

Effects of Montmorency Tart Cherry Supplementation on Cardio-Metabolic Markers in  
Metabolic Syndrome Participants: a pilot study

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**Abstract**

This study compared acute supplementation of Montmorency tart cherries in capsule (MTCC) and juice (MTCJ) forms in MetS humans, as potential prophylactic interventions against cardio-metabolic diseases. In an acute, single-blind, placebo-controlled, randomised, crossover trial, eleven participants with MetS ( $49 \pm 12$  years; 6M/5F), consumed one bolus of MTCC, MTCJ or placebo (PLA) on different occasions. Blood-based and functional cardio-metabolic biomarkers were measured pre-bolus and up to 5 hours post-bolus. MTCJ significantly reduced systolic blood pressure compared to PLA at 2-hours post-bolus ( $P = 0.018$ ). Insulin was significantly lower with MTCC ( $P = 0.016$ ) and MTCJ ( $P = 0.028$ ) than PLA at 1-hour and 3-hours post-bolus, respectively. No significant differences between MTCC and MTCJ were seen. This study demonstrated for the first time that MTCC could reduce insulin concentrations in humans. Importantly, MTCJ induced a clinically relevant reduction in systolic blood pressure and also lowered insulin compared to PLA, in MetS humans.

**Keywords:** polyphenols; anthocyanins; cardiometabolic health; functional foods; hypertension; diabetes

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## **Introduction**

Often accompanied by endothelial dysfunction, a pro-inflammatory, pro-oxidant and pro-thrombotic state (Srikanthan, Feyh, Visweshwar, Shapiro, & Sodhi, 2016), Metabolic Syndrome (MetS) encompasses a cluster of cardio-metabolic conditions including insulin resistance, central adiposity, dyslipidaemia and hypertension (Kaur, 2014). The presence of three or more of these symptoms combined, augments the incidence of chronic diseases such as cardiovascular disease (CVD) by 2-fold and type 2 diabetes (T2D) by 5-fold (Falkner & Cossrow, 2014). Ultimately, this highlights the strong association between MetS and higher rates of cardiovascular and total mortality (Falkner & Cossrow, 2014). Despite the difficulty in measuring MetS prevalence rates, it is accepted that global rates are on the rise (O'Neill & O'Driscoll, 2015). In the United States [~35% MetS prevalence rate (Aguilar, Bhuket, Torres, Liu, & Wong, 2015)], low fruit and vegetable intake accounted for 15.1% of all diet-related cardio-metabolic deaths (Micha et al., 2017). Therefore, fruit and vegetables rich in polyphenols, especially anthocyanins, are attractive interventions against cardio-metabolic disease risk factors (Vendrame, Del Bo, Ciappellano, Riso, & Klimis-Zacas, 2016); as supported by epidemiological evidence (Arts & Hollman, 2005; Cassidy et al., 2013; Wallace, 2011).

Substantial literature (Amiot, Riva, & Vinet, 2016; Basu et al., 2011; Basu et al., 2010; Basu & Lyons, 2012; Basu et al., 2009; Stull et al., 2015; Vendrame et al., 2016) exists suggesting

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Angiotensin Converting Enzyme (ACE); Adenosine Monophosphate-activated Protein Kinase (AMPK); Augmentation Index (AIx); AIx normalised to 75 beats.min<sup>-1</sup> (AIx at HR75); Augmentation Pressure (AP); Blood Pressure (BP); Brachial Diastolic Blood Pressure (bDBP); Brachial Systolic Blood Pressure (bSBP); Carbohydrate (CHO); Cardiac Output (CO); Confidence Interval (CI); Diastolic Blood Pressure (DBP); Energy Expenditure (EE); Glucose Transporter 4 (GLUT-4); Glycated Haemoglobin (HbA1c); Heart Rate (HR); HOMA2-IR (Homeostatic Model Assessment of Insulin Resistance); HOMA2-β (Homeostatic Model Assessment of pancreatic β-cell function); HOMA2-%S (Homeostatic Model Assessment of Insulin Sensitivity); Hydroxypropyl methylcellulose (HPMC); Insulin-receptor Substrate 1 (IRS-1); Mean Arterial Pressure (MAP); Metabolic Syndrome (MetS); Montmorency Tart Cherry (MTC); Montmorency Tart Cherry Capsule (MTCC); Montmorency Tart Cherry Juice (MTCJ); New Zealand Blackcurrant Powder (NZBP); Placebo (PLA); Pulse Pressure (PP); Pulse Wave Analysis (PWA); Resting Metabolic Rate (RMR); RQ (Respiratory Quotient); Sodium-dependent Glucose Transporter-1 (SGLT-1); Stroke Volume (SV); Subendocardial Viability Ratio (SEVR); Systolic Blood Pressure (SBP); Total Peripheral Resistance (TPR); Type 2 Diabetes (T2D).

consumption of anthocyanin-rich dietary interventions are beneficial for mitigating the development of symptoms associated with MetS. Montmorency tart cherries (MTC) is one such intervention not only abundant in anthocyanins, but also other phenolics, resulting in being one of the most potent dietary antioxidant interventions when consumed in habitual portion sizes (Ou, Bosak, Brickner, Iezzoni, & Seymour, 2012). As far as the authors are aware, no study has been published assessing the responses of human participants with MetS, to MTC supplementation; however individual aspects of the MetS cluster have been explored in pathological populations. Keane et al. (2016b) showed significant reductions in systolic blood pressure (SBP) after acute ingestion of Montmorency Tart Cherry Juice (MTCJ) (60 mL concentrate) in hypertensive males. Additionally, Martin, Bopp, Neupane, and Vega-Lopez (2010) demonstrated significant reductions in serum triglycerides, triglyceride/HDL ratio and VLDL (very low-density lipoprotein) concentrations after 4 weeks tart cherry juice (230 mL.day<sup>-1</sup>) supplementation in participants with central adiposity and dyslipidaemia. Moreover, Ataie-Jafari, Hosseini, Karimi, and Pajouhi (2008) reported 6 weeks concentrated sour cherry juice (40 g.day<sup>-1</sup>) supplementation improved glycated haemoglobin (HbA<sub>1c</sub>), in T2D females, along with total cholesterol and LDL (low-density lipoprotein) in those participants with hyperlipidaemia.

Anthocyanin pharmacokinetics after consuming whole Montmorency tart cherries indicated parent anthocyanin concentrations in plasma peaked 2-4 hours post-ingestion, and excretion rates in urine peaked 6-8 hours post-ingestion (Seymour et al., 2014). Similarly, Bell et al. (2014a) reported increased uptake of cyanidin-3-*O*-glucoside in plasma, compared to baseline at 3- and 5-hours post-consumption of 30 mL MTC concentrate in healthy humans. Correspondingly, Keane et al. (2016a) reported the secondary metabolites of cyanidin-3-*O*-glucoside, protocatechuic and vanillic acids were also elevated at 1-hour and 2-hours post-consumption of 30 mL MTC concentrate, respectively, in healthy humans. The

pharmacokinetics of powdered freeze-dried MTC anthocyanins and metabolites are currently unknown when administered in encapsulated form, but bioavailability is anticipated to be enhanced. Anthocyanin bioavailability is notoriously poor (Kay, 2006), hence capsules as a delivery vehicle of anthocyanins to target tissues via systemic circulation may be useful as they selectively degrade once in the gastrointestinal tract, therefore potentially maximising bioavailability (Oidtmann et al., 2012). This is achieved due to protection of anthocyanins by the exterior capsule shell against factors known to influence anthocyanin stability such as pH, temperature, light, enzymes (particularly in saliva) and sugars (Robert & Fredes, 2015). Hydroxypropyl methylcellulose (HPMC) is a material used to form capsule shells, and has been shown to delay the release of green tea catechins (Glube, Moos, & Duchateau, 2013) and mulberry extract anthocyanins (Lown et al., 2017). Consequently, this delayed glucose and insulin responses until 30 minutes after ingestion, coinciding with the disintegration and dissolution of the capsule shell (Lown et al., 2017). Subsequently, Montmorency Tart Cherry Capsules (MTCC) will be provided to assess whether encapsulation, and thus protection of MTC anthocyanins from premature degradation, potentially maximises bioavailability and therefore their beneficial effects on cardio-metabolic function. Furthermore, the high sugar content in polyphenol-rich juices (Vendrame et al., 2016), including MTCJ (Desai, Bottoms, & Roberts, 2018), are purported to nullify and mask some of the beneficial cardio-metabolic effects induced by the polyphenols. Thus, MTCC, with a significantly lower sugar content, may be more efficacious at improving cardio-metabolic markers compared to MTCJ.

Centred around the pharmacokinetics of MTC parent anthocyanins and their metabolites, this study aimed to explore the short-term responses to acute supplementation of MTCJ and MTCC on cardiac haemodynamics, arterial stiffness by pulse wave analysis (PWA), substrate metabolism and various blood-based cardio-metabolic biomarkers in humans with MetS. Given that these subjects present at least three or more risk factors resulting in a holistic state

of cardio-metabolic dysregulation, it was hypothesised that both MTC interventions would induce favourable responses on diagnostic cardio-metabolic markers, in particular glucose, lipids and SBP, based on previous human studies with tart cherry supplementation (Ataie-Jafari et al., 2008; Keane et al., 2016b; Martin et al., 2010). Furthermore, it was hypothesised that MTCC would be more effective than MTCJ or placebo in mediating these benefits, as the bioavailability of anthocyanins and their metabolites were anticipated to be superior.

