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1 **Mouth rinsing with a sweet solution increases energy expenditure and decreases**  
2 **appetite during 60 minutes of self-regulated walking exercise.**

3

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19

20 **Abstract**

21 Carbohydrate mouth rinsing can improve endurance exercise performance and is most  
22 ergogenic when exercise is completed in the fasted state. This strategy may also be beneficial  
23 to increase exercise capacity and the energy deficit achieved during moderate intensity exercise  
24 relevant to weight control when performed after an overnight fast. Eighteen healthy men  
25 (mean(SD); age 23(4)years, body mass index 23.1(2.4)kg.m<sup>-2</sup>) completed a familiarisation trial  
26 and three experimental trials. After an overnight fast, participants performed 60-minutes of  
27 treadmill walking at a speed that equated to a rating of perceived exertion of 13 (“fairly hard”).  
28 Participants manually adjusted the treadmill speed to maintain this exertion. Mouth rinses for  
29 the experimental trials contained either a 6.4% maltodextrin solution with sweetener (CHO), a  
30 taste-matched placebo (PLA) or water (WAT). Appetite ratings were collected using visual  
31 analogue scales and exercise energy expenditure and substrate oxidation were calculated from  
32 online gas analysis. Increased walking distance during CHO and PLA induced greater energy  
33 expenditure compared with WAT (mean difference (90% CI); 79(60)kJ; P=0.035; *d*=0.24 and  
34 90(63)kJ; P=0.024; *d*=0.27, respectively). Appetite area under the curve was lower in CHO and  
35 PLA than WAT (8(6)mm; P=0.042; *d*=0.43 and 6(8)mm; P=0.201; *d*=0.32, respectively).  
36 Carbohydrate oxidation was higher in CHO than PLA and WAT (7.3(6.7)g; P=0.078; *d*=0.47  
37 and 10.1(6.5)g; P=0.015; *d*=0.81, respectively). This study provides novel evidence that mouth  
38 rinsing with a sweetened solution may promote a greater energy deficit during moderate  
39 exertion walking exercise by increasing energy expenditure and decreasing appetite. A placebo  
40 effect may have contributed to these benefits.

41

42 **Key words:** appetite regulation, energy balance, metabolism, substrate oxidation, cephalic  
43 phase response, oral nutrient sensing

## 44 **Introduction**

45 Carbohydrate mouth rinsing has become established as a successful strategy to improve  
46 endurance performance when exercise is completed after an overnight fast or in a post-  
47 absorptive state (> 4 h) (Ataide-Silva et al. 2016; Carter et al. 2004; Chambers et al. 2009;  
48 Fraga et al. 2015; Gam et al. 2013; Rollo et al. 2008; Sinclair et al. 2014). However, the  
49 ergogenic effect of carbohydrate mouth rinsing is reduced by pre-exercise feeding (Ataide-  
50 Silva et al. 2016; Lane et al. 2013) and it remains debated whether there are any beneficial  
51 effects when exercise is performed in the postprandial state (Ataide-Silva et al. 2016; Beelen  
52 et al. 2009; Lane et al. 2013).

53 Although nutritional recommendations for sports performance advocate the consumption of a  
54 high carbohydrate meal within the four hours prior to commencing exercise (ACSM 2016),  
55 low and moderate intensity exercise for weight control is often performed after an overnight  
56 fast in order to enhance fat oxidation (Deighton et al. 2012). The influence of carbohydrate  
57 mouth rinsing on low and moderate intensity exercise is currently unknown but the ergogenic  
58 effects observed during high intensity exercise appear to be due to an increased self-selected  
59 exercise intensity without a corresponding increase in exertion (Carter et al. 2004; Chambers  
60 et al. 2009; Gam et al. 2013; Rollo et al. 2008; Sinclair et al. 2014). This has also been  
61 associated with increased feelings of pleasure during exercise (Rollo et al. 2008) and evidence  
62 suggests that these ergogenic effects are mediated by an increased activation of the brain  
63 centres involved in reward, motivation and motor control (Chambers et al. 2009). It seems  
64 plausible that these mechanisms may also improve performance during low and moderate  
65 intensity exercise, which would increase energy expenditure and the energy deficit associated  
66 with exercise. Based on this rationale, recent review articles have encouraged investigations  
67 into the effects of carbohydrate mouth rinsing on exercise protocols that are relevant for weight  
68 control (Burke & Maughan 2014; Rollo & Williams 2011).

69 In addition to benefitting exercise performance, exposure of the oral cavity to nutrients has also  
70 been demonstrated to have an anorectic effect. In this regard, masticating and expectorating  
71 food from the mouth during modified sham feeding has been shown to reduce appetite  
72 perceptions (Heath et al. 2004; Smeets & Westerterp-Plantenga 2006). Such reductions in  
73 appetite without the ingestion of nutrients represents another potential benefit of carbohydrate  
74 mouth rinsing for weight control but the effect of rinsing a caloric solution rather than  
75 masticating solid foods is currently unknown.

76 Thus, the purpose of this study was to investigate the influence of carbohydrate mouth rinsing  
77 on self-selected exercise intensity, energy expenditure, substrate oxidation and appetite  
78 perceptions during treadmill walking exercise at a fixed moderate exertion. This represents the  
79 first investigation into the effects of carbohydrate mouth rinsing on moderate exertion exercise  
80 performance and appetite regulation. We hypothesised that mouth rinsing with carbohydrate  
81 would improve walking performance and decrease appetite perceptions.

82

83

## 84 **Methods**

### 85 **Participants**

86 This study was conducted according to the guidelines laid down in the Declaration of Helsinki  
87 and all procedures were approved by the Ethics Advisory Committee at Leeds Beckett  
88 University. Eighteen recreationally active male participants were recruited for the study and  
89 written informed consent was obtained from all participants. Participants were non-smokers,  
90 not taking medication, weight stable for at least six months before the study and were not  
91 dieting. The physical characteristics of participants (mean (SD) were as follows: age 23 (4)  
92 years, body mass 72.2 (2.4) kg, body mass index (BMI) 23.1 (2.4) kg.m<sup>-2</sup>). This population  
93 was selected based on evidence that young, healthy men are most sensitive to nutrient  
94 manipulation (Appleton et al. 2011; Davy et al. 2007), thereby maximising the likelihood of  
95 observing an effect in this proof of concept investigation.

