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Effects of Short-Term Continuous Montmorency Tart Cherry Juice Supplementation in Participants with Metabolic Syndrome
--Manuscript Draft--

Abstract:

Purpose

Metabolic Syndrome (MetS) augments the incidence of cardiovascular disease by 2-fold and type II diabetes mellitus by 5-fold. Montmorency tart cherries are rich in phytochemicals shown to improve biomarkers related to cardio-metabolic health in humans. This study aimed to examine cardio-metabolic responses after 7 days Montmorency tart cherry juice (MTCJ) supplementation and also acute on short-term supplementation responses to a single-bolus, in humans with MetS.

Methods

In a randomised, single-blind, placebo-controlled, crossover trial, twelve participants with MetS (50 ±10 years; 6M/6F), consumed MTCJ or placebo (PLA) for 7 days. Blood-based and functional cardio-metabolic biomarkers were measured pre- and post-supplementation, and acute responses measured pre-bolus and up to 5 hours post-bolus on the 7 th day.

Results

24-hour ambulatory systolic (P = 0.016), diastolic (P = 0.009) blood pressure and mean arterial pressure (P = 0.041) were significantly lower after 7 days MTCJ supplementation compared to PLA. Glucose (P = 0.038), total cholesterol (P = 0.036), LDL (P = 0.023) concentrations, total cholesterol:HDL ratio (P = 0.004) and respiratory exchange ratio values (P = 0.009) were significantly lower after 6 days MTCJ consumption compared to PLA.

Conclusions

	This study revealed for the first time in humans that MTCJ significantly improved 24-hour BP, fasting glucose, total cholesterol and total cholesterol:HDL ratio, and also lower resting respiratory exchange ratio compared to a control group. Responses demonstrated clinically relevant improvements on aspects of cardio-metabolic function, emphasising the potential efficacy of MTCJ in preventing further cardio-metabolic dysregulation in participants with MetS. Registered at clinicaltrials.gov (NCT03619941).
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We would like to thank all reviewers for their insightful comments towards our article and their constructive feedback. We have responded to each of their comments below.

Reviewer 3 Comments

Reviewer #3: TPR units should be mmHg x s x mL-1

Response to Reviewer: Thank you for this comment. We have put the units of TPR in table 3 as mmHg·s^{-1}·mL^{-1} as shown by Wright et al (2016).

Wright, S.P., Granton, J.T., Esfandiari, S., Goodman, J.M. and Mak, S., (2016). The relationship of pulmonary vascular resistance and compliance to pulmonary artery wedge pressure during submaximal exercise in healthy older adults. *The Journal of physiology*, *594*(12), pp.3307-3315.

Reviewer 4 Comments

Reviewer #4: The authors have responded to all concerns as needed, and the manuscript quality has improved. One final clarification is needed regarding the "acute" response post-bolus on the 7th day. This would not really be "acute" as the bolus is given to participants following 6 days of supplementation? Could a different description be used? It is confusing to the reader. Perhaps "acute on chronic supplementation" (here chronic would have to be explicitly defined as 6-days) or "acute on short-term supplementation"? In addition, this aspect of the study is not clear, particularly in Figure 2 - suggest incorporating this into the study design for clarity.

Response to Reviewer: Thank you for this comment. We have clarified further at the end of the Introduction, in the Study Design section and at the start of the Discussion, the difference between short-term and acute on short-term supplementation.

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Effects of Short-Term Continuous Montmorency Tart Cherry Juice Supplementation in Participants with Metabolic Syndrome

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6   **Purpose:** Metabolic Syndrome (MetS) augments the incidence of cardiovascular disease by
7   2-fold and type II diabetes mellitus by 5-fold. Montmorency tart cherries are rich in
8   phytochemicals shown to improve biomarkers related to cardio-metabolic health in humans.
9   This study aimed to examine cardio-metabolic responses after 7 days Montmorency tart
10   cherry juice (MTCJ) supplementation and also acute on short-term supplementation
11   responses to a single-bolus, in humans with MetS. **Methods:** In a randomised, single-blind,
12   placebo-controlled, crossover trial, twelve participants with MetS (50 ±10 years; 6M/6F),
13   consumed MTCJ or placebo (PLA) for 7 days. Blood-based and functional cardio-metabolic
14   biomarkers were measured pre- and post-supplementation, and acute responses measured pre-
15   bolus and up to 5 hours post-bolus on the 7th day. **Results:** 24-hour ambulatory systolic ($P =$
16   0.016), diastolic ($P = 0.009$) blood pressure and mean arterial pressure ($P = 0.041$) were
17   significantly lower after 7 days MTCJ supplementation compared to PLA. Glucose ($P =$
18   0.038), total cholesterol ($P = 0.036$), LDL ($P = 0.023$) concentrations, total cholesterol:HDL
19   ratio ($P = 0.004$) and respiratory exchange ratio values ($P = 0.009$) were significantly lower
20   after 6 days MTCJ consumption compared to PLA. **Conclusions:** This study revealed for the
21   first time in humans that MTCJ significantly improved 24-hour BP, fasting glucose, total
22   cholesterol and total cholesterol:HDL ratio, and also lowered resting respiratory exchange
23   ratio compared to a control group. Responses demonstrated clinically relevant improvements
24   on aspects of cardio-metabolic function, emphasising the potential efficacy of MTCJ in
25   preventing further cardio-metabolic dysregulation in participants with MetS. Registered at
26   clinicaltrials.gov (NCT03619941).

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Acknowledgements:

Author Contributions

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Conflicts of Interest

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1. Introduction

The prevention of cardiovascular disease (CVD) and type II diabetes mellitus (T2D) would be a major step in retarding the current exponential rise in global prevalence and incidence rates [1,2]. Metabolic Syndrome (MetS) augments the incidence of CVD by 2-fold and T2D by 5-fold [3]. Dietary interventions to prevent and mitigate MetS are sought after as they can conveniently be implemented into an individual's lifestyle. Anthocyanins, a sub-class of polyphenols, and their metabolites possess potent anti-oxidative and anti-inflammatory properties and have been shown to improve MetS symptoms [4]. Furthermore, Naseri et al. (2018) [5] reported improvements in cardio-metabolic function after consumption of anthocyanin-rich interventions in humans with MetS. Montmorency tart cherries are rich in phytochemicals including anthocyanins, however their bioefficacy may be attributed to downstream metabolites and synergisms with other nutrients within the fruit, which may also induce health benefits [6–9]. Insulin resistance is key to the underlying pathophysiology of MetS and anthocyanin-mediated improvements in insulin and glucose metabolism [10] may ameliorate many of its associated symptoms [11]. Willems *et al.* (2017) [12] reported 7 days continuous consumption of anthocyanin-rich New Zealand blackcurrant powder (NZBP) likely increased insulin sensitivity in healthy individuals.

Acute supplementation of tart [8,13] and sweet [14] cherry juice has been shown to induce clinically-relevant reductions in SBP at 2-hours post-consumption, due to the pharmacokinetic profile and heightened bioavailability of MTC anthocyanins [15] and secondary metabolites [6]. A caveat of recommending MTCJ as an intervention for managing hypertension is that

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Ambulatory Blood Pressure Monitoring (ABPM); Angiotensin-I-converting Enzyme (ACE); Augmentation Index (AIx); AIx normalised to 75 beats.min^{-1} (AIx at HR75); Augmentation Pressure (AP); Blood Pressure (BP); Carbohydrate (CHO); Cardiac Output (CO); Cardiovascular Disease (CVD); Coefficient of Variation (CV); Diastolic Blood Pressure (DBP); Energy Expenditure (EE); Glycated Haemoglobin (HbA1c); HDL (High-density Lipoprotein); 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); LDL (Low-density Lipoprotein); Mean Arterial Pressure (MAP); Metabolic Syndrome (MetS); Montmorency Tart Cherry (MTC); Montmorency Tart Cherry Juice (MTCJ); New Zealand Blackcurrant Powder (NZBP); Pulse Pressure (PP); Pulse Wave Analysis (PWA); Resting Metabolic Rate (RMR); Stroke Volume (SV); Subendocardial Viability Ratio (SEVR); Systolic Blood Pressure (SBP); Total cholesterol:HDL Ratio (TC:HDL); Total Peripheral Resistance (TPR); Type 2 Diabetes Mellitus (T2D).

100   greater clinical evidence is required which can be applied to normal daily-living conditions

101   such as 24-hour ambulatory blood pressure monitoring (ABPM). The mechanism of action for

102   the hypotensive properties of tart and sweet cherries has yet to be elucidated, with nitric oxide

103   bioavailability [7] and modulation of arterial stiffness [7,13,16] not significantly changing after

104   MTCJ consumption. Therefore, an alternative mechanism involving angiotensin-I-converting

105   enzyme (ACE) was hypothesised in the present study, since Kirakosyan *et al.* (2018) [9]

106   observed 88.7% ACE inhibition *in vitro* with Montmorency tart cherry (MTC) extract. The

107   effect of MTC on ACE has not yet been assessed *ex vivo* in humans, therefore this mechanism

108   warrants exploration in a human randomised controlled trial.

109   The present study aimed to be the first to examine cardio-metabolic responses after short-term,

110   continuous MTCJ supplementation (6 days) and acute responses to a single-bolus on the 7th

111   day following short-term supplementation, in humans with MetS. It was hypothesised that

112   MTCJ would improve glycaemic and insulinaemic function through increasing insulin

113   sensitivity. Moreover, MTCJ would maintain acute reductions in SBP and lower 24-hour SBP

114   after 7 days supplementation, through ACE inhibition.

