

Northumbria Research Link

Citation: Kimble, Rachel, Keane, Karen, Lodge, John and Howatson, Glyn (2021) The influence of tart cherry (*Prunus cerasus*, cv Montmorency) concentrate supplementation for 3 months on cardiometabolic risk factors in middle-aged adults: a randomised, placebo-controlled trial. *Nutrients*, 13 (5). p. 1417. ISSN 2072-6643

Published by: MDPI

URL: <https://doi.org/10.3390/nu13051417> <<https://doi.org/10.3390/nu13051417>>

This version was downloaded from Northumbria Research Link:
<http://nrl.northumbria.ac.uk/id/eprint/45957/>

Northumbria University has developed Northumbria Research Link (NRL) to enable users to access the University's research output. Copyright © and moral rights for items on NRL are retained by the individual author(s) and/or other copyright owners. Single copies of full items can be reproduced, displayed or performed, and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided the authors, title and full bibliographic details are given, as well as a hyperlink and/or URL to the original metadata page. The content must not be changed in any way. Full items must not be sold commercially in any format or medium without formal permission of the copyright holder. The full policy is available online: <http://nrl.northumbria.ac.uk/policies.html>

This document may differ from the final, published version of the research and has been made available online in accordance with publisher policies. To read and/or cite from the published version of the research, please visit the publisher's website (a subscription may be required.)

Article

The influence of tart cherry (*Prunus Cerasus*, cv Montmorency) concentrate supplementation for 3 months on cardiometabolic risk factors in middle-aged adults: a randomised, placebo-controlled trial

Rachel Kimble¹, Karen M Keane², John K Lodge¹ and Glyn Howatson^{1,3*}

¹Faculty of Health and Life Sciences, Northumbria University, Newcastle-upon-Tyne, UK

²School of Science and Computing, Galway-Mayo Institute of Technology, Galway, Ireland

³Water Research Group, School of Environmental Sciences and Development, Northwest University, Potchefstroom, South Africa

* Correspondence: Glyn Howatson

Address: Department of Sport, Exercise and Rehabilitation, Faculty of Health and Life Sciences, Northumbria University, Newcastle-upon-Tyne, UK

E-mail: glyn.howatson@northumbria.ac.uk

Citation: Kimble, R.; Keane, K.M.; Lodge, J.K.; Howatson, G. The influence of tart cherry (*Prunus Cerasus*, cv Montmorency) concentrate supplementation for 3 months on cardiometabolic risk factors in middle-aged adults: a randomised, placebo-controlled trial. *Nutrients* **2021**, *13*, x. <https://doi.org/10.3390/xxxxx>

Received: date

Accepted: date

Published: date

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

Abstract: Background: Tart Montmorency cherries (MC) have been shown to be rich in anthocyanins and other phytochemicals known to have anti-inflammatory properties and influence pathways that might improve cardiometabolic health. However, there is limited evidence for the longer-term use of tart cherries on these indices. The aim of the current study was to investigate the influence of MC concentrate on cardiometabolic health indices following a 3-month supplementation period. **Methods:** Fifty middle-aged adults (34 males and 16 females; mean \pm SD age: 48 ± 6 years and BMI: 27.6 ± 3.7 kg/m²) completed a randomised, placebo-controlled parallel study in which they either received MC or an isocaloric placebo. Participants drank 30 ml of their allocated treatment twice per day for 3 months. Vascular function (blood pressure [BP], heart rate [HR], pulse wave velocity and analysis [PWV/A] and flow mediated dilation [FMD]) as well as indices of metabolic health (insulin, glucose, lipid profiles and high sensitivity C reactive protein) were measured following an overnight fast before and after the 3 months. **Results:** No effect of the intervention between groups was observed for vascular function or metabolic health variables following the intervention ($P > 0.05$). However, MC concentrate was shown to be safe and well tolerated and importantly did not have any deleterious effects on these outcomes. In conclusion MC has no influence on cardiometabolic indices in middle-aged adults.

Keywords: Tart cherry; cardiovascular disease; vascular function; metabolic health

1. Introduction

Recent advances in technology and medicine have resulted in increased life expectancy and an aging population; it is now estimated that non-communicable diseases account for 71% of all deaths globally [1]. In particular, cardiovascular disease (CVD) and type 2 diabetes combined are the primary cause of global mortality, increasing in prevalence with age [2]. Midlife risk factors have been proposed to underlie the development of these diseases, which evolve over years or decades before the emergence of clinical manifestations [3–5]. Thus, midlife has consistently been highlighted as a pivotal period for lifestyle interventions to improve health and reduce disease trajectory to promote healthy aging [6,7]. For example, middle-aged individuals who decrease their blood pressure (BP) to normal ranges ($<120/80$ mmHg) have a significantly lower risk of CVD in

their remaining lifetime compared to those with hypertension [8]. Furthermore, oxidative stress and inflammation are key mechanisms of endothelial dysfunction and arterial damage, thus are linked to type 2 diabetes [9] and CVD [4]. Consequently, there has been a research emphasis in into natural dietary sources of antioxidants and foods with anti-inflammatory properties [10].

There is accumulating evidence that tart Montmorency cherries (MC) can positively impact cardiometabolic risk factors that include antioxidant [11], anti-inflammatory [12] and antihypertensive properties [13-15]. Tart MC are a relatively abundant source of anthocyanins [16]; the polyphenols responsible for the red-blue-purple pigmentation in fruit and vegetables. Previous work from our research group has shown that higher intake of dietary anthocyanins is inversely associated with the risk of CVD mortality [17] and cherries have been reported to account for a considerable dietary intake of these compounds [18-20]. *In vitro* anthocyanin metabolites have been shown to interact with vascular smooth muscle cells [21] and upregulate endothelial nitric oxide synthase (eNOS) in endothelial cells [22]. More recently, anthocyanin metabolites following blueberry intake were associated with improved endothelial function and nitric oxide (NO) bioavailability and were shown to influence genes involved in regulation of cell adhesion, cell migration, inflammation, and cell differentiation processes, conferring the cardioprotective properties of these compounds [23].

Furthermore, MC are also rich in other phytochemicals (e.g. phenolic acids, flavonols, flavon-3-ols, melatonin and carotenoids) that might have synergistic and additive effects on any bioactivities including their antioxidant and anti-inflammatory actions [21,24]. Nonetheless, despite some promising epidemiological and *in vitro* studies suggesting a putative role for MC in cardiovascular and metabolic health, clinical trials have provided paradoxical and equivocal findings for any benefits associated with their intake. For example, relatively short-term (4-6 week) MC interventions failed to influence endothelial function, blood pressure and cholesterol in middle-aged populations [11,12,25,26]. However, longer-term (12 week) consumption of MC juice has been shown to reduce systolic BP and low-density lipoprotein (LDL) in older adults [14]. Moreover, Johnson and colleagues [27] demonstrated that 12, but not 6, weeks of MC reduce oxidised LDL in individuals with metabolic syndrome. To date there has been no study investigating the longer-term influence of MC on cardiometabolic risk factors in a middle-aged population, however based on recent findings it was hypothesised that longer-term MC supplementation would improve vascular function and metabolic health parameters. Therefore, the aim of the current study was to investigate the influence of 3-month of MC concentrate supplementation on cardiometabolic health indices in middle-aged adults.

2. Materials and Methods

Participants

Non-smoking males and females between the ages of 40 to 60 years were recruited from Newcastle Upon Tyne and the local area of the city by the use of posters, email distributions, social media and word of mouth. To be included in the study, participants must have reported to consume (on average) less than 5 servings of fruits and vegetables per day, did ≤ 4 hours of moderate-vigorous physical activity per week and additionally had ≥ 1 risk factor for type 2 diabetes. These risk factors included body mass index (BMI) > 25 kg/m²; waist circumference > 102 cm for males and > 88 cm for females; family history of type 1 or type 2 diabetes; were a member of a type 2 diabetes high risk population (Aboriginal, Hispanic, Asian, South Asian, or African decent) or were hypertensive; $> 140/90$ mmHG [28,29]. All participants were otherwise in apparent good health as assessed by a health-screening questionnaire, not regularly taking medication (or stabilised ≥ 3 months, with no adverse symptoms) or antioxidant supplements and willing to report any changes in health status or medication during the study period.

