

Central Lancashire Online Knowledge (CLoK)

Title	Effects of Montmorency tart cherry and blueberry juice on cardiometabolic and other health related outcomes: a 3-arm placebo randomized controlled trial.
Type	Article
URL	https://clock.uclan.ac.uk/id/eprint/41714/
DOI	
Date	2022
Citation	Sinclair, Jonathan Kenneth, Bottoms, Lindsay, Dillon, Stephanie, Allan, Robert, Shadwell, Gareth and Butters, Bobbie (2022) Effects of Montmorency tart cherry and blueberry juice on cardiometabolic and other health related outcomes: a 3-arm placebo randomized controlled trial. International Journal of Environmental Research and Public Health.
Creators	Sinclair, Jonathan Kenneth, Bottoms, Lindsay, Dillon, Stephanie, Allan, Robert, Shadwell, Gareth and Butters, Bobbie

It is advisable to refer to the publisher's version if you intend to cite from the work.

For information about Research at UCLan please go to <http://www.uclan.ac.uk/research/>

All outputs in CLoK are protected by Intellectual Property Rights law, including Copyright law. Copyright, IPR and Moral Rights for the works on this site are retained by the individual authors and/or other copyright owners. Terms and conditions for use of this material are defined in the <http://clock.uclan.ac.uk/policies/>



Article

Effects of Montmorency tart cherry and blueberry juice on cardiometabolic and other health related outcomes: a 3-arm placebo randomized controlled trial.

Jonathan Sinclair ^{1,*}, Lindsay Bottoms ², Stephanie Dillon ¹, Robert Allan ¹, Gareth Shadwell ¹ and Bobbie Butters ¹

¹ Research Centre for Applied Sport, Physical Activity and Performance, School of Sport & Health Sciences, Faculty of Allied Health and Wellbeing, University of Central Lancashire, Lancashire, UK.

² Centre for Research in Psychology and Sport Sciences, School of Life and Medical Sciences, University of Hertfordshire, Hertfordshire, UK.

* jksinclair@uclan.ac.uk

Abstract: The current study aimed to investigate the influence of tart cherry and blueberry juices on cardiometabolic and other health indices following a 20-day supplementation period. Forty-five adults were randomly assigned to receive tart cherry, blueberry, or a placebo; of which they drank 60 mL per day for 20-days. The primary outcome; systolic blood pressure and secondary measures; anthropometric, energy expenditure, substrate oxidation, haematological, diastolic blood pressure/resting heart rate, psychological wellbeing and sleep efficacy were measured before and after the intervention. There were no statistically significant differences ($P>0.05$) for systolic blood pressure, however total and LDL cholesterol were significantly improved with blueberry intake (pre: total cholesterol=4.36mmol/L and LDL cholesterol=2.71mmol/L & post: total cholesterol=3.79mmol/L and LDL cholesterol=2.23mmol/L) compared to placebo (pre: total cholesterol=4.01mmol/L and LDL cholesterol=2.45mmol/L & post: total cholesterol=4.34mmol/L and LDL cholesterol=2.67mmol/L). Furthermore, psychological wellbeing indices measured using the Beck Depression Inventory, State Trait Anxiety Inventory and COOP WONCA improved statistically in the blueberry arm compared to placebo. Given the clear association between lipid concentrations and the risk of cardiovascular disease as well as the importance of psychological wellbeing to health-related quality of life, this investigation indicates that it could be a useful tool to help in managing cardiovascular diseases.

Keywords: tart cherry; blueberry; cardiovascular disease; blood pressure; metabolic health

Citation: Lastname, F.; Lastname, F.; Lastname, F. Title. *Int. J. Environ. Res. Public Health* **2022**, *19*, x. <https://doi.org/10.3390/xxxxx>

Academic Editor: Firstname Lastname

Received: date
Accepted: date
Published: date

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



Copyright: © 2022 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Cardiometabolic disease is now the principal cause of global mortality and healthcare expenditure [1]. Cardiometabolic syndrome itself is characterized by a range of symptoms including hypertension, insulin resistance, atherogenic dyslipidemia, low high-density lipoproteins, high triglycerides, high adiposity, high body mass index, large waist to hip ratio and poor glucose regulation [2, 3]. Within the epidemiological literature, distinct pathophysiological markers of oxidative stress, nitric oxide and inflammation have been cited as being the mechanistic indicators associated with the clinical presentation of cardiometabolic disease [4, 5].

Pharmaceutical interventions represent the predominant treatment modalities for cardiometabolic conditions [6]. However, whilst these medications are efficacious in regards to the management of cardiometabolic disease, their cost-effectiveness remains ambiguous [7] and significant negative side-effects, remain common [8]. Globally, it has been documented that 84% of adults over the age of 57 are prescribed at least one medication

per day [9]. As such, it is clear that further natural and cost-effective remedies are required for cardiometabolic disease management and prevention [3].

Dietary practices are recognized as the most effective natural approach for the treatment of cardiometabolic disease, such that most national/ international medical organizations advocate improved nutrition for the prevention and management of this condition [10]. There is therefore a clear rationale for the implementation of dietary over pharmaceutical interventions; and indeed Chiva-Blanch et al., [11] propose that such approaches are likely to be more cost-effective and safer for the treatment and prevention of the metabolic diseases. Diets rich in fruits and vegetables have been shown to attenuate the risk from cardiometabolic disease [12], although maintaining such approaches over a sustained duration has been shown to be difficult to accomplish [13]. Therefore, dietary supplements represent a potentially more appealing treatment and prevention modality.

Anthocyanins are abundant in dark coloured fruit and vegetable groups [14], and it is proposed that they may be able to confer significant improvements in cardiometabolic health [15]. Montmorency tart cherries, blueberries, strawberries, cranberries and blackcurrants [16] in particular have been shown to possess high levels of anthocyanins [17], although the majority of peer-reviewed literature has focused on tart cherries. Supplementation of anthocyanin rich tart cherries has been shown to improve oxidative stress [18, 19] and inflammation [19–21], and blackcurrant supplementation was also shown to enhance fat oxidation rates [22]. Improved fat oxidation during rest and physical activity is linked to long-term changes in body mass and composition allied to improvements in insulin sensitivity [23]. Therefore, an increased capacity to oxidize fat at rest and during moderate physical activity, initiated via anthocyanin rich supplementation, may be advantageous for yielding improvements in body composition and insulin control, pertinent to cardiometabolic health. Importantly, the aforementioned anti-inflammatory, anti-oxidative and substrate trafficking effects, mediated through supplementation of anthocyanin rich fruits, appear to conveniently target the underlying chronic low-grade inflammation, pro-oxidant and lipid attenuating status that is central to cardiometabolic disease pathophysiology [24].

However, the findings from parallel randomized controlled trials exploring the effects of anthocyanin rich fruit supplementation on cardiometabolic outcomes have yielded equivocal findings. Some studies exploring the effects of Montmorency tart cherry juice supplementation have shown no effect on cardiometabolic indices of blood pressure, triglycerides, insulin tolerance or cholesterol [25–28] and some have revealed improvements in systolic blood pressure, total cholesterol and low-density lipoprotein (LDL) cholesterol [29–34]. Studies exploring the efficacy of other anthocyanin rich supplements present a similarly equivocal picture, with some demonstrating positive effects on cardiometabolic outcomes [35–39] and some showing no such effects [40–43].

1.1. Rationale

At the current time, there has yet to be any randomized intervention studies, comparatively examining the efficacy of different anthocyanin rich fruit supplements on cardiometabolic outcomes. With some food biochemical investigations showing that anthocyanin contents in dark fruits such as blueberries are as high or even greater than in tart cherries [17], further such investigations may be of both practical and clinical relevance.

1.2. Aim

The aim of the current study was to investigate the influence of 20-days of twice daily Montmorency tart cherry or blueberry juice supplementation on cardiometabolic and other health related indices in healthy adults compared to placebo. The primary objective of this randomized trial is to examine the influence of the tart cherry and blueberry supplements on systolic blood pressure relative to placebo. Its secondary objectives are to assess if tart cherry juice and blueberry supplementation impacted on other risk factors associated with and as a function of cardiometabolic disease.

1.3. Hypotheses

In relation to the primary outcome, it is expected that both Montmorency tart cherry and blueberry supplement groups will mediate significant reductions in systolic blood pressure compared to placebo, but no statistically significant differences will be observed between supplement groups. Furthermore, for the secondary outcomes, the Montmorency tart cherry and blueberry groups will produce improvements in cardiometabolic other health related parameters compared to placebo, but there will be no statistically significant differences between the two supplement groups.

2. Materials and Methods

2.1. Study design

This investigation represents a 20-day parallel, single-blind (blinded to participant) randomized placebo-controlled trial (Figure 1). The 20-day supplementation period was adopted in accordance with [28], and the protocol for this 3-arm randomized investigation has been previously published elsewhere [3] and is designed according to the updated guidelines for reporting parallel group randomized trials [44]. The study was registered prospectively (NCT04177238) and approved by an institutional ethical review board (HEALTH 0016).

After screening for eligibility and enrolment, participants were then randomized by a computer program (Random Allocation Software) to either 1. Montmorency tart cherry, 2. Blueberry or 3. placebo group. All experimental variables were assessed at a. baseline (pre) and b. after 20-days (post). In agreement with previous trials of cardiometabolic health, the primary outcome measure was the between-group difference in systolic blood pressure from baseline to post-intervention [27]. Secondary outcome measures were between-group differences in anthropometric, energy expenditure and substrate oxidation (during rest and moderate intensity exercise), haematological, diastolic blood pressure/resting heart rate, psychological wellbeing and sleep efficacy indices. All experimental testing took place in the morning in a ≥ 10 -hour fasted state, with participants having avoided strenuous exercise, alcohol, and nutritional supplements for 24 h and caffeine for 12 h prior to data collection [28].