## **2. Methods**

### 2.1. Participants

Eleven (6 males and 5 females) participants (mean  $\pm$ SD, age  $49 \pm 12$  years, stature  $1.72 \pm 0.11$  m, body mass  $99.53 \pm 20.49$  kg) with Metabolic Syndrome (Table 1) volunteered for this pilot study. All participants were screened for MetS prior to formal inclusion onto the study according to the harmonised criteria outlined by Alberti et al. (2009), where 3 of the 5 qualifying criteria [waist circumference: ethnicity and sex specific criteria; fasting triglycerides:  $\geq 1.69$  mmol.L<sup>-1</sup>; fasting high-density lipoprotein:  $< 1.03$  mmol.L<sup>-1</sup> (men),  $< 1.29$  mmol.L<sup>-1</sup> (women); brachial blood pressure:  $\geq 130$  mmHg SBP or  $\geq 85$  mmHg DBP and/or fasting glucose:  $\geq 6.1$  mmol.L<sup>-1</sup>] had to be met. Recruitment (Figure 1) was conducted via word of mouth, flyers and email advertisements. Participants were excluded from the study if they did not meet the criteria for MetS at screening, were smokers, pregnant, heavy alcohol consumers ( $> 14$  units per week), current or previous diagnosis of chronic disease (gastrointestinal, cardiovascular, hepatic or renal), or were on statins, hyperlipidaemic, anti-hypertensive, anti-diabetic, anti-inflammatory or steroidal medication. All participants were instructed to abstain from consuming any other multivitamin, antioxidant, fish oil, dietary or herbal supplementation two weeks prior to and for the duration of the study. Verbal and written information was provided to all participants regarding the study purpose and procedures. Ethical approval was obtained from the University of Hertfordshire HSET Ethics Committee

(LMS/PGT/UH/02843) and the study's experimental procedures followed the principles outlined in the Declaration of Helsinki. Written informed consent was provided by all participants prior to enrolment. This study was registered prospectively as a clinical trial on [clinicaltrials.gov](https://clinicaltrials.gov) (NCT03615885).

Table 1. Baseline characteristics obtained during screening of all participants (n = 11).

Characteristics	Mean (95% CI)	Participant										
		1	2	3	4	5	6	7	8	9	10	11
BMI (kg.m <sup>-2</sup> )	33 (30 – 37)	30	39	28	29	44	36	28	36	32	36	29
Waist Circumference (cm)	106 (97 – 115)	102.2*	133*	93	90.8	119*	119*	95	104.1*	94	112*	104*
Fasting Glucose (mmol.L <sup>-1</sup> )	5.63 (5.12 – 6.14)	6.20*	5.33	6.72*	4.48	5.93	6.50*	6.21*	5.12	5.78	4.85	4.79
Fasting Triglycerides (mmol.L <sup>-1</sup> )	1.9 (1.57 – 2.23)	1.9*	2.0*	2.6*	1.7*	0.9	1.5	2.6*	2.1*	2.0*	2.1*	1.5
Fasting HDL (mmol.L <sup>-1</sup> )	1.23 (0.99 – 1.48)	2.23	1.25	1.22*	0.94*	1.23*	0.83*	1.19*	1.30	1.06*	1.28*	1.01*
bSBP (mmHg)	132 (127 – 138)	122	146*	130*	130*	136*	143*	129	132*	142*	120	126
bDBP (mmHg)	80 (74 – 87)	67	90*	86*	87*	79	91*	72	72	88*	67	85*
Fasting Insulin (pmol.L <sup>-1</sup> )	115.85 (64.37 – 167.34)	53.10	81.65	182.85	72.37	216.88	280.16	116.33	56.81	91.83	62.09	60.29
HOMA2-IR (AU)	2.2 (1.2 – 3.2)	1.0	1.5	3.5	1.3	4.0	5.3	2.3	1.1	1.8	1.1	1.1
HOMA2-β (%)	121.3 (97.4 – 145.2)	61.8	111.7	126.2	145.2	179.2	181.9	106.1	94.5	103.4	111.8	112.4
HOMA2-%S (%)	61.8 (42.3 – 81.4)	95.5	64.9	28.2	76.5	24.7	19.0	44.3	93.6	56.7	87.0	89.8

\*Denotes value met threshold for MetS inclusion criteria. AU (Arbitrary Units); BMI (Body Mass Index); bDBP (Brachial Diastolic Blood Pressure); bSBP (Brachial Systolic Blood Pressure); HDL (High-density Lipoprotein); HOMA2-IR (Homeostatic Model Assessment of Insulin Resistance); HOMA2- $\beta$  (Homeostatic Model Assessment of pancreatic  $\beta$ -cell function); HOMA2-%S (Homeostatic Model Assessment of Insulin Sensitivity).



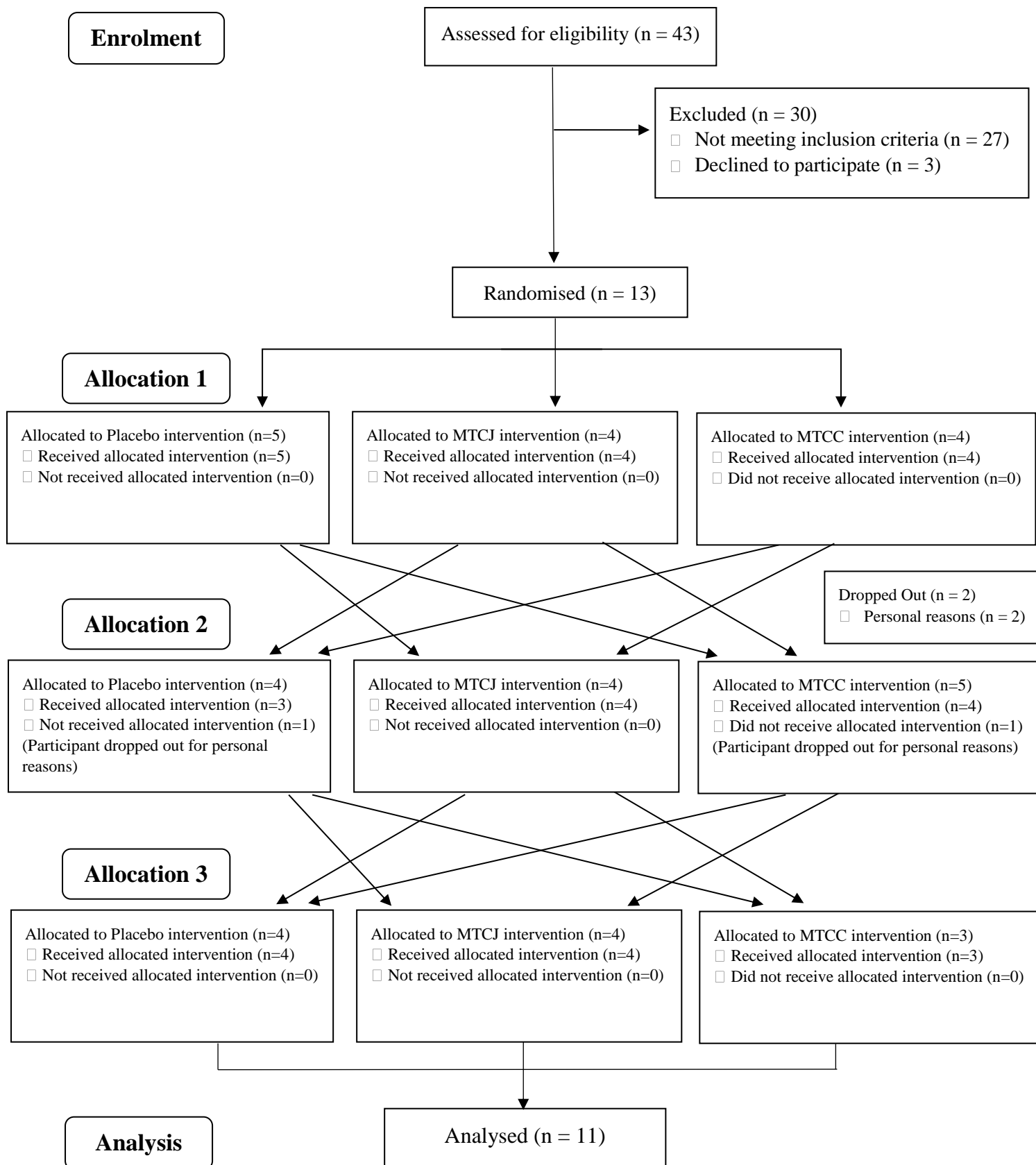


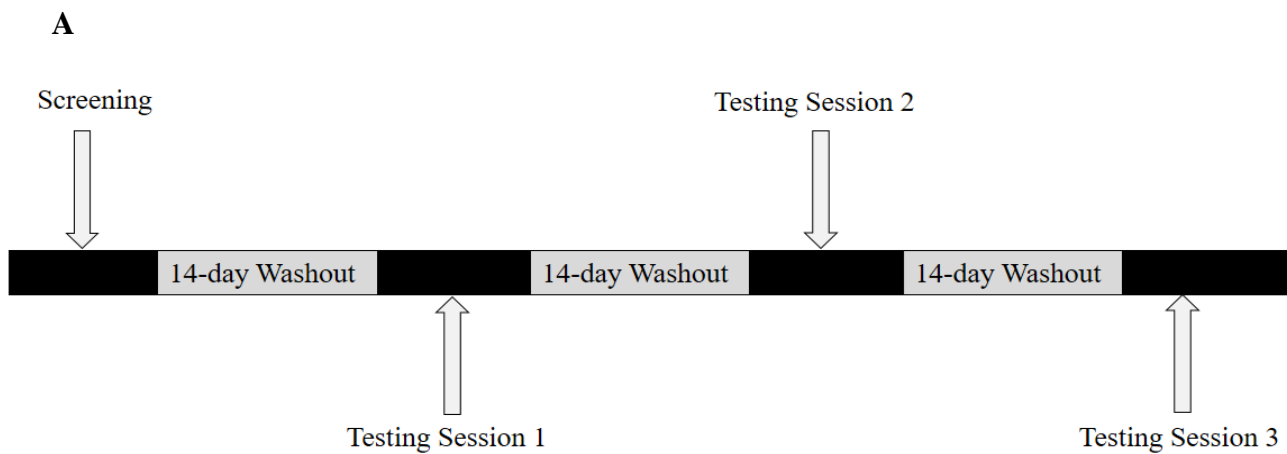
Figure 1. CONSORT flow diagram of the participants recruited, screened, tested, analysed and excluded during the course of the study.

