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Effects of Matcha green tea on heart rate variability and physiological and metabolic responses in young adult females

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Submission Date: September 10th, 2023; Acceptance Date: January 18th, 2024; Publication Date: January 23rd, 2024

Please cite this article as: Willems M. E. T., Foster C. T. Effects of Matcha green tea on heart rate variability and physiological and metabolic responses in young adult females. *Dietary Supplements and Nutraceuticals* 2024; 3(1): 1-12. DOI: https://www.doi.org/10.31989/dsn.v3i1.1230

ABSTRACT

Introduction: Compared to other green teas, higher intake of multiple phytochemicals is achieved with Matcha green tea consumption. Green tea consumption is known to have metabolic effects but is also consumed for supposed calming effects. The aim of the present study was to examine the effects of encapsulated Matcha green tea on heart rate variability metrics during supine rest, as well as on physiological and metabolic responses during both supine rest and moderate-intensity exercise.

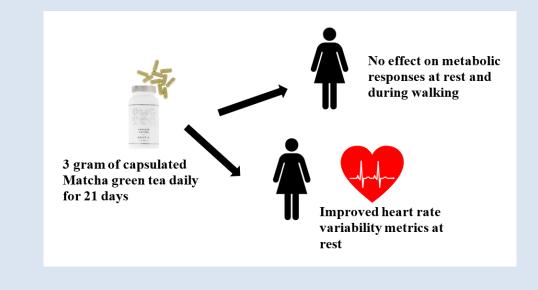
Methods: Healthy females (n=8, age: 22±3 yrs, body mass: 68 ± 11 kg, height: 162 ± 5 cm) volunteered. The study employed a placebo-controlled, randomised cross-over design. Time-domain heart rate variability metrics during supine rest (n=5) and physiological and metabolic responses using indirect calorimetry techniques during supine rest and 60-min of moderate-intensity (~ 4-METs) treadmill walking (speed: 4.4 ± 0.5 km·h⁻¹) were measured following 3 weeks of 3 g·day⁻¹ of Matcha green tea or placebo.

Results: During supine rest with Matcha green tea, all participants had lower heart rates by $13\pm7\%$ (P=0.01, d= -1.45), higher mean beat-to-beat RR intervals by $16\pm9\%$ (P=0.03, d=1.25), higher SDNN by $44\pm32\%$ (P=0.01, d=0.76) and higher pNN50 by $139\pm139\%$ (P<0.01, d=1.28). Matcha green tea had no effects on the physiological and metabolic responses during supine rest and moderate-intensity treadmill walking (e.g. respiratory exchange ratio, placebo: 0.78 ± 0.04 ; Matcha: 0.78 ± 0.03 , P=0.87). Fat oxidation during supine rest was correlated (r=0.75, P<0.01) with the moderate-intensity

walking induced fat oxidation.

Conclusions: In young adult healthy females, Matcha green tea beneficially effects heart rate variability metrics during supine rest indicating an alteration in parasympathetic nervous activity and therefore suggestive of a relaxing effect. Matcha green tea did not change the metabolic responses during supine rest and exercise possibly due to the low respiratory exchange ratio in the female cohort. Future work should address the effectiveness of Matcha green tea during conditions of psychological stress.

Key words: Matcha green tea, heart rate variability, rest, walking, substrate oxidation



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INTRODUCTION

Matcha green tea powder is derived from the grounding of unfermented leaves from tea plants (*Camellia Sinensus*) exposed to shading a few weeks before harvest. The limited light exposure of the tea plants provides the leaves with a high content of flavonoids [especially epigallocatechin gallate making up 50-80% of the total catechin content [1], theanine, chlorophyl and caffeine [2, 3]. Matcha green tea consumption involves the digestion of the phytochemical-rich powder. Recent reviews highlighted the association between the unique Matcha composition and health benefits that were associated with the anti-oxidant and anti-inflammatory properties [3-5]. In general, supplementation with antioxidant properties may affect the acute and chronic *invivo* physiological, cardiovascular and metabolic responses at rest and during exercise [6-7].

Studies on the *in-vivo* effects of Matcha green tea are limited. In untrained men, 1.5 g of Matcha intake daily for 12 weeks enhanced the resistance-training induced hypertrophy with a trivial change for lower body fat% [8]. Matcha intake (1.5 gram daily) for two weeks in young adult healthy males and females provided beneficial changes in fecal microbiota composition [9]. In healthy female cohorts, Matcha green tea drinks over a 24-hr period (4 x 1-gram drinks) and 3-weeks of capsulated Matcha green tea intake (3 x 1 gram/day) enhanced walking-induced fat oxidation during a 30-min moderate-intensity walk [10, 11]. Several studies on the effects of green tea on metabolic responses have provided observations on the enhanced fat oxidation in rest (for a review see Hodgson et al [12]). It was likely that the enhanced walking-induced fat oxidation by Matcha green tea [10, 11] was due to the combined effects of the catechins and caffeine. The effects of Matcha green tea on the metabolic responses at rest have not been addressed.