2.2. Inclusion criteria:

- 18 years of age and above
- Non-smoker
- BMI < 30
- Able to give informed consent

Exclusion criteria:

- Pregnancy
- 65 years of age and above
- Diabetes or any other metabolic/ uncontrolled hypertensive conditions
- Food allergies to cherries or blueberries
- Habitual consumption of blueberries/ cherries and/or blueberry/ cherry products
- Not regularly taking medication or antioxidant supplements

2.3. Participants

Power calculations were performed for the primary outcome variable i.e. the between groups difference in systolic blood pressure. This showed that a total sample size of 45 was necessary to provide 80% power to detect a minimally important clinical difference (MCID) of 6 mmHg between groups [45], with a projected standard deviation of 5.5 mmHg in each group [46], accounting for a loss to follow up rate of 10%. Participants attended an eligibility, enrolment and familiarization session prior to the commencement of formal data collection at the University of Central Lancashire. All participants provided informed consent in written form and completed a Par-Q screening form before taking

part, in compliance with principles outlined in the declaration of Helsinki and the Oviedo Convention.

2.4. Dietary intervention

After the conclusion of their baseline data collection session, participants were provided with either Montmorency tart cherry, blueberry or placebo concentrate. Participants were required to consume 30 mL of supplementation diluted in 100 mL of water twice daily: once in the morning and again in the evening [27]. All supplementation was kept refrigerated throughout the 20-days. According to the manufacturer (ActiveEdge, UK), a 30 mL dose of Montmorency tart cherry concentrate (Energy: 102 Kcal, carbohydrates: 25 g of which sugars: 18 g, protein: 1.10 g and fibre: 2.6 g) is equivalent to approximately 320 mg of anthocyanins. Similarly, taking into account the manufacturers (ActiveEdge, UK) guidelines, a 30 mL dose of blueberry concentrate (Energy: 103 Kcal, carbohydrates: 22 g of which sugars: 22 g, protein: 0.2 g and fibre: 0.2 g) is equivalent to approximately 387 mg of anthocyanins.

Preparation of the placebo was undertaken in accordance with that outlined previously within the literature; this method of placebo preparation has been shown by previous intervention trials to provide an effective blinding strategy [47]. Placebo preparation involved mixing 100% un-flavoured maltodextrin carbs (MyProtein, UK) into drinking water using a magnetic stirrer (Stuart Scientific, UK) and stir bar (Fisher Scientific, USA). 666g of maltodextrin was added to water to create a litre of placebo concentrate, working out as 20g of maltodextrin per 30 mL serving: closely matching the Montmorency tart cherry or blueberry supplementation. Even amounts of red and black food colouring were added to match the colour of the Montmorency tart cherry concentrate and even amounts of red, blue and black colouring were utilized to match the colour of the blueberry supplement. Either cherry or blueberry flavdrops (1 mL) (MyProtein, UK) were then added to match the required flavour. Irrespective of flavour, a 30 mL dose of placebo concentrate (100 Kcal, carbohydrates 25 g of which sugars: 0 g, protein: 0 g and Fibre 0g) contained 0 mg of anthocyanins.

Throughout the study, the participants were encouraged to maintain their habitual diet and exercise routines; and asked to refrain from consuming any multivitamin, or antioxidant supplements [25]. For their post-intervention data collection session, all participants were asked to return any un-used supplementation to determine the actual amount of supplement/ placebo that was consumed (mL) and their % compliance. Furthermore, in order to explore the total quantity of supplementary ingested anthocyanins (mg), experimental average daily energy intake (Kcal/day) and supplementary average daily sugars (g/day), the amount of supplementation that was consumed was multiplied by the anthocyanin, energy and sugar contents established by the manufacturer. Finally, in order to examine blinding efficacy, each participant was asked whether they felt that they had been allocated to the supplement or placebo group at the conclusion of their post-intervention data collection session. In all three trial arms, loss to follow up was monitored, as were any adverse events.

2.5. Data collection

Laboratory visit data

All measurements were undertaken at the University of Central Lancashire's physiology laboratory and undertaken in an identical manner on two occasions i.e. baseline and post-intervention. The laboratories housed by the University of Central Lancashire are fully accredited by the British Association for Sport & Exercise Sciences, illustrating that they have undergone meticulous inspection and evidenced that; all instrumentation is well maintained in terms of reliability, validity and routine servicing, staff have the appropriate professional and vocational qualifications and that the requisite operational procedures for health and safety are met.

Anthropometric measurements

Anthropometric measures of mass (kg) and stature (m) (without shoes) were used to calculate the body mass index (BMI) (kg/m^2). Stature was measured using a stadiometer (Seca, Hamburg, Germany) and mass using weighing scales (Seca 875, Hamburg, Germany). In addition, body composition was examined using a phase-sensitive multifrequency bioelectrical impedance analysis device (Seca mBCA 515, Hamburg, Germany) [48], allowing percentage body fat (%) and fat mass (kg) to be quantified. Finally, waist circumference was measured at the midway point between the inferior margin of the last rib and the iliac crest and hip circumference around the pelvis at the point of maximum protrusion of the buttocks, without compressing the soft tissues [49]; allowing the waist-to-hip ratio to be quantified.

Energy expenditure and substrate oxidation

Respiratory gases were collected using a gas analysis system (MetaLyser 3B system, Cortex Biophysic, Leipzig, Germany). The experimental laboratory was maintained using an air-conditioning system at a fixed ambient temperature of 20 °C. To quantify resting energy expenditure and substrate oxidation, participants laid supine for a period of 20 minutes and data was extracted and averaged over the final 17 minutes [50]. Resting fat and carbohydrate oxidation rates (g/min) were quantified using the stoichiometric formulae outlined by Freyn, [51] (EQ1-2), assuming negligible protein utilization. To quantify resting metabolic rate (RMR) (kcal/day) the formula of Weir, [52] was adopted (EQ3).

$$\text{Carbohydrate (g/min)} = (4.55 \times \text{VCO}_2) - (3.21 \times \text{VO}_2) \quad [\text{EQ1}]$$

$$\text{Fat (g/min)} = (1.67 \times \text{VO}_2) - (1.67 \times \text{VCO}_2) \quad [\text{EQ2}]$$

$$\text{RMR (kcal/day)} = [(3.941 \times \text{VO}_2) + (1.1106 \times \text{VCO}_2)] \times 1440 \quad [\text{EQ3}]$$

In addition, carbohydrate and fat oxidation rates (g/min) and energy expenditure per minute (kcal/min) were also examined during moderate intensity physical activity. Participants walked on a treadmill (hp Cosmos Pulsar, Nussdorf Germany) at a velocity of 4.5 km/h for a period of 6-minutes. This walking velocity has reliably been shown to correspond to moderate exercise intensities [53]. Data was averaged over the last minute of the 6-minute test. Fat and carbohydrate oxidation rates (g/min) as well as energy expenditure (kcal/min) during the exercise test were quantified using stoichiometric formulae outlined by Jeukendrup & Wallis, [54] specifically developed for the exercise intensity examined in this study (EQ4-6).

$$\text{Carbohydrate (g/min)} = (4.21 \times \text{VCO}_2) - (2.962 \times \text{VO}_2) \quad [\text{EQ4}]$$

$$\text{Fat (g/min)} = (1.695 \times \text{VO}_2) - (1.701 \times \text{VCO}_2) \quad [\text{EQ5}]$$

$$\text{Energy expenditure (kcal/min)} = (0.550 \times \text{VCO}_2) - (4.471 \times \text{VO}_2) \quad [\text{EQ6}]$$