## 2.2. Procedures

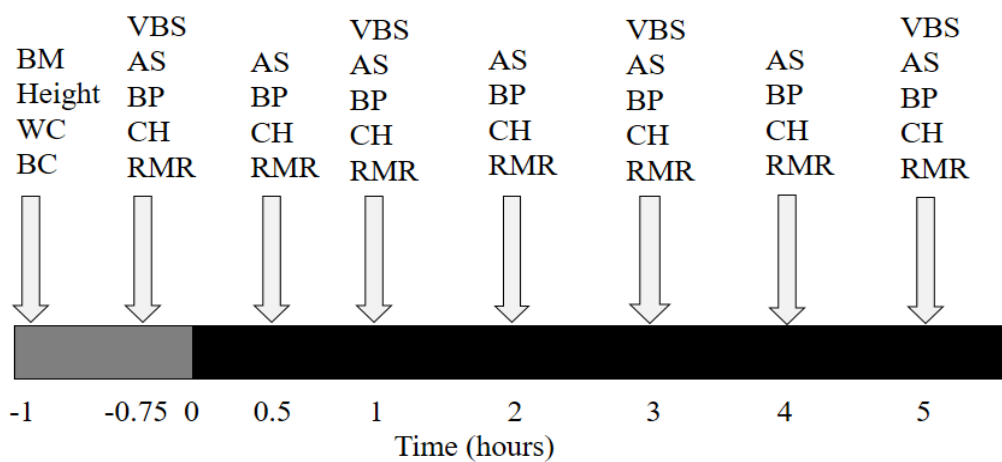
### 2.2.1. Research Design

A single-blind (blinded to participant), placebo-controlled, randomised, crossover design was implemented; each participant acted as their own control. During the 6-week study duration, all participants completed three testing sessions during which one of three [placebo (PLA) or MTCJ or MTCC] different supplements were provided each time. GraphPad software (GraphPad, QuickCalcs, San Diego, California, USA) was used to randomise supplementation order. A 14-day washout period (Cook, Myers, Blacker, & Willems, 2015; Howatson et al., 2012; Keane et al., 2016c) was incorporated between testing sessions and between the screening and first testing sessions.

Testing sessions lasted 6 hours and were identical in terms of design and testing procedures. Figure 2 depicts schematics of the overall study design and specific procedures during a testing session. Baseline anthropometric (stature, body mass, waist circumference) and functional (PWA, cardiac haemodynamics, RMR) measurements were obtained prior to consumption of the supplement. Functional measurements were recorded at 30 minutes, 1, 2, 3, 4- and 5-hours post-bolus. Venous blood sampling was performed at baseline, 1, 3- and 5-hours post-bolus only.



**B**



**Key:**

AS – Arterial Stiffness; BC – Body Composition; BM – Body Mass; BP – Blood Pressure; CH – Cardiac Haemodynamics; RMR – Resting Metabolic Rate; VBS – Venous Blood Sampling; WC – Waist Circumference

■ = Pre-Bolus  
 ■ = Post-Bolus

Figure 2. (A) Schematic representation of the overall study design. (B) Schematic representation of the specific procedures during each testing session.

### 2.2.2. Dietary Guidelines

All participants arrived at the laboratory between 7 – 10am, after an overnight fast of a minimum of 10 hours, to account for circadian variation (Bell, Walshe, Davison, Stevenson, & Howatson, 2014c). Participants were instructed to maintain their habitual polyphenol intake, including anthocyanins, as opposed to complete restriction throughout the study. This was to ensure that the polyphenols provided by MTCJ were supplementary to the existing habitual polyphenol intake of each participant representing normal daily activity and therefore upholding ecological validity. Fluids or food were not provided during testing sessions, but participants were able to drink water *ad libitum*, where volume of consumption was monitored during the first session and repeated in subsequent sessions (Keane et al., 2016b).

Total energy, macronutrient and polyphenol intake of participants' 'Western' habitual diet was analysed through food diaries. This was to assess compliance of replicating dietary intake for the 3 days prior to each testing session (Alkhatib, Seijo, Larumbe, & Naclerio, 2015; Nordby, Saltin, & Helge, 2006; Roberts, Roberts, Tarpey, Weekes, & Thomas, 2015). Participants were given instructions on how to complete the food diaries, including portion sizes. Dietary analysis software (Dietplan 7.0, Forestfield Software, UK) was used to monitor compliance of the 3-day food diaries before each session. All participants complied with dietary guidelines upon analysis for percentage contributions of macronutrients to total energy intake [mean  $\pm$  SD (protein 16  $\pm$  23%), (CHO 45  $\pm$  56%), (fat 39  $\pm$  38%)], total polyphenols (70  $\pm$  83 mg) and anthocyanins (26  $\pm$  22 mg).

### 2.2.3. Supplementation

This study acutely administered three different supplements including a placebo which acted as the control condition and two experimental conditions, MTCJ and MTCC. The placebo was composed of 30 mL commercially available fruit-flavoured cordial (Cherries and Berries, Morrisons, Bradford, UK) mixed with 100 mL water. The placebo drink was also matched

against MTCJ for energy content, taste and visual appearance by adding a flavourless carbohydrate (Maltodextrin, My Protein Ltd, Northwich, UK), citric acid (100% Pure Citric Acid, VB and Sons, UK), red and black food colouring (Morrisons, Bradford, UK), respectively. MTCJ consisted of 30 mL Montmorency tart cherry concentrate (Cherry Active, Active Edge Ltd, Hanworth, UK) and 100 mL water. Each 30 mL serving of MTC concentrate provided a total anthocyanin content of 270 mg (9 mg.mL<sup>-1</sup>) (Howatson et al., 2010). In MTC concentrate, the most abundant anthocyanins include, cyanidin-3-glucosylrutinoside, cyanidin-3-rutinoside and peonidin-3-rutinoside (Bell et al., 2014a). In order to match the anthocyanin content of MTCJ, participants consumed 10 Montmorency tart cherry capsules (Cherry Active Capsules, Active Edge, Ltd, Hanworth, UK) with 130 mL water. Each capsule contained 435 mg of freeze-dried Montmorency tart cherry skin powder (Table 2). Independent analysis (AtlasBiosciences, 2018) showed total anthocyanin content in each capsule was 27 mg. The 30 mL serving of Montmorency tart cherry concentrate contained ~90-110 whole Montmorency tart cherries whilst 10 capsules were made from ~100 whole cherries. Anonymity of supplementation was ensured by blinding the participants to the source of anthocyanins. This was achieved by explaining that an ‘anthocyanin-rich supplement’ would be provided rather than disclosing Montmorency tart cherries as the specific source.

Table 2. Nutritional information of each supplement provided.

	Montmorency tart cherry concentrate (per 30 mL)	Montmorency tart cherry capsules (per capsule – 435 mg)	Placebo (per 30 mL)
Energy (kcal)	102	1.3	102
Carbohydrate (g)	24.5	0.3	25.35
of which sugars (g)	17.9	0.1	25.32
Protein (g)	1.1	Trace	0.03
Fat (g)	0	Trace	0

of which saturates (g)	0	0	0
Fibre (g)	2.6	Trace	Trace
Total Anthocyanins (mg)	270	27	0

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#### 2.2.4. Measures and Equipment

##### 2.2.4.1. Blood Pressure

Brachial systolic (bSBP) and diastolic (bDBP) blood pressure (Omron MX3, Omron, Japan) were measured four times in an upright seated position, with an average of the final three being taken as BP (Cook et al., 2015).

##### 2.2.4.2. Cardiac Haemodynamics

Beat-to-beat resting cardiac haemodynamic parameters including heart rate (HR), cardiac output (CO), stroke volume (SV), mean arterial pressure (MAP) and total peripheral resistance (TPR) were measured non-invasively (Finometer MIDI Model-2, Finapres Medical Systems BV, Amsterdam, The Netherlands) at all time points, using the arterial volume clamp method (Penaz, 1992). To avoid data selection, recordings were averaged over 10 consecutive beats with the lowest values for each parameter taken for statistical analysis (Cook, Myers, Gault, Edwards, & Willems, 2017).

##### 2.2.4.3. Pulse Wave Analysis

Pulse wave analysis (PWA) was used to monitor arterial stiffness non-invasively using applanation tonometry (SphygmoCor, ScanMed Medical, UK), as there is a strong association between PWA and cardiovascular disease incidents, independent of traditional risk factors (Choi et al., 2004; Keane et al., 2016b). A pencil-type probe was placed over the radial artery on the right wrist which was slightly extended and rested on a pillow (Doupis, Papanas, Cohen,

McFarlan, & Horton, 2016). Aortic pulse waveforms, aortic SBP, aortic DBP, pulse pressure (PP), augmentation pressure (AP), augmentation index (AIx), AIx normalised to 75 beats.min<sup>-1</sup> (AIx at HR75) and subendocardial viability ratio (SEVR) were determined by the in-built software (SphygmoCor version 7.0, ScanMed, UK) using generalised transfer functions. Recordings with a quality-index  $\geq 80\%$ , as determined by the manufacturer, were selected for statistical analysis.

