Title: Three weeks daily intake of Matcha green tea powder affects substrate oxidation during moderate-intensity exercise in females

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Abstract

Artificial green tea extracts may enhance exercise-induced fat oxidation. Natural Matcha green tea consumption involves the ingestion of the powdered green tea leaves. We examined the effects of three weeks daily intake of Matcha green tea powder on substrate oxidation during moderate-intensity exercise in females. Females with a regular menstrual cycle (n=12, age: 28±10 yr, body mass: 69±17 kg, height: 163±6 cm) volunteered to complete an incremental walking test to determine the individual moderate exercise intensity (4 metabolic equivalent) for the subsequent 30-min treadmill walk. The study had a randomized placebo-controlled cross-over design with participants tested between day 9 and 11 of the menstrual cycle (follicular phase). Participants consumed 3x1 gram capsules of Matcha premium grade, (OMGTea Ltd, UK) per day for three weeks, with the final dose (1 gram) two hours before the 30-min walk (walking speed: 5.8±0.4 km·h^{-1}). Matcha had no effect on physiological responses (e.g. heart rate, placebo: 127±14; Matcha: 124±14 beats·min^{-1}, P=0.154), but resulted in lower respiratory exchange ratio (placebo: 0.872±0.040; Matcha: 0.839±0.035) (*P*=0.033), higher fat oxidation by 35±47% (placebo: 0.21±0.08; Matcha: 0.26±0.06 g·min^{-1}) (*P*=0.034), and lower carbohydrate oxidation (placebo: 0.75±0.21; Matcha: 0.60±0.18 g·min^{-1}) (*P*=0.048) during the 30-min moderate-intensity walk. Energy expenditure was similar for both conditions. There was no significant correlation between body fat % and the absolute or relative change in Matcha-induced fat oxidation during exercise. Continuous intake of Matcha green tea effects exercise-induced metabolic responses by enhancing fat oxidation during moderate-intensity exercise in adult females, seemingly independent of body composition.

Introduction

Matcha green tea is typically consumed in the form of finely powdered unfermented tea leaves which are treated with restricted light exposure in the final weeks before harvesting, affecting the polyphenolic catechin and caffeine content (Komes et al. 2010). Compared to common brewed green tea, the ingestion of the finely powdered Matcha tea leaves results in the highest intake of epigallocatechin-3-gallate (EGCG), one of the most abundant and potent bioactive compounds found in green tea (Weiss and Anderton 2003).

The anti-oxidant and anti-inflammatory properties of green tea polyphenols are known to provide various health benefits, including reduction of risk for chronic medical conditions such as type 2 diabetes and obesity (Xing et al. 2019). The polyphenols present in green tea are primarily composed of catechins, a subgroup of the flavonoid flavanols such as; epicatechin, epigallocatechin, epicatechin-3-gallate, and epigallocatechin-3-gallate (EGCG) (Graham 1992). Because of the bioactivity of EGCG (for a review see Singh et al. 2011), both *in-vitro* (e.g. Grube et al. 2018) and *in-vivo* (e.g. Lorenz et al. 2014) experimental studies have examined the mechanisms and effects of the polyphenolic compound EGCG on human health. In addition, many *in-vivo* studies have used manufactured green tea extracts containing multiple catechins and caffeine to examine the effects on human metabolism at rest and during exercise (for a review see

Hodgson et al. 2013). The positive effects on human metabolism with intake of green tea polyphenols may contribute to the reported health benefits (Hanhineva et al. 2010). Understanding of the effectiveness of bioactive compounds in natural food sources in improving human health is key to informing public health policies (e.g. Presseau et al. 2020), and the daily consumption of food and drinks with natural high EGCG content could be recommended as an essential part of a healthy lifestyle.