Matcha green tea also contains L-theanine [3] that has been shown to have anti-stress effects potentially by inhibiting cortical neuron excitation [13]. For example, the daily consumption of Matcha-enriched cookies over 8 days, formulated with specific molar ratios of certain components (i.e., caffeine, epigallocatechin-gallate, theanine, and arginine), resulted in a reduction of anxiety levels among university students, as assessed by a state-trait anxiety inventory test conducted during pharmacy practice [14]. In general, however, inhibition of cortical neuron excitation can reduce sympathetic activation and increase parasympathetic activation, promoting a "rest and digest" state [13]. Heart rate variability metrics can detect changes in the balance between the sympathetic and parasympathetic output (for a review see [15]), with studies reporting effects by nutritional interventions. For example, daily intake of watermelon juice for two weeks affected heart rate variability metrics during an oral glucose challenge test [16]. It is surprising that studies in humans on the effects of green tea consumption on heart rate variability metrics are absent considering that studies showed green tea components can lower stress and anxiety levels (for a review see [17]). No studies have addressed the effect of Matcha green tea on heart rate variability metrics during rest.

Therefore, the primary aims of the present study were to examine in healthy young adult females the effects of Matcha green tea on 1) heart rate variability metrics during supine rest, 2) physiological and metabolic responses during supine rest and during a 60-min moderate-intensity walk. In males, it was observed that fat oxidation at rest showed a significant correlation with exercise-induced maximal fat oxidation [18]. As far as we know, the relationship between fat oxidation during rest and exercise has not been examined in young-adult healthy females. A secondary aim was therefore to examine whether there was a significant correlation between whole-body fat oxidation in rest and during moderate-intensity exercise in young-adult healthy females.

METHODS

Participants: Healthy females (University students and staff, age: 22±3 yrs, height: 162±5 cm, body mass: 68±11 kg, skeletal muscle mass: 26.1±3.2 kg, body fat%: 30.5±9.4) volunteered for the study. Participants had to have a regular menstrual cycle (>3 months) with accepted contraceptive methods the combined pill, diaphragm, and intrauterine device. Ethical approval was obtained from the University of Chichester research ethics committee (number: 22231905455). Participants provided written informed consent after being provided with written and verbal information on the purpose and study requirements and after completion of a health history questionnaire.

Experimental design and procedures: The study used a placebo-controlled double-blind, randomised crossover design with a familiarisation and two experimental sessions (i.e. placebo and Matcha green tea), all between 9 and 11 am. For all sessions, participants were instructed

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to abstain from strenuous or unaccustomed exercise for 48 hours and not to brisk walk or cycle with high-intensity during commute to the sessions. In addition, participants had to abstain from alcohol for 24 hours and avoid caffeine/nicotine intake for 140-240 minutes preceding the testing sessions.

Familiarization session: In the familiarisation session, participants underwent anthropometric measurements using a stadiometer (Holtain Counter VR High Speed Metal, Holtain Ltd, Crymmych, UK), digital scales (Seca Model 876, Seca Ltd, Birmingham, UK) and a body composition analyser (InBody 770, InBody USA, California, USA) for height, body mass and body composition. Then, the participants rested on a portable couch for 10 minutes. For the measurements during seated rest, 2 x 10 min of expired air was collected using Douglas bags (volume: 220 L) with 5 min between the expired air collections. The lowest oxygen consumption for the 2 x 10 min collection was taken as the one metabolic equivalent [i.e. 1-MET: 3.48±0.44 mL·kg⁻¹·min⁻ ¹ (mean SD)]. Then, participants underwent a 5 x 8 min treadmill walking test, starting at 2 km·h⁻¹ with a stage increase of 1 km·h⁻¹ until 6 km·h⁻¹. Expired air collections were taken in the last 3 minutes of each stage. Expired air collections were analysed with a two-point oxygen and carbon dioxide calibrated gas analyser (Servomex Series 1400 Gas Analyser, Servomex, Crowborough, UK). The expired air volumes were measured using a calibrated gas meter (Harvard Dry Gas Meter, Harvard Apparatus, Edenbridge, Kent, UK). Gas volumes for oxygen and carbon dioxide were corrected to standard temperature and pressure and dry gas conditions and calculated using Haldane transformation with the consideration of inspired fractions of oxygen and carbon dioxide at the time of expired air collections. The inspired fractions of oxygen and carbon dioxide of the laboratory air were measured mid-way during an expired air collection. The oxygen uptakes during the incremental walking test were used to determine a linear relationship between treadmill walking speed and oxygen uptake expressed as METs. The linear relationship between treadmill walking speed and METs ($r^2=0.9492\pm0.0194$, mean \pm SD) allowed for each individual the calculation of the treadmill walking speed at the moderate-intensity of 4-METs (walking speed: 4.4 ± 0.5 km·h⁻¹, mean \pm SD).

