with 200 ml/day of QGPJ and placebo drink for 4 weeks. Supplementation with QGPJ significantly reduces body weight and BMI ($p < 0.05$) with the average decrease of 0.6 kg in body weight and 0.2 units in BMI over the period of 4 weeks. Furthermore, consumption of QGPJ significantly increases adiponectin blood levels ($p < 0.05$) (average increase of 3.83 μg/mL) and decreases leptin blood levels (average decrease of 1.31 ng/mL). QGPJ may have potential to be used as a functional food for preventing obesity and obesity related disorders.

1. Introduction

The prevalence of human obesity and its related metabolic disorders has escalated dramatically in the past decades worldwide and it has become a leading global public health problem (Gregor & Hotamisligil, 2011; Hotamisligil, 2006; Trayhurn & Wood, 2005). It has been accepted for over a decade that obesity is connected with chronic low-grade inflammation (Gregor & Hotamisligil, 2011; Hotamisligil, 2006; Trayhurn & Wood, 2005). The basis for this view is that the circulatory levels of several cytokines and acute phase proteins associated with inflammation are increased in obesity (Trayhurn & Wood, 2005).

Previously viewed as a simple, passive site of energy storage and accumulation of excess triacylglycerols, adipose tissue proves itself as a secretory organ which plays a major role in regulating energy homeostasis, glucose and lipids metabolism as well as inflammation, coagulation and blood pressure regulation (Hajer, van Haeften, & Visseren, 2008; Trayhurn, 2007; Trayhurn & Wood, 2005). Apart from releasing free fatty acids during the fasting state and some digestive enzymes such as lipoprotein lipase, adipocytes synthesise and secrete several naturally active molecules known as adipokines including hormones such as leptin, adiponectin, visfatin, apelin, vaspin, hepcidine, chemerin, omentin and inflammatory cytokines such as tumor necrosis factor alpha (TNF-alpha), monocyte chemoattractant protein-1 (MCP-1), and plasminogen activator protein (PAI), which have regulatory functions in the metabolism and as such may play important role in combating obesity and obesity induce inflammation (Hajer et al., 2008; Trayhurn, 2007). The hypersecretion of pro-atherogenic, pro-inflammatory and pro-diabetic adipokines as well as decreased secretion of adiponectin as seen in obesity results in chronic mild inflammation (Hajer et al., 2008; Maury & Brichard, 2010; Trayhurn & Wood, 2005). This state of adipose tissue dysfunction will further lead to insulin resistance and an increased risk of diabetes and vascular disease development (Hajer et al., 2008).

Leptin, which is often called an anti-obesity hormone, plays a key role in regulating energy intake and energy expenditure by spreading a satiety signal through the bloodstream to the brain (Zhang et al., 1994). It stimulates fatty acid oxidation and glucose uptake, and prevents lipid accumulation in adipose and other tissues (Haque et al., 1999; Minokoshi et al., 2002). Its plasma levels increase in obesity, analogous with adipose tissue mass, and mirrors immediate changes in nutritional status decreasing soon after the beginning of fasting (Becker, Ongemba, Brichard, Henquin, & Brichard, 1995). Hyperleptinaemia leads to leptin resistance which is commonly present in obese individuals (Correia & Rahmouni, 2006). Furthermore, the low-grade inflammation present in

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obese subjects may be the result of increased leptin levels as leptin stimulates production of several inflammatory markers and cells, such as TNF-alpha, interleukin 6 (IL-6) and interleukin 12 (IL-12), as it was shown in the study of Loffreda et al. (1998). Reducing or stabilising leptin levels could result in reduction of obesity induced inflammation in general.

Specifically and highly secreted by adipocytes, adiponectin (ApN) is one of the most important adipokines, a modulator of food intake and energy expenditure (Breitfeld, Stumvoll, & Kovacs, 2012; Guerre-Millo, 2008). In addition to this, adiponectin has an array of anti-inflammatory, anti-atherogenic, anti-diabetic and anti-obesity properties making it one of most intensively studied adipokines (Lee et al., 2009; Ouchi et al., 1999, 2000; Yokota et al., 2000). In contrast to most other adipokines, plasma levels of ApN are negatively correlated with BMI and are decreased in obese and/or insulin resistant individuals (Hajer, van der Graaf, Olijhoek, Edlinger, & Visseren, 2007; Lindsay et al., 2002). This downregulation of ApN secretion is probably due to higher oxidative stress and the pro-inflammatory state present in obese individuals (Bruun et al., 2003; Furukawa et al., 2004). It is known that enhanced adiponectin bioactivity could be beneficial in reducing metabolic risk factors in conditions such as obesity, where hypoadiponectinemia is present (Guerre-Millo, 2008). Furthermore, prospective studies on healthy individuals and Pima Indians have revealed that an elevated ApN plasma concentration is strongly related with reduced risk of type 2 diabetes (Lindsay et al., 2002; Spranger et al., 2003). ApN with its insulin-sensitizing, anti-inflammatory and anti-obesity properties may modify obesity-induced inflammation and prevent development of obesity and insulin resistance.

