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## **A Randomised trial to investigate the effects of acute consumption of a blackcurrant juice drink on markers of vascular reactivity and bioavailability of anthocyanins in human subjects**

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1 **A randomised trial to investigate the effects of acute consumption of a**  
2 **blackcurrant juice drink on markers of vascular reactivity and bioavailability of**  
3 **anthocyanins in human subjects**

4

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12

13 **Abstract:**

14

15 **Objective:** To study the bioavailability of anthocyanins and the effects of a 20%  
16 blackcurrant juice drink on vascular reactivity, plasma antioxidant status and other  
17 CVD risk markers.

18 **Subjects/Methods:** The study was a randomised, cross over, double blind, placebo  
19 controlled acute meal study. Twenty healthy volunteers (11 females 9 males) were  
20 recruited, and all subjects completed the study. Fasted volunteers consumed a 20%  
21 blackcurrant juice drink (250 ml) or a control drink following a low-flavonoid diet for  
22 the previous 72 hours. Vascular reactivity was assessed at baseline and 120 mins after  
23 juice consumption by Laser Doppler Imaging (LDI). Plasma and urine samples were  
24 collected periodically over an 8 hour period for analysis, with a final urine sample  
25 collected at 24h. The cross over was performed after a 4-week washout.

26 **Results:** There were no significant effects of the 20% blackcurrant juice drink on  
27 acute measures of vascular reactivity, biomarkers of endothelial function or lipid risk  
28 factors.

29 Consumption of the test juice caused increases in plasma vitamin C ( $P=0.006$ ), and  
30 urinary anthocyanins ( $P<0.001$ ). Delphinidin-3-rutinoside and cyanidin-3-rutinoside

31 were the main anthocyanins excreted in urine with delphinidin-3-glucoside also  
32 detected. The yield of anthocyanins in urine was  $0.021 \pm 0.003\%$  of the dietary intake  
33 of delphinidin glycosides and  $0.009 \pm 0.002\%$  of the dietary intake of cyanidin  
34 glycosides.

35 **Conclusions:** The juice consumption did not have a significant effect on vascular  
36 reactivity. Anthocyanins were present at low concentrations in the urine, and microbial  
37 metabolites of flavonoids were detected in plasma after juice consumption.

38 **Keywords:** anthocyanin, antioxidant, bioavailability, blackcurrant juice, vascular  
39 reactivity.

40 Running title: Effects of blackcurrant juice drink on vascular reactivity

41

## 42 **Introduction**

43 Several epidemiological studies have reported an inverse relationship between  
44 flavonoid intake and mortality risk due to CVD (Keli *et al.*, 1996; Knekt *et al.*, 1996;  
45 Hertog *et al.*, 1995).

46 The main flavonoids in blackcurrants are the anthocyanins cyanidin-3-glucoside,  
47 cyanidin-3-rutinoside, delphinidin -3-glucoside, and delphinidin-3-rutinoside, which  
48 comprise 98% of the anthocyanins present (Slimestad & Solheim, 2002). Several  
49 studies have demonstrated possible beneficial effects of anthocyanins on chronic  
50 degenerative diseases including CVD and cancer (Prior & Cao, 2000; Tsuda *et al.*,  
51 1996; Kay & Holub, 2002; Dalgard *et al.*, 2009; Xu *et al.*, 2004; Rechner & Kroner,  
52 2005; Nakamura *et al.*, 2002; Hou, 2003; Kang *et al.*, 2003). Anthocyanins have low  
53 bioavailability (Kay, 2006; Hollands *et al.*, 2008) and are metabolized in humans into  
54 methylated derivatives, glucuronides and sulfoconjugates (Felgines *et al.*, 2003;  
55 Felgines *et al.*, 2005; Kay *et al.*, 2004; Kay *et al.*, 2005; Wu *et al.*, 2002), but phenolic  
56 acids are the major stable metabolites of anthocyanins *in vivo* (Fleschhut *et al.*, 2006;  
57 Keppler & Humpf, 2005). Endothelial cell dysfunction is a critical initiating event in  
58 the progression of atherosclerosis (Ross, 1999), but the effects of anthocyanins on

59 endothelial function at normal intake levels have not been reported previously. The  
60 present investigation was an acute meal study in which the effects of consumption of  
61 a blackcurrant juice drink on physiological and biochemical measures of vascular  
62 function, antioxidant status and other markers of cardiovascular disease risk were  
63 assessed.

