

**Effects of an acute bout of aerobic exercise on immediate and subsequent three-day food intake and energy expenditure in active and inactive pre-menopausal women taking oral contraceptives**

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1 **Effects of an acute bout of aerobic exercise on**  
2 **immediate and subsequent three-day food intake and**  
3 **energy expenditure in active and inactive pre-**  
4 **menopausal women taking oral contraceptives**

5

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### 30 **Abstract (314 words)**

31 This study examined the effects of an acute bout of exercise of low-intensity on food  
32 intake and energy expenditure over four days in women taking oral contraceptives.  
33 Twenty healthy, active (n=10) and inactive (n=10) pre-menopausal women taking oral  
34 contraceptives completed two conditions (exercise and control), in a randomised,  
35 crossover fashion. The exercise experimental day involved cycling for one hour at an  
36 intensity equivalent to 50% of maximum oxygen uptake and two hours of rest. The  
37 control condition comprised three hours of rest. Participants arrived at the laboratory  
38 fasted overnight; breakfast was standardised and an *ad libitum* pasta lunch was  
39 consumed on each experimental day. Participants kept a food diary to measure food  
40 intake and wore an Actiheart to measure energy expenditure for the remainder of the  
41 experimental days and over the subsequent 3 days. There was a condition effect for  
42 absolute energy intake (exercise vs. control:  $3363 \pm 668$  kJ vs.  $3035 \pm 752$  kJ;  $p =$   
43  $0.033$ ,  $d = 0.49$ ) and relative energy intake (exercise vs. control:  $2019 \pm 746$  kJ vs.  
44  $2710 \pm 712$  kJ;  $p < 0.001$ ,  $d = -1.00$ ) at the *ad libitum* lunch. There were no significant  
45 differences in energy intake over the four days in active participants and there was a  
46 suppression of energy intake on the first day after the exercise experimental day  
47 compared with the same day of the control condition in inactive participants (mean

48 difference = -1974 kJ; 95% CI -1048 to -2900 kJ,  $p = 0.002$ ,  $d = -0.89$ ). There was a  
49 group effect ( $p = 0.001$ ,  $d = 1.63$ ) for free-living energy expenditure, indicating that  
50 active participants expended more energy than inactive participants during this period.  
51 However, there were no compensatory changes in daily physical activity energy  
52 expenditure. These results support the use of low-intensity aerobic exercise as a  
53 method to induce a short-term negative energy balance in inactive women taking oral  
54 contraceptives.

55

56 **Keywords:** Exercise, Food intake, Energy expenditure, Oral contraceptives, Appetite, Active, Inactive.

## 57 **Introduction**

58 Regular exercise is prophylactic and promotes metabolic adaptations that improve  
59 physical and mental health (Bertheussen et al., 2011; Chaput et al., 2010; Tremblay &  
60 Therrien, 2006). In addition, the ability of exercise to disrupt energy balance through  
61 its effects on food intake and energy expenditure makes it important for the  
62 maintenance of adequate body mass and composition.

63

64 Exercise-induced behavioural and physiological compensatory responses in energy  
65 intake and/or non-exercise energy expenditure (King et al., 2007) might explain the  
66 high inter-variability responses of exercise interventions that are designed to reduce  
67 body mass. Additionally, these responses differ according to participants' habitual  
68 physical activity (Martins et al., 2008) and sex (Hagobian et al., 2010), therefore, it is  
69 important to control for these variables. Indeed, results from a recently published  
70 meta-analysis on the effect of acute exercise on subsequent (within 24 hours post-  
71 exercise) energy intake (Schubert et al., 2013) suggested that individuals who engage  
72 in less physical activity are more likely to experience an anorexic effect of exercise.

73 In addition, findings from our previous study (Rocha et al., 2013) suggest that active  
74 men compensate for an acute exercise-induced energy deficit quicker than inactive  
75 men. However, it is still not known if these findings occur in women.

76

77 Most studies investigating the effects of an acute bout of exercise on hunger and food  
78 intake in active (Finlayson et al., 2009; Hagobian et al., 2012; Lluch et al., 1998;  
79 Lluch et al., 2000; Larson-Meyer et al., 2012) and inactive women (George &  
80 Morganstein, 2003; Maraki et al., 2005; Reger et al., 1984; Tsofliou et al., 2003;  
81 Unick et al., 2010) have reported no changes in hunger and/or energy intake. Despite  
82 the majority of studies reporting a consistent lack of an acute effect of exercise on  
83 energy intake, most of these studies have assessed energy intake in only one  
84 subsequent meal one to two hours post exercise (Finlayson et al., 2009; George &  
85 Morganstein, 2003; Hagobian et al., 2012; Larson-Meyer et al., 2012; Tsofliou et al.,  
86 2003; Unick et al., 2010), so any compensation that may have occurred later on the  
87 day or during subsequent days was not measured.