115   **2. Methods**

116   2.1. Participants

117   Twelve (6 males and 6 post-menopausal females) participants [mean ± SD, age 50 ± 10 years

118   (range 28-62 years), stature 1.73 ± 0.12 m, body mass 94.1 ± 23.1 kg, body mass index 31 ± 7

119   kg.m^{-2}] with MetS (Tables 1A and 1B) volunteered for this study. All participants were

120   screened for MetS prior to formal inclusion onto the study according to the harmonised criteria

121   outlined by Alberti *et al.* (2009) [17], where 3 of the 5 qualifying criteria [Waist Circumference:

122   ethnicity and sex specific criteria; Fasting Triglycerides: ≥1.69 mmol.L^{-1}; Fasting High-

123   Density Lipoprotein: <1.03 mmol.L^{-1} (men), <1.29 mmol.L^{-1} (women); Blood Pressure: ≥130

124   mmHg SBP or ≥85 mmHg diastolic blood pressure (DBP); Fasting Glucose: ≥5.6 mmol.L^{-1}]

125   had to be met. Recruitment (Figure 1) was conducted via word of mouth, flyers and email

126   advertisements. Participants were excluded from the study if they did not meet the criteria for

127 MetS at screening, were smokers, pregnant, heavy alcohol consumers (>14 units per week),

128 current or previous diagnosis of chronic disease (gastrointestinal, cardiovascular, hepatic or

129 renal), or were on statins, hyperlipidaemic, anti-hypertensive, anti-diabetic, anti-inflammatory

130 or steroidal medication. All participants were instructed to abstain from consuming any other

131 multivitamin, anti-oxidant, fish oil, dietary or herbal supplementation two weeks prior to and

132 for the duration of the study. Verbal and written information was provided to all participants

133 regarding the study purpose and procedures. Ethical approval was obtained from the University

134 of Hertfordshire HSET Ethics Committee (LMS/PGR/UH/03319) and the study's experimental

135 procedures followed the principles outlined in the Declaration of Helsinki. Written informed

136 consent was provided by all participants prior to enrolment. This study was registered as a

137 clinical trial on clinicaltrials.gov (NCT03619941). ***Table 1A and 1B near here***

138   ***Figure 1 near here***

139   2.2 Procedures

140   2.2.1. Research Design

141   A single-blind (blinded to participant), placebo-controlled, randomised, crossover design was

142   utilised; each participant acted as their own control. During the 6-week study duration,

143   participants completed both testing sessions during which placebo (PLA) or Montmorency Tart

144   Cherry Juice (MTCJ) supplements were consumed for a continuous period of 7 days. A 14-day

145   washout period [8] was incorporated prior to crossover to the opposing condition.

146   Schematics are shown of the overall study design (Figure 2A) and specific procedures during

147   a testing session. The first and third testing sessions lasted 1 hour; pre-supplementation

148   anthropometric (stature, body mass, waist circumference), functional [pulse wave analysis

149   (PWA), cardiac haemodynamics, resting metabolic rate (RMR)] and fasting blood-based

150   biomarkers were measured. Testing sessions two and four lasted 6 hours; anthropometric,

151   functional and fasting blood-based biomarkers were measured prior to consumption of the

152   supplement (6 days post-supplementation) and up to 5-hours post-consumption of the 7th bolus

153   (Figure 2B). Testing sessions two and four were conducted following 6 days short-term

154   supplementation of either MTCJ or PLA, during which the 7th bolus of supplement was

155   consumed, and acute measurements taken thereafter which we have termed acute on short-term

156   supplementation (Figure 2A).

157

158   ***Figure 2 near here***

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. 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.

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. Dietary analysis software (Dietplan 7.0, Forestfield Software, UK) was used to monitor compliance of the 3-day food diaries for macronutrient, polyphenol and anthocyanin intake, before each session. All participants complied with dietary guidelines upon analysis for percentage contributions of macronutrients to total energy intake [(protein $14 \pm 22\%$), (carbohydrate $48 \pm 40\%$), (fat $38 \pm 41\%$)], total polyphenols (62 ± 70 mg) and anthocyanins (20 ± 17 mg).

2.2.3. Supplementation

This study acutely administered two different supplements including a placebo which acted as the control condition and one experimental condition, MTCJ. MTCJ consisted of 30 mL Montmorency tart cherry concentrate (Cherry Active, Active Edge Ltd, Hanworth, UK) diluted in 100 mL water. It was identified that the concentrate contained glucose (55.73% of total sugars) and fructose (44.27% of total sugars). The 30 mL serving of Montmorency tart cherry concentrate contained ~90-110 whole Montmorency tart cherries. 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 matched against MTCJ for percentage contribution of carbohydrate to total energy and contribution of sugars to total carbohydrate, energy content, taste and visual appearance by adding dextrose (My

185 Protein Ltd, Northwich, UK), fructose (Fruit Sugar, Morrisons, Bradford, UK), cornflour

186 (Morrisons, Bradford, UK), citric acid (100% Pure Citric Acid, VB and Sons, UK), red and

187 black food colouring (Morrisons, Bradford, UK). Nutritional analysis for MTCJ (Volume:

188 130 mL, Energy: 102 kcal, Carbohydrates: 24.5 g of which Sugars: 17.9 g, Glucose: 9.98 g,

189 Fructose: 7.92 g, Protein: 1.1 g, Fat: 0 g, Fibre: 2.6 g, Total Anthocyanins: 270 mg) and

190 placebo (Volume: 130 mL, Energy: 102 kcal, Carbohydrates: 25 g of which Sugars: 18 g,

191 Glucose: 10.03 g, Fructose: 7.97 g, Protein: 0.5 g, Fat: 0 g, Fibre: Trace, Total Anthocyanins:

192 0 mg). Anonymity of the supplementation was ensured by blinding the participants to the

193 source of anthocyanins. This was achieved by explaining that an 'anthocyanin-rich

194 supplement' would be provided rather than disclosing Montmorency tart cherries as the

195 specific source. Only 2/12 participants correctly distinguished the supplements provided

196 (intervention or placebo) and none identified the intervention as 'cherry' or 'containing

197 cherries (either sweet or tart)'.

198 2.2.4. Measures and Equipment

199 Serum insulin was regarded as the primary endpoint for the present study. HOMA2-IR, acute

200 SBP and 24-hour SBP were regarded as secondary variables. Tertiary endpoints focused on

201 other aspects of MetS including hyperlipidaemia, hyperglycaemia and cardiovascular

202 dysfunction.

203 Blood pressure, cardiac haemodynamics and PWA measurements were all conducted in a quiet,

204 temperature-controlled laboratory maintained between 21-24ºC. All measurements were

205 performed in an upright seated position with 10 minutes prior rest.

206 2.2.4.1. Blood Pressure

207 Brachial systolic (SBP) and diastolic (DBP) blood pressure (Omron MX3, Omron, Japan)

208 were measured four times, with an average of the final three being taken as BP.

209 2.2.4.2. Cardiac Haemodynamics

210 Beat-to-beat resting cardiac haemodynamic parameters including heart rate (HR), cardiac

211 output (CO), stroke volume (SV), mean arterial pressure (MAP) and total peripheral

212 resistance (TPR) were measured non-invasively (Finometer MIDI Model-2, Finapres Medical

213 Systems BV, Amsterdam, The Netherlands) at all time points, using the arterial volume

214 clamp method.

215 2.2.4.3. 24-hour Ambulatory Blood Pressure

216 In addition to clinic BP measurements in the laboratory using an automated

217 sphygmomanometer, 24-hour ambulatory blood pressure monitoring (ABPM) (Meditech

218 ABPM-04, Meditech, Hungary) was also conducted through an oscillometric method. ABPM-

219 04 monitors have been clinically validated against British Hypertension Society guidelines

220 [18]. ABPM provides a more accurate representation of BP as measurements are obtained

221 under normal daily-living conditions, negating white coat syndrome; and mean pressures are

222 taken from multiple readings over a 24-hour period, thus ABPM has become the reference

223 standard for measuring BP non-invasively and diagnosing hypertension [19]. Moreover, 24-

224 hour ABPM is deemed to be the reference standard for accurate assessment of cardiovascular

225 risk in adults [19].

226 Participants underwent a familiarisation period of wearing the ABPM device prior to data

227 collection. A day before and after the 7-day supplementation period, participants were fitted

228 with a 24-hour ABPM on the non-dominant arm. The ABPM was programmed (CardioVisions

229 Software 1.15.2, Meditech, Hungary) to take readings every 30 minutes during the day (07:00-

230 22:00) and every hour during the night (22:00-07:00) [20]. A minimum of 14 day-time readings

231 and 7 night-time readings were considered for a valid 24-hour ABPM assessment [21].

232 Participants were advised to be seated upright, keep still and relax their arm whenever the

233 monitor recorded measurements. The following data was obtained from the ABPM monitor:

234 mean 24-hour, day-time and night-time SBP, DBP, MAP and PP. The difference between mean

235 day and night SBP, DBP, MAP and PP was also calculated.

236 ### 2.2.4.4. Pulse Wave Analysis

237 Aortic pulse waveforms, aortic SBP, aortic DBP, pulse pressure (PP), augmentation pressure

238 (AP), augmentation index (AIx), AIx normalised to 75 beats.min^{-1} (AIx at HR75) and

239 subendocardial viability ratio (SEVR) were determined by pulse wave analysis (PWA) using

240 applanation tonometry (SphygmoCor, ScanMed Medical, UK) as previously described in

241 [13].

242 ### 2.2.4.5. Resting Metabolic Rate (RMR)

243 RMR, resting energy expenditure (EE), substrate oxidation rates and respiratory exchange

244 ratio (RER) were measured using an open-circuit indirect calorimetry system (GEM Nutrition

245 Ltd, Cheshire, UK) as previously described in [13].

246 Resting EE was determined by application of the Weir equation [22] below.

247 $$\textit{Energy Expenditure} \; (kcal.day^{-1}) = [(3.94 * \dot{V}O_2) + (1.106 * \dot{V}CO_2)] * 1.44$$

248 Equations outlined by Frayn (1983), were used to determine fat oxidation and carbohydrate

249 (CHO) oxidation rates at rest.