Belonging to a family of polyphenols, anthocyanins are responsible for red, purple and blue colour in plants (Davinelli et al., 2016; Kwon et al., 2007). There are currently 600 naturally occurring anthocyanins and while they all vary slightly in the structure of their molecule, they all play an important role as dietary antioxidants in the prevention of oxidative damage (Prior & Wu, 2006). They also have several other biological activities, such as being an anticonvulsant, anticarcigenic, anti-atherosclerotic and anti-inflammatory agent, and playing a role in lowering the risk of coronary heart disease (Kwon et al., 2007; Tsoyi et al., 2008). Recent studies have reported that anthocyanins exhibit an anti-obesity effect as well and they display potential to regulate adipocyte function and adipokine expression (Kwon et al., 2007; Tsuda, 2008). Human adipocyte response to anthocyanins was observed by microarray assay revealing up-regulation of adiponectin levels and down-regulation of PAI-1 and IL-6 levels (Tsuda, 2008). While high expression of PAI-1 and IL-6 in adipose tissue and low expression of adiponectin is connected with obesity and type-2 diabetes, regulation of their gene expression may become a therapeutic target for treating obesity and its relevant disorders.

Queen Garnet plum juice (QGPJ) was made from a new anthocyanin-rich Japanese plum (Prunus salicina) cultivar, Queen Garnet plum (QGP), which was developed in a Queensland Government breeding program. It was demonstrated that high anthocyanins content (up to 272 mg anthocyanins/100 g fresh weight) in QGP has a significant effect on thrombosis through reduction of platelet activation and oxidative stress (Netzel et al., 2012; Santhakumar et al., 2015).

In the present study the effect of anthocyanin-rich Queen Garnet plum juice (QGPJ) on body weight and secretion of adipocyte hormones such as leptin and adiponectin as well as levels of their gene expression was examined. It was hypothesised that anthocyanin-rich QGPJ may reduce body weight by modifying gene expression of these hormones

2. Materials and methods

2.1. Recruitment of study subjects

Study subjects were recruited from the healthy general population, aged between 18 and 65 years, by means of advertisements placed around Griffith University, Gold Coast, Australia. Each volunteer gave his or her written informed consent prior to participation in the study. Twenty-one volunteers (11 men and 10 women) initially recruited and screened by questionnaires, were non-smokers, with no history of medical conditions such as cardio vascular disease (CVD), high blood pressure/cholesterol, diabetes, stroke, renal disease or allergies; and not under daily health/energy supplements, anti-platelet or anti-inflammatory medications during the duration of the study. Volunteers on a high antioxidant diet, screened using dietary antioxidant questionnaires, were excluded from the study. Volunteer baseline full blood counts (FBC), biochemical profile, body mass index (BMI) and blood pressure (BD) were within normal reference ranges (Table 1). One of the participant dropped out due to physical injury.

Investigations were approved by Griffith University Human Research Ethics Committee (MSC/21/12/HREC).

Table 1
Primer sequences, NCBI gene ID and amplicon size.

Gene	NCBI gene ID		Sequence (5′-3′)	Amplicon size (bp)
Leptin	3952	Forward	GCGGATTCTTGTGGCTTTG	137
		Reverse	GGAGACTGACTGCGTGTG	
ApN	9370	Forward	CAGGTCTTATTGGTCCTAAGGGA	200
		Reverse	GTAGAAGATCTTGGTAAAGCGAATG	
GAPDH	2597	Forward	TGCACCACCAACTGCTTAGC	87
		Reverse	GGCATGGACTGTGGTCATGAG	

2.2. Study design

A randomized, double blinded, placebo-controlled, cross-over trial was performed (Fig. 1). Dietary-antioxidant, medical history questionnaires; full blood examination and biochemical profile were used for initial screening. Volunteers were randomly assigned into two different supplement groups: (1) Queen Garnet plum juice (QGPJ), and (2) sugar and colour matched placebo (PBO) using computer-generated arbitrary numbers. Juice supplementation bottles, 1 L each, were identical non-see through plastic bottles labelled with respective volunteer codes, juice codes (A and B) to prevent volunteers from determining what product they were consuming. This also helped maintain the blinding of the researchers who supplied the bottles to the volunteers. The labelling of the juice bottles was carried out by a research assistant independent to the investigators of this study.

During the study period, volunteers followed their usual diet, but consumed 200 ml of either QGPJ or PBO for 28 days. On day 1, baseline fasting blood samples and anthropometric measures were collected. On day 29, after 4 weeks of supplementation, the above assays were performed again. This was followed by a two-week wash out period in which no juice supplements were consumed before the start of the next round of supplementation. This was implemented to avoid potential antioxidant carryover from previous supplementation. The second supplementation and cross-overs were performed on day 44 for their respective juice supplement. Total time of involvement in the study was 73 days.

2.3. Juice supplements

QGPJ was made from Queen Garnet plum, which is a Japanese plum (Prunus salicina) cultivar that was developed through a Queensland Government breeding program and shown to contain very high levels of anthocyanins of up to 272 mg/100 g fruit with cyanidin-3-glucoside and cyanidin-3-rutinoside as the main pigments. The juice was prepared using fruit harvested in February 2012. The placebo drink (PBO) was prepared by diluting a commercial raspberry cordial (Coles, Australia) 1:4 with water. All juices were heated to 72 °C and held for 5 min prior