88

89 According to the United Nations, oral contraceptives are the most common modern  
90 contraceptive method (including both reversible and non-reversible methods) in  
91 developed countries and the third most common in developing countries (United  
92 Nations Department of Economic and Social Affairs, 2009). Oral contraceptives  
93 (OCs) have now become a feature of everyday life, with globally, nearly 200 million  
94 women taking the “pill” packet on a daily basis (Chadwick et al., 2012). However,  
95 there is little evidence of the effects of exercise on appetite and energy intake in  
96 women taking OCs. For instance, only one study has provided information on the use  
97 of oral contraceptives by all participants (Hagobian et al., 2012), while several of

98 these studies (George & Morganstein, 2003; Kissileff et al., 1990; Maraki et al., 2005;  
99 Reger et al., 1984) examined premenopausal women without controlling variables  
100 such as the regularity of the menstrual cycles, premenstrual or unusual menstrual  
101 symptoms, menstrual phase when testing and the use of hormonal contraceptive  
102 preparations. This is despite research suggesting higher energy intakes at the luteal  
103 phase than follicular phase and that women prone to premenstrual or unusual  
104 menstrual symptoms have greater fluctuations of energy intake and appetite (Dye &  
105 Blundell, 1997). Moreover, some studies examining the effects of OCs on energy  
106 intake reported an increase (Eck et al., 1997; Naessen et al., 2007) and others no  
107 difference (Bancroft & Rennie, 1993; McVay et al., 2011; Tucci et al., 2010).

108

109 Other limitations include the use of *ad libitum* buffet-style meals (George &  
110 Morganstein, 2003; Reger et al., 1984, Tsofliou et al., 2003; Unick et al., 2010), the  
111 lack of definition of participants' inactivity (Reger et al., 1984; Tsofliou et al., 2003),  
112 the estimation of energy expenditure using heart rate equations (George &  
113 Morganstein, 2003; Maraki et al., 2005) and the lack of measurement of energy  
114 expenditure (Tsofliou et al., 2003). Therefore, the present study sought to overcome  
115 some of these limitations by controlling for participants' premenstrual or unusual  
116 menstrual symptoms, menstrual phase when testing and the use of hormonal  
117 contraceptive preparations. In addition, this study increased the observation period to  
118 four days and used well-controlled and validated methods to measure *ad libitum*  
119 energy intake in the laboratory and free-living energy expenditure.

120

121 No study has examined acute effects of an acute bout of exercise on food intake and  
122 physical activity energy expenditure while directly comparing active and inactive

123 women taking oral contraceptives. Findings from this study will inform whether an  
124 exercise challenge will alter these groups' physical activity and energy intake over a  
125 number of days.

126

## 127 **Methods**

### 128 **Participants**

129 With institutional ethics approval, twenty-nine healthy women were recruited. Nine  
130 participants withdrew from the study stating personal reasons (n=4), not able to find  
131 suitable dates for the experimental days (n=3), not liking the breakfast provided (n=1)  
132 and feeling uncomfortable wearing the Actiheart (n=1). Therefore, 20 healthy, active  
133 (n=10; age  $22.6 \pm 3.6$  years; body mass  $61.4 \pm 4.4$  kg; body mass index  $21.9 \pm 1.3$   
134  $\text{kg}\cdot\text{m}^{-2}$ ) and inactive (n=10; age  $22.3 \pm 3.2$  years; body mass  $60.1 \pm 4.3$  kg; body mass  
135 index  $21.6 \pm 2.0$   $\text{kg}\cdot\text{m}^{-2}$ ) women completed the study. Participants were non-smokers,  
136 had regular menstrual cycles (21-35 days), were not pregnant or lactating, had no  
137 known history of cardiovascular or metabolic diseases, were not dieting, had a stable  
138 body mass ( $\pm 2$  kg) for 6 months before the study and were not taking any medication  
139 except oral contraceptives (16 participants were taking combined oral contraceptives  
140 and 4 progesterone-only pills). Severity of premenstrual symptoms was assessed  
141 through the shortened premenstrual assessment form (SPAF; Allen et al., 1991) that  
142 consists of 10 items rated on a scale from 1 (not present or no change from usual) to 6  
143 (extreme change, perhaps noticeable even to casual acquaintances). The mean score  
144 for the SPAF for the active and inactive groups were  $16.8 \pm 6.8$  and  $17.6 \pm 5.8$ ,  
145 respectively with no participant scoring greater than 28 (scores greater than 30 are  
146 indicative of moderate premenstrual symptoms) (Allen et al., 1991). Participants

147 mean score for cognitive restraint based on the revised version of the Three-Factor  
148 Eating Questionnaire (Karlsson et al., 2000) was  $11.6 \pm 3.1$  for the active and  $10.5 \pm$   
149  $3.3$  for the inactive group with all participants having a cognitive restraint score lower  
150 than 18. Self-reported weekly physical activity assessed by a modified version of  
151 Godin Leisure-Time Exercise Questionnaire (GLTEQ) (Godin & Shepard, 1985) was  
152 used to allocate participants to the active (engaged in regular exercise and undertaken  
153 at least 150 minutes per week of moderate-intensity physical activity i.e., physical  
154 activity that noticeably increases breathing, sweating and heart rate and is between  
155 12-14 in the 6-20 rating of perceived exertion scale), and inactive groups (did not  
156 engage in regular exercise and did not meet the minimum physical activity  
157 recommendation guidelines of 150 minutes of moderate-intensity physical activity per  
158 week) (Department of Health, 2004). Veracity of self-reported measures of physical  
159 activity was confirmed with a posteriori analysis of the Actiheart data. These data  
160 calculated individual Physical Activity Level (PAL) by dividing participants' total  
161 energy expenditure in a 24-hour period by their basal metabolic rate. The active group  
162 had a mean PAL of  $1.79 \pm 0.13$  and the inactive  $1.56 \pm 0.15$ , which according to the  
163 classification of lifestyles in relation to PAL in adults (WHO, 2004) identified them as  
164 having an active to moderately active lifestyle (1.70-1.99) and a sedentary to light  
165 activity lifestyle (1.40-1.69), respectively.