Experimental visits with placebo and Matcha green tea: For the two experimental visits with the intake of placebo or Matcha green tea (see below for details), participants arrived in a fasted state and were allowed to have water. On arrival, participants rested for 20-min on a portable couch in a dimly lit physiology laboratory (21°C), wearing comfortable clothing. Participants were instructed to avoid talking and fidgeting and were not allowed to read, use mobile phones, or listen to music. Following the 20min adjustment period, 2 x 10 min expired air collections were taken with 5 min between collections. The average for the two 2 x 10 min collections were used for analysis. One 10-min collection was recorded using a smartwatch (Polar v800, Polar Electro UK Ltd, Warwick, UK) for heart rate variability metrics.

Heart rate variability measurements from the Polar v800 were downloaded with Kubios software (Kubios HRV Standard, version 3.5, Kuopio, Finland) for the following time-domain parameters, i.e. mean RR (i.e. the inter-beat intervals between successive heart beats), SDNN (i.e. standard deviation of inter-beat interval of normal sinus beats) and pNN50 (% of adjacent NN intervals that differ from each by more than 50 ms) [19]. The analysis was limited to the time-domain parameters due to equipment issues for three participants. Following the rest protocol, a 60-min treadmill walk was conducted for each participant at the calculated walking speed of 4 METs that was determined in the familiarization visit (see above). Expired air collections were collected from 8-10, 18-20, 28-30, 38-40, 48-50, and 58-60 minutes. Wholebody fat and carbohydrate oxidation rates were calculated using equations by Frayn et al [20] with the assumption of negligible protein oxidation. Participants recorded their dietary intake for 48 hours before the first session in the placebo or Matcha green tea condition. Participants were advised to replicate the dietary intake of the first session over the time period of 48 hours before the subsequent session. Energy intake (kcal) and intake of carbohydrates, fat, and protein were quantified with Nutritics (Nutritics LTD, Dublin, Ireland) (Table 1). There were no differences between conditions for carbohydrate (P=0.37), fat (P=0.42), protein (P=0.45) and energy (P=0.37). The placebo and Matcha green tea testing sessions for each participant were in the same phase of the menstrual cycle, as confirmed by the participants.

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Supplementation: Placebo and Matcha green tea were consumed for three weeks. Matcha green tea supplementation (premium grade, OMGtea, Brighton, United Kingdom) consisted of 3 grams·day⁻¹ (3 grams containing 429 mg total catechin, 90 mg caffeine, 37 mg L-theanine). The supplementation was taken orally via six 500 mg capsules evenly throughout meals, with the last two consumed two hours before the test, following an overnight fast. The placebo comprised of 6x500 mg capsules with cellulose and food colouring with similar dosing instructions.

Table 1. Daily absolute values for carbohydrate, fat, protein, and total energy intake for placebo and Matcha green tea conditions.

Parameter	Placebo	Matcha green tea
Carbohydrate (g)	212 ± 57	195 ± 43
Fat (g)	78 ± 32	70 ± 27
Protein (g)	95 ± 36	87 ± 26
Total energy intake (kcal)	1936 ± 574	1805 ± 438

Statistical analysis: Data analysis was completed using Graphpad Prism version 5.00 for Windows (GraphPad Software, San Diego, California, USA). The heart rate variability metrics and the mean physiological and metabolic responses during supine rest and the 60-min moderate-intensity treadmill walk were analysed with paired samples t-tests. A two-way ANOVA was used to analyse minute ventilation, oxygen uptake, carbon dioxide production, carbohydrate oxidation, fat oxidation and respiratory exchange ratio for condition and time effects during the moderate-intensity treadmill walk. Means were calculated for all parameter values collected from 8 to 10, 18 to 20, 28 to 30, 38 to 40, 48 to 50 and 58 to 60 minutes during the 60-min treadmill walk. Pearson correlation coefficients were calculated for the relationship between fat oxidation during supine rest and during the 60-min moderate-intensity treadmill walk for the placebo and Matcha green tea conditions. Cohens'd

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effect size was calculated as small ($0.2 \le d < 0.5$), moderate ($0.5 \le d \le 0.79$) and large ($d \ge 0.8$) for those parameters that showed a significant change with Matcha green tea intake. Statistical significance was accepted at P<0.05.