#### 2.2.4.4. Resting Metabolic Rate (RMR)

RMR, resting energy expenditure, substrate oxidation rates and respiratory quotient (RQ) were measured using an open-circuit indirect calorimetry system (GEM Nutrition Ltd, Cheshire, UK). Participants lay supine for 20 minutes with data averaged for the final 17 minutes only, to achieve steady-state and account for any initial short-term variances in respiration (Kelly, King, Goerlach, & Nimmo, 2013). A ventilated hood was placed over the head with a flexible plastic seal around the neck and shoulders to prevent air inside and outside the hood from mixing.

Resting fat and carbohydrate oxidation rates were calculated using stoichiometric equations, assuming negligible protein oxidation (Jeukendrup & Wallis, 2005).

$$\text{Fat Oxidation Rate (g.min}^{-1}\text{)} = (1.695 \cdot \dot{V}O_2) - (1.701 \cdot \dot{V}CO_2) \quad (1)$$

$$\text{CHO Oxidation Rate (g.min}^{-1}\text{)} = (4.344 \cdot \dot{V}CO_2) - (3.061 \cdot \dot{V}O_2) \quad (2)$$

#### 2.2.5. Blood Sampling and Analysis

##### 2.2.5.1. Blood Sampling

Venous blood was sampled through 4 individual venepunctures (one at each time point: pre-bolus and 1, 3, 5 hours post-bolus), using the butterfly method (BD Vacutainer Safety-Lok Blood Collection Set 21G with Luer Adapter, Becton Dickinson and Co., Oxford, UK). A total

of 5 mL venous blood was collected into serum-separation tubes (BD Vacutainer, Becton Dickinson and Co., Oxford, UK) for each sample. Tubes were centrifuged at 4000 rev.min<sup>-1</sup>, 4°C for 10 minutes (Sorvall ST 8R, Thermo Fisher Scientific, USA). Serum supernatants were aliquoted and stored at -80°C for later analysis.

#### 2.2.5.2. Glucose

Serum samples were assessed for glucose (range 0.5-50 mmol.L<sup>-1</sup>, CV ≤ 1.5%) (Biosen C-Line, EKF Diagnostics, Cardiff, UK) in duplicates.

#### 2.2.5.3. Insulin Assay

Serum insulin samples were measured in duplicates using a human 96-well colorimetric insulin enzyme-linked immunosorbent assay (ELISA) (Insulin Human ELISA KAQ1251, Invitrogen, Thermo Fisher Scientific, USA), with a common sample measured on each plate for determining inter-plate CV. A four-parameter algorithm standard curve was generated using standard curve fitting software (SigmaPlot, Systat Software Inc, San Jose, USA), where values were converted from μIU.mL<sup>-1</sup> to pmol.L<sup>-1</sup> by multiplying by 6.945 (Bos et al., 2010). Inter- and intra-plate precision were 8.8% and 7.69%, respectively.

#### 2.2.5.4. Insulin Resistance and Sensitivity Indexes

Homeostatic Model Assessment (HOMA) was used to estimate fasting steady-state pancreatic β-cell function (HOMA2-β) and insulin resistance (HOMA2-IR index) through the HOMA2 spreadsheet model (HOMA2-IR, available from <https://www.dtu.ox.ac.uk/homacalculator/>) (Levy, Matthews, & Hermans, 1998). *Equations 1, 2 and 3* (Matthews et al., 1985), outline the determination of pancreatic β-cell function, insulin sensitivity (HOMA2-%S) and insulin resistance, respectively. As recommended by Wallace, Levy, and Matthews (2004), HOMA2-%S was calculated to assist in the correct interpretation of HOMA2-β.



$$\text{HOMA2-}\beta \text{ (\%)} = \frac{(20 \cdot \text{Fasting Insulin } (\mu\text{IU.mL}^{-1}))}{(\text{Fasting Glucose } (\text{mmol.L}^{-1}) - 3.5)} \cdot 100 \quad (3)$$

$$\text{HOMA2-}\%S \text{ (\%)} = \left( \frac{1}{\text{HOMA-IR}} \right) \cdot 100 \quad (4)$$

$$\text{HOMA2-IR} = \frac{[\text{Fasting Glucose } (\text{mmol.L}^{-1}) \cdot \text{Fasting Insulin } (\mu\text{IU.mL}^{-1})]}{22.5} \quad (5)$$

#### 2.2.5.5. Lipid Assays

Serum lipids were determined in duplicates using commercially available colorimetric assays on a semi-automated spectrophotometer (RX Monza, Randox Laboratories Ltd, Antrim, UK), according to manufacturer's guidelines. Triglyceride (Triglycerides TR210, Randox) values were corrected for free glycerol by subtracting 0.11 mmol.L<sup>-1</sup> (Stinshoff et al., 1977), according to the manufacturer's guidelines. Intra-assay CV for triglycerides, total cholesterol and HDL were 2.33%, 4.45% and 3.63%, respectively. LDL was determined indirectly using the formula below (Ahmadi, Boroumand, Gohari-Moghaddam, Tajik, & Dibaj, 2008).

$$\text{LDL (mmol.L}^{-1}\text{)} = \left( \frac{\text{Total Cholesterol}}{1.19} \right) + \left( \frac{\text{Triglycerides}}{0.81} \right) - \left( \frac{\text{HDL}}{1.1} \right) - 0.98 \quad (6)$$

#### 2.3. Data Analysis

Statistical analysis was performed using SPSS v22.0 (IBM, Chicago, USA) where data are reported as means [95% Confidence Intervals] (CI). Data normality was checked using a Shapiro-Wilk test. Greenhouse-Geisser correction was applied upon violation of Mauchly's test of sphericity for ANOVAs. Statistical significance was set at  $P < 0.05$ . Due to this being a pilot study and the lack of prior data on the effects of Montmorency tart cherry products in humans with MetS from which to conduct power calculations, a minimum sample size of 10 was established (Udani, Singh, Singh, & Barrett, 2011). Subsequently, the statistics performed are exploratory given the small sample size.

To account for day-to-day physiological variances at pre-bolus between conditions for each variable, data was analysed as change from pre-bolus for each time point measured post-bolus. This enabled a fair assessment of the post-bolus responses to each condition from pre-bolus across all variables. The pre-bolus time point was not included as a covariate, as one-way ANOVA analysis indicated no significant differences between conditions for all variables at the pre-bolus time point, hence two-way repeated-measures ANOVA was performed.

A within-group two-way, 3 x 6, condition (PLA vs MTCC vs MTCJ) x time (30 minutes, 1, 2, 3, 4- and 5-hours post-bolus), repeated-measures ANOVA design with *post-hoc* Bonferroni's adjustment, measured differences for bSBP, bDBP, RMR, cardiac haemodynamic and PWA parameters on change from pre-bolus values for each condition.

Blood-based biomarkers were analysed using the same model but with a 3 x 3, condition (PLA vs MTCJ vs MTCC) by time (1, 3- and 5-hours post-bolus) design on change from pre-bolus values for each condition. A paired-samples t-test was used to identify differences between individual data points within conditions and between conditions for the corresponding time point.

Partial Eta-Squared ( $\eta^2_{partial}$ ) was used to report effect sizes for ANOVA where effects were classified as small (0.01-0.08), moderate (0.09-0.25) and large (>0.25) (Cohen, 1998). Cohen's *d* effect size was used for paired-samples t-test and *post-hoc* interaction comparisons where effects were classified as no effect (0-0.1), small (0.2-0.4), moderate (0.5-0.7) and high ( $\geq 0.8$ ) (Cohen, 1998).

### **3. Results**

The present study measured variables relevant to MetS, and as the defining aspect is insulin resistance, serum insulin and glucose were regarded as primary variables. Other variables

focused on the other two main aspects of MetS, cardiovascular dysfunction and lipidaemia. Raw data for all variables is provided in Supplemental Tables 1-4.

### 3.1. Blood Biomarkers

Analysis of change from pre-bolus responses for serum insulin (Figure 3) showed a significant main effect for condition ( $F_{(2, 20)} = 3.653$ ;  $P = 0.044$ ,  $\eta^2_{partial} = 0.27$ ), time ( $F_{(1.07, 10.67)} = 13.411$ ;  $P = 0.000$ ,  $\eta^2_{partial} = 0.57$ ) and interaction ( $F_{(4, 40)} = 5.837$ ;  $P = 0.001$ ,  $\eta^2_{partial} = 0.37$ ). The change from pre-bolus to 1-hour post-bolus between PLA (60.84 [24.33 – 97.34] pmol.L<sup>-1</sup>) and MTCC (-9.86 [-25.16 – 5.46] pmol.L<sup>-1</sup>) was significantly different ( $P = 0.016$ ,  $d = 1.70$ ); 7/11 participants lowered insulin with MTCC compared to PLA. The change from pre-bolus to 3-hours post-bolus between PLA (-8.99 [-35.55 – 17.57] pmol.L<sup>-1</sup>) and MTCJ (-48.80 [-82.40 – -15.20] pmol.L<sup>-1</sup>) was significantly different ( $P = 0.028$ ,  $d = 0.88$ ); 9/11 participants had lower insulin with MTCJ compared to PLA. Mean post-bolus responses showed PLA (12.01 [-4.42 – 28.44] pmol.L<sup>-1</sup>) increased more than MTCJ (-18.01 [-40.33 – 4.31] pmol.L<sup>-1</sup>) ( $P = 0.039$ ). The change from 1- to 3-hours post-bolus was significant between PLA and MTCC ( $t_{(10)} = -2.295$ ;  $P = 0.045$ ,  $d = 0.85$ ) and between MTCJ and MTCC ( $t_{(10)} = 3.361$ ;  $P = 0.007$ ,  $d = 1.06$ ).

