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Queen Garnet plum juice and raspberry cordial in mildly hypertensive obese or overweight subjects: A randomized, double-blind study

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ABSTRACT

The anthocyanin, cyanidin 3-glucoside, in Queen Garnet (QG) plums reduced cardiovascular parameters, obesity and inflammation in diet-induced metabolic syndrome in rats. We have now assessed whether QG juice improves cardiovascular and metabolic risk factors and markers of inflammation in mildly hypertensive overweight or obese humans. The 32 subjects were randomly divided into two groups consuming either QG juice or Placebo (raspberry cordial) drinks for 12 weeks. QG juice decreased systolic blood pressure by 12 ± 3 mmHg, diastolic blood pressure by 9 ± 2 mmHg, insulin by 6 ± 3 pmol/L, and leptin by 4 ± 2.5 ng/ml, and increased adiponectin by 3.62 ± 0.28 μg/ml. Cyanidin 3-glucoside is the likely active component of the plum juice, with possible additive effects of other flavonoids such as quercetin glycosides. Thus, QG juice decreased blood pressure and attenuated some risk factors of metabolic syndrome after 12 weeks suggesting that daily consumption could attenuate the development of cardiovascular and metabolic diseases.

1. Introduction

Sedentary lifestyles with reduced physical or recreational activities together with diets with increased fat and sugars are the most important environmental factors for the progression of signs of metabolic syndrome in humans, especially obesity, diabetes, cardiovascular diseases, and liver damage (McGill, 2014). Since a lower intake of fruits and vegetables is associated with a higher risk of metabolic syndrome (Miller et al., 2016; Nguyen et al., 2016; Wang et al., 2014), diets with increased fruits and vegetables are among the recommended lifestyle modifications to decrease the risk of cardiovascular diseases, but they can also reduce the complications associated with disturbed metabolic states or already established disorders (Tian, Su, Wang, Duan, & Jiang, 2018; Wang et al., 2014). Anthocyanins are flavonoids commonly found in dark-coloured fruits and vegetables, and increased consumption of diets containing anthocyanins reduced the risk of developing cardiovascular diseases (Wallace, 2011).

In patients with metabolic syndrome, chokeberry extract containing cyanidin 3-glucoside decreased systolic and diastolic blood pressures, endothelin-1, total cholesterol, LDL-cholesterol, triglycerides, TBARS concentrations, and catalase activity, and increased superoxide dismutase activity without changes in body weight (Broncel et al., 2010). In hypercholesterolemic patients, anthocyanins isolated from bilberries and blackcurrants increased brachial artery flow-mediated dilation, and cGMP and HDL-cholesterol concentrations and decreased serum soluble vascular adhesion molecule-1 and LDL cholesterol concentrations (Zhu et al., 2011). Further, Queen Garnet (QG) plum juice containing cyanidin 3-glycosides and other flavonoids attenuated thrombosis (Santhakumar et al., 2015) and acutely decreased blood pressure with no effect on cognitive function (Igwe et al., 2017) in humans.

In this study, we have used QG juice to investigate whether our previous findings including antihypertensive and body fat lowering effects in a diet-induced obese rat model (Bhaswant et al., 2015) can be replicated in mildly hypertensive, over-weight or obese humans. Measurements included body weight, blood pressure, abdominal and total body fat mass, fasting plasma glucose, insulin, lipid markers and other inflammatory cytokines. We hypothesized that an adequate intake of cyanidin-containing juice such as QG juice for 12 weeks would improve blood pressure, fasting plasma insulin, blood lipids, and anti-inflammatory mediators in these patients.

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Table 1
Composition of the study drinks by analysis.

Variables	Raspberry cordial drink	QG drink
Cyanidin 3-glucoside (mg C3GE/100 ml)[a,b]	0.1	78
Cyanidin 3-rutinoside (mg C3GE/100 ml)[a,b]	0.1	24
Other cyanidin-based anthocyanins (mg C3GE/100 ml)[b]	0.2	ND
Total anthocyanins (mg C3GE/100 ml)[b]	0.4	102
Quercetin glycosides (mg/100 ml)[b,c]	0.4	36
Vitamin C (mg/100 ml)[d]	< 0.05	< 0.05
Energy (kJ/100 g)[d]	146	239
Protein (g/100 ml)[d]	0.7	0.8
Fat (g/100 ml)[d]	0.1	0.3
Total sugars (g/100 ml)[d]	7.3	12
Fibre (g/100 ml)[d]	< 0.1	0.6
pH[d]	2.7	3.25

Values are represented as mean of duplicate analyses.
[a] Cyanidin-3-glucoside equivalents (C3GE).
[b] Analyzed by authors.
[c] Sum of quercetin 3-glucoside, quercetin 3-rutinoside and quercetin 3-galactoside; calculated as quercetin 3-glucoside equivalents.
[d] Analyzed by a commercial laboratory (Symbio Alliance, Brisbane, QLD, Australia).

