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

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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 7th 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 lowered 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).

Keywords: ambulatory blood pressure; anthocyanins; cardio-metabolic health; diabetes; functional foods; hypertension

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Acknowledgements:

The authors would like to thank the participants for partaking in this study.

Author Contributions

Conceptualization, TD, LB and MR.; Methodology, TD; Formal Analysis, 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.

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

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).

greater clinical evidence is required which can be applied to normal daily-living conditions such as 24-hour ambulatory blood pressure monitoring (ABPM). The mechanism of action for the hypotensive properties of tart and sweet cherries has yet to be elucidated, with nitric oxide bioavailability [7] and modulation of arterial stiffness [7,13,16] not significantly changing after MTCJ consumption. Therefore, an alternative mechanism involving angiotensin-I-converting enzyme (ACE) was hypothesised in the present study, since Kirakosyan *et al.* (2018) [9] observed 88.7% ACE inhibition *in vitro* with Montmorency tart cherry (MTC) extract. The effect of MTC on ACE has not yet been assessed *ex vivo* in humans, therefore this mechanism warrants exploration in a human randomised controlled trial.

The present study aimed to be the first to examine cardio-metabolic responses after short-term, continuous MTCJ supplementation (6 days) and acute responses to a single-bolus on the 7th day following short-term supplementation, in humans with MetS. It was hypothesised that MTCJ would improve glycaemic and insulinaemic function through increasing insulin sensitivity. Moreover, MTCJ would maintain acute reductions in SBP and lower 24-hour SBP after 7 days supplementation, through ACE inhibition.

2. Methods

2.1. Participants

Twelve (6 males and 6 post-menopausal females) participants [mean ± SD, age 50 ± 10 years (range 28-62 years), stature 1.73 ± 0.12 m, body mass 94.1 ± 23.1 kg, body mass index 31 ± 7 kg.m^{-2}] with MetS (Tables 1A and 1B) volunteered for this 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) [17], where 3 of the 5 qualifying criteria [Waist Circumference: ethnicity and sex specific criteria; Fasting Triglycerides: ≥1.69 mmol.L^{-1}; Fasting High-Density Lipoprotein: <1.03 mmol.L^{-1} (men), <1.29 mmol.L^{-1} (women); Blood Pressure: ≥130 mmHg SBP or ≥85 mmHg diastolic blood pressure (DBP); Fasting Glucose: ≥5.6 mmol.L^{-1}] 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, anti-oxidant, 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/PGR/UH/03319) 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 as a clinical trial on clinicaltrials.gov (NCT03619941). ***Table 1A and 1B near here***

Figure 1 near here

2.2 Procedures

2.2.1. Research Design

A single-blind (blinded to participant), placebo-controlled, randomised, crossover design was utilised; each participant acted as their own control. During the 6-week study duration, participants completed both testing sessions during which placebo (PLA) or Montmorency Tart Cherry Juice (MTCJ) supplements were consumed for a continuous period of 7 days. A 14-day washout period [8] was incorporated prior to crossover to the opposing condition.

Schematics are shown of the overall study design (Figure 2A) and specific procedures during a testing session. The first and third testing sessions lasted 1 hour; pre-supplementation anthropometric (stature, body mass, waist circumference), functional [pulse wave analysis (PWA), cardiac haemodynamics, resting metabolic rate (RMR)] and fasting blood-based biomarkers were measured. Testing sessions two and four lasted 6 hours; anthropometric, functional and fasting blood-based biomarkers were measured prior to consumption of the supplement (6 days post-supplementation) and up to 5-hours post-consumption of the 7th bolus (Figure 2B). Testing sessions two and four were conducted following 6 days short-term supplementation of either MTCJ or PLA, during which the 7th bolus of supplement was consumed, and acute measurements taken thereafter which we have termed acute on short-term supplementation (Figure 2A).

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 ± 22%), (carbohydrate 48 ± 40%), (fat 38 ± 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

Protein Ltd, Northwich, UK), fructose (Fruit Sugar, Morrisons, Bradford, UK), cornflour (Morrisons, Bradford, UK), citric acid (100% Pure Citric Acid, VB and Sons, UK), red and black food colouring (Morrisons, Bradford, UK). Nutritional analysis for MTCJ (Volume: 130 mL, Energy: 102 kcal, Carbohydrates: 24.5 g of which Sugars: 17.9 g, Glucose: 9.98 g, Fructose: 7.92 g, Protein: 1.1 g, Fat: 0 g, Fibre: 2.6 g, Total Anthocyanins: 270 mg) and placebo (Volume: 130 mL, Energy: 102 kcal, Carbohydrates: 25 g of which Sugars: 18 g, Glucose: 10.03 g, Fructose: 7.97 g, Protein: 0.5 g, Fat: 0 g, Fibre: Trace, Total Anthocyanins: 0 mg). Anonymity of the 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. Only 2/12 participants correctly distinguished the supplements provided (intervention or placebo) and none identified the intervention as 'cherry' or 'containing cherries (either sweet or tart)'.

2.2.4. Measures and Equipment

Serum insulin was regarded as the primary endpoint for the present study. HOMA2-IR, acute SBP and 24-hour SBP were regarded as secondary variables. Tertiary endpoints focused on other aspects of MetS including hyperlipidaemia, hyperglycaemia and cardiovascular dysfunction.

Blood pressure, cardiac haemodynamics and PWA measurements were all conducted in a quiet, temperature-controlled laboratory maintained between 21-24ºC. All measurements were performed in an upright seated position with 10 minutes prior rest.

2.2.4.1. Blood Pressure

Brachial systolic (SBP) and diastolic (DBP) blood pressure (Omron MX3, Omron, Japan) were measured four times, with an average of the final three being taken as BP.

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.

2.2.4.3. 24-hour Ambulatory Blood Pressure

In addition to clinic BP measurements in the laboratory using an automated sphygmomanometer, 24-hour ambulatory blood pressure monitoring (ABPM) (Meditech ABPM-04, Meditech, Hungary) was also conducted through an oscillometric method. ABPM-04 monitors have been clinically validated against British Hypertension Society guidelines [18]. ABPM provides a more accurate representation of BP as measurements are obtained under normal daily-living conditions, negating white coat syndrome; and mean pressures are taken from multiple readings over a 24-hour period, thus ABPM has become the reference standard for measuring BP non-invasively and diagnosing hypertension [19]. Moreover, 24-hour ABPM is deemed to be the reference standard for accurate assessment of cardiovascular risk in adults [19].