64

## 65 **Experimental methods**

66

67 *Materials and reagents.* All chemicals were purchased from Sigma Aldrich,  
68 Gillingham, UK. A 20% blackcurrant juice drink and a control drink were supplied by  
69 GlaxoSmithKline, Coleford, UK. Both drinks contained citric acid solution (50%),  
70 aspartame (1%), and acesulfame K (8%).

71

72

## 73 *Subjects*

74

75 Healthy volunteers (11 female and 9 male) with a mean ( $\pm$  SD) age of  $44.55 \pm 13.34$   
76 years and body mass index of  $23.81 \pm 2.46 \text{ kg/m}^2$  were recruited from members of the  
77 University of Reading and the surrounding area. Exclusion criteria included suffering  
78 from liver disease, diabetes mellitus, stroke, myocardial infarction, gall bladder  
79 problems or abnormalities of fat metabolism, on a weight reducing dietary regimen or  
80 taking any dietary supplements. Subjects who vigorously exercised (3 times/week, 20  
81 minutes each session) or consumed more than 15 units of alcohol per week were  
82 rejected from the study. Females who were pregnant or lactating, or likely to become  
83 pregnant were also excluded. All subjects were asked to complete a health and  
84 lifestyle questionnaire, and suitable subjects were asked to sign an informed consent  
85 form and were screened for blood, anthropometric, blood pressure and dietary  
86 parameters, and were given an information sheet. The study protocol was given a  
87 favourable ethical opinion to proceed by the Research Ethics Committee of University

88 of Reading.

89

90 *Study design*

91

92 The study was a randomised, double blind, placebo controlled cross-over acute meal  
93 study conducted in University of Reading. Twenty healthy volunteers (11 females 9  
94 males) were recruited, and all subjects completed the study. The 20 subjects were  
95 asked to follow a low-flavonoid diet for 3 days by omitting foods from a prescribed  
96 list and to fast overnight prior to each visit to the human investigation unit, when  
97 blood pressure and anthropometric measurements were determined. Afterwards,  
98 subjects lay down for 30 minutes, after which the vascular reactivity to acetylcholine  
99 (endothelium dependent) and sodium nitroprusside (endothelium independent) was  
100 determined non-invasively by laser Doppler imaging with iontophoresis (LDI). The  
101 subjects were cannulated and baseline blood samples were taken. The subjects then  
102 consumed 250 ml of either the 20% blackcurrant juice drink or the control drink, and  
103 blood samples were collected periodically up to 480 minutes after ingestion of juice.  
104 All urine produced during the study day was collected every 2 hours and at 24 hours  
105 after juice consumption. A second LDI scan of the blood vessels of volunteers was  
106 taken 2 hours after juice consumption. The subjects consumed a standard meal  
107 comprising bread, soft cheese, cucumber, crisps and white marshmallows at 180  
108 minutes. This process was then repeated by the volunteers one month later in their  
109 second visit when 250 ml of the other juice (either control or 20% blackcurrant juice  
110 drink) was consumed.

111 The clinical trial number was ISRCTN26166772.

112

113 *Sampling procedures*

114

115 Blood samples were taken from subjects via an inlaying cannula in the antecubital  
116 vein and collected into vacuum lithium heparin and EDTA tubes (Greiner Bio-One

117 GmbH, Bad Haller Str. 32, Austria). The tubes were wrapped in foil and kept on ice  
118 for transport to the laboratory. Following centrifugation at 4°C at 1560 g, plasma was  
119 stored at -80°C. Urine samples were collected, and centrifuged at 1560 g. Clear  
120 supernatant was aliquoted and stored at -20°C.

121

122

### 123 *Laser Doppler Imaging (LDI) with iontophoresis*

124

125 LDI measurements were conducted before juice consumption, and at 120 mins after  
126 juice consumption using a Moor Instruments combined iontophoresis and LDI system,  
127 as described by Vauzour *et al.* (2010). The number of scans was 20 with the area of  
128 flux vs time curve indicating the microvascular response.

129

### 130 *Analysis of flavonoids and their metabolites from urine*

131

132 Anthocyanins were isolated from urine samples (20 ml) as described by Hollands *et al.*  
133 (2008). The extract was analysed by LC-MS using an Agilent 1100 with an ACE 5  
134 C18-AR (2.1mm i.d. x 15cm) column. The mobile phase was a binary gradient  
135 comprising (A) water/1% formic acid and (B) acetonitrile /1% formic acid, flow  
136 200µl/min, 40°C with 97%A-3%B changing to 70%A-30%B (15 min), then to  
137 5%A-95%B (5 min). A Bruker MicroTof QII high resolution TOFMS was used in the  
138 positive ion mode scanning the range m/z 50-1100 Da.