2. Materials and methods

2.1. Queen Garnet plum juice and raspberry cordial

QG plums were harvested in February 2015 and QG plum juice was prepared (Netzel et al., 2012). A commercial raspberry-flavoured cordial (ingredients: water, sugar, acidity regulator [E330], flavor, natural color [E163], preservatives [E211, E223] Coles Smart Buy – Raspberry flavoured cordial; Coles, Brisbane, QLD, Australia) was used as a control drink. The cordial was diluted 1:4 with water. All study beverages were heated to 72 °C, held for 5 min prior to packing into identical 2 L plastic bottles and stored at 4 °C prior to distribution to participants. The drinks were analyzed for anthocyanins, quercetin glycosides, energy, protein, fat, total sugar, fibre, vitamin C, and pH (Table 1).

2.2. Experimental design, participant recruitment and randomisation

This study was a randomized, double-blinded, placebo-controlled trial, completed by 29 human volunteers (15 males and 14 females) residing in Melbourne, between the ages of 20 and 60 years, with a mean age of 45 years. The trial was conducted at Victoria University, St. Albans, Melbourne, Australia. Potential volunteers were recruited from the general public (by newspaper advertisement) and staff members at Victoria University (by University-wide emails and posters) after attaining study approval from Victoria University Human Research Ethics Committee (HRE14281) and registration with the Australia New Zealand Clinical Trial Registry (ANZCTR no: 12614001270606). Volunteers were recruited who were overweight or obese with body mass index (BMI) greater than 25 Kg/m^2 or a waist circumference > 94 cm (male) or > 80 cm (female) along with high-normal or mild hypertension defined as systolic blood pressures between 130 and 159 mmHg and diastolic blood pressures between 85 and 99 mmHg with no medication (Gabb et al., 2016). Volunteers were excluded from the study if they were under medication for blood pressure or using weight loss supplements or having systolic blood pressure > 160 mmHg or diastolic blood pressure > 100 mmHg. Potential participants with systolic blood pressure > 160 mmHg were recommended to make an appointment with their doctor. Baseline characteristics of the 29 participants who completed the study are shown in Table 2.

After recruitment, each participant was assigned a computer-generated arbitrary code and randomly allocated into one of the two groups by an experienced investigator who worked independently of the study investigators. Randomization of participants into the groups took account of their physical characteristics including age, body weight, and BMI. Stratified randomization was used to ensure all baseline variables associated with the outcome were evenly distributed and assigned to two groups, treatment with either QG juice (n = 16; 8 male, 8 female) or raspberry cordial (n = 16; 8 male, 8 female) (Fig. 1). Both drinks were packed in opaque brown bags labelled with a respective code. The labelling of the bags was carried out by a researcher independent of the clinical investigators of this study, so as to maintain the blinding of the study investigators. Participants were informed that they were part of a research project testing the effects of two different fruit drinks. At the beginning of the study, eligible volunteers were informed about the details of the study including that they would be randomly assigned into one of 2 intervention groups. Formal written consent was obtained from all participants before beginning the study. Staff and participants involved in the intervention process of the trial were blinded to group assignment. The randomization code was broken only after data collection and analysis were completed. The inclusion and exclusion criteria for the study are presented in Fig. 1.

Table 2
Baseline measurements.

Variables	Raspberry cordial group (n = 14)	QG group (n = 15)	p Value
Body weight (kg)	91 ± 15	86 ± 19	0.89
Body mass index (kg/m^2)	32 ± 5	31 ± 5	0.46
Age (years)	38 ± 14	47 ± 11	0.37
Systolic blood pressure (mmHg)	139 ± 5	142 ± 7	0.27
Diastolic blood pressure (mmHg)	91 ± 3	92 ± 4	0.37

Values are expressed as mean ± standard deviation (SD), n = number of subjects in group.

2.3. Administration of juice

Every fortnight for 12 weeks, participants came to the Victoria University nutrition clinics for measurements, and also collection of 2 × 2 L bottles with blinded label. Participants were requested to drink 250 ml every morning. The ingestion of the drinks was monitored fortnightly during the general consultations and a drink calendar was also given out with the drinks and collected every fortnight. The dietary intake was monitored through 3-day food diaries fortnightly and participation in physical activity was also noted in the food diary. The food diaries were analyzed using Food Works Professional 2009, version 6 (Xyris Software Pty Ltd, Brisbane, Australia).

2.4. Anthropometric measurements

Anthropometric measures were taken during fortnightly consultation with standard equipment and techniques. Three measurements were taken with the mean used as the final reading. Height was measured to the nearest millimetre after the removal of shoes using a stadiometer. Body weight was taken using digital scales (Tanita Inner Scan, BC-545, Cloverdale, WA, Australia) when heavy clothing was removed. BMI was calculated using the following formula: BMI = weight (in kg)/height^2 (in m). Waist circumference was measured to the nearest millimetre at the midway point between the lowest costal border and the iliac crest in a horizontal plane (above the umbilicus). Hip circumference was measured in a horizontal plane at the maximum posterior protuberance of the buttocks. Waist to hip ratio (WHR) was calculated using the following formula: WHR = waist circumference (mm)/hip circumference (mm).

