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1 **Effect of a tart cherry juice supplement on arterial stiffness and inflammation in**
2 **healthy adults: a randomised controlled trial.**

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11 **Abbreviations used:** BP, blood pressure; BMI, body mass index; CV, coefficient of variation; CRP,
12 c-reactive protein; DBP, diastolic blood pressure; FRAP, ferric reducing-antioxidant power; HDL,
13 high density lipoprotein; PWV, pulse wave velocity; SBP, systolic blood pressure; SD, standard
14 deviation.

15

16 **Keywords:** cherry, arterial stiffness, inflammation, blood pressure.

17

18 **Abstract**

19 Tart cherries are a particularly rich source of anthocyanins. Evidence indicates that dietary
20 intake of anthocyanins is inversely associated with arterial stiffness. We conducted an open-
21 label randomised placebo controlled study to determine whether a tart cherry juice
22 concentrate (Cherry Active®) reduced arterial stiffness, inflammation and risk markers for
23 cardiovascular disease in 47 healthy adults (30 – 50 y). Participants consumed 30 ml of
24 cherry concentrate diluted to a volume of 250 ml with water or the same volume of an energy
25 matched control drink daily for 6 weeks. Measurements were taken at baseline and at the end
26 of the intervention. There was no effect of the intervention on arterial stiffness ($P=0.218$), c-

27 reactive protein ($P=0.220$), systolic blood pressure ($P=0.163$), diastolic blood pressure
28 ($P=0.121$), total cholesterol ($P=0.342$) and high density lipoprotein cholesterol ($P=0.127$). At
29 the end of the intervention, plasma antioxidant capacity (measured as the ferric reducing
30 ability of plasma (FRAP) was significantly higher in the intervention group than the control
31 group ($P=0.012$). We conclude that a tart cherry juice concentrate rich in anthocyanins has no
32 effect on arterial stiffness, c-reactive protein and risk markers for cardiovascular disease, but
33 evokes a minor increase in antioxidant status in healthy adults.

34

35 **Introduction**

36 Epidemiological studies indicate that the consumption of foods rich in flavonoids reduces the
37 risk of cardiovascular disease, with some evidence that anthocyanins may be particularly
38 protective [1-3]. Cherries are a rich source of flavonoids, especially anthocyanins [4-5]. In
39 cell culture and animal models, cherry extracts or isolated anthocyanins have been shown to
40 exert a range of potentially cardioprotective effects including, increasing nitric oxide
41 production and antioxidant status, reducing lipid oxidation and inhibiting inflammation [6-9].
42 Anti-inflammatory effects have also been reported in a limited number of human studies [10-
43 13]. In healthy middle-aged adults, the daily consumption of sweet cherries for 4 weeks
44 reduced several markers of inflammation [10, 11]. Also, supplementation with tart cherry
45 juice reduced exercise-induced inflammation in marathon runners and in adults completing a
46 resistance exercise task [12, 13].

47 There is interest in whether diets rich in dietary anthocyanins can reduce arterial stiffness
48 and therefore risk of cardiovascular disease [14]. Arterial stiffness is determined by the
49 structural properties of the arterial wall, blood pressure and endothelial function [15]. Some
50 experimental evidence indicates that anthocyanins can improve endothelial function via
51 induction of endothelial nitric oxide synthase, so by extrapolation anthocyanins may reduce

52 arterial stiffness [16, 17]. In patients with hypercholestromia, endothelial function was
53 improved by a single dose of anthocyanins (320 mg) and a period of chronic supplementation
54 (320 mg/d x 12 weeks) [17]. In the chronic supplementation study, endothelial function was
55 improved > 12 hours after consumption of the last dose of anthocyanins when intact
56 anthocyanins and their metabolites could not be detected in the plasma of participants. The
57 authors speculated that anthocyanins may have accumulated in tissues [17].

58 In a cross-sectional study of women from the TwinsUK registry, arterial stiffness was
59 inversely associated with anthocyanin intake, but not with total flavonoid intake [14]. The
60 risk of residual confounding means that casual inference cannot be ascertained from cross-
61 sectional studies, so intervention studies are needed to demonstrate that anthocyanin rich
62 foods can reduce arterial stiffness [14]. In a recent study, daily supplementation with
63 cranberry juice (rich in anthocyanins; 94 mg/d) for 4 weeks reduced arterial stiffness in
64 overweight adults with coronary artery disease [18]. The effect of anthocyanin rich foods on
65 arterial stiffness in healthy adults remains largely unexplored. In the present study we aimed
66 to determine whether a commercial tart cherry juice concentrate rich in anthocyanins reduced
67 arterial stiffness, inflammation and other markers of cardiovascular risk in healthy adults.