### 96 **Experimental design**

97 Each participant performed one familiarisation trial and three experimental trials separated by  
98 approximately one week. All trials were identical and the experimental trials were completed  
99 using a double-blind, counterbalanced Latin Square design. The mouth rinse solutions for the  
100 experimental trials consisted of a 6.4% maltodextrin solution sweetened with saccharin (CHO),  
101 water sweetened with saccharin (PLA), or plain water (WAT). The addition of saccharin to the  
102 CHO and PLA solutions ensured that they were matched for taste. To investigate the possibility  
103 of a placebo effect with carbohydrate mouth rinsing, participants were told that both flavoured  
104 solutions contained carbohydrate and that the purpose of the study was to compare this with  
105 rinsing a water solution (Hulston & Jeukendrup 2009).

### 106 **Physical activity and dietary standardisation**

107 Participants completed a food diary during the 24-h before the first experimental trial and  
108 replicated this before each subsequent trial. Alcohol, caffeine and strenuous physical activity  
109 were not permitted during this period. Participants arrived at the laboratory between 0700 h  
110 and 0800 h after an overnight fast of at least 10 h and exerted themselves minimally when  
111 travelling to the laboratory using motorised transport when possible. Verbal confirmation of  
112 dietary and exercise standardisation was obtained at the beginning of each trial.

### 113 **Experimental trials**

114 During each trial, participants completed 60-min of walking exercise on a motorised treadmill  
115 using a 1% gradient (Model ELG 70, Woodway, Germany). Participants were asked to  
116 manually increase their speed using the buttons available on the arm of the treadmill until they  
117 reached a speed corresponding to a rating of perceived exertion (RPE) of 13 indicating “fairly  
118 hard” (Borg 1973). Participants were required to achieve this speed within 2-min and the 60-  
119 min walk commenced from this point. Throughout the trial, participants were permitted to alter  
120 the speed of the treadmill in order to maintain an RPE of 13. The prescription of exercise at a  
121 self-regulated RPE of 13 is supported by previous research that has demonstrated this to  
122 produce positive affective responses as well as improvements in fitness and cardiovascular  
123 health markers (Parfitt et al. 2012).

124 Participants were able to view the elapsed time during the walk but were blinded from the  
125 speed and distance. The only interaction between researchers and participants was during the  
126 provision of mouth rinses and collection of subjective scales. No other encouragement or  
127 distraction was permitted and the participants did not receive any feedback about the distance  
128 covered until the completion of the study. Heart rate was measured at 15-min intervals during  
129 the exercise (Polar FT1, Finland). The temperature and relative humidity of the laboratory were  
130 maintained at 20 (1) °C and 39 (9) %, respectively.

131 **Expired gas analysis**

132 Expired gas was collected continuously throughout the walk using an online gas analysis  
133 system (Metalyzer 3B, Cortex, Germany) and averaged over the 60-min period. During rinsing,  
134 the facemask was unclipped and gas analysis data for the subsequent 60-s period was excluded.  
135 Energy expenditure and substrate oxidation rates were calculated from  $VO_2$  and  $VCO_2$  values  
136 using the equations of Frayn (1983).

137 **Mouth rinse protocol**

138 Participants were required to rinse their mouth with one of the experimental solutions (CHO,  
139 PLA or WAT) at -10, -5, 0, and every 7.5 min thereafter throughout the 60-min walk. In  
140 accordance with previous research, 25 mL of solution was rinsed around the oral cavity for 10  
141 seconds before being expectorated into a pre-weighed cup (Sinclair et al. 2014).

142 **Subjective scales**

143 During each trial, appetite perceptions (hunger, satisfaction, fullness and prospective food  
144 consumption) were assessed at -10, 0, 30 and 60 min using 100 mm visual analogue scales  
145 (Flint et al. 2000). A composite appetite rating was calculated as the mean value of the four  
146 appetite perceptions after inverting the values for satisfaction and fullness (Stubbs et al. 2000).  
147 Feelings of thirst were assessed at -10, 0, and every 15 min during exercise using an adapted  
148 Borg scale (Ispoglou et al. 2015).

149 Affective responses to exercise were assessed at -10, 0 and every 15 min during exercise using  
150 the Feeling Scale to assess pleasure-displeasure (Hardy & Rejeski 1989) and the Felt Arousal  
151 Scale to assess perceived activation (Svebak & Murgatroyd 1985). All subjective scales were  
152 completed immediately before the administration of mouth rinses. Participants were reminded  
153 to maintain an RPE of 13 after the completion of each mouth rinse.



154 **Statistical analysis**

155 Data were analysed using IBM SPSS statistics version 22 for Windows. Time-averaged area  
156 under the curve (AUC) values were calculated for all subjective scales using the trapezoidal  
157 method. One-way repeated measures ANOVA was used to assess trial based differences in  
158 total distance walked, energy expenditure, substrate oxidation and mean heart rate during  
159 exercise, as well as time-averaged AUC values for appetite, thirst and affective responses. Area  
160 under the curve values were used for statistical analysis rather than individual timepoints in  
161 accordance with the statistical guidance provided by Matthews and colleagues (1990) and the  
162 subject-specific guidance provided by Blundell et al. (2010). Where significant main effects of  
163 trial were observed, post-hoc analysis was performed using unadjusted Student's paired t-tests.  
164 Pearson's product-moment correlation coefficient was used to examine the relationship  
165 between BMI and the response to carbohydrate and placebo mouth rinsing compared with  
166 water rinsing for the primary outcome variables (distance walked, energy expenditure,  
167 substrate oxidation, appetite perceptions). Null-hypothesis significance testing was performed  
168 with an alpha value of 5 % in accordance with current convention.