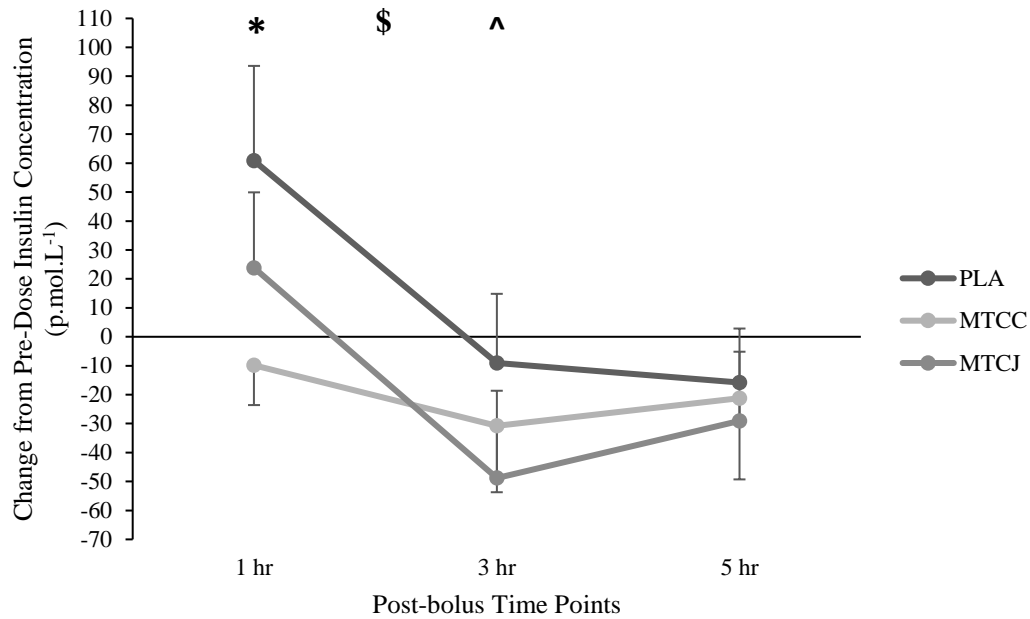


Figure 3. Response curves for the mean (error bars indicate 95% CI) change in insulin concentrations from pre-bolus values to time points post-bolus for each condition. \*Denotes significant difference between PLA and MTCC at 1-hour time point. ^Denotes significant difference between PLA and MTCJ at 3-hour time point. §Denotes significant difference for change in insulin from 1-3 hours post-bolus between PLA and MTCJ against MTCC.

Individual responses showed 10/11 participants responded with an increase in LDL concentrations at 5-hours post-bolus ( $P = 0.057$ ,  $d = 0.77$ ) with PLA (0.71 [0.33 – 1.09] mmol.L<sup>-1</sup>) compared to MTCC (0.10 [-0.27 – 0.47] mmol.L<sup>-1</sup>). Change from pre-bolus responses for serum glucose did not show significant main effects for condition, time or interaction ( $P > 0.05$ ). Statistical analysis for total cholesterol (TC) ( $F_{(1,159, 11.587)} = 6.470$ ;  $P = 0.023$ ,  $\eta^2_{partial} = 0.39$ ), indicated a main effect for time only (Table 3). No main effects for condition, time or interaction were detected for triglycerides and HDL ( $P > 0.05$ ) (Table 3).

Table 3. Mean [95% CI] change from pre-bolus values to post-bolus time points for selected blood-based biomarkers per treatment condition.

		Post-bolus Time Points		
		1 hr	3 hr	5 hr
Glucose (mmol.L <sup>-1</sup> )	PLA	0.35 [-0.77 – 1.46]	-0.58 [-1.16 – -0.01]	-0.63 [-1.07 – -0.18]
	MTCC	-0.11 [-0.37 – 0.12]	-0.22 [-0.59 – 0.19]	-0.28 [-0.80 – 0.25]
	MTCJ	-0.22 [-0.66 – 0.24]	-0.79 [-1.42 – -0.13]	-0.72 [-1.55 – 0.10]
Triglycerides (mmol.L <sup>-1</sup> )	PLA	0.09 [-0.06 – 0.26]	0.11 [-0.15 – 0.38]	0.21 [-0.08 – 0.49]
	MTCC	-0.05 [-0.09 – 0.08]	-0.06 [-0.31 – 0.20]	-0.05 [-0.29 – 0.18]
	MTCJ	-0.05 [-0.10 – 0.15]	0.03 [-0.10 – 0.19]	0.09 [-0.17 – 0.33]
Total Cholesterol* <sup>§</sup> (mmol.L <sup>-1</sup> )	PLA	0.04 [-0.16 – 0.23]	0.16 [-0.15 – 0.48]	0.29 [0.03 – 0.55]
	MTCC	-0.16 [-0.50 – 0.19]	-0.16 [-0.55 – 0.26]	0.15 [-0.31 – 0.57]
	MTCJ	-0.05 [-0.21 – 0.11]	-0.03 [-0.16 – 0.10]	0.13 [-0.02 – 0.27]
HDL (mmol.L <sup>-1</sup> )	PLA	-0.12 [-0.15 – 0.06]	-0.10 [-0.12 – 0.02]	-0.18 [-0.26 – 0.09]
	MTCC	-0.05 [-0.16 – 0.05]	-0.04 [-0.11 – 0.03]	-0.03 [-0.15 – 0.09]
	MTCJ	-0.07 [-0.15 – 0.01]	0.01 [-0.06 – 0.09]	-0.09 [-0.21 – 0.03]
LDL (mmol.L <sup>-1</sup> )	PLA	0.25 [-0.08 – 0.57]	0.36 [-0.04 – 0.76]	0.71 [0.33 – 1.09]
	MTCC	0.30 [-0.44 – 0.51]	-0.07 [-0.64 – 0.51]	0.06 [-0.38 – 0.51]
	MTCJ	0.08 [-0.27 – 0.43]	0.12 [-0.10 – 0.34]	0.24 [-0.20 – 0.68]

AU (Arbitrary Units); HDL (High-density Lipoprotein); LDL (Low-density Lipoprotein); TC (Total Cholesterol). \*Denotes significant main effect for time with *post-hoc* identifying differences between 1-hour and 3-hours post-bolus. <sup>§</sup>Denotes significant main effect for time with *post-hoc* identifying differences between 3-hour and 5-hour post-bolus.

### 3.2. Cardiac Haemodynamics

No significant main effects for condition, time or the condition by time interaction were detected for bDBP, MAP, SV, CO, TPR ( $P > 0.05$ ) (Table 4).

The change from pre-bolus to 2-hours post-bolus between PLA and MTCJ tended to be significantly different ( $P = 0.051$ ,  $d = 1.48$ ); 9/11 participants had lower MAP with MTCJ compared to PLA.

Despite there being no main effects for condition, time or interaction, pairwise comparisons revealed a significant difference ( $P = 0.018$ ,  $d = 1.75$ ) between PLA (3 [-1 – 8] mmHg) and

MTCJ (-8 [-13 – -4] mmHg) at 2-hours post-bolus for bSBP (Figure 4). Individual responses showed 10/11 participants responded with a lower bSBP after MTCJ consumption compared to PLA. Moreover, the mean difference (-11 [-19 – -4] mmHg) in responses between PLA and MTCJ at 2-hours post-bolus was greater than the clinically relevant threshold of 5 mmHg in 8/11 participants.

Heart rate responses revealed a significant main effect for time ( $F_{(1,992, 19,917)} = 6.854$ ;  $P = 0.005$ ,  $\eta^2_{partial} = 0.41$ ) and the condition by time interaction ( $F_{(10, 100)} = 5.301$ ;  $P = 0.000$ ,  $\eta^2_{partial} = 0.35$ ). *Post-hoc* analysis displayed a tendency towards significance for HR at 30 minutes post-bolus between PLA (-1 [-3 – 1] beats.min<sup>-1</sup>) and MTCC (-5 [-9 – -2] beats.min<sup>-1</sup>) ( $P = 0.080$ ,  $d = 0.27$ ), where all 11 participants responded with lower HR at 30 minutes post-bolus with MTCC compared to PLA. Individual responses at 30 minutes post-bolus showed SV increased in 8/11 participants with MTCC compared to PLA.