68

69 **METHODS**

70 *Participants* A total of 47 (29 female and 18 male) apparently healthy, non-smoking
71 volunteers (age 30-50 y) were recruited through University email distribution lists, posters
72 and personal contacts. Exclusion criteria included taking medication for heart disease,
73 hypertension or diabetes, regular consumption of cherries or cherry juice and the use of
74 antioxidant supplements. The study was approved by Sheffield Hallam University Ethics
75 Committee and all volunteers gave written informed consent. The study was conducted
76 between May 2010 and August 2011.

77 **Study design.** The study was a 6-week parallel open-label intervention of a daily supplement
78 of cherry juice concentrate (30 ml diluted with 220 ml of water; Cherry Active®) *versus* a
79 control drink of lemonade (250 ml/d; Sprite®). Both drinks were delivered to participants at
80 the start of the intervention; those consuming the cherry juice were instructed to store the
81 juice in their refrigerators before consumption. The concentrated cherry juice was dispensed
82 automatically using a pump-action dispenser from a 950 ml bottle, whilst the lemonade was
83 supplied in individual 250 ml bottles. Participants consumed the drinks during the day,
84 according to their preference.

85 Participants were stratified by gender and age (30-39 years and > 40 years) and then
86 block randomised to treatment by an investigator who was not involved in recruitment.
87 Concealed envelopes were used to inform the researchers responsible for recruitment of the
88 treatment allocation; this information was not made available until after baseline
89 measurements were completed. Compliance to the intervention was recorded through
90 participants keeping a daily tick-sheet of drinks consumed. It was emphasised to all
91 participants that they should adhere to their usual diet and maintain usual exercise patterns in
92 conjunction with daily consumption of the test drinks.

93 Our primary outcome variable was change in pulse wave velocity (PWV); our
94 secondary outcome variable was change in c-reactive protein (CRP). Power calculations
95 were based on a 10% change in PWV. A sample size of 50 (25 per arm) was calculated to
96 detect a 10% change in PWV with 80% power and an alpha value of 0.05, using variance
97 estimates of PWV (SD = 1.06, n=57) from a study of PWV (unpublished data).

98 ***Tart cherry juice concentrate and placebo beverage***

99 The tart cherry juice concentrate was supplied by Cherry Active, Sunbury UK. A 30 ml
100 serving of tart cherry juice was diluted with 220 ml of water prior to consumption. Each
101 serving was estimated to contain the equivalent of 90-100 Montmorency tart cherries. The

102 composition of the juice was analysed by an independent laboratory (Atlas Bioscience Inc,
103 Tuscan, AZ, USA). Each 30 ml serving of the concentrate contained 102 kcal, 24.5 g
104 carbohydrate, 1.1 g protein, trace amounts of fat, 2.6 g fibre, 9.72 mg ascorbic acid, 408 µg β-
105 carotene and trace amounts of sodium. The total anthocyanin content per serving was 273.5
106 mg. The predominant anthocyanins normally present in tart cherries are cyanidin derivatives
107 with smaller amounts of peonidin-3-rutinoside. Other compounds typically present include
108 hydroxycinnamic acids, flavonols and the indoleamine, melatonin [4, 19, 20]. The very
109 distinctive taste of the cherry concentrate made it difficult to blind the study. The placebo
110 beverage (Sprite®, The Coca-Cola Company) was chosen because it closely matched the
111 energy and macronutrient composition of cherry juice (109 kcal, 26.4 g CHO, 0 g fat, 0.1 g
112 sodium per 250 ml) and was devoid of plant compounds, antioxidants and vitamins.

113 **Measurements** Participants attended Sheffield Hallam University on two occasions for
114 vascular measurements, anthropometric measurements and blood sampling. All these were
115 taken in the early morning after an overnight fast. On both occasions, PWV, systolic blood
116 pressure (SBP), diastolic blood pressure (DBP) and body weight were measured and a blood
117 sample was taken. Height was also measured at the baseline visit. Measurements were taken
118 after a 15 min rest in the supine position to allow time for BP, cardiac function and
119 vasomotor tone to reach resting levels.