Participants underwent a familiarisation period of wearing the ABPM device prior to data collection. A day before and after the 7-day supplementation period, participants were fitted with a 24-hour ABPM on the non-dominant arm. The ABPM was programmed (CardioVisions Software 1.15.2, Meditech, Hungary) to take readings every 30 minutes during the day (07:00-22:00) and every hour during the night (22:00-07:00) [20]. A minimum of 14 day-time readings and 7 night-time readings were considered for a valid 24-hour ABPM assessment [21]. Participants were advised to be seated upright, keep still and relax their arm whenever the monitor recorded measurements. The following data was obtained from the ABPM monitor: mean 24-hour, day-time and night-time SBP, DBP, MAP and PP. The difference between mean day and night SBP, DBP, MAP and PP was also calculated.

2.2.4.4. Pulse Wave Analysis

Aortic pulse waveforms, aortic SBP, aortic DBP, pulse pressure (PP), augmentation pressure (AP), augmentation index (AIx), AIx normalised to 75 beats.min^{-1} (AIx at HR75) and subendocardial viability ratio (SEVR) were determined by pulse wave analysis (PWA) using applanation tonometry (SphygmoCor, ScanMed Medical, UK) as previously described in [13].

2.2.4.5. Resting Metabolic Rate (RMR)

RMR, resting energy expenditure (EE), substrate oxidation rates and respiratory exchange ratio (RER) were measured using an open-circuit indirect calorimetry system (GEM Nutrition Ltd, Cheshire, UK) as previously described in [13].

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

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

Equations outlined by Frayn (1983), were used to determine fat oxidation and carbohydrate (CHO) oxidation rates at rest.

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

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

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). Tubes were centrifuged at 4000 rev.min^{-1}, 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^{-1}, coefficient of variation (CV) ≤ 1.5%) (Biosen C-Line, EKF Diagnostics, Cardiff, UK) in duplicates.

2.2.5.3. Insulin

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). Inter- and intra-plate precision were 6.1% and 5.5%, 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), insulin sensitivity (HOMA2-%S) and insulin resistance (HOMA2-IR index) through the HOMA2 spreadsheet model (HOMA2-IR, available from https://www.dtu.ox.ac.uk/homacalculator/) [24].

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^{-1}, according to the manufacturer's guidelines. Intra-assay CV for triglycerides, total cholesterol and HDL were 2.94%, 4.45% and 4.22%, respectively. LDL was determined indirectly using the formula below [25].

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

2.2.5.6. Angiotensin-I-converting Enzyme

Human ACE (CD 143) (peptidyl-dipeptidase A, EC 3.4.15.1) protein concentrations were measured according to manufacturer's guidelines, from serum samples in duplicates using a

96-well ELISA [Human ACE ELISA (CD 143) ab119577, Abcam, Cambridge, UK]. Inter- and intra-plate precision were 12.3% and 9.2%, respectively.

2.3. Data Analysis

Statistical analysis was performed using SPSS v22.0 (IBM, Chicago, USA) where data are reported as means ±standard deviation (±SD). Data normality was checked using a Shapiro-Wilk test. Greenhouse-Geisser correction was applied upon violation of Mauchly's test of sphericity for ANOVAs ($P < 0.05$). Statistical significance was set at $P < 0.05$. Based on data from Desai et al. (2019) [13] for the interaction effect between condition (PLA and MTCJ) and time for serum insulin, *a priori* power ($\alpha = 0.05$; $1\text{-}\beta = 0.8$) analysis indicated a sample size of 8 would be sufficient to detect a significant difference pre-post 6 days supplementation for serum insulin. Therefore, a minimum of 12 participants were recruited assuming a 20% dropout rate. *Post-hoc* power analysis indicated sufficient power ($1\text{-}\beta = 0.99$; $\alpha = 0.05$; $n = 12$) to detect a significant main effect for the interaction between condition and time (pre-post 6 days supplementation) for insulin, HOMA2-IR and 24-hour SBP. Acute SBP also demonstrated sufficient power ($1\text{-}\beta = 0.86$; $\alpha = 0.05$; $n = 12$) to detect a significant interaction effect.

A within-group two-way, 2 x 2, condition (PLA vs MTCJ) x time (Pre-Supplementation and Post-Supplementation), repeated-measures ANOVA design with *post-hoc* Bonferroni adjustment, measured differences for all blood-based biomarkers, cardiac haemodynamic, PWA, RMR and 24-hour BP parameters.

To account for day-to-day physiological variances at pre-bolus between conditions for each variable, data were analysed as change from pre-bolus for each time point measured post-bolus during testing sessions 2 and 4. 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 ($P > 0.05$) between conditions for all variables at the pre-bolus time point, hence two-way repeated-measures ANOVA was performed. A within-group two-way, 2 x 6, condition (PLA vs MTCJ)

x time (30 minutes, 1, 2, 3, 4- and 5-hours post-bolus), repeated-measures ANOVA design with *post-hoc* Bonferroni adjustment, measured differences of cardiac haemodynamic, PWA and RMR parameters on change from pre-bolus values. Blood-based biomarkers were analysed using the same model but with a 2 x 3, condition (PLA vs MTCJ) by time (1, 3- and 5-hours post-bolus) design on change from pre-bolus values for each condition.

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) [26]. 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 (≥0.8) [26].

3. Results

3.1. Blood Biomarkers

3.1.1. Glucose

Responses to glucose pre and post 6 days supplementation (Figure 3) demonstrated a significant interaction effect ($F_{(1, 11)} = 5.534$; $P = 0.038$, $\eta^2_{partial} = 0.34$) and main effect for time ($F_{(1, 11)} = 8.077$; $P = 0.016$, $\eta^2_{partial} = 0.42$) only. *Post-hoc* indicated a significant difference between PLA and MTCJ at pre-supplementation time point ($P = 0.023$; $d = 2.85$). Fasting glucose was significantly ($t_{(11)} = 3.506$; $P = 0.005$, $d = 0.56$) lower 6 days after supplementation (5.39 ± 0.23 mmol.L^{-1}) compared to pre-supplementation (5.90 ± 0.86 mmol.L^{-1}) with MTCJ. Individual responses showed 10/12 individuals with lower fasting glucose after 6 days supplementation of MTCJ compared to PLA.