139

140

### 141 *Analysis of phenolic compounds in blackcurrant juice*

142

143 Juice (5 ml) containing added methyl gallate as internal standard was extracted with  
144 ethyl acetate (4 x 5 ml). The combined ethyl acetate extracts were washed with water  
145 and concentrated to 2 ml for GC-MS analysis of phenolics.

146 *HPLC analysis of anthocyanins in juice.*

147

148 Juice (1 ml) was mixed with methanol (1 ml) and heated at 90 °C for an hour with  
149 hydrochloric acid (2 ml, 5M) to hydrolyze the anthocyanins.

150 The samples were then analyzed by HPLC-DAD using an ACE5 C18 column (150 x  
151 4.6 mm; 5 M), with CH<sub>3</sub>OH, 1% HCOOH (aq) as mobile phase, 1.0 ml flow rate. The  
152 HPLC gradient was CH<sub>3</sub>OH: 1% HCOOH (aq) 5:95 (5 min) changing to 70:30 by 30  
153 min, isocratic for 10 min, then changing to 60: 40 in 10 min, to 50:50 in 5 min, to  
154 10:90 in 5 min, to 5:95 in 10 min.

155

156 *GC-MS analysis of plasma flavonoids and phenolic acids, and juice phenolic acids*

157

158 The flavonoids and phenolic acids in plasma and phenolic acids in blackcurrant juice  
159 were determined as described by Zhang and Zuo (2004) with methyl gallate and  
160 *m*-toluic acid as internal standards.

161

162

163 *Antioxidant capacity analysis of plasma*

164

165 The antioxidant capacity of the plasma was determined by the Oxygen Radical  
166 Antioxidant Capacity (ORAC) assay as described by Cao & Prior (1999) using a 96  
167 well black flat-bottomed microtitre plate, with fluorescence determined with a Genios  
168 spectrophotometer (Tecan Ltd, Dorset, UK). The ORAC values were calculated as  
169 described by Prior *et al.* (2003). The Ferric Reducing Ability of Plasma (FRAP) assay  
170 was determined according to Bub *et al.* (2000).

171

172 *Determination of plasma ascorbic acid and uric acid concentrations*

173

174 The plasma ascorbic acid and uric acid concentrations were measured by high

175 pressure liquid chromatography with UV detection (Liau *et al.*, 1993).

176

177 *Plasma total nitrate and nitrite*

178 Plasma nitrate and nitrite were analyzed using Nitric Oxide Quantitation Kit (Active  
179 Motif Europe, 104 Avenue Franklin Roosevelt, B-1330 Rixensart, Belgium).

180

181 *Plasma lipid profiles, glucose, insulin and urinary creatinine*

182

183 Analyses of plasma lipids, glucose and creatinine were performed using an Instrument  
184 Laboratory ILAB 600 autoanalyzer. Standard kits and appropriate sero-normal, low,  
185 and high, quality control standards were purchased from Instrument Laboratories Ltd  
186 (Warrington, UK) and included in all batches. Low density lipoprotein (LDL) was  
187 calculated from Friedwald's equation. Insulin was assessed by ELISA (Dako  
188 Cytomation, Ely, Cambs, UK) with in-house pooled plasma controls in each batch.

189

190 *Plasma vascular cell adhesion molecule-1 (VCAM), endothelial intercellular*  
191 *adhesion molecule-1 (ICAM)*

192

193 Both VCAM and ICAM were analyzed using quantitative sandwich enzyme  
194 immunoassay (EIA) (R & D Systems, Abingdon, Oxon, UK). In-house pooled plasma  
195 controls were included in each batch.

196

197 *Statistical analysis*

198 All statistical analyses were performed using SPSS 15.0 for Microsoft Windows. The  
199 data was checked for normality using the Shapiro-Wilk test. Data that were not  
200 normally distributed were log transformed and reassessed. A repeated measure  
201 ANOVA with Bonferroni correction was used to look for significant differences  
202 between treatment groups. The incremental area under the curve (IAUC) for each  
203 parameter across the study timeline, and anthocyanin excretion for the two treatment



204 groups were compared using a paired t-test.  $P$  values  $\leq 0.05$  were considered as  
205 significant.

206

## 207 **Results**

208

### 209 *Effect of juice consumption on CVD risk factors*

210

211 The volunteers for the study had CVD risk factors within the normal range (Table 1).

212 There were no significant changes in BMI, blood pressure, fasting plasma glucose,  
213 cholesterol or triacylglycerol concentrations between the first and second visits.