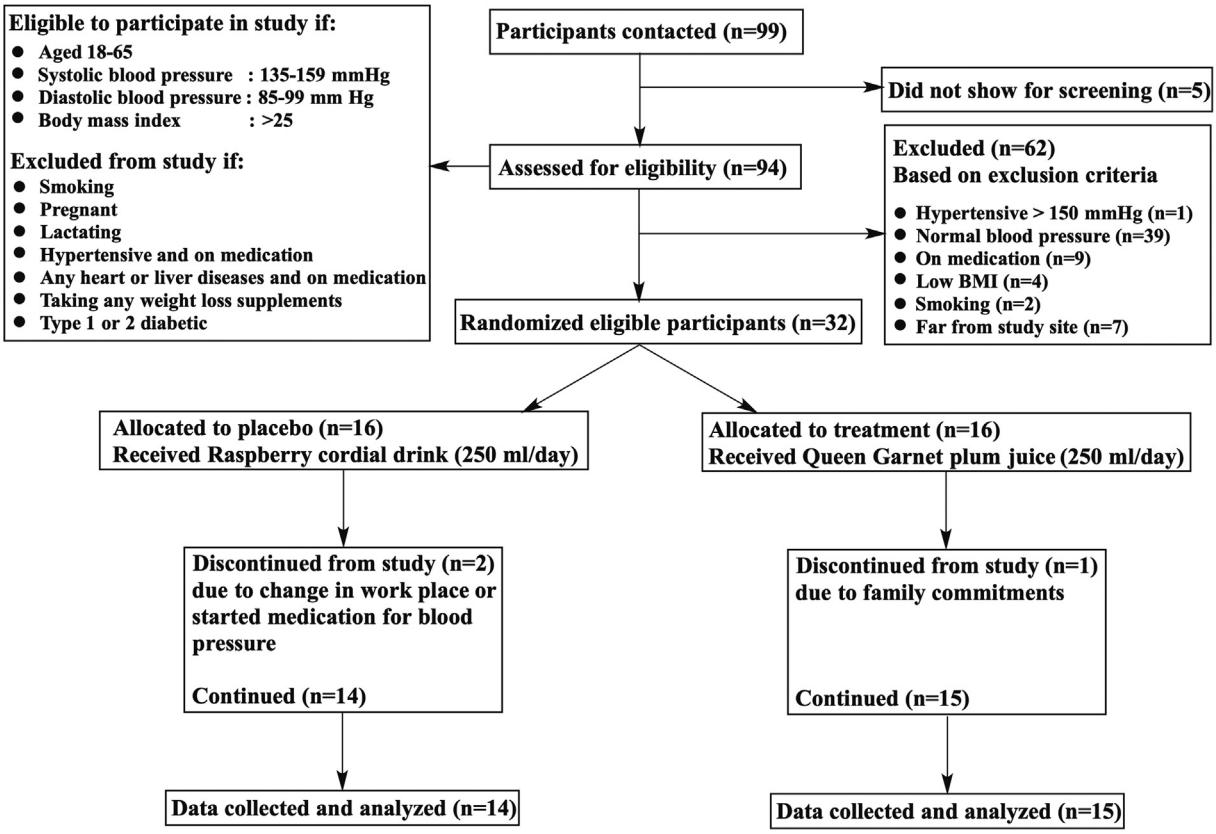

Fig. 1. Eligibility criteria and study design.

2.5. Blood pressure measurement

Blood pressure was measured in a seated position using an oscillometric device Omron HEM-7320 (Omron Healthcare Co., Ltd., Kyoto, Japan), an automated digital blood pressure monitor, where the inflatable cuff of the sphygmomanometer was positioned at the brachial artery in the right upper arm of each subject. Blood pressure was measured three times with the final reading obtained by calculating the mean of the three readings (Stevens, McManus, & Stevens, 2018). Systolic and Diastolic blood pressure measurement for individual participant are shown in Supplementary Table 1. Heart rate was also recorded using the same automated digital blood pressure monitor.

2.6. Body composition

Dual-energy X-ray absorptiometric measurements were performed on participants at the beginning and end of the study using a GE-Lunar iDXA scanner (Silverwater, NSW, Australia). Participants were requested to wear light clothing and to remove jewellery before the scan was performed. Scans were analyzed using the manufacturer's recommended software.

2.7. Basal metabolic rate

Participants were requested to fast overnight for at least 10 h and respiratory exchange ratio was measured using a metabolic cart (closed circuit spirometry). The gas exchange was measured by indirect calorimetry by comparing room air with exhaled air samples. Participants were asked to rest for 15–25 min while the test was performed. The participant breathed through a mouthpiece and valve attached to the volume displacement spirometer. The equipment is a closed system because the subjects rebreathe only the gas in the spirometer. A canister of soda lime in the breathing circuit absorbs the carbon dioxide in exhaled air. A drum attached to the spirometer revolves at a known speed and records oxygen uptake from the changes in the system's volume.

2.8. Blood collection and plasma analysis

Following an overnight fast of at least 10 h, 10 ml of forearm venous blood was collected using the Vacutainer System at baseline and post-intervention (BD vacutainer tubes, Becton, Dickinson and Company, USA). Using Samsung LABGEO^{PT10} Analyser (Point of Care Diagnostics, Sydney, Australia), blood concentrations of glucose, gamma-glutamyl transferase (GGT), alanine transaminase (ALT), aspartate transaminase (AST), total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein were determined by adding 75 µl of blood to Samsung Labgeo PT10 Biochem 9 test cartridge. Remaining blood was then centrifuged for 15 min at 3000 *g* at 4 °C. Plasma was transferred to Eppendorf tubes for storage at −20 °C before analysis. Plasma concentrations of insulin, C-peptide, glucagon-like peptide-1 (GLP-1), gastric inhibitory polypeptide (GIP), and adipokines and cytokines were determined using commercial multiplex kits (Bio-Plex Pro™ human diabetes and inflammatory assay kits purchased from Bio-Rad, Australia) according to manufacturer-provided standards and protocols.