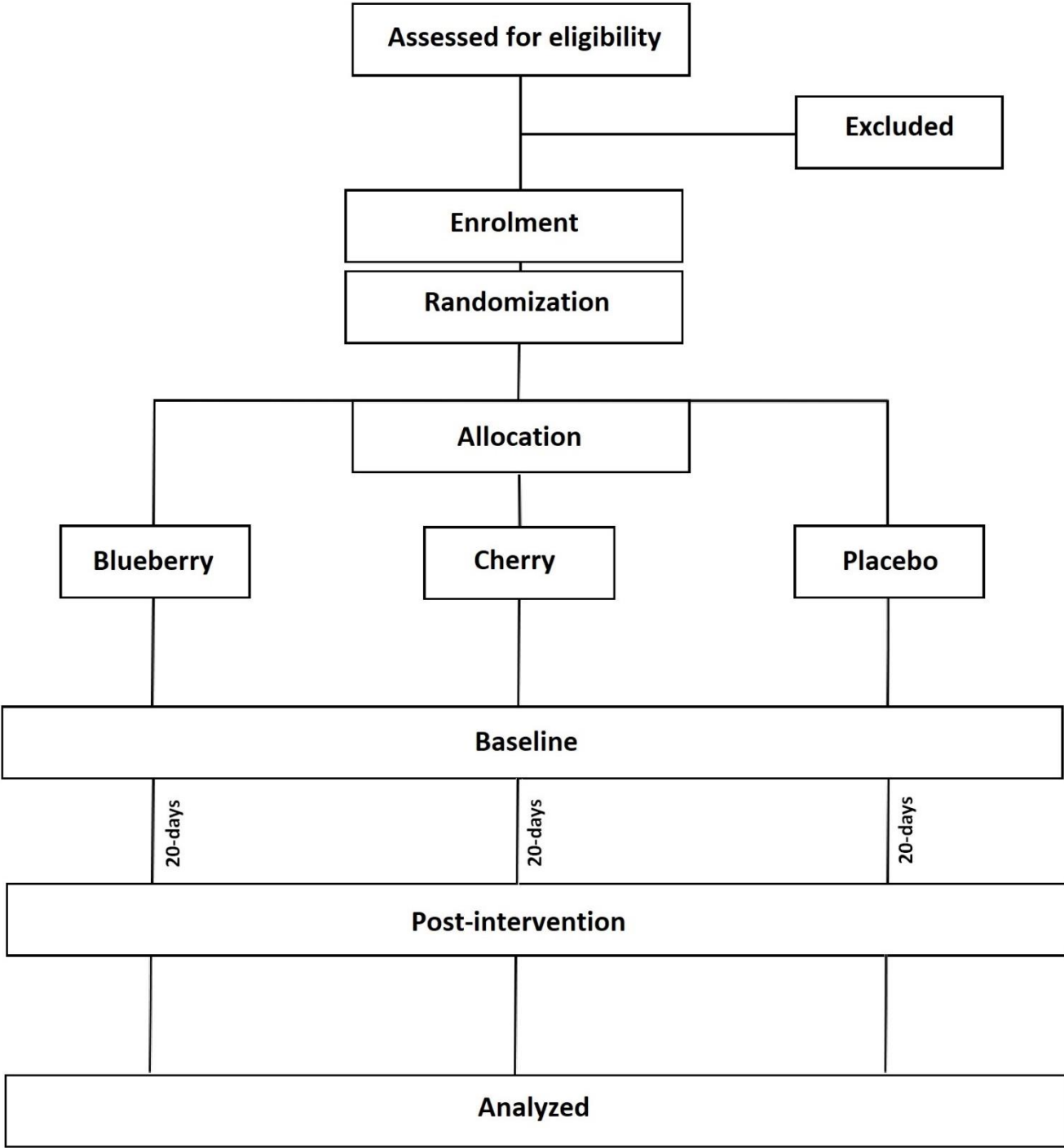


Figure 1. Consort diagram showing the study design.

Haematological testing

Capillary blood samples were also collected via finger-prick using a disposable lancet after cleaning with a 70% ethanol wipe. Capillary triglyceride, total cholesterol and glucose levels (mmol/L) were immediately obtained using three handheld analyzers (MulticareIn, Multicare Medical, USA) and capillary heamoglobin levels (g/L) using a single handheld analyzer (HemoCue, Ångelholm, Sweden). From these outcomes’ LDL cholesterol (mmol/L) was firstly quantified using the Anandaraja et al., [55] formula with total cholesterol and triglycerides as inputs. In addition, high-density lipoprotein (HDL) cholesterol (mmol/L) was also calculated by re-arranging the Chen et al., [56] equation to make HDL the product of the formulae. Both of these approaches have been shown to

have excellent similarity to their associated lipoprotein values examined using immunoassay techniques $r=0.948-0.970$ [55, 56] The ratios between total and HDL cholesterol and between LDL and HDL cholesterol levels were also determined in accordance with Millán et al., [57].

Blood pressure and resting heart rate

Blood pressure (mmHg) and resting heart rate (beats.min⁻¹) measurements were undertaken in an up-right seated position at the end of the above-described resting energy expenditure test. Both peripheral measures of systolic and diastolic blood pressure and resting heart rate were be measured via a non-invasive, automated blood pressure monitor (OMRON M2, Kyoto, Japan), adhering to the recommendations specified by the European Society of Hypertension [58]. Three readings were undertaken, each separated by a period of 1 min [59], and the mean of the last 2 readings used for analysis.

Questionnaires

Sleep quality is diminished in patients with cardiometabolic disease [60] and intake of dietary polyphenols [61] and supplementation of Montmorency tart cherry has been demonstrated to enhance sleep quality and symptoms of insomnia [62, 63]. Therefore, general sleep quality was examined using the Pittsburgh sleep quality index (PSQI) [64], daytime sleepiness using the Epworth Sleepiness Scale [65] and symptoms of insomnia via the Insomnia Severity Index [66]. These questionnaires were utilized co-operatively to provide a collective representation of sleep efficacy.

Furthermore, psychological wellbeing is lower in those with cardiometabolic disease [67] and a high intake of dietary polyphenols has been shown to enhance indices of psychological wellbeing [68]. Therefore, general psychological wellbeing was examined using the COOP WONCA questionnaire [69], depressive symptoms using the Beck Depression Inventory [65] and state/ trait anxiety with the State Trait Anxiety Inventory (STAI) [70]. Once again, these scales were utilized conjunctively to provide a collective depiction of psychological wellbeing.

2.6. Statistical analysis

All continuous experimental variables are presented as mean and standard deviations. Comparisons between participant characteristics and all experimental variables were undertaken at baseline, as were the % compliance levels, experimental anthocyanins, experimental energy intake and experimental sugars (g/ day) between the groups using linear mixed models, with group modelled as a fixed factor and random intercepts by participants. All analyses of the intervention-based data were performed using on an intention to treat basis. To determine the effects of the intervention on all of the outcome measures, differences between the three groups were examined using linear mixed models with group modelled as a fixed factor and random intercepts by participants adopted, adjusted for baseline values modelled as a continuous fixed covariate. For linear mixed models the mean difference (b), t -value and 95% confidence intervals of the difference are presented. Effects sizes for all statistically significant comparisons were quantified using partial eta squared (η^2). Blinding efficacy was examined using a one-way chi-squared (X^2) goodness of fit test. Finally, changes from baseline to 20-days in the experimental parameters were used to create binary variables i.e. improve/ didn't improve for each participant. Pearson chi-square tests of independence were also used to undertake bivariate cross-tabulation comparisons between the three trial groups, specifically to test differences in the number of participants who exhibited improvements in the experimental outcomes, the number lost to follow-up and the number of adverse outcomes in each group. Probability values for all chi-square analyses in this trial were calculated using Monte-Carlo simulation. All analyses were conducted using SPSS v27 (IBM, SPSS), and statistical significance for all analyses was accepted as the $P \leq 0.05$ level. In the interests of conciseness and clarity only experimental variables that presented with statistical significance as a function of the intervention are presented in the results section.

3. Results

3.1. Baseline characteristics

All of the experimental measurements were contrasted at baseline for the participants who completed the trial, and no significant differences between groups were found ($p=0.06$ – 0.98 – Table 1).

Table 1. Baseline characteristics of completed study participants.

	All		Placebo		Cherry		Blueberry	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	34.02	12.97	35.13	16.84	32.80	7.89	34.13	13.03
Mass (kg)	68.41	10.74	67.62	10.47	69.44	11.38	68.17	9.43
Stature (m)	1.68	0.09	1.67	0.10	1.69	0.10	1.68	0.08
BMI (kg/m ²)	24.26	2.90	23.82	2.98	24.90	2.35	24.07	3.10
Sex (m/f)	24/ 20		8/ 7		7/ 7		9/ 6	

3.2. Loss to follow up, compliance, ingested anthocyanins & adverse events

Total trial completion numbers in each group were cherry ($n=14$), placebo ($n=15$) and blueberry ($n=15$) and number of adverse effects were cherry ($n=1$), placebo ($n=0$) and blueberry ($n=0$). The chi-squared tests were non-significant ($X^2_{(2)} = 2.05$, $p=0.36$ & $X^2_{(2)} = 2.05$, $p=0.36$) indicating that there were no statistically significant differences between trial arms in either loss to follow up or adverse events (Figure 2).

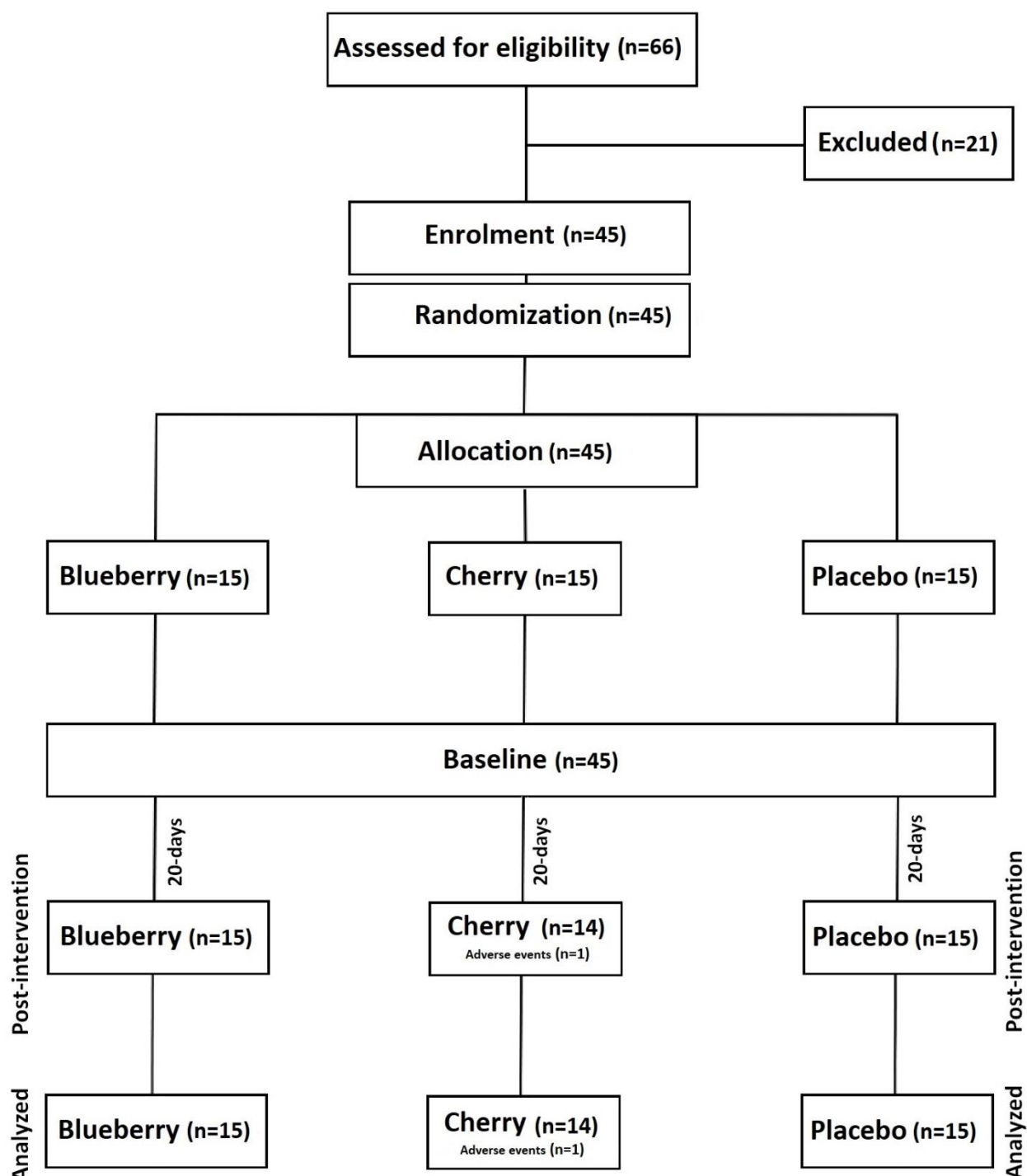


Figure 2. Consort diagram showing of participant flow throughout the study.