214 The juice contained a range of bioactive components from blackcurrants (Table 2).

215 LDI measures of vascular reactivity in response to acetylcholine (endothelial  
216 dependent) and sodium nitroprusside (endothelial independent) were taken at baseline  
217 and 120 minutes after consumption of test and control juices. The changes in vascular  
218 reactivity in response to acetylcholine or sodium nitroprusside of the test and control  
219 samples ( $52 \pm 149$  and  $-189 \pm 129$  for acetylcholine and  $-251 \pm 273$  and  $-209 \pm 472$   
220 for nitroprusside) were not significantly different

221 There was no significant change in plasma nitrate and nitrite after juice consumption.

222 The plasma concentrations of ICAM and VCAM tended to fall after the consumption  
223 of juices, but no significant difference was detected between test and control. An  
224 increase in plasma insulin level was observed after test juice consumption and  
225 confirmed as a significant treatment effect both by a significant increase in the IAUC  
226 ( $P=0.016$ ) and by RM ANOVA on percentage change from baseline ( $P=0.013$ )  
227 (Table 3). There was no significant treatment effect on plasma glucose concentrations,  
228 plasma TAG and NEFA concentrations (Table 3).

229

230

231

232

233 *Flavonoids and Phenolic acids in plasma*

234

235 Six phenolic acids, namely salicylic acid, benzoic acid, *p*-hydroxyphenylacetic acid,  
236 hippuric acid, phenylacetic acid and 3-(4-hydroxyphenyl)-2-hydroxypropanoic acid  
237 were detected in plasma, but no flavonoids were detected.

238

239 Plasma concentrations of benzoic acid, hippuric acid, salicylic acid and phenylacetic  
240 acid did not increase significantly more after juice consumption than after control  
241 (Table 3). Since phenolic acids are colonic metabolites of flavonoids, the sum of the  
242 plasma concentrations of benzoic acid, hippuric acid, salicylic acid and phenylacetic  
243 acid was calculated. The sums of the concentrations for these four phenolic acids  
244 peaked at approximately 30 and 180 minutes post juice consumption (Figure. 1).  
245 There was a significant time effect and a trend for a time\*treatment effect on the sum  
246 of these 4 phenolic acids ( $p=0.099$ ; Table 3).

247

248

249 *Vitamin C and uric acid levels in plasma*

250

251 Plasma vitamin C was  $66.92 \pm 12.61$   $\mu\text{M}$  at baseline. An increase in plasma vitamin C  
252 was observed after juice consumption and confirmed as a significant treatment effect  
253 both by the IAUC ( $P=0.006$ ) and by RM ANOVA on percentage change from  
254 baseline ( $P=0.001$ ) (Table 3). The increase in plasma vitamin C due to juice  
255 consumption was significantly above that of the control value at 60 and 90 minutes  
256 ( $P<0.01$ ,  $P<0.05$ ) and remained higher than the control value throughout the study  
257 day (Figure. 2).

258

259 The plasma uric acid concentration tended to decrease from baseline after the  
260 consumption of the test juice, as shown by the significant time effect, but there was no  
261 significant treatment\*time interaction (Table 3).

262

263

264 *Urinary excretion of anthocyanins and phenolic acids*

265

266 Delphinidin-3-rutinoside and cyanidin-3-rutinoside were the main anthocyanins  
267 excreted in urine with delphinidin-3-glucoside also detected (Table 4). However, the  
268 concentrations of the anthocyanins corresponded to only  $0.021 \pm 0.003\%$  of the  
269 dietary intake of delphinidin glycosides and  $0.009 \pm 0.002 \%$  of the dietary intake of  
270 cyanidin glycosides.

271

272 Hippuric acid was detected as the major phenolic metabolite in urine. The total  
273 phenolics in urine were quantified by summing the peak areas of all the peaks on the  
274 chromatogram detected at 260 nm.

275

276 The concentrations of urinary hippuric acid and total phenolics after test juice  
277 consumption both peaked at 120 minutes. The total urinary phenolics increased from  
278 240 or 360 mins until 1440 minutes after juice consumption, but this effect was  
279 mainly attributed to foods consumed overnight after the intervention period. Both  
280 urinary hippuric acid and total phenolics tended to show an increase after test juice  
281 consumption; which was above the control group. However, no significant difference  
282 was observed between the two treatment groups.