169 In addition to null-hypothesis significance testing, magnitude-based inferences were calculated  
170 to examine whether the observed differences were meaningful. This approach has been  
171 supported within biomedical and exercise sciences, in addition to other fields such as computer  
172 studies (van Schaik & Weston 2016), due to reduced inferential error rates compared with null-  
173 hypothesis significance testing (Hopkins & Batterham 2016). This approach also promotes  
174 direct interpretation of the magnitude of changes and whether these are meaningful (Buchheit  
175 2016). Subsequently, this approach was utilised and prioritised for the appropriate variables.  
176 Using the spreadsheet by Hopkins (2007), the P value was converted into 90% confidence  
177 intervals (CI) for inferences about the true value of the effect statistic (Hopkins 2007). An effect  
178 was deemed unclear when the upper and lower confidence limits represented meaningful

179 increases and decreases, respectively. All other effects were deemed clear, and the probabilities  
180 that the true effect was a substantial increase, a trivial change, and a substantial decrease were  
181 calculated via the sampling t-distribution of the effect in relation to the smallest worthwhile  
182 change. The smallest worthwhile change in walking distance was set at 1% to allow  
183 comparisons with previous research that has used this threshold to investigate changes in self-  
184 regulated running exercise with carbohydrate mouth rinsing (Rollo et al. 2011). An 8 mm  
185 difference in composite appetite time-averaged AUC was set as the smallest worthwhile change  
186 in appetite perceptions (Blundell et al. 2010). Meaningful changes in the remaining variables  
187 are not well-established, which limited the analysis of beneficial, trivial or negative effect  
188 magnitudes to walking distance and appetite perceptions only.

189 All results in the text are presented as mean (SD) or 90% confidence intervals where  
190 appropriate. Graphical representations of results are presented as mean (SEM) to avoid  
191 distortion of the graphs. Effect sizes are presented as Cohen's *d* and interpreted as  $\leq 0.2$  trivial,  
192  $> 0.2$  small,  $> 0.6$  moderate,  $> 1.2$  large,  $> 2$  very large and  $> 4$  extremely large (Hopkins 2004).  
193 Individual responses are presented within figures to allow further examination of the findings.  
194 The inclusion of null-hypothesis significance testing and magnitude-based inferences within  
195 the results was intended to support the interpretation of the findings via the most commonly  
196 employed methods within the subject area.

197

198

199

## 200 **Results**

### 201 **Distance walked**

202 One-way ANOVA revealed no significant differences in the total distance walked during the  
203 CHO, PLA and WAT trials ( $P = 0.204$ ; Figure 1). The mean distance walked during the three  
204 trials was 5814 m; therefore a threshold value of 58 m was selected as a 1 % meaningful  
205 difference in walking distance for magnitude-based inferences. The mean difference in distance  
206 covered between the CHO and WAT trials was 163 m (90% CI 3–323 m; 2.9%;  $P = 0.095$ ;  $d$   
207 = 0.21). The mean difference in distance covered between the PLA and WAT trials was 134 m  
208 (90% CI 7–261 m; 2.3%;  $P = 0.084$ ;  $d = 0.17$ ). The mean difference in distance covered  
209 between the CHO and PLA trials was 29 m (90% CI -170-230 m; 0.5%;  $P = 0.806$ ;  $d = 0.03$ ).  
210 The chance that the true value of the effect has a beneficial, trivial or negative influence on  
211 walking distance is 86.5%, 12.1%, and 1.4% for the CHO versus WAT trial; and 84.4%, 14.7%,  
212 and 0.9% for the PLA versus WAT trial. The effect between CHO and PLA was unclear due  
213 to the upper and lower confidence limits representing meaningful increases and decreases,  
214 respectively.

### 215 **Energy expenditure and substrate oxidation**

216 One-way ANOVA revealed a significant difference between trials for energy expenditure  
217 during walking exercise ( $P = 0.049$ ). Post-hoc analysis demonstrated lower energy expenditure  
218 in WAT compared with CHO ( $P = 0.035$ ;  $d = 0.24$ ) and PLA ( $P = 0.024$ ;  $d = 0.27$ ), respectively  
219 (Figure 2).

220 One-way ANOVA revealed a significant difference in carbohydrate oxidation between trials  
221 ( $P = 0.040$ ). Post-hoc analysis demonstrated significantly higher carbohydrate oxidation during  
222 the CHO trial compared with WAT ( $P = 0.015$ ;  $d = 0.81$ ) and a trend towards higher  
223 carbohydrate oxidation during CHO than PLA ( $P = 0.078$ ;  $d = 0.47$ ) (Figure 3a). Contrastingly,

224 there was no difference between trials for fat oxidation ( $P = 0.126$ ; Figure 3b). There was no  
225 significant difference between trials for heart rate during the exercise bout ( $P = 0.572$ ).

## 226 **Appetite and affective responses**

227 There were no baseline differences between trials for composite appetite scores (CHO: 63 (22);  
228 PLA: 64 (18); WAT: 67 (17);  $P = 0.653$ ), pleasure-displeasure (CHO: 2 (1); PLA: 2 (1); WAT:  
229 2 (2);  $P = 0.719$ ) or perceived activation (CHO: 3 (1); PLA: 3 (1); WAT: 3 (2);  $P = 0.432$ ).  
230 Baseline thirst perceptions tended to be different between trials ( $P = 0.091$ ), indicating higher  
231 thirst in WAT and PLA than CHO (CHO: 9 (2); PLA: 10 (3); WAT: 9 (2)).

232 One-way ANOVA revealed no significant differences in composite appetite time-averaged  
233 AUC scores between trials ( $P = 0.101$ ; Figure 4). The mean difference in composite appetite  
234 time-averaged AUC between the CHO and WAT trials was 8 mm (90% CI 2 - 15 mm;  $P =$   
235  $0.042$ ;  $d = 0.43$ ). The mean difference in composite appetite time-averaged AUC between the  
236 PLA and WAT trials was 6 mm (90% CI -2 - 15 mm;  $P = 0.201$ ;  $d = 0.32$ ). The mean difference  
237 in composite appetite time-averaged AUC between the CHO and PLA trials was 2 mm (90%  
238 CI -3 - 7 mm;  $P = 0.524$ ;  $d = 0.08$ ). Based on a threshold value of 8 mm as a meaningful  
239 difference in appetite, the probability that the true value of the effect has a beneficial, trivial or  
240 negative influence is 53.5%, 46.5%, and 0% for the CHO versus WAT trial; 37.1%, 62.5%,  
241 and 0.4% for the PLA versus WAT trial; and 2.9%, 96.9% and 0.2% for CHO versus PLA,  
242 respectively. Time-averaged area under the curve for thirst perception tended to be different  
243 between trials ( $P = 0.055$ ), indicating higher thirst in WAT than CHO and PLA (WAT: 13 (3),  
244 CHO: 12 (3), PLA: 12 (3)).

