The effects of Montmorency tart cherry juice supplementation and FATMAX exercise on fat oxidation rates and cardio-metabolic markers in healthy humans

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Montmorency tart cherries (*Prunus cerasus* L.) are rich in anthocyanins, compounds capable of augmenting fat oxidation and regulating metabolic dysfunction. The present study examined whether Montmorency tart cherry juice (MTCJ) supplementation could augment fat oxidation rates at rest and during FATMAX exercise, thus improve cardio-metabolic health. Eleven, healthy participants consumed MTCJ or placebo (PLA) twice daily, in a randomised, counterbalanced order for 20 days. Participants cycled at FATMAX for 1-hour pre-, mid- (10 days) and post-supplementation whilst substrate oxidation rates were measured. Before exercise anthropometrics and resting metabolic rate were measured. Blood pressure, serum triglycerides, cholesterol, HDL, total antioxidant status (TAS) and glucose were measured immediately before and after exercise. No significant differences between conditions or interactions were observed for any functional and blood-based cardio-metabolic markers or fat oxidation during exercise or rest ($P > 0.05$). Pre-exercise TAS ($P = 0.036$) and HDL ($P = 0.001$) were significantly reduced from mid- to post-supplementation with MTCJ only. Twenty days MTCJ supplementation had no effect on fat oxidation, therefore it is unnecessary for individuals in this participant cohort to consume MTCJ with exercise to improve cardio-metabolic biomarkers.

Keywords: Anthocyanins; Exercise; Polyphenols; Cardiometabolic Health; Fat Oxidation

Abbreviations

Blood Pressure (BP); Carbohydrate (CHO); Calorie Restrictive Mimetic (CRM); Diastolic Blood Pressure (DBP); Energy Expenditure (EE); Heart Rate (HR); Maximal Fat Oxidation (MFO); Montmorency Tart Cherry Juice (MTCJ); New Zealand Blackcurrant Extract (NZBE); Placebo (PLA); Plasma Volume Change (PVC); Peroxisome Proliferator-Activated Receptor (PPAR); RER (Respiratory Exchange Ratio); Resting Metabolic Rate (RMR); Ratings of Perceived Exertion (RPE); Systolic Blood Pressure (SBP); Total Antioxidant Status (TAS); Volume of Maximal Oxygen Uptake ($\dot{V}O_2max$); $\dot{V}CO_2$ (Volume of Carbon Dioxide Production); $\dot{V}O_2$ (Volume of Oxygen Uptake).

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Introduction

Cardiovascular disease, type 2 diabetes mellitus and associated diseases combined are the leading health burden and cause of mortality worldwide (Guo & Ling, 2015), therefore the necessity for an intervention is paramount. Dietary interventions to improve cardio-metabolic health are highly sought after as they possess less risk than pharmacological drugs (Vendrame, Del Bo, Ciappellano, Riso, & Klimis-Zacas, 2016). Substantial evidence now exists demonstrating the relationship between high consumption of vegetables and fruits, improvements in disease symptoms and the reduced risk of disease development (Li, Wang, Luo, Zhao, & Chen, 2017). However, concerns remain over the feasibility of maintaining a high fruit and vegetable intake over a prolonged time period, therefore daily dietary supplementation containing health-promoting phytonutrients is appealing. Subsequently, the present study supplemented small volumes of highly concentrated Montmorency tart cherry juice (MTCJ), to boost consumption of anthocyanins and thus provide sufficient amounts at physiologically relevant concentrations in a more practical and efficient method (Zheng et al., 2017).

Anthocyanins, a major polyphenolic sub-class of flavonoids, are predominantly responsible for the dark red, blue, black and purple pigments found in various fruits and vegetables (Wu et al., 2006). Anthocyanins are powerful antioxidants also capable of ameliorating cardio-metabolic dysfunction (He & Giusti, 2010). A recent epidemiological study highlighted a significant correlation with anthocyanin consumption and improved long-term weight management (Bertoia et al., 2016). Tart cherries (*Prunus cerasus* L.) and thus tart cherry juice possess a high phytochemical content particularly rich in specific anthocyanins, flavonols and phenolic acids (Kirakosyan, Seymour, Llanes, Kaufman, & Bolling, 2009; Kirakosyan et al., 2010). These phytochemicals are thought to contribute to effectively combating oxidative stress, inflammation and repairing muscle damage post-exercise (Bell, Walshe, Davison, Stevenson, & Howatson, 2014b; Bell, Walshe, Davison, Stevenson, & Howatson, 2015). In rodents, tart cherries have shown significant improvements at the molecular, cellular and systemic level predominantly due to modulation of peroxisome proliferator-activated receptor (PPAR) signalling pathways (Seymour et al., 2009; Seymour et al., 2008). Consequently, percentage fat mass, hyperinsulinaemia, hyperlipidaemia and inflammation were all reduced (Seymour et al., 2009; Seymour et al., 2008). Observations in humans have been more equivocal, with tart cherry supplementation showing no effect on blood pressure and blood-based cardio-metabolic markers in healthy participants (Lynn et al., 2014). More favourable findings have been

reported in studies (Ataie-Jafari, Hosseini, Karimi, & Pajouhi, 2008; Keane et al., 2016a; Keane, Haskell-Ramsay, Veasey, & Howatson, 2016b; Martin, Bopp, Neupane, & Vega-Lopez, 2010) examining 'at risk' or diseased patients with tart cherry juice, indicating amelioration of cardio-metabolic function.