Exclusion criteria was defined as a history of cardiometabolic disease, uncontrolled hypertension (SBP >159 mmHg or DBP >99 mmHg), gastrointestinal disease or malabsorption syndromes, reported changes in dietary or physical activity patterns within 3 months prior or intention to change during the study period, vegetarians, vegans or had known eating disorders, excessive alcohol intake, or a BMI ≥ 40 kg/m². Additionally, participants who were pregnant or planning to become pregnant during the study, lactating, or initiating or changing a hormone replacement therapy regimen within 3 months of the start of the study were also excluded. The study was conducted in accordance with the Declaration of Helsinki and ratified by the University's Research Ethics Committee prior to participants providing written, informed consent. This study was registered as a clinical trial with clinicaltrials.gov [NCT04021342].

Study design

This study employed a randomised, double-blind, placebo-controlled, parallel design. After screening and recruitment (visit 1), participants were familiarised with the testing equipment and procedures. Following this they were randomly assigned to receive either MC concentrate or an isocaloric placebo for 3 months using a computer-generated plan (<https://www.randomization.com>), stratified by sex. The study comprised of two experimental visits, following a minimum of a 7-day low anthocyanin run-in. Vascular function and metabolic health variables, as described below, were assessed at baseline (visit 2; pre-supplementation) and at 3 months (visit 3; post-supplementation). All experimental visits took place between 8:00 and 10:00 am and were preceded by an overnight fast (≥ 10 h). Participants were also asked to arrive hydrated and to avoid strenuous exercise, alcohol, nutritional supplements 24 hours and caffeine 12 hours prior.

Dietary intervention

A concentrated MC juice stored at 4°C was used in this study. The concentrate was provided by Cherry Marketing Institute (USA). Participants were instructed to consume either 30 ml of MC concentrate diluted in 240 ml of water or the same volume of placebo twice daily, once in the morning and again in the evening. According to the manufacturers this is the equivalent of ~180 cherries per day. Previous batch analysis by our laboratory using pH differential method and modified Folin-Ciocalteu colorimetric method, 60 ml of MC concentrate is the equivalent to 68-73.5 mg of anthocyanins and 160.8-178.8 mg of total phenolics, respectively [13,30]. The placebo was prepared by mixing unsweetened black cherry flavoured Kool-Aid (Kraft Foods, United States), dextrose (MyProtein Ltd., Northwich, UK), fructose (Sports Supplements Ltd., Essex, UK) with bottled water to best match the calorie content of the MC concentrate (Energy = 102 kcal, volume = 30 ml, carbohydrates = 25.5 g, protein = 0 g and fat = 0 g). Additional lemon juice (which is not known to contain anthocyanins [31]), for tartness and preservation, and artificial food colouring was added so the final product had the same visual properties [14,32]. The assigned treatment and a 30 ml measuring cup were supplied to the participants by a researcher (independent to the project) to ensure the study remained double-blinded. Compliance was measured by daily tick sheets and return of any unconsumed juice. To evaluate blinding efficacy, participants were asked to guess which treatment they thought they had been taking following the supplementation period.

Throughout the study participants were encouraged to maintain their habitual diet and exercise routines, however they were asked to refrain from consuming cherries, cherry products, or any antioxidant supplements. Additionally, they were given verbal and written instructions to limit berry fruits, red grapes (including extracts and juices) and red wine (which are the highest contributors of dietary anthocyanins [18,19]) to ≤ 1 portion per day throughout the study period. Participants recorded their pre-evening meal before experimental visit one and were asked to replicate this before the second experimental visit. To monitor dietary intake, participants recorded a 3-day food and

exercise diary (two consecutive weekdays and one weekend day), which was analysed retrospectively (Nutritics software, v5.09, Dublin, Ireland). Participants also completed the International Physical Activity Questionnaire (IPAQ) and a short form quality-of-life survey (SF-36 [33]) at the beginning and end of the study to determine physical activity levels and tolerance to the intervention, respectively.

Anthropometry and aerobic capacity

Stature was measured to the nearest 0.1 cm using a stadiometer and body mass measured to the nearest 0.1 kg using the same digital scale (Seca Scales 703, Seca Ltd. Birmingham, UK). Body composition (fat mass, fat percentage, android/gynoid ratio and lean body mass) was measured by dual-energy X-ray absorptiometry (DXA; Hologic, Horizon, Manchester, UK). The scanner was calibrated before each assessment in accordance with manufacturer's guidelines and participants were instructed to wear the same clothing for each visit. Exercise capacity (VO_{2max}) was also assessed before and after the intervention using a sub-maximal cycle test; the Astrand-Rhyming single-stage, 6-min test [34,35]. All measurements were performed at baseline and on the return visit, 3 months later.

Vascular function

Vascular function was assessed as previously described with participant in the supine position [36]. Briefly, BP and heart rate (HR) were measured using non-invasive, automated vital signs monitor (Carescape V100; Dinamap) closely adhering to the guidelines specified by the European Society of Hypertension [37]. Peripheral BP measurements were taken in triplicate, each separated by 1 minute [38], and the mean of the last 2 readings of systolic (SBP), diastolic (DBP) and HR used for analysis (coefficient of variation; $CV < 6\%$).

The pulse wave velocity and analysis (PWV/A) were determined through arterial tonometry using the SphygmoCor CPV system (ScanMed Medical, UK). The PWV (in m/s) was determined between carotid and femoral sites with electrocardiogram gating. The PWA was recorded at the radial artery and the corresponding augmentation index (AIx) derived using a generalised transfer function [39]. The AIx is influenced by heart rate [40], therefore AIx normalised for a standard heart rate of 75 bpm (AIx@75) was also measured. The SphygmoCor software (version 9.0, ScanMed Medical, UK) provides indices of quality control, if the measurement did not meet these control criteria, it was discarded and replaced by a new measurement. A minimum of two acceptable readings were obtained for both PWV and PWA and the average used for analysis.

Brachial artery flow mediated dilation (FMD) was acquired using ultrasonography (HDI-5000 SONO CT ultrasound machine; Philips Medical System). The resting baseline diameter was recorded for 1 minute prior to occlusion of the artery which was obtained by inflating a manual sphygmomanometer >50 mmHg above SBP. Occlusion of the artery was maintained for 5 minutes. The recording was resumed 1 minute before deflating the cuff and 3 minutes thereafter. The average baseline resting diameter and peak diameter post-occlusion were determined using semi-automated computer software (Brachial Analyzer; Medical Imaging Applications) and used to calculate the percentage FMD.

Haematological samples

Venous blood samples (~12 ml) were collected in lithium-heparin vacutainers (Becton, Dickinson and Company, USA). Due to sampling errors, samples were available for 40 participants (Cherry $n = 19$; Placebo $n = 21$). These were centrifuged at $3000 \times g$ ($4^\circ C$) for 10 min and the plasma aliquoted and stored at $-80^\circ C$ to be analysed later. Plasma samples were analysed for cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides and glucose using a colorimetric enzymatic method ($CV < 3.7\%$). Insulin was analysed using enzyme-linked immunosorbent assay (Merckodia, Sweden; $CV = 12\%$). High sensitivity CRP (hs-CRP) was analysed using particle enhanced

immunoturbidimetric assay (CV = 7.8%). Non-HDL cholesterol was calculated as HDL subtracted from total cholesterol. The LDL cholesterol was calculated using the Friedewald equation [41]. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated according to Matthews et al. [42] using the following formula:

$$\text{HOMA-IR} = (\text{Fasting insulin} \times \text{Fasting glucose}) / 405.$$

Power calculation and statistical analysis

Power calculations were performed for the primary end point: change in SBP after 3 month consumption. The power was based on the inter-individual variability for SBP measurement [36], assuming an 80% power, and a 0.05 significance level, the total number of subjects required to provide sufficient power to detect a 5 mmHg, a clinically meaningful amount [43] in a 2-arm, parallel study was estimated to be 50. A total of 60 participants were needed to allow for a 20% drop-out, however only 56 were recruited before the study end.

