

1 Matcha green tea drinks enhance fat oxidation during brisk walking in females

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3 Authors: Mark Elisabeth Theodorus Willems¹, Mehmet Akif Şahin², Matthew David Cook³

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5 Affiliation: ¹Department of Sport and Exercise Sciences, University of Chichester,
6 College Lane, Chichester, PO19 6PE, United Kingdom

7 ²Department of Nutrition and Dietetics, Faculty of Health Sciences, Hacettepe
8 University, Sıhhiye, Ankara, Turkey

9 ³University of Worcester, Institute of Sport and Exercise Sciences, Henwick
10 Grove, Worcester, WR2 6AJ, United Kingdom

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14 Corresponding author: Professor Mark Willems

15 Phone: +44 (0)1243 816468

16 Email: m.willems@chi.ac.uk

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19 **ABSTRACT**

20 Intake of the catechin epigallocatechin gallate and caffeine has been shown to enhance
21 exercise-induced fat oxidation. Matcha green tea powder contains catechins and caffeine and
22 is consumed as a drink. We examined the effect of Matcha green tea drinks on metabolic,
23 physiological and perceived intensity responses during brisk walking. Thirteen females (age:
24 27±8 yr, body mass: 65±7 kg, height: 166±6 cm) volunteered. Resting metabolic equivalent
25 (1-MET) was measured using Douglas bags (1-MET: 3.4±0.3 ml·kg⁻¹·min⁻¹). Participants

26 completed an incremental walking protocol to establish the relationship between walking
27 speed and oxygen uptake and individualize the walking speed at 5- or 6-MET. A randomized
28 cross-over design was used with participants tested between day 9 and 11 of the menstrual
29 cycle (follicular phase). Participants consumed 3 drinks (each drink made with 1 gram of
30 Matcha premium grade, OMGTea Ltd UK) the day before, and 1 drink 2 hours before the 30-
31 min walk at 5- (n=10) or 6-METs (walking speed: $5.8\pm 0.4 \text{ km}\cdot\text{h}^{-1}$) with responses measured
32 at 8-10, 18-20 and 28-30 min. Matcha had no effect on physiological and perceived intensity
33 responses. Matcha resulted in lower respiratory exchange ratio (control: 0.84 ± 0.04 ; Matcha:
34 0.82 ± 0.04) ($P < 0.01$) and enhanced fat oxidation during a 30-min brisk walk (control:
35 0.31 ± 0.10 ; Matcha: $0.35\pm 0.11 \text{ g}\cdot\text{min}^{-1}$) ($P < 0.01$). Matcha green tea drinking can enhance
36 exercise-induced fat oxidation in females. However, when regular brisk walking with 30-min
37 bouts is being undertaken as part of a weight loss program, the metabolic effects of Matcha
38 should not be overstated.

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40 Key words: catechins; health promotion; treadmill walking

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42 INTRODUCTION

43 The polyphenol composition of green tea leaves is characterised by the flavonoid catechins
44 i.e. catechin gallate, epicatechin gallate, epigallocatechin gallate, epicatechin epigallate,
45 gallocatechin and gallocatechin gallate (Xu et al., 2004). Due to the processing methods of
46 the leaves, green tea has a high content of catechins compared to oolong and black tea. The
47 green tea components contribute to the antioxidant capacity (Peluso and Serafini, 2017), with
48 epigallocatechin gallate (EGCG) considered the bioactive compound (Khan et al., 2006). The
49 antioxidant capacity of green tea likely contributed to reported health benefits by regular
50 intake of green tea such as a reduced risk for some cancers (Guo et al., 2017) and

51 cardiovascular and ischemic-related diseases (Pang et al., 2016). Green tea has also been
52 implicated in body-weight management (Janssens et al., 2016) by promoting fat oxidation.

53 EGCG is considered the bioactive compound to promote fat oxidation (Kapoor et al.,
54 2017). Chronic intake of green tea extract enhanced fat oxidation during swimming (Murase
55 et al., 2005) and running in mice (Murase et al., 2006). In addition, EGCG has been shown to
56 reduce body weight in diet-induced obese mice (Lee et al., 2009), indicative of a change in
57 energy balance. In humans, observations on fat oxidation during exercise with short term
58 intake of green tea or EGCG intake were inconsistent. Randell et al (2013) did not observe
59 enhanced fat oxidation during cycling at 50% of maximum power in men after 1 and 7-day
60 intake with no intake on the day of testing. During 2 hr of cycling at 50% of maximum
61 power, green tea extract had no effect on the respiratory exchange ratio (Eichenberger et al.,
62 2009). However, Venables et al (2008) showed enhanced fat oxidation with green tea extract
63 during 30-min cycling exercise at 60% of maximum oxygen uptake in men with the
64 supplement taken on the day before and on the day 1 hr before testing.