A main effect for time was found for change from pre-bolus data for glucose ($F_{(2, 22)} = 12.641$; $P < 0.001$, $\eta^2_{partial} = 0.54$) (Table 2). *Post-hoc* showed glucose to be significantly higher at 1-hour post-bolus compared to both 3-hours ($P = 0.003$, $d = 1.11$) and 5-hours post-bolus ($P = 0.021$, $d = 0.92$).

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***

348 3.1.3. HOMA2

349 As with insulin, HOMA2-IR ($F_{(1, 11)} = 8.115$; $P = 0.016$, $\eta^2_{partial} = 0.43$) and HOMA2-%S ($F_{(1,}$

350 $_{11)} = 6.332$; $P = 0.029$, $\eta^2_{partial} = 0.37$) demonstrated a significant interaction after 6 days

351 supplementation (Figure 3), with pairwise comparisons showing a significant difference

352 between PLA and MTCJ at pre-supplementation time point for HOMA2-IR ($P = 0.039$; $d =$

353 0.43). Individual responses for HOMA2-IR showed insulin resistance increased in 10/12

354 participants with PLA, whereas insulin resistance was reduced in 8/12 participants after 6 days

355 MTCJ consumption. Moreover, HOMA2-%S showed 9/12 participants increased insulin

356 sensitivity with 6 days continuous MTCJ consumption; 2/12 increased with PLA.

357 A main effect for time ($F_{(1, 11)} = 7.720$; $P = 0.018$, $\eta^2_{partial} = 0.41$) only was observed for

358 HOMA2-β with pre-post 6 days supplementation data. *Post-hoc* analysis highlighted greater

359 pancreatic β-cell function post-supplementation (PLA: 152 ± 49%, MTCJ: 148 ± 56%)

360 compared to pre-supplementation (PLA: 131 ± 43%, MTCJ: 132 ± 53%) ($P = 0.018$, $d = 0.37$).

361 No significant main effects were found on change from pre-bolus data for HOMA2-β ($P >$

362 0.05).

363 ***Figure 3 near here***

364 3.1.4. Lipids

365 Reponses to total cholesterol after 6 days supplementation showed significant main effects for
366 time ($F_{(1, 11)}$ = 5.097; P = 0.045, $\eta^2_{partial}$ = 0.32) and interaction ($F_{(1, 11)}$ = 5.700; P = 0.036,
367 $\eta^2_{partial}$ = 0.34) (Figure 4). *Post-hoc* identified a significant difference between PLA and MTCJ
368 at pre-supplementation (P = 0.030; d = 2.14). Total cholesterol was significantly ($t_{(11)}$ = 3.724;
369 P = 0.003, d = 0.39) lower post-supplementation (4.1 ± 1.0 mmol.L^{-1}) compared to pre-
370 supplementation (4.5 ± 1.0 mmol.L^{-1}) with MTCJ.

371 A significant interaction effect was observed for HDL ($F_{(1, 11)}$ = 5.212; P = 0.043, $\eta^2_{partial}$ =
372 0.32) and LDL ($F_{(1, 11)}$ = 7.004; P = 0.023, $\eta^2_{partial}$ = 0.39) after 6 days supplementation (Figure
373 4). A significant difference between PLA and MTCJ was found at post-supplementation time
374 point for HDL (P = 0.049, d = 0.42), while pre-supplementation was significantly different for
375 LDL (P = 0.031, d = 0.42). LDL was significantly ($t_{(11)}$ = 3.681; P = 0.004, d = 0.21) lower
376 post-supplementation (2.71 ± 1.62 mmol.L^{-1}) compared to pre-supplementation (3.07 ± 1.69
377 mmol.L^{-1}) with MTCJ. Individual responses showed 6/12 participants increased HDL
378 concentrations after 6 days MTCJ supplementation, whereas 2/12 increased with PLA.
379 Moreover, 10/12 participants reduced LDL concentrations after MTCJ supplementation,
380 compared to 5/12 with PLA.

381 After 6 days supplementation, an interaction effect was observed for TC:HDL ($F_{(1, 11)}$ = 13.681;
382 P = 0.004, $\eta^2_{partial}$ = 0.55) (Figure 4). *Post-hoc* comparisons showed PLA to be significantly
383 higher than MTCJ at pre-supplementation time point (P = 0.011; d = 2.67). TC:HDL ratio was
384 significantly ($t_{(11)}$ = 2.690; P = 0.021, d = 0.30) lower post-supplementation (3.11 ± 1.13
385 mmol.L^{-1}) compared to pre-supplementation (3.47 ± 1.28 mmol.L^{-1}) with MTCJ. No significant
386 interaction or main effects for condition and time were observed for triglycerides with pre-post
387 6 days supplementation data (P > 0.05) (Table 2).

Change from pre-bolus data showed a main effect of time ($F_{(2, 22)} = 11.649$; $P < 0.001$, $\eta^2_{partial} = 0.51$) and a tendency for a significant interaction ($F_{(2, 22)} = 3.148$; $P = 0.063$, $\eta^2_{partial} = 0.22$) for triglyceride concentrations (Table 2). Change from pre-bolus data indicated a significant main effect of condition ($F_{(1, 11)} = 5.874$; $P = 0.034$, $\eta^2_{partial} = 0.35$) and time ($F_{(2, 22)} = 4.110$; $P = 0.030$, $\eta^2_{partial} = 0.27$) for LDL, with higher LDL concentrations at pre-supplementation compared to post-supplementation ($P = 0.034$; $d = 0.08$) (Table 2). No main effects for condition, time or interaction were found for HDL or TC:HDL ratio with change from pre-bolus data ($P > 0.05$) (Table 2).

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

3.3. 24-hour ABPM

A significant interaction ($F_{(1, 11)}$ = 9.941; P = 0.016, $\eta^2_{partial}$ = 0.59) and main effect for condition ($F_{(1, 11)}$ = 7.916; P = 0.026, $\eta^2_{partial}$ = 0.53) was observed for mean 24-hour SBP after 7 days of supplementation (Figure 5). A significant difference between PLA and MTCJ was identified with *post-hoc* analysis at the post-supplementation time point (P = 0.024; d = 0.44). Individual responses showed 11/12 participants reduced 24-hour SBP after 7 days MTCJ supplementation, compared to 2/12 with PLA.