## 166 **Design and procedure**

167 To minimise participant-expectancy effects, participants were blinded about the true  
168 purpose of the study (effects of an acute bout of exercise on immediate and  
169 subsequent three days energy intake and expenditure) and were informed that the  
170 investigation was assessing how food and physical activity affected mood.



171 Before the experimental days, participants attended the laboratory for one preliminary  
172 session consisting of two exercise tests (submaximal and maximal cycling tests),  
173 screening and habituation with all procedures. After the preliminary session,  
174 participants were allocated either to the active or inactive group and completed the  
175 study in a randomised, crossover fashion with approximately 4 weeks (time varied  
176 according to participants' menstrual cycle) between both conditions (exercise and  
177 control). Experimental days were booked during the first week they restarted taking  
178 the oral contraceptives or, if continuous, when a new pack was started. This control  
179 means that findings are limited to the mechanisms operating at the examined stages,  
180 however, this was undertaken to minimise possible effects of sex hormones in energy  
181 intake (Dye & Blundell, 1997) and expenditure (Bowen et al., 2011). The  
182 experimental days were completed on the same day of the week to control for dietary  
183 and physical activity habits. Additionally, participants were asked to refrain from  
184 consuming alcohol or caffeine and taking part in vigorous physical activity in the 24  
185 hours prior to each experimental day and to record their food intake for two days  
186 before the first experimental day. This allowed participants to keep their activity  
187 patterns consistent between conditions and replicate their food intake during the two  
188 days before the second experimental day.

189

190 On the experimental days, participants arrived at the laboratory between 8.00 and  
191 9.30am after a 10-hour overnight fast with only water consumption permitted (Figure  
192 1). On arrival participants consumed a standard breakfast within 15 minutes. On the  
193 exercise experimental day, participants rested for one hour, cycled for one hour at  
194 50% of maximum oxygen uptake and then rested for another hour. On the control  
195 experimental day this was equivalent to three hours of rest (participants had to remain

196 seated while working, reading or listening to music and were monitored to ensure that  
197 they abstained from any food related cues) from the end of breakfast until the  
198 beginning of lunch. After eating the *ad libitum* lunch participants were fitted with an  
199 Actiheart and given a food diary that was used to estimate three-day food intake and  
200 energy expenditure. At the end of the study participants were debriefed about the true  
201 purpose of the study.

## 202 **Measures**

### 203 **Anthropometry**

204 Procedures adhered to recommendations of the International Society for the  
205 Advancement of Kinanthropometry (ISAK). Stature, body mass, waist and hip  
206 circumference were measured as previously described (Rocha et al., 2013). Body  
207 Mass Index (BMI) was calculated as body mass in kilograms divided by the square of  
208 stature in meters. Waist circumference was divided by hip circumference to determine  
209 waist to hip ratio. Percentage of body fat was obtained via a bioelectrical impedance  
210 body composition analyser InBody720 (Derwent Healthcare Ltd, Newcastle upon  
211 Tyne, UK) according to the manufacturer's instructions. Measurements were  
212 performed without shoes and socks with participants being instructed to slightly  
213 abduct their arms and remain still in the upright position. All bioelectrical impedance  
214 measurements were performed with the participants having fasted for at least two  
215 hours and without having engaged in any kind of exercise during that day.

### 216 **Submaximal and maximal cycling tests**

217 Before the tests, participants were allowed some time (no longer than 15 minutes) to  
218 warm-up and accustom themselves to cycle-ergometer exercise (model 874E,  
219 Monark, Sweden). The submaximal–incremental cycling test was completed to

220 determine the relationship between exercise intensity and oxygen consumption. The  
221 test consisted of a maximum of 16 min of continuous cycling divided into four, 4-min  
222 stages. The pedalling rate was initially set at 60 rpm but participants were allowed to  
223 choose a different rpm if they felt uncomfortable or could not maintain this cadence.  
224 Initial exercise intensity was adjusted to individual activity status with inactive  
225 participants starting at 60 W and active at 60 W or 90 W. At the end of each 4-min  
226 stage, exercise intensity was increased by 30 W. Participants were required to  
227 undertake the entire test whilst seated. A calibrated MedGraphics CPX Ultima  
228 (Medical Graphics Ltd, Gloucester, UK) gas analysis system determined oxygen  
229 consumption and carbon dioxide production. A heart rate monitor (Polar F4, Polar  
230 Electro, Kempele, Finland) was used to assess heart rate continuously which was  
231 recorded every 15 s during the last minute of each stage. In addition, ratings of  
232 perceived exertion (Borg, 1973) were assessed during the same time periods.  
233 After allowing for sufficient recovery from the sub-maximal test participants began  
234 the maximal oxygen uptake cycling test. The test involved cycling continuously  
235 through 3-min stages until volitional exhaustion. The initial pedalling rate was the  
236 same as the one chosen for the submaximal test and initial intensity of exercise was  
237 set equal to the last stage of the submaximal cycling test. At the end of each 3-min  
238 stage exercise intensity was increased by 30 W. Strong verbal encouragement was  
239 given to all participants throughout the test which was terminated when the participant  
240 failed to maintain cycling cadence for 20 consecutive seconds or signalled that they  
241 could not continue. To confirm that a true cycling-specific maximal oxygen  
242 consumption had been attained, two or more of the criteria were met: participant heart  
243 rate within 15 beats/min of age-predicted maximum heart rate ( $205.8 - 0.685(\text{age})$ )  
244 (InBar et al., 1994), an increase in oxygen consumption ( $\dot{V}O_2$ ) of less than 100 ml

245 min<sub>1</sub> despite an increase in exercise intensity, and a RER greater than 1.15. The  
246 participants maximal oxygen consumption and oxygen cost of cycling was used to  
247 ascertain the exercise intensity necessary to elicit 50% of maximal oxygen  
248 consumption.