250 $$\textit{Fat Oxidation Rate} \; (g.min^{-1}) = (1.67 * \dot{V}O_2) - (1.67 * \dot{V}CO_2)$$

251 $$\textit{Carbohydrate Oxidation Rate} \; (g.min^{-1}) = (4.55 * \dot{V}CO_2) - (3.21 * \dot{V}O_2)$$

252 ### 2.2.5. Blood Sampling and Analysis

253 ### 2.2.5.1. Blood Sampling

254 Venous blood was sampled through 4 individual venepunctures (one at each time point: pre-

255 bolus and 1, 3, 5 hours post-bolus), using the butterfly method (BD Vacutainer Safety-Lok

256 Blood Collection Set 21G with Luer Adapter, Becton Dickinson and Co., Oxford, UK).

257 Tubes were centrifuged at 4000 rev.min^{-1}, 4°C for 10 minutes (Sorvall ST 8R, Thermo Fisher

258 Scientific, USA). Serum supernatants were aliquoted and stored at -80°C for later analysis.

259 ### 2.2.5.2. Glucose

260   Serum samples were assessed for glucose (range 0.5-50 mmol.L^{-1}, coefficient of variation

261   (CV) $\leq$ 1.5%) (Biosen C-Line, EKF Diagnostics, Cardiff, UK) in duplicates.

262   2.2.5.3. Insulin

263   Serum insulin samples were measured in duplicates using a human 96-well colorimetric

264   insulin enzyme-linked immunosorbent assay (ELISA) (Insulin Human ELISA KAQ1251,

265   Invitrogen, Thermo Fisher Scientific, USA). Inter- and intra-plate precision were 6.1% and

266   5.5%, respectively.

267   2.2.5.4. Insulin Resistance and Sensitivity Indexes

268   Homeostatic Model Assessment (HOMA) was used to estimate fasting steady-state

269   pancreatic β-cell function (HOMA2-β), insulin sensitivity (HOMA2-%S) and insulin

270   resistance (HOMA2-IR index) through the HOMA2 spreadsheet model (HOMA2-IR,

271   available from https://www.dtu.ox.ac.uk/homacalculator/) [24].

272   2.2.5.5. Lipid Assays

273   Serum lipids were determined in duplicates using commercially available colorimetric assays

274   on a semi-automated spectrophotometer (RX monza, Randox Laboratories Ltd, Antrim, UK),

275   according to manufacturer's guidelines. Triglyceride (Triglycerides TR210, Randox) values

276   were corrected for free glycerol by subtracting 0.11 mmol.L^{-1}, according to the

277   manufacturer's guidelines. Intra-assay CV for triglycerides, total cholesterol and HDL were

278   2.94%, 4.45% and 4.22%, respectively. LDL was determined indirectly using the formula

279   below [25].

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

281   2.2.5.6. Angiotensin-I-converting Enzyme

282   Human ACE (CD 143) (peptidyl-dipeptidase A, EC 3.4.15.1) protein concentrations were

283   measured according to manufacturer's guidelines, from serum samples in duplicates using a

284   96-well ELISA [Human ACE ELISA (CD 143) ab119577, Abcam, Cambridge, UK]. Inter-

285   and intra-plate precision were 12.3% and 9.2%, respectively.

286   2.3. Data Analysis

287   Statistical analysis was performed using SPSS v22.0 (IBM, Chicago, USA) where data are

288   reported as means ±standard deviation (±SD). Data normality was checked using a Shapiro-

289   Wilk test. Greenhouse-Geisser correction was applied upon violation of Mauchly's test of

290   sphericity for ANOVAs ($P < 0.05$). Statistical significance was set at $P < 0.05$. Based on data

291   from Desai et al. (2019) [13] for the interaction effect between condition (PLA and MTCJ) and

292   time for serum insulin, *a priori* power ($\alpha = 0.05$; 1-$\beta = 0.8$) analysis indicated a sample size of

293   8 would be sufficient to detect a significant difference pre-post 6 days supplementation for

294   serum insulin. Therefore, a minimum of 12 participants were recruited assuming a 20% dropout

295   rate. *Post-hoc* power analysis indicated sufficient power (1-$\beta = 0.99$; $\alpha = 0.05$; n = 12) to detect

296   a significant main effect for the interaction between condition and time (pre-post 6 days

297   supplementation) for insulin, HOMA2-IR and 24-hour SBP. Acute SBP also demonstrated

298   sufficient power (1-$\beta = 0.86$; $\alpha = 0.05$; n = 12) to detect a significant interaction effect.

299   A within-group two-way, 2 x 2, condition (PLA vs MTCJ) x time (Pre-Supplementation and

300   Post-Supplementation), repeated-measures ANOVA design with *post-hoc* Bonferroni

301   adjustment, measured differences for all blood-based biomarkers, cardiac haemodynamic,

302   PWA, RMR and 24-hour BP parameters.

303   To account for day-to-day physiological variances at pre-bolus between conditions for each

304   variable, data were analysed as change from pre-bolus for each time point measured post-bolus

305   during testing sessions 2 and 4. This enabled a fair assessment of the post-bolus responses to

306   each condition, from pre-bolus across all variables. The pre-bolus time point was not included

307   as a covariate, as one-way ANOVA analysis indicated no significant differences ($P > 0.05$)

308   between conditions for all variables at the pre-bolus time point, hence two-way repeated-

309   measures ANOVA was performed. A within-group two-way, 2 x 6, condition (PLA vs MTCJ)

310   x time (30 minutes, 1, 2, 3, 4- and 5-hours post-bolus), repeated-measures ANOVA design with

311   *post-hoc* Bonferroni adjustment, measured differences of cardiac haemodynamic, PWA and

312   RMR parameters on change from pre-bolus values. Blood-based biomarkers were analysed

313   using the same model but with a 2 x 3, condition (PLA vs MTCJ) by time (1, 3- and 5-hours

314   post-bolus) design on change from pre-bolus values for each condition.

315   Partial Eta-Squared ($\eta^2_{partial}$) was used to report effect sizes for ANOVA where effects were

316   classified as small (0.01-0.08), moderate (0.09-0.25) and large (>0.25) [26]. Cohen's *d* effect

317   size was used for paired-samples *t*-test and *post-hoc* interaction comparisons where effects

318   were classified as no effect (0-0.1), small (0.2-0.4), moderate (0.5-0.7) and high (≥0.8) [26].

319   **3. Results**

320   3.1. Blood Biomarkers

321   3.1.1. Glucose

322   Responses to glucose pre and post 6 days supplementation (Figure 3) demonstrated a

323   significant interaction effect ($F_{(1, 11)}$ = 5.534; P = 0.038, $\eta^2_{partial}$ = 0.34) and main effect for

324   time ($F_{(1, 11)}$ = 8.077; P = 0.016, $\eta^2_{partial}$ = 0.42) only. *Post-hoc* indicated a significant difference

325   between PLA and MTCJ at pre-supplementation time point (P = 0.023; d = 2.85). Fasting

326   glucose was significantly ($t_{(11)}$ = 3.506; P = 0.005, d = 0.56) lower 6 days after supplementation

327   (5.39 ± 0.23 mmol.L^{-1}) compared to pre-supplementation (5.90 ± 0.86 mmol.L^{-1}) with MTCJ.

328   Individual responses showed 10/12 individuals with lower fasting glucose after 6 days

329   supplementation of MTCJ compared to PLA.

330   A main effect for time was found for change from pre-bolus data for glucose ($F_{(2, 22)}$ = 12.641;

331    $P < 0.001$, $\eta^2_{partial}$ = 0.54) (Table 2). *Post-hoc* showed glucose to be significantly higher at 1-

332   hour post-bolus compared to both 3-hours (P = 0.003, d = 1.11) and 5-hours post-bolus (P =

333   0.021, d = 0.92).

334   3.1.2. Insulin

335 Insulin responses to 6 days supplementation (Figure 3) only showed a significant interaction

336 effect ($F_{(1, 11)} = 7.293$; $P = 0.021$, $\eta^2_{partial} = 0.40$). Pairwise comparisons indicated a significant

337 difference between PLA and MTCJ at pre-supplementation time point ($P = 0.049$; $d = 0.41$).

338 However, physiological responses showed after 6 days the mean change pre- to post-

339 supplementation was 27.05 ± 42.42 pmol.L^{-1} with PLA and -12.42 ± 30.50 pmol.L^{-1} with

340 MTCJ. Moreover, individual responses showed 10/12 individuals with lower fasting insulin

341 after 6 days supplementation of MTCJ compared to PLA, indicating a tendency for lower

342 fasting insulin after MTCJ consumption compared to PLA.

343 Similar to glucose, serum insulin only demonstrated a main effect of time ($F_{(2, 22)} = 16.828$; P

344 < 0.001, $\eta^2_{partial} = 0.61$) on change from pre-bolus data (Table 2). *Post-hoc* showed insulin to

345 be significantly higher at 1-hour post-bolus compared to both 3-hours ($P = 0.005$, $d = 1.27$)

346 and 5-hours post-bolus ($P = 0.004$, $d = 1.34$).

347 *** Table 2 near here***

3.1.3. HOMA2

As with insulin, HOMA2-IR ($F_{(1, 11)} = 8.115$; $P = 0.016$, $\eta^2_{partial} = 0.43$) and HOMA2-%S ($F_{(1, 11)} = 6.332$; $P = 0.029$, $\eta^2_{partial} = 0.37$) demonstrated a significant interaction after 6 days supplementation (Figure 3), with pairwise comparisons showing a significant difference between PLA and MTCJ at pre-supplementation time point for HOMA2-IR ($P = 0.039$; $d = 0.43$). Individual responses for HOMA2-IR showed insulin resistance increased in 10/12 participants with PLA, whereas insulin resistance was reduced in 8/12 participants after 6 days MTCJ consumption. Moreover, HOMA2-%S showed 9/12 participants increased insulin sensitivity with 6 days continuous MTCJ consumption; 2/12 increased with PLA.

A main effect for time ($F_{(1, 11)} = 7.720$; $P = 0.018$, $\eta^2_{partial} = 0.41$) only was observed for HOMA2-β with pre-post 6 days supplementation data. *Post-hoc* analysis highlighted greater pancreatic β-cell function post-supplementation (PLA: 152 ± 49%, MTCJ: 148 ± 56%) compared to pre-supplementation (PLA: 131 ± 43%, MTCJ: 132 ± 53%) ($P = 0.018$, $d = 0.37$). No significant main effects were found on change from pre-bolus data for HOMA2-β ($P > 0.05$).