283

284

285 *Antioxidant capacity of plasma expressed as ORAC and FRAP*

286

287 There was no significant treatment effect on the oxidative stability of plasma assessed  
288 by both the ORAC and FRAP assays. However, it was found that plasma uric acid  
289 concentration correlated significantly with the FRAP value of plasma at times of 0 -  
290 480 minutes after juice consumption ( $P < 0.001$ ). Also, the plasma uric acid  
291 concentrations at 150 - 180 minutes correlated significantly with the plasma ORAC

292 values at 180 minutes in the test group ( $P < 0.05$ ).

293 **Discussion**

294

295 The aim of the study was to determine the acute effects of ingestion of a 20%  
296 blackcurrant juice drink on vascular reactivity, biomarkers of endothelial function,  
297 bioavailability of anthocyanins, antioxidant status and other CVD risk factors in a  
298 healthy human population. The dose of bioactive components is much less than those  
299 used in many studies, but it is realistic in terms of a normal dietary intake from juice  
300 drinks. Differences in vascular reactivity assessed by LDI measurements between 0  
301 and 120 mins, and between test and control were not significant. These observations  
302 are consistent with the non-significant changes in plasma total nitrate and nitrite,  
303 ICAM and VCAM, indicating that the consumption of the 20% blackcurrant juice  
304 drink did not exert any significant changes on vascular reactivity and biomarkers of  
305 endothelial function. This outcome is considered to be partly attributable to the low  
306 concentrations of polyphenolic compounds and vitamin C in the blackcurrant juice.  
307 However, any effects after longer times due to components absorbed in the  
308 gastrointestinal tract were not investigated.

309 The blackcurrant juice drink contained delphinidin and cyanidin glucosides and  
310 rutinosides, with delphinidin and cyanidin rutinosides detected as the main  
311 anthocyanins in urine together with a small concentration of delphinidin glucoside.  
312 The urinary yield of anthocyanins excreted within 4 hours of consuming the juice was  
313 calculated as  $0.021 \pm 0.003\%$  of the dietary intake of delphinidin glycosides and  $0.009$   
314  $\pm 0.002\%$  of the dietary intake of cyanidin glycosides. The urinary yield of  
315 anthocyanins was reported as  $0.036 \pm 0.043\%$  of the ingested dose after  
316 consumption of a blackcurrant juice similar to that consumed in this study (Hollands  
317 *et al.*, 2008). Vasodilatory effects of cyanidin-3-glucoside, delphinidin and  
318 cyanidin-3-glucoside-rich juices have been reported but only at higher levels of intake  
319 (Xu *et al.*, 2004; George *et al.*, 2009; Fitzpatrick *et al.*, 2000; Stein *et al.*, 1999).

320 A significant increase in plasma vitamin C concentration was found in the current  
321 study, but the dose of vitamin C was 20 to 80 fold less than the dose used in

322 supplementation studies that have shown effects on endothelium-dependent  
323 vasodilation (Chambers *et al.*, 1999; Gokce *et al.*, 1999; Levine *et al.*, 1996;  
324 Kugiyama *et al.*, 1998; Hornig *et al.*, 1998).

325 No significant changes in antioxidant status of plasma, assessed by both ORAC and  
326 FRAP assays, were observed post juice consumption. The FRAP values of plasma  
327 showed a significant fall with time but there was no treatment effect. This fall reflects  
328 the significant reduction in plasma uric acid after juice consumption. Plasma uric acid  
329 levels correlated with FRAP values at times of 0-480 min post juice consumption, and  
330 with the plasma ORAC value at 180 min in the test group, which is consistent with  
331 the important contribution of uric acid to the antioxidant capacity of plasma  
332 (Glantzounis *et al.*, 2005). Lotito and Frei (2006) suggested that acute effects of  
333 consumption of flavonoid-rich foods on plasma antioxidant capacity may be due to  
334 changes in uric acid concentration.

335 Ascorbic acid is known as an antioxidant in plasma (Frei *et al.*, 1990), but the  
336 ingested dose of vitamin C (25.5 mg) and the increase of 6 - 8% in maximum plasma  
337 vitamin C concentration were not sufficient to contribute a significant increase in  
338 antioxidant capacity. The increase in plasma ascorbic acid concentration after test  
339 juice consumption was small compared with other studies where significant effects on  
340 antioxidant capacity were reported (Pedersen *et al.*, 2000; Volkovova *et al.*, 2005;  
341 Samman *et al.*, 2003; Cao *et al.*, 1998).