2.9. Statistical analysis

Data were presented as the mean ± standard deviation (SD) and were analyzed using SPSS package, version 22 (SPSS, Chicago, IL, USA). Target sample size was calculated to include a minimum of 14 participants per group to detect significant differences in systolic blood pressure, the major measured outcome with 90% power (two tailed *t*-test at the 0.05 significance level) to determine whether anthocyanins reduce blood pressure. We based our calculation on the systolic blood pressure and variation in the data reported previously (Broncel et al.,

2010) to calculate the required number of participants for the study to achieve statistical power. Independent *t*-tests were performed to compare baseline data between groups. Mixed model ANOVA was used to analyze the effects of the intervention, time, and the interaction (time*group) between the intervention and time with pairwise comparisons adjusted for multiple comparisons by Bonferroni's *post hoc* test. When the interaction and/or the main effects were significant, means were compared using Tukey's multiple comparison *post hoc* test. The significance level was set as $p < 0.05$. The precision of the primary and secondary outcomes for each group were calculated using 95% confidence intervals.

3. Results

3.1. Study participants

94 volunteers were screened for enrolment into the study, with 62 volunteers excluded from study based on the exclusion criteria as either obese or overweight but not hypertensive or taking blood pressure medication (Fig. 1). The 32 volunteers registered into the trial based on eligibility criteria were randomized into two equal groups to receive QG juice or raspberry cordial. Two participants from raspberry cordial group and one participant from QG juice group discontinued from the study due to change in work place, recommendation for medication and family commitments, respectively, so that the data analysis was completed for 14 participants in raspberry cordial group and 15 participants in QG juice group (Fig. 1). In addition, no breach of the blinding process was identified during the intervention period.

3.2. Participant parameters

There were no significant differences in age, body weight, body mass index, systolic blood pressure, and diastolic blood pressure between the two intervention groups.

Baseline and post-intervention parameters such as body weight, BMI, waist circumference, hip circumference, WHR, percentage body fat and resting respiratory exchange ratio (Table 3) showed no significant changes between groups for post-intervention variables. No significant changes were observed in energy intake or physical activity (Supplementary Table 2).

The cardiovascular parameters including heart rate, and systolic and diastolic blood pressures are presented in Table 3. There were significant differences between groups and decreases over time in systolic and diastolic blood pressure with QG juice treatment but not with raspberry cordial treatment (Fig. 2). Heart rate over time significantly decreased in both groups.

3.3. Fasting plasma analyses

At the end of 12 weeks, intervention with QG juice decreased fasting plasma LDL with no change in HDL concentration, whereas raspberry cordial treatment decreased fasting plasma HDL with no change in LDL concentration (Table 4). Fasting plasma glucose, insulin, and C-peptide concentrations were decreased with QG juice, with no change in GGT and glucagon (Table 4). The raspberry cordial intervention decreased GGT and increased glucagon and insulin concentrations compared to baseline and QG juice intervention (Table 4). Both raspberry cordial and QG juice interventions decreased GLP-1 compared to respective baseline (Table 5). QG juice intervention decreased fasting plasma leptin and increased adiponectin concentrations compared to raspberry cordial (Table 5). Further, QG juice intervention decreased fasting plasma interleukins such as IL-2, IL-6, and IL-13. and TNF-α compared to raspberry cordial (Table 5). No changes were observed in fasting plasma total cholesterol, triglycerides, ALT, AST, PAI-1, creatinine, and other cytokines with either raspberry cordial or QG juice interventions (Tables 4 and 5).

4. Discussion

Animal studies using anthocyanins as dietary interventions suggest that high anthocyanin intake may prevent increases in blood pressure, glucose, lipids, adiposity, oxidative stress, and inflammation. As examples, purple carrot, chokeberry, purple maize and QG plums decreased body weight, improved glucose metabolism and cardiovascular and liver structure and function in rats fed a high-carbohydrate, high-fat diet, which mimics the human metabolic syndrome (Bhaswant et al., 2015; Bhaswant, Shafie, Mathai, Mouatt, & Brown, 2017; Poudyal, Panchal, & Brown, 2010). Blueberries, tart cherries and black rice extract also lowered body weight gain, fasting insulin, HOMA-IR, and serum triglycerides in rats (Seymour et al., 2009, 2011; Yang et al., 2011). Dietary administration of anthocyanin-containing foods such as purple maize, purple sweet potato and red radish decreased systolic and mean blood pressures in Spontaneously Hypertensive Rats by preservation of endothelial nitric oxide production and prevention of serum lipid oxidation, but inhibition of angiotensin converting enzyme (ACE) activity was not found (Lai, Hsu, Huang, & Wu, 2012). In contrast, some of the anthocyanin-containing foods and the anthocyanin, delphinidin, have shown ACE inhibition (Actis-Goretta, Ottaviani, & Fraga, 2006; Lacaille-Dubois, Franck, & Wagner, 2001; Persson, Persson, & Andersson, 2009). This evidence from animal studies suggests that anthocyanin-containing foods are beneficial in reversing cardiovascular diseases or metabolic disorders by several, possibly additive, mechanisms (Azzini, Giacometti, & Russo, 2017; Brown, Poudyal, & Panchal, 2015; Wallace, Slavin, & Frankenfeld, 2016). However, translation of these results from rodent studies to humans remains an important question to address. While epidemiological studies show that increased consumption of dietary anthocyanins reduced the risk of developing cardiovascular diseases and obesity (Wallace, 2011), few long-term intervention studies have been reported for anthocyanins.