120 Brachial-knee PWV was measured in the supine position using a Nicolet Vasoguard
121 Microlight system (VIASYS Healthcare, USA) as previously described [21]. BP and heart
122 rate were measured in triplicate at 2.5 min intervals in the supine position using a semi-
123 automated Accutorr Plus™ sphygmomanometer (Datascope®, USA). The first reading was
124 disregarded to prevent readings being affected by the defence mechanism (the rise in BP
125 associated with anxiety of measurement, that tends to subside with subsequent
126 measurements) [22] and mean SBP, DBP and heart rate were calculated from the second and

127 third measurements. The Accutorr Plus™ sphygmomanometer was calibrated prior to the
128 start of the study. The device meets the US Association for the Advancement of Medical
129 Instrumentation and British Hypertension Society criteria for accuracy and has been
130 recommended by the European Society of Hypertension [23].

131 Weight was measured to the nearest 0.1 kg using a SECA 709 mechanical column
132 scale and height was measured to the nearest 0.1 cm with a SECA 220 telescopic measuring
133 rod (SECA, Birmingham, UK).

134 Fasting capillary blood samples were collected for the determination of total
135 cholesterol, high density lipoprotein (HDL) cholesterol, CRP and total antioxidant activity
136 (ferric reducing ability of plasma; FRAP). Lipid variables were measured on a Reflotron Plus
137 reflectance photometer (Roche Diagnostics Ltd, Burgess Hill, UK). For the analysis of total
138 cholesterol a 30 µl sample of whole blood was collected into a capillary pipette and applied
139 to a Reflotron reagent strip (Inverness Medical, Stockport, UK). The within day precision
140 was 0.8% and the between day precision was 1.2%. For the determination of HDL, a 60 µl
141 sample of whole blood was collected into an EDTA-potassium coated microcentrifuge tube
142 (Sarstedt Ltd, Leicester, UK) and spun at 2000 g x 5 min. Plasma was then extracted and
143 applied to a Reflotron HDL cholesterol strip (Inverness Medical, Stockport, UK) for
144 immediate analysis. The within day precision was 7.0% and between day precision was 8.3%.
145 A 250 µl whole blood sample was also collected into a lithium-heparin coated
146 microcentrifuge tube (Sarstedt Ltd, Leicester, UK) and spun at 2000 g for 10 min at 4°C to
147 extract plasma. The plasma was divided into two aliquots. One aliquot was stored at -80°C
148 for the later analysis of CRP and the second aliquot was used for the immediate
149 determination of plasma antioxidant activity (FRAP). FRAP was measured as described by
150 Benzie and Strain [24] on a Cecil Series 1000 UV/Vis spectrophotometer (Cecile Instruments
151 Ltd, Cambridge, UK). The intra-assay coefficient of variation (CV) was 1.4% and the inter-

152 assay CV was 4.2%. Plasma CRP was measured using a commercially available ELISA kit
153 (MP Biomedicals UK, Cambridge, UK). The intra-assay CV was 11.5% and the inter-assay
154 CV was 12.3%.

155 **Statistical analysis** For each variable, analysis was restricted to participants for whom
156 baseline and end of study data were available (see Table 2 for *n* values). The effect of
157 treatment on post-intervention measures was analysed by a one way ANCOVA, with
158 adjustment for baseline [25]. The residuals from the model were tested for normality using
159 the Shapiro-Wilk test and log transformation was used when there was deviation from
160 normality. The criterion for significance was a $P < 0.05$. All analyses were conducted using
161 SPSS version 20.0 (SPSS UK Ltd. Woking, UK).

162

163 **Results**

164 **Baseline characteristics, retention and compliance** Anthropometric, biochemical and
165 vascular measures were similar in randomisation groups at baseline (Table 1 & 2) and the
166 groups were balanced for sex and age. One participant withdrew from the study because they
167 did not like the taste of the cherry juice; all other participants completed the intervention
168 without report of adverse effects. Compliance to the intervention was good with 97% of
169 drinks consumed by the cherry juice group and 98% of drinks consumed by the placebo
170 group.

171 **Effect of intervention** There was no significant difference between the groups for the
172 primary outcome variable of PWV (Table 2). Cherry juice did not have any effect on CRP,
173 SBP, DBP, total cholesterol or HDL cholesterol relative to the placebo drink (Table 2).
174 Antioxidant status was significantly elevated at the end of the intervention in the cherry juice
175 group compared to the placebo group ($P = 0.012$). In comparison to the cherry juice group

176 there was a weak trend towards an increase in bodyweight in the placebo group (adjusted
177 mean difference 0.69 (95% CI -0.07, 1.15) kg; $P=0.073$).