245 There were no differences between trials for time-averaged AUC ratings of pleasure-  
246 displeasure (CHO: 2 (1); PLA: 2 (1); WAT: 2 (2);  $P = 0.297$ ) or perceived activation (CHO: 4  
247 (1); PLA: 4 (2); WAT: 4 (1);  $P = 0.123$ ).

248 **Blinding efficacy, rinse ingestion and correlations**

249 Five out of the 18 participants were able to correctly distinguish between the CHO and PLA  
250 solutions (i.e. less than what would be predicted by pure chance), indicating successful  
251 blinding. The mean difference in the volume of rinse and expectorate was 0.5, 0.4 and 0.3 mL  
252 for each rinse in the WAT, PLA and CHO trials respectively. This resulted in a mean total  
253 consumption of 5 mL, 4 mL and 3 mL across the ten rinses for WAT, PLA and CHO,  
254 respectively. Body mass index was not significantly correlated with distance walked, energy  
255 expenditure, substrate oxidation or appetite perceptions in response to carbohydrate or placebo  
256 mouth rinsing compared with rinsing the water solution (all  $r < 0.5$ ;  $P > 0.05$ ).

257

## 258 **Discussion**

259 This is the first experiment to investigate the effects of carbohydrate mouth rinsing on self-  
260 selected exercise intensity and physiological responses during moderate exertion exercise. The  
261 findings demonstrate that mouth rinsing with a sweet solution (either carbohydrate or  
262 sweetened placebo) increased self-selected walking speed, which induced a small but  
263 significant increase in energy expenditure during the exercise bout. Mouth rinsing with a sweet  
264 solution also suppressed appetite during exercise compared with a water solution but increases  
265 in carbohydrate oxidation occurred only with carbohydrate mouth rinsing. This study provides  
266 novel evidence that the stimulation of carbohydrate and sweet receptors in the oral cavity may  
267 have physiological effects beyond those previously reported.

268 In the present study, mouth rinsing with carbohydrate increased the distance walked during 60-  
269 min of treadmill exercise at a fixed RPE of 13 in comparison with rinsing a water solution.  
270 This is the first study to reveal a beneficial effect of carbohydrate mouth rinsing for moderate  
271 intensity exercise and the mean improvement of 2.9 % is comparable with previously reported  
272 improvements in endurance time trial performance of 1.7 – 3.1 % (Carter et al. 2004; Chambers  
273 et al. 2009; Rollo et al. 2008). However, the performance improvements in the present study  
274 were only observed in comparison with a water solution and similar benefits occurred with a  
275 taste-matched placebo. This contrasts with the previously reported benefits of carbohydrate  
276 mouth rinsing, which have been observed in relation to a placebo solution (Ataide-Silva et al.  
277 2016; Carter et al. 2004; Chambers et al. 2009; Gam et al. 2013; Rollo et al. 2008; Sinclair et  
278 al. 2014) and suggests that a placebo effect may be responsible for the ergogenic benefits  
279 observed in the current study. The reasons for this effect are unclear as the participants were  
280 not made aware of any hypothesised benefits of carbohydrate mouth rinsing on walking  
281 performance. However, although performance improvements were not attributed to the  
282 presence of carbohydrate, mouth rinsing with a sweet solution successfully increased self-

283 selected exercise intensity and walking distance. It seems plausible that the recreationally  
284 active participants may have been aware that carbohydrate ingestion improves exercise  
285 performance but this was not communicated from the research team. The novelty of the current  
286 experiment also demonstrates that the effects of mouth rinsing on the measured variables was  
287 unknown for both the researchers and participants. The factors contributing to a potential  
288 placebo effect require further investigation, in addition to substantiation of this intervention as  
289 a potential nutritional strategy to increase exercise intensity during moderate exertion exercise  
290 relevant for weight control. The inclusion of sweetened and unsweetened carbohydrate and  
291 placebo solutions in future experiments may be beneficial to elucidate any separate effects of  
292 sweetness and carbohydrate content on the variables assessed within the present experiment.

293 The increase in walking distance during the carbohydrate and placebo trials resulted in a  
294 corresponding increase in energy expenditure compared with the water trial. Although these  
295 effects are small, the observed mean increases of 79 kJ and 90 kJ during exercise in CHO and  
296 PLA are greater than that previously reported with carbohydrate mouth rinsing during 30-min  
297 of running exercise (Rollo et al. 2008) and similar to the 76 kJ increase in eight-hour energy  
298 expenditure during a sit-to-stand intervention in the workplace (Thorp et al. 2016). The  
299 practical relevance of such small increases in energy expenditure are unclear but previous  
300 physical activity interventions have reported the potential for a cumulative effect that may  
301 induce meaningful deficits (Thorp et al. 2016). In this regard, mouth rinsing with a sweetened  
302 solution during the completion of five 60-min bouts of walking exercise per week would  
303 hypothetically yield an additional weekly energy expenditure of ~425 kJ (~100 kcal). It remains  
304 unknown whether the effects observed during the present study would continue with repeated  
305 exposures to mouth rinsing during multiple exercise bouts and this requires further  
306 investigation. These findings also suggest that increases in walking distance and energy  
307 expenditure may not be associated with differences in heart rate between trials. In this regard,

308 the present study supports previous evidence that the ergogenic effect of carbohydrate mouth  
309 rinsing is not associated with any differences in heart rate during the exercise bout (Lane et al.  
310 2013; Rollo et al. 2008; Sinclair et al. 2014). This accords with the fixed effort of the exercise  
311 bout but may also represent low sensitivity to detect differences in heart rate during exercise.