There was no statistically significant difference in % compliance between the placebo and cherry ($b = 1.47$, (95% CI = -1.01 – 3.95), $t = 1.21$, $p=0.24$), placebo and blueberry ($b = 0.80$, (95% CI = -1.70 – 3.30), $t = 0.66$, $p=0.52$) or between cherry and blueberry groups ($b = 0.67$, (95% CI = -1.45 – 2.79), $t = 0.64$, $p=0.53$). For supplementary anthocyanins however there were significant differences between the placebo and cherry ($b = 6059.52$, (95% CI = 5966.31 – 6152.73), $t = 132.77$, $p<0.001$, $\eta^2 = 0.98$), placebo and blueberry ($b = 14759.66$, (95% CI = 14538.12 – 14981.21), $t = 136.06$, $p<0.001$, $\eta^2 = 0.99$) and between cherry and blueberry groups ($b = 2640.62$, (95% CI = 2351.08 – 2930.17), $t = 18.63$, $p<0.001$, $\eta^2 = 0.92$). For supplementary daily sugars, there were significant differences between the placebo and cherry ($b = 17.04$, (95% CI = 16.78 – 17.30), $t = 132.66$, $p<0.001$, $\eta^2 = 0.98$), placebo and blueberry ($b = 41.95$, (95% CI = 41.32 – 42.58), $t = 136.57$, $p<0.001$, $\eta^2 = 0.99$) and between

cherry and blueberry groups ($b = 7.87$, (95% CI = 7.05 – 8.69), $t = 19.61$, $p < 0.001$, $\eta^2 = 0.93$). Finally, for supplementary daily energy intake, there were significant differences between the placebo and cherry ($b = 2.69$, (95% CI = 0.27 – 5.12), $t = 2.27$, $p = 0.031$, $\eta^2 = 0.26$), placebo and blueberry ($b = 8.65$, (95% CI = 3.82 – 13.49), $t = 3.66$, $p = 0.001$, $\eta^2 = 0.31$), but no differences between cherry and blueberry groups ($b = 3.27$, (95% CI = -0.92 – 7.45), $t = 1.59$, $p = 0.121$) (Table 2).

Table 2. Supplementary compliance and consumption throughout the intervention.

	Placebo		Cherry		Blueberry	
	Mean	SD	Mean	SD	Mean	SD
Amount consumed (mL)	1127	45	1136	34	1144	34
Compliance (%)	94	4	95	3	95	3
Experimental anthocyanins (mg)	0	8	12119	366	14760	435
Experimental energy intake (Kcal/ day)	188	8	193	6	196	6
Experimental sugars (g/ day)	0	0.	34	1	42	1

3.3 Blinding efficacy

Of the 44 participants that completed the trial 52% ($n = 23$) correctly identified their designated trial arm, the chi-squared test was non-significant ($X^2_{(2)} = 0.02$, $p = 0.89$) indicating that an effective blinding strategy was adopted.

3.4. Anthropometric measurements

No statistically significant differences ($p > 0.05$) in anthropometric parameters were found (Table 3).

Table 3. Anthropometric measurements as a function of each trial arm.

	Placebo				Cherry				Blueberry			
	Pre		Post		Pre		Post		Pre		Post	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Mass (kg)	67.62	10.47	69.27	12.55	69.44	11.38	69.62	9.94	68.17	9.43	66.46	7.87
Fat mass (kg)	15.55	5.07	16.61	6.07	17.97	6.18	17.85	5.36	17.02	5.18	15.83	4.11
BMI (kg/m ²)	23.82	2.98	24.46	3.25	24.90	2.35	24.69	2.23	24.07	3.10	23.80	2.89
Body fat (%)	23.21	5.61	22.62	5.58	25.33	7.03	26.30	6.36	24.70	5.64	23.72	4.73
Waist circumference (m)	0.79	0.08	0.80	0.10	0.80	0.07	0.79	0.06	0.79	0.08	0.78	0.09
Waist: hip ratio	0.81	0.07	0.79	0.09	0.79	0.05	0.79	0.05	0.82	0.06	0.82	0.09

3.5. Energy expenditure and substrate oxidation

No statistically significant differences ($p > 0.05$) in energy expenditure and substrate oxidation parameters were found (Table 4).

Table 4. Energy expenditure and substrate oxidation measurements as a function of each trial arm.

	Placebo				Cherry				Blueberry			
	Pre		Post		Pre		Post		Pre		Post	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Rest												
Carbohydrate oxidation (g/min)	0.23	0.06	0.29	0.11	0.23	0.05	0.24	0.05	0.21	0.06	0.21	0.08
Fat oxidation (g/min)	0.04	0.03	0.03	0.03	0.02	0.01	0.03	0.02	0.04	0.03	0.03	0.02
% Carbohydrate	69.24	16.23	70.26	17.82	71.01	8.67	69.66	12.63	68.65	18.15	68.55	21.53
% Fat	30.76	16.23	29.74	17.82	28.99	8.67	30.34	12.63	31.35	18.15	31.45	21.53
RMR (kcal/day)	1816.84	416.87	1797.67	580.28	1627.67	347.90	1683.43	306.60	1631.41	421.40	1638.71	388.42
Moderate intensity exercise												
Carbohydrate oxidation (g/min)	0.40	0.20	0.52	0.26	0.69	0.26	0.68	0.24	0.45	0.26	0.45	0.25
Fat oxidation (g/min)	0.23	0.12	0.17	0.11	0.11	0.08	0.12	0.08	0.20	0.09	0.18	0.10
% Carbohydrate	75.18	22.27	76.73	23.01	72.00	20.10	71.59	19.23	73.12	25.46	72.23	26.71
% Fat	24.82	22.27	23.27	23.01	28.00	20.10	28.42	19.23	26.88	25.46	27.77	26.71
Energy expenditure (kcal/min)	3.72	0.88	3.71	0.84	3.91	0.78	3.87	0.66	3.71	0.87	3.49	0.75

3.6. Haematological values

Adjusted for baseline, total cholesterol ($b = 0.72$, (95% CI = 0.19 – 1.24), $t = 2.79$, $p = 0.009$, $\eta^2 = 0.21$) and LDL cholesterol ($b = 0.53$, (95% CI = 0.09 – 0.97), $t = 2.56$, $p = 0.020$, $\eta^2 = 0.17$) were significantly reduced in the blueberry arm compared to placebo. Furthermore, adjusted for baseline glucose was significantly lower in the placebo ($b = 0.61$, (95% CI = 0.22 – 1.01), $t = 3.18$, $p = 0.003$, $\eta^2 = 0.08$) and cherry ($b = 0.41$, (95% CI = 0.10 – 0.72), $t = 2.68$, $p = 0.012$, $\eta^2 = 0.11$) arms compared to blueberry. Finally, adjusted for baseline haemoglobin was significantly reduced in the placebo ($b = 10.96$, (95% CI = 1.61 – 20.32), $t = 2.39$, $p = 0.023$, $\eta^2 = 0.16$) arm compared to blueberry (Table 5).

For total cholesterol the chi-squared test was significant ($X^2_{(2)} = 8.92$, $p = 0.012$) and 80%, 86.7% and 40% of participants exhibited improvements in the cherry, blueberry and placebo groups respectively. Similarly, for LDL cholesterol the chi-squared test was significant ($X^2_{(2)} = 8.89$, $p = 0.011$) and 60%, 86.7% and 33.3% of participants exhibited improvements in the cherry, blueberry and placebo groups respectively. Finally, for triglycerides the chi-squared test was also significant ($X^2_{(2)} = 6.01$, $p = 0.049$) and 80%, 73.3% and 40% of participants exhibited improvements in the cherry, blueberry and placebo groups respectively.

Table 5. Haematological values as a function of each trial arm.

	Placebo				Cherry				Blueberry			
	Pre		Post		Pre		Post		Pre		Post	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cholesterol (mmol/L)	4.01	0.68	4.34	0.90	4.04	0.70	4.10	0.64	4.36	0.50	3.79 ^A	0.58
LDL (mmol/L)	2.45	0.56	2.67	0.75	2.47	0.72	2.55	0.59	2.71	0.48	2.23 ^A	0.45
HDL (mmol/L)	1.19	0.10	1.25	0.19	1.18	0.15	1.17	0.10	1.23	0.15	1.19	0.20
Total: HDL ratio	3.42	0.46	3.51	0.69	3.49	0.82	3.59	0.68	3.64	0.60	3.21	0.48
LDL: HDL ratio	2.11	0.40	2.17	0.62	2.14	0.75	2.23	0.64	2.29	0.55	1.91	0.45
Glucose (mmol/L)	4.71	0.80	4.36	0.64	4.60	0.60	4.54	0.42	4.55	0.56	4.93 ^{AB}	0.48
Triglycerides (mmol/L)	1.06	0.25	1.17	0.55	1.07	0.37	0.96	0.28	1.21	0.44	1.20	0.56
Haemoglobin (g/L)	141.40	18.77	136.05	12.58	140.07	11.30	142.45	13.17	145.37	12.38	146.93	13.18

Note: A = significant difference from baseline compared to placebo & B = significant difference from baseline compared to cherry.