178

179 **Discussion**

180 There is some evidence from dietary observational studies that anthocyanin intake is
181 associated with PWV – a large study of 1872 women from the TwinsUK registry reported
182 that women with a high intake of dietary anthocyanins had lower PWV [14]. The present
183 study was designed to test the hypothesis that short-term dietary intervention with
184 anthocyanins supplied as tart cherry juice would reduce PWV and a range of other risk
185 markers for CVD in healthy adults. To our knowledge no other study has investigated the
186 effect of tart cherry juice on PWV. However, Dohadwala et al. [18] reported that a 4-week
187 intervention with double strength cranberry juice (supplying 94 mg/d of anthocyanins)
188 reduced carotid-femoral PWV (by approximately 0.5 m/s) in overweight adults with coronary
189 artery disease. Despite providing approximately 3 times the quantity of anthocyanins to our
190 participants we failed to observe a reduction in PWV. Differences in study design may
191 explain this discrepancy. Dohadwala et al. [18] studied a group of overweight adults with
192 elevated risk markers for cardiovascular disease, whereas our volunteers had a normal body
193 mass index (BMI) (approx. 24 kg/m²) and were seemingly healthy. It is possible that in
194 healthy individuals, arterial stiffness is less responsive to a short-term increase in the intake
195 of anthocyanins. Moreover we measured brachial-knee PWV, which because it includes
196 segments of peripheral muscular arteries may be less amenable to dietary intervention than
197 carotid-femoral PWV. It is also possible that constituents of cranberry juice other than
198 anthocyanins may have driven the reduction in PWV observed by Dohadwala et al. [18].
199 The influence of supplementation with drinks rich in other polyphenolic compounds such as
200 green tea (rich in flavanols) [26] and pomegranate juice (rich in elagitannins but also a source

201 of anthocyanins) have also shown no effect on arterial stiffness [21], whilst supplementation
202 with isolated clover isoflavone over a 6-week period lowered PWV in postmenopausal
203 women and older men [27].

204 Cherry juice appeared to have no influence on BP in our sample of apparently
205 healthy middle-aged men and women. There are no directly comparable studies, and those
206 testing purified anthocyanins and extracts/juices rich in anthocyanins are inconsistent. A 4-
207 wk intervention with a twice-daily supplement of 320 mg of purified anthocyanins failed to
208 alter BP in middle-aged men with raised baseline BP [28]. Similarly, in elderly women
209 supplementation with an elderberry extract (500 mg/d of anthocyanins) for 12 weeks failed to
210 alter BP [29]. However, Chong et al [30] suggested that chokeberries may have hypotensive
211 effects based on a single intervention study in patients with cardiovascular disease.

212 We also observed no change in CRP as a result of cherry juice supplementation.
213 CRP is a recognised biomarker of systemic inflammation and a risk marker for
214 cardiovascular events [31]. This lack of effect on CRP contrasts with the studies of Kelley et
215 al. [10, 11] who reported that 280 g/d of sweet cherries (supplying approx. 100 mg/d
216 anthocyanins) reduced CRP by 8% after 2 weeks of supplementation and by 25% after four
217 weeks of supplementation. Although these changes in CRP seem to indicate that sweet
218 cherries elicit a reduction in inflammation, the study lacked a control group so interpretation
219 must be cautious. Our results also contrast with studies demonstrating that tart cherry juice
220 attenuates the inflammatory response to marathon running and resistance exercise [12, 13].
221 This discrepancy may be explained by differences in dosing schedule (1 serving/d v 2
222 servings/d) or indicate that in healthy adults, tart cherry juice attenuates the response to an
223 inflammatory challenge, but does not modulate baseline levels of inflammation.

224 Cherry juice has potent antioxidant effects [4, 32]. At the end of the current
225 intervention, plasma antioxidant status (measured by the FRAP assay) was modestly elevated

226 in the tart cherry group in comparison to the control group, however, this was largely driven
227 by a fall in antioxidant status in the control group. This may reflect the displacement of
228 antioxidant containing beverages by the placebo drink. The FRAP assay probably has limited
229 sensitivity to detect an increase in antioxidant status in response to the tart cherry juice. The
230 main determinants of plasma FRAP are uric acid, which may be lowered by cherries and
231 ascorbate which is only present in low amounts in tart cherry juice [24, 33]. The contribution
232 of polyphenols to plasma FRAP activity is likely to be low [24].