RESULTS

Physiological and metabolic responses during supine rest: Table 2 provides the physiological and metabolic responses during supine rest for placebo and Matcha green tea conditions. During supine rest, there were no differences for minute ventilation (placebo: 95%CI [5.88, 7.35 L·min⁻¹], Matcha: 95%CI [6.05, 7.03 L·min⁻¹], oxygen uptake (placebo: 95%CI [0.20, 0.25 L·min⁻¹]; Matcha: 95%CI [0.20, 0.24 L·min⁻¹], carbon dioxide production (placebo: 95%CI [0.16, 0.21 L·min⁻¹]; Matcha: 95%CI [0.16, 0.19 L·min⁻¹], carbohydrate oxidation (placebo: 95%CI [0.06, 0.15 g·min⁻¹]; Matcha: 95%CI [0.07, 0.14 g·min⁻¹], fat oxidation (placebo: 95%CI [0.05, 0.09 g·min⁻¹]; Matcha: 95%CI [0.05, 0.08 g·min⁻¹] and respiratory exchange ratio (placebo: 95%CI [0.77, 0.85]; Matcha: 95%CI [0.77, 0.84] (Table 2).

Table 2. Physiological and metabolic responses during supine rest for placebo and Matcha green tea conditions. Data aremean ± SD.

Parameter	placebo	Matcha green tea	p-value
Minute ventilation (L·min ⁻¹)	6.62 ± 0.88	6.54 ± 0.59	0.73
Oxygen uptake (L·min⁻¹)	0.23 ± 0.03	0.22 ± 0.02	0.38
Carbon dioxide production (L·min ⁻¹)	0.18 ± 0.03	0.18 ± 0.02	0.42
Carbohydrate oxidation (g·min ⁻¹)	0.11 ± 0.06	0.11 ± 0.04	0.95
Fat oxidation (g·min⁻¹)	0.07 ± 0.02	0.07 ± 0.02	0.74
Respiratory exchange ratio	0.81 ± 0.05	0.81 ± 0.04	0.90

Heart rate and heart rate variability metrics during

supine rest: Table 3 provides heart rate and heart rate variability metrics during supine rest for placebo and Matcha green tea conditions. During supine rest, all five participants in the Matcha condition exhibited changes in heart rate and heart rate variability metrics, with moderate and large effect sizes observed for the cohort. Participants had lower heart rates by 13±7% (placebo: 95%CI [59, 73 beats·min⁻¹], Matcha: 95%CI [50, 65 beats·min⁻¹] (d= -1.45), higher mean beat-to-beat RR intervals by 16±9% (placebo: 95%CI [819, 1032 ms], Matcha: 95%CI [895, 1254 ms] (d=1.25), higher SDNN (standard deviation of inter-beat interval of normal sinus beats) by 44±32% (placebo: 95%CI [41, 158 ms], Matcha: 95%CI [71, 202 ms] (d=0.76) and higher pNN50 (% of adjacent NN intervals that differ from each by more than 50 ms) by 139±139% (placebo: 95%CI [6, 53%], Matcha: 95%CI [32, 73%] (d=1.28). **Table 3.** Heart rate and heart rate variability metrics during supine rest for placebo and Matcha green tea conditions. Data are mean ± SD.

Parameter	Placebo	Matcha green tea	p-value
Heart rate (beats·min ⁻¹)	66 ± 5	58 ± 6	0.01
Beat-to-beat RR intervals (ms)	925 ± 86	1074 ± 145	0.03
SDNN (ms)	99 ± 47	137 ± 53	0.01
pNN50 (%)	29 ± 19	52 ± 17	<0.01

SDNN, standard deviation of inter-beat interval of normal sinus beats. pNN50%, (% of adjacent NN intervals that differ from each by more than 50 ms.

Physiological and metabolic responses during moderate-intensity walking: The actual MET values during the 60-min treadmill walk were not different between conditions (placebo: 4.00±0.39, 95%CI [3.67, 4.32]; Matcha: 4.12±0.26, 95%CI [3.90, 4.34] (P=0.47).