65 In human studies on exercise-induced fat oxidation, the delivery mode of green tea
66 supplementation has been in capsule form. No studies examined the effect of *traditional*
67 brewed green tea drinks with leaves on fat oxidation during exercise. Matcha green tea
68 powder contains catechins and caffeine and when it is consumed as a drink it ensures an oral
69 intake of all the green tea leaf components. In addition, the process of powdering green tea
70 leaves adds to the potential beneficial effects of Matcha (Fujioka et al., 2016). Therefore, the
71 intake of green tea components by Matcha drinking may be higher than brewed green tea
72 without the leaf consumption and guarantees intake of water-soluble and water-insoluble
73 parts (Xu et al., 2016). In mice fed a high-fat diet, Matcha intake promoted lipid metabolism
74 (Xu et al., 2016). No studies have examined the effect of Matcha drinks on substrate
75 oxidation during exercise in humans.

76 Regular exercise that is performed to obtain health benefits is recommended to have an
77 exercise intensity between 3 and 6 metabolic equivalents i.e. 3 to 6 times the resting energy
78 expenditure according to physical activity guidelines (Haskell et al., 2007). Walking is a
79 popular physical activity (Paul et al., 2015) and for most people brisk walking meets intensity
80 requirements (Fitzsimons et al., 2005). No studies have examined the effect of a nutritional
81 ergogenic aid on substrate oxidation during brisk walking. Dietary changes and regular
82 exercise may result in a negative energy balance and reduce body weight and body fat.
83 Nutritional ergogenic aids could enhance these effects (Arent et al., 2017). For example, a
84 decaffeinated green tea extract was associated with a decrease in body fat and enhanced fat
85 oxidation during cycling (Roberts et al., 2015). Fat oxidation during brisk walking with green
86 tea drinking has not been examined. According to Weiss and Anderton (2003), the
87 concentration of EGCG from drinking Matcha green tea is at least 3 times the highest intake
88 of EGCG compared to other green teas.

89 Therefore, the aim of the present study was to examine the effect of the consumption of
90 Matcha on substrate oxidation, physiological responses and perceived intensity during brisk
91 walking in females.

92

93 **METHODS**

94

95 *Participants*

96 A randomised, cross-over experimental design was used. Thirteen recreationally active
97 healthy women [age: 27±8 yr, height: 166±6 cm, body mass: 65±7 kg, BMI: 23.5±2.6 kg·m⁻²
98 (range: 19.1-30.2 eleven with 18.9 < BMI < 24.9), means±SD] volunteered and provided
99 written informed consent. All participants were non-smokers. Accepted contraceptive
100 methods were combined pill, diaphragm or intrauterine device. Ethics approval was obtained

101 from the University of Chichester Research Ethics Committee (ethical approval code
102 1617_24).

103

104 *Experimental design and preliminary testing*

105 Participants visited the laboratory on three occasions between 9 and 11 o'clock in the
106 morning. During the first visit, height and body mass were measured. Subsequently,
107 participants rested in a chair for 30 minutes with 2 x 10 min expired air collections separated
108 by 5 minutes using the Douglas bag technique to determine the oxygen consumption at rest
109 (i.e. the one metabolic equivalent 1-MET) with the lowest value taken as the 1-MET.

110 Subsequently, participants completed an incremental-intensity walking test on a treadmill
111 (HP Cosmos Pulsar Bodycare Products UK) with 5 x 8-min stages. Starting speed was 2
112 km·h⁻¹ with a stage increment of 1 km·h⁻¹ until speed reached 6 km·h⁻¹. During each stage,
113 expired air was collected in the last 3 minutes. The incremental-intensity walking test was
114 performed to determine the linear relationship between walking speed and oxygen
115 consumption expressed as the metabolic equivalent. For each individual, the linear
116 relationship between walking speed and metabolic equivalent ($r^2 = 0.9353 \pm 0.0383$) was used
117 to calculate the walking speed at 5- or 6-METs (i.e. moderate intensity exercise). For visits
118 two and three with either having Matcha or no supplement, participants were tested in the
119 follicular phase of the menstrual cycle (i.e. 9-11 days following start of the menstruation).
120 Hormonal levels were not measured and determination of the follicular phase was based on
121 verbal information provided by the participants. In preparation for all testing sessions,
122 participants abstained from strenuous and unaccustomed exercise for 48 hours, no alcohol for
123 24 hours before testing and no other caffeine-containing products on the day of testing.

124

125 *Exercise testing and supplementation*

126 For the Matcha condition, participants consumed 3 x 1 gram of Matcha powder (Matcha
127 premium grade OMGTea Ltd, UK) mixed with water at meal times on the day before testing.
128 On the day of testing, participants consumed 1 gram of Matcha with water two hours before
129 arrival and arrived following an overnight fast. The supplementation strategy was based on
130 Venables et al (2008). One gram of Matcha premium grade contains 143 mg total catechins
131 and 30 mg caffeine (composition data from OMGTea Ltd, UK). Before visit two, participants
132 recorded their dietary intake for 48 hours and were instructed to match the same dietary
133 intake 48 hours before arrival for visit three. The intake before visit three was recorded on a
134 new food diary. Carbohydrate fat and protein intake and total energy intake (kJ) were
135 quantified with Nutritics (Nutritics LTD Dublin Ireland).