Skeletal muscle is a critical organ involved in lipid metabolism and dysfunction of cellular and molecular cascades which are implicated in the manifestation of cardiovascular disease, insulin resistance, inflammation and oxidative stress (Stump, Henriksen, Wei, & Sowers, 2006). During sub-maximal exercise at FATMAX intensities [intensity eliciting maximal fat oxidation rate, conveyed as percentage of maximal oxygen uptake (%$\dot{V}O_2$max)], the primary source of energy is derived from oxidation of free fatty acids and intramuscular triglycerides in skeletal muscle (Romijn, Coyle, Sidossis, Gastaldelli, Horowitz, Endert & Wolfe, 1993). Therefore, FATMAX exercise, as an intervention, was included in the present study to facilitate fat oxidation and subsequently improve cardio-metabolic function. Perez-Martin et al. (2001) conducted a study comparing substrate utilisation between healthy obese and normal-weight participants during a FATMAX determination. An earlier shift to CHO oxidation and significantly lower maximal fat oxidation rates (MFO) were observed with obese participants compared to normal-weight indicating metabolic inflexibility amongst the obese cohort (Perez-Martin et al., 2001). However, in healthy participants Robinson, Hattersley, Frost, Chambers, and Wallis (2015) demonstrated a significant positive correlation between MFO and both 24-hour fat oxidation and insulin sensitivity. Furthermore, in obese yet metabolically healthy participants, FATMAX training elicited a 44% increase in fat oxidation rates and 27% increase in insulin sensitivity index (Venables & Jeukendrup, 2008). A meta-analysis of FATMAX training in obese, Metabolic Syndrome and Type 2 diabetic patients confirmed a shift of fat oxidation to higher exercise intensities and reductions in body weight, fat mass, waist circumference and cholesterol, thus advocating its use to improve health (Romain et al., 2012). Overall, these findings suggest FATMAX exercise encourages enhancements in fat oxidation rate leading to improved body composition, cholesterol and insulin sensitivity (Brun, Romain, & Mercier, 2011).

Consequently, the purpose of specifically incorporating aerobic FATMAX exercise in the present study were twofold. Firstly, an individualised approach to achieve maximal fat oxidation rates during moderate-intensity exercise has been suggested as the best method to reduce glycated haemoglobin (HbA_{1c}), insulin-dependent glucose, fat mass and total cholesterol (Brun et al., 2011). Secondly, it is an appropriate methodological test to measure the

effects of an intervention on fat oxidation rates during exercise and when combined with a potential calorie restrictive mimetic (CRM), such as MTCJ, may induce additional improvements on cardio-metabolic pathways and therefore overall health (Besnier et al., 2015).

Rationale for the present study is based on previous work demonstrating the benefits of anthocyanin supplementation on fat oxidation rates during exercise (Cook, Myers, Gault, Edwards, & Willems, 2017; Cook, Myers, Blacker, & Willems, 2015) and the positive results regarding the effects of tart cherry supplementation against cardio-metabolic dysfunction in rodents (Seymour et al., 2009; Seymour et al., 2008) and humans (Ataie-Jafari, 2008; Keane et al., 2016a; Keane et al., 2016b; Martin et al., 2010). However, to date, no research has explored the cardio-metabolic responses to tart cherry supplementation and exercise in tandem, thus healthy participants were recruited to assess for any adverse effects prior to investigating responses in clinical populations. Subsequently, this study set out to examine the physiological responses of MTCJ supplementation with FATMAX exercise on fat oxidation rates, body composition and cardio-metabolic markers in healthy participants. It was hypothesised that MTCJ supplementation would augment fat oxidation rates at rest and during exercise, thus proving more efficacious at improving body composition, functional and in sera cardio-metabolic markers than previous research conducted with tart (Lynn et al., 2014; Martin et al., 2010) and sweet (Kelley, Rasooly, Jacob, Kader, & Mackey, 2006) cherry supplementation at rest in healthy participants.

Methods

Participants

Eleven (7 males and 4 females) healthy, recreationally active (150 minutes moderate-intensity aerobic exercise per week), participants (mean ± SD age 30 ± 10 years, height 1.76 ± 0.09 m, body mass 76.42 ± 13.19 kg, BMI 24.43 ± 3.23 $kg.m^{-2}$, $\dot{V}O_{2peak}$ 35.87 ± 4.78 $mL.kg^{-1}.min^{-1}$) volunteered for the study. All participants were non-smokers, BMI <30, injury-free and not diagnosed with any cardio-metabolic or renal diseases at the time of testing but had a family history of cardio-metabolic disease. Participants were instructed to cease consumption of any other supplementation two weeks before and for the duration of the study. All participants provided written informed consent to participate in the study and completed health screen questionnaires before the study commenced. Ethical approval was obtained from the University of Hertfordshire Health and Human Sciences Ethics Committee and the study's experimental

procedures followed the principles outlined in the 1964 Declaration of Helsinki. The study was registered as a clinical trial on clinicaltrials.gov (NCT02999256).

As this was the first study to examine fat oxidation with cherries, it was difficult to confidently predict a sample size using power analyses. Previous studies that had researched the effects of fat oxidation (Cook, Myers, Blacker, & Willems, 2015; Roberts, Roberts, Tarpey, Weekes, & Thomas, 2015) or cherry supplementation (Bell, McHugh, Stevenson, & Howatson, 2014a; Bell et al., 2014b; Bowtell et al., 2011) had a total sample size between 10-16 participants.

Procedures

Research Design

This study utilised a single-blind (blinded to participants), placebo-controlled, randomised, counterbalanced design, where each participant acted as their own control. Participants were required to complete two conditions over 10 weeks, differing only in supplementation, Montmorency tart cherry juice (MTCJ) and placebo (PLA). Participants were randomised to start consumption of either MTCJ or PLA first (6 received PLA first), followed by a 14-day washout period (Cook et al., 2015; Howatson et al., 2012; Keane et al., 2016a; Keane et al., 2016b) and then consumption of the opposite supplement to the first condition.

Both conditions were identical in terms of design and testing procedures and comprised of 5 sessions each, with sessions lasting approximately 2.5 hours. Figure 1 shows a timeline for testing sessions and the supplementation period. ***Figure 1 near here***

Baseline measurements were obtained during the first FATMAX/$\dot{V}O_2$max session in the first condition. Other than blood sampling and the type of exercise itself, all other testing procedures pre- and post-exercise were identical across each session. Blood sampling was conducted pre- and post-exercise to ascertain the acute differences induced by one-hour of FATMAX exercise and pre-, mid- and post-supplementation to assess the longer-term effects of supplementation on cardio-metabolic biomarkers, within and between conditions.

Dietary and Exercise Guidelines

Participants were instructed to refrain from consuming water and conducting vigorous exercise 3 and 24 hours before each testing session, respectively. All participants arrived at the laboratory between 7 – 10am, after an overnight fast of a minimum of 10 hours, to account for circadian variation (Bell et al., 2014b). Participants were instructed to drink fluids *ad libitum* and maintain their habitual polyphenol intake, particularly 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 (Meyer, Gassler, & Kindermann, 2007).