3.7. Blood pressure and resting heart rate

No statistical differences ($P > 0.05$) in blood pressure or resting heart rate were found (Table 6).

Table 6. Blood pressure and resting heart rate measurements as a function of each trial arm.

	Placebo				Cherry				Blueberry			
	Pre		Post		Pre		Post		Pre		Post	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Systolic blood pressure (mmHg)	123	20	120	16	118	9	119	9	120	12	122	14
Diastolic blood pressure (mmHg)	82	9	80	11	74	7	78	7	80	8	80	8
Resting heart rate (beats.min ⁻¹)	66	13	65	9	65	6	64	9	65	11	67	13

3.8. Questionnaires

Adjusted for baseline, Beck Depression Inventory ($b = 1.90$, (95% CI = 0.09 – 3.72), $t = 2.14$, $p = 0.041$, $\eta^2 = 0.13$), COOP WONCA ($b = 0.31$, (95% CI = 0.06 – 0.56), $t = 2.49$, $p = 0.019$, $\eta^2 = 0.17$), state ($b = 5.76$, (95% CI = 1.04 – 10.49), $t = 2.49$, $p = 0.018$, $\eta^2 = 0.17$) and trait ($b = 7.18$, (95% CI = 1.05 – 13.32), $t = 2.39$, $p = 0.023$, $\eta^2 = 0.16$) anxiety scores were significantly reduced in the blueberry arm compared to placebo. Furthermore, adjusted for baseline trait anxiety ($b = 6.64$, (95% CI = 0.40 – 12.89), $t = 2.17$, $p = 0.038$, $\eta^2 = 0.15$) scores were significantly reduced in the blueberry arm compared to cherry (Table 7).

Table 7. Questionnaire measurements as a function of each trial arm.

	Placebo				Cherry				Blueberry			
	Pre		Post		Pre		Post		Pre		Post	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Beck depression inventory	7.00	9.79	7.01	7.97	6.13	5.26	5.27	5.74	3.67	3.33	2.53 ^A	2.92
COOP WONCA	1.95	0.58	2.06	0.45	1.92	0.55	1.93	0.54	1.88	0.36	1.71 ^A	0.38
STAI state	32.60	10.76	35.87	11.10	33.47	7.68	32.73	8.39	30.67	9.55	28.87	6.83
STAI trait	40.20	10.66	40.73	11.43	36.87	8.72	39.20	8.67	39.33	9.98	33.07 ^A	8.94
PSQI	4.47	2.20	4.40	2.06	5.67	2.23	5.27	1.87	5.40	3.07	5.07	3.61
Insomnia severity index	5.93	4.35	3.16	4.29	7.13	4.00	5.40	2.72	6.67	5.42	4.73	4.59
Epworth sleepiness scale	5.47	3.50	5.73	3.10	5.80	4.23	6.07	3.59	6.67	5.00	5.67	4.30

Note: A = significant difference from baseline compared to placebo

4. Discussion

The current study aimed to investigate the influence of 20-days of twice daily Montmorency tart cherry or blueberry juice supplementation on cardiometabolic and other health related indices in healthy adults compared to placebo. To date this represents the first investigation to explore the effects of these supplementary interventions in a 3-arm parallel placebo-controlled trial. The primary aim was to determine whether systolic blood pressure was improved as a function of these supplements whereas the secondary aim(s) were to explore the effects of supplementation on other risk factors for cardiometabolic disease.

In relation to the primary outcome, the current investigation does not support our hypothesis in that there were significant reductions in systolic blood pressure in either the cherry or blueberry supplementation groups compared to placebo (Table 6). This result is in line with those of Lynn et al., [25], Desai et al., [28] and Kimble et al., [27] who showed that tart cherry supplementation had no effect on systolic blood pressure in healthy patients, whilst the current investigation also confirms a similar lack of efficacy for supplemental blueberry ingestion. It could therefore be speculated that in healthy individuals, arterial stiffness which governs systolic blood pressure is less responsive to short-term increases in anthocyanin intake via both tart cherry and blueberry supplementation. However, in healthy patients Chai et al., [33] and Kent et al., [34] observed significantly lower

systolic blood pressure in the first 3 hours after ingestion, as did Desai et al., [30] and Keane et al., [29] in those with metabolic syndrome and early onset hypertension. Notably, Keane et al., [31] showed that plasma anthocyanin metabolites peak in the first two hours following ingestion with rapid clearance and return to basal within 4 hours. This coincides with the aforementioned previously observed statistical reductions in postprandial blood pressure and suggests that the current and previous analyses adopting longitudinal rather than acute study designs may have missed the peak effects of tart cherry and blueberry supplementation. Importantly, as no statistically significant differences in blood pressure were observed, the current investigation lends further support to the concept that anthocyanin rich supplementation mediates a transient rather than sustained attenuation of systolic blood pressure. Although physiologically important, the associated long-term clinical benefits of an acute reduction in blood pressure from both prophylactic and treatment standpoints has not yet been explored. Therefore, future analyses should seek to establish the enduring clinical efficacy of transient reductions in systolic blood pressure mediated through anthocyanin rich supplementation.

Although no statistically significant differences in the primary outcome were evident, linear mixed model and chi-square analyses support our hypothesis in that both total and LDL cholesterol were significantly improved in the blueberry arm compared to placebo (Table 5), and a larger number of participants experienced reductions in triglycerides in the cherry and blueberry groups. As no changes in HDL cholesterol were evident, it is clear that reductions in total cholesterol were mediated as a function of the corresponding attenuation in LDL values. Previous trials have shown that consuming tart cherry mediated statistical reductions in both total and LDL cholesterol in older patients [32] and those with metabolic syndrome [33]; although this is the first investigation to show similar effects in healthy participants ingesting blueberry supplementation. It is proposed that the reductions in cholesterol mediated via the blueberry trial arm are a reflection of the statistically greater anthocyanin concentrations in this supplement (Table 2), lending support to the concept of a dose response to supplementary anthocyanins in cardiometabolic disease [72]. In relation to triglycerides, our observations concur with those in pathological patients [33] and the current investigation notably shows similar effects in healthy participants and efficacy also those ingesting blueberry supplementation. Owing to the greater anthocyanin content in the blueberry supplement, it is proposed that the reductions in LDL cholesterol in this condition, were mediated via the inhibition of plasma cholesteryl ester transfer protein (CETP). Several studies have indicated that CETP inhibition is a crucial mechanism for the attenuation of LDL cholesterol [73, 74] and both human and animal analyses have shown that anthocyanins decrease plasma CETP activity [73, 75]. Taking into account the long-standing and well-established association between lipid concentrations and the risk of cardiovascular disease [76], these observations may have considerable clinical relevance. Whilst lipid lowering pharmaceuticals have been shown to exhibit a high level of efficacy, they are associated with significant side-effects [7], impose significant monetary restrictions on healthcare budgets and contribute to the worldwide overreliance on prescription medications [8]. Therefore, the findings from the current trial lend support the concept that in particular blueberry supplementation may be important in the management of cardiometabolic disease.

Further to the improvements in total and LDL cholesterol shown in the blueberry trial arm, the current investigation also importantly showed that this supplemental condition was able to mediate statistical improvements in all indices of psychological wellbeing compared to placebo. This observation concurs with those of Khalid et al., [77] who showed improvements in mood state in both children and young adults ingesting a blueberry concentrate compared to placebo. Previous analyses have linked cardiometabolic disease to reduced levels of psychological wellbeing [67], so taking into account the aforementioned improvements noted in the blueberry trial arm, this observation makes intuitive sense. The mechanism responsible for the improvements in psychological wellbeing is not currently known and requires further consideration given the global incidence of

depression and other psychological disorders [78]. There are several conceivable mechanisms which may explain our findings, including increased cerebral blood flow where cognitive and emotional control is located [79] and reduced Monoamine Oxidase activity causing increasing levels circulating monoamines, some of which are neurotransmitters associated with mood regulation [80]. Regardless, the observations from the current trial lend further support to the concept that blueberry supplementation may be important in the management of psychological disorders.

However, it is important to also acknowledge that despite the potentially exciting improvements in blood lipid and psychological wellbeing profiles in the blueberry trial arm, this supplement was associated with increased resting glucose values in relation to both the placebo and cherry trial arms (Table 5). It is apparent that this observation was caused by the increased sugar content (Table 3) in the blueberry supplement, and the statistically greater daily sugar intakes in this arm compared to the others. This finding allied with the previously outlined reductions in blood lipids, concurs with those of Chai et al., [32] with tart cherries. This observation is biologically interesting as typically increased blood glucose is met with corresponding increases in LDL cholesterol [81]. It is not within the scope of the biological measurements examined in this trial to accurately determine the mechanisms responsible for this finding. However, it can be speculated that the unique nature of the anthocyanin rich blueberry supplementation may be responsible. Firstly, through the aforementioned CETP inhibition pathway, as although potentially responsible for the attenuation of LDL cholesterol, animal models have shown strong correlations between CETP expression and bile acid signaling that may result in increased glucose disposal [82]. In addition, new information has shown that the influence of anthocyanins themselves on glucose and lipid metabolism in humans may be affected by their distinct chemical composition [83]. Blueberries are characterized by a greater number of hydroxyl groups [84] and belong to the malvidin variety of anthocyanins [85]. The malvidin group is associated with greater antioxidant capacity, which may explain their greater potential for improvements in dyslipidemia [86], though there is insufficient evidence concerning the effects of different anthocyanin compositions on blood glucose regulation. As such it is important for further investigation to be conducted into the biological influence of anthocyanin chemical composition. Nonetheless, it is important to note that the mean fasting blood glucose values remained within normal ranges [87], and the long-term effects of elevated blood glucose levels remain unknown in healthy individuals. However, in patients with cardiometabolic conditions or diabetes mellitus characterized by poor glucose control, the findings from the current investigation do not currently support habitual utilization of this supplement, despite the improvements in blood lipids. Therefore, it is important for future analyses to examine the longer-term effects of blueberry supplementation and to explore continuous blood glucose control, as well as insulin and haemoglobin A1c indices in both healthy and pathological populations.