342

343 Benzoic acid, hippuric acid, salicylic acid, phenylacetic acid,  
344 *p*-hydroxyphenylacetic acid and 3-(4-hydroxyphenyl)-2-hydroxypropanoic acid were  
345 detected in plasma within 3 h of juice consumption. The sum of benzoic acid, hippuric  
346 acid, salicylic acid and phenylacetic acid showed a tendency ( $P= 0.099$ ) to increase  
347 after juice consumption with two peaks at 30 and 180 minutes, even though these  
348 phenolic acids were not present in the juice. The peak at 30 minutes indicated that  
349 metabolites of phenolic components in the juice, which were absorbed in the small  
350 intestine, contributed to the concentrations of these phenolic acids. Benzoic acid and

351 hippuric acid are known to be colonic metabolites of anthocyanins (Kim *et al.*, 1998).  
352 However, concentrations of the four phenolic acids decreased gradually after peaking  
353 at 30 min, indicating further chemical or microbial degradation of the phenolic  
354 compounds, as suggested by Keppler & Humpf (2005). The quantity of hippuric acid  
355 formed by biosynthesis is reported to directly correlate with the concentrations of its  
356 precursors, primarily phenolic acids (benzoic acid and salicylic acid) (Toromanović *et*  
357 *al.*, 2008). There was no treatment effect on the concentration of urinary hippuric acid  
358 in the period up to 24 hours.

359

360 The increase in plasma insulin can be ascribed to the presence of sugars in the juice  
361 (Table 2).

362

363 In summary, ingestion of 250 ml of the 20% blackcurrant juice drink caused  
364 significant increases in plasma ascorbic acid, insulin and urinary anthocyanins, and a  
365 trend for an increase in plasma phenolic acids. There was no significant effect on  
366 vascular reactivity or biomarkers of endothelial function.

367

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370

371 **Conflict of interest:**

372 The authors declare no conflict of interest.

373

374

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559 **List of Figures.**

560 **Figure. 1.** Percentage change from baseline of the sum of the plasma levels of  
561 benzoic acid, hippuric acid, salicylic acid and phenylacetic acid following the  
562 consumption of control and test juices. There was a significant time effect.

563 **Figure. 2.** Percentage change from baseline of plasma vitamin C following the  
564 consumption of control and test juices. The different symbols on the graph indicate  
565 different levels of statistical significance: \* $P < 0.05$ , † $P < 0.01$ .

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Table 1 Baseline characteristics of volunteers (Mean  $\pm$  SEM)

	Males (n=9)	Females (n=11)
Age (years)	44 $\pm$ 5.2	45 $\pm$ 3.7
Weight (kg)	75.7 $\pm$ 3.32	64.8 $\pm$ 3.1
BMI (kg/m <sup>2</sup> )	24.0 $\pm$ 0.83	23.0 $\pm$ 0.91
Sys. BP (mm Hg)	121 $\pm$ 2.47	110 $\pm$ 2.41
Dia. BP (mm Hg)	72 $\pm$ 2.15	68 $\pm$ 1.74
Glucose (mmol/L)	5.5 $\pm$ 0.14	5.1 $\pm$ 0.1
Total cholesterol (mmol/L)	4.6 $\pm$ 0.24	4.6 $\pm$ 0.34
Triacylglycerols (mmol/L)	0.9 $\pm$ 0.08	0.6 $\pm$ 0.07

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578 **Table 2** Composition of test and control Juice (mean  $\pm$  sd unless specified)

Composition	Test juice	Control juice
Delphinidin (mg/100 ml)	12.2 $\pm$ 0.1	0
Cyanidin (mg/100 ml)	8.0 $\pm$ 0.1	0
Vitamin C* (mg/100 ml)	10.2	0
Phenolic acids* (mg/100 ml)	81.5	<10
Nitrate + Nitrite ( $\mu$ M)	28.6 $\pm$ 8.9	0
Glucose ( $\mu$ M)**	3906.96 $\pm$ 6.88	0
Fructose ( $\mu$ M)**	385.67 $\pm$ 14.4	0
<i>p</i> -hydroxybenzoic acid ( $\mu$ M)**	47.75 $\pm$ 0.67	0
Sinapic acid ( $\mu$ M)**	65.83 $\pm$ 0.62	0
Caffeic acid ( $\mu$ M)**	98.66 $\pm$ 1.18	0
Ferulic acid ( $\mu$ M)**	62.3 $\pm$ 0.48	0
<i>p</i> -coumaric acid ( $\mu$ M)**	158.94 $\pm$ 1.21	0
Gallic acid ( $\mu$ M)**	186.58 $\pm$ 10.29	0

579 \* Analytical data provided by RSSL, Reading, UK

580 \*\* Mean  $\pm$  range of duplicate determinations

581

582 **Table 3** Effects on plasma and urinary variables assessed by incremental area  
583 under the curve of each parameter against time (IAUC value.h) and Repeated  
584 Measures ANOVA P values for within-subject effects (on percentage change from  
585 baseline; mean  $\pm$  SEM).