All data were analysed using IBM SPSS statistics (v 26.0 for Windows; SPSS, Chicago, IL), measures are reported as means \pm standard deviation (SD) in tables and standard error (SE) in figures unless otherwise stated. Normality of distribution for outcome measures was tested using the Shapiro Wilks test and assumptions were tested prior to analysis. Baseline characteristics were compared by Wilcoxon signed-rank test where data were continuous, and Chi-square test where data were categorical. Dietary, physical activity and SF-36 data were analysed using a two-way (treatment \times time) analysis of variance (ANOVA). Treatment guess data was analysed by Chi-square test. The effect of the intervention on vascular function and metabolic health variables was evaluated using a one-way analysis of covariance (ANCOVA) adjusted for baseline [44]. Additional covariates of sex and use of medication were added into the analysis for the primary outcome (vascular function). For blood samples where values fell below the limits of detection the sensitivity threshold of the assay were used to maintain participant numbers. The hs-CRP, HOMA-IR and insulin values were non-normally distributed so were log transformed before analysis. Sidak adjusted *post-hoc* comparisons were then carried out between cherry juice and placebo as appropriate

3. Results

A total of 56 individuals were enrolled in the study and randomised to the intervention (Figure 1). There was no difference between the group characteristics at baseline (Table 1). Three participants from each group did not complete the study as shown in Figure 1. One participant in the cherry group discontinued the juice and withdrew from the study due to gastrointestinal discomfort and bloating. The treatments were otherwise well tolerated as suggested by the SF-36 which showed no treatment, time or interaction differences between groups (data not shown). The mean (\pm SD) self-reported compliance (as assessed by tick sheets) was $94 \pm 9\%$ and $98 \pm 4\%$ in the cherry and placebo group, respectively. Five participants (20%) correctly guessed they were in the placebo group, but Chi squared suggested successful blinding ($P = 0.386$).

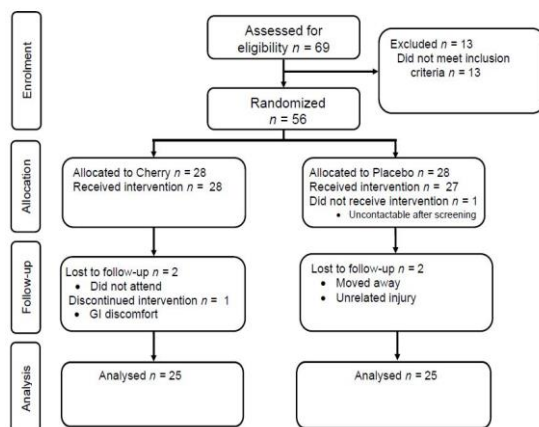


Figure 1. Consort flow diagram of participant enrolment and analysis in the study.

237

238

239

240

241

Table 1. Baseline characteristics (Mean ± SD).

Characteristic	All (n = 56)	Cherry (n = 28)	Placebo (n = 28)	P-Value
Age (y)	48 ± 6	49 ± 6	47 ± 6	0.160
Sex (m/f)	37/19	19/9	18/10	0.778
Stature (cm)	173.1 ± 8.8	173.7 ± 8.9	172.4 ± 9.0	0.494
Body Mass (kg)	81.8 ± 12.9	81.7 ± 14.0	82.0 ± 11.9	0.793
BMI (kg/m ²)	27.3 ± 3.7	27.0 ± 3.8	27.4 ± 3.7	0.569
Ethnicity (n; %)				0.368
White	54 (96.4)	27 (96.4)	27 (96.4)	
Education (n; %)				0.798
Less than high school	-	-	-	
High school or equivalent	24 (43)	11 (39)	13 (46)	
Bachelor's degree	19 (34)	9 (32)	10 (36)	
Postgraduate degree	13 (23)	8 (29)	5 (18)	
Medication (n; %)	17 (30)	10 (36)	7 (25)	0.771

254

255

256

257

258

259

260

261

262

Table 2. Physical activity, diet and body composition during the study.

	Cherry	Placebo	ANOVA		
			Treatment	Time	Interaction
METs (min/week)					
Baseline	2462 ± 2038	1953 ± 1527	0.267	0.959	0.961
3 months	2464 ± 2336	1978 ± 1217			
Sitting time (h)					
Baseline	6.6 ± 3.1	6.7 ± 3.0	0.856	0.852	0.486
3 months	6.7 ± 2.8	6.3 ± 3.0			
VO_{2max} (ml·kg⁻¹·min⁻¹)					
Baseline	35.9 ± 11.1	37.3 ± 7.5	0.319	0.187	0.386
3 months	33.7 ± 9.6	37.1 ± 6.2			
Energy (Kcal)					
Baseline	1921 ± 340	1896 ± 439	0.997	0.212	0.673
3 months	1977 ± 439	2001 ± 423			
Carbohydrates (g)					
Baseline	208.8 ± 38.6	196.1 ± 47.3	0.671	<0.001	0.350
3 months	237.3 ± 70.0 [†]	239.6 ± 66.7 [†]			
Fat (g)					
Baseline	76.5 ± 18.5	78.4 ± 24.2	0.681	0.231	0.999
3 months	72.1 ± 18.1	74.1 ± 21.2			
Saturated fat (g)					
Baseline	28.2 ± 8.6	27.5 ± 8.0	0.849	0.224	0.836
3 months	25.8 ± 8.3	25.7 ± 10.6			
Protein (g)					
Baseline	80.2 ± 21.9	92.3 ± 16.9 [*]	0.193	0.051	0.006
3 months	80.3 ± 15.7	78.5 ± 19.3 [†]			
Body mass (kg)					
Baseline	82.8 ± 13.9	82.4 ± 12.3	0.968	0.007	0.048
3 months	83.4 ± 14.2	84.1 ± 13.2 [†]			
BMI (kg/m²)					
Baseline	27.3 ± 3.8	27.5 ± 3.8	0.757	0.001	0.057
3 months	27.5 ± 3.8	28.1 ± 4.0 [†]			
Body fat (%)					
Baseline	37.1 ± 7.9	36.1 ± 6.8	0.642	0.031	0.862
3 months	37.7 ± 6.8 [†]	36.7 ± 6.8			
Fat mass (kg)					
Baseline	30.2 ± 9.1	29.6 ± 7.1	0.856	0.007	0.691
3 months	31.1 ± 9.5 [†]	30.7 ± 7.5 [†]			
Lean mass (kg)					
Baseline	48.1 ± 9.4	49.8 ± 8.5	0.360	0.248	0.199
3 months	48.1 ± 8.5	50.4 ± 9.2			
Android/gynoid ratio					
Baseline	1.09 ± 0.17	1.13 ± 0.18	0.394	0.403	0.529
3 months	1.08 ± 0.17	1.13 ± 0.18			

Mean ± SD; body mass index (BMI); [†]significantly different from baseline; * significantly different between groups (P < 0.05).