233 The inertia in response of total and HDL cholesterol concurs with one short-term
234 intervention study using sweet cherries [10]. A lack of impact on blood lipids has also been
235 noted in relation to diet intervention with other anthocyanin-rich berries [30].

236 The current study has several limitations. It would have been informative to have
237 checked compliance to the intervention through measurement of plasma or urinary
238 anthocyanins. As an adjunct to this, a detailed dietary record would have been informative as
239 to any dietary displacement effects. We narrowly failed to meet our recruitment target of 25
240 participants in each arm. This slightly reduced the power of the study to identify a change in
241 PWV. For CRP, the low sensitivity of the assay (CV 11.5%) may have masked a small anti-
242 inflammatory effect. All biochemical measures were performed in capillary samples collected
243 by finger prick. Limited blood volume meant that we failed to collect complete data for some
244 analytes; this may have contributed to our null effect. It is also possible that our null effect
245 arose because participants may have been too healthy to detect a beneficial effect of cherry
246 juice. We cannot rule out the possibility that cherry juice may be more effective in
247 individuals with existing hypertension or cardio-metabolic disease.

248 In conclusion our study provides no evidence that a short-term intervention with tart
249 cherry juice reduces arterial stiffness or improves other markers of cardiovascular risk in

250 apparently healthy adults. Confirmation of this null effect is needed, perhaps using longer
251 intervention periods and examining individuals at high risk of cardiovascular disease.

252

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254 AL and MEB designed the study and wrote the manuscript. JR carried out the statistical
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260

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348

349 **Table 1** Participant characteristics according to randomisation group at study inclusion (means and SDs)

	Cherry juice (n=25)	Placebo (n=21)
351 Age (years)	38.3 (6.16)	37.2 (5.78)
352 Weight (kg)	70.4 (12.75)	69.2 (14.55)
353 BMI (kg/m ²)	24.6 (3.63)	23.5 (3.00)
354 Sex (Female/Male)	16/9	13/8

355

356

357 **Table 2** Change in outcome variables in the tart cherry juice and control groups over the 6 week intervention period

358

	Cherry juice		Placebo		Adjusted	
	Baseline	End	Baseline	End	difference#	
	(mean (SD))	(mean (SD))	(mean (SD))	(mean (SD))	(95% CI)	
363 PWV (m/s)	8.22 (1.69)	8.18 (1.60)	7.98 (1.21)	7.74 (1.06)	0.243	<i>F</i> (1, 43) =1.564
364 n	25		21		(-0.149, 0.634)	<i>P</i> = 0.218

365	hsCRP (mg/L)*	1.13 (0.92, 1.38)	1.13 (0.93, 1.03)	1.14 (0.91, 1.43)	1.26 (1.01, 1.59)	-10%**	$F(1, 36) = 1.559$
366	n	22		17		(-14%, 7%)	$P = 0.220$
367	SBP (mm Hg)	110.5 (14.38)	110.2 (12.58)	110.43 (12.28)	113.36 (11.88)	-3.21	$F(1, 43) = 2.019$
368	n	25		21		(-7.763, 1.346)	$P = 0.163$
369	DBP (mm Hg)	70.3 (10.04)	69.2 (9.86)	67.4 (8.28)	69.9 (7.62)	-2.814	$F(1, 43) = 2.496$
370	n	25		21		(-6.406, 0.778)	$P = 0.121$
371	Cholesterol (mmol/L)						
372	Total	4.25 (0.79)	4.22 (0.77)	3.76 (0.67)	4.12 (0.67)	-0.183	$F(1, 40) = 0.926$
373	n	24		19		(-0.569, 0.202)	$P = 0.342$
374	HDL*	0.96 (0.87, 1.05)	1.09 (1.02, 1.16)	1.37 (0.92, 1.15)	1.04 (0.97, 1.11)	7%**	$F(1, 36) = 2.436$
375	n	22		17		(-2%, 16%)	$P = 0.127$
376	FRAP (μ M)	1205 (339.45)	1294 (269.03)	1298 (323.05)	1165 (255.55)	173.58	$F(1, 39) = 6.909$
377	n	23		19		(40.002, 307.159)	$P = 0.012$

378

379 #Effect of intervention was assessed by ANCOVA adjusted for baseline. *Data was log transformed prior to analysis so baseline and end of
380 study data is expressed as geometric mean and 95% CI. **Adjusted mean difference for log transformed data is expressed as a percentage
381 (Bland 2000).