Figure 3 near here

3.1.4. Lipids

Reponses to total cholesterol after 6 days supplementation showed significant main effects for time ($F_{(1, 11)}$ = 5.097; P = 0.045, $\eta^2_{partial}$ = 0.32) and interaction ($F_{(1, 11)}$ = 5.700; P = 0.036, $\eta^2_{partial}$ = 0.34) (Figure 4). *Post-hoc* identified a significant difference between PLA and MTCJ at pre-supplementation (P = 0.030; d = 2.14). Total cholesterol was significantly ($t_{(11)}$ = 3.724; P = 0.003, d = 0.39) lower post-supplementation (4.1 ± 1.0 mmol.L^{-1}) compared to pre-supplementation (4.5 ± 1.0 mmol.L^{-1}) with MTCJ.

A significant interaction effect was observed for HDL ($F_{(1, 11)}$ = 5.212; P = 0.043, $\eta^2_{partial}$ = 0.32) and LDL ($F_{(1, 11)}$ = 7.004; P = 0.023, $\eta^2_{partial}$ = 0.39) after 6 days supplementation (Figure 4). A significant difference between PLA and MTCJ was found at post-supplementation time point for HDL (P = 0.049, d = 0.42), while pre-supplementation was significantly different for LDL (P = 0.031, d = 0.42). LDL was significantly ($t_{(11)}$ = 3.681; P = 0.004, d = 0.21) lower post-supplementation (2.71 ± 1.62 mmol.L^{-1}) compared to pre-supplementation (3.07 ± 1.69 mmol.L^{-1}) with MTCJ. Individual responses showed 6/12 participants increased HDL concentrations after 6 days MTCJ supplementation, whereas 2/12 increased with PLA. Moreover, 10/12 participants reduced LDL concentrations after MTCJ supplementation, compared to 5/12 with PLA.

After 6 days supplementation, an interaction effect was observed for TC:HDL ($F_{(1, 11)}$ = 13.681; P = 0.004, $\eta^2_{partial}$ = 0.55) (Figure 4). *Post-hoc* comparisons showed PLA to be significantly higher than MTCJ at pre-supplementation time point (P = 0.011; d = 2.67). TC:HDL ratio was significantly ($t_{(11)}$ = 2.690; P = 0.021, d = 0.30) lower post-supplementation (3.11 ± 1.13 mmol.L^{-1}) compared to pre-supplementation (3.47 ± 1.28 mmol.L^{-1}) with MTCJ. No significant interaction or main effects for condition and time were observed for triglycerides with pre-post 6 days supplementation data (P > 0.05) (Table 2).

388   Change from pre-bolus data showed a main effect of time ($F_{(2, 22)} = 11.649$; $P < 0.001$, $\eta^2_{partial}$

389    $= 0.51$) and a tendency for a significant interaction ($F_{(2, 22)} = 3.148$; $P = 0.063$, $\eta^2_{partial} = 0.22$)

390   for triglyceride concentrations (Table 2). Change from pre-bolus data indicated a significant

391   main effect of condition ($F_{(1, 11)} = 5.874$; $P = 0.034$, $\eta^2_{partial} = 0.35$) and time ($F_{(2, 22)} = 4.110$;

392    $P = 0.030$, $\eta^2_{partial} = 0.27$) for LDL, with higher LDL concentrations at pre-supplementation

393   compared to post-supplementation ($P = 0.034$; $d = 0.08$) (Table 2). No main effects for

394   condition, time or interaction were found for HDL or TC:HDL ratio with change from pre-

395   bolus data ($P > 0.05$) (Table 2).

396   ***Figure 4 near here***

3.1.5. ACE

ACE did not show any main effects for condition, time or interaction with pre-post 6 days supplementation data or change from pre-bolus data (Table 2) ($P > 0.05$).

3.2. Cardiac Haemodynamics

No main effects for condition, time or interaction were detected for SBP, DBP, MAP, HR, SV, CO and TPR ($P > 0.05$) for pre-post 6 days supplementation data (Table 3).

There were no main effects for condition, time or interaction on change from pre-bolus data with DBP, MAP, HR, SV, CO and TPR ($P > 0.05$) (Table 3). A tendency towards significance was detected for the interaction ($F_{(5, 55)} = 2.128$; $P = 0.076$, $\eta^2_{partial} = 0.16$) with SBP. Individual responses for the change from pre-bolus to 2-hours post-bolus showed 4/12 participants decreased SBP with PLA (4 ± 13 mmHg), while 10/12 participants reduced with MTCJ (-7 ± 10 mmHg).

Table 3 near here

410 3.3. 24-hour ABPM

411 A significant interaction ($F_{(1, 11)}$ = 9.941; P = 0.016, $\eta^2_{partial}$ = 0.59) and main effect for

412 condition ($F_{(1, 11)}$ = 7.916; P = 0.026, $\eta^2_{partial}$ = 0.53) was observed for mean 24-hour SBP

413 after 7 days of supplementation (Figure 5). A significant difference between PLA and MTCJ

414 was identified with *post-hoc* analysis at the post-supplementation time point (P = 0.024; d =

415 0.44). Individual responses showed 11/12 participants reduced 24-hour SBP after 7 days MTCJ

416 supplementation, compared to 2/12 with PLA.

417 Likewise, mean 24-hour DBP only showed significant main effects for condition ($F_{(1, 11)}$ =

418 12.321; P = 0.010, $\eta^2_{partial}$ = 0.64) and interaction ($F_{(1, 11)}$ = 12.789; P = 0.009, $\eta^2_{partial}$ = 0.65)

419 (Figure 5). At pre-supplementation (P = 0.049; d = 0.14) and post-supplementation (P = 0.008;

420 d = 0.66) time points, *post-hoc* demonstrated a significant difference between PLA and MTCJ.

421 Individual responses showed 10/12 participants reduced mean 24-hour DBP after 7 days MTCJ

422 supplementation, compared to 2/12 with PLA.

423 A significant interaction ($F_{(1, 11)}$ = 6.236; P = 0.041, $\eta^2_{partial}$ = 0.47) and tendency towards

424 significance for the main effect of condition ($F_{(1, 11)}$ = 5.122; P = 0.058, $\eta^2_{partial}$ = 0.42) was

425 detected for mean 24-hour MAP (Figure 5). *Post-hoc* analysis indicated PLA was significantly

426 higher than MTCJ at the post-supplementation time point (P = 0.010; d = 0.58). Individual

427 responses showed 9/12 participants reduced mean 24-hour MAP after 7 days MTCJ

428 supplementation, compared to 1/12 with PLA. A tendency towards a significant interaction

429 effect ($F_{(1, 11)}$ = 3.995; P = 0.086, $\eta^2_{partial}$ = 0.36) was found for mean 24-hour PP. No main

430 effects for time or condition were detected for mean 24-hour PP (P > 0.05).

431

432   Mean day-time SBP demonstrated a tendency towards significance for the interaction ($F_{(1, 11)}$

433    $= 4.499$; $P = 0.072$, $\eta^2_{partial} = 0.39$) and a significant main effect for condition only ($F_{(1, 11)} =$

434   6.507; $P = 0.038$, $\eta^2_{partial} = 0.48$) (Table 4). Three of 12 participants were found to have lower

435   mean day-time SBP with PLA after 7 days supplementation, whilst 11/12 participants were

436   found to have lower mean day-time SBP with MTCJ.

437   Significant main interaction ($F_{(1, 11)} = 5.725$; $P = 0.048$, $\eta^2_{partial} = 0.45$) and condition ($F_{(1, 11)}$

438    $= 5.876$; $P = 0.046$, $\eta^2_{partial} = 0.46$) effects were observed for mean day-time DBP (Table 4).

439   Pairwise comparisons for the interaction effect showed significantly higher mean day-time

440   DBP with PLA compared to MTCJ at post-supplementation time point ($P = 0.020$; $d = 0.67$).

441   Individual responses showed 8/12 participants had lower mean day-time DBP after 7 days

442   MTCJ supplementation, compared to 2/12 with PLA.

443   There were no significant main effects for condition, time or interaction with mean day-time

444   MAP ($P > 0.05$) (Table 4). A main effect for time was observed for mean day-time PP ($F_{(1, 11)}$

445    $= 13.661$; $P = 0.008$, $\eta^2_{partial} = 0.66$), with *post-hoc* showing pre-supplementation to be

446   higher than post-supplementation ($P = 0.008$; $d = 0.42$) (Table 4). Analysis on night-time

447   SBP, DBP, MAP and PP demonstrated no significant main effects for condition, time or

448   interaction ($P > 0.05$) (Table 4).

449   The day-night difference for SBP ($F_{(1, 11)} = 7.355$; $P = 0.030$, $\eta^2_{partial} = 0.51$) and PP ($F_{(1, 11)} =$

450   7.199; $P = 0.031$, $\eta^2_{partial} = 0.51$) only showed a significant main effect for condition, where

451   PLA was larger than MTCJ (Table 4). Day-night differences for DBP and MAP indicated no

452   significant main effects for condition, time or interaction ($P > 0.05$) (Table 4).

453   ***Figure 5 and Table 4 near here***

454   3.4. Pulse Wave Analysis

455   No significant main effects for condition, time or interaction were detected for aortic SBP,

456   aortic DBP, AP, PP, AIx, AIx at HR75 and SEVR ($P > 0.05$) for pre-post 6 days

457   supplementation data (Table 3).

458   No main effects for condition, time or interaction ($P > 0.05$) were found on change from pre-

459   bolus data for aortic SBP. However, individual responses for the change from pre-bolus to 2-

460   hours post-bolus showed 4/12 participants decreased aortic SBP with PLA (3 ± 9 mmHg),

461   while 9/12 participants responded with lower aortic SBP after consuming MTCJ (-4 ± 8

462   mmHg). There were no main effects for condition, time or interaction on change from pre-

463   bolus data for aortic DBP, AP, PP, AIx and SEVR ($P > 0.05$) (Table 3). A main effect of

464   condition was found for AIx at HR75 ($F_{(1, 11)} = 4.929$; $P = 0.048$, $\eta^2_{partial} = 0.31$).