#### 249 **Breakfast and *ad libitum* lunch meal**

250 Breakfast was standardised across conditions and quantities were determined based on  
251 individual body mass (23.6 kJ/kg of body mass). This meal consisted of a bowl of  
252 cereal (CornFlakes, Kellogg's, UK) with fresh semi-skimmed milk (Sainsbury, UK)  
253 and a glass of UHT orange juice (Drink Fresh, DCB Foodservice, UK). The *ad*  
254 *libitum* lunch meal consisted of durum wheat semolina conchiglie pasta  
255 (Granaria, Favellatos.r.l, Italy) served with tomato and mascarpone cheese sauce  
256 (FratelliSacla, S.p.A., Asti, Italy). This meal comprised 10.1% energy from protein,  
257 67.2% carbohydrate and 22.7% fat, with an energy density of 7.4 kJ/g. Cooking and  
258 cooling times were standardised across conditions and the pasta and sauce meal was  
259 served on both experimental days at a temperature of 60-65°C.

#### 260 **Hunger ratings**

261 Hunger ratings were assessed during the experimental trials with 100-mm paper  
262 version visual analogue scales (VAS) before and after breakfast, and at 30 min  
263 intervals thereafter until the end of lunch. The VAS was preceded by the question  
264 "how hungry do you feel?" anchored on the left by "not at all hungry" and on the right  
265 by "very hungry" (Flint et al., 2000). Participants placed a vertical mark through the  
266 line at the point which best matched their present feeling of hunger. The distance from  
267 the left anchor to the vertical mark was then measured with a ruler and used as the  
268 hunger score.

269 **Laboratory energy expenditure**

270 Expired air samples were collected in 150 L Douglas Bags (Harvard Apparatus,  
271 Edenbridge, Kent, UK) at 15 min intervals during the 60-minute exercise and rest  
272 period of the experimental days. Samples were analysed using an oxygen/carbon  
273 dioxide gas analyser (Dual Gas Analyser GIR250, Hitech Instruments, Luton, UK)  
274 which was calibrated before each analysis. A dry gas meter (Harvard Apparatus,  
275 Edenbridge, Kent, UK) determined expired air volumes that were corrected to STPD  
276 (standard temperature, pressure and dry gas). This method was used to ensure that  
277 participants cycled at 50% of their  $\dot{V}O_{2\max}$  and to estimate energy expenditure by  
278 indirect calorimetry (Frayn, 1983).

279 **Free-living energy expenditure**

280 Free-living energy expenditure was estimated using an Actiheart (Cambridge  
281 Neurotechnology, Cambridge, UK) that was attached to each participant's chest  
282 (lower position described in Brage et al., 2006) using electrocardiogram (ECG)  
283 electrodes (E4 T815 Telectrode, Surrey, UK). The Actihearts were set up to collect  
284 data in "HR variability" and record activity every 15 seconds. Participants were told  
285 to wear the monitor at all times, when awake or asleep including when washing or  
286 swimming. At the end of the three-day period, participants returned the Actihearts and  
287 the data were downloaded using a docking station and analysed using its commercial  
288 software. Heart rate and accelerometer data were converted to energy expenditure  
289 using the revised branched group calibration equation (Brage et al., 2007).

290 **Laboratory energy intake**

291 On each experimental day, participants ate their breakfast and *ad libitum* lunch alone  
292 in individual air-conditioned testing cubicles equipped with Sussex Ingestion Pattern

293 Monitors (SIPM). During lunch, participants were not given a specific time to finish  
294 eating but were instructed to “eat as much or as little as they wanted”. Food intake (in  
295 grams) was covertly monitored using the SIPM, which consists of a concealed digital  
296 balance (KMB-TM, Kern, Germany) connected to a PC computer. To ensure  
297 participants did not use the empty plate as an external cue to end their meal, the SIPM  
298 was programmed to prompt the participant to call the experimenter, using a call  
299 button, once at least 300 g were consumed to receive a refill. This process was  
300 repeated until the participants indicated that they had finished eating. A separate side  
301 plate was provided for participants to place cutlery when not eating with them (e.g.  
302 still chewing food) to ensure the weight of cutlery did not interfere with the food  
303 weighing process.

#### 304 **Free-living energy intake**

305 Participants were instructed to weigh and record all items of food and drink consumed  
306 both at home and outside the home in food diaries for the remainder of the  
307 experimental days and subsequent three days. All participants received guidance on  
308 how to complete the dietary record and measure food portions. When weighing was  
309 not possible, participants were asked to estimate portion sizes using standard  
310 household measures. Immediately upon receipt, food diaries were reviewed in the  
311 presence of the participant to ensure completeness and legibility, with any missing or  
312 unclear items being corrected. Food diaries were analysed to estimate energy and  
313 macronutrient intake using the dietary analysis software NetWisp (version 3.0;  
314 Tinuviel Software, Warrington, UK).

315 **Percentage of energy compensation**

316 Percentage of energy compensation was calculated for the *ad libitum* lunch meal, and  
317 for each one of the daily energy intakes (i.e. experimental day and subsequent 3 days).