Overall, the current investigation exhibited a very effective level of blinding efficacy, a low number of adverse incidences, a high retention rate, very good compliance levels as well as improvements in blood lipids and psychological wellbeing, predominantly in the blueberry trial arm. However, owing to the statistically greater mean daily sugar and associated kilocalorie intake (Table 3) in comparison to the cherry and placebo groups; in agreement with Kimble et al., [27], those seeking to utilize blueberry juice as a dietary supplement should utilize caution and seek to modify their daily dietary intake to account for the increase in daily Kcal. Furthermore, recent analyses have shown that some fruit phenolics constrain the formation of advanced glycation end products (AGE) [88], and thus mediate cellular and tissue impairment by damaging protein function and clearance [89]. Therefore, to better understand its potential biological effects, further exploration of the effects of blueberry juice should seek to examine the effects of this supplement on AGE formation. As with all research, this trial is not without limitations. Firstly, the experimental anthocyanin, energy and sugar contents were reported according to the manufacturer's guidelines, which for anthocyanins in particular have been shown to exhibit

variability from sample to sample owing to differences in growing conditions [27]. Whilst the current investigation observed positive effects of blueberry supplementation on cardiometabolic and psychological wellbeing indices, the mechanistic bases for these improvements was not elucidated. Therefore, future investigations should seek to explore and perhaps better utilize and exploit the mechanistic pathways of blueberry supplementation in order to improve health related outcomes. Furthermore, as participants were randomized into their designated trial arms without consideration for their previous anthocyanin intake, a stratified random sampling approach should be adopted for future interventions exploring the effects of anthocyanin rich fruits on cardiometabolic health indices. Finally, as many of the experimental variables are positively influence by exercise, that physical activity was not monitored may serve as a limitation to this trial. Therefore, subsequent randomized interventions may seek to quantify physical activity throughout the intervention period via continuous actigraphy.

5. Conclusions

The current study aimed to investigate the influence of Montmorency tart cherry or blueberry juice supplementation on cardiometabolic, and other health related indices compared to placebo. The current study did not support the primary hypothesis in that neither cherry or blueberry supplementation improved systolic blood pressure compared to placebo. However, the secondary hypothesis was supported in that 20-days of blueberry supplementation was able to mediate improvements in blood lipid concentrations and psychological wellbeing indices in relation to placebo. Given the clear and long-standing association between lipid concentrations and the risk of cardiovascular disease and the paramount importance of psychological wellbeing to health-related quality of life, the current investigation indicates that blueberry juice could represent a useful means to enhance cardiometabolic and psychological health. Future intervention trials and studies should consider exploring the longer-term effects of blueberry juice, the effects of increased supplemental sugar intake as well as its efficacy in populations with cardiometabolic abnormalities at baseline.

Author Contributions: Conceptualization, JS & LB; methodology, JS, RA, GS, BB & SD; writing—original draft preparation, JS & LB writing—review and editing, JS, RA, GS, SD, BB & LB; funding acquisition, JS & GS. All authors have read and agreed to the published version of the manuscript.

Funding: The sponsor of this research is the University of Central Lancashire, UK. This research received no external funding, but the experimental supplementation has been provided by ActiveEdge (Nutrition) Ltd UK, who did not have any input into the research design.

Institutional Review Board Statement: The study was be conducted according to the guidelines of the Declaration of Helsinki and has been granted ethical approval by the University of Central Lancashire Health Research Ethics Committee (ref: HEALTH 0016).

Informed Consent Statement: All participants provided written informed consent in accordance with the Declaration of Helsinki and the Oviedo Convention.

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

References

1. Danaei, G., Lu, Y., Singh, G. M., Carnahan, E., Stevens, G. A., Cowan, M. J., & Kobayashi, J. (2014). Cardiovascular disease, chronic kidney disease, and diabetes mortality burden of cardiometabolic risk factors from 1980 to 2010: a comparative risk assessment. *Lancet Diabetes & Endocrinology*, 2(8):634-47.
2. Ahmed, C. M., Jiang, H., Chen, J. Y., & Lin, Y. H. (2018). Traffic-related particulate matter and cardiometabolic syndrome: a review. *Atmosphere*, 9(9), 336-346.
3. Sinclair, J., Shadwell, G., Dillon, S., Allan, R., Butters, B., & Bottoms, L. (2021). Effects of Montmorency tart cherry and blueberry juice on cardiometabolic outcomes in healthy individuals: protocol for a 3-arm placebo randomized controlled trial. *International Journal of Environmental Research and Public Health*, 18(18), 9759.
4. Keane, K. M., George, T. W., Constantinou, C. L., Brown, M. A., Clifford, T., & Howatson, G. (2016). Effects of Montmorency tart cherry (*Prunus Cerasus* L.) consumption on vascular function in men with early hypertension. *The American Journal of Clinical Nutrition*, 103(6), 1531-1539.

5. Odegaard, A. O., Jacobs, D. R., Sanchez, O. A., Goff, D. C., Reiner, A. P., & Gross, M. D. (2016). Oxidative stress, inflammation, endothelial dysfunction and incidence of type 2 diabetes. *Cardiovascular diabetology*, 15(1), 1-12.
6. Chai, S. C., Davis, K., Wright, R. S., Kuczmarski, M. F., & Zhang, Z. (2018). Impact of tart cherry juice on systolic blood pressure and low-density lipoprotein cholesterol in older adults: a randomized controlled trial. *Food & function*, 9(6), 3185-3194.
7. Qato, D. M., Alexander, G. C., Conti, R. M., Johnson, M., Schumm, P., & Lindau, S. T. (2008). Use of prescription and over-the-counter medications and dietary supplements among older adults in the United States. *JAMA*, 300(24), 2867-2878.
8. Nguyen, Q., Dominguez, J., Nguyen, L., & Gullapalli, N. (2010). Hypertension management: an update. *American Health & Drug Benefits*, 3(1), 47-56.
9. Oyebode, O., Gordon-Dseagu, V., Walker, A., & Mindell, J. S. (2014). Fruit and vegetable consumption and all-cause, cancer and CVD mortality: analysis of Health Survey for England data. *Journal of Epidemiology & Community Health*, 68(9), 856-862.
10. Anand, S. S., Hawkes, C., De Souza, R. J., Mente, A., Dehghan, M., Nugent, R., & Popkin, B. M. (2015). Food consumption and its impact on cardiovascular disease: importance of solutions focused on the globalized food system: a report from the workshop convened by the World Heart Federation. *Journal of the American College of Cardiology*, 66(14), 1590-1614.
11. Chiva-Blanch, G., & Visioli, F. (2012). Polyphenols and health: Moving beyond antioxidants. *Journal of Berry Research*, 2(2), 63-71.
12. Aune, D., Giovannucci, E., Boffetta, P., Fadnes, L. T., Keum, N., Norat, T., & Tonstad, S. (2017). Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality—a systematic review and dose-response meta-analysis of prospective studies. *International journal of epidemiology*, 46(3), 1029-1056.
13. Zheng, J., Zhou, Y., Li, S., Zhang, P., Zhou, T., Xu, D. P., & Li, H. B. (2017). Effects and mechanisms of fruit and vegetable juices on cardiovascular diseases. *International Journal of Molecular Sciences*, 18(3), 555-562.
14. Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2006). Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. *Journal of Agricultural and Food Chemistry*, 54(11), 4069-4075.
15. Reis, J. F., Monteiro, V. V. S., de Souza Gomes, R., do Carmo, M. M., da Costa, G. V., Ribera, P. C., & Monteiro, M. C. (2016). Action mechanism and cardiovascular effect of anthocyanins: a systematic review of animal and human studies. *Journal of Translational Medicine*, 14(1), 1-16.
16. Kevers, C., Pincemail, J., Defraigne, J. O., & Dommes, J. (2014). Antioxidant capacity of small dark fruits: Influence of cultivars and harvest time. *Journal of Berry Research*, 4(2), 97-105.
17. Nemzer, B., Vargas, L., Xia, X., Sintara, M., & Feng, H. (2018). Phytochemical and physical properties of blueberries, tart cherries, strawberries, and cranberries as affected by different drying methods. *Food Chemistry*, 262, 242-250.
18. Traustadóttir, T., Davies, S. S., Stock, A. A., Su, Y., Heward, C. B., Roberts, L. J., & Harman, S. M. (2009). Tart cherry juice decreases oxidative stress in healthy older men and women. *The Journal of Nutrition*, 139(10), 1896-1900.
19. Bell, P. G., Walshe, I. H., Davison, G. W., Stevenson, E., & Howatson, G. (2014). Montmorency cherries reduce the oxidative stress and inflammatory responses to repeated days high-intensity stochastic cycling. *Nutrients*, 6(2), 829-843.
20. Howatson, G., McHugh, M. P., Hill, J. A., Brouner, J., Jewell, A. P., Van Someren, K. A., & Howatson, S. A. (2010). Influence of tart cherry juice on indices of recovery following marathon running. *Scandinavian Journal of Medicine & Science in Sports*, 20(6), 843-852.
21. Martin, K. R., Bopp, J., Burrell, L., & Hook, G. (2011). The effect of 100% tart cherry juice on serum uric acid levels, biomarkers of inflammation and cardiovascular disease risk factors. *FASEB*, S1, 339-340.
22. Cook, M. D., Myers, S. D., Blacker, S. D., & Willems, M. E. T. (2015). New Zealand blackcurrant extract improves cycling performance and fat oxidation in cyclists. *European Journal of Applied Physiology*, 115(11), 2357-2365.
23. Robinson, S. L., Hattersley, J., Frost, G. S., Chambers, E. S., & Wallis, G. A. (2015). Maximal fat oxidation during exercise is positively associated with 24-hour fat oxidation and insulin sensitivity in young, healthy men. *Journal of Applied Physiology*, 118(11), 1415-1422.
24. Minihane, A. M., Vinoy, S., Russell, W. R., Baka, A., Roche, H. M., Tuohy, K. M., & Calder, P. C. (2015). Low-grade inflammation, diet composition and health: current research evidence and its translation. *British Journal of Nutrition*, 114(7), 999-1012.
25. Lynn, A., Mathew, S., Moore, C. T., Russell, J., Robinson, E., Soumpasi, V., & Barker, M. E. (2014). Effect of a tart cherry juice supplement on arterial stiffness and inflammation in healthy adults: a randomised controlled trial. *Plant Foods for Human Nutrition*, 69(2), 122-127.
26. Martin, K. R., Burrell, L., & Bopp, J. (2018). Authentic tart cherry juice reduces markers of inflammation in overweight and obese subjects: A randomized, crossover pilot study. *Food & Function*, 9(10), 5290-5300.
27. Kimble, R., Keane, K. M., Lodge, J. K., & Howatson, G. (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), 1417-1423.
28. Desai, T., Bottoms, L., & Roberts, M. (2018). The effects of Montmorency tart cherry juice supplementation and FATMAX exercise on fat oxidation rates and cardio-metabolic markers in healthy humans. *European Journal of Applied Physiology*, 118(12), 2523-2539.
29. Keane, K. M., George, T. W., Constantinou, C. L., Brown, M. A., Clifford, T., & Howatson, G. (2016). Effects of Montmorency tart cherry (*Prunus Cerasus* L.) consumption on vascular function in men with early hypertension. *The American Journal of Clinical Nutrition*, 103(6), 1531-1539.