Likewise, mean 24-hour DBP only showed significant main effects for condition ($F_{(1, 11)}$ = 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) (Figure 5). At pre-supplementation (P = 0.049; d = 0.14) and post-supplementation (P = 0.008; d = 0.66) time points, *post-hoc* demonstrated a significant difference between PLA and MTCJ. Individual responses showed 10/12 participants reduced mean 24-hour DBP after 7 days MTCJ supplementation, compared to 2/12 with PLA.

A significant interaction ($F_{(1, 11)}$ = 6.236; P = 0.041, $\eta^2_{partial}$ = 0.47) and tendency towards significance for the main effect of condition ($F_{(1, 11)}$ = 5.122; P = 0.058, $\eta^2_{partial}$ = 0.42) was detected for mean 24-hour MAP (Figure 5). *Post-hoc* analysis indicated PLA was significantly higher than MTCJ at the post-supplementation time point (P = 0.010; d = 0.58). Individual responses showed 9/12 participants reduced mean 24-hour MAP after 7 days MTCJ supplementation, compared to 1/12 with PLA. A tendency towards a significant interaction effect ($F_{(1, 11)}$ = 3.995; P = 0.086, $\eta^2_{partial}$ = 0.36) was found for mean 24-hour PP. No main effects for time or condition were detected for mean 24-hour PP (P > 0.05).

Mean day-time SBP demonstrated a tendency towards significance for the interaction ($F_{(1, 11)}$ = 4.499; P = 0.072, $\eta^2_{partial}$ = 0.39) and a significant main effect for condition only ($F_{(1, 11)}$ = 6.507; P = 0.038, $\eta^2_{partial}$ = 0.48) (Table 4). Three of 12 participants were found to have lower mean day-time SBP with PLA after 7 days supplementation, whilst 11/12 participants were found to have lower mean day-time SBP with MTCJ.

Significant main interaction ($F_{(1, 11)}$ = 5.725; P = 0.048, $\eta^2_{partial}$ = 0.45) and condition ($F_{(1, 11)}$ = 5.876; P = 0.046, $\eta^2_{partial}$ = 0.46) effects were observed for mean day-time DBP (Table 4). Pairwise comparisons for the interaction effect showed significantly higher mean day-time DBP with PLA compared to MTCJ at post-supplementation time point (P = 0.020; d = 0.67). Individual responses showed 8/12 participants had lower mean day-time DBP after 7 days MTCJ supplementation, compared to 2/12 with PLA.

There were no significant main effects for condition, time or interaction with mean day-time MAP (P > 0.05) (Table 4). A main effect for time was observed for mean day-time PP ($F_{(1, 11)}$ = 13.661; P = 0.008, $\eta^2_{partial}$ = 0.66), with *post-hoc* showing pre-supplementation to be higher than post-supplementation (P = 0.008; d = 0.42) (Table 4). Analysis on night-time SBP, DBP, MAP and PP demonstrated no significant main effects for condition, time or interaction (P > 0.05) (Table 4).

The day-night difference for SBP ($F_{(1, 11)}$ = 7.355; P = 0.030, $\eta^2_{partial}$ = 0.51) and PP ($F_{(1, 11)}$ = 7.199; P = 0.031, $\eta^2_{partial}$ = 0.51) only showed a significant main effect for condition, where PLA was larger than MTCJ (Table 4). Day-night differences for DBP and MAP indicated no significant main effects for condition, time or interaction (P > 0.05) (Table 4).

Figure 5 and Table 4 near here

3.4. Pulse Wave Analysis

No significant main effects for condition, time or interaction were detected for aortic SBP, aortic DBP, AP, PP, AIx, AIx at HR75 and SEVR ($P > 0.05$) for pre-post 6 days supplementation data (Table 3).

No main effects for condition, time or interaction ($P > 0.05$) were found on change from pre-bolus data for aortic SBP. However, individual responses for the change from pre-bolus to 2-hours post-bolus showed 4/12 participants decreased aortic SBP with PLA (3 ± 9 mmHg), while 9/12 participants responded with lower aortic SBP after consuming MTCJ (-4 ± 8 mmHg). There were no main effects for condition, time or interaction on change from pre-bolus data for aortic DBP, AP, PP, AIx and SEVR ($P > 0.05$) (Table 3). A main effect of condition was found for AIx at HR75 ($F_{(1, 11)} = 4.929$; $P = 0.048$, $\eta^2_{partial} = 0.31$).

3.5. Resting Metabolic Rate

No significant main effects for condition, time or the condition by time interaction were detected for resting EE after 6 days MTCJ consumption ($P > 0.05$). Significant interaction effects were found for resting RER ($F_{(1, 11)} = 10.045$; $P = 0.009$, $\eta^2_{partial} = 0.48$), fat oxidation rate ($F_{(1, 11)} = 9.394$; $P = 0.011$, $\eta^2_{partial} = 0.46$) and carbohydrate oxidation rate ($F_{(1, 11)} = 5.644$; $P = 0.037$, $\eta^2_{partial} = 0.34$) between PLA and MTCJ after 6 days supplementation (Table 3). *Post-hoc* identified a significant difference between conditions at pre-supplementation time point for fat ($P = 0.024$; $d = 0.68$) and carbohydrate ($P = 0.027$; $d = 0.81$) oxidation rates. RER was significantly ($t_{(11)} = 2.823$; $P = 0.017$, $d = 0.70$) lower post-supplementation (0.83 ± 0.04) compared to pre-supplementation (0.86 ± 0.04) with MTCJ.

A significant main effect for time only was observed for resting EE ($F_{(5, 55)} = 2.788$; $P = 0.026$, $\eta^2_{partial} = 0.20$), RER ($F_{(5, 55)} = 43.536$; $P < 0.001$, $\eta^2_{partial} = 0.80$), fat ($F_{(5, 55)} = 14.183$; $P < 0.001$, $\eta^2_{partial} = 0.56$) and carbohydrate ($F_{(5, 55)} = 15.936$; $P < 0.001$, $\eta^2_{partial} = 0.59$) oxidation 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)

[12] demonstrated a reduction in fasting insulin by 14.3% (-9.5 pmol.L^{-1}) after 7 days consumption of NZBP and attributed this to heightened insulin sensitivity. Similarly, fasting insulin was reduced by 9.3% (-12.42 pmol.L^{-1}) after 6 days consumption of MTCJ and reduced by 14% when comparing against placebo. Individual responses suggested insulin resistance tended to be improved after 6 days intake of MTCJ (HOMA2-IR change: -0.27 ± 0.56) compared to PLA (HOMA2-IR change: 0.48 ± 0.78), likewise Willems *et al.* (2017) [12] indicated 7 days anthocyanin-rich NZBP consumption tended to improve insulin resistance. Together, these findings highlight short-term continuous supplementation of cyanidin- (MTC) and delphinidin-rich (NZBP) interventions may have the capacity to improve insulin sensitivity/resistance in healthy [12] and MetS populations. However, larger datasets are required to justify these initial observations. Moreover, it remains to be seen how long any beneficial effects last, and whether intermittent supplementation for 6-7 days over a longer duration may be a more physiologically effective, ecologically valid and economically viable supplementation strategy. As fasting glucose and insulin responses were complementary of each other, the change after 6 days indicates MTCJ may normalise glucoregulatory control [10]. This may be of significance when considering insulin resistance is the underlying cause of impaired cardio-metabolic function in humans with MetS.