318

319 To calculate the percentages compensation for the *ad libitum* lunch meals and for  
320 each day the following formulas were applied:

321

322 [(lunch energy intake in exercise condition - lunch energy intake in control condition)  
323 / (net exercise-induced energy expenditure)] x 100

324

325 [(energy intake of day A in exercise condition - energy intake of day A in control  
326 condition) / (net exercise-induced energy expenditure)] x 100

327

328 In the latter, A denotes the day for which the percentage compensation is being  
329 calculated.

330

331 When positive, the percentage compensation values indicated that over the analysed  
332 period of time, energy intake was greater in the exercise than in the control condition  
333 while negative values indicated a greater intake in the control than in the exercise  
334 condition. A value of 100% indicated complete compensation of the net exercise-  
335 induced energy expenditure (i.e. the excess energy intake at the exercise compared  
336 with the control condition matched for the net exercise-induced energy expenditure).  
337 A value of 0% indicates no compensation (i.e. energy intake was the same in both  
338 conditions).

339 **Statistical analyses**

340 Statistical Package for the Social Sciences program for windows (SPSS 19.0,  
341 Chicago, IL) was used for all analyses. Data were checked for normal distribution  
342 using histograms and Shapiro-Wilk tests. Homogeneity of variance and sphericity  
343 were checked using Levene's and Mauchley's test, respectively. Area under the curve  
344 (AUC) values for hunger were calculated using the trapezoidal rule. Net exercise-  
345 induced energy expenditure was calculated as (energy expenditure during the 60 min  
346 cycling period - energy expended during equivalent control period). Relative energy  
347 intake was calculated as lunch energy intake minus the net exercise-induced energy  
348 expenditure or the resting energy expenditure for the exercise and control condition,  
349 respectively.

350

351 Differences between groups for baseline characteristics, work rate, relative intensity  
352 of exercise (% of  $\dot{V}O_{2max}$ ), ratings of perceived exertion (RPE) during exercise and net  
353 exercise-induced energy expenditure were assessed by independent Student's t-tests.  
354 Percentages of energy compensation were compared between groups using a one-way  
355 ANOVA with the Welch test (when homogeneity of variance was violated). Two-way  
356 mixed-model ANOVAs (Group  $\times$  Condition) compared the experimental day's lunch  
357 energy intake, energy expenditure, heart rate and respiratory exchange ratio (RER).  
358 Three-way mixed-model ANOVAs (Group  $\times$  Condition  $\times$  Time) compared subjective  
359 hunger ratings, body mass on the experimental days, daily energy intake and  
360 expenditure and macronutrient intakes. In these analyses energy intake on the  
361 experimental day was calculated by summing participants' energy intake throughout  
362 the day (breakfast + *ad libitum* lunch + remainder of experimental day). However, the  
363 same formula could not be applied to macronutrient intake because the macronutrient



364 values for breakfast and lunch of the experimental day were fixed. Therefore,  
365 macronutrient intake for the experimental day is limited to the free-living period of  
366 that day (i.e. remainder of the experimental day). Post hoc tests were performed using  
367 Bonferroni adjustments when statistical significance or large effect sizes were present.  
368 Cohen's d (standardised mean difference) effect sizes were calculated by dividing the  
369 difference between means by the pooled standard deviation thus reflecting differences  
370 expressed in standard deviation units. According to Cohen's (1988) guidelines, effect  
371 sizes were conservatively interpreted as small (0.2), medium (0.5), and large (0.8)  
372 effects. In addition, 95% confidence intervals were determined for energy intake,  
373 macronutrient intake, energy expenditure and percentage of energy compensation.  
374 Means and standard deviations (mean  $\pm$  SD) are presented for all outcomes unless  
375 otherwise stated. Statistical significance was accepted at the 5% level.

376

## 377 **Results**

### 378 **Baseline characteristics**

379 Participant baseline characteristics are presented in Table 1. Active participants had  
380 greater  $\dot{V}O_{2max}$  and lower percentage of body fat than inactive participants ( $p < 0.05$ ).  
381 There were no differences in age, stature, body mass, BMI and waist-to-hip ratio.

### 382 **Body mass during the experimental days**

383 There were no main or interaction effects ( $p > 0.05$ ) for body mass on the exercise  
384 (active start vs. end:  $61.1 \pm 5.6$  kg vs.  $61.0 \pm 5.6$  kg; inactive start vs. end:  $61.1 \pm 4.3$   
385 kg vs.  $61.0 \pm 4.4$  kg) and control experimental days (active start vs. end:  $61.1 \pm 5.5$  kg  
386 vs.  $61.0 \pm 5.5$  kg; inactive start vs. end:  $60.6 \pm 4.2$  kg vs.  $60.6 \pm 4.2$  kg).

### 387 **Hunger ratings**

388 There was a main effect of time ( $p < 0.001$ ) for hunger ratings but there were no  
389 interactions or other main effects ( $p > 0.05$ ) (Fig. 2). Differences in hunger ratings  
390 were also evaluated using AUC values for the time before and after breakfast (08:45-  
391 09:00), the following hours until lunch (09:00-12:00), and the time before and after  
392 lunch (12:00-12:20). There was a main effect of time ( $p < 0.001$ ) for hunger AUC  
393 values but no interactions or other main effects ( $p > 0.05$ ).