136 Participants walked on a treadmill at a speed to elicit 5- or 6-MET for 30 minutes with
137 expired air collected from 8 to 10, 18 to 20 and 28 to 30 minutes with recording of heart rate
138 (Polar Vantage NV Polar Electro Oy Kempele Finland) and rating of perceived exertion
139 (Borg 6 to 20 scale) (Borg, 1982). Expired air was analyzed with a three-point calibrated gas
140 analyser (Servomex Series 1400 gas analyser Servomex Crowborough United Kingdom) and
141 volume measured (Harvard Apparatus Ltd. Edenbridge United Kingdom). Gas volumes were
142 corrected to standard temperature and pressure and dry gas conditions (STPD) and calculated
143 using Haldane transformation with consideration of inspired fractions of oxygen and carbon
144 dioxide at the time of expired air collections. Rates of whole body fat and carbohydrate
145 oxidation were calculated with equations 1 and 2 from Jeukendrup and Wallis (2005) and the
146 assumption of negligible protein oxidation:

147

$$148 \quad \textit{Fat oxidation} (g \cdot \textit{min}^{-1}) = 1.695 \times \dot{V}O_2 - 1.701 \times \dot{V}CO_2 \quad (1)$$

149

$$150 \quad \textit{Carbohydrate oxidation} (g \cdot \textit{min}^{-1}) = 4.210 \times \dot{V}CO_2 - 2.962 \times \dot{V}O_2 \quad (2)$$

151

152 *Statistical analysis*

153 Analyses were completed using Graphpad Prism version 5.00 for Window (GraphPad
154 Software, San Diego, California, USA). A power analysis indicated that a sample size of 13
155 was required to allow a detection of a 15% increase in fat oxidation from a baseline value
156 of fat oxidation of $0.25 \text{ g} \cdot \text{min}^{-1}$ (Dasilva et al., 2011) with a SD of 0.07 for both groups with
157 a high statistical power ($1-\beta = 0.80$: $0.05 = \alpha$ level). A two-way ANOVA was used to
158 analyse oxygen consumption, carbon dioxide production and substrate oxidation for time
159 effects with post-hoc paired samples t-tests. Means were calculated for all parameter values
160 collected from 8 to 10, 18 to 20 and 28 to 30 minutes during the 30-min treadmill walk. Data
161 normality was assessed with D'Agostino-Pearson normality tests. Paired samples t-tests were
162 conducted to compare parameter values between control and Matcha conditions. Statistical
163 significance was accepted at $P < 0.05$.

164

165 **RESULTS**

166 The 1-MET was $3.4 \pm 0.3 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, means \pm SD range: 2.9–3.8 $\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). There
167 were no differences in absolute values of daily dietary intake parameters (i.e. carbohydrate,
168 control: $182 \pm 71 \text{ g}$, Matcha: $157 \pm 49 \text{ g}$; fat, control: $67 \pm 23 \text{ g}$, Matcha: $72 \pm 22 \text{ g}$, protein,
169 control: $70 \pm 43 \text{ g}$, Matcha: $75 \pm 35 \text{ g}$; total energy intake, control: $6697 \pm 2302 \text{ kJ}$, Matcha:
170 $6604 \pm 1796 \text{ kJ}$). Participants were low caffeine consumers (control: $48 \pm 57 \text{ mg}$, Matcha:
171 $40 \pm 45 \text{ mg}$).

172

173 *Matcha vs control*174 *Physiological responses and rating of perceived exertion*

175 Participants walked in the control and Matcha condition at an individualized walking speed
176 for 30 minutes with an exercise intensity of 5- or 6-MET. Ten participants walked at 5-MET
177 (walking speed: $5.7 \pm 0.4 \text{ km} \cdot \text{h}^{-1}$) to avoid those participants willing to jog at the treadmill
178 speed of 6-MET. For the three participants walking at 6-MET, the walking speed was 6.0 ± 0.5
179 $\text{km} \cdot \text{h}^{-1}$. Oxygen uptake (control: 18.1 ± 2.8 ; Matcha: $18.1 \pm 2.8 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), minute
180 ventilation (control: 25.9 ± 3.3 ; Matcha: $25.2 \pm 3.3 \text{ L} \cdot \text{min}^{-1}$) and heart rate (control: 119 ± 18 ;
181 Matcha: $120 \pm 17 \text{ beats} \cdot \text{min}^{-1}$) were not different. Figure 1 shows the oxygen (A) and carbon
182 dioxide (B) values over time with no time effects. Rating of perceived exertion during
183 walking at an intensity of 5- or 6-MET was not different compared to the Matcha condition
184 (control: 11 ± 1 ; Matcha: 12 ± 1).