3.2. Physical activity, diet and body composition

There was no treatment, time or treatment × time interaction effects observed for physical activity, sitting time or exercise capacity (Table 2). Analysis of 3-day diet records showed that there were no differences between the two groups for mean intake of total energy, fat or saturated fat intake. Protein intake showed a treatment × time interaction (F = 7.8, P = 0.011). Planned post hoc tests revealed protein intake at baseline was on average 12 g higher in the placebo group compared to the cherry group (F = 6.7, P = 0.016). There was also an increase in carbohydrate (36 g) intake at 3 months in both groups, main effect of time (F = 17.3, P < 0.001). Body mass (F = 8.5, P = 0.007), BMI (F = 12.6, P = 0.002) and fat mass (F = 4.8, P = 0.040) increased relative to baseline in the placebo group, main

263

264

265

266

267

268

269

270

271

272

effect of time. Fat mass ($F= 7.7, P = 0.011$) and fat percentage ($F= 4.38, P = 0.047$) also increased after 3 months in the cherry group. There was no treatment effect observed at baseline or 3 months for body mass. There was no treatment, time or treatment \times time interaction effects between lean mass or android/gynoid ratio (Table 2).

3.3. Influence on MC on vascular function

After adjusting for baseline (pre-treatment) values, sex and medication there were no group differences between MC and placebo at 3 months for SBP (117 ± 14 vs 118 ± 11 mmHg; $P = 0.8$; Figure 2). After 3 months there was also no difference was DBP, HR, arterial stiffness (PWV, AIx and AIx@75) or endothelial function (FMD) compared to the placebo (Table 3).

3.4. Influence on MC on metabolic health indices

After adjusting for baseline (pre-treatment) values, sex and medication there were no group differences between cherry juice and placebo for lipid profiles, insulin, glucose, HOMA-IR or hs-CRP (Table 4).

273
274
275
276
277
278
279
280
281
282
283
284
285
286
287

Commented [GH1]: This figure looks like it has been squashed (top to bottom) can you reset it?

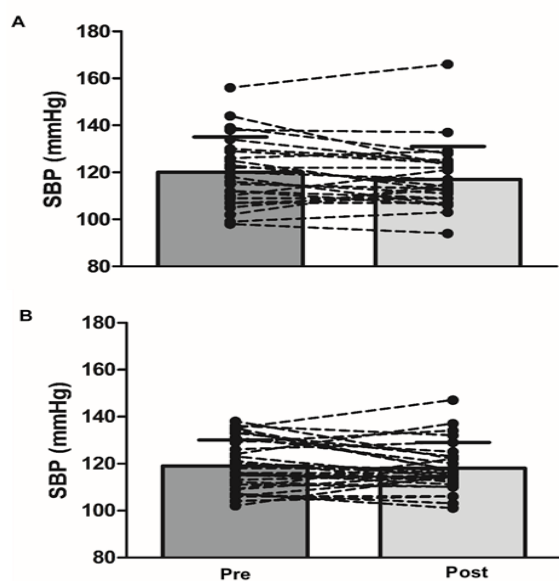


Figure 2. Systolic blood pressure before (left) and after 3-month supplementation (right) with cherry juice (A) and placebo (B).

288
289
290
291

Table 3. Influence of tart Montmorency cherries on vascular function compared to a placebo

	Cherry Juice	Placebo	ANCOVA adjusted for baseline			adjusted for baseline, sex and medication		
			Difference (95%CI)	F	P-Value	Difference (95%CI)	F	P-Value
SBP (mmHg)								
Baseline	120 ± 15	119 ± 11	-0.5 (-4.9, 3.8)	0.056	0.814	-0.6 (-5.1, 3.9)	0.079	0.780
3 months	117 ± 14	118 ± 11						
DBP (mmHg)								
Baseline	73 ± 10	73 ± 8	-0.4 (-3.0, 2.3)	0.086	0.770	-0.5 (-3.1, 2.2)	0.127	0.723
3 months	73 ± 9	73 ± 8						
HR (BPM)								
Baseline	59 ± 11	59 ± 10	-0.3 (-3.7, 3.1)	0.033	0.858	-0.2 (-3.6, 3.2)	0.014	0.908
3 months	59 ± 12	59 ± 10						
PWV (m/s)								
Baseline	6.7 ± 1.0	6.4 ± 0.8	0.3 (-0.3, 0.8)	1.051	0.312	0.2 (-0.2, 0.7)	0.967	0.332
3 months	6.8 ± 1.3	6.2 ± 0.8						
AIx (%)								
Baseline	22.1 ± 8.9	17.8 ± 11.4	0.3 (-3.6, 4.2)	0.021	0.886	0.3 (-3.6, 4.2)	0.022	0.884
3 months	20.4 ± 9.6	17.0 ± 10.0						
AIx@75 (%)								
Baseline	13.4 ± 8.1	9.6 ± 12.9	0.02 (-3.6, 3.6)	<0.001	0.991	-0.06 (-3.6, 3.5)	0.001	0.937
3 months	12.3 ± 9.2	9.3 ± 11.0						
FMD (%)								
Baseline	8.3 ± 3.5	9.3 ± 3.5	1.1 (-1.1, 3.3)	0.972	0.330	1.2 (-1.0, 3.3)	1.256	0.269
3 months	9.7 ± 3.5	9.0 ± 4.0						

Mean ± SD; Abbreviations; augmentation index (AIx); AIx normalised for a heart rate of 75 bpm (AIx@75); diastolic blood pressure (DBP); flow-mediated dilation (FMD); heart rate (HR); pulse wave velocity (PWV).

292

293

Table 4. Influence of tart Montmorency cherries on metabolic health indices

	Cherry (n = 19)	Placebo (n = 21)	ANCOVA adjusted for baseline		
			Difference (95% CI)	F	P-Value
Insulin[‡] (pmol/L)					
Baseline	20.3 ± 15.4	19.6 ± 10.2	-0.01 (-0.18, 0.15)	0.020	0.888
3 months	17.8 ± 10.0	19.0 ± 13.0			
Glucose (mmol/L)					
Baseline	5.4 ± 0.5	5.5 ± 0.5	-0.01 (-0.21, 0.18)	0.016	0.899
3 months	5.4 ± 0.5	5.4 ± 0.3			
HOMA-IR[‡]					
Baseline	0.7 ± 0.6	0.7 ± 0.4	0.04 (-0.13, 0.21)	0.239	0.629
3 months	0.7 ± 0.4	0.7 ± 0.5			
hs-CRP[‡] (mg/L)					
Baseline	1.6 ± 2.3	1.2 ± 1.2	-0.003 (-0.14, 0.14)	0.111	0.741
3 months	1.4 ± 1.7	1.2 ± 1.0			
Triglycerides (mmol/L)					
Baseline	1.2 ± 0.6	1.2 ± 0.7	-0.06 (-0.29, 0.18)	0.241	0.627
3 months	1.2 ± 0.7	1.3 ± 0.7			
Cholesterol (mmol/L)					
Baseline	5.3 ± 1.2	5.0 ± 1.0	-0.15 (-0.51, 0.21)	0.728	0.399
3 months	5.2 ± 1.1	5.1 ± 0.9			
LDL cholesterol (mmol/L)					
Baseline	3.2 ± 1.0	3.0 ± 1.0	-0.09 (-0.38, 0.20)	0.411	0.525
3 months	3.1 ± 1.0	3.1 ± 1.0			
HDL cholesterol (mmol/L)					
Baseline	1.6 ± 0.3	1.5 ± 0.4	-0.02 (-0.15, 0.12)	0.061	0.806
3 months	1.5 ± 0.4	1.5 ± 0.4			
Non-HDL cholesterol (mmol/L)					
Baseline	3.7 ± 1.2	3.5 ± 1.2	-0.17 (-0.49, 0.16)	1.08	0.305
3 months	3.7 ± 1.1	3.6 ± 1.1			
Total/HDL cholesterol ratio					
Baseline	3.5 ± 1.0	3.7 ± 1.3	-0.076, -0.41, 0.26)	0.209	0.650
3 months	3.6 ± 1.2	3.8 ± 1.4			

Mean ± SD. Abbreviations: high-density lipoproteins (HDL); homeostatic model assessment of insulin resistance (HOMA-IR); high-sensitivity C-reactive protein (hs-CRP) [‡] log transformed before analysis, raw values are presented