465   3.5. Resting Metabolic Rate

466   No significant main effects for condition, time or the condition by time interaction were

467   detected for resting EE after 6 days MTCJ consumption ($P > 0.05$). Significant interaction

468   effects were found for resting RER ($F_{(1, 11)} = 10.045$; $P = 0.009$, $\eta^2_{partial} = 0.48$), fat oxidation

469   rate ($F_{(1, 11)} = 9.394$; $P = 0.011$, $\eta^2_{partial} = 0.46$) and carbohydrate oxidation rate ($F_{(1, 11)} = 5.644$;

470    $P = 0.037$, $\eta^2_{partial} = 0.34$) between PLA and MTCJ after 6 days supplementation (Table 3).

471   *Post-hoc* identified a significant difference between conditions at pre-supplementation time

472   point for fat ($P = 0.024$; $d = 0.68$) and carbohydrate ($P = 0.027$; $d = 0.81$) oxidation rates. RER

473   was significantly ($t_{(11)} = 2.823$; $P = 0.017$, $d = 0.70$) lower post-supplementation (0.83 ± 0.04)

474   compared to pre-supplementation (0.86 ± 0.04) with MTCJ.

475   A significant main effect for time only was observed for resting EE ($F_{(5, 55)} = 2.788$; $P = 0.026$,

476    $\eta^2_{partial} = 0.20$), RER ($F_{(5, 55)} = 43.536$; $P < 0.001$, $\eta^2_{partial} = 0.80$), fat ($F_{(5, 55)} = 14.183$; $P <$

477   0.001, $\eta^2_{partial} = 0.56$) and carbohydrate ($F_{(5, 55)} = 15.936$; $P < 0.001$, $\eta^2_{partial} = 0.59$) oxidation

478   on change from pre-bolus data (Table 3).

4. Discussion

This study examined blood-based and functional cardio-metabolic responses to short-term continuous (6 days) MTCJ supplementation and acute on short-term supplementation (acute bolus on 7th day following 6 days supplementation) of MTCJ in humans with MetS. The hypotheses of the study were partially accepted as individual responses suggested a tendency for potential improvements in the underlying pathophysiology of MetS, insulin resistance and sensitivity, after 6 days MTCJ consumption compared to PLA. Findings also indicated a significant reduction in glucose, total cholesterol and LDL concentrations with concomitant lower resting RER values after 6 days MTCJ consumption compared to PLA. Of great clinical relevance, MTCJ significantly improved 24-hour BP after 7 days consumption compared to PLA. However, the present study was unable to confirm the hypothesis that the anti-hypertensive effect of MTCJ was due to changes in ACE concentrations.

4.1. Metabolic Responses

The present study showed MTCJ significantly reduced fasting glucose by 9% (-0.51 mmol.L^{-1}) after 6 days consumption, highlighting the potential efficacy of tart cherries to improve glycaemic function in insulin resistant individuals. Similarly, Ataie- Jafari *et al.* (2008) [27] reported an 8% (-0.7 mmol.L^{-1}) reduction in fasting glucose and reduced HbA$_{1c}$ after 6 weeks tart cherry concentrate consumption in individuals with T2D. This study therefore suggests short-term supplementation may be as effective, but more practical and economically viable than prolonged supplementation strategies.

After 6 days supplementation of MTCJ compared to PLA, there was a tendency for lower fasting insulin concentrations with concomitant normal fasting glucose concentrations. As mentioned by Willems *et al.* (2017) [12] with anthocyanin-rich NZBP, this may be suggestive of improved insulin sensitivity. However, HOMA2-%S was not found to be higher after 6 days MTCJ intake, although 9/12 participants did report a physiological increase in HOMA2-%S. Hence, with a larger sample size these findings may corroborate the physiological theory postulated that MTCJ may confer improvements in insulin sensitivity. Willems *et al.* (2017)

506　[12] demonstrated a reduction in fasting insulin by 14.3% (-9.5 pmol.L^{-1}) after 7 days

507　consumption of NZBP and attributed this to heightened insulin sensitivity. Similarly, fasting

508　insulin was reduced by 9.3% (-12.42 pmol.L^{-1}) after 6 days consumption of MTCJ and reduced

509　by 14% when comparing against placebo. Individual responses suggested insulin resistance

510　tended to be improved after 6 days intake of MTCJ (HOMA2-IR change: -0.27 ± 0.56)

511　compared to PLA (HOMA2-IR change: 0.48 ± 0.78), likewise Willems *et al.* (2017) [12]

512　indicated 7 days anthocyanin-rich NZBP consumption tended to improve insulin resistance.

513　Together, these findings highlight short-term continuous supplementation of cyanidin- (MTC)

514　and delphinidin-rich (NZBP) interventions may have the capacity to improve insulin

515　sensitivity/resistance in healthy [12] and MetS populations. However, larger datasets are

516　required to justify these initial observations. Moreover, it remains to be seen how long any

517　beneficial effects last, and whether intermittent supplementation for 6-7 days over a longer

518　duration may be a more physiologically effective, ecologically valid and economically viable

519　supplementation strategy. As fasting glucose and insulin responses were complementary of

520　each other, the change after 6 days indicates MTCJ may normalise glucoregulatory control

521　[10]. This may be of significance when considering insulin resistance is the underlying cause

522　of impaired cardio-metabolic function in humans with MetS.

523　4.2. Lipid Responses

524　This study suggests MTCJ may improve aspects of the lipid profile in individuals with MetS.

525　The significant reduction in total cholesterol found in the present study agrees with findings

526　from Ataie- Jafari *et al.* (2008) [27], but similar responses were not observed in other studies

527　providing tart cherries [28,29], although a trend for lower concentrations was observed in MetS

528　adults [30]. The reduction in total cholesterol with MTCJ may be explained by lower LDL

529　fractions after 6 days supplementation. Similarly, reductions in LDL were reported in subjects

530　with elevated baseline LDL after tart cherry juice consumption [27,29]; aligning with findings

531　from other human trials supplementing anthocyanin-rich interventions that hyperlipidaemia is

532   a prerequisite to observe improvements [31]. Clinically, LDL responses after 6 days MTCJ

533   consumption may correspond to an 8% relative risk reduction of major vascular events [32].

534   Bing sweet cherry consumption had no effect on TC:HDL ratio in healthy adults [33]. In the

535   present study, TC:HDL ratio was found to be lower after 6 days consumption of MTCJ and

536   individual responses showed 10/12 participants with lower TC:HDL ratios after MTCJ

537   supplementation compared to 1/12 participants with placebo. Furthermore, TC:HDL ratio was

538   shown to be a good predictor of cardiovascular risk reduction when assessing interventions

539   [34], thus improvements in TC:HDL after 6 days MTCJ ingestion highlight its clinical efficacy

540   against cardiovascular events.

541   4.3. Cardiovascular Responses

542   The efficacy of sweet and tart cherry interventions on improving BP, particularly SBP, had

543   been demonstrated numerous times in various populations [7,8,14,27,35], including MetS [13].

544   However, these studies used lab-based measurements which is clinically inferior to 24-hour

545   ABPM [19,36]. Therefore, the present study was the first to demonstrate reductions in mean

546   24-hour SBP, DBP and MAP after cherry supplementation in any population.

547   A clinically significant reduction in mean 24-hour SBP was observed after 7 days MTCJ

548   consumption (-5 mmHg); which would be associated with prevention of all-cause and

549   cardiovascular mortality by 20% [37]. This finding adds greater clinical and biological

550   relevance to the individual responses reported for acute SBP reductions in the present study,

551   by Keane *et al.* (2016b) [8] and Desai *et al*. (2019) [13] after an acute, single-bolus of MTCJ.

552   In this study, a reduction of 11 mmHg was observed after 6 days MTCJ consumption compared

553   to PLA for acute SBP at 2-hours post-bolus. Comparative reductions of 8 mmHg were seen

554   with 7 days blueberry juice consumption compared to PLA in individuals with pre- and stage

555   1 hypertension [38], highlighting the efficacy of 6-7 days consumption of anthocyanin-rich

556   juices (~270-314 mg.day^{-1} total anthocyanins). Importantly, the magnitude of acute SBP

557   reduction with MTCJ was comparable to approved anti-hypertensive drugs, associated with

558   harmful side effects [39]. Moreover, the 2 mmHg reduction in mean 24-hour DBP after 7 days

559   MTCJ consumption would be associated with a risk reduction of coronary heart disease and

560   stroke by 6 and 15%, respectively [40]. This response was facilitated by significant day-time

561   DBP reductions with MTCJ, which has been shown to be a significant predictor of CVD,

562   coronary heart disease and stroke [41]. Despite supplementing a similar daily anthocyanin

563   dosage as the present study (270 mg.day^{-1}), Stull *et al.* (2015) [20] reported no effect on 24-

564   hour BP after consuming anthocyanin-rich (290.3 mg.day^{-1} anthocyanins) blueberry smoothie

565   for 6 weeks compared to PLA, in individuals with MetS. Hence the above literature supports

566   the hypothesis that short-term, low-dose anti-oxidant supplementation derived from

567   anthocyanins may be a more pragmatic method to induce clinically relevant improvements in

568   lab-based and 24-hour BP. However, it should be noted that long-term supplementation of

569   anthocyanin-rich interventions [31,42], including tart cherries [27,29], have been shown to

570   reduce BP through overlapping but different mechanisms (increased arterial compliance and/or

571   improved endothelial function) [43] to short-term supplementation.