During moderate-intensity walking, there were no condition effects for minute ventilation (Figure 1a), oxygen uptake (Figure 1b), carbon dioxide production (Figure 1c), carbohydrate oxidation (Figure 1d), fat oxidation (Figure 1e) and respiratory exchange ratio (Figure 1f). For carbon dioxide production, carbohydrate oxidation, fat oxidation and respiratory exchange, a time effect was present with no differences for these parameters between time points in the last 30 min of the walk. No further analysis of the time effects was pursued in the absence of a condition effect. During the 60-min moderate-intensity treadmill walk, there were no differences for minute ventilation (placebo: 20.55±3.17 L·min⁻¹, 95%CI [17.90, 23.20]; Matcha: 20.40±2.53 L·min⁻ ¹, 95%CI [18.29, 22.52] (P=0.79); oxygen uptake (placebo: 0.91±0.16 L·min⁻¹, 95%CI [0.77, 1.05]; Matcha: 0.90±0.12 L·min⁻¹, 95%CI [0.80, 1.00] (P=0.75); carbon dioxide production (placebo: 0.71 ± 0.14 L·min⁻¹, 95%CI [0.60, 0.82]; Matcha: 0.70 ± 0.09 L·min⁻¹, 95%CI [0.62, 0.78] (P=0.72); carbohydrate oxidation (placebo: 0.32 ± 0.18 g·min⁻¹, 95%CI [0.17, 0.48]; Matcha: 0.31 ± 0.11 g·min⁻¹, 95%CI [0.21, 0.40] (P=0.77); fat oxidation (placebo: 0.33 ± 0.08 g·min⁻¹, 95%CI [0.26, 0.40]; Matcha: 0.33 ± 0.06 g·min⁻¹, 95%CI [0.28, 0.38] (P=0.94) and respiratory exchange ratio (placebo: 0.78 ± 0.04 , 95%CI [0.75, 0.81]; Matcha: 0.78 ± 0.03 , 95%CI [0.76, 0.80] (P=0.87).

Fat oxidation at rest and during moderate-intensity treadmill walking: Due to the absence of an effect of Matcha on fat oxidation at rest and during moderateintensity treadmill walking, data on fat oxidation for the placebo and Matcha condition are plotted in Figure 2. The fat oxidation during the 60-min moderate-intensity treadmill walk was significantly correlated with the fat oxidation during supine rest (Figure 2). The relationship indicates that higher exercise-induced fat oxidation by moderate-intensity treadmill walking was associated with high fat oxidation during supine rest in healthy females.

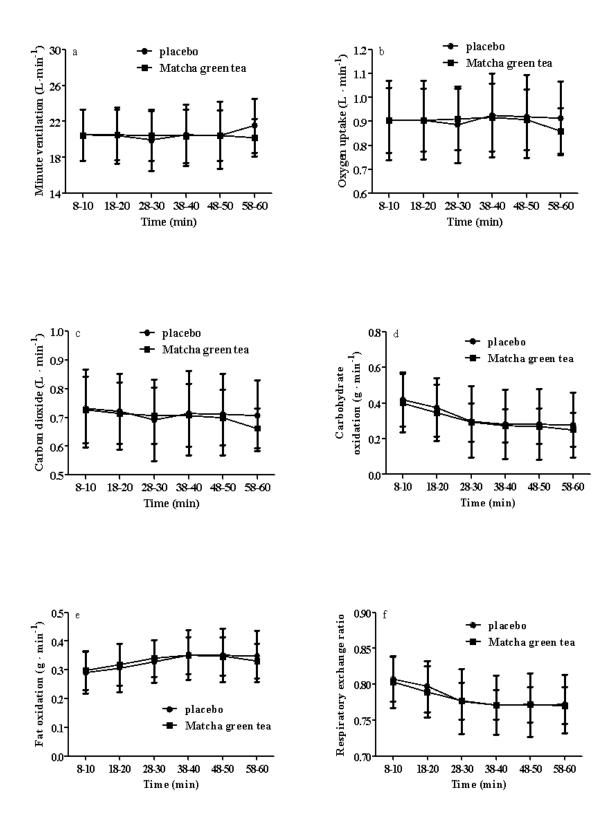


Figure 1. Minute ventilation (a), oxygen uptake (b), carbon dioxide production (c), carbohydrate oxidation (d), fat oxidation (e) and respiratory exchange ratio (f) during the 60-min moderate-intensity treadmill walk for placebo and Matcha conditions (see methods for details of dosing strategy and measurements).

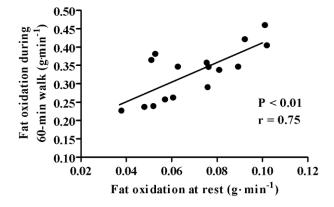


Figure 2. Relationship between whole-body fat oxidation at rest and during a moderate-intensity treadmill walk for 60 minutes. The pearson correlation coefficient was significant.