4.2. Lipid Responses

This study suggests MTCJ may improve aspects of the lipid profile in individuals with MetS. The significant reduction in total cholesterol found in the present study agrees with findings from Ataie-Jafari *et al.* (2008) [27], but similar responses were not observed in other studies providing tart cherries [28,29], although a trend for lower concentrations was observed in MetS adults [30]. The reduction in total cholesterol with MTCJ may be explained by lower LDL fractions after 6 days supplementation. Similarly, reductions in LDL were reported in subjects with elevated baseline LDL after tart cherry juice consumption [27,29]; aligning with findings from other human trials supplementing anthocyanin-rich interventions that hyperlipidaemia is

a prerequisite to observe improvements [31]. Clinically, LDL responses after 6 days MTCJ consumption may correspond to an 8% relative risk reduction of major vascular events [32].

Bing sweet cherry consumption had no effect on TC:HDL ratio in healthy adults [33]. In the present study, TC:HDL ratio was found to be lower after 6 days consumption of MTCJ and individual responses showed 10/12 participants with lower TC:HDL ratios after MTCJ supplementation compared to 1/12 participants with placebo. Furthermore, TC:HDL ratio was shown to be a good predictor of cardiovascular risk reduction when assessing interventions [34], thus improvements in TC:HDL after 6 days MTCJ ingestion highlight its clinical efficacy against cardiovascular events.

4.3. Cardiovascular Responses

The efficacy of sweet and tart cherry interventions on improving BP, particularly SBP, had been demonstrated numerous times in various populations [7,8,14,27,35], including MetS [13]. However, these studies used lab-based measurements which is clinically inferior to 24-hour ABPM [19,36]. Therefore, the present study was the first to demonstrate reductions in mean 24-hour SBP, DBP and MAP after cherry supplementation in any population.

A clinically significant reduction in mean 24-hour SBP was observed after 7 days MTCJ consumption (-5 mmHg); which would be associated with prevention of all-cause and cardiovascular mortality by 20% [37]. This finding adds greater clinical and biological relevance to the individual responses reported for acute SBP reductions in the present study, by Keane *et al.* (2016b) [8] and Desai *et al*. (2019) [13] after an acute, single-bolus of MTCJ. In this study, a reduction of 11 mmHg was observed after 6 days MTCJ consumption compared to PLA for acute SBP at 2-hours post-bolus. Comparative reductions of 8 mmHg were seen with 7 days blueberry juice consumption compared to PLA in individuals with pre- and stage 1 hypertension [38], highlighting the efficacy of 6-7 days consumption of anthocyanin-rich juices (~270-314 $mg.day^{-1}$ total anthocyanins). Importantly, the magnitude of acute SBP reduction with MTCJ was comparable to approved anti-hypertensive drugs, associated with

harmful side effects [39]. Moreover, the 2 mmHg reduction in mean 24-hour DBP after 7 days MTCJ consumption would be associated with a risk reduction of coronary heart disease and stroke by 6 and 15%, respectively [40]. This response was facilitated by significant day-time DBP reductions with MTCJ, which has been shown to be a significant predictor of CVD, coronary heart disease and stroke [41]. Despite supplementing a similar daily anthocyanin dosage as the present study (270 $mg.day^{-1}$), Stull *et al.* (2015) [20] reported no effect on 24-hour BP after consuming anthocyanin-rich (290.3 $mg.day^{-1}$ anthocyanins) blueberry smoothie for 6 weeks compared to PLA, in individuals with MetS. Hence the above literature supports the hypothesis that short-term, low-dose anti-oxidant supplementation derived from anthocyanins may be a more pragmatic method to induce clinically relevant improvements in lab-based and 24-hour BP. However, it should be noted that long-term supplementation of anthocyanin-rich interventions [31,42], including tart cherries [27,29], have been shown to reduce BP through overlapping but different mechanisms (increased arterial compliance and/or improved endothelial function) [43] to short-term supplementation.

Aviram and Dornfeld (2001) [44] demonstrated reductions in SBP were correlated with significantly lower ACE activity after 2 weeks pomegranate juice consumption. Furthermore, Kirakosyan *et al.* (2018) [9] demonstrated 88.7% ACE inhibition *in vitro,* with MTC extract. However, in the present study, as no correlations were observed between serum ACE concentrations and changes in cardiovascular parameters, the hypotensive effects of MTCJ may not be explained by alterations in ACE concentrations. Yet, individual responses showed 8/12 participants had lower ACE concentrations 6 days after MTCJ compared to 4/12 with PLA. The high inter-individual variation of ACE may contribute to the null results, particularly given the variation in age between participants. Hence, it is possible that the action of ACE may only be prominent in certain individuals, and other mechanisms such as increased arterial stiffness and endothelial dysfunction in older participants may contribute to elevated BP. 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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Tables and Figures