572   Aviram and Dornfeld (2001) [44] demonstrated reductions in SBP were correlated with

573   significantly lower ACE activity after 2 weeks pomegranate juice consumption. Furthermore,

574   Kirakosyan *et al.* (2018) [9] demonstrated 88.7% ACE inhibition *in vitro,* with MTC extract.

575   However, in the present study, as no correlations were observed between serum ACE

576   concentrations and changes in cardiovascular parameters, the hypotensive effects of MTCJ

577   may not be explained by alterations in ACE concentrations. Yet, individual responses showed

578   8/12 participants had lower ACE concentrations 6 days after MTCJ compared to 4/12 with

579   PLA. The high inter-individual variation of ACE may contribute to the null results, particularly

580   given the variation in age between participants. Hence, it is possible that the action of ACE

581   may only be prominent in certain individuals, and other mechanisms such as increased arterial

582   stiffness and endothelial dysfunction in older participants may contribute to elevated BP.

583   Overall, MTCJ may still inhibit ACE and explain the hypotensive effects, however future

584 research should assess this through ACE activity assays to accurately elucidate this mechanism
585 of action.

586 Despite the prospective nature of the study, a limitation would be the small sample size on
587 which conclusions are based, therefore larger clinical trials are required assessing individuals
588 with MetS, but also other clinical populations. Moreover, surrogate indices of pancreatic β-cell
589 function, insulin resistance and sensitivity were used. Future work should consider using
590 tolerance tests or hyperinsulinaemic-euglycaemic clamps to assess the efficacy of MTCJ on
591 post-prandial responses, which provides more ecologically valid conclusions. A key strength
592 of the study was the use of 24-hour ABPM as a clinically relevant measure of assessing the
593 effect of MTCJ on blood pressure and subsequently cardiovascular risk. Another strength was
594 the use of a dietary supplement made of a whole food; upholding ecological validity due to the
595 simplicity of incorporating such an intervention into habitual diets.

596 **5. Conclusion**
597 The present study has provided novel findings and demonstrated clinically relevant
598 improvements on aspects of cardio-metabolic function, emphasising the potential efficacy of
599 MTCJ in preventing further cardio-metabolic dysregulation in an 'at risk' population. Of
600 particular clinical relevance, 24-hour ABPM was significantly improved after 7 days MTCJ
601 consumption. Further work is required to elucidate mechanisms for BP responses, but also
602 other cardio-metabolic improvements shown here. It remains to be seen how long the
603 reductions in 24-hour BP last with MTCJ, however with further research MTCJ could perhaps
604 replace or be used as an adjuvant to anti-hypertensive drugs in the future. Nevertheless, the
605 evidence presented is promising for individuals with elevated cardiovascular risk particularly,
606 pre-hypertension. Future clinical trials with larger sample sizes assessing individuals with
607 MetS, but also other clinical populations are required to affirm these conclusions.

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Table 1A. Selected baseline characteristics obtained during screening (n = 12).

Characteristics	Mean ± SD
Fasting Total Cholesterol (mmol.L^{-1})	4.17 ± 1.21
24-hour SBP (mmHg)	128 ± 10
24-hour DBP (mmHg)	77 ± 8
Fasting Insulin (pmol.L^{-1})	118.99 ± 68.14
HOMA2-IR (AU)	2.2 ± 1.4
HOMA2-β (%)	137.9 ± 49.5
HOMA2-%S (%)	63.2 ± 40.5
ACE (pg.mL^{-1})	8627 ± 8702

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754

755   **Tables and Figures**

756

757   Table 1B. Individual baseline characteristics of MetS criteria obtained during screening (n = 12).

Characteristics	Mean ± SD	Participant											
		1 (M)	2 (M)	3 (F)	4 (M)	5 (M)	6 (F)	7 (F)	8 (F)	9 (F)	10 (F)	11 (M)	12 (M)
Waist Circumference (cm)	101.0 ± 19.3	102*	125*	80*	85	125.4*	119*	88*	82*	74.5	100*	104*	127*
Fasting Glucose (mmol.L^{-1})	5.32 ± 1.04	6.15*	4.89	5.42	4.20	6.90*	6.38*	3.94	4.90	3.95	5.04	5.26	6.08*
Fasting Triglycerides (mmol.L^{-1})	1.6 ± 0.3	1.7*	2.0*	1.2	1.8*	2.2*	1.3	1.7*	1.9*	1.8*	1.7*	1.1	1.3
Fasting HDL (mmol.L^{-1})	1.41 ± 0.32	1.95	1.32	1.38	1.69	1.09	1.31	1.19*	1.58	1.88	1.52	1.01*	1.01*
SBP (mmHg)	135 ± 16	122	131*	131*	158*	149*	161*	104	131*	136*	135*	131*	125
DBP (mmHg)	77 ± 8	66	75	88*	91*	85*	70	75	70	85*	79	69	76

758   *Denotes meeting MetS criteria for respective characteristic. (F) denotes female participant. (M) denotes male participant.

759

760

Enrolment

Assessed for eligibility (n = 39)

Excluded (n = 24)
- Not meeting inclusion criteria (n = 23)
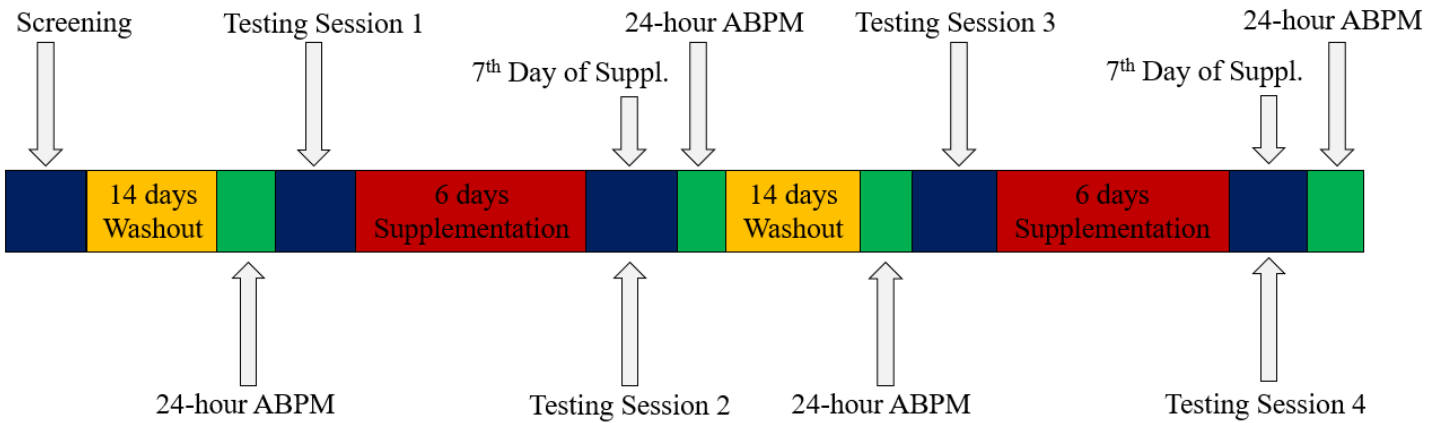- Declined to participate (n = 1)

Randomised (n = 15)

Allocation

Allocated to Placebo intervention (n = 8)
- Received allocated intervention (n = 7)
- Not received allocated intervention (n = 1)
(Participant dropped out for personal reasons)

Allocated to MTCJ intervention (n = 7)
- Received allocated intervention (n = 7)
- Not received allocated intervention (n = 0)

Allocation

Allocated to Placebo intervention (n = 7)
- Received allocated intervention (n = 6)
- Not received allocated intervention (n = 1)
(Participant dropped out for personal reasons)

Allocated to MTCJ intervention (n = 7)
- Received allocated intervention (n = 6)
- Not received allocated intervention (n = 1)
(Participant dropped out for personal reasons)

Analysis

Analysed (n = 12)

761
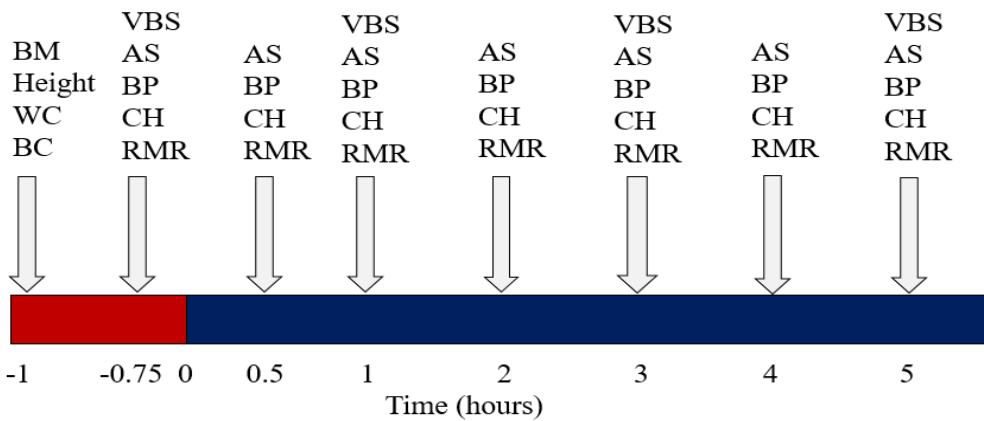
762   Figure 1. CONSORT flow diagram of the participants recruited, screened, tested, analysed and

763   excluded during the course of the study.