DISCUSSION

The present study provides novel observations on the effects of three weeks intake of capsulated Matcha green tea on heart rate variability metrics and physiological and metabolic responses during supine rest in young-adult healthy females. Over a three-week period of Matcha green tea intake (3 grams per day), resting heart rate decreased by 13%, and time-domain heart rate variability metrics, including mean beat-to-beat intervals (increased by 16%), SDNN (increased by 44%), and pNN50 (increased by 139%), showed significant changes in all females during supine rest. The observed effect sizes were large, except for SDNN, which had a moderate effect size. No effects were observed for other physiological and metabolic responses during supine rest. As far as we know, the beneficial changes of heart rate variability parameters with intake of green tea have not been shown in humans. In Wistar rats, ad libitum drinking of green tea for 8 weeks lowered heart rate similar to the present study by 13%, and the beat-to-beat interval by 15% [21]. In the present study, the observed reduction in heart rate and time-domain heart rate variability metrics during supine rest may be due to an enhanced parasympathetic activity or decreased

sympathetic activity of the autonomic nervous system. Studies have focused on the acute relaxing effects by the green tea component L-theanine. Acute intake of ~50 mg of L-theanine increased alpha wave activity in the brain and indicative of relaxation [22]. An acute intake of 200 mg of L-theanine did not affect resting heart rate and heart rate variability metrics [23], and a systematic review by Williams et al [17] suggested an intake of 200-400 mg·day⁻¹ to reduce stress and anxiety in human exposed to stressful conditions. In contrast, Unno et al [14] observed that daily intake for 15 days of Matcha green tea enriched cookies (4.5 gram·day⁻¹ with 78.4 mg theanine) reduced the stress marker salivary α -amylase. However, it needs to be noted that in the present study the final dose of 1 gram of Matcha green tea was taken on the testing day and contained only 12.4 mg of Ltheanine. The effects of the preceding 3 weeks intake of Matcha green tea with L-theanine are not known. In addition, Matcha green tea contains caffeine which has an antagonistic effect against L-theanine [24]. Nevertheless, the beneficial effects of Matcha green tea on the time-domain heart rate variability metrics during supine rest warrants future studies to address the effects of Matcha green tea in humans exposed to stressful conditions. It also needs to be noted that the dose of 1 gram of Matcha green tea in the present study is a recommended amount to make a drink. Therefore, the total daily intake can be obtained with three Matcha drinks [10]. Matcha green tea may therefore be a promising functional food considering the potential health benefits [3-5]. Generally, green tea consumption is acknowledged for its role in disease prevention [25, 26]. However, it is important to note that studies involving longer durations of Matcha green tea intake are warranted. This consideration arises from findings suggesting that even a two-week intake can influence fecal microbiota, potentially leading to health consequences [9]. The present study, focusing on youngadult healthy females, confirmed an association between fat oxidation during supine rest and exercise-induced fat oxidation. This finding aligns with observations made by Robinson et al. [18] and Rosenkilde et al. [27]. Robinson et al [18] confirmed that the 24-hour fat oxidation measured in a dual-respiration whole-room metabolic chamber system correlated with treadmill runninginduced maximal fat oxidation in males. In addition, a relationship between fat oxidation in rest (5-10 min in supine position) and cycling-induced peak fat oxidation was established in overweight males [27]. For supplementation studies that examine fat oxidation during rest and exercise, a significant correlation for the observations as observed in the present study provides validity of the indirect calorimetry measurements to quantify fat oxidation. Interestingly, the correlation between fat oxidation at rest and during moderateintensity exercise in the present study was 0.75 and comparable to Robinson et al [18] who had a correlation of 0.65. However, in contrast to our previous studies in females by Willems et al [10, 11], fat oxidation during moderate-intensity exercise was not enhanced by the intake of Matcha green tea. In the present study, the

respiratory exchange ratio during a moderate-intensity exercise of 4-MET was 0.78, suggesting a high habitual preference for a contribution of fat oxidation to the energy demands in our female cohort. In the cohort of females investigated for the effects of Matcha green tea on walking-induced fat oxidation, the respiratory exchange ratios were reported as 0.84 [10] and 0.87 [11]. Our observations in the present study along with the observations in the previous studies [10, 11] on Matcha green tea and fat oxidation would suggest that the lower respiratory exchange ratio may have contributed to the absence of an effect of Matcha green tea due to the already existing high preference of fat oxidation to the energy demands. In females, a study by Ishikawa et al [28], observed an effect of 3 doses of green tea extract in females with comparable cohort respiratory exchange ratio (i.e. 0.77-0.78) but with higher total catechin intake (i.e. 968.4 mg per dose), compared to the total catechin intake in the present study of 429 mg per dose. Therefore, it is also possible that higher doses of Matcha green tea are required to enhance fat oxidation for females with relatively low respiratory exchange ratios. Future work on green tea effects on fat oxidation in recreationally active females should examine whether there is a relationship between the respiratory exchange ratio and responsiveness to enhance fat oxidation during rest and exercise.

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CONCLUSIONS

It is concluded that 21-day daily intake of Matcha green tea was able to beneficially alter time-domain heart rate variability metrics during supine rest in young-adult healthy females. The absence of an effect on fat oxidation during supine rest and moderate-intensity exercise may be due to the existing high habitual preference of fat oxidation in the cohort. Future studies on the alteration of substrate oxidation by dietary supplementation may need to recognize the intrinsic ability by individuals of preferred substrate use for energy metabolism.