312 Despite similar improvements in self-selected walking speed with carbohydrate and placebo  
313 mouth rinsing, changes in substrate oxidation occurred only when rinsing with the carbohydrate  
314 solution. To the authors' knowledge, this is the first study to demonstrate this effect and the  
315 paucity of research investigating substrate oxidation in response to oral nutrient stimulation  
316 (both at rest and during exercise) makes it difficult to draw comparisons with previous  
317 literature. However, it remains plausible to speculate that changes in substrate oxidation may  
318 be related to the activation of brain centres that has been observed in response to carbohydrate  
319 mouth rinsing (Chambers et al. 2009). This includes activation of the insula cortex, which has  
320 been shown to increase sympathetic activity (Oppenheimer et al. 1992) and may subsequently  
321 increase carbohydrate utilisation (Brooks & Mercier 1994). The investigation of low to  
322 moderate intensity exercise in the present study may also have increased the likelihood to detect  
323 changes in substrate use associated with this pathway as sympathetic activation during exercise  
324 is expected to have been lower than previous studies that have measured substrate oxidation in  
325 response to more strenuous protocols (Ataide-Silva et al. 2016; Ispoglou et al. 2015). A mean  
326 total of 3 mL of rinse solution (equating to < 0.2 g of carbohydrate) was ingested during the  
327 carbohydrate trial, which is insufficient to have mediated the change in substrate oxidation.

328 The concept that changes in substrate oxidation may occur without the ingestion of nutrients is  
329 also supported in rodent studies as increases in carbohydrate oxidation have been observed in  
330 response to the anticipation of a high carbohydrate meal (McGregor & Lee 1998). This suggests  
331 that increases in energy expenditure in response to carbohydrate mouth rinsing may be solely  
332 due to increased carbohydrate oxidation without any changes in fat oxidation. Although the



333 potential mechanisms remain speculative, the current findings support further investigation  
334 into the effects of oral nutrient stimulation on substrate oxidation. Such investigations may help  
335 to increase our understanding of the potential physiological effects of carbohydrate mouth  
336 rinsing, as well as the relationship between oral nutrient sensing and cephalic phase responses  
337 in determining physiological changes with feeding.

338 Oral nutrient sensing has also been demonstrated to increase satiety during modified sham  
339 feeding whereby a meal of mixed nutrient composition is masticated and expectorated without  
340 ingestion (Heath et al. 2004; Smeets & Westerterp-Plantenga 2006). The present study  
341 represents the first investigation into the effects of mouth rinsing a liquid solution on appetite  
342 perceptions and demonstrates that mouth rinsing with a sweet solution decreases appetite  
343 during 60-min of walking exercise. Although some authors have suggested that oral rinsing of  
344 a liquid solution is insufficient to stimulate a cephalic phase response (Teff et al. 1995), others  
345 have demonstrated cephalic phase insulin release after rinsing the oral cavity with either a  
346 sucrose or artificially sweetened solution for 45-seconds (Just et al. 2008). Such changes in  
347 circulating insulin concentrations and the appetite suppression observed during modified sham  
348 feeding are thought to be primarily mediated via vagal stimulation from oral nutrient sensing  
349 (Zafra et al. 2006). The findings of the present study extend evidence of a previously observed  
350 cephalic phase insulin response to suggest that mouth rinsing with a sweet solution can also  
351 reduce appetite perceptions. Although it was beyond the scope of the present study, it would  
352 be beneficial for future experiments to incorporate measurements of circulating insulin and  
353 gastrointestinal hormone concentrations as potential mediators of the observed reductions in  
354 appetite when mouth rinsing with a sweetened solution. It remains unclear whether this effect  
355 would be observed with mouth rinsing in isolation or whether a combination of mouth rinsing  
356 and exercise is required. In this regard, it seems plausible that exercise may have enhanced the  
357 anorectic effect of mouth rinsing in the present study as previous research has demonstrated

358 significant appetite suppression during 60-min of moderate intensity walking exercise  
359 compared with a resting control (Farah et al. 2012). Thirst perceptions were also lower during  
360 the carbohydrate and placebo trials in comparison with the water trial but it seems unlikely that  
361 this would have influenced appetite perceptions based on current evidence (Corney et al. 2015;  
362 McKiernan et al. 2008).

363 Although the findings of the present study have provided novel insights into the effects of  
364 carbohydrate mouth rinsing on moderate exertion exercise performance and appetite  
365 regulation, this study also contains some notable limitations. Firstly, the use of a manually  
366 operated treadmill may have reduced the opportunity for participants to change their speed  
367 spontaneously based on how they feel and required conscious decision making. Subsequently  
368 the use of an automated treadmill as used by Rollo et al. (2008) may have allowed for more  
369 sensitive detection of ergogenic effects and revealed greater changes in walking distance and  
370 energy expenditure. Secondly, although this is the first study to investigate a placebo effect  
371 with carbohydrate mouth rinsing, a no-rinse control condition was not provided. In this regard,  
372 evidence suggests that a no-rinse condition can result in better time trial performance than  
373 mouth rinsing with a placebo solution as a result of potential breathing interference and  
374 distraction during mouth rinsing (Gam et al. 2013). However, these effects are likely to be of  
375 smaller consequence in the present study due to the lower demands of moderate exertion  
376 walking exercise in comparison with time trial performance. Thirdly, although meaningful  
377 changes in appetite perceptions occurred when mouth rinsing with a sweetened solution, ad  
378 libitum energy intake also needs to be assessed to understand whether these changes transpire  
379 into reduced energy intake and an enhanced energy deficit from the intervention. Appetite  
380 perceptions should also be assessed during the post-exercise period as this is the period when  
381 energy compensation may occur and therefore this period is the most relevant for weight  
382 management. However, the reduction in appetite during and immediately after the exercise

383 bout in the present study demonstrates a proof of concept that can be developed with future  
384 experiments. Finally, the population sample for this study was comprised of young healthy  
385 males; therefore the findings may not generalise to overweight and obese populations where  
386 weight management strategies have the most clinical relevance. Although further research is  
387 required in different populations, understanding appetite regulation and energy balance in  
388 normal weight participants remains important as the prevention of weight gain has been  
389 highlighted as a major public health priority (Lawlor & Chaturvedi 2006).