764

765

A

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
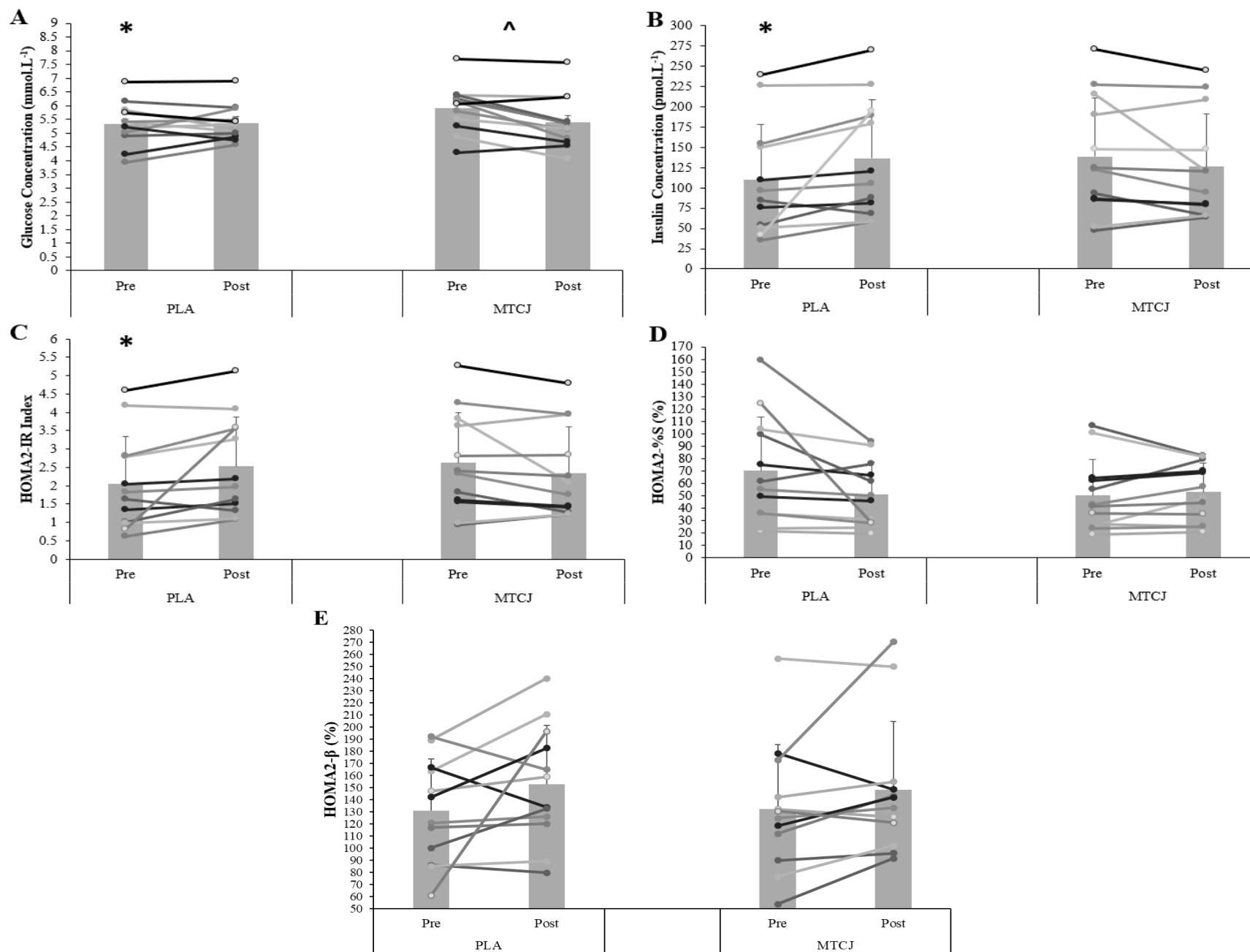
■ = Pre-Bolus
■ = Post-Bolus

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

Table 2. Mean ± SD absolute raw data for blood-based biomarkers per treatment condition.

		Pre 6 days Supplementation	Post 6 days Supplementation	Post-bolus Time Points		
				1 hr	3 hr	5 hr
Glucose$^{\$}$	PLA	5.33 ± 0.18*	5.36 ± 0.25	5.83 ± 0.24	5.28 ± 0.13	5.49 ± 0.19
(mmol.L^{-1})	MTCJ	5.90 ± 0.86^	5.39 ± 0.23	5.88 ± 0.24	5.13 ± 0.22	5.14 ± 0.13
Insulin$^{\$}$	PLA	109.55 ± 68.95*	136.59 ± 72.18	146.29 ± 76.90	104.26 ± 59.70	97.77 ± 64.19
(pmol.L^{-1})	MTCJ	138.39 ± 72.28	125.96 ± 65.57	153.23 ± 87.21	97.43 ± 54.57	93.76 ± 65.29
Triglycerides$^{\$}$	PLA	1.2 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.6 ± 0.1
(mmol.L^{-1})	MTCJ	1.4 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.5 ± 0.1	1.6 ± 0.1
Total Cholesterol	PLA	3.99 ± 0.22*	4.03 ± 0.33	4.17 ± 0.34	4.23 ± 0.24	4.25 ± 0.19
(mmol.L^{-1})	MTCJ	4.55 ± 0.30^	4.14 ± 0.41	4.17 ± 0.39	4.03 ± 0.45	3.96 ± 0.17
HDL	PLA	1.43 ± 0.01	1.30 ± 0.01	1.30 ± 0.02	1.27 ± 0.02	1.27 ± 0.02
(mmol.L^{-1})	MTCJ	1.39 ± 0.03	1.40 ± 0.02§	1.40 ± 0.03	1.40 ± 0.01	1.36 ± 0.03
LDL	PLA	2.39 ± 0.21	2.65 ± 0.31	2.99 ± 0.37	3.14 ± 0.30	3.30 ± 0.28
(mmol.L^{-1})	MTCJ	3.07 ± 0.32^	2.71 ± 0.46	2.77 ± 0.35	2.91 ± 0.36	2.83 ± 0.31
ACE	PLA	8706 ± 8748	9334 ± 10363	10609 ± 12780	10743 ± 12267	10538 ± 11417
(pg.mL^{-1})	MTCJ	10161 ± 11474	9127 ± 10915	9548 ± 11486	9460 ± 11385	9150 ± 11521

*Denotes significant difference between conditions at respective time point. ^Denotes significant difference between pre- and 6 days post-supplementation time points for MTCJ. §Denotes significant difference between conditions at post-supplementation time point. $^{\$}$Denotes significant main effect for time with post-hoc identifying differences between 1-hour and both 3-hours and 5-hours post-bolus ($P < 0.05$).

Figure 3. (A) Glucose, (B) Insulin, (C) HOMA2-IR, (D) HOMA2-%S and (E) HOMA2-β responses before and after supplementation of PLA and MTCJ. Bar graphs depict mean ($\pm$SD) group values for each condition, pre and post 6 days supplementation. Lines depict individual responses for all 12 participants. *Denotes significant difference between conditions at respective time point. ^Denotes significant difference between pre- and post-supplementation time points for MTCJ.
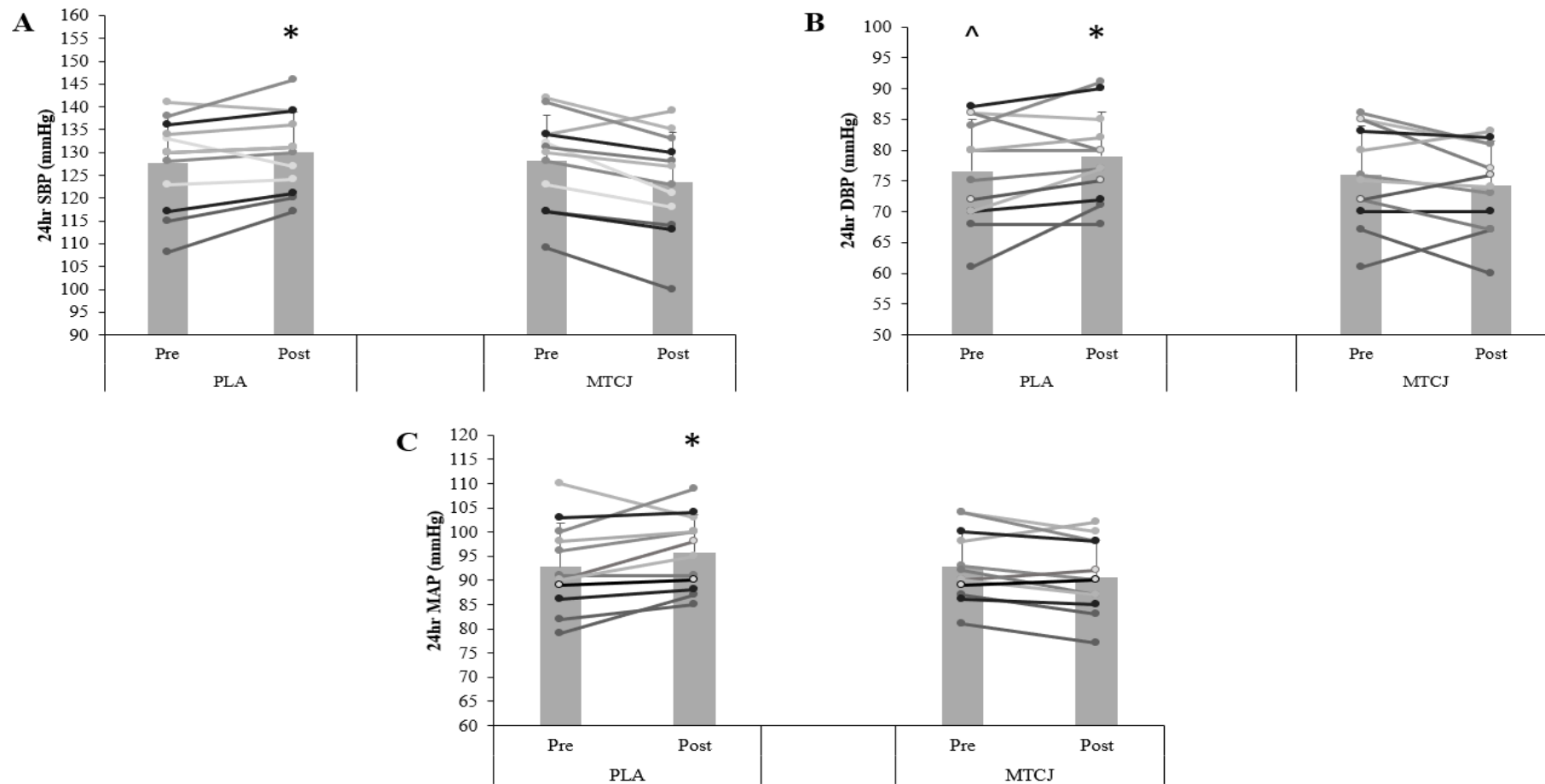
Table 3. Mean ± SD absolute raw data for cardiac haemodynamic, PWA and RMR parameters per treatment condition.