Abbreviations: MET, metabolic equivalent; SDNN, standard deviation of the beat-to-beat intervals; pNN50, % of adjacent of beat-to-beat intervals greater than 50ms.

Author Contributions: Mark Willems and Cameron Foster conceived and designed research. Cameron Foster conducted the experiments. Mark Willems and Cameron Foster analyzed the data. Mark Willems and Cameron Foster wrote and approved the manuscript.

Data Availability: Data is available on request to the corresponding author.

Acknowledgement: OMGtea (Brighton, United Kingdom) provided capsulated placebo and Matcha green tea.

Declaration of Interest: OMGtea provided capsulated placebo and Matcha green tea but had no role in any aspect of the study and manuscript.

REFERENCES

- Weiss DJ, Anderton CR: Determination of catechins in matcha green tea by micellar electrokinetic chromatography. J Chromatography A 2003, 1011(1–2):173-180. DOI: https://doi.org/10.1016/s0021-9673(03)01133-6
- Komes D, Horžić D, Belščak A, Ganić KK, Vulić I: Green tea preparation and its influence on the content of bioactive compounds. Food Res Int 2010, 43(1):167-176. DOI: https://doi.org/10.1016/j.foodres.2009.09.022
- Phuah YQ, Chang SK, Ng WJ, Lam MQ, Ee KY: A review on matcha: Chemical composition, health benefits, with insights on its quality control by applying chemometrics and multi-omics. Food Res Int 2023, 179:113007. DOI: https://doi.org/10.1016/j.foodres.2023.113007
- Kochman J, Jakubczyk K, Antoniewicz J, Mruk H, Janda K: Health Benefits and Chemical Composition of Matcha Green Tea: A Review. Molecules 2020, 26(1):85.

DOI: https://doi.org/10.3390/molecules26010085

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- El-Elimat T, Qasem WM, Al-Sawalha NA, Abu Al Samen MM, Munaiem RT, Al-Qiam R, Al Sharie AK: A Prospective Non-Randomized Open-Label Comparative Study on The Effects of Matcha Tea on Overweight and Obese Individuals: A Pilot Observational Study. Plant Foods Hum Nutr 2022, 77(3):447-454. DOI: https://doi.org/10.1007/s11130-022-00998-9
- Margaritelis NV, Nastos GG, Vasileiadou O, Chatzinikolaou PN, Theodorou AA, Paschalis V, Vrabas IS et al: Interindividual variability in redox and performance responses after antioxidant supplementation: A randomized double blind crossover study. Acta Physiol (Oxf) 2023, 238(4): e14017. DOI: https//doi.org/10.1111/apha.14017
- Purdy Lloyd KL, Wasmund W, Smith L, Raven PB. Clinical Effects of a Dietary Antioxidant Silicate Supplement, Microhydrin[®], on Cardiovascular Responses to Exercise. J Med Food 4(3):151-159.

DOI: https://doi.org/10.1089/109662001753165738

- Shigeta M, Aoi W, Morita C, Soga K, Inoue R, Fukushima Y, Kobayashi Y, Kuwahata M: Matcha green tea beverage moderates fatigue and supports resistance training-induced adaptation. Nutr J 2023, 22(1):32.
 DOI: <u>https://doi.org/10.1186/s12937-023-00859-4</u>
- Morishima S, Kawada Y, Fukushima Y, Takagi T, Naito Y, Inoue R: A randomized, double-blinded study evaluating effect of matcha green tea on human fecal microbiota. J Clin Biochem Nutr 2023, 72(2):165-170.

DOI: https://doi.org/10.3164/jcbn.22-81

- Willems MET, Şahin MA, Cook MD: Matcha green tea drinks enhance fat oxidation during brisk walking in females. Int J Sport Nutr Exerc Metab 2018, 28(5):536-541.
 DOI: https://doi.org/10.1123.ijsnem.2017-0237
- Willems MET, Fry HL, Belding MA, Kaviani M: Three weeks daily intake of Matcha green tea powder affects substrate oxidation during moderate-intensity exercise in females. J Diet Suppl 2021, 18(5):566-576.

DOI: https://doi.org/10.1080/19390211.2020.1811443

 Hodgson AB, Randell RK, Jeukendrup AE: The effect of green tea extract on fat oxidation at rest and during exercise: evidence of efficacy and proposed mechanisms. Adv Nutr 2013, 4(2):129-140.

DOI: https://doi.org/10.3945/an.112.003269

 Kimura K, Ozeki M, Juneja LR, Ohira H. L-Theanine reduces psychological and physiological stress responses. Biol Psych 2007, 74(1):39-45.