390 In conclusion, this study has demonstrated that mouth rinsing with a sweet solution can increase  
391 self-selected exercise intensity and energy expenditure, in addition to reducing appetite, during  
392 60-min of treadmill walking at a fixed moderate exertion. Mouth rinsing with a carbohydrate  
393 solution also increased carbohydrate oxidation during the exercise bout in comparison with  
394 placebo and water rinses. These findings provide novel insights into the effects of mouth  
395 rinsing on physiological variables during an exercise mode relevant to weight control. Future  
396 investigations into the energy intake response to mouth rinsing with a sweetened solution is  
397 required to understand whether the observed anorectic effects translate into reductions in  
398 energy intake. Understanding the maintenance of the observed effects during multiple exercise  
399 bouts is also required but these initial findings demonstrate the potential for mouth rinsing to  
400 increase the energy deficit associated with exercise through increased energy expenditure and  
401 reduced appetite perceptions.

402

403

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508

509 **Figure 1.** Distance walked in the carbohydrate (CHO), placebo (PLA) and water (WAT)  
510 trials expressed as mean (SEM) (a), and the difference in walking distance between trials as  
511 individual responses (circles represent values for individual participants) (b). N = 18.

512

513 **Figure 2.** Energy expenditure in the carbohydrate (CHO), placebo (PLA) and water (WAT)  
514 trials expressed as mean (SEM) (a), and the difference in energy expenditure between trials  
515 as individual responses (circles represent values for individual participants) (b). N = 18.

516 \*Significantly different from WAT,  $P < 0.05$ .

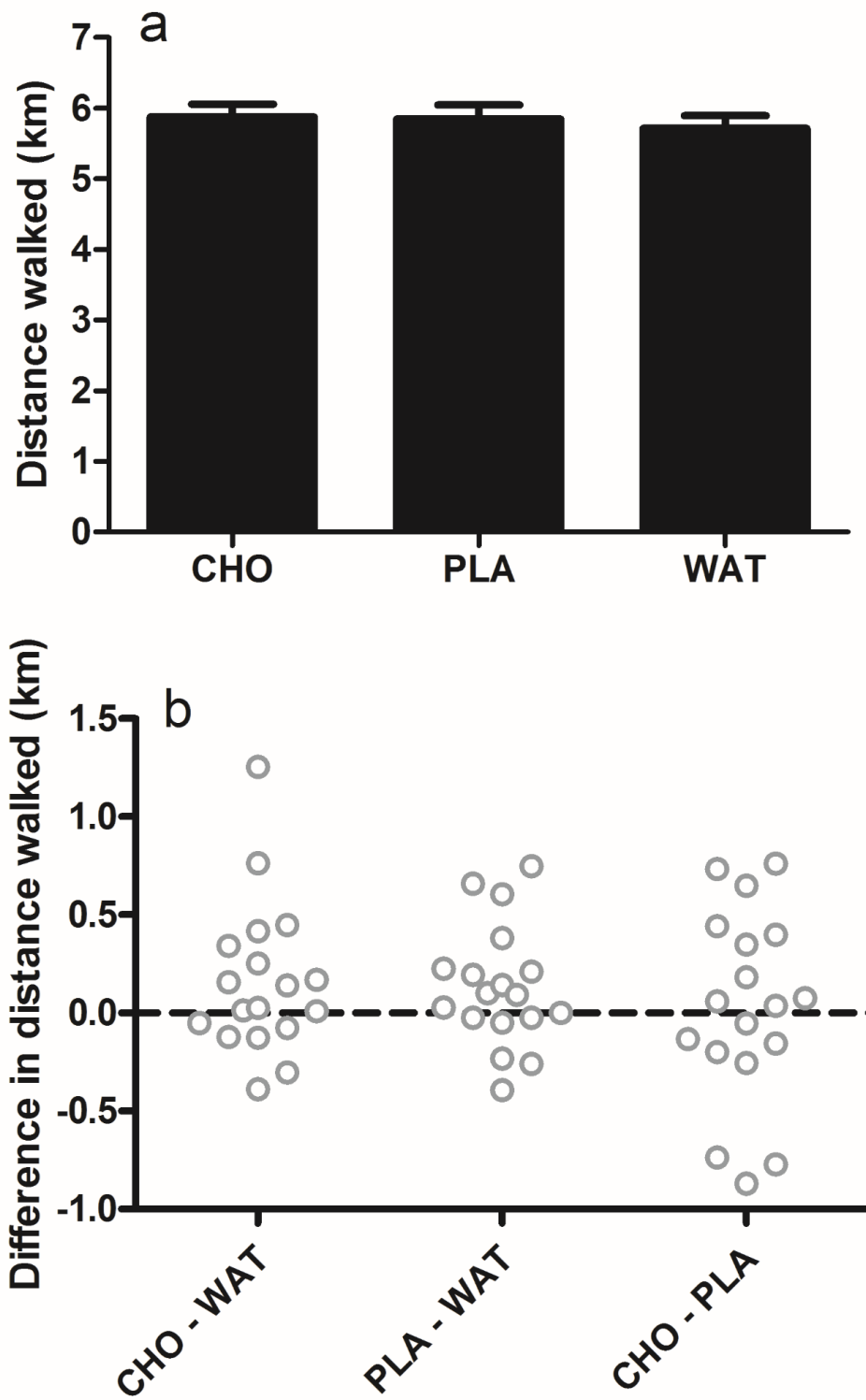
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518 **Figure 3.** Carbohydrate (a) and fat oxidation (b) in the carbohydrate (CHO), placebo (PLA)  
519 and water (WAT) trials. Values are mean (SEM). N = 18. \*Significantly different from WAT,  
520  $P < 0.05$ ; #Trend towards being significantly different from PLA,  $P < 0.10$ .