Therefore, this clinical trial evaluated the responses of QG juice on metabolic syndrome risk factors, especially hypertension and altered metabolic parameters, in over-weight or obese men or women. Previous studies have shown blood pressure lowering effects of anthocyanins. In hypercholesterolemic patients, 320 mg/day of purified anthocyanins isolated from bilberries and blackcurrants increased brachial artery flow-mediated dilation, cGMP and HDL-cholesterol concentrations, and decreased the serum soluble vascular adhesion molecule-1 and LDL cholesterol concentrations (Zhu et al., 2011). Similar to the current study, *Aronia melanocarpa* extract 300 mg/day containing mainly cyanidin 3-galactoside for 2 months decreased systolic blood pressure by ~12 mmHg, and diastolic blood pressure by ~5 mmHg, as well as decreasing total cholesterol, LDL, and triglycerides (Broncel et al., 2010). Anthocyanins improved endothelial function which was abolished by NO-cGMP inhibitors suggesting the role of NO-cGMP signaling pathway in anthocyanin-mediated vasodilation (Zhu et al., 2011). Further, 300 ml/day of the same QG juice as in this trial in both young and old participants showed an acute blood pressure lowering effect with no change in cognitive function (Igwe et al., 2017). Similarly, a double-blind placebo-controlled parallel study with *Aronia melanocarpa* (100 mg of anthocyanins/day) for six weeks in subjects with myocardial infarction decreased systolic and diastolic blood pressures (Zapolska-Downar, Bryk, Malecki, Hajdukiewicz, & Sitkiewicz, 2012).

QG plums contain both anthocyanins and quercetin glycosides, suggesting that either could be responsible for the physiological responses. The anthocyanin dose from QG juice in the current study was ~255 mg/day, similar to published studies with this and other anthocyanins, while the dose of quercetin glycosides was ~77 mg/day. Roughly doubling this dose of quercetin in QG juice to 150 mg/day for 6 weeks was required to reduce systolic blood pressure by 3.7 mmHg, also reducing LDL and plasma inflammatory markers in humans with metabolic syndrome (Egert et al., 2009; Pfeuffer et al., 2013). These published results suggest that cyanidin 3-glucoside is the major

Table 3
Anthropometric & cardiovascular parameters baseline and post-intervention.

Variable	Group	Baseline	Week 12	Group	Time	Interaction
Anthropometry and body composition measurements						
Body weight (kg)	Raspberry cordial	92.5 ± 14.7	92.7 ± 14.0	0.3	0.8	0.4
	QG juice	86.6 ± 20.7	86.2 ± 21.9			
Waist (cm)	Raspberry cordial	107.5 ± 14.0	107.5 ± 13.3	0.5	0.3	0.4
	QG juice	103.7 ± 14.9	104.3 ± 14.9			
Hip (cm)	Raspberry cordial	115.2 ± 12.2	116.2 ± 12.1	0.1	0.2	0.2
	QG juice	109.2 ± 9.7	109.2 ± 9.8			
WHR	Raspberry cordial	0.93 ± 0.06	0.92 ± 0.05	0.3	0.8	0.08
	QG juice	0.94 ± 0.06	0.95 ± 0.06			
Respiratory exchange ratio	Raspberry cordial	0.86 ± 0.06	0.84 ± 0.07	0.8	0.5	0.5
	QG juice	0.85 ± 0.7	0.85 ± 0.5			
Total body lean mass (kg)	Raspberry cordial	50.1 ± 9	50.1 ± 9	0.7	0.8	0.3
	QG juice	51.1 ± 11	51.3 ± 11			
Total body fat mass (kg)	Raspberry cordial	37.3 ± 13	37.4 ± 13	0.2	0.9	0.6
	QG juice	32.4 ± 14	32.4 ± 14			
Total fat (%)	Raspberry cordial	42.2 ± 9.8	42.2 ± 9.9	0.5	0.3	0.4
	QG juice	37.7 ± 10.3	37.8 ± 9.9			
Android (% fat)	Raspberry cordial	48.8 ± 10.7	48.8 ± 11.2	0.3	0.9	1.0
	QG juice	45.1 ± 11.4	45.1 ± 10.3			
Gynoid (% fat)	Raspberry cordial	43.3 ± 10.1	43.4 ± 10.1	0.1	0.3	0.5
	QG juice	37.5 ± 11.1	37.4 ± 10.9			
Android to gynoid ratio (% fat)	Raspberry cordial	1.1 ± 0.2	1.14 ± 0.2	0.1	0.4	0.3
	QG juice	1.2 ± 0.2	1.42 ± 0.8			
Bone mineral content (g)	Raspberry cordial	2799 ± 552	2807 ± 531	0.4	0.8	0.4
	QG juice	2965 ± 598	2951 ± 580			
Cardiovascular measurements						
Systolic blood pressure (mmHg)	Raspberry cordial	140 ± 3	139 ± 3	0.05	< 0.001	< 0.0001
	QG juice	142 ± 6	130 ± 4$^{*\#}$			
Diastolic blood pressure (mmHg)	Raspberry cordial	92 ± 6	91 ± 4	0.006	< 0.0001	< 0.0001
	QG juice	92 ± 5	83 ± 3$^{*\#}$			
Heart rate (bpm)	Raspberry cordial	77 ± 8	74 ± 7$^{\#}$	0.4	0.013	0.6
	QG juice	76 ± 8	71 ± 7$^{\#}$			