185

186 *Respiratory exchange ratio and substrate oxidation*

187 Figure 2 shows substrate oxidation as a function of time during the 30-min walk. Time effects
188 for carbohydrate oxidation showed a trend to be lower at 28-30 min compared to 8-10 min in
189 the placebo condition ($P = 0.07$) and lower in the Matcha condition ($P = 0.01$) (Figure 2A).
190 In the placebo condition, there was a trend for fat oxidation at 28-30 min to be higher than fat
191 oxidation at 8-10 min ($P = 0.06$) (Figure 2B). Fat oxidation at 28-30 min was higher than fat
192 oxidation at 18-20 min ($P = 0.04$) (Figure 2B). In the Matcha condition, fat oxidation at 28-
193 30 min was higher than fat oxidation at 8-10 min ($P = 0.04$) and 18-20 min ($P < 0.01$) (Figure
194 2B). The respiratory exchange ratio was 0.02 units lower in the Matcha condition (Figure 3)
195 indicating a larger contribution of fat as an energy source. In the Matcha condition,
196 carbohydrate oxidation was lower (control: 0.69 ± 0.18 ; Matcha: $0.56 \pm 0.20 \text{ g} \cdot \text{min}^{-1}$, $P < 0.05$)
197 (Figure 4) and fat oxidation was higher (control: 0.31 ± 0.10 ; Matcha: $0.35 \pm 0.11 \text{ g} \cdot \text{min}^{-1}$, $P <$
198 0.05) (Figure 5) over the full 30-min of the walk. The individual observations on
199 carbohydrate (Figure 4) (i.e. 11 participants lower values) and fat oxidation (Figure 5) (i.e. 10

200 participants higher values) seem to indicate that the absolute changes in substrate oxidation
201 were not related to the values observed in the control condition.

202

203 **4. Discussion**

204 With Matcha green tea drinking, polyphenol and caffeine intake occurs by whole
205 consumption of the powdered green tea leaves. Previous studies on the effects of green tea on
206 exercise responses used capsulated intake of green tea extract or EGCG (Dean et al., 2009;
207 Eichenberger et al., 2009; Martin et al., 2014; Venables et al., 2008) or enriched canned
208 drinks with green tea catechins and caffeine (Hodgson et al., 2013; Randell et al., 2013). We
209 are not aware of studies on the effects on exercise responses by traditionally brewed green tea
210 drinking by which the intake of catechins and caffeine is not by the consumption of green
211 tea leaves. Females in our study consumed 4 normal cups of Matcha green tea in 24 hours.
212 We observed an enhanced fat oxidation with Matcha green tea drinking during 30 min of
213 brisk walking in females. Our observation is similar to that in a study by Venables et al
214 (2008) with effects of green tea extract in enhancing fat oxidation during 30-min cycling at
215 60% VO_{2max} in males. In the study by Venables et al (2008) participants were dosed 2 times
216 the day before and 1 h before testing with a green tea extract that contained in total 890 mg of
217 polyphenols and 408 mg EGCG but was without caffeine. EGCG intake in the present study
218 with 4 cups of Matcha green tea over a 24 hour period amounted to a total intake of 292 mg
219 EGCG and 120 mg caffeine. The final intake in the present study provided 73 mg of EGCG
220 and 30 mg of caffeine, whereas the dose in Venables et al (2008) was 86% higher, i.e. 136
221 mg EGCG but no caffeine. It is possible that the components of Matcha provide a synergistic
222 effect on exercise-induced fat oxidation. A comparison with other studies on the effects of the
223 intake of green tea extract or EGCG is problematic due to differences in dosing strategy i.e.
224 amounts, intake duration, intake composition, training status of participants and fed or fasted

225 status testing. For example, Martin et al (2014) did not observe an effect of green tea during
226 exercise but as participants were provided with a standardized breakfast 90 min before the
227 exercise test, this may have affected the observed substrate oxidation during the exercise.
228 Eichenberger et al (2009) examined green tea extract effects during 2 hr cycling in endurance
229 trained men cycling > 6 hours per week, and the absence of a green tea effect could be due to
230 training status of the subjects. However, observations of enhanced fat oxidation in the present
231 study seem to indicate that it is possible to have by a cup of Matcha an intake of essential
232 catechins, e.g. EGCG, and caffeine, in amounts that cannot be achieved with a cup of
233 *traditional* brewed green tea. The caffeine intake in our study was very small: a total of 120
234 mg over 24 hours. An acute intake of 6 mg/kg of body mass of caffeine reduced the
235 respiratory exchange ratio during exercise (Cruz et al., 2015). In the present study, the intake
236 of 30 mg of caffeine by Matcha on the day of testing was less than 0.5 mg/kg of body mass,
237 an amount for which there is no evidence for affecting exercise-induced fat oxidation.