294

295

296

297

298

299

300

301

302

303

304

305

306

307

308

309

310

311

312

313

314

315

316

317

318

319

320

321

4. Discussion

To date this is the largest and longest duration study to determine the influence of MC on vascular function and metabolic health in free-living, middle-aged adults. The primary aim was to assess any change in vascular function, specifically SBP which was shown to be modulated in previous work [14,30]. However, on the basis of the vascular function variables measured in the current study, and contrary to the hypothesis, there was no effect on BP, endothelial function or arterial stiffness (Table 3). While the reasons for this could be manifold it might be explained by the kinetics of tart cherry phenolics; we and others have previously shown that tart cherry anthocyanin metabolites peak in the plasma within 1-2 hours, a time course which coincides with the greatest reductions in postprandial SBP [13,15,45]. Moreover, there is a rapid clearance in these metabolites, with SBP returning to basal within 3-4 hours [13,15]. In the current study vascular function was measured after an overnight fast, therefore peak vasomodulatory properties of the MC might have been missed. This represents a limitation of the current study as acute responses were not investigated, however the study was ultimately interested in the cumulative influence of MC consumption. In a recent addition to the literature Desai and colleagues [46] demonstrated that 6-day supplementation with 30 ml MC had no influence on fasted or post bolus laboratory SBP, however did influence 24-hour ambulatory SBP after the 7th day. These data could suggest a first dose phenomenon similar to that of antihypertensive medications, in that the large fall in SBP is as an initial response to the peak tart cherry phenolics but the magnitude of which is less pronounced over time [47].

In contrast to the current study, two randomised controlled studies have shown that tart [14] and sweet cherry juice [48] reduce SBP in older adults following a 12 week supplementation. The reason for sustained changes in vascular function following chronic anthocyanin and other polyphenol supplementation are currently unknown, but are possibly mediated via modulation of the microbiome and complex gene expression alterations [23,49,50]. Therefore, the genetic changes and reductions in microbial diversity that occur in older adults [51,52], might explain why the aforementioned [14,48] found a beneficial effect of MC. Whereas, in the current study middle-aged adults likely have higher numbers and diversity of many protective microbial species [53,54], hence less amenable to these changes [25]. Other potential reasons for these discrepancies could be due to inter-individual differences in the response or initial vascular function of the cohort. A recent review of the factors that influence the efficacy of anthocyanins on BP regulation highlighted that baseline BP was an important factor, with changes only evident in those with elevated initial BP [55]. Despite recruiting middle-aged individuals with additional risk factors for CVD, the participants in the current study were either pre-symptomatic or had controlled hypertension, thus had BP readings within the normal range whereas the older adults had higher BP at baseline. Moreover, the data in the present study is in line with others that longer tart cherry supplementation does not influence resting SBP in normotensive individuals [11,56], even those with increased CVD risk [12,57], suggesting the latter reason is the most probable reason for disparity between studies.

Endothelial dysfunction and arterial stiffness are indicators of subclinical atherosclerosis and precede hypertension, thus are a major risk factors for CVD [58], but also modifiable by diet [3]. Here we found no noteworthy changes in FMD, PWV or PWA. However, it should be acknowledged that the participants in the current study were on medications and these could have had an influence on these variables and although included in the statistical analysis could have confounded the results. Nonetheless, according to the HSE [59] nearly half (48%) of adults in England are regularly taking at least one prescription drug therefore the cohort in this study has general applicability to the wider population. Importantly, our findings are consistent with the literature, for example, Lynn and colleagues [11] reported no effect on brachial-knee PWV in healthy middle-aged adults, following 6-week supplementation with 30 ml MC concentrate in an open-labelled randomised controlled trial. Similarly, Johnson et al. [27] reported no effect of 12-week bi-daily MC consumption on A1x when corrected for a HR of 75 beats/min or PWV in

322

323

324

325

326

327

328

329

330

331

332

333

334

335

336

337

338

339

340

341

342

343

344

345

346

347

348

349

350

351

352

353

354

355

356

357

358

359

360

361

362

363

364

365

366

367

368

369

370

371

372

373

374

375

individuals with metabolic syndrome. Moreover, Aboo-Bakker et al. [26] demonstrated that 4-week supplementation of MC powder (256 mg/day anthocyanins split into two doses, morning and evening) did not improve resting FMD, but did restore FMD and enhance recovery of plasma nitrite following occlusion induced ischemia reperfusion. Likewise, in clinical populations with greater impairment to vascular function, anthocyanin-rich foods have been shown to improve these parameters [60-62]. Therefore, while early intervention in at-risk individuals (such as those in the current study) remains a research priority in reversing or reducing the disease risk trajectory preventing CVD, it is plausible that improvements following MC intake in these individuals might require more longitudinal observations. Those with pathological conditions might benefit more from MC supplementation due to the systemic pro-inflammatory and pro-oxidative state associated with these [63], likewise those with hypertension or other cardiovascular dysfunction might also benefit more, and hence future work could focus on these sorts of pathology to examine this idea.

The current study also identified no changes in markers of metabolic health or inflammation following the intervention (Table 4). These findings are not alone, given that tart cherries have failed to influence cholesterol [11], insulin concentrations/resistance [64] and markers of systemic inflammation [25] in similar populations. In the current study the levels of these markers before the intervention were all within normal ranges and thus, similar to BP, less likely to benefit from an intervention, relative to those with elevated baseline values, i.e. older individuals, those with metabolic or inflammatory conditions [12,14,15,65]. Notably, despite the relatively high sugar content of the concentrate and subsequent increase in carbohydrate intake over the intervention there were no deleterious influence on these outcomes in response to the additional glycaemic stress [66]. Moreover, there is epidemiological evidence that suggests higher intake of dietary anthocyanins is associated with reduced inflammation, insulin resistance, risk of type 2 diabetes and CVD [17,67,68], therefore again we cannot rule out the influence of longer study durations.

It should be acknowledged that marked effect of the intervention on body composition in both groups. After 3 months both body mass and BMI were higher in the placebo group, whereas fat percentage was higher in the cherry group and fat mass had increased in both groups (Table 2). In the current study the MC concentrate was given as an adjunct to the diet, as previous studies had suggested little influence on body composition [12,27]. A limitation of the present study is that dietary records and IPAQ were only collected at the beginning and end of the study as opposed to throughout, although total energy intake, physical activity or exercise capacity did not appear to change, we cannot rule out that changes may be seasonal and un-related to the intervention [69]. However, future studies should adjust diet to accommodate calories from juice, particularly since interventions demonstrate that energy from beverages leads to little dietary compensation and results in weight gain because beverages possess poor satiating properties compared to their solid equivalents [70]. Moreover, changes in body composition following MC intake are not in isolation. For example, Chai and colleagues [14] reported a higher BMI (1.06 kg/m²) in those consuming MC concentrate for 12 weeks. More recently, Dodier, *et al.* [71] reported an increase in body mass and BMI following the intake of 240 ml/day of MC for 90 days, warranting careful consideration in future research designs.

Due to the low number of adverse events, good compliance levels reported and no effect on quality-of-life indices, it is reasonable to suggest that cherry juice is a safe and tolerable intervention, although future studies should consider dietary modification to accommodate for the increase in Kcal from the juice. This study has several strengths such that it was successfully blinded, sufficiently powered, of a longer-term duration and well controlled compared to other studies of a similar nature [11,14,15,63,72]. However, there are other limitations that warrant discussion. Firstly, compliance to the intervention was self-reported; as these were free living adults, there was no control over whether they adhered to the intervention, how they stored the concentrate or when they consumed the

376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429

MC. Secondly, anthocyanin content is reported based on previous analysis which was conducted in our laboratory, but batch-to-batch variation can naturally occur due to growing conditions (e.g. soil, use of fertiliser, time of year, weather), storage, and processing and the lack of specific analytics [73] in the current study needs to be considered while interpreting the findings. Lastly, BP was measured in the laboratory which was shown to be reliable, but 24-hour ambulatory BP could be more advantageous in establishing small changes in BP following MC supplementation [46] due to the large number of readings and avoidance of ‘white-coat hypertension’.