Values are expressed in mean ± SD. $^{*}p < 0.05$, compared to other group, $^{\#}p < 0.05$, compared to baseline of each intervention.

bioactive compound in our study with QG juice, but that quercetin may produce additive responses. Further investigation is required to determine the possible interactions of these compounds.

In our study, participants were requested to maintain usual dietary habits and physical activity during the intervention time, and there were no changes reported in either QG juice or raspberry cordial groups for all domains. This is similar to previous trials of anthocyanin ingestion and berry consumption where participants were asked to maintain habitual diet and lifestyle during the intervention period, with no change observed in mean daily intakes of nutrients reported over 8 or 12 weeks of treatment (Erlund et al., 2008; Qin et al., 2009).

The decrease in blood pressure with QG juice is similar to reductions with standard antihypertensive drugs in similar populations. In 120 patients aged 18–60 years with BMI ≥ 27 kg/m^2 and systolic blood pressure > 140 to < 160 mmHg, perindopril 10 mg/day, enalapril 20 mg/day, losartan 100 mg/day and telmisartan 80 mg/day for 168 days decreased systolic blood pressure by 22, 11, 12, and 15 mmHg, and diastolic blood pressure by 13, 6, 13, and 12 mmHg, respectively (Nedogoda et al., 2013). Further, treatment of mildly hypertensive, overweight patients with perindopril 5 mg/day or barnipine 20 mg/day for 6 months followed by addition of simvastatin 20 mg/day for a further 6 months reduced TNF-α, IL-6, and high-sensitivity C

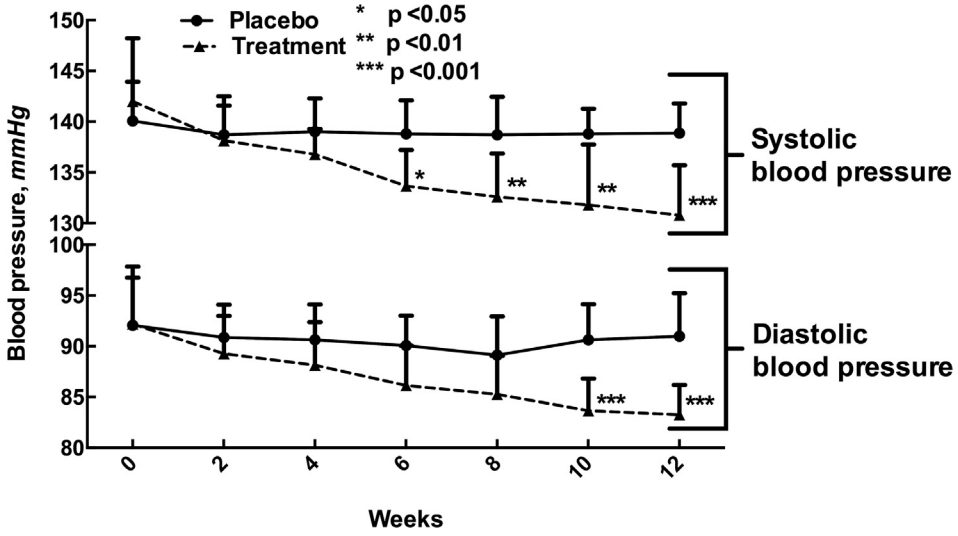

Fig. 2. Effect of raspberry cordial and QG on systolic and diastolic blood pressure. Data are shown as mean ± SD.

Table 4
Fasting plasma analysis at baseline and post-intervention.