### 394 **Exercise responses and energy expenditure on the experimental days**

395 The active participants exercised at a higher work rate than the inactive ( $70.3 \pm 11.4$   
396 W vs.  $57.4 \pm 14.2$  W;  $p = 0.039$ ), however, the relative intensity of exercise and  
397 ratings of perceived exertion were not different between the active and inactive  
398 groups ( $51.2 \pm 2.2\%$  vs.  $54.0 \pm 7.5\%$  of  $\dot{V}O_{2max}$ ;  $p = 0.27$ ; RPE:  $11.9 \pm 1.6$  vs.  $11.7 \pm$   
399  $1.2$ ;  $p = 0.79$ ). There were no main or interaction effects for RER ( $p > 0.05$ ) and only a  
400 condition ( $F(1,18) = 709.5$ ;  $p < 0.001$ ) effect for heart rate that, as anticipated, was  
401 different between the control and the exercise experimental day ( $72 \pm 11$  bpm vs.  $131$   
402  $\pm 14$  bpm,  $p < 0.001$ ). Similarly, there was only a condition ( $p < 0.001$ ) effect for the  
403 energy expenditure during the 60 minutes of exercise ( $1345 \pm 195$  kJ) and equivalent  
404 resting period ( $325 \pm 41$  kJ) and no differences between the net exercise-induced  
405 energy expenditure of active and inactive participants ( $1078 \pm 132$  kJ vs.  $964 \pm 239$   
406 kJ;  $p = 0.227$ ,  $d = 0.60$ ). There was a group effect for total energy expenditure (active  
407 vs. inactive:  $6389 \pm 1036$  kJ vs.  $4949 \pm 841$  kJ,  $p = 0.001$ ,  $d = 1.61$ ) and physical  
408 activity energy expenditure (active vs. inactive:  $2780 \pm 857$  kJ vs.  $1571 \pm 727$  kJ,  $p <$   
409  $0.001$ ,  $d = 1.60$ ) during the remainder of the experimental days. There were no other  
410 main or interaction effects ( $p > 0.05$ ).

411 ***Ad libitum* lunch energy intake on experimental days**

412 The energy intake at the *ad libitum* lunch meal for active and inactive participants on  
413 both experimental days is presented in Table 2. There was only a condition ( $p =$   
414  $0.033$ ,  $d = 0.49$ ) effect for absolute energy intake at the *ad libitum* lunch with a higher  
415 absolute energy intake in the exercise than the control condition (exercise vs. control:  
416  $3363 \pm 668$  kJ vs.  $3035 \pm 752$  kJ). After adjustment of absolute energy intake for the  
417 energy expended during the 60 min of exercise/rest (relative energy intake, REI),  
418 there was a condition effect ( $F(1,18) = 19.723$ ;  $p < 0.001$ ,  $d = -1.00$ ) with a lower REI  
419 in the exercise than the control condition ( $2019 \pm 746$  kJ vs.  $2710 \pm 712$  kJ).

420 **Daily energy expenditure**

421 Total free-living energy expenditure indicated that active participants expended more  
422 energy than inactive participants over the course of the three days ( $F(1,18) = 15.817$ ;  
423  $p = 0.001$ ,  $d = 1.63$ , mean difference = 1573 kJ; 95% CI 597 to 2548 kJ). This  
424 difference can be explained by the differences in physical activity energy expenditure  
425 during this period, which was higher in the active than the inactive group ( $3639 \pm 787$   
426 kJ vs.  $2363 \pm 767$  kJ,  $p < 0.001$ ). There were no other main effects or interactions ( $p >$   
427  $0.05$ ) for daily energy expenditure.

428 **Daily energy intake**

429 Daily energy intake for both groups is shown in Figure 3. One participant in the  
430 inactive group did not complete the full four-day food diary, therefore analyses were  
431 made with 10 active and 9 inactive participants. There was a time ( $p = 0.003$ ) and  
432 group ( $p = 0.036$ ) effect and a trend with a large effect size for a condition x group x  
433 time interaction ( $p = 0.056$ ;  $d = 0.80$ ) for daily energy intake. Pairwise comparisons  
434 showed that energy intake was greater on the experimental days ( $10180 \pm 1670$  kJ)

435 than the subsequent first ( $8535 \pm 2511$  kJ,  $p = 0.027$ ,  $d = 0.81$ ), second ( $8531 \pm 2330$   
436 kJ,  $p = 0.022$ ,  $d = 0.84$ ) and third ( $8364 \pm 2459$  kJ,  $p = 0.024$ ,  $d = 0.91$ ) days and that  
437 inactive participants had a higher mean energy intake over the four days than the  
438 active group ( $9431 \pm 1168$  kJ vs.  $8385 \pm 1364$  kJ,  $p = 0.036$ ,  $d = -0.86$ ). Post hoc  
439 analysis did not show any differences in the active group and inactive participants had  
440 only a decrease in energy intake on the first day after the exercise experimental day  
441 compared with the same day of the control condition (mean difference =  $-1974$  kJ;  
442 95% CI  $-1048$  to  $-2900$  kJ,  $p = 0.002$ ,  $d = -0.89$ ).

#### 443 **Daily macronutrient intake**

444 There were no main or interaction effects for the percentage of energy consumed from  
445 protein, fat and carbohydrate ( $p > 0.05$ ) but there was a trend with a large effect size  
446 for condition x group interaction for energy consumed from fat ( $p = 0.055$ ,  $d = -1.35$ ).  
447 Post hoc analysis demonstrated that only the active group consumed less fat in the  
448 exercise than the control condition (mean difference =  $-6\%$ ; 95% CI  $-11$  to  $-2\%$ ,  $p =$   
449  $0.012$ ,  $d = -1.10$ ). No other differences were observed in the inactive and active group.