In conclusion the current study found no effect of 3-month MC concentrate on vascular function and metabolic health indices. Given the current interest in the facilitation of healthy aging, investigation into the intake of anthocyanin-rich foods such as MC in pre-symptomatic and at-risk individuals are important as a means to preserve cardiometabolic health, but more longitudinal observations might be required. Future studies should consider utilising populations with greater risk factors and impairments in vascular and metabolic function at baseline, which provide a suitable population to examine the potential efficacy of foods rich in these compounds.

Author Contributions: RK: KMK, JKL and GH: conceived and designed the research; RK: conducted the research; RK and GH analyzed and interpreted the data; RK and GH: drafted the manuscript and had primary responsibility for the final content; and all authors: read and approved the final manuscript.

Funding: This study was supported by Northumbria University and the Cherry Research Committee, a not-for-profit organisation, for a Collaborative Doctoral Research Studentship Scheme. The external funders had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

Institutional Review Board Statement: This study was conducted according to the guidelines of the Declaration of Helsinki. Ethical approval for the trial was obtained from the University of Northumbria, Department of Sport, Exercise and Rehabilitation (number 4240; approved January 2018).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Anonymised data described in the manuscript, code book, and analytic code will be made available upon reasonable request to the Principal Investigator.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. WHO. *World health statistics 2019: monitoring health for the SDGs sustainable development goals*; World Health Organization: 2019.
2. Danaei, G.; Lu, Y.; Singh, G.; Stevens, G.; Cowan, M.; Farzadfar, F.; Lin, J.; Finucane, M.; Rao, M.; Khang, Y. Cardiovascular disease, chronic kidney disease, and diabetes mortality burden of cardiometabolic risk factors from 1980 to 2010: a comparative risk assessment. *The Lancet* **2014**, *2*, 634-647.
3. Houston, M. The role of noninvasive cardiovascular testing, applied clinical nutrition and nutritional supplements in the prevention and treatment of coronary heart disease. *Therapeutic advances in cardiovascular disease* **2018**, *12*, 85-108.
4. Seals, D.R.; Jablonski, K.L.; Donato, A.J. Aging and vascular endothelial function in humans. *Clinical science* **2011**, *120*, 357-375.
5. Pandey, A.; Patel, M.R.; Willis, B.; Gao, A.; Leonard, D.; Das, S.R.; Defina, L.; Berry, J.D. Association between midlife cardiorespiratory fitness and risk of stroke: The Cooper Center Longitudinal Study. *Stroke* **2016**, *47*, 1720-1726.
6. Samieri, C.; Sun, Q.; Townsend, M.K.; Rimm, E.B.; Grodstein, F. Dietary flavonoid intake at midlife and healthy aging in women. *The American journal of clinical nutrition* **2014**, *100*, 1489-1497.

7. King, D.E.; Mainous III, A.G.; Geesey, M.E. Turning back the clock: adopting a healthy lifestyle in middle age. *The American journal of medicine* **2007**, *120*, 598-603. 476
477
8. Allen, N.; Berry, J.D.; Ning, H.; Van Horn, L.; Dyer, A.; Lloyd-Jones, D.M. Impact of blood pressure and blood pressure change during middle age on the remaining lifetime risk for cardiovascular disease: the cardiovascular lifetime risk pooling project. *Circulation* **2012**, *125*, 37-44. 478
479
480
9. Odegaard, A.O.; Jacobs, D.R.; Sanchez, O.A.; Goff, D.C.; Reiner, A.P.; Gross, M.D. Oxidative stress, inflammation, endothelial dysfunction and incidence of type 2 diabetes. *Cardiovascular diabetology* **2016**, *15*, 1-12. 481
482
10. Wallace, T.; Slavin, M.; Frankenfeld, C. Systematic review of anthocyanins and markers of cardiovascular disease. *Nutrients* **2016**, *8*, 32. 483
484
11. Lynn, A.; Mathew, S.; Moore, C.T.; Russell, J.; Robinson, E.; Soumpasi, V.; Barker, M.E. Effect of a tart cherry juice supplement on arterial stiffness and inflammation in healthy adults: a randomised controlled trial. *Plant foods for human nutrition (Dordrecht, Netherlands)* **2014**, *69*, 122-127, doi:<http://dx.doi.org/10.1007/s11130-014-0409-x>. 485
486
487
12. Martin, K.R.; Burrell, L.; Bopp, J. Authentic tart cherry juice reduces markers of inflammation in overweight and obese subjects: a randomized, crossover pilot study. *Food & function* **2018**, *9*, 5290-5300. 488
489
13. Keane, K.M.; George, T.W.; Constantinou, C.L.; Brown, M.A.; Clifford, T.; Howatson, G. Effects of Montmorency tart cherry (*Prunus Cerasus* L.) consumption on vascular function in men with early hypertension. *The American journal of clinical nutrition* **2016**, *103*, 1531-1539, doi:<http://dx.doi.org/10.3945/ajcn.115.123869>. 490
491
492
14. Chai, S.C.; Davis, K.; Wright, R.S.; Kuczmarski, M.F.; Zhang, Z. Impact of tart cherry juice on systolic blood pressure and low-density lipoprotein cholesterol in older adults: a randomized controlled trial. *Food & function* **2018**. 493
494
15. Desai, T.; Roberts, M.; Bottoms, L. Effects of Montmorency tart cherry supplementation on cardio-metabolic markers in metabolic syndrome participants: A pilot study. *Journal of Functional Foods* **2019**, *57*, 286-298. 495
496
16. Kirakosyan, A.; Seymour, E.; Llanes, D.E.U.; Kaufman, P.B.; Bolling, S.F. Chemical profile and antioxidant capacities of tart cherry products. *Food Chem.* **2009**, *115*, 20-25. 497
498
17. Kimble, R.; Keane, K.M.; Lodge, J.K.; Howatson, G. Dietary intake of anthocyanins and risk of cardiovascular disease: A systematic review and meta-analysis of prospective cohort studies. *Crit. Rev. Food Sci. Nutr.* **2018**, 1-12. 499
500
18. Tresserra-Rimbau, A.; Rimm, E.B.; Medina-Remón, A.; Martínez-González, M.A.; De la Torre, R.; Corella, D.; Salas-Salvadó, J.; Gómez-Gracia, E.; Lapetra, J.; Arós, F. Inverse association between habitual polyphenol intake and incidence of cardiovascular events in the PREDIMED study. *Nutrition, Metabolism and Cardiovascular Diseases* **2014**, *24*, 639-647. 501
502
503
19. Adriouch, S.; Lampuré, A.; Nechba, A.; Baudry, J.; Assmann, K.; Kesse-Guyot, E.; Hercberg, S.; Scalbert, A.; Touvier, M.; Fezeu, L. Prospective Association between Total and Specific Dietary Polyphenol Intakes and Cardiovascular Disease Risk in the Nutrinet-Santé French Cohort. *Nutrients* **2018**, *10*, 1587. 504
505
506
20. Igwe, E.; Charlton, K.; Probst, Y. Usual dietary anthocyanin intake, sources and their association with blood pressure in a representative sample of Australian adults. *Journal of Human Nutrition and Dietetics* **2019**. 507
508
21. Keane, K.M.; Bell, P.G.; Lodge, J.K.; Constantinou, C.L.; Jenkinson, S.E.; Bass, R.; Howatson, G. Phytochemical uptake following human consumption of Montmorency tart cherry (*L. Prunus cerasus*) and influence of phenolic acids on vascular smooth muscle cells in vitro. *European journal of nutrition* **2016**, *55*, 1695-1705, doi:<http://dx.doi.org/10.1007/s00394-015-0988-9>. 509
510
511
512
22. Edwards, M.; Czank, C.; Woodward, G.M.; Cassidy, A.; Kay, C.D. Phenolic metabolites of anthocyanins modulate mechanisms of endothelial function. *J. Agric. Food Chem.* **2015**, *63*, 2423-2431. 513
514
23. Rodriguez-Mateos, A.; Ista, G.; Boschek, L.; Feliciano, R.P.; Mills, C.E.; Boby, C.; Gomez-Alonso, S.; Milenkovic, D.; Heiss, C. Circulating anthocyanin metabolites mediate vascular benefits of blueberries: insights from randomized controlled trials, metabolomics, and nutrigenomics. *The Journals of Gerontology: Series A* **2019**. 515
516
517