Observations from *in-vivo* studies in humans on the effects of Matcha are limited (e.g. Dietz et al. 2017, Willems et al. 2018). Dietz et al (2017) reported acute effects of Matcha green tea powder (4 grams) on specific attentional tasks. As far as we know, Willems et al (2018) is the only study that examined the effects of Matcha consumption and exercise-induced metabolic responses. It was observed that the drinking of four cups of Matcha (3 cups the day before and 1 cup on the day of testing, each cup made up with 1 gram of Matcha) enhanced fat oxidation by 18% during brisk walking in females and lowered the respiratory exchange ratio (Willems et al. 2018). The respiratory exchange ratio provides the relative contribution of carbohydrate and fat oxidation to total energy expenditure, and a lower value by an intervention is considered to be beneficial as there is more reliance of fat as a substrate. An increase in exercise-induced fat oxidation by a nutritional ergogenic may affect body composition (e.g. decaffeinated green tea extract, Roberts et al. 2015). While the study by Willems et al (2018) demonstrated short term benefits, the mechanisms for enhanced fat oxidation by green tea extract may differ between short- and long-term intake (Hodgson et al. 2013). In addition, for individuals to respond to supplement-induced fat oxidation during exercise may affect their metabolic flexibility (see Hogdson et al. 2013). No studies have examined the effects of long-term intake of Matcha on the metabolic and physiological responses during moderate-intensity exercise. In addition, resting fat oxidation has been correlated with body mass index after 4-weeks intake of capsinoids (Inoue et al. 2007). However, the relationship between body fat percentage and potential to enhance exercise-induced fat oxidation by green tea intake has not been examined. The primary aim of the present study was to examine the effects of intake of Matcha green tea powder over 3 weeks on fat oxidation during moderate-intensity exercise in females. The secondary aim was to examine whether body fat percentage was related to the Matcha response on exercise-induced fat oxidation.

Methods

Twelve females [age: 28±10 yr (means±SD), height: 163±6 cm, body mass: 69±17 kg, body fat%: 31.8±7.2%, BMI: 25.8±6.0 kg·m^{-2} (range: 21.6-42.5, eight with 18.9 < BMI < 24.9, two 25.0 < BMI < 29.9, and two with a BMI > 30)] volunteered, provided written informed consent after being provided with requirements and purpose of the study, and completed a Physical Activity Readiness Questionnaire Plus. Female participants were healthy and non-smokers. Females were having a regular menstrual cycle (> 3 months), not pregnant or intending to get pregnant during the study, not breast feeding, never had a hysterectomy, and had no known allergy to green tea or green tea extracts. Accepted contraceptive methods were combined pill, diaphragm or intrauterine device. Ethics approval was obtained from the Acadia University Research Ethics Committee (ethical approval code 18-51).

Experimental design, testing and measurements

This study used a randomised placebo-controlled, cross-over experimental design. Female participants were required to visit the exercise physiology laboratory on three occasions, all visits at the same time of day. During the first visit, females arrived at least 3 hours postprandial in the laboratory. Height and body mass were measured using the Healthometer Professional scale (McCook, IL, USA), and body composition using the Maltron Body Composition Analyzer (Model BF-907, Maltron International Ltd., United Kingdom). Females were then seated for 10 min. Subsequently, 2 x 10 min expired air collections were completed while seated and resting using the PARVO Medics TrueONE 2400 Metabolic Measurement System (Sandy, Utah, USA). A 5-min rest time separated the 10-min collections and the lowest oxygen consumption was recorded as one metabolic equivalent (1-MET). To individualize the exercise intensity of the 30-min treadmill walk (TMX428CP Trackmaster® Treadmill, Full Vision Inc, Newton, KS, USA), females completed an incremental walking protocol with walking speeds of 2, 3, 4, 5 and 6 km·h^{-1}, remaining at each speed for 8 min with expired air collection during the last 3 min of each interval at speeds of 2-4 km·h^{-1}, and the last 2 min for 5 and 6 km·h^{-1}. For each participant, the linear relationship between the walking speed and oxygen consumption allowed calculation of the walking speed at the moderate-intensity of 4-MET. Exercise with an intensity between 3 and 6 METs is considered moderate-intensity exercise (Haskell et al. 2007).