Variable	Group	Baseline	Week 12	Group	Time	Interaction
ALT (U/L)	raspberry cordial	34.6 ± 11.3	35.6 ± 13.8	0.9	0.5	0.7
	QG juice	33.6 ± 20.4	36.6 ± 24.2			
AST (U/L)	raspberry cordial	18.7 ± 5.8	21.3 ± 10.1	0.4	0.5	0.6
	QG juice	22.1 ± 13.5	22.4 ± 8.8			
Creatinine (μmol/L)	raspberry cordial	65.9 ± 22.9	76.8 ± 17	0.5	0.1	0.2
	QG juice	66.9 ± 22.3	67.5 ± 24.4			
Cholesterol (mmol/L)	raspberry cordial	5.1 ± 1.1	5.1 ± 0.8	0.7	0.2	0.3
	QG juice	5.2 ± 0.9	4.8 ± 0.8			
Triglycerides (mmol/L)	raspberry cordial	1.5 ± 0.6	1.5 ± 0.8	0.8	0.9	0.9
	QG juice	1.4 ± 0.7	1.4 ± 0.5			
HDL (mmol/L)	raspberry cordial	1.1 ± 0.3	0.9 ± 0.2*	0.1	0.8	0.001
	QG juice	1.1 ± 0.2	1.2 ± 0.2			
LDL (mmol/L)	raspberry cordial	3.3 ± 0.9	3.4 ± 0.7	0.5	0.1	0.034
	QG juice	3.4 ± 0.9	2.9 ± 0.6*			
PAI-1 (ng/ml)	raspberry cordial	3.6 ± 1.7	3.5 ± 1.5	0.76	0.74	0.57
	QG juice	3.2 ± 1.2	3.3 ± 1.4			
GGT (U/L)	raspberry cordial	21.9 ± 15.4	14.6 ± 5.7*	0.2	0.3	0.031
	QG juice	26.2 ± 28.2	29.2 ± 25.1			
Glucose (mmol/L)	raspberry cordial	5.5 ± 0.3	5.6 ± 0.3	0.7	0.016	0.0005
	QG juice	5.8 ± 0.7	5.2 ± 0.4*#			
Glucagon (pmol/L)	raspberry cordial	39.2 ± 23.8	64.5 ± 24.0*#	0.26	0.01	0.004
	QG juice	43.9 ± 21.3	42.6 ± 22.8			
Insulin (pmol/L)	raspberry cordial	28.9 ± 19.1	54.3 ± 33.6#	0.01	0.04	0.001
	QG juice	28.1 ± 10.5	22.1 ± 7.1*#			
C-Peptide (pmol/L)	raspberry cordial	340.2 ± 146.6	377.7 ± 142.6	0.02	0.87	0.21
	QG juice	284.5 ± 107.3	255.7 ± 75.7*			

Values are expressed in mean ± SD. *$p < 0.05$, compared to other group, #$p < 0.05$, compared to baseline of each intervention.

reactive protein (Hs-CRP) (Derosa, Mugellini, Pesce, D'Angelo, & Maffioli, 2015), similar to the changes in this study with QG juice, indicating that QG juice is as effective as standard medications for lowering blood pressure and improving metabolic and inflammatory parameters in this patient group.

The adipocyte-derived hormone, adiponectin, improved insulin sensitivity in liver and skeletal muscle and its plasma concentration was inversely related to adiposity (Lee & Shao, 2014). Adiponectin may be the link between obesity and metabolic syndrome, as therapeutic strategies for weight loss increase adiponectin concentrations (Frankenberg, Reis, & Gerchman, 2017). In our study of 12 weeks duration, QG juice increased adiponectin concentrations with no

Table 5
Fasting plasma analysis of hormones and cytokines at baseline and post-intervention.

Variable	Group	Baseline	Week 12	Group	Time	Interaction
Ghrelin (pmol/L)	raspberry cordial	321.9 ± 192.8	307.1 ± 121.9	0.64	0.98	0.66
	QG juice	333.4 ± 231.8	350 ± 164.9			
GIP (pmol/L)	raspberry cordial	43.3 ± 31.8	47.7 ± 27.5	0.46	0.68	0.24
	QG juice	43.5 ± 28.4	34.5 ± 22.5			
GLP-1 (pmol/L)	raspberry cordial	74.5 ± 26.7	89.8 ± 16.6#	0.08	0.02	0.31
	QG juice	65.1 ± 25.2	71.7 ± 24.2#			
Leptin (ng/ml)	raspberry cordial	7.5 ± 4.4	7.9 ± 5.9	0.19	0.09	0.04
	QG juice	7.7 ± 5.9	3.7 ± 2.5*#			
Adiponectin (ng/ml)	raspberry cordial	4977 ± 2332	5198 ± 1862	0.01	0.02	0.03
	QG juice	4138 ± 1979	7719 ± 1341*#			
Resistin (ng/ml)	raspberry cordial	3.6 ± 1.7	3.5 ± 1.5	0.95	0.99	0.84
	QG juice	3.4 ± 1.3	3.3 ± 1.3			
IL-ß (pg/ml)	raspberry cordial	24.2 ± 10.7	25.4 ± 15	0.12	0.50	0.60
	QG juice	22.9 ± 9.6	21.8 ± 12.9			
IL-2 (pg/ml)	raspberry cordial	216.5 ± 149.1	285.6 ± 146.3	0.04	0.83	0.09
	QG juice	190.7 ± 132.7	141.1 ± 53*			
IL-4 (pg/ml)	raspberry cordial	18.9 ± 15.8	18.9 ± 11.9	0.51	0.55	0.57
	QG juice	13.7 ± 10.7	13.5 ± 6.7			
IL-5 (pg/ml)	raspberry cordial	124.0 ± 70.1	122.3 ± 82.8	0.11	0.56	0.13
	QG juice	116.8 ± 61.5	117.2 ± 101.2			
IL-6 (pg/ml)	raspberry cordial	275.1 ± 157.6	274.6 ± 156.1	0.04	0.04	0.05
	QG juice	265.8 ± 110.3	141.5 ± 86.7*#			
IL-10 (pg/ml)	raspberry cordial	138.5 ± 81.9	132.8 ± 68.9	0.15	0.15	0.76
	QG juice	116.5 ± 98.5	126.1 ± 63.9			
IL-13 (pg/ml)	raspberry cordial	22.2 ± 18.1	21.4 ± 16.4	0.35	0.05	0.99
	QG juice	16.7 ± 25.5	6.7 ± 9.7*#			
IFN-γ (pg/ml)	raspberry cordial	118.7 ± 102.1	110.8 ± 87.5	0.33	0.43	0.87
	QG juice	119.5 ± 60.5	115.5 ± 89.2			
TNF-α (pg/ml)	raspberry cordial	7.2 ± 5.9	7.0 ± 3.2	0.05	0.03	0.05
	QG juice	5.6 ± 2.6	3.4 ± 3.4*#			