24. Kirakosyan, A.; Mitchell Seymour, E.; Noon, K.R.; Urcuyo Llanes, D.E.; Kaufman, P.B.; Warber, S.L.; Bolling, S.F. Interactions of antioxidants isolated from tart cherry (*Prunus cerasus*) fruits. *Food Chem.* **2010**, *122*, 78–83, doi:10.1016/j.foodchem.2010.02.017. 518
519
520
25. Lear, R.; O'Leary, M.; O'Brien Andersen, L.; Holt, C.C.; Stensvold, C.R.; van der Giezen, M.; Bowtell, J.L. Tart Cherry Concentrate Does Not Alter the Gut Microbiome, Glycaemic Control or Systemic Inflammation in a Middle-Aged Population. *Nutrients* **2019**, *11*, 1063. 521
522
523
26. Aboo-Bakkar, Z.; Fulford, J.; Gates, P.E.; Jackman, S.R.; Jones, A.M.; Bond, B.; Bowtell, J.L. Montmorency cherry supplementation attenuates vascular dysfunction induced by prolonged forearm occlusion in overweight middle-aged men. *J. Appl. Physiol.* **2018**. 524
525
526
27. Johnson, S.A.; Navaei, N.; Pourafshar, S.; Jaime, S.J.; Akhavan, N.S.; Alvarez-Alvarado, S.; Proaño, G.V.; Litwin, N.S.; Clark, E.A.; Foley, E.M. Effects of montmorency tart cherry juice consumption on cardiometabolic biomarkers in adults with metabolic syndrome: a randomized controlled pilot trial. *J. Med. Food* **2020**, *23*, 1238–1247. 527
528
529
28. Ekoé, J.-M.; Punthakee, Z.; Ransom, T.; Prebtani, A.P.; Goldenberg, R. Screening for type 1 and type 2 diabetes. *Canadian journal of diabetes* **2013**, *37*, S12–S15. 530
531
29. Lindström, J.; Tuomilehto, J. The diabetes risk score: a practical tool to predict type 2 diabetes risk. *Diabetes care* **2003**, *26*, 725–731. 532
533
30. Keane, K.M.; Haskell-Ramsay, C.F.; Veasey, R.C.; Howatson, G. Montmorency Tart cherries (*Prunus cerasus* L.) modulate vascular function acutely, in the absence of improvement in cognitive performance. *The British journal of nutrition* **2016**, *116*, 1935–1944, doi:<http://dx.doi.org/10.1017/S0007114516004177>. 534
535
536
31. Neveu, V.; Perez-Jiménez, J.; Vos, F.; Crespy, V.; Du Chaffaut, L.; Mennen, L.; Knox, C.; Eisner, R.; Cruz, J.; Wishart, D. Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *Database* **2010**, *2010*. 537
538
32. Losso, J.N.; Finley, J.W.; Karki, N.; Liu, A.G.; Prudente, A.; Tipton, R.; Yu, Y.; Greenway, F.L. Pilot Study of the Tart Cherry Juice for the Treatment of Insomnia and Investigation of Mechanisms. *American journal of therapeutics* **2017**, doi:<http://dx.doi.org/10.1097/MJT.0000000000000584>, doi:<http://dx.doi.org/10.1097/MJT.0000000000000584>. 539
540
541
33. Ware, J.E.; Kosinski, M. Interpreting SF&-36 summary health measures: A response. *Quality of life research* **2001**, *10*, 405–413. 542
34. Noonan, V.; Dean, E. Submaximal exercise testing: clinical application and interpretation. *Physical therapy* **2000**, *80*, 782–807. 543
35. Astrand. Aerobic Work Capacity in Men and Women: With Special Reference to Age. Norstedt: 1960. 544
36. Kimble, R.; Keane, K.; Lodge, J.; Howatson, G. Methodological Considerations for a Vascular Function Test Battery. *International journal of sports medicine* **2019**, *40*, 601–608. 545
546
37. O'Brien, E.; Asmar, R.; Beilin, L.; Imai, Y.; Mallion, J.-M.; Mancia, G.; Mengden, T.; Myers, M.; Padfield, P.; Palatini, P. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. *Journal of hypertension* **2003**, *21*, 821–848. 547
548
549
38. Pickering, T.G.; Hall, J.E.; Appel, L.J.; Falkner, B.E.; Graves, J.; Hill, M.N.; Jones, D.W.; Kurtz, T.; Sheps, S.G.; Roccella, E.J. Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. *Circulation* **2005**, *111*, 697–716. 550
551
552
553
39. Pauca, A.L.; O'rouke, M.F.; Kon, N.D. Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure waveform. *Hypertension* **2001**, *38*, 932–937. 554
555
40. Wilkinson, I.B.; MacCallum, H.; Flint, L.; Cockcroft, J.R.; Newby, D.E.; Webb, D.J. The influence of heart rate on augmentation index and central arterial pressure in humans. *The Journal of physiology* **2000**, *525*, 263–270. 556
557
41. Friedewald, W.T.; Levy, R.I.; Fredrickson, D.S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **1972**, *18*, 499–502. 558
559