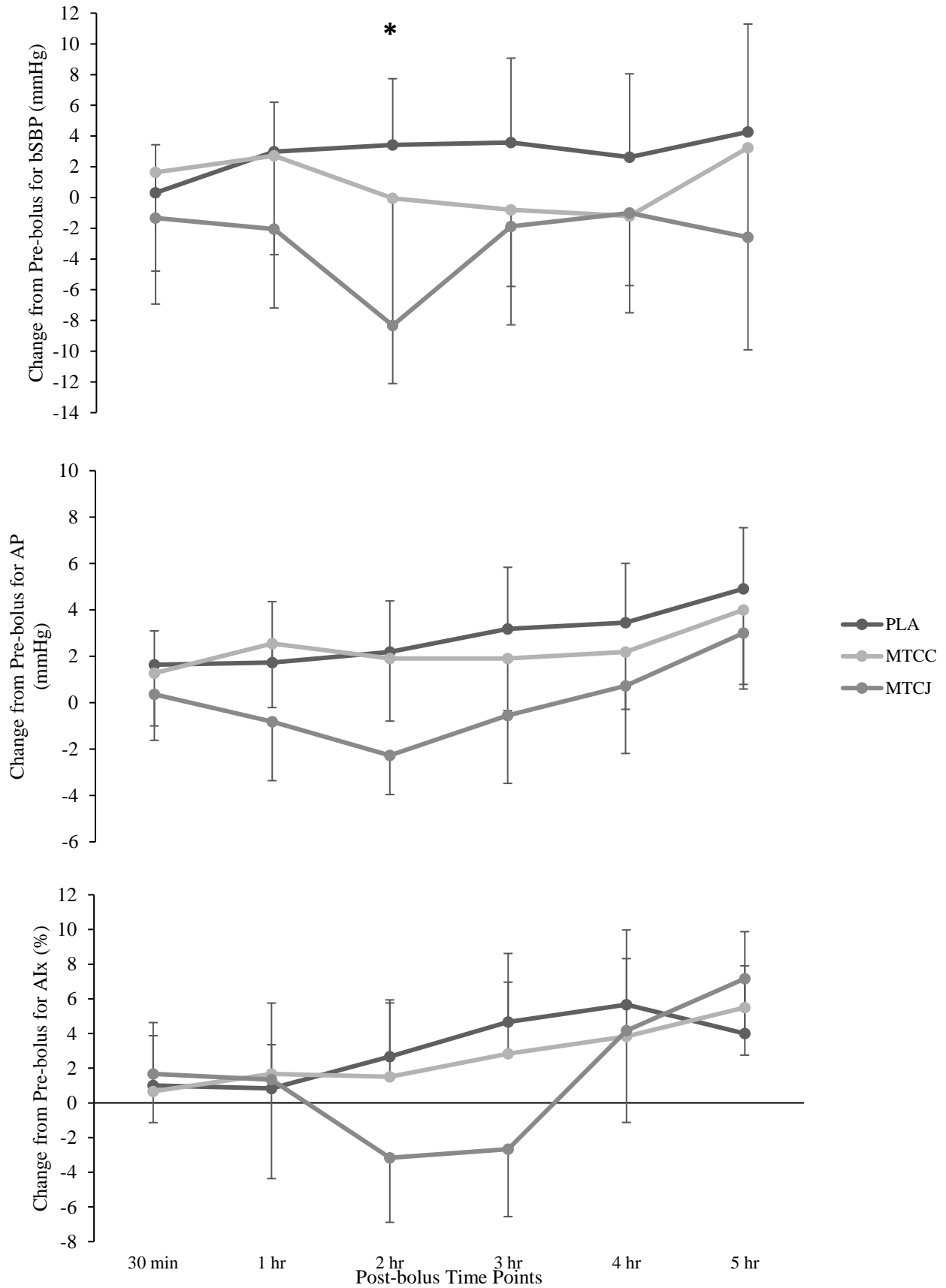


Figure 4. Mean (error bars indicate 95% CI) bSBP, augmentation pressure (AP) and augmentation index (AIx) response curves from respective pre-bolus time points for each condition. \*Denotes significant difference between PLA and MTCJ at 2-hours post-bolus.

Table 4. Mean [95% CI] change from pre-bolus values to post-bolus time points for selected cardiac haemodynamic and PWA parameters per condition.

		Post-bolus Time Points					
		30 minutes	1 hr	2 hr	3 hr	4 hr	5 hr
Brachial DBP (mmHg)	PLA	3 [0 – 5]	5 [0– 10]	5 [2 – 9]	8 [3 – 13]	5 [0 – 9]	4 [0 – 8]
	MTCC	1 [-5 – 7]	4 [-2 – 11]	2 [-4 – 9]	0 [-4 – 4]	1 [-5 – 6]	3 [-2 – 9]
	MTCJ	3 [-1 – 7]	6 [3 – 9]	3 [0 – 6]	7 [3 – 11]	6 [1 – 11]	6 [2 – 10]
MAP (mmHg)	PLA	1 [-2 – 5]	5 [0 – 10]	5 [0 – 9]	7 [1 – 13]	5 [-1 – 11]	5 [-1 – 11]
	MTCC	3 [-4 – 11]	6 [0 – 13]	2 [-5 – 9]	2 [-2 – 7]	1 [-5 – 7]	5 [0 – 11]
	MTCJ	2 [0 – 4]	2 [-2 – 6]	-4 [-7 – 1]	4 [0– 8]	4 [-2 – 9]	-2 [-10 – 6]
Cardiac Output (L.min <sup>-1</sup> )	PLA	0.11 [-0.53 – 0.75]	-0.02 [-1.08 – 1.03]	-0.31 [-1.20 – 0.58]	-0.58 [-1.27 – 0.11]	-0.53 [-1.64 – 0.57]	-0.35 [-1.32 – 0.61]
	MTCC	-0.10 [-0.56 – 0.35]	-0.24 [-0.91 – 0.43]	-0.13 [-0.90 – 0.64]	0.03 [-0.69 – 0.74]	-0.31 [-1.31 – 0.68]	-0.21 [-1.13 – 0.71]
	MTCJ	-0.33 [-1.54 – 0.87]	-0.46 [-1.45 – 0.52]	-0.04 [-1.37 – 1.28]	-0.34 [-1.64 – 0.97]	-0.62 [-1.62 – 0.39]	-0.61 [-1.66 – 0.43]
Stroke Volume (L)	PLA	1 [-9 – 12]	-1 [-14 – 11]	-1 [-11 – 9]	-5 [-17 – 8]	-5 [-21 – 12]	2 [-15 – 18]
	MTCC	6 [-1 – 13]	-1 [-12 – 11]	4 [-1 – 10]	6 [-1 – 17]	1 [-12 – 13]	3 [-10 – 17]
	MTCJ	1 [-17 – 19]	-4 [-16 – 9]	1 [-17 – 19]	-4 [-24 – 16]	1 [-14 – 17]	2 [-14 – 17]
TPR (mmHg.min <sup>-1</sup> .L)	PLA	0.00 [-0.14 – 0.14]	0.15 [-0.10 – 0.40]	0.14 [-0.06 – 0.35]	0.26 [-0.05 – 0.57]	0.16 [-0.02 – 0.34]	0.09 [-0.09 – 0.26]
	MTCC	0.01 [-0.10 – 0.13]	0.09 [-0.10 – 0.28]	0.02 [-0.23 – 0.26]	0.08 [-0.14 – 0.29]	0.09 [-0.17 – 0.35]	0.12 [-0.11 – 0.35]
	MTCJ	0.08 [-0.14 – 0.31]	0.07 [-0.11 – 0.25]	-0.03 [-0.29 – 0.23]	0.10 [-0.13 – 0.32]	0.10 [-0.09 – 0.28]	0.11 [-0.13 – 0.34]
HR (beats.min <sup>-1</sup> )	PLA	-1 [-3 – 1]	-3 [-6 – -1]	-5 [-8 – -1]	-5 [-10 – -1]	-7 [-12 – -1]	-7 [-11 – -2]
	MTCC	-5 [-9 – -2]	-4 [-7 – 0]	-4 [-9 – 1]	-6 [-11 – -2]	-5 [-12 – 1]	-4 [-10 – 3]
	MTCJ	0 [-3 – 4]	-1 [-5 – 2]	-5 [-9 – -1]	-7 [-11 – -4]	-7 [-10 – -4]	-8 [-11 – -4]
Aortic SBP (mmHg)	PLA	1 [-4 – 6]	3 [-3 – 8]	4 [-2 – 10]	2 [-5 – 10]	4 [-1 – 10]	5 [2 – 8]
	MTCC	2 [-2 – 5]	4 [-3 – 10]	4 [-6 – 13]	2 [-5 – 8]	2 [-4 – 7]	4 [0 – 7]
	MTCJ	-1 [-5 – 5]	-1 [-6 – 4]	-2 [-7 – 2]	1 [-2 – 3]	2 [-3 – 6]	3 [-2 – 8]



Aortic DBP (mmHg)	PLA	0 [-4 – 4]	0 [-5 – 5]	3 [-6 – 12]	3 [0 – 6]	4 [-1 – 8]	2 [-3 – 6]
	MTCC	5 [1 – 9]	3 [-1 – 6]	4 [-2 – 9]	0 [-6 – 6]	3 [-2 – 8]	6 [1 – 10]
	MTCJ	-2 [-6 – 2]	-2 [-6 – 2]	2 [-1 – 4]	1 [-4 – 6]	1 [-4 – 6]	1 [-2 – 4]
Pulse Pressure (mmHg)	PLA	1 [-2 – 9]	3 [-2 – 9]	1 [-5 – 7]	-1 [-4 – 7]	0 [-1 – 8]	3 [1 – 8]
	MTCC	-3 [-6 – 4]	1 [-5 – 7]	0 [-3 – 6]	2 [-3 – 3]	-1 [-5 – 4]	-2 [-4 – 6]
	MTCJ	1 [-6 – 2]	1 [-5 – 2]	-4 [-7 – 2]	0 [-3 – 7]	1 [-4 – 4]	2 [-1 – 6]
AIx at HR75* <sup>^</sup> <sup>§</sup> (%)	PLA	0 [-2 – 2]	-1 [-3 – 1]	2 [-2 – 6]	2 [-1 – 5]	3 [-1 – 6]	5 [2 – 8]
	MTCC	0 [-4 – 4]	-1 [-5 – 3]	-2 [-6 – 2]	0 [-3 – 4]	2 [-2 – 5]	3 [-2 – 8]
	MTCJ	0 [-2 – 3]	-1 [-6 – 4]	-2 [-7 – 2]	-2 [-7 – 2]	0 [-5 – 5]	2 [-2 – 7]
SEVR (%)	PLA	-1 [-24 – 21]	11 [-3 – 24]	15 [-3 – 33]	10 [-4 – 24]	16 [-1 – 34]	3 [-11 – 17]
	MTCC	15 [0 – 30]	9 [-1 – 20]	17 [3 – 31]	16 [1 – 31]	19 [3 – 35]	9 [-7 – 26]
	MTCJ	6 [-11 – 24]	8 [-7 – 23]	31 [10 – 52]	24 [3 – 46]	21 [0 – 41]	12 [-3 – 27]