30. Desai, T., Roberts, M., & Bottoms, L. (2019). Effects of Montmorency tart cherry supplementation on cardio-metabolic markers in metabolic syndrome participants: A pilot study. *Journal of Functional Foods*, 57, 286-298.
31. Keane, K. M., Haskell-Ramsay, C. F., Veasey, R. C., & Howatson, G. (2016). Montmorency Tart cherries (*Prunus cerasus* L.) modulate vascular function acutely, in the absence of improvement in cognitive performance. *British Journal of Nutrition*, 116(11), 1935-1944.
32. Johnson, S. A., Navaei, N., Pourafshar, S., Jaime, S. J., Akhavan, N. S., Alvarez-Alvarado, S., & Figueroa, A. (2020). Effects of montmorency Tart Cherry juice consumption on cardiometabolic biomarkers in adults with metabolic syndrome: a randomized controlled pilot trial. *Journal of Medicinal Food*, 23(12), 1238-1247.
33. Chai, S. C., Davis, K., Wright, R. S., Kuczmarski, M. F., & Zhang, Z. (2018). Impact of tart cherry juice on systolic blood pressure and low-density lipoprotein cholesterol in older adults: a randomized controlled trial. *Food & function*, 9(6), 3185-3194.
34. Kent, K., Charlton, K. E., Jenner, A., & Roodenrys, S. (2016). Acute reduction in blood pressure following consumption of anthocyanin-rich cherry juice may be dose-interval dependant: a pilot cross-over study. *International journal of food sciences and nutrition*, 67(1), 47-52.
35. Stull, A. J., Cash, K. C., Johnson, W. D., Champagne, C. M., & Cefalu, W. T. (2010). Bioactives in blueberries improve insulin sensitivity in obese, insulin-resistant men and women. *The Journal of Nutrition*, 140(10), 1764-1768.
36. Dohadwala, M. M., Holbrook, M., Hamburg, N. M., Shenouda, S. M., Chung, W. B., Titas, M., & Vita, J. A. (2011). Effects of cranberry juice consumption on vascular function in patients with coronary artery disease. *The American Journal of Clinical Nutrition*, 93(5), 934-940.
37. Chew, B., Mathison, B., Kimble, L., McKay, D., Kaspar, K., Khoo, C. & Blumberg, J. (2019). Chronic consumption of a low calorie, high polyphenol cranberry beverage attenuates inflammation and improves glucoregulation and HDL cholesterol in healthy overweight humans: a randomized controlled trial. *European Journal of Nutrition*, 58(3), 1223-1235.
38. Barati Boldaji, R., Akhlaghi, M., Sagheb, M. M., & Esmailinezhad, Z. (2020). Pomegranate juice improves cardiometabolic risk factors, biomarkers of oxidative stress and inflammation in hemodialysis patients: A randomized crossover trial. *Journal of the Science of Food and Agriculture*, 100(2), 846-854.
39. Khan, F., Ray, S., Craigie, A. M., Kennedy, G., Hill, A., Barton, K. L., & Belch, J. J. (2014). Lowering of oxidative stress improves endothelial function in healthy subjects with habitually low intake of fruit and vegetables: a randomized controlled trial of antioxidant-and polyphenol-rich blackcurrant juice. *Free Radical Biology and Medicine*, 72, 232-237.
40. Stote, K. S., Sweeney, M. I., Kean, T., Baer, D. J., Novotny, J. A., Shakerley, N. L., & Gottschall-Pass, K. T. (2017). The effects of 100% wild blueberry (*Vaccinium angustifolium*) juice consumption on cardiometabolic biomarkers: A randomized, placebo-controlled, crossover trial in adults with increased risk for type 2 diabetes. *BMC nutrition*, 3(1), 1-11.
41. Basu, A., Betts, N. M., Ortiz, J., Simmons, B., Wu, M., & Lyons, T. J. (2011). Low-energy cranberry juice decreases lipid oxidation and increases plasma antioxidant capacity in women with metabolic syndrome. *Nutrition Research*, 31(3), 190-196.
42. Sumner, M. D., Elliott-Eller, M., Weidner, G., Daubenmier, J. J., Chew, M. H., Marlin, R., & Ornish, D. (2005). Effects of pomegranate juice consumption on myocardial perfusion in patients with coronary heart disease. *The American Journal of Cardiology*, 96(6), 810-814.
43. González-Ortiz, M., Martínez-Abundis, E., Espinel-Bermúdez, M. C., & Pérez-Rubio, K. G. (2011). Effect of pomegranate juice on insulin secretion and sensitivity in patients with obesity. *Annals of Nutrition and Metabolism*, 58(3), 220-223.
44. Moher, D., Hopewell, S., Schulz, K. F., Montori, V., Gøtzsche, P. C., Devereaux, P. J., & Altman, D. G. (2012). CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *International journal of surgery*, 10(1), 28-55.
45. Charrois, T. L., McAlister, F. A., Cooney, D., Lewanczuk, R., Kolber, M. R., Campbell, N. R., & Tsuyuki, R. T. (2011). Improving hypertension management through pharmacist prescribing; the rural Alberta clinical trial in optimizing hypertension (Rural RxACTION): trial design and methods. *Implementation Science*, 6(1), 1-9.
46. Lee, C. L., & Wang, J. S. (2020). Systolic blood pressure trajectory and cardiovascular outcomes: An analysis using data in the Systolic Blood Pressure Intervention Trial. *International Journal of Clinical Practice*, 74(3), e13450.
47. Bottoms, L., Hobbs, S. J., Dillon, S., Taylor, P., Allan, R., Stainton, P., & Sinclair, J. (2021). Efficacy Of Montmorency Tart Cherry Juice For The Treatment Of Patellofemoral Pain In Recreational Athletes: 1365. *Medicine & Science in Sports & Exercise*, 53(8), 449-450.
48. Bosy-Westphal, A., Jensen, B., Braun, W., Pourhassan, M., Gallagher, D., & Müller, M. J. (2017). Quantification of whole-body and segmental skeletal muscle mass using phase-sensitive 8-electrode medical bioelectrical impedance devices. *European Journal of Clinical Nutrition*, 71(9), 1061-1067.
49. Czernichow, S., Kengne, A. P., Huxley, R. R., Batty, G. D., De Galan, B., Grobbee, D., & Chalmers, J. Group. (2011). Comparison of waist-to-hip ratio and other obesity indices as predictors of cardiovascular disease risk in people with type-2 diabetes: a prospective cohort study from ADVANCE. *European Journal of Preventive Cardiology*, 18(2), 312-319.
50. Kelly, B., King, J. A., Goerlach, J., & Nimmo, M. A. (2013). The impact of high-intensity intermittent exercise on resting metabolic rate in healthy males. *European Journal of Applied Physiology*, 113(12), 3039-3047.
51. Frayn, K. N. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology*, 55(2), 628-634.
52. Weir, J. D. V. (1949). New methods for calculating metabolic rate with special reference to protein metabolism. *The Journal of Physiology*, 109(1-2), 1.