#### 450 **Percentages of energy compensation**

451 Percentages of energy compensation are presented in Figure 4. There were no  
452 significant between group differences for the percentages of energy compensation for  
453 the *ad libitum* lunch (active:  $43 \pm 67\%$  vs. inactive:  $16 \pm 67\%$ ;  $p = 0.63$ ,  $d = 0.42$ ),  
454 experimental day (active:  $109 \pm 208\%$  vs. inactive:  $-49 \pm 216\%$ ;  $p = 0.08$ ,  $d = 0.78$ ),  
455 day one (active:  $53 \pm 346\%$  vs. inactive:  $-176 \pm 150\%$ ;  $p = 0.053$ ,  $d = 0.90$ ), day two  
456 (active:  $-91 \pm 293\%$  vs. inactive:  $161 \pm 371\%$ ;  $p = 0.21$ ,  $d = -0.80$ ) or day three  
457 (active:  $34 \pm 267\%$  vs. inactive:  $1 \pm 253\%$ ;  $p = 0.40$ ,  $d = 0.13$ ) after the experimental  
458 day. Nevertheless, the moderate to large effect sizes for the experimental day, day one

459 and day two after the experimental day suggest possible between group compensatory  
460 differences on these days. The cumulative percentage of energy compensation over  
461 the four days was not significantly different between groups (active:  $104 \pm 718\%$  vs.  
462 inactive:  $-62 \pm 631\%$ ;  $p = 0.32$ ,  $d = 0.26$ ).

## 463 **Discussion**

464 The present study is the first to examine the effects of an acute bout of low-intensity  
465 aerobic exercise on immediate and subsequent three-day energy intake and  
466 expenditure in active and inactive women taking oral contraceptives. The main  
467 findings arising from this study are that an acute bout of low-intensity aerobic  
468 exercise elicited an increase in *ad libitum* energy intake, did not induce significant  
469 changes in energy intake over the free-living period in active participants and induced  
470 a suppression of energy intake on the first day after the experimental day in inactive  
471 participants. Additionally, groups did not differ in physical activity energy  
472 expenditure between conditions suggesting that there were no acute compensatory  
473 changes to physical activity.

474

475 In contrast to our previous study (Rocha et al., 2013), there were no differences  
476 between the net exercise-induced energy expenditure in active and inactive women  
477 participants in the present study. This occurred despite both groups exercising at the  
478 same relative intensity and is possibly explained by the differences between groups'  
479 aerobic capacity being less in the present study. There were no changes in body mass  
480 between conditions suggesting that participants remained in energy balance during the  
481 period of time between the first and the second experimental day. Moreover,  
482 participants remained in fluid balance during the laboratory period of the

483 experimental days as no changes were observed in body mass from the start to the end  
484 of the exercise/rest periods.

485

486 There were no differences in subjective hunger ratings either between groups or  
487 conditions in this study. This finding is in agreement with recent studies in active  
488 (Finlayson et al., 2009) and inactive women (Unick et al., 2010) and men (Rocha et  
489 al., 2013). However, the relationship between exercise intensity and hunger has not  
490 been consistently reported in women making it difficult to ascertain if this finding is  
491 attributable to the low exercise intensity ( $\approx 50\%$  of  $\dot{V}O_{2\max}$ ) used in this study. For  
492 instance, previous studies have reported no effects on hunger using cycling (King et  
493 al., 1996), decreases after running (Reger et al., 1984) and an increase after a  
494 combination of aerobic and resistance exercise (Maraki et al., 2005), suggesting that,  
495 in women, the acute effect of exercise on hunger is also determined by the type of  
496 exercise undertaken.

497

498 There was an overall condition effect on absolute energy intake at the *ad libitum*  
499 lunch meal that was greater during the exercise than the control experimental day.  
500 This finding is not supported by previous studies in active (Hagobian et al., 2012;  
501 Lluch et al., 2000; Larson-Meyer et al., 2012) and inactive women (George &  
502 Morganstein, 2003; Maraki et al., 2005; Unick et al., 2010) which have reported a  
503 lack of an exercise-induced effect on absolute energy intake at the meal immediately  
504 after exercise. However, as previously discussed, different research designs and  
505 methodological limitations make comparisons difficult. Nevertheless, findings from  
506 the present study could be explained by a psychological drive to use food as a reward  
507 for exercising (King *et al.*, 2007) or by exercise-induced changes in the hedonic

508 response to food (Finlayson et al., 2009). In contrast, adjustment of energy intake for  
509 the energy expended during the exercise/rest period showed that both groups had a  
510 lower REI after exercise than control, suggesting that, similar to previous research in  
511 active (Hagobian et al., 2012, Pomerleau et al., 2004) and inactive women (Unick et  
512 al., 2010), participants maintained a short-term negative energy balance.