Total energy, macronutrient and polyphenol intake was assessed through food diaries which were completed three days prior to each session. In addition to replicating their 3-day diary before each testing session, a standardised menu for the final 24-hours of the three-day period, was provided to each participant based on their healthy spontaneous habitual diet to reduce day-to-day intra-individual variability in fat oxidation (Roberts et al., 2015). Standardisation of macronutrients was set at 15% protein, 55% carbohydrate (CHO) and 30% fat of energy intake (Ben Ounis et al., 2009; Melanson et al., 2002) with 20% of the three-day average for habitual energy intake added to account for underreporting dietary consumption with food diaries (Black et al., 1993; Mertz et al., 1991). All participants reported 100% adherence when food diaries were assessed using dietary analysis software (Dietplan 7.0, Forestfield Software Ltd, West Sussex, UK) for percentage contributions of macronutrients to total energy intake, total polyphenols and anthocyanins.

Supplementation

The supplementation period lasted 20 days, similar to Roberts et al. (2015), where participants ceased consumption following the 20th day and during the washout period. The experimental condition included supplementation with MTCJ (Volume: 30 mL, Energy: 102 kcal, Carbohydrates: 24.50 g of which Sugars: 17.90 g, Protein: 1.10 g and Fat: 0 g) whilst the control involved supplementation of an energy matched placebo (Volume: 30 mL, Energy: 102 kcal, Carbohydrates: 25.35 g of which Sugars: 25.32 g, Protein: 0.03 g and Fat: 0 g). The MTCJ was made with 30 mL Montmorency tart cherry concentrate (Cherry Active, Active Edge Ltd, Hanworth, UK) and 100 mL water. Placebo composition consisted of 30 mL commercially available fruit-flavoured cordial (Cherries and Berries, Morrisons, Bradford, UK), with anthocyanins used only for colouring and negligible antioxidant content, mixed with 100 mL water. In order to match placebo for energy, taste and visual appearance, a flavourless carbohydrate

(Maltodextrin, My Protein Ltd, Northwich, UK), citric acid (100% Pure Citric Acid, VB and Sons, UK) and black food colouring (Morrisons, Bradford, UK) were added, respectively. Participants consumed two 130 mL servings per day, once in the morning immediately before breakfast, then again in the evening before dinner (Bell, Stevenson, Davison, & Howatson, 2016; Bell et al., 2014b). Due to fasting restrictions on testing days, participants delayed consumption of the morning serving until lunchtime. Each 30 mL serving of Montmorency tart cherry concentrate provided juice from approximately 90-110 whole Montmorency tart cherries with a total anthocyanin content of 270 mg (9 $mg.mL^{-1}$) (Howatson et al., 2012).

Testing Protocol

All testing sessions were conducted in a temperature-controlled laboratory kept between 21-24ºC and 38-45% relative humidity (dry-bulb) (AWS888N, Oregon Scientific, USA).

During all testing sessions, stature (Seca 217 Stadiometer, Seca, Hamburg, Germany), body mass (Seca 799, Germany) and waist circumference (Seca 201, Germany) were measured initially, immediately followed by segmental body composition analysis (Body Composition Analyser BC-418, Tanita, Japan) via bioelectrical impedance. Body composition was measured according to the manufacturer's guidelines and specific pre-measurement conditions to reduce variability between measurements. Pilot work showed test-retest intra-individual variability (measurements taken in the morning from 5 participants according to the timeline of the study) of the body composition analyser to have coefficients of variation of 2.06% for percentage body fat and 1.91% for fat mass. Participants were not on any weight loss or weight gain regimen during this period.

After 10 minutes' rest, resting heart rate (HR) (Polar T31c and FT1, Polar Electro Oy, Finland) and blood pressure (BP) (Omron MX3, Omron, Japan) were obtained followed by resting metabolic rate (RMR). Pre-exercise blood sampling was then performed followed by post-exercise blood sampling during the one-hour sub-maximal sessions only. Heart rate and BP were measured after post-exercise blood sampling, approximately 5-8 minutes after the cessation of exercise (Figure 2A and 2B). ***Figure 2A and 2B near here***

Exercise Protocols

During the first and fourth testing sessions of each condition, participants conducted FATMAX and maximal oxygen uptake ($\dot{V}O_2max$) tests.

The FATMAX (expressed as % of $\dot{V}O_2$peak) determination protocol, adapted from Achten, Gleeson, and Jeukendrup (2002) and Alkhatib, Seijo, Larumbe, and Naclerio (2015), required participants to cycle on an electromagnetically braked cycle ergometer (Excalibur, Lode, Groningen, The Netherlands) at 70 rev.min^{-1} at an initial intensity of 30W with increments of 10W every 3 minutes. The test was terminated once respiratory exchange ratio (RER) exceeded 1 for a continuous period of 30 seconds (Croci et al., 2014).

The $\dot{V}O_2$max test was performed 15 minutes following the FATMAX test. Due to the low-intensity nature of the FATMAX test, 15 minutes provided sufficient recovery time before the $\dot{V}O_2$max test. The protocol for determination of $\dot{V}O_2$max consisted of cycling at 70-80 rev.min^{-1}, at an initial intensity of 100W with wattage increasing by 20W every minute until volitional exhaustion. The test was terminated according to BASES Sport and Exercise Physiology testing guidelines (Winter, 2006).

Heart rate and differentiated (overall and legs) ratings of perceived exertion (RPE) on a 6-20 scale (Borg, 1973) were recorded 15 seconds before the end of each stage for FATMAX and $\dot{V}O_2$max tests. RPE was also measured every 5 minutes during the one-hour sub-maximal exercise.

FATMAX Analysis

FATMAX was determined through visual inspection of the fat oxidation curve generated from the incremental sub-maximal test and confirmed by identifying the highest fat oxidation rate for the final minute of each stage calculated from a 15 second rolling average of each 3 minute stage. The corresponding intensity (power output, W) at the peak of this curve was deemed to be FATMAX and was implemented during the one-hour sub-maximal exercise.

The second $\dot{V}O_2$max and FATMAX tests were used to assess changes in fitness status (MacRae & Mefferd, 2006) and any differences in FATMAX which may have occurred due to the previous exercise sessions and supplementation. FATMAX was adjusted to the new exercise intensity for the post-supplementation one-hour sub-maximal exercise if the change in FATMAX was greater than a pre-determined threshold (>6%).

Measures and Equipment

Blood Pressure and Heart Rate

Systolic (SBP) and diastolic (DBP) blood pressure was monitored pre- and post-exercise during all testing sessions where four measurements were recorded in an upright seated position, with an average of the final three being taken as BP (Cook et al., 2015). Resting HR was also recorded in the same anatomical position.