238 EGCG is considered the bioactive compound in green tea acting by inhibition of
239 catechol-*O*-methyltransferase (i.e. COMT). In general, inhibition of COMT would reduce the
240 breakdown of catecholamines and promote an internal cellular environment for enhanced fat
241 oxidation. However, a study by Hodgson et al (2013) observed that 8-day intake of green tea
242 extract did not enhance adrenergic stimulation during exercise. Therefore, due to the absence
243 of differences in the adrenergic system with intake of green tea extract *in-vivo*, the inhibition
244 of COMT may not be the cause for the observed enhanced fat oxidation. Alternatively,
245 EGCG has been linked with activation of the transient receptor potential vanilloid type 1 (i.e.
246 TRPV1) and eNOS activation (Guo et al., 2015). In addition, TRPV1 is also linked with
247 eNOS activation and NO production (Yu et al., 2017). Activation of eNOS would result in
248 increased production of nitric oxide and enhanced blood flow with improved delivery of free
249 fatty acids. Interestingly, activation of TRPV1 was associated with enhanced fat oxidation in

250 male mice by capsiate supplementation (Haramizu et al., 2006), possible by contributing to
251 functional sympatholysis during exercise (Ives et al., 2017). However, differences in both the
252 metabolite profiles in bioavailability of plasma catechins in animal studies and the amount of
253 catechins used to examine effects in endothelial cells (Guo et al., 2015), warrants caution to
254 generalize from these findings to observations with green tea extracts or powder in humans.

255 The exercise modality in the present study was walking in females with an intensity
256 known to provide health benefits. In addition, females were tested during the follicular phase
257 but evidence on hormonal effects on fat oxidation in females during exercise is inconsistent
258 (Kanaley et al., 1992; Vaiksaar et al., 2011; Wenz et al., 1997). In addition, we had no
259 objective measurement of the follicular phase by hormonal observations and cannot exclude
260 that the variation in individual responses may be due to intra-individual differences in
261 hormonal levels. We also did not control the physical activity status of the participants.
262 Future studies may want to examine the effects of Matcha green tea drinking for longer
263 duration and combined with an exercise intervention in normal weight, overweight, and obese
264 individuals. In addition, future studies on nutritional interventions that enhance fat oxidation
265 during exercise should address the causality of high responders. It is of interest also to
266 explore in future studies whether enhanced fat oxidation by Matcha green tea would affect
267 insulin sensitivity. A study by Robinson et al (2015) observed that maximal fat oxidation
268 during exercise was associated with insulin sensitivity.

269 In summary, Matcha green tea drinking, just 4 cups in 24 hours enhanced fat oxidation
270 during brisk walking in healthy females. The composition of Matcha green tea leaves is
271 sufficient for habitual Matcha drinking to provide beneficial metabolic responses during brisk
272 walking. However, when regular moderate intensity exercise is undertaken as part of a weight
273 loss program, the effects of Matcha should not be overstated.