		Pre 6 days Suppl.	Post 6 days Suppl.	Post-bolus Time Points					
				30 minutes	1 hr	2 hr	3 hr	4 hr	5 hr
Brachial SBP	PLA	127 ± 16	128 ± 17	133 ± 13	130 ± 14	132 ± 19	132 ± 19	128 ± 10	129 ± 13
(mmHg)	MTCJ	134 ± 17	128 ± 15	127 ± 13	128 ± 13	121 ± 10	127 ± 12	129 ± 18	133 ± 14
Brachial DBP	PLA	75 ± 10	74 ± 7	76 ± 8	72 ± 5	74 ± 5	73 ± 8	74 ± 7	75 ± 5
(mmHg)	MTCJ	75 ± 10	72 ± 7	73 ± 4	74 ± 5	70 ± 7	74 ± 7	73 ± 6	75 ± 4
MAP	PLA	98 ± 12	93 ± 10	99 ± 9	94 ± 8	97 ± 8	96 ± 10	97 ± 10	97 ± 9
(mmHg)	MTCJ	98 ± 12	93 ± 9	95 ± 4	96 ± 7	93 ± 6	95 ± 8	95 ± 8	96 ± 8
HR	PLA	65 ± 12	67 ± 14	68 ± 16	67 ± 16	66 ± 17	66 ± 16	65 ± 15	65 ± 5
(beats.min^{-1})	MTCJ	65 ± 14	63 ± 11	66 ± 11	64 ± 12	62 ± 13	61 ± 11	62 ± 12	60 ± 11
Cardiac Output	PLA	6.85 ± 2.46	6.64 ± 2.37	6.86 ± 2.46	7.05 ± 2.74	7.00 ± 4.07	7.10 ± 3.23	6.02 ± 2.22	6.44 ± 2.03
(L.min^{-1})	MTCJ	6.19 ± 2.81	5.85 ± 2.02	5.69 ± 2.97	5.46 ± 2.64	5.52 ± 2.66	5.70 ± 2.36	5.60 ± 2.47	5.87 ± 2.72
Stroke Volume	PLA	104 ± 26	98 ± 21	100 ± 22	104 ± 21	101 ± 34	105 ± 23	103 ± 26	98 ± 17
(mL)	MTCJ	102 ± 20	110 ± 29	105 ± 25	98 ± 22	101 ± 27	100 ± 26	103 ± 29	109 ± 29
TPR	PLA	1.03 ± 0.48	0.91 ± 0.23	0.96 ± 0.33	0.89 ± 0.27	1.04 ± 0.44	0.93 ± 0.32	1.09 ± 0.38	0.99 ± 0.29
(mmHg·s^{-1}·mL^{-1})	MTCJ	0.93 ± 0.37	0.79 ± 0.28	1.00 ± 0.42	0.98 ± 0.29	0.95 ± 0.29	0.95 ± 0.31	0.97 ± 0.34	0.94 ± 0.38
Aortic SBP	PLA	124 ± 12	119 ± 15	120 ± 12	119 ± 12	121 ± 14	124 ± 13	119 ± 15	120 ± 13
(mmHg)	MTCJ	124 ± 15	120 ± 15	121 ± 14	118 ± 14	116 ± 13	120 ± 14	119 ± 15	120 ± 13

Aortic DBP (mmHg)	PLA	80 ± 7	80 ± 9	82 ± 11	80 ± 11	83 ± 9	84 ± 11	84 ± 8	83 ± 7
	MTCJ	81 ± 9	79 ± 10	79 ± 9	80 ± 9	78 ± 8	83 ± 10	81 ± 7	80 ± 8
AP (mmHg)	PLA	11 ± 6	9 ± 6	8 ± 5	7 ± 5	9 ± 6	11 ± 8	9 ± 6	10 ± 8
	MTCJ	12 ± 7	9 ± 7	9 ± 8	9 ± 8	9 ± 7	10 ± 8	10 ± 8	11 ± 8
Pulse Pressure (mmHg)	PLA	38 ± 9	35 ± 11	38 ± 7	39 ± 7	38 ± 7	39 ± 13	35 ± 8	37 ± 10
	MTCJ	39 ± 11	38 ± 10	42 ± 10	38 ± 11	38 ± 10	37 ± 11	38 ± 11	40 ± 10
AIx (%)	PLA	26 ± 13	23 ± 12	23 ± 12	24 ± 12	25 ± 13	26 ± 15	26 ± 14	27 ± 14
	MTCJ	25 ± 14	24 ± 12	24 ± 14	24 ± 15	22 ± 14	23 ± 14	24 ± 14	26 ± 14
AIx at HR75 (%)*	PLA	22 ± 10	19 ± 11	20 ± 11	21 ± 12	21 ± 11	22 ± 12	23 ± 11	23 ± 12
	MTCJ	21 ± 12	20 ± 11	20 ± 10	20 ± 12	19 ± 11	18 ± 12	19 ± 11	19 ± 12
SEVR (%)	PLA	180 ± 30	192 ± 36	187 ± 37	189 ± 35	192 ± 39	194 ± 43	197 ± 39	191 ± 37
	MTCJ	183 ± 39	178 ± 25	175 ± 29	177 ± 25	187 ± 40	189 ± 33	181 ± 29	177 ± 25
Resting EE$ (kcal.day^{-1})	PLA	1835 ± 509	1847 ± 437	1895 ± 397	1890 ± 360	1835 ± 411	1827 ± 394	1785 ± 394	1785 ± 356
	MTCJ	1793 ± 572	1794 ± 489	1871 ± 467	1892 ± 408	1781 ± 439	1795 ± 459	1790 ± 443	1865 ± 451
Resting RER^$ (AU)	PLA	0.85 ± 0.04	0.86 ± 0.06	0.96 ± 0.10	0.93 ± 0.08	0.87 ± 0.08	0.84 ± 0.08	0.82 ± 0.08	0.81 ± 0.05
	MTCJ	0.86 ± 0.04	0.83 ± 0.04§	0.98 ± 0.08	0.94 ± 0.08	0.89 ± 0.08	0.86 ± 0.08	0.82 ± 0.07	0.81 ± 0.05
Resting Fat Oxidation^$ (g.min^{-1})	PLA	0.08 ± 0.04	0.06 ± 0.03	0.04 ± 0.07	0.08 ± 0.09	0.06 ± 0.04	0.08 ± 0.05	0.08 ± 0.04	0.09 ± 0.04
	MTCJ	0.05 ± 0.03#	0.06 ± 0.02	0.01 ± 0.03	0.02 ± 0.04	0.05 ± 0.04	0.07 ± 0.05	0.08 ± 0.04	0.09 ± 0.05
Resting CHO Oxidation^$ (g.min^{-1})	PLA	0.14 ± 0.08	0.20 ± 0.13	0.25 ± 0.15	0.16 ± 0.20	0.18 ± 0.08	0.14 ± 0.08	0.13 ± 0.09	0.12 ± 0.09
	MTCJ	0.22 ± 0.12#	0.17 ± 0.08	0.33 ± 0.11	0.30 ± 0.14	0.21 ± 0.08	0.18 ± 0.09	0.14 ± 0.08	0.13 ± 0.05

795 *Denotes significant main effect of condition for change from pre-bolus data. ^Denotes significant main effect for interaction between PLA and MTCJ pre
796 and post 6 days supplementation. #Denotes significant difference between conditions at pre-supplementation time point. §Denotes significant difference
797 between pre-supplementation and post-supplementation time points for MTCJ. $Denotes significant main effect of time on change from post 6 days
798 supplementation data.

799

Figure 5. (A) Mean 24-hour SBP, (B) Mean 24-hour DBP and (C) Mean 24-hour MAP responses before and after supplementation of PLA and MTCJ. Bar graphs depict mean (±SD) group values for each condition, pre and post 7 days supplementation. Lines depict individual responses for all 12 participants. ^Denotes significant difference between conditions at pre-supplementation time point. *Denotes significant difference between conditions at post-supplementation time point.

Table 4. Mean ± SD day-time, night-time and day-night differences from 24-hour ABPM responses before and after 7 days supplementation of PLA and MTCJ.

		Pre-Supplementation	Post-Supplementation
Day SBP*	PLA	132 ± 8	133 ± 8
(mmHg)	MTCJ	132 ± 9	127 ± 11
Day DBP$	PLA	79 ± 7	81 ± 7^
(mmHg)	MTCJ	79 ± 6	76 ± 6
Day MAP	PLA	92 ± 8	97 ± 7
(mmHg)	MTCJ	92 ± 10	94 ± 7
Day PP$	PLA	53 ± 6	51 ± 5
(mmHg)	MTCJ	53 ± 5	50 ± 8
Night SBP	PLA	113 ± 13	117 ± 7
(mmHg)	MTCJ	117 ± 12	117 ± 13
Night DBP	PLA	68 ± 9	69 ± 8
(mmHg)	MTCJ	69 ± 10	68 ± 8
Night MAP	PLA	82 ± 11	85 ± 7
(mmHg)	MTCJ	81 ± 14	84 ± 11
Night PP	PLA	46 ± 8	48 ± 5
(mmHg)	MTCJ	48 ± 7	49 ± 7
D/N SBP*	PLA	19 ± 12	16 ± 6
(mmHg)	MTCJ	15 ± 10	10 ± 8
D/N DBP	PLA	11 ± 8	12 ± 8
(mmHg)	MTCJ	10 ± 9	9 ± 7
D/N MAP	PLA	10 ± 8	13 ± 7
(mmHg)	MTCJ	11 ± 11	10 ± 9
D/N PP*	PLA	7 ± 7	4 ± 4
(mmHg)	MTCJ	5 ± 5	1 ± 3

804 D/N (Day/Night Difference). *Denotes significant main effect for condition. $Denotes significant main effect for
805 time. $Denotes significant main effect for interaction. ^Denotes significant difference between conditions at
806 corresponding time point.

807