Resting Metabolic Rate

RMR, resting energy expenditure, substrate oxidation rates and RER were measured pre-exercise, based on an open-circuit indirect calorimetry system (GEM Nutrition Ltd, Cheshire, UK). Participants lay supine for 30 minutes with data averaged for the final 20 minutes only, to achieve steady-state and account for any initial short-term variances in respiration (Kelly, King, Goerlach, & Nimmo, 2013). A ventilated hood was placed over the head with a flexible plastic seal around the neck and shoulders to prevent air inside and outside the hood from mixing. Participants remained silent and lay as still as possible, whilst music was played to prevent sleeping.

Respiratory Gas Analysis

Real-time breath-by-breath gaseous exchange data (Metalyzer 3B, Cortex Biophysik, Leipzig, Germany) of $\dot{V}O_2$ ($L.min^{-1}$), $\dot{V}CO_2$ ($L.min^{-1}$) and RER were recorded during all exercise tests. Consequently, indirect calorimetry was used to calculate EE ($kJ.min^{-1}$) and substrate oxidation rates ($g.min^{-1}$) using stoichiometric equations, assuming negligible protein oxidation, specifically developed for exercise intensities between 40-50% $\dot{V}O_2$peak, as shown below (Jeukendrup & Wallis, 2005).

$$\text{Fat Oxidation Rate} = (1.695 \cdot \dot{V}O_2) - (1.701 \cdot \dot{V}CO_2)$$

$$\text{CHO Oxidation Rate} = (4.344 \cdot \dot{V}CO_2) - (3.061 \cdot \dot{V}O_2)$$

$$\text{Energy Expenditure} = [(0.575 \cdot \dot{V}CO_2) - (4.435 \cdot \dot{V}O_2)]$$

Exported data was analysed only from the final 50 minutes of all one-hour sub-maximal tests to ensure participants reached steady-state. Data was averaged for every 15 second period during the entire 50 minutes of exercise.

Blood Sampling

Venous blood, was sampled pre- and post-exercise, using the butterfly method (BD Vacutainer Safety-Lok Blood Collection Set 21G with Luer Adapter, Becton Dickinson and Co., Oxford, UK) from veins located in the antecubital fossa region, into one lithium heparin tube (4 mL) and one serum separator tube (4 mL) (BD Vacutainer,

Becton Dickinson and Co., Oxford, UK). Tubes were centrifuged at 4000 rev.min^{-1}, 4ºC for 10 minutes (Sorvall ST 8R, Thermo Fisher Scientific, USA). Serum and plasma supernatants were aliquoted and stored at -80ºC for later analysis. Blood samples were obtained from 10 participants, as one subject was uncomfortable with the venepuncture method.

Biochemical Analysis

Plasma Volume Change

Whole blood from lithium heparin tubes, was analysed for haemoglobin (HemoCue Hb 201^{+} Reader, Sweden) and haematocrit to calculate plasma volume change (PVC). Haematocrit was analysed by pipetting 60µL of whole blood into micro-haematocrit capillary tubes (Hawksley, Lancing, Sussex, UK). Tubes were then centrifuged (Haematospin 1300, Hawksley, Lancing, Sussex, UK) at 1300 rev.min^{-1} for 3 minutes. Percentage packed cell volume was determined from a slide reader (Micro Haematocrit Tube Reader, Hawksley, Lancing, Sussex, UK).

Assay results were corrected for PVC as a result of exercise-induced changes in haemoconcentration (Allgrove, Farrell, Gleeson, Williamson, & Cooper, 2011) by the equation shown below.

$$\text{Corrected Assay Results} = \%\text{PVC} \times \text{Measured Post-Exercise Assay Value}$$

PVC was determined using the method and equations of Dill and Costill (1974) by means of comparing the pre- and post-exercise haemoglobin and haematocrit values.

Glucose

Pre- and post-exercise serum samples were assessed for glucose (range 0.5-50 mmol.L^{-1}, CV ≤ 1.5%) (Biosen C-Line, EKF Diagnostics, Cardiff, UK) in duplicates.

Total Antioxidant Status Assay

Total antioxidant capacity within serum samples was assessed in duplicates according to the manufacturers guidelines using the total antioxidant status (Total Antioxidant Status NX2332, Randox Laboratories Ltd, Antrim, UK) colorimetric assay on a semi-automated spectrophotometer (RX Monza, Randox). Intra-assay CV was 3.49%.

Lipid Assays

Serum lipids were determined in duplicates using commercially available colorimetric assays on a semi-automated spectrophotometer, according to manufacturer's guidelines. Triglyceride (Triglycerides TR210, Randox) values were corrected for free glycerol by subtracting 0.11 $mmol.L^{-1}$ (Stinshoff et al., 1977), according to the manufacturer's guidelines. Intra-assay CV for triglycerides, total cholesterol and HDL were 4.96%, 1.99% and 4.66%, respectively. The ratio between total cholesterol and HDL was also determined. LDL was determined indirectly using the formula below (Ahmadi, Boroumand, Gohari-Moghaddam, Tajik, & Dibaj, 2008).

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

Data Analysis

Statistical analysis was performed using SPSS v22.0 (IBM, Chicago, USA) where data are reported as means ±SD. Data normality was checked using a Shapiro-Wilk test. Greenhouse-Geisser correction was applied upon violation of Mauchly's test of sphericity for analyses of variance (ANOVA). Statistical significance was set at $P < 0.05$.

A within-group 3-way, 2 x 3 x 2, condition (MTCJ vs PLA), time (pre-, mid-, post-supplementation), exercise (pre- and post-exercise), repeated-measures ANOVA with post-hoc Bonferroni's adjustment, measured differences of body mass, BMI, HR, BP, glucose, TAS, triglycerides, total cholesterol, HDL, total cholesterol:HDL, haematocrit and haemoglobin. Waist circumference, body composition and resting EE, RER, fat and CHO oxidation were analysed using a 2-way, 2 x 3 (condition x time) repeated-measures ANOVA design with post-hoc Bonferroni's adjustment. A paired-samples t-test was used to identify differences between individual data points within conditions and between conditions for the corresponding time point.