AIx at HR75 (Augmentation Index at Heart Rate 75 bpm); DBP (Diastolic Blood Pressure); HR (Heart Rate); MAP (Mean Arterial Pressure); mmHg (millimetres of Mercury); mmHg (millimetres of Mercury); MTCC (Montmorency Tart Cherry Capsules); MTCJ (Montmorency Tart Cherry Juice); PLA (Placebo); SEVR (Subendocardial Viability Ratio); TPR (Total Peripheral Resistance). \*Denotes significant main effect for time with *post-hoc* identifying differences between 1-hour and 3-hours post-bolus. <sup>^</sup>Denotes significant main effect for time with *post-hoc* identifying differences between 1-hour and 5-hours post-bolus. <sup>§</sup>Denotes significant main effect for time with *post-hoc* identifying differences between 3-hour and 5-hours post-bolus.

### 3.3. Pulse Wave Analysis (PWA)

A main effect for time ( $F_{(5, 50)} = 7.839$ ;  $P = 0.000$ ,  $\eta^2_{\text{partial}} = 0.44$ ) was observed for the change in AP from pre-bolus. No main effects for condition or interaction ( $P > 0.05$ ) were found, yet *post-hoc* revealed a difference ( $P = 0.001$ ,  $d = 1.37$ ) between PLA (2 [0 – 5] mmHg) and MTCJ (-2 [-4 – 0] mmHg) at 2-hours post-bolus (Figure 4). Individual responses showed 10/11 participants responded with a lower AP, 2-hours after MTCJ consumption compared to PLA.

A main effect for time ( $F_{(5, 50)} = 10.090$ ;  $P = 0.000$ ,  $\eta^2_{\text{partial}} = 0.50$ ) was detected for AIx, but not for condition or interaction ( $P > 0.05$ ). However as with AP, *post-hoc* comparisons identified a significant difference ( $P = 0.016$ ,  $d = 1.22$ ) at 2-hours post-bolus between PLA (4 [1 – 8] %) and MTCJ (-3 [-7 – 2] %). This indicates more compliant arteries with MTCJ at this time point whereby 9/11 participants reduced arterial stiffness compared to PLA. There were no significant correlations between bSBP and AIx ( $\rho = 0.105$ ;  $P = 0.401$ ) or AP ( $\rho = 0.145$ ;  $P = 0.247$ ) at 2-hours post-bolus for MTCJ.

No significant main effects for condition, time or the condition by time interaction were detected for aortic SBP, aortic DBP, and pulse pressure ( $P > 0.05$ ). A main effect for time was detected for AIx at HR75 ( $F_{(5, 50)} = 7.747$ ;  $P = 0.000$ ,  $\eta^2_{\text{partial}} = 0.44$ ) and SEVR ( $F_{(5, 50)} = 3.903$ ;  $P = 0.005$ ,  $\eta^2_{\text{partial}} = 0.28$ ), however no other main effects for condition or interaction were found ( $P > 0.05$ ) (Table 4).

### 3.4. Resting Metabolic Rate

Resting RQ did not show a significant main effect for condition or interaction ( $P > 0.05$ ). However, a main effect for time ( $F_{(1.938, 19.376)} = 28.261$ ;  $P = 0.000$ ,  $\eta^2_{\text{partial}} = 0.74$ ) was found, *post-hoc* analysis for the main effect of time showed all post-bolus time points to be lower than 30 minutes post-bolus ( $P < 0.05$ ).

No main effects for condition or interaction ( $P > 0.05$ ) were seen, however significant main effects for time were observed for resting fat ( $F_{(5, 50)} = 18.096$ ;  $P = 0.000$ ,  $\eta^2_{\text{partial}} = 0.64$ ) and carbohydrate oxidation ( $F_{(5, 50)} = 16.750$ ;  $P = 0.000$ ,  $\eta^2_{\text{partial}} = 0.63$ ). Specifically, significant differences were observed between all post-bolus time points against 30 minutes post-bolus for fat and carbohydrate oxidation ( $P < 0.05$ ). Finally, no significant main effects for condition, time or interaction were seen for resting energy expenditure ( $P > 0.05$ ).

#### **4. Discussion**

The present study examined cardio-metabolic responses of humans with MetS to an acute bolus of MTCC and MTCJ in a randomised, placebo-controlled trial, for the first time. The hypotheses were partially accepted as the main findings from this research demonstrated significantly lower post-prandial responses to serum insulin with MTCJ compared to PLA and a blunted insulin response with MTCC compared to PLA, 1-hour post-bolus. In accordance with previous research (Keane et al., 2016b), bSBP was significantly lower with MTCJ compared to PLA, 2-hours post-bolus. Finally, reported for the first time, responses between MTCC and MTCJ were not statistically different, thus refuting the hypothesis that MTCC may be superior than MTCJ; although physiological differences were apparent at particular time points for certain markers.

A novel finding from the present research was mean post-bolus insulin responses were significantly lower with MTCJ than PLA, and also significantly lower at 3-hours post-bolus. Although not statistically significant, insulin responses between PLA and MTCC were also physiologically different. The mean change from pre-bolus across all post-bolus time points increased with PLA, but decreased by  $20.61 \text{ pmol.L}^{-1}$  with MTCC. However, this was likely due to the carbohydrate content in the placebo beverage (99.88% of which sugars), where maltodextrin was added to match the energy content of the placebo to MTCJ. Consequently, this provoked a contrived response of the placebo on insulin, making comparisons against MTCC and MTCJ difficult. Nevertheless, as this was the first study to assess acute glycaemic and insulinaemic tolerability to MTCC and MTCJ consumption, it is possible to discern novel findings. A blunted insulin secretion response throughout the 5-hour post-bolus period with MTCC was observed, as participants only consumed 1 gram of carbohydrate from the 10 capsules ingested. Additionally, in alignment with MTC pharmacokinetics (Keane et al., 2016a), absolute insulin concentrations at 3-hours post-bolus were similar between MTCC

(77.42 [54.44 – 100.39] pmol.L<sup>-1</sup>) and MTCJ (76.53 [49.07 – 103.99] pmol.L<sup>-1</sup>). Despite differences in glycaemic load, this highlights a potential action of MTC anthocyanins and their metabolites given the only similarity between MTCC and MTCJ was the total anthocyanin content. Interestingly, the similar insulin concentrations suggest no difference between the delivery of MTC anthocyanins in capsule or juice form, indicating enhanced bioavailability of anthocyanins with capsules likely did not occur. The data indicates both MTC interventions effectively blunted insulin responses when parent anthocyanin and secondary metabolite plasma concentrations were likely elevated.

The high glucose load in the placebo elicited a significantly higher insulin secretion response 1-hour post-bolus compared to MTCC. Although not statistically different, a similar physiological response was found with MTCJ compared to MTCC (7/11 participants had increased insulin with MTCJ, 3/11 increased with MTCC). This may potentially be due to the lower sugar content in MTCJ than PLA, along with the effect of anthocyanins inhibiting glucose absorption across the apical membrane of enterocytes into systemic circulation via suppression of sodium-dependent glucose transporter-1 (SGLT-1) (Alzaid, Cheung, Preedy, & Sharp, 2013). Likewise, insulinaemic and glycaemic responses to 7 days of New Zealand blackcurrant powder (NZBP) supplementation suggested a 14.3% reduction in fasting insulin compared to control (Willems, Silva, Cook, & Blacker, 2017). Crucially, the authors measured fasting insulin 1-hour post-consumption of the final bolus, implying an acute effect of NZBP anthocyanins and metabolites on insulin. Willems et al. (2017) explained that an increased glycaemic and insulinaemic response was expected, however the carbohydrate load was not sufficient to induce a glycaemic or insulinaemic response and ascribed the decline in insulin to improved insulin sensitivity. A similar mechanism may be cited to explain the smaller changes from baseline for insulin with MTCJ and MTCC in the present study, given baseline HOMA2-

IR (2.2 [1.2 – 3.2]) and HOMA2-%S (61.8 [42.3 – 81.4] %) values indicated insulin resistance (Geloneze et al., 2009) and low insulin sensitivity respectively.

The effect of lower insulin concentrations with normal glucose suggests MTC improved insulin sensitivity (Willems et al., 2017) which may prevent pancreatic  $\beta$ -cell glucotoxicity (Amiot et al., 2016), reducing the burden on  $\beta$ -cells by averting excess insulin secretion (Alvarado et al., 2016). Additionally, the acute nature of this study limits the mechanisms through which MTC phytochemicals can operate. Hence, improved insulin sensitivity within 5-hours of ingesting MTC may have been mediated through enzyme-interactions involving adenosine monophosphate-activated protein kinase (AMPK) (Tsuda, 2016) and/or receptor-interactions along the insulin signalling cascade. Specifically, binding of insulin to insulin-receptor substrate 1 (IRS-1) prompting glucose transporter 4 (GLUT-4) translocation to the cell membranes of adipocytes and myocytes (Belwal, Nabavi, Nabavi, & Habtemariam, 2017). Subsequently, adaptations may include greater expression of AMPK and GLUT-4 mRNA; more efficient insulin receptor activity through greater insulin receptors on hepatocytes, myocytes and adipocytes; and peroxisome proliferator-activated receptor agonism (Seymour et al., 2009). Through the physiological mechanisms hypothesised above, improvements in insulin sensitivity may manifest to lower insulin resistance and theoretically reverse MetS; in the long-term. The direction of the insulinaemic response and its low carbohydrate content, provides evidence that MTCC may be a more tolerable intervention for individuals with dysfunctional glycaemic control and insulin resistance, such as type 2 diabetics. Moreover, the findings even hint at short-term improvements in insulin control with both MTC interventions, which may develop into long-term adaptations with consistent use; however, a direct investigation of this is required.