42. Matthews, D.; Hosker, J.; Rudenski, A.; Naylor, B.; Treacher, D.; Turner, R. Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **1985**, *28*, 412–419. 560
43. Makai, P.; Int'Hout, J.; Deinum, J.; Jenniskens, K.; van der Wilt, G.J. A network meta-analysis of clinical management strategies for treatment-resistant hypertension: making optimal use of the evidence. *J. Gen. Intern. Med.* **2017**, *32*, 921–930. 561
44. Vickers, A.J.; Altman, D.G. Analysing controlled trials with baseline and follow up measurements. *BMJ* **2001**, *323*, 1123–1124, doi:10.1136/bmj.323.7321.1123. 562
45. Seymour, E.M.; Warber, S.M.; Kirakosyan, A.; Noon, K.R.; Gillespie, B.; Uhley, V.E.; Wunder, J.; Urcuyo, D.E.; Kaufman, P.B.; Bolling, S.F. Anthocyanin pharmacokinetics and dose-dependent plasma antioxidant pharmacodynamics following whole tart cherry intake in healthy humans. *Journal of Functional Foods* **2014**, *11*, 509–516, doi:10.1016/j.jff.2014.08.007. 563
46. Desai, T.; Roberts, M.; Bottoms, L. Effects of short-term continuous Montmorency tart cherry juice supplementation in participants with metabolic syndrome. *European Journal of Nutrition* **2020**, 1–17. 564
47. Larochelle, P.; du Souich, P.; Hamet, P.; Larocque, P.; Armstrong, J. Prazosin plasma concentration and blood pressure reduction. *Hypertension* **1982**, *4*, 93–101. 565
48. Kent, K.; Charlton, K.; Roodenrys, S.; Batterham, M.; Potter, J.; Traynor, V.; Gilbert, H.; Morgan, O.; Richards, R. Consumption of anthocyanin-rich cherry juice for 12 weeks improves memory and cognition in older adults with mild-to-moderate dementia. *European journal of nutrition* **2017**, *56*, 333–341, doi:<http://dx.doi.org/10.1007/s00394-015-1083-y>. 566
49. Ista, G.; Wood, E.; Le Sayec, M.; Rawlings, C.; Yoon, J.; Dandavate, V.; Cera, D.; Rampelli, S.; Costabile, A.; Fromentin, E. Effects of aronia berry (poly) phenols on vascular function and gut microbiota: a double-blind randomized controlled trial in adult men. *The American journal of clinical nutrition* **2019**. 567
50. Krga, I.; Milenkovic, D.; Morand, C.; Monfoulet, L.-E. An update on the role of nutrigenomic modulations in mediating the cardiovascular protective effect of fruit polyphenols. *Food & function* **2016**, *7*, 3656–3676. 568
51. Filosa, S.; Di Meo, F.; Crispi, S. Polyphenols-gut microbiota interplay and brain neuromodulation. *Neural regeneration research* **2018**, *13*, 2055. 569
52. Barrera-Reyes, P.K.; de Lara, J.C.-F.; González-Soto, M.; Tejero, M.E. Effects of Cocoa-Derived Polyphenols on Cognitive Function in Humans. Systematic Review and Analysis of Methodological Aspects. *Plant Foods for Human Nutrition* **2020**, 1–11. 570
53. Mariat, D.; Firmesse, O.; Levenez, F.; Guimaraes, V.; Sokol, H.; Doré, J.; Corthier, G.; Furet, J. The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiol.* **2009**, *9*, 123. 571
54. de la Cuesta-Zuluaga, J.; Kelley, S.T.; Chen, Y.; Escobar, J.S.; Mueller, N.T.; Ley, R.E.; McDonald, D.; Huang, S.; Swafford, A.D.; Knight, R. Age- and sex-dependent patterns of gut microbial diversity in human adults. *Msystems* **2019**, *4*. 572
55. Vendrame, S.; Klimis-Zacas, D. Potential Factors Influencing the Effects of Anthocyanins on Blood Pressure Regulation in Humans: A Review. *Nutrients* **2019**, *11*, 1431. 573
56. Desai, T.; Bottoms, L.; Roberts, M. The effects of Montmorency tart cherry juice supplementation and FATMAX exercise on fat oxidation rates and cardio-metabolic markers in healthy humans. *Eur. J. Appl. Physiol.* **2018**, *118*, 2523–2539. 574
57. Johnson, S.A.; Navaei, N.; Pourafshar, S.; Jaime, S.J.; Akhavan, N.S.; Alvarez-Alvarado, S.; Proaño, G.V.; Litwin, N.S.; Clark, E.A.; Foley, E.M. Effects of Montmorency Tart Cherry Juice Consumption on Cardiometabolic Biomarkers in Adults with Metabolic Syndrome: A Randomized Controlled Pilot Trial. *Journal of Medicinal Food* **2020**. 575
58. Mattace-Raso, F.U.; van der Cammen, T.J.; Hofman, A.; van Popele, N.M.; Bos, M.L.; Schalekamp, M.A.; Asmar, R.; Reneman, R.S.; Hoeks, A.P.; Breteler, M.M. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. *Circulation* **2006**, *113*, 657–663. 576
59. Osborne, B.; Cooper, V. Health Survey for England 2017 Adult health related behaviours. Available online: <https://digital.nhs.uk/data-and-information/publications/statistical/health-survey-for-england/2017> (accessed on 2019). 577

60. Dohadwala, M.M.; Holbrook, M.; Hamburg, N.M.; Shenouda, S.M.; Chung, W.B.; Titas, M.; Kluge, M.A.; Wang, N.; Palmisano, J.; Milbury, P.E., et al. Effects of cranberry juice consumption on vascular function in patients with coronary artery disease. *The American journal of clinical nutrition* **2011**, *93*, 934-940, doi:10.3945/ajcn.110.004242. 602
603
604
61. Zhu, Y.; Xia, M.; Yang, Y.; Liu, F.; Li, Z.; Hao, Y.; Mi, M.; Jin, T.; Ling, W. Purified anthocyanin supplementation improves endothelial function via NO-cGMP activation in hypercholesterolemic individuals. *Clin. Chem.* **2011**, *57*, 1524-1533. 605
606
62. Chou, E.J.; Keevil, J.G.; Aeschlimann, S.; Wiebe, D.A.; Folts, J.D.; Stein, J.H. Effect of ingestion of purple grape juice on endothelial function in patients with coronary heart disease. *American Journal of Cardiology* **2001**, *88*, 553-555. 607
608
63. Ataie-Jafari, A.; Hosseini, S.; Karimi, F.; Pajouhi, M. Effects of sour cherry juice on blood glucose and some cardiovascular risk factors improvements in diabetic women: A pilot study. *Nutr. Food Sci.* **2008**, *38*, 355-360. 609
610
64. Martin, K.R.; Coles, K.M. Consumption of 100% Tart Cherry Juice Reduces Serum Urate in Overweight and Obese Adults. *Current Developments in Nutrition* **2019**, *3*, doi:10.1093/cdn/nzz011. 611
612
65. Schumacher, H.R.; Pullman-Moore, S.; Gupta, S.R.; Dinnella, J.E.; Kim, R.; McHugh, M.P. Randomized double-blind crossover study of the efficacy of a tart cherry juice blend in treatment of osteoarthritis (OA) of the knee. *Osteoarthritis and cartilage* **2013**, *21*, 1035-1041, doi:<http://dx.doi.org/10.1016/j.joca.2013.05.009>. 613
614
615
66. Vendrame, S.; Del Bo, C.; Ciappellano, S.; Riso, P.; Klimis-Zacas, D. Berry fruit consumption and metabolic syndrome. *Antioxidants* **2016**, *5*, 34. 616
617
67. Wedick, N.M.; Pan, A.; Cassidy, A.; Rimm, E.B.; Sampson, L.; Rosner, B.; Willett, W.; Hu, F.B.; Sun, Q.; van Dam, R.M. Dietary flavonoid intakes and risk of type 2 diabetes in US men and women. *The American journal of clinical nutrition* **2012**, *95*, 925-933. 618
619
620
68. Jennings, A.; Welch, A.A.; Spector, T.; Macgregor, A.; Cassidy, A. Intakes of anthocyanins and flavones are associated with biomarkers of insulin resistance and inflammation in women. *The Journal of nutrition* **2014**, *144*, 202-208. 621
622
69. Ma, Y.; Olendzki, B.C.; Li, W.; Hafner, A.R.; Chiriboga, D.; Hebert, J.R.; Campbell, M.; Sarnie, M.; Ockene, I.S. Seasonal variation in food intake, physical activity, and body weight in a predominantly overweight population. *European journal of clinical nutrition* **2006**, *60*, 519-528. 623
624
625
70. DiMeglio, D.P.; Mattes, R.D. Liquid versus solid carbohydrate: effects on food intake and body weight. *Int. J. Obesity* **2000**, *24*, 794-800. 626
627
71. Dodier, T.; Anderson, K.L.; Bothwell, J.; Hermann, J.; Lucas, E.A.; Smith, B.J. US Montmorency Tart Cherry Juice Decreases Bone Resorption in Women Aged 65–80 Years. *Nutrients* **2021**, *13*, 544. 628
629
72. Johnson, S.A.; Figueroa, A.; Navaei, N.; Wong, A.; Kalfon, R.; Ormsbee, L.T.; Feresin, R.G.; Elam, M.L.; Hooshmand, S.; Payton, M.E. Daily blueberry consumption improves blood pressure and arterial stiffness in postmenopausal women with pre-and stage 1-hypertension: a randomized, double-blind, placebo-controlled clinical trial. *Journal of the Academy of Nutrition and Dietetics* **2015**, *115*, 369-377. 630
631
632
633
73. Sabou, V.R.; O'Leary, M.F.; Liu, Y.; Brown, P.N.; Murch, S.; Bowtell, J.L. Review of Analytical Methods and Reporting of the Polyphenol Content of Tart Cherry Supplements in Human Supplementation Studies Investigating Health and Exercise Performance Effects: Recommendations for Good Practice. *Frontiers in Nutrition* **2021**, *8*, doi:10.3389/fnut.2021.652094. 634
635
636
637
- 1.