Parameters measured during the FATMAX and $\dot{V}O_2$max tests, including MFO (maximal fat oxidation) and power output at FATMAX, FATMAX (% of $\dot{V}O_{2peak}$), RER at FATMAX, percentage of maximal HR (%HRmax) at FATMAX and $\dot{V}O_2$peak were analysed using a 2 x 2 (condition x time) repeated-measures ANOVA. A paired samples t-test was used to identify differences between conditions for only the first and second FATMAX tests.

Variables measured during the one-hour sub-maximal exercise at FATMAX including, RER, HR, RPE (overall), RPE (legs), EE, CHO oxidation, fat oxidation and percentage contribution of fat and CHO to total EE, were

analysed using a 2 x 3 (condition x time) repeated-measures ANOVA with post-hoc Bonferroni's adjustment, when averaged for the final 50 minutes.

Partial Eta-Squared ($\eta^2_{partial}$) was used to report effect sizes for ANOVAs where effects were classified as small (0.01-0.08), moderate (0.09-0.25) and large (>0.25) (Cohen, 1988). Cohen's *d* effect size was used for paired-samples t-test where effects were classified as no effect (0-0.1), small (0.2-0.4), moderate (0.5-0.7) and high (≥0.8) (Cohen, 1988).

In order to determine whether independent variables could explain the variance observed for MFO, bivariate correlations were conducted between MFO values obtained during the final minute of the stage corresponding to FATMAX at baseline and independent variables affecting FATMAX, including age, anthropometrics, body composition, dietary intake and $\dot{V}O_{2peak}$. No significant correlations (P>0.05) were found for any independent variables, thus regression analysis was not performed.

Results

Exercise Results

One-hour Sub-Maximal Cycling Tests

No significant interactions or main effects for condition and time (P>0.05) were observed for mean EE, percentage contributions of fat and CHO to EE, $\dot{V}O_2$ and $\dot{V}CO_2$, HR, overall RPE, legs RPE and serum glucose during one-hour cycling at individual FATMAX suggesting exercise intensities and physiological responses were similar between conditions over the supplementation period (Table 1). ***Table 1 near here***

Mean fat oxidation rates during the final 50 minutes of the one-hour cycling exercise at individual FATMAX were not significantly different between conditions (PLA: 0.25 ± 0.10 g.min^{-1} and MTCJ: 0.26 ± 0.09 g.min^{-1}; $F_{(1, 10)}$ = 0.35; P = 0.567, $\eta^2_{partial}$ = 0.034), time (Pre-Supplementation: 0.25 ± 0.09 g.min^{-1}, Mid-Supplementation: 0.27 ± 0.08 g.min^{-1}, Post-Supplementation: 0.25 ± 0.11 g.min^{-1}; $F_{(2, 20)}$ = 1.22; P = 0.318, $\eta^2_{partial}$ = 0.108) or the interaction between condition and time ($F_{(2, 20)}$ = 0.273; P = 0.764, $\eta^2_{partial}$ = 0.027) (Figure 3). ***Figure 3 near here***

There were also no main effects (P>0.05) for condition, time or the interaction for mean CHO oxidation rates.

A significant main effect for time (Pre-Supplementation: 0.88 ± 0.04, Mid-Supplementation: 0.87 ± 0.04, Post-Supplementation: 0.89 ± 0.04; $F_{(2, 20)} = 4.14$; $P = 0.031$, $\eta^2_{partial} = 0.293$) was detected for mean RER but not between conditions or the interaction ($P>0.05$). *Post-hoc* analysis identified a trend towards significance between mid-supplementation and post-supplementation ($P = 0.070$).

FATMAX and $\dot{V}O_2$max Determination Tests

No significant differences between conditions (PLA: 0.26 ± 0.05 g.min^{-1} and MTCJ: 0.23 ± 0.04 g.min^{-1}; $F_{(1, 10)} = 2.79$; $P = 0.126$, $\eta^2_{partial} = 0.22$) or time (Test 1: 0.25 ± 0.08 g.min^{-1} and Test 2: 0.25 ± 0.10 g.min^{-1}; $F_{(1,10)} = 0.06$; $P = 0.807$, $\eta^2_{partial} = 0.01$) were found for MFO during the FATMAX determination tests. A tendency ($t_{(10)} = 2.21$; $P = 0.052$, $d = 0.59$) towards significance was observed between conditions for MFO during the second FATMAX test (PLA: 0.28 ± 0.12 g.min^{-1} and MTCJ: 0.22 ± 0.08 g.min^{-1}).

No main effects ($P>0.05$) for condition, time or interaction were detected for FATMAX (%$\dot{V}O_2$peak) (Test 1 – PLA: 42.77 ± 8.69% and MTCJ: 45.94 ± 9.28%, Test 2 – PLA: 45.28 ± 12.07% and MTCJ: 47.91 ± 11.52%), MFO (Test 1 – PLA: 0.25 ± 0.08 g.min^{-1} and MTCJ: 0.24 ± 0.08 g.min^{-1}, Test 2 – PLA: 0.28 ± 0.12 g.min^{-1} and MTCJ: 0.22 ± 0.08 g.min^{-1}), RER at FATMAX (Test 1 – PLA: 0.87 ± 0.04 and MTCJ: 0.88 ± 0.04, Test 2 – PLA: 0.87 ± 0.03 and MTCJ: 0.90 ± 0.03), %HR_{max} at FATMAX (Test 1 – PLA: 60.27 ± 9.59% and MTCJ: 62.58 ± 7.38%, Test 2 – PLA: 59.84 ± 10.43% and MTCJ: 63.04 ± 8.38%), power output at FATMAX (Test 1 – PLA: 66 ± 20 W and MTCJ: 72 ± 18 W, Test 2 – PLA: 70 ± 19 W and MTCJ: 78 ± 18 W) and $\dot{V}O_2$peak (Test 1 – PLA: 36.10 ± 5.17 mL.kg^{-1}.min^{-1} and MTCJ: 36.33 ± 5.34 mL.kg^{-1}.min^{-1}, Test 2 – PLA: 37.07 ± 5.77 mL.kg^{-1}.min^{-1} and MTCJ: 36.94 ± 5.89 mL.kg^{-1}.min^{-1}). Values tended to be greater with MTCJ ($F_{(1,10)} = 3.65$; $P = 0.085$, $\eta^2_{partial} = 0.27$) for RER at FATMAX, particularly during the second test (0.90 ± 0.03) compared to the first test (0.88 ± 0.04) ($t_{(10)} = -2.04$; $P = 0.068$, $d = 0.63$). RER at FATMAX was significantly greater ($t_{(10)} = -2.77$; $P = 0.020$, $d = 1.1$) during the second test with MTCJ (0.90 ± 0.03) compared to PLA (0.87 ± 0.03).