For the experimental visits including the 30-min treadmill walk, in both the placebo and Matcha condition, females arrived in the laboratory in a rested, fasted and hydrated state. For the Matcha condition, participants had supplemented with Matcha green tea capsules for 3 weeks, taking 3x1 gram a day equally distributed throughout the day, and were advised to take the capsules with water. Each capsule contained 500 mg of Matcha premium grade powder (OMGTea Ltd, United Kingdom). One gram of Matcha premium grade powder contained 30 mg caffeine and 143 mg total catechins (73 mg EGCG) (composition data provided by OMGTea Ltd, United Kingdom). The last two capsules were taken 90 min before the 30-min treadmill walk, in the fasted state. Color-matched placebo capsules contained cellulose and food colouring. For both the placebo and Matcha conditions, visits were between day 9 and 11 of the menstrual cycle in the follicular phase. Hormonal levels were not measured, and determination of the follicular phase was based on verbal information provided by the female participants. Because the time period between placebo and Matcha testing was aligned with subsequent follicular phases, no wash-out was needed. In preparation for all testing sessions, participants abstained from strenuous and unaccustomed exercise for 48 hours, consumed no alcohol for 24 hours before testing, and no other caffeine-containing products on the day of testing. For the placebo and Matcha conditions, participants were advised to consume a similar diet for 48 hours before testing. Table 1 provides the dietary intake recorded with MyFitnessPal. In the experimental visits, the female participants walked for 30-min at the walking speed which provided an exercise intensity of 4-METs. During the walk, expired air was collected and heart rate was recorded (Polar 610i HR monitor, Polar Electro OY, Kempele, Finland). Rates of whole-body fat and carbohydrate oxidation were calculated using equations from Frayn (1983), and energy expenditure was calculated using the equation from Weir (1949).

Statistical analysis

Analyses were completed using Graphpad Prism version 5.00 for Window (GraphPad Software, San Diego, California, USA). Data normality was assessed with D'Agostino-Pearson normality tests. Paired sample t-tests were conducted to compare parameter values between placebo and Matcha conditions for normally distributed data, otherwise data was tested with the Mann-Whitney test. As the primary aim of the study was to examine effects of Matcha on exercise-induced fat oxidation, pearson correlation coefficient was calculated and tested for relationships between body fat % and absolute and percentual changes of exercise-induced fat oxidation. Data are provided as mean±SD and 95% confidence intervals. Statistical significance was accepted at $P<0.05$. When significance was obtained, Cohen's d effect sizes were calculated (small: $0.2 \leq d < 0.5$; moderate: $0.5 \leq d \leq 0.79$; large: $d \geq 0.8$).

Results

Physiological responses

During the placebo and Matcha condition, the walking speed for the 30-min treadmill walk was individualized to a moderate-exercise intensity of 4-MET using the oxygen consumption at rest (1-MET: 3.73±0.38 mL·kg^{-1}·min^{-1} (mean±SD), 95% CI [3.49, 3.97]). In the placebo and Matcha condition, the oxygen consumption during the 30-min treadmill walk was 14.95±1.91 mL·kg^{-1}·min^{-1} (95% CI [13.74, 16.16]) and 14.94±2.25 mL·kg^{-1}·min^{-1} (95% CI [13.51, 16.37]), with no difference (Mann-Whitney, P=0.75). The MET values for placebo and Matcha were 4.01±0.34 (range 3.77 to 4.74, 95% CI [3.80, 4.22]) and 4.00±0.47 (range 3.40 to 5.05, 95% CI [3.70, 4.30]) with no difference (P=0.91), confirming matched moderate-intensity exercise for both conditions. In addition, heart rates during the 30-min treadmill walk were not different (placebo: 127±14 beats·min^{-1}, 95% CI [118, 136], Matcha: 124±14 beats·min^{-1}, 95% CI [116, 133], P=0.15). There were also no differences for carbon dioxide production between conditions during the 30-min treadmill walk (placebo: 13.04±1.75 mL·kg^{-1}·min^{-1}, 95% CI [11.93, 14.15]); Matcha: 12.58±2.17 mL·kg^{-1}·min^{-1}; 95% CI [11.20, 13.96]) (Mann-Whitney, P=0.67). There were no differences for energy expenditure between conditions during the 30-min treadmill walk (placebo: 146±20 kcal (95% CI [133, 158]; Matcha: 144±019 (95% CI [132, 156]) (P=0.55).