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

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

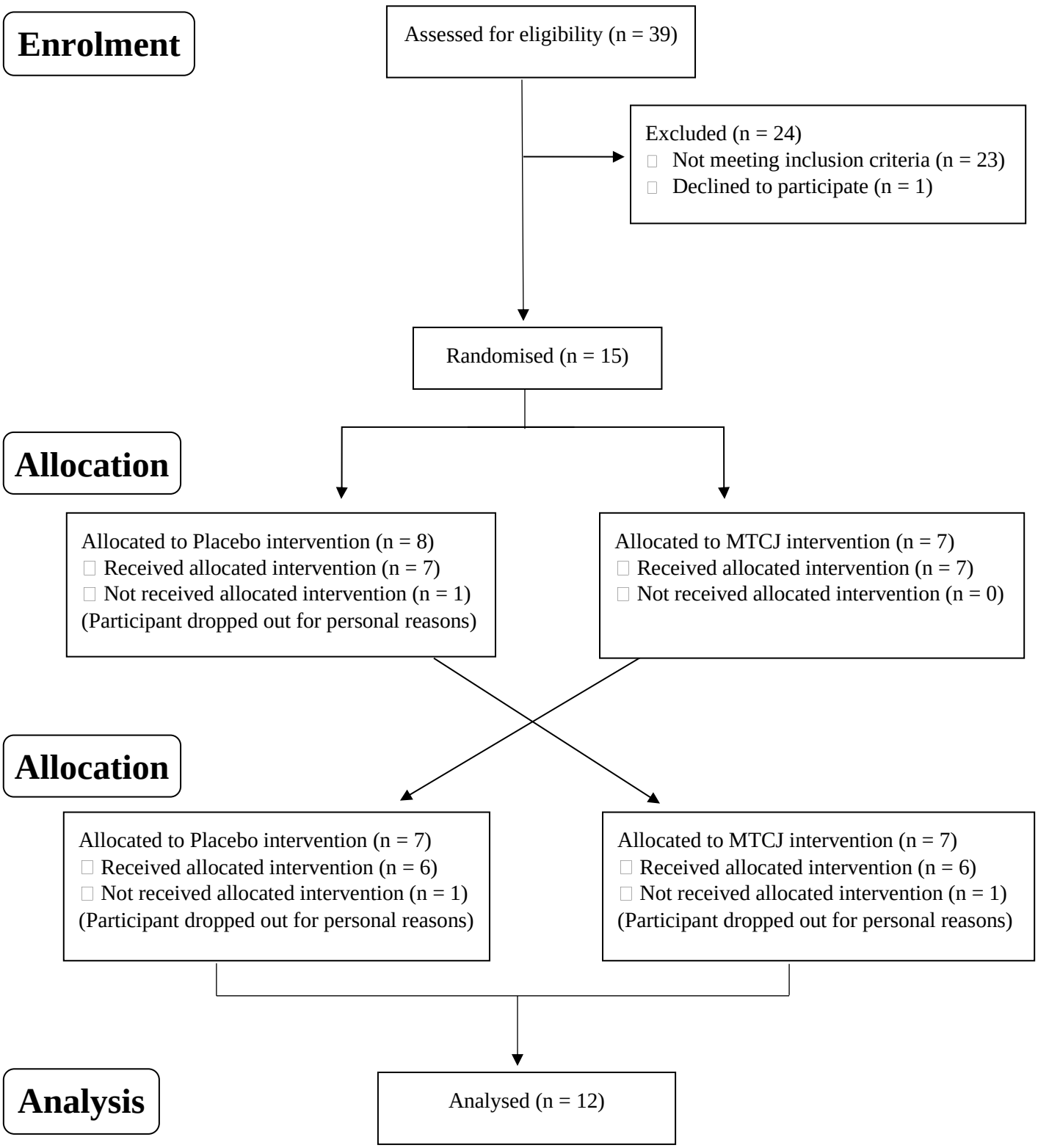
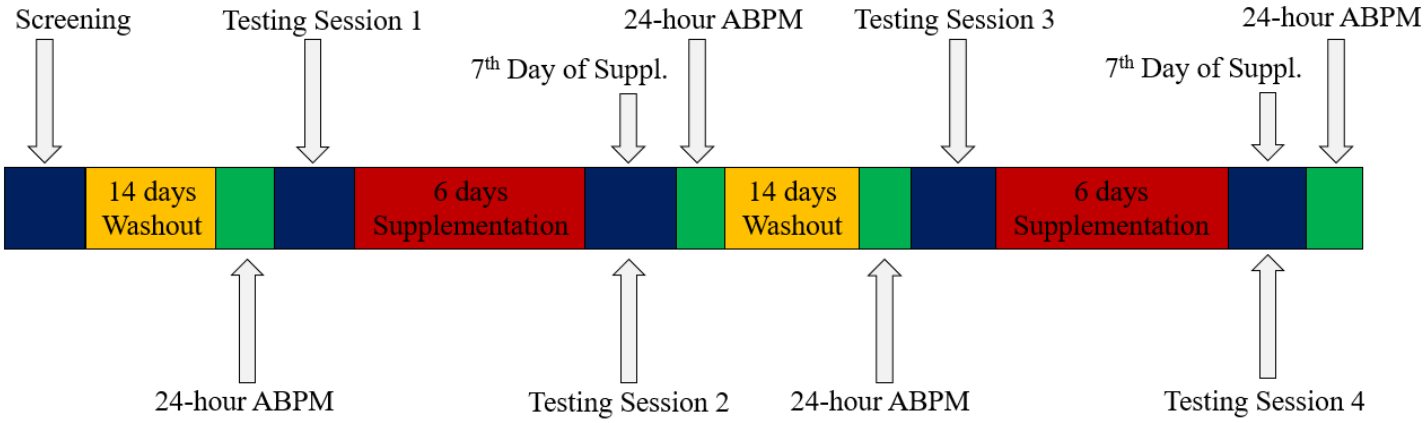


Figure 1. CONSORT flow diagram of the participants recruited, screened, tested, analysed and excluded during the course of the study.

A



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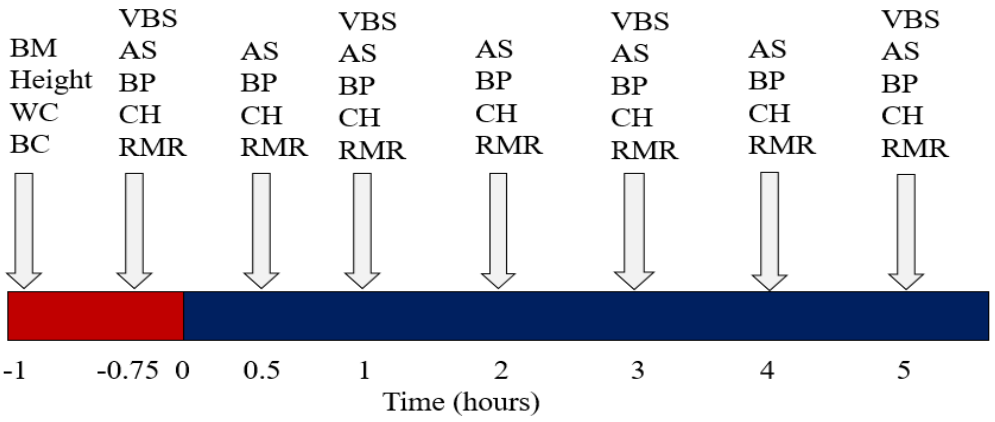

Key:
AS – Arterial Stiffness; BC – Body Composition; BM – Body Mass; BP – Blood Pressure; CH – Cardiac Haemodynamics; RMR – Resting Metabolic Rate; VBS – Venous Blood Sampling; WC – Waist Circumference

= 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.