DOI: https://doi.org/10.1016/j.biopsycho.2006.06.006

- Unno K, Furushima D, Hamamoto S, Iguchi K, Yamada H, Morita A, Pervin M et al: Stress-reducing effect of cookies containing matcha green tea: essential ratio among theanine, arginine, caffeine and epigallocatechin gallate. Heliyon 2019, 5(5):e01653.
 DOI: <u>https://doi.org/10.1016/j.heliyon.2019.e01653</u>
- Shaffer F, McCraty R, Zerr CL: A healthy heart is not a metronome: an integrative review of the heart's anatomy. Front Psychol 2014, 5:1040.
 DOI: https://doi.org/10.3389/fpsyg.2014.01040
- Matthews R, Early KS, Vincellette CM, Losso J, Spielmann G, Irving BA, Allerton TD: The Effect of Watermelon Juice Supplementation on Heart Rate Variability and Metabolic Response during an Oral Glucose Challenge: A Randomized, Double-Blind Placebo-Controlled Crossover Trial. Nutrients 2023, 15(4):810. DOI: https://doi.org/10.3390/nu15040810
- Williams JL, Everett JM, D'Cunha NM, Sergi D, Georgousopoulou EN, Keegan RJ, McKune AJ et al: The Effects of Green Tea Amino Acid L-Theanine Consumption on the Ability to Manage Stress and Anxiety Levels: a Systematic Review. Plant Foods Hum Nutr 2020, 75(1):12-23. DOI: <u>https://doi.org/10.1007/s11130-019-00771-5</u>
- Robinson SL, Hattersley J, Frost GS, Chambers ES, Wallis GA: Maximal fat oxidation during exercise is positively associated with 24-hour fat oxidation and insulin sensitivity in young, healthy men. J Appl Physiol (1985) 2015, 118(11):1415-1422.

DOI: https://doi.org/10.1152/japplphysiol.00058.2015

- Shaffer F, Ginsberg JP: An Overview of Heart Rate Variability Metrics and Norms. Front Public Health 2017, 5:258. DOI: <u>https://doi.org/10.3389/fpubh.2017.00258</u>
- Frayn KN: Calculation of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol Respir Environ Exerc Physiol 1983, 55(2):628-634.
 DOI: https://doi.org/10.1152/jappl.1983.55.2.628
- Santos WLMD, Eustáquio JMJ, Ferreira ICR, Perez JO, Policarpo RP, Chriguer RS, Barbosa Neto O: DOES GREEN TEA ENHANCE THE EFFECTS OF PHYSICAL TRAINING ON HEART RATE VARIABILITY? Rev Bras Med Esporte 2022, 28:256-260. DOI: <u>https://doi.org/10.1590/1517-8692202228042021 0065</u>
- Nobre AC, Rao A, Owen GN: L-theanine, a natural constituent in tea, and its effect on mental state. Asia Pac J Clin Nutr 2008, 17 Suppl 1:167-168.
- Williams J, McKune AJ, Georgousopoulou EN, Kellett J, D'Cunha NM, Sergi D, Mellor D et al: The Effect of L-Theanine Incorporated in a Functional Food Product (Mango

Sorbet) on Physiological Responses in Healthy Males: A Pilot Randomised Controlled Trial. Foods 2020, 9(3):371. DOI: https://doi.org/10.3390/foods9030371

- Haskell CF, Kennedy DO, Milne AL, Wesnes KA, Scholey AB: The effects of L-theanine, caffeine and their combination on cognition and mood. Biol Psychol 2008, 77(2):113-122. DOI: <u>https://doi.org/10.1016/j.biopyscho.2007.09.008</u>
- Zhang M, Li L, Liu P, Holman CDJ. Green tea for the prevention of cancer: evidence of field epidemiology. FFHD 2012, 2(10):339-350. DOI:

https://doi.org/10.31989/ffhd. v2i10.79

- Norouzi M, Pour PM, Asgary S. Camellia sinensis in the form of green and black tea for the primitive prophylactic effect on cardiovascular disease. FBCCD 2022, 1(11):1-3. DOI: https://doi.org/10.31989/fbccd.v1i11.1026
- Rosenkilde M, Nordby P, Nielsen LB, Stallknecht BM, Helge JW: Fat oxidation at rest predicts peak fat oxidation during exercise and metabolic phenotype in overweight men. Int J Obes (Lond) 2010, 34(5):871-877.

DOI: https://doi.org/10.1038/ijo.2010.11

 Ishikawa A, Matsuda T, Gam H, Kanno M, Yamada M, Ikegami N, Funaki A et al: Effect of Green Tea Extract Ingestion on Fat Oxidation during Exercise in the Menstrual Cycle: A Pilot Study. Nutrients 2022, 14(19):3896.
 DOI: https://doi.org/10.3390/nu14193896