Metabolic responses

Whole-body fat oxidation during the 30-min treadmill walk was 35±47% higher (P=0.034) in the Matcha condition with a moderate effect size (d=0.71) (placebo: 0.21±0.08 $g{\cdot}min^{-1}$, 95% CI [0.16, 0.26]; Matcha: 0.26±0.06 $g{\cdot}min^{-1}$, 95% CI [0.22, 0.30]). Eight participants had higher values of whole-body fat oxidation with intake of Matcha (Fig. 1A). There was no significant correlation between body fat % and the absolute change (r^2=0.0215, P=0.68) and percentual change (r^2 =0.0033, P=0.86) in whole-body fat oxidation during the 30-min treadmill walk (Fig. 2AB).

Whole-body carbohydrate oxidation during the 30-min treadmill walk was 16±30% lower (P=0.048) in the Matcha condition with a moderate effect size (d=-0.79) (placebo: 0.75±0.21 $g{\cdot}min^{-1}$, 95% CI [0.62, 0.88]; Matcha: 0.60±0.17 $g{\cdot}min^{-1}$, 95% CI [0.49, 0.71]). Nine participants had lower values of whole-body carbohydrate oxidation with intake of Matcha (Fig. 1B).

The respiratory exchange ratio during the 30-min treadmill walk (Fig. 3) for the group was 0.033±0.047 lower (P=0.033) in the Matcha condition with a large effect size (d=-0.85) (placebo: 0.872±0.040 (95% CI [0.847, 0.898]; Matcha: 0.839±0.035 (95% CI [0.817, 0.862]).

Discussion

Matcha green tea has gained popularity among the general population possibly due to the potential health benefits, however long-term studies on intake of Matcha green tea and health remain absent. The present study provides novel observations of the long-term effects (i.e. 3 weeks) of Matcha green tea capsule intake on substrate oxidation during moderate-intensity walking in females. Previous research on green tea consumption has primarily examined the effects of manufactured green tea extracts on substrate oxidation during exercise (for a review, see Hodgson et al. 2013). The Matcha green tea capsules in the present study did not contain extracts, but Matcha green tea powder, as is normally used in the preparation of Matcha green tea drinks. In the present study, the group data showed that exercise-induced fat oxidation was significantly enhanced by 35%, albeit with a moderate effect size (d=0.71). There were no relationships between the body fat percentage and the absolute or percentual changes in exercise-induced fat oxidation by Matcha green tea.

Recent studies have detailed the complex composition of Matcha green tea, with particular focus on the link between compositional elements and anti-oxidant capacity (Jakubczyk et al. 2020; Koláčková et al. 2020). However, our understanding of the potential of Matcha green tea requires knowledge on the plasma bioavailability of Matcha-derived metabolites as it is likely those will be linked with adaptive changes in metabolic function over time. For ECGC, for example, plasma concentrations depend on whether intake is in a fasted state or in combination with food (Andreu-Fernández et al. 2020). In the present study, participants were instructed to take the Matcha green tea capsules throughout the day without specific instructions, the final intake of Matcha green tea capsules, however, were in a fasted state.