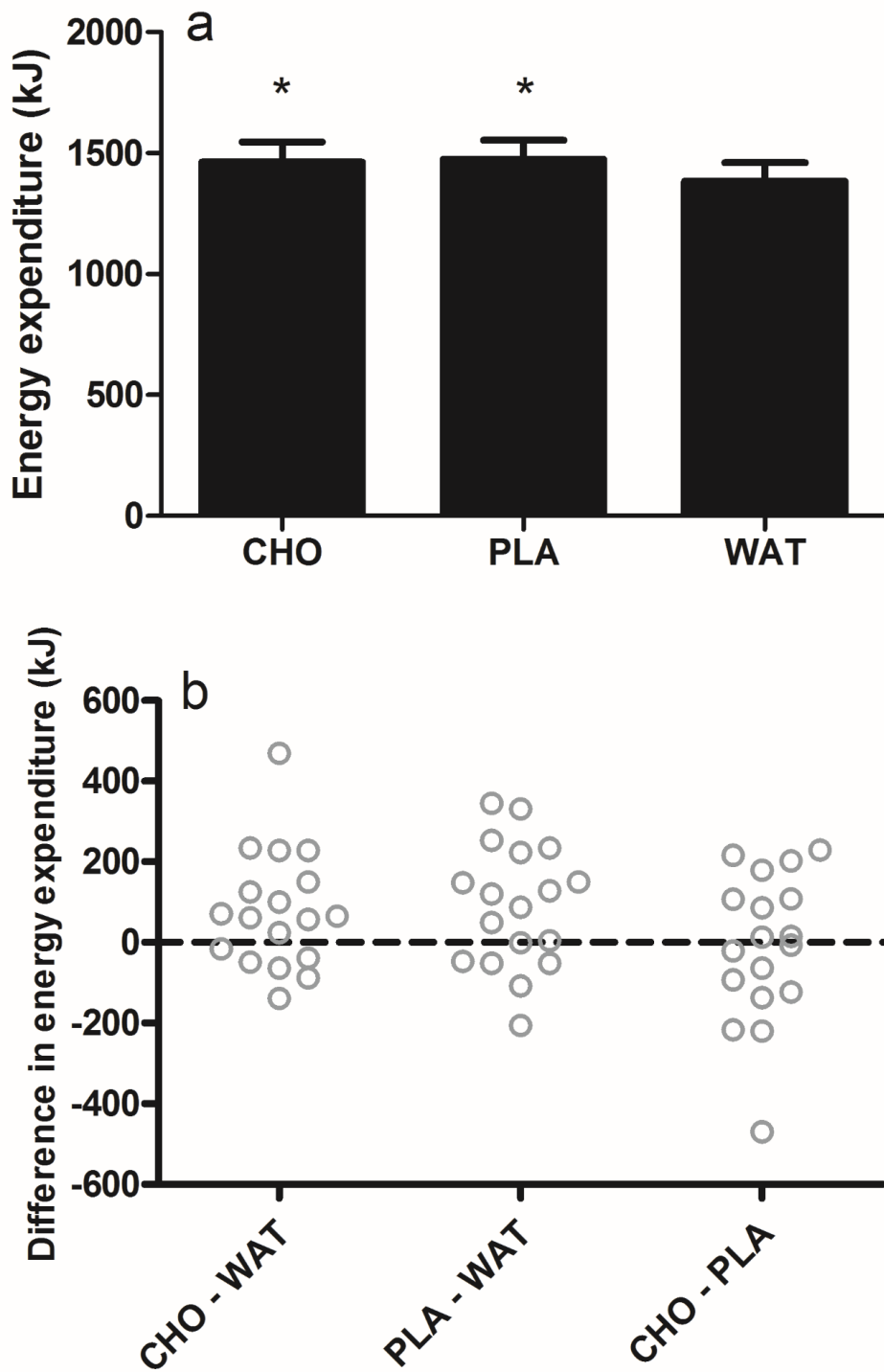
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522 **Figure 4.** Composite appetite scores in the carbohydrate (CHO) (●), placebo (PLA) (○) and  
523 water (WAT) (▼) trials expressed as mean (SEM) (a), and the difference in composite  
524 appetite time-averaged AUC between trials as individual responses (circles represent values  
525 for individual participants) (b). N = 18.