Paired-samples t-test between conditions did not outline any significant differences ($t_{(10)} = -2.04$; $P = 0.256$, $d = 0.63$) for the shift in wattage for FATMAX, where 3 participants increased wattage with PLA whilst 4 participants increased with MTCJ. Thus, the shift in wattage between conditions was considered negligible and not a limitation of the research.

Anthropometric and Functional Variables

No interactions or main effects for condition and time were detected for anthropometric measurements in Table 2 (P>0.05). However, there was a tendency towards significantly lower percentage body fat ($t_{(10)}$ = 1.887; P = 0.08, d = 0.05) and fat mass ($t_{(10)}$ = 1.903; P = 0.08, d = 0.05) values pre- to post-supplementation with PLA compared to no difference with MTCJ for percentage body fat ($t_{(10)}$ = -0.841; P = 0.42, d = 0.02) and fat mass ($t_{(10)}$ = -1.386; P = 0.196, d = 0.04).

No interactions or main effects for condition, time and exercise (P>0.05) were obtained for functional variables presented in Table 2, apart from a main effect of exercise for HR ($F_{(1,10)}$ = 25.493; P < 0.001, $\eta^2_{partial}$ = 0.718), as expected.

The change in resting EE from the mid- to post-supplementation trial was significantly different between conditions ($t_{(10)}$ = -2.602; P = 0.026, d = 0.86) suggesting different responses during days 10-20 of supplementation. A tendency towards significance was detected with MTCJ where resting CHO oxidation increased from mid- to post-supplementation but not with PLA ($t_{(10)}$ = -2.213; P = 0.051, d = 0.77).

*** Table 2 near here***

Glucose and TAS Biomarkers

No significant interactions or main effects for condition, time and exercise were detected for glucose (P>0.05).

A main effect for time ($F_{(2, 18)}$ = 11.137; P = 0.001, $\eta^2_{partial}$ = 0.55) and a significant interaction ($F_{(1, 9)}$ = 19.122; P = 0.002, $\eta^2_{partial}$ = 0.68) between condition and exercise were detected for TAS (Figure 4). *Post-hoc* analysis revealed significantly lower concentrations post-supplementation compared to mid-supplementation. There was also a tendency towards significance for the interaction between time and exercise ($F_{(2, 18)}$ = 3.466; P = 0.053, $\eta^2_{partial}$ = 0.28).

Pre-exercise TAS with MTCJ was significantly lower post-supplementation (1.18 ± 0.02 mmol.L^{-1}; 98.92% of baseline) compared to mid-supplementation (1.33 ± 0.05 mmol.L^{-1}; 111.93% of baseline) by 11.45%. In comparison, values were statistically similar with PLA ($t_{(9)}$ = 1.464; P = 0.177, d = 0.47) (Table 3). There was a tendency towards significance ($t_{(9)}$ = -1.998; P = 0.077, d = 0.71) for the difference between pre-supplementation (1.17 ± 0.03 mmol.L^{-1}; 101.26% of baseline) and mid-supplementation (1.33 ± 0.05 mmol.L^{-1}; 111.93% of baseline) values prior to exercise with MTCJ, but not for PLA ($t_{(9)}$ = -0.556; P = 0.585, d = 0.24). After the onset of supplementation, the change in TAS during one-hour FATMAX exercise was significantly different ($t_{(19)}$ = 2.291; P = 0.034, d = 0.64), where an average increase of 1.75% was seen with MTCJ and a decrease of 5.17% with PLA. ***Figure 4 near here***

Lipid Biomarkers

No significant interactions or main effects for condition, time and exercise were detected for triglycerides and LDL (P>0.05).

A main effect for time only was found for HDL ($F_{(1.3, 11.703)}$ = 7.098; P = 0.016, $\eta^2_{partial}$ = 0.441). *Post-hoc* analysis showed post-supplementation concentrations were significantly lower than mid-supplementation (P < 0.001) (Figure 5). As with TAS, a significant ($t_{(9)}$ = 3.123; P = 0.012, d = 0.68) reduction in pre-exercise HDL concentrations was observed from mid-supplementation (1.84 ± 0.47 mmol.L^{-1}; 114.48% of baseline) to post-supplementation (1.56 ± 0.34 mmol.L^{-1}; 98.62% of baseline) with MTCJ whereas no difference was found for PLA ($t_{(9)}$ = 0.719; P = 0.490, d = 0.15) (Table 3). ***Figure 5 near here***

Mean post-exercise values for total cholesterol (main effect for exercise: $F_{(1, 9)}$ = 8.951; P = 0.015, $\eta^2_{partial}$ = 0.5) and total cholesterol:HDL ratio (main effect for exercise: $F_{(1, 9)}$ = 8.951; P = 0.015, $\eta^2_{partial}$ = 0.5) were significantly lower

than pre-exercise values. In addition, both total cholesterol ($F_{(1.23, 11.154)} = 6.092$; $P = 0.026$, $\eta^2_{partial} = 0.404$) and total cholesterol:HDL ($F_{(2, 18)} = 10.995$; $P = 0.001$, $\eta^2_{partial} = 0.55$) demonstrated main effects for time, however no main effect for condition, or significant interactions were detected ($P>0.05$).

Table 3 near here

Discussion

This was the first study in any population to combine exercise and tart cherry supplementation whilst examining cardio-metabolic biomarkers. The main findings of this study were that 20 days of MTCJ supplementation did not significantly increase fat oxidation rates at rest or during FATMAX exercise, nor did it alter waist circumference, body composition or cardio-metabolic biomarkers compared to PLA.

The present study did not find significance between conditions for RER, EE, fat and CHO oxidation rates, contrasting previously reported results regarding the effect of dietary anthocyanin supplementation on substrate metabolism with exercise. Cook et al. (2015) observed a 16% increase in fat oxidation with 7 days encapsulated, anthocyanin-rich, New Zealand blackcurrant extract (NZBE) supplementation compared to placebo during cycling exercise at 65% $\dot{V}O_2$max. Crucially, Cook et al. (2015) instructed participants to consume one NZBE capsule, containing 105 mg anthocyanins, 2 hours prior to commencing exercise thus coinciding peak bioavailability and concentrations of anthocyanins, secondary metabolites and phase II conjugates in plasma and target tissues with the time of measurement of substrate oxidation rates during exercise. This provides evidence that fat oxidation can be increased with short-term (7 days) and acute (2 hours before exercise) supplementation of dietary anthocyanins and highlights the importance of timing of consumption to maximise bioavailability and concentrations in plasma and target tissues. Subsequently, the inclusion of an overnight fast may explain why Montmorency tart cherry anthocyanins and its metabolites were not able to significantly augment fat oxidation rates.