A study by Willems et al (2018) observed an increase in exercise-induced fat oxidation by 18% following short term intake consisting of 3 cups of Matcha on the day before and one cup of Matcha on the day of testing. In the present study, the exercise-induced fat oxidation after 3-weeks intake of Matcha green tea capsules was almost double, by 35%. However, although the present study and the study by Willems et al (2018) were similar in experimental approach, the studies were not performed in the same testing environment. Though this study provides novel findings on long term impacts of Matcha supplementation, other studies examining long term intake of green tea extract have reported inconsistent observations. Eichenberger et al (2009) observed no effects on fat oxidation during 2 hours of exercise at 50%Wmax after intake of 160 mg$\cdot$day^{-1} total catechins over three weeks, although a much lower dosage of total catechins was used than in the present study (i.e. 462 mg$\cdot$day^{-1} and 154 mg on testing day). Though we are not aware of studies that examined just the effects of 3-weeks daily intake of green tea on exercise-induced metabolic responses, a study by Ota et al (2005) supplemented daily for 2 months with green tea beverage containing antioxidants and 570 mg of catechins in combination with an exercise program. Authors observed an increase in exercise-induced fat oxidation of 32% during treadmill walking at 5km$\cdot$hr^{-1}, a percentual increase corresponding to our observations (i.e. 35%). Although the present study did not incorporate structured exercise in combination with Matcha green tea intake, there are additional methodological differences between Ota et al (2005) and the present study. Intake duration, composition of green tea intake, non-personalized exercise intensity, could have contributed as well to the similarity for observation for exercise-induced fat oxidation by Ota et al (2005) compared with the present study. Future work should address the effects of intake duration of Matcha green tea capsules in combination with the plasma bioavailability of Matcha green tea components and Matcha-derived metabolites. The bioavailability of Matcha components and Matcha-derived metabolites is not known.

Exercise-induced fat oxidation involves many steps, starting with lipolysis in adipocytes (Duncan et al. 2007). Caffeine present in Matcha green tea may have contributed to enhanced lipolysis, however some studies have shown that enhanced lipolysis does not necessarily enhance fat oxidation, at least during rest (Arciero et al. 1995). The effects of Matcha green tea on fat oxidation at rest is not known, though potential effects of Matcha green tea on fat oxidation at rest are probably more important to consider for weight management than exercise-induced responses in the present study. It would be of interest to perform studies on the effects of Matcha green tea at rest, as well as during longer duration walks, and in combination with a structured exercise program. Our experimental approach did not allow for examination of potential mechanisms for the enhanced exercise-induced fat oxidation following 3-weeks intake of Matcha green tea. In addition, we do not know whether the observed enhanced exercise-induced fat oxidation was due to the intake on the day of testing. However, no effect of a single dose intake on exercise-induced fat oxidation was observed by Randell et al (2013) (560 mg total catechins and 120 mg caffeine) and Sugita et al (2016) (780 mg of total catechins). The amount of total catechins in these studies (Randell et al. 2013, Sugita et al. 2016) was lower than the final intake (154 mg total catechins) on the day of testing in the present study. Furthermore, future work should consider repeated measurements of Matcha-induced fat oxidation during treadmill walking as large day-to-day variability in peak fat oxidation (within subject coefficient of variation of 21%) has been reported (Chrzanowski-Smith et al. 2020). In the present study, the eight participants that had higher fat oxidation with Matcha had changes >24%. However, the within subject coefficient of variation for fat oxidation during a 30-min treadmill walk is not known.

One of the limitations of the present study was that the female participants had a broad range of BMI values, but most were within a narrow range of normal weight. Inoue et al (2007) observed in 29 individuals that 4-weeks intake of capsinoids affected resting fat oxidation and correlated with BMI. Our narrow range of BMI values may be why no significant correlations were observed between body fat percentage and exercise-induced fat oxidation. Future studies should examine the effects of Matcha on fat oxidation in rest and exercise for females with a broad range of BF%s. Another limitation was that we did not control dietary intake and physical activity levels between follicular phases of menstrual cycle at which female participants were tested for placebo and Matcha conditions.

The results of this study provide evidence that long term intake of Matcha green tea powder does affect the metabolic response to moderate-intensity exercise by enhancing fat oxidation and lowering carbohydrate oxidation. Such metabolic adaptations are normally obtained through endurance exercise training. Therefore, future studies should address whether Matcha green tea has performance-enhancing effects, to determine what implications this could have for recommendation of Matcha intake by athletes.

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Declaration of interest

OMGTea Ltd, United Kingdom provided the Matcha capsules but had no role in any aspect of the study and manuscript.