513

514 In this study, there were no significant differences in energy intake during the  
515 remainder of the experimental day or subsequent three days in the active group, a  
516 finding consistent with the only study examining the effects of exercise on daily  
517 energy intake in women (Pomerleau et al., 2004). Conversely, the inactive group had  
518 a lower energy intake on the first day after the exercise experimental day compared  
519 with control and no other differences in the remaining days. This is a novel finding  
520 and suggests that, as with our previous study in men (Rocha et al., 2013), an acute  
521 bout of exercise elicits a delayed response in inactive individuals. Despite the lack of  
522 significant differences in free-living energy intake in the active group, the mean  
523 percentages of energy compensation in the current study elicited a similar pattern to  
524 those previously observed in men (Rocha et al., 2013) suggesting that active  
525 participants may compensate quicker than inactive participants. In the present study,  
526 the energy compensation of active women was close to 100% within the experimental  
527 day (109%) whilst the same was not observed in inactive women (-49%). In addition,  
528 inactive women reduced their energy intake (-176%) on day one after experimental  
529 day before increasing it (161%) on day two after the experimental day providing  
530 further support to a more sensitive short-term appetite control in active than inactive  
531 individuals. When examining the cumulative percentage of energy compensation over  
532 the four days there were no statistical significant differences between groups.

533 Nevertheless, these values still provides important information regarding each group's  
534 overall energy compensation over the 4 days with the active group compensating for  
535 approximately all their net exercise-induced energy expenditure (104%) whereas the  
536 inactive group increased their exercise-induced energy deficit (-62%). For weight  
537 management, the latter values would, if sustained over greater durations, translate to  
538 weight loss. However, it is important to acknowledge that it is still not known what  
539 threshold, if one exists, separates active from inactive individuals. Hence, these  
540 results might not be applicable in the long-term as inactive participants will eventually  
541 become active and be able to immediately compensate for the exercise-induced  
542 energy deficits.

543

544 There were no differences between daily macronutrient intake in the exercise and  
545 control condition in the inactive group. However, the active group consumed less  
546 energy from fat over the four days of the exercise than the control condition, which is  
547 possibly explained by being more motivated to eat foods associated with restoring the  
548 expended energy (Blundell et al., 2003). Total energy expenditure and physical  
549 activity energy expenditure during the free-living period of the study were not  
550 different between conditions suggesting that both groups maintained their physical  
551 activity. These results agree with our previous findings in men (Rocha et al., 2013)  
552 suggesting that an acute bout of low-intensity aerobic exercise does not elicit  
553 compensatory changes in daily physical activity energy expenditure in premenopausal  
554 women taking oral contraceptives.

555

556 Limitations in this study should be acknowledged. Participants were young healthy  
557 women taking oral contraceptives, therefore the findings might not apply to women



558 not taking oral contraceptives, and older or obese adults. Controlling for participants  
559 menstrual cycle means that findings are limited to the mechanisms operating at the  
560 examined stages, however, this was undertaken to minimise possible effects of sex  
561 hormones in energy intake (Dye & Blundell, 1997) and expenditure (Bowen et al.,  
562 2011). Energy intake is affected by other factors that could not be controlled in the  
563 free-living so it may be that observed differences in energy intake did not arise from  
564 physiological regulatory mechanisms but from behavioural/psychological (e.g.  
565 emotional states) and/or environmental factors (e.g. presence of other people at meal  
566 times). Despite not being statistically significant the percentage of energy  
567 compensation group differences on the experimental day and subsequent day one and  
568 day two elicited moderate to large effect sizes and therefore it is possible that the low  
569 sample size in our study could have limited the statistical power to detect differences  
570 in free-living energy intake. Finally, caution should be taken when interpreting energy  
571 intake and expenditure data collected in the free-living because this is highly  
572 dependent on participants' compliance with methods and instructions making it more  
573 susceptible to errors in data collection.

574

## 575 **Conclusions**

576 This study demonstrated that an acute bout of low-intensity aerobic exercise did not  
577 elicit changes in hunger but increased energy intake at the meal immediately after  
578 exercise. Moreover, it induced a decrease in relative energy intake after exercise in  
579 both active and inactive pre-menopausal women taking oral contraceptives. There  
580 were no significant differences in active participants' daily energy intake over the four  
581 days whereas the inactive group decreased their daily energy intake on the first day

582 after the exercise experimental day compared to control suggesting a delayed  
583 exercise-induced suppression of energy intake. The percentages of energy  
584 compensation have also provided further support to a more sensitive short-term  
585 appetite control in active than inactive individuals. Moreover, there were no  
586 concomitant compensatory changes in daily physical activity energy expenditure.  
587 These findings support the use of low-intensity aerobic exercise to induce a short-term  
588 negative energy balance in inactive women, which if sustained, would translate to  
589 weight loss.

590

## 591 **Conflict of interest**

592 None of the authors had any conflict of interest regarding any aspect of this study.

## 593 **Authors' contributions**

594 J.R. conceived the study, recruited the participants, collected the data, performed the  
595 data analysis and wrote the manuscript. D.B., J.P, E.W. and C.D. obtained the  
596 funding, contributed to the design of the study and critically revised the manuscript.  
597 All authors read and approved the final manuscript.

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732

### 733 **Figures captions**

734 **Figure 1.** Schematic representation of the laboratory period of the experimental days.

735

736 **Figure 2.** Subjective feelings of hunger (n=10 per group; means  $\pm$  SEM). Hatched  
737 rectangles are consumption of meals; dark rectangle is equivalent to the 60 minutes  
738 cycling period.

739

740 **Figure 3.** Daily energy intake (n=10 for active and n=9 for inactive; means  $\pm$  SEM).

741 \*Means significantly different between conditions ( $p = 0.002$ ,  $d = -0.89$ ).

742

743 **Figure 4.** Percentages of energy compensation (N=10 for active and N=9 for inactive;  
744 means  $\pm$  SEM); Exp. = Experimental. Dashed line indicates complete compensation  
745 (100%) of the exercise-induced energy expenditure.