<b>Parameter</b>	<b>IAUC (control)</b>	<b>IAUC (test)</b>	<b>Treatment</b>	<b>Time</b>	<b>Interv* Time</b>
Plasma ORAC ( $\mu$ M TE.h)	2099 $\pm$ 118	2122 $\pm$ 157	0.921	0.012*	0.231
Plasma FRAP ( $\mu$ M TE.h)	-253 $\pm$ 60	-163 $\pm$ 151	0.896	0.006**	0.885
Plasma Vitamin C ( $\mu$ M.h)	-5.16 $\pm$ 5.34	17.2 $\pm$ 6.6	0.001**	0.975	0.083
Plasma Uric acid ( $\mu$ M.h)	-57.8 $\pm$ 11.5	-113.3 $\pm$ 41.4	0.031*	0.000**	0.274
Plasma Benzoic acid ( $\mu$ M.h)	-1.79 $\pm$ 2.75	2.81 $\pm$ 3.13	0.830	0.006**	0.275
Plasma Hippuric acid ( $\mu$ M.h)	-3.71 $\pm$ 2.29	-1.86 $\pm$ 1.28	0.857	0.067	0.795
Plasma Salicylic acid ( $\mu$ M.h)	-0.39 $\pm$ 1.00	0.26 $\pm$ 0.69	0.345	0.078	0.633
Plasma p-hydroxyphenyl acetic acid ( $\mu$ M.h)	-0.01 $\pm$ 0.05	-0.14 $\pm$ 0.08	0.527	0.639	0.157
Plasma 3-(4-hydroxyph enyl)-2- hydroxypropanoi c acid ( $\mu$ M.h)	-0.08 $\pm$ 0.20	-0.26 $\pm$ 0.23	0.668	0.821	0.383

Plasma phenylacetic acid ( $\mu\text{M.h}$ )	$-0.37 \pm 1.12$	$1.80 \pm 1.22$	0.888	0.304	0.441
Sum of benzoic acid, hippuric acid, salicylic acid and phenylacetic acid ( $\mu\text{M.h}$ )	$-6.19 \pm 4.35$	$3.17 \pm 4.10$	0.365	0.003	0.099
Plasma triacylglycerols ( $\text{mM.h}$ )	$0.37 \pm 0.21$	$0.36 \pm 0.26$	0.842	0.000**	0.544
Plasma insulin ( $\mu\text{IU/ml.h}$ )	$-1.17 \pm 0.35$	$1.53 \pm 0.81$	0.013*	0.173	0.186
Plasma ICAM (Endothelial Intercellular Adhesion Molecule-1) ( $\text{ng.h/mL}$ )	$-34.2 \pm 15.3$	$-28.6 \pm 14.6$	0.102	0.159	0.480
Plasma VCAM (Vascular Cell Adhesion Molecule-1) ( $\text{ng.h/mL}$ )	$-80.2 \pm 27.9$	$-128.3 \pm 60.8$	0.403	0.070	0.689
Plasma NEFA ( $\mu\text{M.h}$ )	$-763 \pm 457$	$-794 \pm 385$	0.637	0.059	0.102
Plasma glucose	$4.66 \pm 1.15$	$3.52 \pm 0.88$	0.716	0.000**	0.390

(mM.h)					
total urinary phenolics (24 hours data) (area.h)	85551.30 ± 29864.02	87922.45 ± 18376.08	0.301	0.000**	0.542
Urinary hippuric acid (24 hours data) (mg/μmol creatinine.h)	-1009.95 ± 392.65	-617.34 ± 590.09	0.784	0.293	0.327

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588 **Table 4 Urinary content of anthocyanins excreted within 4 hours of juice**  
 589 **consumption**

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	Urinary anthocyanins ( $\mu\text{g}$ ) (Mean $\pm$ SEM)			
Compounds	Test juice baseline	Test juice (2-4 h excretion)	Control juice baseline	Control juice (2-4 h excretion)
Total anthocyanins	0.04 $\pm$ 0.04	15.61 $\pm$ 2.16*	0.10 $\pm$ 0.07	0.32 $\pm$ 0.21
Delphinidin-3 -rutinoside	0.04 $\pm$ 0.04	11.05 $\pm$ 1.42*	0.10 $\pm$ 0.07	0.25 $\pm$ 0.20
Delphinidin-3 -glucoside	0	0.81 $\pm$ 0.39 ( <i>p</i> = 0.052)	0	0
Cyanidin-3- rutinoside	0	3.76 $\pm$ 0.68*	0	0.07 $\pm$ 0.07