Differences in fat oxidation responses to anthocyanin supplementation may be explained by the different types of anthocyanins present compared to MTCJ. The main anthocyanins in NZBE (35-50% delphinidin-3-rutinoside and 5-20% delphinidin-3-glucoside) in NZBE are derived from the anthocyanidin, delphinidin (Cook et al., 2017), whereas cyanidin based anthocyanins (cyanidin-3-glucosylrutinoside and cyanidin-3-rutinoside) comprise 93% of the total anthocyanin content in Montmorency tart cherries (Kirakosyan et al., 2009). It has been previously reported that delphinidin based anthocyanins provide more effective cardio-metabolic protection than cyanidin based anthocyanins (Overall, Bonney, Wilson, Beermann, Grace, Esposito, Lila & Komarnytsky, 2017). Additionally, cyanidin is less stable in the gastrointestinal environment, although it is not dependent on gut bacteria for metabolism or absorption whereas delphinidin is dependent on gut bacteria (Overall et al., 2017). Healthy individuals are expected to have greater diversity and concentrations of 'good' gut bacteria, acting to increase the bioavailability of anthocyanins and their metabolites (Fernandes, Faria, Calhau, de Freitas, & Mateus, 2014; Hidalgo et al., 2012). Since gut bacteria do not modulate cyanidin-based

anthocyanin metabolism and absorption, it is not surprising that cardio-metabolic markers or fat oxidation rates were not improved in the healthy participants from this study.

It is feasible that consumption of a fruit extract (NZBE) in capsule form would not influence substrate oxidation rates to a greater extent than supplements in juice form (MTCJ) when ingested immediately before exercise, given the likely differences in macronutrient content and metabolism such as anthocyanin degradation and absorption rates. Perhaps this explains why Cook et al. (2015) were able to observe greater fat oxidation with NZBE. Cook et al. (2015) reported NZBE anthocyanins contributed 30.70% of fat to total EE during exercise, whilst MTCJ peaked during the mid-supplementation test at 24.06% and averaged 21.48% throughout the present study. Similar results were reported with the placebo, with the most likely explanation being the high carbohydrate content of the MTCJ and placebo drinks, compared to the capsules provided in the NZBE study which were completely devoid of carbohydrate. The significant increase in resting EE was facilitated by upregulation of CHO oxidation signifying that the higher CHO content of MTCJ did induce greater glycolytic flux from days 10-20 of supplementation. This provides an explanation for the lower MFO rates and percentage contributions of fat to total EE in the present study compared to Cook et al. (2015). The absorption of maltodextrin, present in the placebo, from the small intestine into systemic circulation, has not been proven to be different from pure glucose, therefore the magnitude and time course of the glycaemic and insulinaemic response are expected to be similar either at rest or during exercise (Hofman, van Buul, & Brouns, 2016). However, as MTCJ contained fructose and glucose the significant elevation in CHO oxidation, at rest with MTCJ during the final 10 days of supplementation is likely due to fructose accumulation. In support of these findings, co-ingestion of glucose and fructose as opposed to either alone has been shown to significantly increase CHO oxidation rates in healthy participants (Jentjens & Jeukendrup, 2005).

The tendency towards significance for the 13.35% increase in TAS from pre-supplementation to mid-supplementation with MTCJ, may explain the tendency for greater fat oxidation with MTCJ during one-hour FATMAX exercise between these time points. The increase in oxygen flux and therefore generation of ROS during exercise (Radak, Zhao, Koltai, Ohno, & Atalay, 2013), as a by-product of fatty acid oxidation at FATMAX, may have been mitigated by MTCJ antioxidants. These antioxidant may have retarded mitophagy and preserved mitochondrial function, thus enabling the continuation of fat oxidation (Montgomery & Turner, 2015). The 12.76% decrease in TAS from mid-supplementation to post-supplementation prior to exercise suggests TAS does not increase linearly with further ingestion of MTCJ after 10 days but returns to baseline. As far as the authors are aware, this is the first study to demonstrate such an effect. Consequently, this may have

contributed to the different responses observed between conditions for HDL and resting EE during days 10-20 of supplementation. Endogenously derived antioxidants are the primary contributors to the antioxidant balance, thus supplementation of exogenous antioxidants may inhibit synthesis of endogenous antioxidants in order to maintain a homeostatic balance (Poljsak, Suput, & Milisav, 2013). Thus, it is plausible that the synthesis of endogenous antioxidants was reduced during the final 10 days of exogenous antioxidant supplementation with MTCJ resulting in a net reduction of TAS. A potential increase in oxidative stress from mid- to post-supplementation may have contributed to inefficient oxidative metabolism and thus greater resting EE (Frisard & Ravussin, 2006). These findings are supported by Timmers et al. (2011) who reported significantly lower basal EE due to improved mitochondrial function and therefore metabolic efficiency after 30 days resveratrol supplementation , a potent antioxidant, in healthy, obese humans. Furthermore, the reduced synthesis/uptake of antioxidants may be a defensive mechanism against an excessively elevated antioxidant balance which may affect hormetic responses. Such is the complexity of the interactions of free radicals, pro-oxidant species, antioxidants and the cellular mechanisms involved, that no one reason is liable to be responsible for this potential antioxidant effect.

In relation to lipid responses, Ataie-Jafari et al. (2008) reported tart cherry juice significantly reduced total cholesterol and LDL, whilst Martin et al. (2010) reported a significant improvement in triglycerides and VLDL. In both studies, participants were found to be hyperlipidaemic for the specific variables in which a treatment effect was observed. Likewise, studies (Kelley et al., 2006; Lynn et al., 2014) reporting non-significant differences with the cherry treatment recruited healthy participants, therefore it is reasoned the lack of cardio-metabolic dysregulation does not provide sufficient scope for a cherry intervention to further regulate cardio-metabolic function at rest. Despite the addition of an exercise intervention, a similar reasoning can be applied to the present study, given the healthy baseline lipid concentrations presented by the participants in this study. This observation is further supported by findings presented in rodents susceptible to dyslipidaemia responding significantly better to cherry consumption than those without dyslipidaemia (Seymour et al., 2009; Seymour et al., 2008; Wu et al., 2006). The molecular mechanisms associated with these responses were suggested to be via increased mRNA transcription of peroxisome proliferator-activated receptor alpha (PPARα), peroxisome proliferator-activated receptor gamma (PPARγ) and hepatic activation of these isoforms by cherry anthocyanins (Seymour et al., 2009; Seymour et al., 2008). Thus enhancing skeletal muscle insulin sensitisation due to increased fat oxidation through sirtuin-1 mediated PPARα and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) activation (Huffman, 2010).