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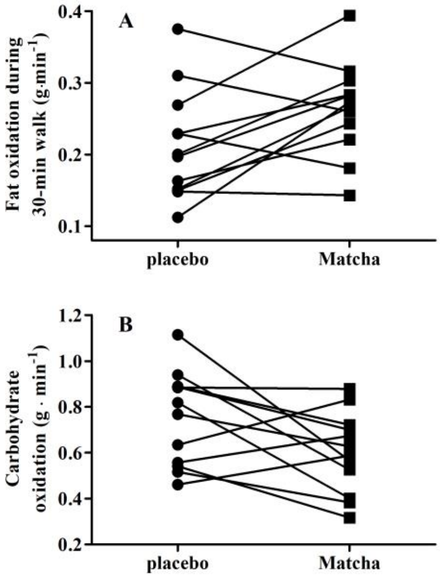

Figure 1. Individual responses of fat oxidation (A) and carbohydrate oxidation (B) during 30-min of moderate-intensity walking exercise in the placebo and Matcha condition. Data from 12 female participants.

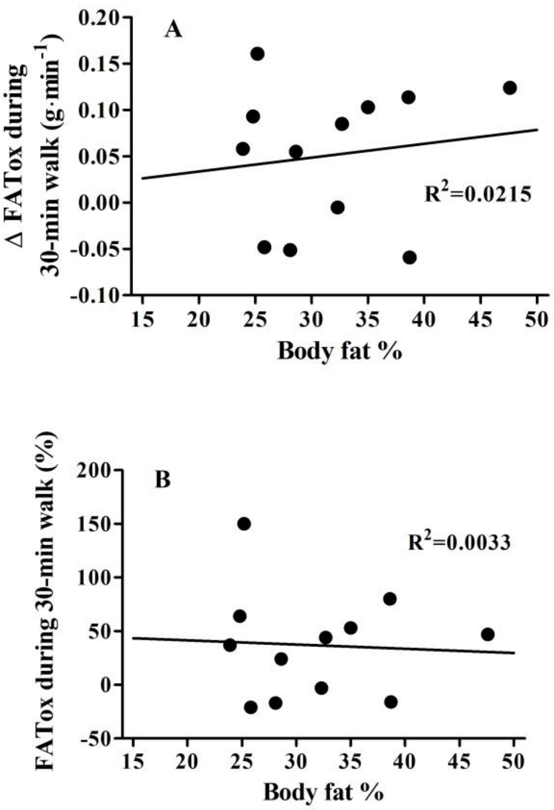

Figure 2. Relationship between body fat % and the absolute change (A) and percentual change (B) in whole-body fat oxidation during the 30-min treadmill walk. Correlations were not significant.

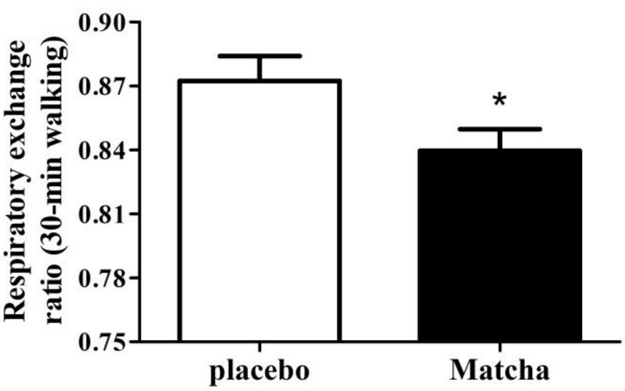

Figure 3. Respiratory exchange ratio during 30-min of moderate-intensity walking exercise. Data reported as mean ± SD from 12 female participants. * indicates difference with placebo (p<0.05).

Table 1. Daily dietary and energy intake before each experimental visit.

Carbohydrate (g)	218±65
Fat (g)	73±19
Protein (g)	93±35
Fiber (g)	24±4
Sugar (g)	84±33
Total energy intake (kcal)	1843±346