53. Tudor-Locke, C., Han, H., Aguiar, E. J., Barreira, T. V., Schuna Jr, J. M., Kang, M., & Rowe, D. A. (2018). How fast is fast enough? Walking cadence (steps/min) as a practical estimate of intensity in adults: a narrative review. *British Journal of Sports Medicine*, 52(12), 776-788. 692
54. Jeukendrup, A. E., & Wallis, G. A. (2005). Measurement of substrate oxidation during exercise by means of gas exchange measurements. *International journal of sports medicine*, 26(S 1), 28-37. 693
55. Anandaraja, S., Narang, R., Godeswar, R., Lakshmy, R., & Talwar, K. K. (2005). Low-density lipoprotein cholesterol estimation by a new formula in Indian population. *International Journal of Cardiology*, 102(1), 117-120. 694
56. Chen, Y., Zhang, X., Pan, B., Jin, X., Yao, H., Chen, B., & Chen, H. (2010). A modified formula for calculating low-density lipoprotein cholesterol values. *Lipids in Health and Disease*, 9(1), 1-5. 695
57. Millán, J., Pintó, X., Muñoz, A., Zúñiga, M., Rubiés-Prat, J., Pallardo, L. F., & Pedro-Botet, J. (2009). Lipoprotein ratios: physiological significance and clinical usefulness in cardiovascular prevention. *Vascular Health and Risk Management*, 5, 757-765. 696
58. O'Brien, E., Asmar, R., Beilin, L., Imai, Y., Mallion, J. M., Mancia, G., & European Society of Hypertension Working Group on Blood Pressure Monitoring. (2003). European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. *Journal of Hypertension*, 21(5), 821-848. 697
59. Pickering, T. G., Hall, J. E., Appel, L. J., Falkner, B. E., Graves, J., Hill, M. N., & Roccella, E. J. (2005). 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. *Hypertension*, 45(1), 142-161. 698
60. Matricciani, L., Paquet, C., Frayssé, F., Grobler, A., Wang, Y., Baur, L., & Olds, T. (2021). Sleep and cardiometabolic risk: a cluster analysis of actigraphy-derived sleep profiles in adults and children. *Sleep*, 44(7), zsab014. 699
61. Godos, J., Ferri, R., Castellano, S., Angelino, D., Mena, P., Del Rio, D., & Grosso, G. (2020). Specific dietary (poly) phenols are associated with sleep quality in a cohort of Italian adults. *Nutrients*, 12(5), 1226-1330. 700
62. Howatson, G., Bell, P. G., Tallent, J., Middleton, B., McHugh, M. P., & Ellis, J. (2012). Effect of tart cherry juice (*Prunus cerasus*) on melatonin levels and enhanced sleep quality. *European Journal of Nutrition*, 51(8), 909-916. 701
63. Pigeon, W. R., Carr, M., Gorman, C., & Perlis, M. L. (2010). Effects of a tart cherry juice beverage on the sleep of older adults with insomnia: a pilot study. *Journal of Medicinal Food*, 13(3), 579-583. 702
64. Buysse, D. J., Reynolds III, C. F., Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Research*, 28(2), 193-213. 703
65. Smith, S. S., Oei, T. P., Douglas, J. A., Brown, I., Jorgensen, G., & Andrews, J. (2008). Confirmatory factor analysis of the Epworth Sleepiness Scale (ESS) in patients with obstructive sleep apnoea. *Sleep Medicine*, 9(7), 739-744. 704
66. Morin, C. M., Belleville, G., Bélanger, L., & Ivers, H. (2011). The Insomnia Severity Index: psychometric indicators to detect insomnia cases and evaluate treatment response. *Sleep*, 34(5), 601-608. 705
67. Gheshlagh, R. G., Parizad, N., & Sayehmiri, K. (2016). The relationship between depression and metabolic syndrome: systematic review and meta-analysis study. *Iranian Red Crescent Medical Journal*, 18(6), e26523. 706
68. Kontogianni, M. D., Vijayakumar, A., Rooney, C., Noad, R. L., Appleton, K. M., McCarthy, D., & Woodside, J. V. (2020). A high polyphenol diet improves psychological well-being: The polyphenol intervention trial (pphit). *Nutrients*, 12(8), e2445. 707
69. Bentsen, B. G., Natvig, B., & Winnem, M. (1999). Questions you didn't ask? COOP/WONCA Charts in clinical work and research. *Family practice*, 16(2), 190-195. 708
70. Wang, Y. P., & Gorenstein, C. (2013). Psychometric properties of the Beck Depression Inventory-II: a comprehensive review. *Brazilian Journal of Psychiatry*, 35, 416-431. 709
71. Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., & Jacobs, G. A. (1983). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press. 710
72. Yang, L., Ling, W., Du, Z., Chen, Y., Li, D., Deng, S., & Yang, L. (2017). Effects of anthocyanins on cardiometabolic health: a systematic review and meta-analysis of randomized controlled trials. *Advances in nutrition*, 8(5), 684-693. 711
73. Inazu, A., Brown, M. L., Hesler, C. B., Agellon, L. B., Koizumi, J., Takata, K., & Tall, A. R. (1990). Increased high-density lipoprotein levels caused by a common cholesteryl-ester transfer protein gene mutation. *New England Journal of Medicine*, 323(18), 1234-1238. 712
74. Qin, Y., Xia, M., Ma, J., Hao, Y., Liu, J., Mou, H., & Ling, W. (2009). Anthocyanin supplementation improves serum LDL-and HDL-cholesterol concentrations associated with the inhibition of cholesteryl ester transfer protein in dyslipidemic subjects. *The American Journal of Clinical Nutrition*, 90(3), 485-492. 713
75. Lam, C. K., Zhang, Z., Yu, H., Tsang, S. Y., Huang, Y., & Chen, Z. Y. (2008). Apple polyphenols inhibit plasma CETP activity and reduce the ratio of non-HDL to HDL cholesterol. *Molecular Nutrition & Food Research*, 52(8), 950-958. 714
76. Nelson, R. H. (2013). Hyperlipidemia as a risk factor for cardiovascular disease. *Primary Care: Clinics in Office Practice*, 40(1), 195-211. 715
77. Khalid, S., Barfoot, K. L., May, G., Lamport, D. J., Reynolds, S. A., & Williams, C. M. (2017). Effects of acute blueberry flavonoids on mood in children and young adults. *Nutrients*, 9(2), 158. 716
78. Zuberi, A., Waqas, A., Naveed, S., Hossain, M. M., Rahman, A., Saeed, K., & Fuhr, D. C. (2021). Prevalence of mental disorders in the WHO Eastern Mediterranean Region: A systematic review and meta-analysis. *Frontiers in Psychiatry*, 12, 665019. 717
79. Vauzour, D., Vafeiadou, K., Rodriguez-Mateos, A., Rendeiro, C., & Spencer, J. P. (2008). The neuroprotective potential of flavonoids: a multiplicity of effects. *Genes & nutrition*, 3(3), 115-126. 718

80. Watson, A. W., Haskell-Ramsay, C. F., Kennedy, D. O., Cooney, J. M., Trower, T., & Scheepens, A. (2015). Acute supplementation with blackcurrant extracts modulates cognitive functioning and inhibits monoamine oxidase-B in healthy young adults. *Journal of functional foods*, 17, 524-539. 752-754
81. Jameel, F., Phang, M., Wood, L. G., & Garg, M. L. (2014). Acute effects of feeding fructose, glucose and sucrose on blood lipid levels and systemic inflammation. *Lipids in Health and Disease*, 13(1), 1-7. 755-756
82. Cappel, D. A., Palmisano, B. T., Emfinger, C. H., Martinez, M. N., McGuinness, O. P., & Stafford, J. M. (2013). Cholesteryl ester transfer protein protects against insulin resistance in obese female mice. *Molecular Metabolism*, 2(4), 457-467. 757-758
83. Araki, R., Yada, A., Ueda, H., Tominaga, K., & Isoda, H. (2021). Differences in the Effects of Anthocyanin Supplementation on Glucose and Lipid Metabolism According to the Structure of the Main Anthocyanin: A Meta-Analysis of Randomized Controlled Trials. *Nutrients*, 13(6), 2003. 759-761
84. Habtemariam S. Chapter 7—Bilberries and blueberries as potential modulators of type 2 diabetes and associated diseases. In: Habtemariam S., editor. *Medicinal Foods as Potential Therapies for Type-2 Diabetes and Associated Diseases*. Academic Press; Cambridge, MA, USA: 2019. pp. 135–175. 762-764
85. Castañeda-Ovando, A., de Lourdes Pacheco-Hernández, M., Páez-Hernández, M. E., Rodríguez, J. A., & Galán-Vidal, C. A. (2009). Chemical studies of anthocyanins: A review. *Food chemistry*, 113(4), 859-871. 765-766
86. Medina-Vera, I., Gómez-de-Regil, L., Gutiérrez-Solis, A. L., Lugo, R., Guevara-Cruz, M., Pedraza-Chaverri, J., & Avila-Nava, A. (2021). Dietary strategies by foods with antioxidant effect on nutritional management of dyslipidemias: a systematic review. *Antioxidants*, 10(2), 225. 767-769
87. Tirosh, A., Shai, I., Tekes-Manova, D., Israeli, E., Pereg, D., Shochat, T., & Rudich, A. (2005). Normal fasting plasma glucose levels and type 2 diabetes in young men. *New England Journal of Medicine*, 353(14), 1454-1462. 770-771
88. Jariyapamornkoon, N., Yibchok-anun, S., & Adisakwattana, S. (2013). Inhibition of advanced glycation end products by red grape skin extract and its antioxidant activity. *BMC Complementary and Alternative Medicine*, 13(1), 1-9. 772-773
89. Thornalley PJ. (1996). Formation, modification of proteins and nucleic acids, and enzymatic detoxification—a role in pathogenesis and antiproliferative chemotherapy. *General Pharmacology*, 27(4), 565-573. 774-775