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

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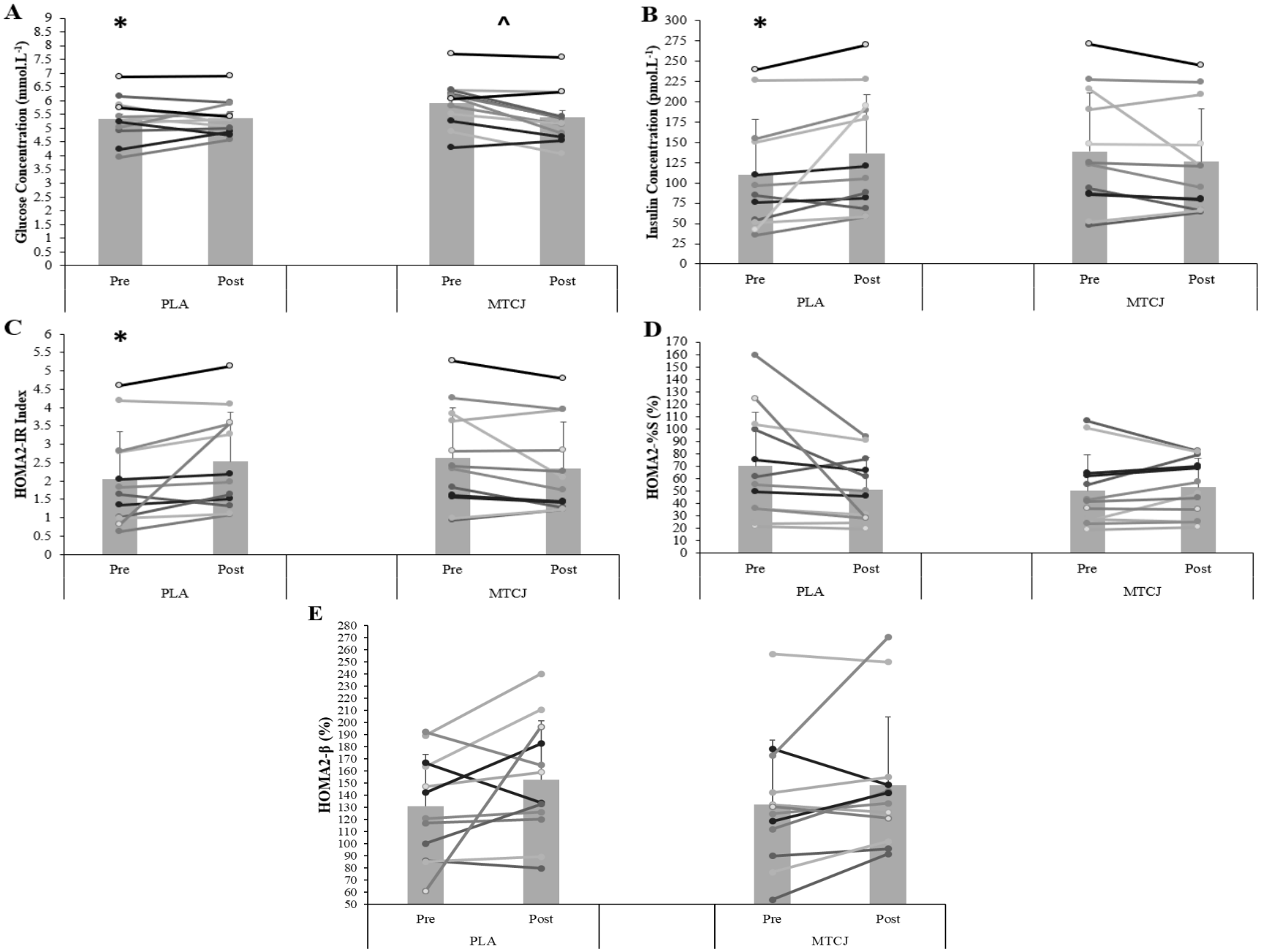
A
Glucose Concentration (mmol.L-1)
*
^
Pre
Post
PLA
MTCJ
B
Insulin Concentration (pmol.L-1)
*
C
HOMA2-IR Index
*
D
HOMA2-%S (%)
E
HOMA2-β (%)

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 (±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.

				Post-bolus Time Points					
		Pre 6 days Suppl.	Post 6 days Suppl.	30 minutes	1 hr	2 hr	3 hr	4 hr	5 hr
Brachial SBP (mmHg)	PLA	127 ± 16	128 ± 17	133 ± 13	130 ± 14	132 ± 19	132 ± 19	128 ± 10	129 ± 13
	MTCJ	134 ± 17	128 ± 15	127 ± 13	128 ± 13	121 ± 10	127 ± 12	129 ± 18	133 ± 14
Brachial DBP (mmHg)	PLA	75 ± 10	74 ± 7	76 ± 8	72 ± 5	74 ± 5	73 ± 8	74 ± 7	75 ± 5
	MTCJ	75 ± 10	72 ± 7	73 ± 4	74 ± 5	70 ± 7	74 ± 7	73 ± 6	75 ± 4
MAP (mmHg)	PLA	98 ± 12	93 ± 10	99 ± 9	94 ± 8	97 ± 8	96 ± 10	97 ± 10	97 ± 9
	MTCJ	98 ± 12	93 ± 9	95 ± 4	96 ± 7	93 ± 6	95 ± 8	95 ± 8	96 ± 8
HR (beats.min^{-1})	PLA	65 ± 12	67 ± 14	68 ± 16	67 ± 16	66 ± 17	66 ± 16	65 ± 15	65 ± 5
	MTCJ	65 ± 14	63 ± 11	66 ± 11	64 ± 12	62 ± 13	61 ± 11	62 ± 12	60 ± 11
Cardiac Output (L.min^{-1})	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
	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 (mL)	PLA	104 ± 26	98 ± 21	100 ± 22	104 ± 21	101 ± 34	105 ± 23	103 ± 26	98 ± 17
	MTCJ	102 ± 20	110 ± 29	105 ± 25	98 ± 22	101 ± 27	100 ± 26	103 ± 29	109 ± 29
TPR (mmHg·s^{-1}·mL^{-1})	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
	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 (mmHg)	PLA	124 ± 12	119 ± 15	120 ± 12	119 ± 12	121 ± 14	124 ± 13	119 ± 15	120 ± 13
	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$^{\wedge\$}$ (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$^{\wedge\$}$ ($g.min^{-1}$)	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$^{\wedge\$}$ ($g.min^{-1}$)	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