The most noteworthy cardiovascular response observed in the present study was the significant reduction in bSBP at 2-hours post-bolus with MTCJ compared to PLA. This finding was

consistent with previous literature assessing bSBP with cherry interventions (Chai, Davis, Wright, Kuczmarski, & Zhang, 2018; Keane et al., 2016b; Keane et al., 2016c; Kent, Charlton, Jenner, & Roodenrys, 2016). Keane et al. (2016bc) observed significantly lower bSBP with MTCJ compared to PLA for the first 3-hours after ingestion, with peak reductions of 7 mmHg occurring at 2-hours post-bolus (Keane et al., 2016b) and 6 mmHg at 1-hour post-bolus (Keane et al., 2016c). Similarly, the present study observed the greatest reduction in bSBP (-8 mmHg) also at 2-hours post-bolus, thus it can be hypothesised that MTCJ modulates bSBP during this time period as it likely coincides with parent anthocyanin and metabolite pharmacokinetics (Keane et al., 2016a). Likewise, sweet cherry juice also had its greatest effect on bSBP (-5.50 mmHg) 2-hours post-prandially, in old and young adults (Kent et al., 2016), with the authors attributing the improvement to the parent anthocyanins but not phenolic metabolites, as peak metabolite concentrations did not coincide with the time course of the observed effect. Interestingly, modulation of bSBP with MTCC did not occur, despite administering the same total anthocyanin content. Based on Keane et al. (2016b) explaining that phenolic metabolites (protocatechuic and vanillic acids) from MTCJ, likely exerted the dampened bSBP response, it may be that these metabolites were not present at physiologically relevant concentrations within systemic circulation with MTCC. This could be due to the variable mechanics of capsule shell disintegration impeding anthocyanin metabolism.

Hypertension and isolated systolic hypertension increases risk of CVD, the leading cause of morbidity and mortality worldwide (Feresin et al., 2017), however mean bSBP reductions of at least 5-6 mmHg over 5 years has been linked with a lower risk of coronary heart disease and stroke by 20-25% and 35-40%, respectively (Collins et al., 1990). Bundy et al. (2017) reported that a reduction in bSBP of greater than 5 mmHg was clinically relevant and associated with lower CVD/mortality risk. Thus, the magnitude of change with MTCJ indicates clinically relevant reductions in bSBP; mean difference between MTCJ and PLA at 2-hours post-bolus

was -11 mmHg. Consequently, this emphasises the highly potent properties of MTCJ acutely improving bSBP and the effect of a lower MTCJ dosage compared to Keane et al. (2016b). This finding is of high importance in the context that approved anti-hypertensive drugs, with associated harmful side effects, comparably lower bSBP by a similar magnitude (Bramlage & Hasford, 2009) as MTCJ, which currently has no known side effects. A limitation of examining acute cardio-metabolic responses is low ecological validity and biological significance of the results (Rodriguez-Mateos et al., 2013). However, long-term studies supplementing anthocyanins from tart (Keane et al., 2016c) and sweet (Kent et al., 2017) cherries have also been shown to lower bSBP, although only in subjects presenting elevated baseline bSBP. Furthermore, the significance of short-term decrements of bSBP in a MetS population may mean subsequent long-term improvements in arterial compliance and endothelial function. Consequently, this may reverse the systemic pro-inflammatory and pro-oxidative state of MetS and reduce the risk of developing cardiovascular disease (Yanai et al., 2008); although this requires direct investigation.

Arterial stiffness, particularly of the aorta, is an independent predictor of cardiovascular morbidity and mortality (Cecelja & Chowienczyk, 2012). Evidence exists that a higher consumption of anthocyanins is associated with reductions in aortic blood pressure and arterial stiffness (Jennings et al., 2012). Previous research pertaining to supplementation of MTCJ in healthy (Lynn et al., 2014), early-hypertensive (Keane et al., 2016b) and MetS (Johnson et al., 2017) participants had not demonstrated improvements in arterial stiffness. Individual responses and statistical analysis from the present study demonstrated an improvement in arterial stiffness (AIx) only at 2-hours post-bolus with MTCJ compared to PLA. However, this finding remains dubious given that PP, aortic (AP) and peripheral (TPR) indicators of vascular stiffness were not improved with MTCJ. Furthermore, AIx and AP were not significantly



correlated to bSBP at 2-hours post-bolus with MTCJ; suggesting modulation of arterial stiffness does not explain the reduction in bSBP at that time point.

As the results from this research seem to not support a mechanical mechanism of action through improved arterial compliance, the findings may be explained by a chemical mechanism of action involving nitric oxide (NO) and/or inhibition of angiotensin-converting enzyme (ACE). However, Keane et al. (2016b) were unable to demonstrate augmented plasma nitrate/nitrite concentrations after 60 mL MTCJ consumption, suggesting NO mediated vasodilation may not be the responsible pathway. Conversely, Kirakosyan, Gutierrez, Ramos Solano, Seymour, and Bolling (2018) demonstrated MTC extract to inhibit ACE, potentially explaining reductions in bSBP from the present study; future work should assess ACE inhibition in humans following MTC consumption. However, concomitant stimulation of endothelial nitric oxide synthase and ACE inhibition should not be ruled out.

MTCC induced vasodilation may have facilitated greater venous return thus explaining HR, SV and CO responses at 30 minutes post-bolus. A similar mechanism was provided by Willems et al. (2017) in response to acute, encapsulated NZBP supplementation. The observed response with MTCC was remarkable, as disintegration of the capsule shell requires 30 minutes (Lown et al., 2017), indicating that responses on HR and SV were immediate after dissolution. Subsequently, it was thought that provision of MTC in liquid form (MTCJ) would facilitate faster absorption of parent anthocyanins and metabolites (Bohn, 2014; McGhie & Walton, 2007) than MTCC. However, the lack of response with MTCJ would suggest either extensive degradation by salivary amylase (Kamonpatana et al., 2012) and/or a delayed uptake of secondary metabolites and phase II conjugates, due to gut metabolism of anthocyanins (Fernandes, Faria, Calhau, de Freitas, & Mateus, 2014). This delayed uptake of metabolites may be the rate-limiting step.

In agreement with responses observed in MetS subjects during the present investigation, Johnson et al. (2017) reported 12 weeks MTCJ consumption had no significant effect on lipids. However, Johnson et al. (2017) observed a trend for lower total cholesterol with MTCJ compared to PLA. This suggests lipids may be more sensitive to improvement at higher anthocyanin, secondary metabolite and phase II conjugate concentrations; through higher dosages and/or longer supplementation length.

As with all research, certain limitations are apparent with the present study. Firstly, the artificial effect of the placebo on glycaemic and insulinaemic responses influenced the data and nullified comparisons against MTCC and MTCJ. Subsequent studies should ensure the placebo is matched for relative percentage of each macronutrient to total energy or alternatively use a water placebo if aiming to evaluate the intervention as a whole, including detrimental effects of juices such as sugars (Peluso & Palmery, 2014). Furthermore, the parent anthocyanin and secondary metabolite pharmacokinetics of MTCC in humans was and remains unknown. Thus, there is a necessity to address this gap in the literature, to enable better understanding of the data from this study and future work incorporating MTCC. Additionally, any advantageous responses observed with MTC interventions may be due to a residual synergistic effect of habitually consumed polyphenols, as this study aimed to uphold ecological validity by permitting habitual polyphenol intake. The beneficial responses observed with MTCJ compared to MTCC and PLA, may be a consequence of other nutrients, such as fibre which was shown to reduce bSBP, total cholesterol and post-prandial glucose and insulin (Hodgson, 2004); aligning with findings from the present study. Although much attention has been given to the beneficial effects of anthocyanins and their metabolites, the synergistic influence of other phytonutrients within MTC require consideration. Finally, acute cardio-metabolic responses were monitored during this study, however to enhance ecological validity and clinical relevance, monitoring data over a longer time frame is required.

## **5. Conclusion**

This research is the first to present data that acute administration of MTC interventions in capsule and juice form can modulate certain cardio-metabolic markers in humans with MetS.

Further evidence is provided that MTCJ is an effective, low-risk intervention for reducing bSBP in various populations and should be considered for individuals with isolated systolic hypertension. Despite recruiting humans with MetS, the effects of MTC on cardio-metabolic markers seemed to only apply to certain variables which were abnormal at baseline. The physiological responses to either MTC intervention differed depending on the cardio-metabolic marker examined, although there were no statistically significant differences between MTCC and MTCJ. Consequently, recommendations for which MTC intervention is more suitable should be based on the cardio-metabolic marker that requires most attention, with consideration of the individuals' overall cardio-metabolic health.

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## **Author Contributions**

Conceptualization, TD, LB and MR.; methodology, TD; writing—original draft preparation, TD.; writing—review and editing, TD, LB and MR; supervision, LB and MR. All authors read and approved the final manuscript.

## **Conflicts of Interest**

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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