274

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277

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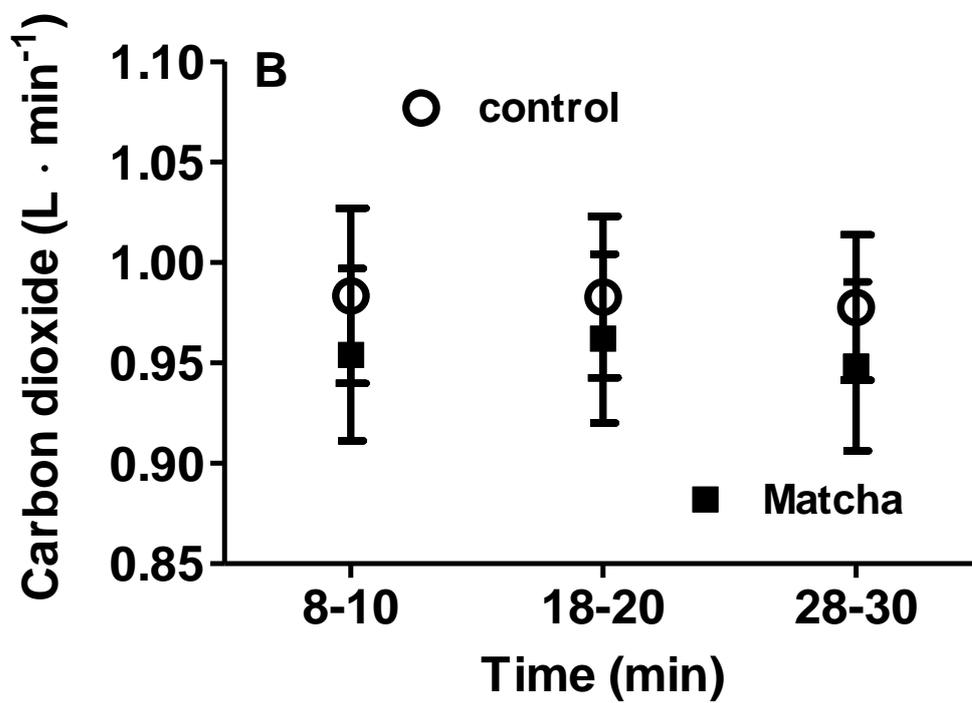
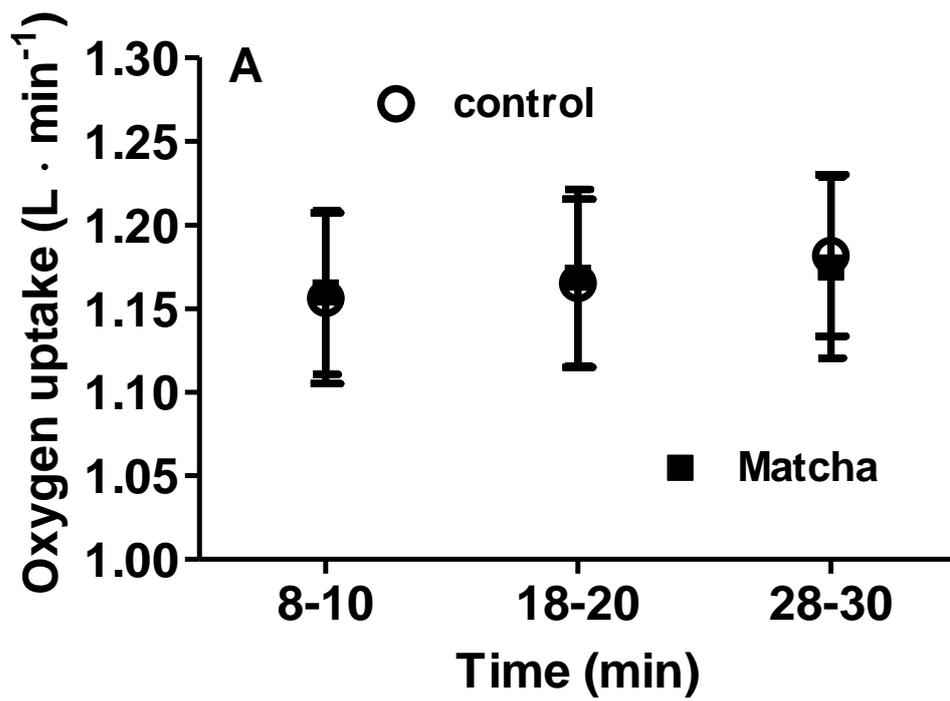
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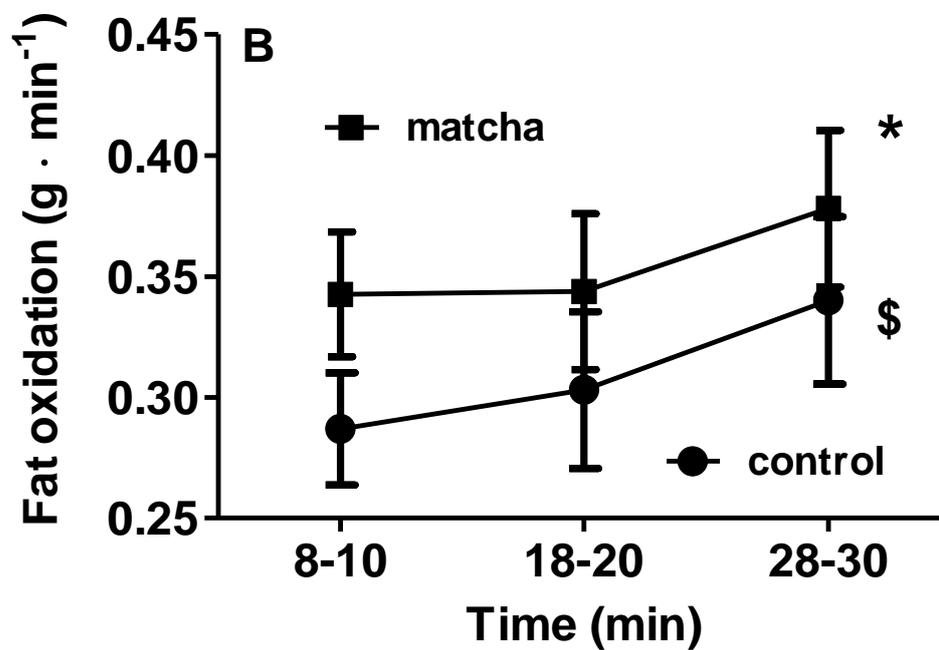
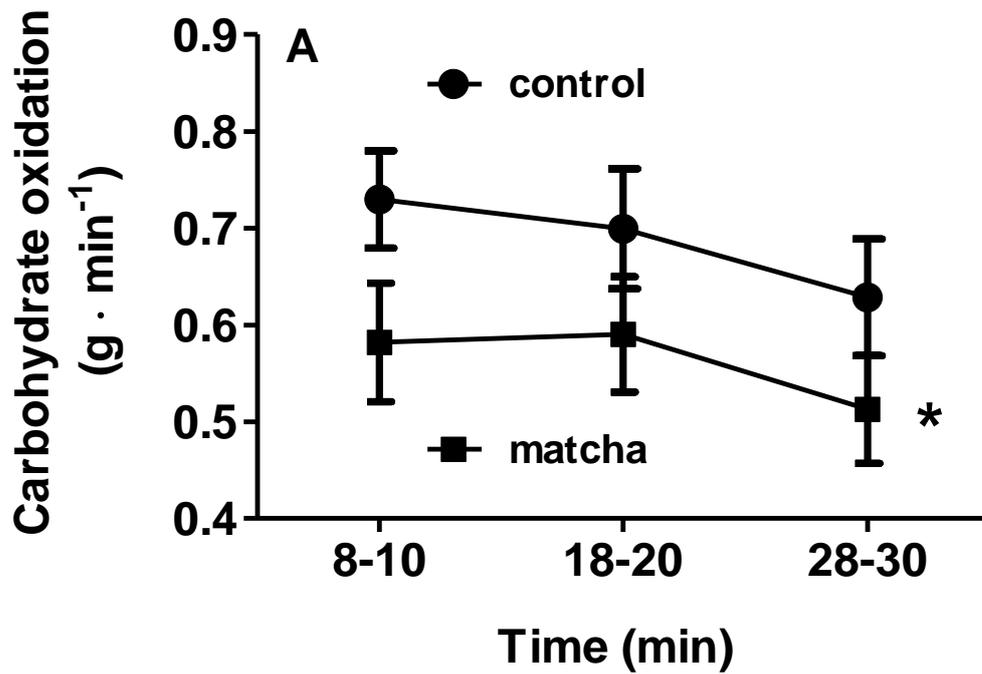