It is apparent accumulation of anthocyanins and their metabolites with long-term supplementation likely did not occur in the present study. This is likely due to the poor bioavailability, intestinal absorption and rapid elimination rates of anthocyanins (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004). Alternatively, it may be that the anthocyanin and secondary metabolite concentrations in target tissues reached a ceiling level below a physiologically relevant threshold concentration (Krga et al., 2016) to affect serum lipids, thus explaining pre-exercise HDL responses to MTCJ consumption. A biphasic response was observed where a non-significant, yet clinically relevant, increase in HDL from pre- to mid-supplementation was followed by a significant decline from mid- to post-supplementation with MTCJ but not PLA. Based on data from epidemiological studies with middle-age and high-risk subjects, suggesting an increase of 0.026 $mmol.L^{-1}$ could reduce the risk of coronary heart disease by 2% in males (Gordon et al., 1989), the change in HDL from pre- to mid-supplementation would result in a risk reduction of 12% with MTCJ. However, this finding should be interpreted with caution as the translation across to healthy subjects of both sexes and the lack of statistical and clinically significant improvements for other lipid profile markers with MTCJ, renders the increase in HDL less important. The decline from mid- to post-supplementation suggests that administering cherry interventions longer than 10 days does not maintain elevated HDL concentrations, but rather a return to baseline as supported by findings from Kelley et al. (2006), Ataie-Jafari et al. (2008), Martin et al. (2010) and Lynn et al. (2014).

Given the variability in study design and supplementation strategy in the present and previous studies (Ataie-Jafari, Hosseini, Karimi, & Pajouhi, 2008; Kelley et al., 2006; Lynn et al., 2014; Martin et al., 2010) it is possible to conclude that the length of supplementation, volume and concentration of juice are unlikely to be factors responsible for the equivocal results regarding lipids after cherry consumption. Rather, the initial physiological status of participants is thought to be more influential regarding the efficacy of a cherry intervention.

Finally, limitations associated with this study include the overnight fast dietary restriction put in place to reduce intra- and inter-individual variability, but at the expense of upholding ecological validity. Secondly, previous research has shown improvements in body mass, fat mass and metabolic function required participants to exercise at FATMAX 2-4 times per week for 2-12 months (Brun et al., 2011). Thus, Montmorency tart cherry anthocyanins were not able to compound upon any cellular and molecular adaptations promoting fat oxidation which may have occurred with FATMAX training. Thirdly, the small sample size did not assist the ability to find significant differences with MTCJ as *post-hoc* power analysis suggested the study was underpowered. Additionally, due to the differences in viscosity of the placebo and MTCJ, 9 out of 11 participants correctly

identified which supplement they were provided when asked at the end of the study. Consequently, this may have contributed to the lack of significance between conditions as participants could manipulate their activity during the study. This highlights a limitation of juice concentrate as a form of supplementation and perhaps alternative forms such as capsules are preferable to uphold anonymity between conditions.

Future work should attempt to elucidate the cellular and molecular mechanisms, including epigenetic changes, in humans to provide a basis upon which theories explaining the obtained responses can be either accepted or refuted. This would provide crucial information by which supplementation strategies can be altered to maximise the efficacy of MTCJ. Based on Cook et al. (2015) findings, it would be appropriate to suggest that any effect of anthocyanin supplementation on fat oxidation is short-term and provides a rationale to acutely supplement Montmorency tart cherry anthocyanins in future studies. The augmentation of fat oxidation with acute supplementation may then mitigate the development of cardio-metabolic symptoms in clinical populations such as those with Metabolic Syndrome.

Conclusion

This was the first study to examine the effect of cherry supplementation on fat oxidation rates at rest and during exercise. Findings showed that MTCJ did not significantly increase fat oxidation rates at rest or during FATMAX exercise. Additionally, secondary cardio-metabolic markers were also not significantly different with MTCJ supplementation, the primary reason being that this intervention does not indicate CRM properties in healthy participants. Consequently, it is unnecessary for healthy participants to supplement MTCJ to reduce body fat percentage and improve both functional and blood-based cardio-metabolic markers. Previous studies to report a significant response to cherry supplementation in animal (Seymour et al., 2009; Seymour et al., 2008; Wu et al., 2006) and human (Ataie-Jafari et al., 2008; Keane et al., 2016a; Keane et al., 2016b; Martin et al., 2010) studies occurred when initial values were abnormal, thus further research is warranted in clinical populations.

Conflicts of Interest

Conflicts of interest: none.

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Figure Captions

Fig 1. Schematic of testing protocol for each condition (MTCJ and PLA).

Fig 2. Schematic of testing procedure during FATMAX and VO_2max testing sessions (A) and during 1-hour sub-maximal exercise at individually determined FATMAX sessions (B).

Fig 3. Mean (±SD) fat oxidation rates measured during final 50 minutes exercise at individual FATMAX for PLA and MTCJ.

Fig 4. Mean (±SD) TAS concentrations presented at all measured time points for PLA and MTCJ. *Denotes significant difference between corresponding time point during post-supplementation for MTCJ.

Fig 5. Mean (±SD) HDL concentrations presented at all measured time points for PLA and MTCJ. *Denotes significant difference between corresponding time point during post-supplementation for MTCJ.

Table Captions

Table 1. Mean (±SD) values of final 50 minutes for variables measured during one-hour exercise at individual FATMAX throughout the study duration for PLA and MTCJ.

Table 2. Mean (±SD) for anthropometric, body composition and resting functional variables obtained pre- and/or post-exercise in both conditions at baseline and during pre-, mid- and post-supplementation trials.

Table 3. Mean (±SD) for all blood-based biomarkers measured for PLA and MTCJ during pre-, mid-, post-supplementation, before and after one-hour FATMAX exercise.