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592 \* significant increase from baseline ( $p < 0.001$ )

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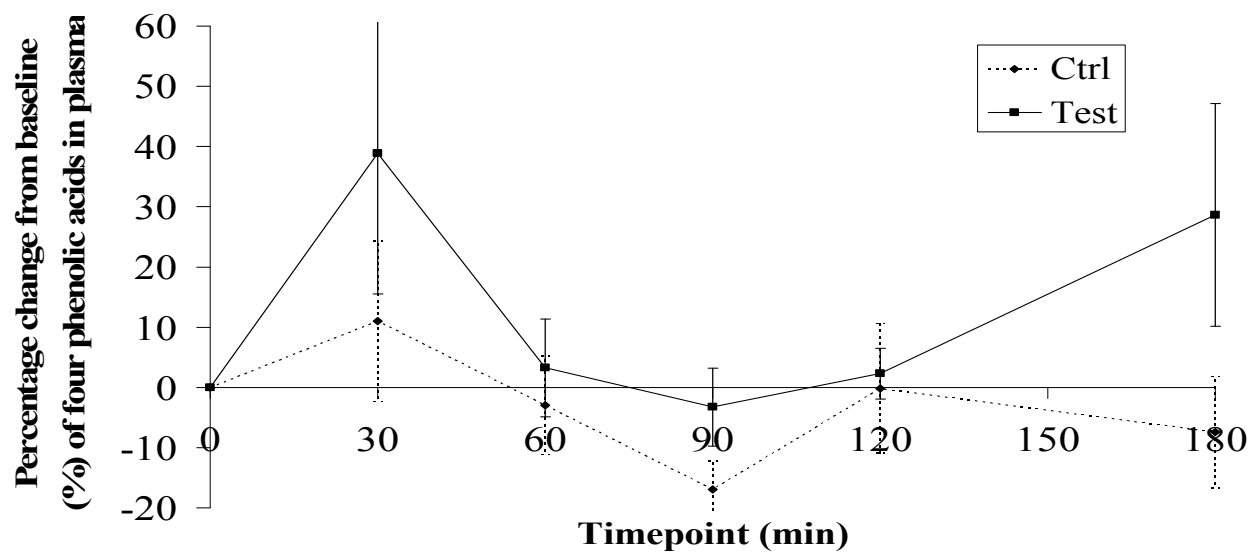


Figure 1

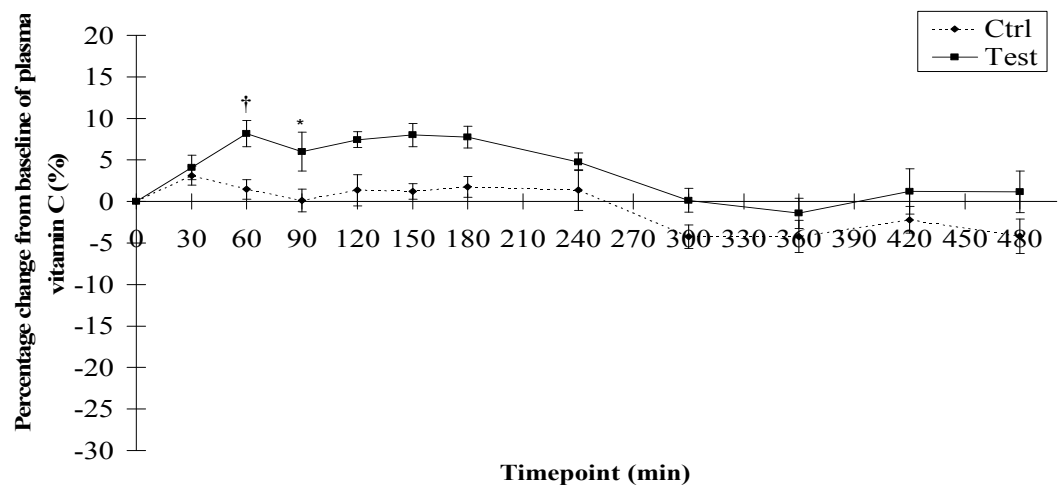


Figure 2