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400 **Figure 1.** Oxygen consumption (A) and carbon dioxide production (B) at different time
401 points during the 30-min treadmill walk individualized walking speeds at 5-MET (10
402 participants) or 6-MET (3 participants). Data are presented as mean \pm SEM.
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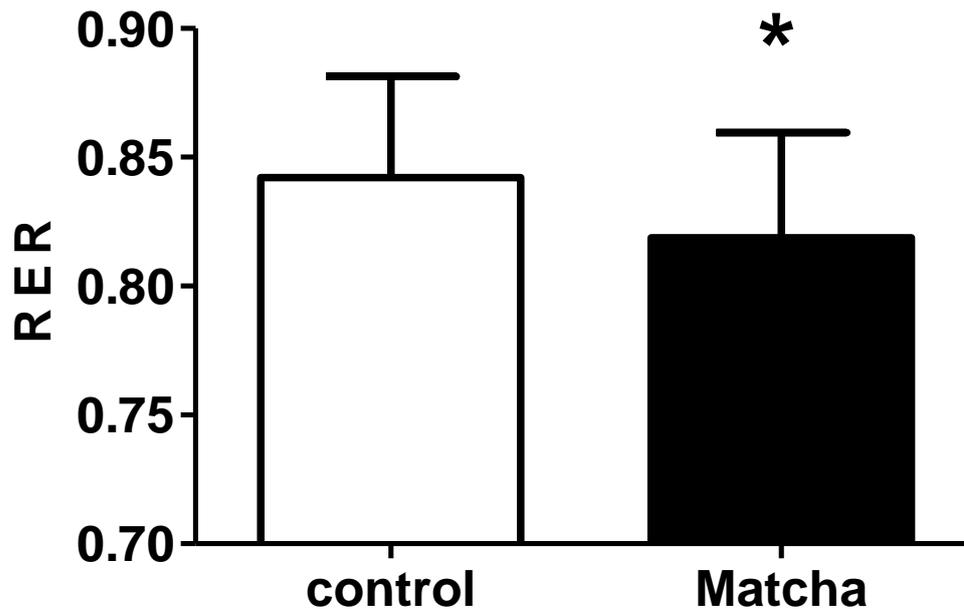