526



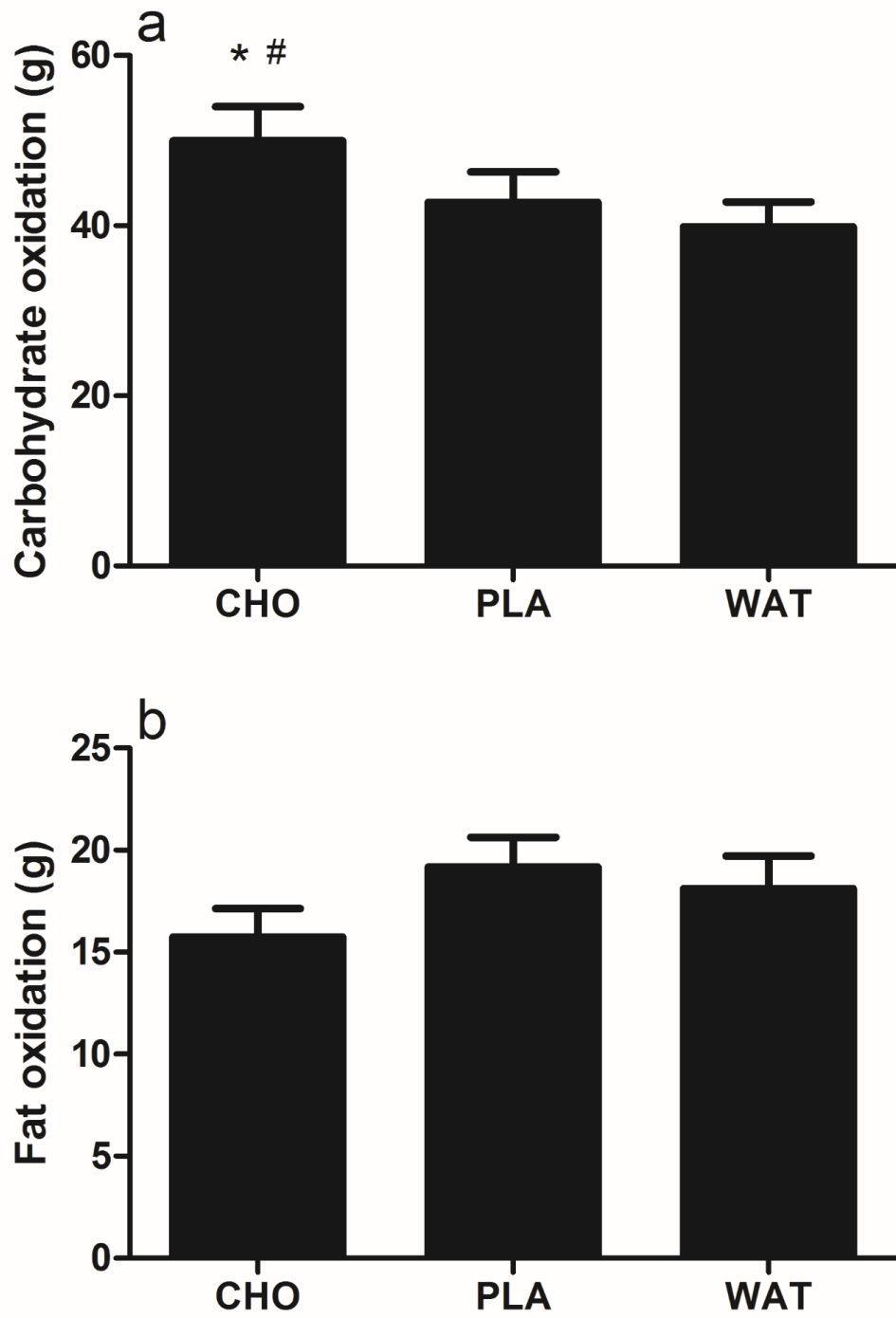
530 **Figure 2**



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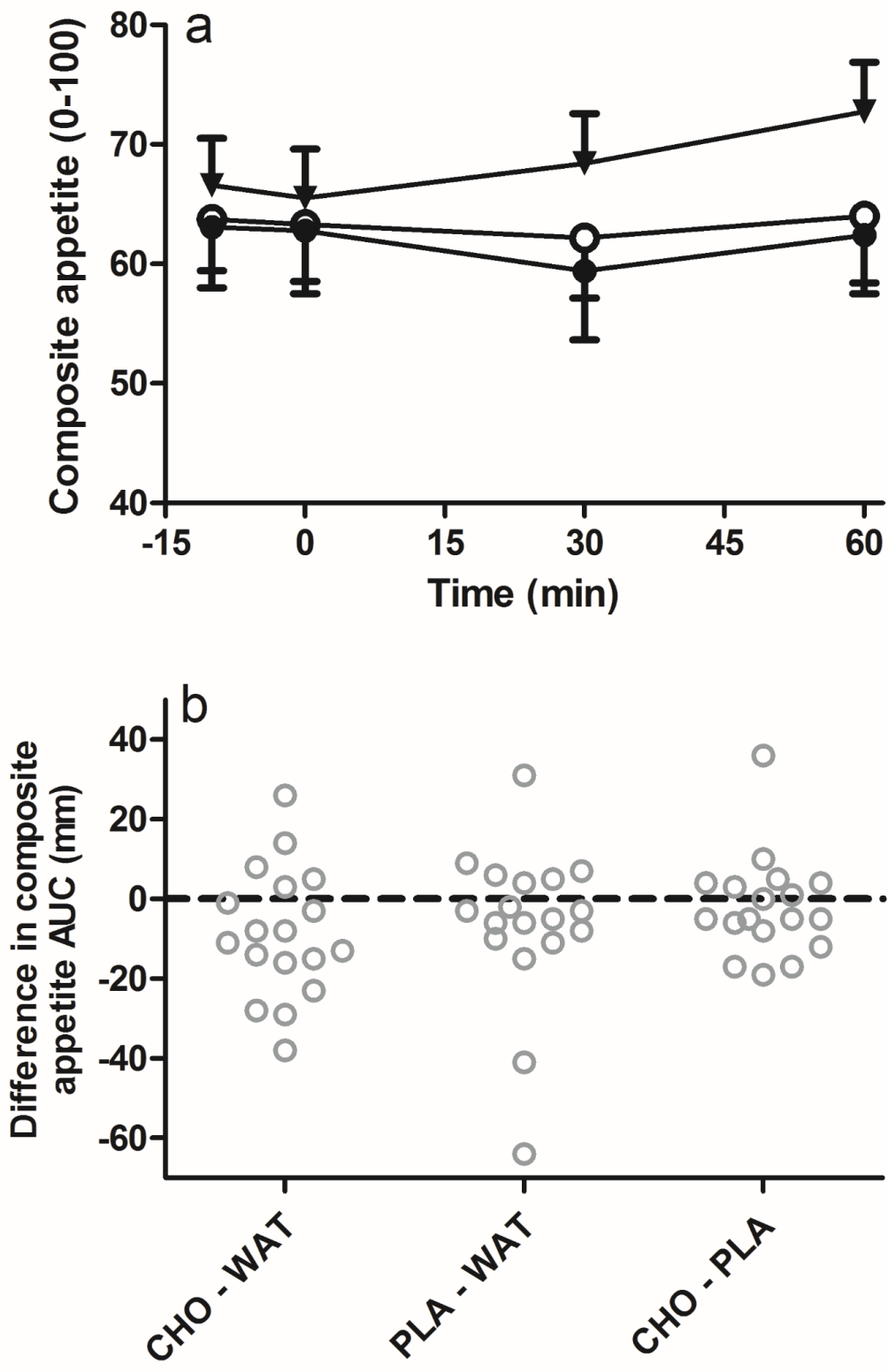
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540 **Figure 4**



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