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

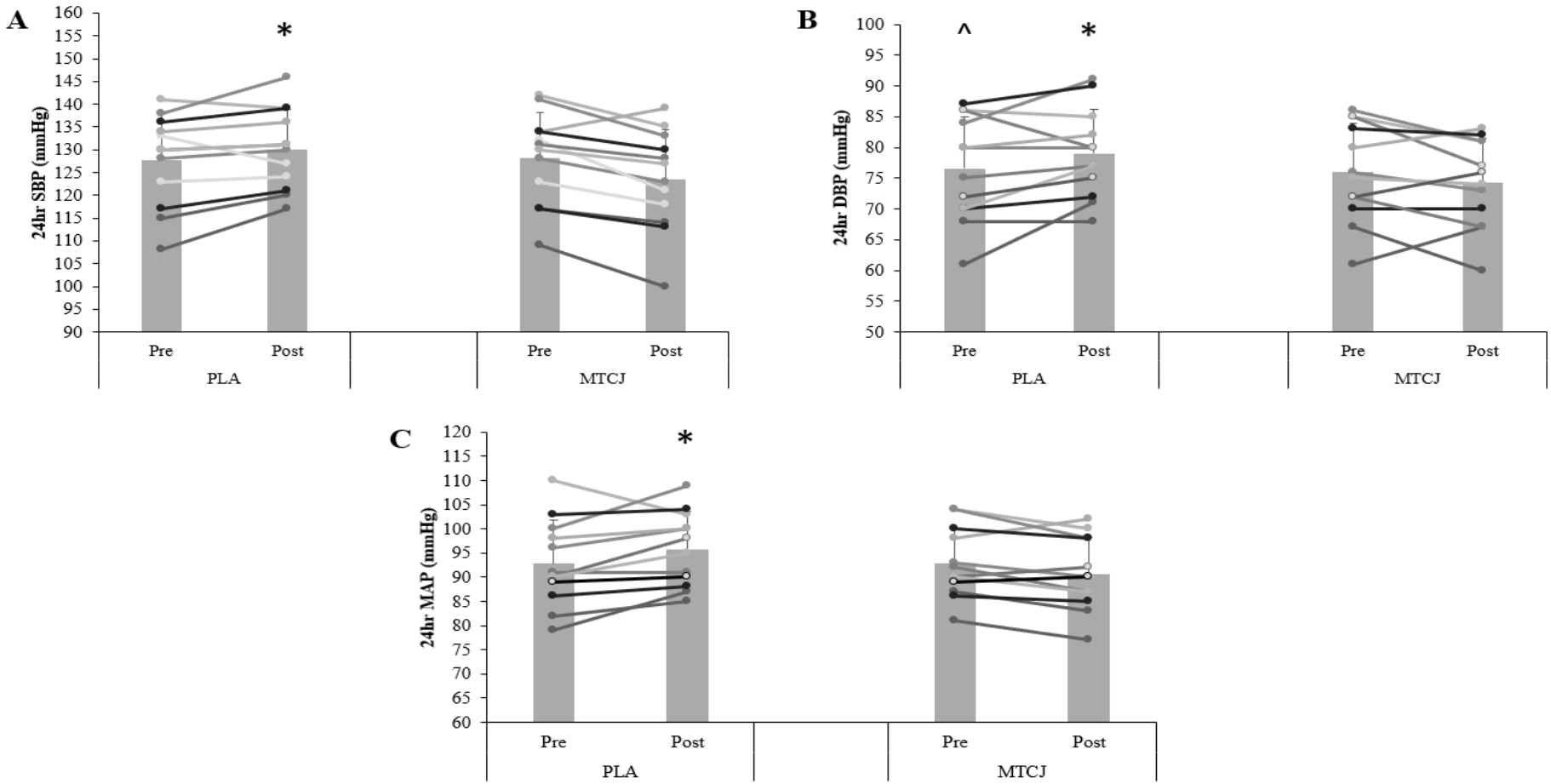

Figure 5. (A) Mean 24-hour SBP, (B) Mean 24-hour DBP and (C) Mean 24-hour MAP responses before and after supplementation of PLA and MTCJ. Bar graphs depict mean (±SD) group values for each condition, pre and post 7 days supplementation. Lines depict individual responses for all 12 participants. ^Denotes 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* (mmHg)	PLA	132 ± 8	133 ± 8
	MTCJ	132 ± 9	127 ± 11
Day DBP$ (mmHg)	PLA	79 ± 7	81 ± 7^
	MTCJ	79 ± 6	76 ± 6
Day MAP (mmHg)	PLA	92 ± 8	97 ± 7
	MTCJ	92 ± 10	94 ± 7
Day PP§ (mmHg)	PLA	53 ± 6	51 ± 5
	MTCJ	53 ± 5	50 ± 8
Night SBP (mmHg)	PLA	113 ± 13	117 ± 7
	MTCJ	117 ± 12	117 ± 13
Night DBP (mmHg)	PLA	68 ± 9	69 ± 8
	MTCJ	69 ± 10	68 ± 8
Night MAP (mmHg)	PLA	82 ± 11	85 ± 7
	MTCJ	81 ± 14	84 ± 11
Night PP (mmHg)	PLA	46 ± 8	48 ± 5
	MTCJ	48 ± 7	49 ± 7
D/N SBP* (mmHg)	PLA	19 ± 12	16 ± 6
	MTCJ	15 ± 10	10 ± 8
D/N DBP (mmHg)	PLA	11 ± 8	12 ± 8
	MTCJ	10 ± 9	9 ± 7
D/N MAP (mmHg)	PLA	10 ± 8	13 ± 7
	MTCJ	11 ± 11	10 ± 9
D/N PP* (mmHg)	PLA	7 ± 7	4 ± 4
	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.

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