Values are expressed in mean ± SD. *$p < 0.05$, compared to other group, #$p < 0.05$, compared to baseline of each intervention.

change in body weight. Similar changes in adiponectin concentrations but without changes in body weight were reported in 58 diabetic non-hypertensive patients with borderline overweight given 320 mg cyanidin 3-glucoside/day for 12 weeks, together with improved diabetes-related endothelial dysfunction (Liu, Li, Zhang, Sun, & Xia, 2014). Further, the satiety signal, GLP-1, may be impaired in obese humans (Madsbad, 2014); thus, the increase in GLP-1 concentrations following QG juice intervention may lead to decreased fat mass in the longer term. Leptin is unable to treat typical obesity, but the characteristic hyperleptinemia in obesity (Farr, Gavrieli, & Mantzoros, 2015) could be reversed with QG juice intervention as in this study, leading to an improvement in leptin tolerance and possible weight loss. Thus, we suggest that the combination of changes in incretins, pro-inflammatory cytokines, and adipokines in our study could lead to decreased fat mass in these overweight or obese patients with prolonged QG juice interventions by improving metabolic parameters including glucose metabolism and insulin resistance.

Additionally, in diabetic patients, consumption of anthocyanins in *Aronia melanocarpa* juice decreased mean fasting blood glucose concentrations (Simeonov et al., 2002). Similar observations of decreased fasting glucose concentrations were seen in this study with QG juice. *In vitro* studies using anthocyanin-containing foods showed that beneficial effects for regulating glucose are associated with insulin-releasing stimulatory properties and protective effects on pancreatic β-cells (Hong et al., 2013; Rugina et al., 2015). The volunteers in our study were free of a history of type 2 diabetes and thus, a separate investigation on the glucose-reducing properties of QG juice in diabetic subjects is justified.

Recent studies have focused on anthocyanin metabolites in plasma and tissues after ingestion of anthocyanin-rich food. Bioavailability of anthocyanin metabolites was 45-fold higher than for the parent anthocyanins (Czank et al., 2013). In humans, concentration of anthocyanins or their metabolites in tissues have not been thoroughly studied due to ethical concerns and intrinsic methodologic limitations, so the mechanisms of action of anthocyanins have not been clearly determined. Therefore, *in vivo* and *in vitro* studies using animals and cellular models are relevant for determining mechanisms of anthocyanin bioactivity (Fang, 2014; Jiang et al., 2018). In isolated thoracic aortae of db/db mice, cyanidin 3-glucoside induced endothelium-dependent relaxation (Liu et al., 2014). Additionally, cyanidin 3-glucoside reduced inducible nitric oxide synthase, reactive oxygen species, vascular NADPH oxidase 1 and 4 and up-regulated mitochondrial electron transport chain complex I and III in vascular endothelial cells (Sivasinprasasn et al., 2016; Xie, Zhao, & Shen, 2012). Further, wild berry-rich diet fed to obese and lean Zucker rats downregulated the expression of retinol binding protein 4 (RBP4) in abdominal adipose tissue (Vendrame, Zhao, Merrow, & Klimis-Zacas, 2015). RBP4 may be important as serum concentrations were increased in insulin-resistant mice and in humans with obesity and diabetes; genetic deletion of RBP4 in mice enhanced insulin sensitivity (Q. Yang et al., 2005). Anthocyanin treatment decreased pro-inflammatory cytokines mediated by I kappa B alpha degradation, suppressed the translocation of the p65 subunit of NF-κB from cytosol to nucleus by mitogen-activated protein kinase pathway and inhibited cyclooxygenase activity (Sivasinprasasn et al., 2016; Yoon-Mi et al., 2017).

5. Perspective

The results from this study show that anthocyanins from QG juice may be of benefit to human subjects with cardiometabolic risk factors to reduce blood pressure and fasting plasma glucose, insulin and LDL concentrations. Further investigations on QG juice are important to determine the possible beneficial effects in improving glucose metabolism in type 2 diabetic humans. The 12-week duration of the current study could be a limitation in reversing obesity as an important sign of metabolic syndrome. Hence, it is important to study the potential benefits of long-term intervention with QG juice, because incretins, cytokines and adipokines play major roles in energy intake and expenditure, insulin resistance and glucose utilization which are key factors initiating and maintaining obesity.

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Author contributions

M.B., L.B., and M.M. developed the original study aims. M.B. conducted the experiments analysed and interpreted the data. M.B., L.B. and M.M. prepared manuscript drafts, with all authors contributing to the final version. M.M. has been the corresponding author throughout the writing process. All authors read and approved the final manuscript.

Conflict of interest

None declared.

Ethics statement

Recruitment for this trial was initiated after the trial was registered with the Australia New Zealand Clinical Trial Registry (ANZCTR no: 12614001270606) and ethical approval was granted by the Victoria University Human Ethics Committee (HRE14281). Recruits were informed about the requirement of the study and informed consent was obtained prior to their participation.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2019.03.011.

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