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405 **Figure 2.** Carbohydrate oxidation (A) and fat oxidation (B) in the control and Matcha
 406 condition at different time points during a 30-min treadmill walk at individualized walking
 407 speeds at 5-MET (10 participants) or 6-MET (3 participants). A, * different between time

408 points 28-30 and 8-10 min in the Matcha condition. B, \$ different between time points 28-30
409 min and 8-10 in the control condition and * different between 28-30 min and 8-10 min and
410 18-20 min in the Matcha condition.

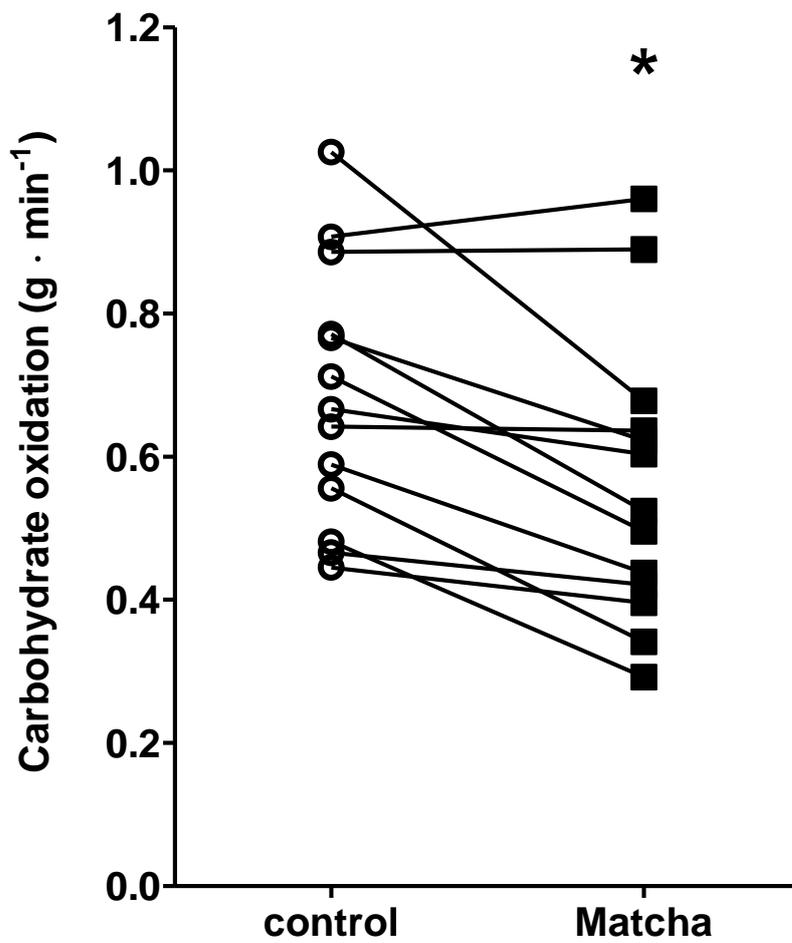
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413 **Figure 3.** Respiratory exchange ratio (RER) in the control and Matcha condition during a 30-
414 min treadmill walk at individualized walking speeds at 5-MET (10 participants) or 6-MET (3
415 participants). Data are presented as mean \pm SD. *, difference between conditions.

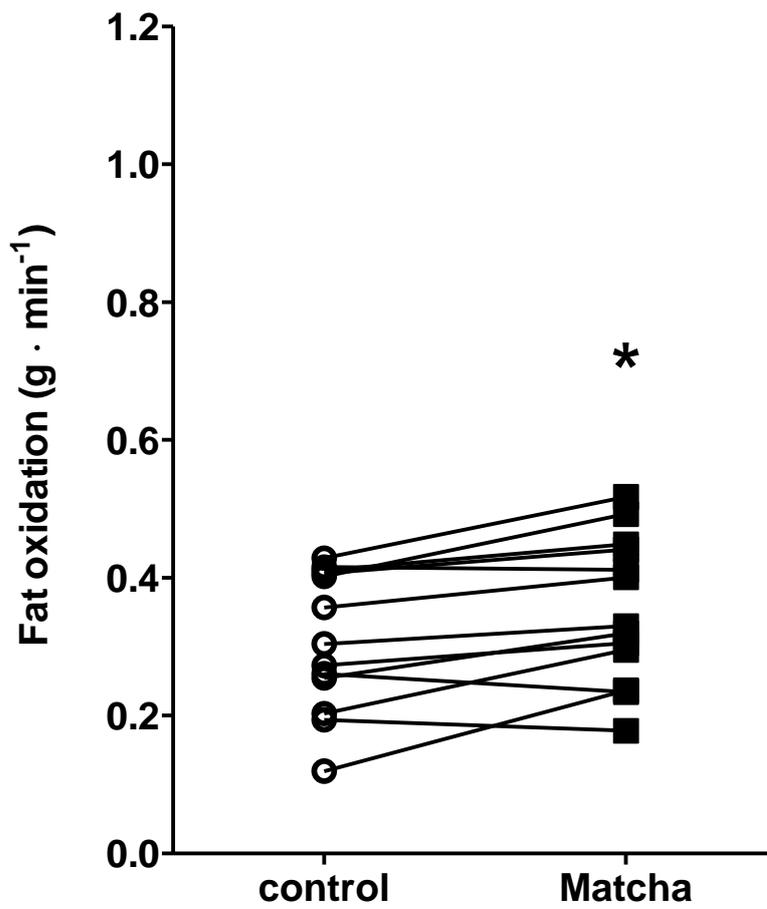
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418 **Figure 4.** Carbohydrate oxidation in the control and Matcha condition during a 30-min
419 treadmill walk at individualized walking speeds at 5-MET (10 participants) or 6-MET (3
420 participants). Columns represent mean values. * different between conditions.

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424 **Figure 5.** Fat oxidation in the control and Matcha condition during a 30-min treadmill walk
425 at individualized walking speeds at 5-MET (10 participants) or 6-MET (3 participants).

426 Columns represent mean values. * different between conditions.