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1 **Appetite, food intake and gut hormone responses to intense aerobic exercise of different duration**

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16

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30

31 **ABSTRACT (250 words)**

32 **Purpose:** To investigate the effect of acute bouts of high-intensity aerobic exercise of differing durations on  
33 subjective appetite, food intake and appetite-associated hormones in endurance-trained males.

34 **Method:** Twelve endurance-trained males (age=21±2 years; BMI=21.0±1.6 kg•m<sup>-2</sup>; VO<sub>2max</sub>=61.6±6.0 mL•kg<sup>-1</sup>•  
35 min<sup>-1</sup>) completed four trials, within a maximum 28-day period, in a counterbalanced order: resting (REST); 15-  
36 minutes exercise bout (15-MIN); 30-minute exercise bout (30-MIN) and 45-minute exercise bout (45-MIN). All  
37 exercise was completed on a cycle ergometer at an intensity of ~76% VO<sub>2max</sub>. Sixty minutes post-exercise,  
38 participants consumed an *ad libitum* meal. Measures of subjective appetite and blood samples were obtained  
39 throughout the morning, with plasma analysed for acylated ghrelin, total polypeptide tyrosine-tyrosine (PYY)  
40 and total glucagon-like peptide 1 (GLP-1) concentrations.

41 **Results:** Neither subjective appetite nor absolute food intake differed between trials. Relative energy intake  
42 (intake – expenditure) was significantly greater after REST (2641±1616 kJ) compared with both 30-MIN  
43 (1039±1520 kJ) and 45-MIN (260±1731 kJ), and significantly greater after 15-MIN (2699±1239 kJ) compared  
44 with 45-MIN (condition main effect, p<0.001). GLP-1 concentration increased immediately post-exercise in 30-  
45 MIN and 45-MIN, respectively (condition-x-time interaction, p<0.001). Acylated ghrelin was transiently  
46 suppressed in all exercise trials (condition-x-time interaction, p=0.011); the greatest, most enduring suppression  
47 was observed in 45-MIN. PYY concentration was unchanged with exercise.

48 **Conclusion:** High-intensity aerobic cycling lasting up to 45 minutes did not suppress subjective appetite or  
49 affect absolute food intake, but did reduce relative energy intake, in well-trained endurance athletes. Findings  
50 question the role of appetite hormones in regulating subjective appetite in the acute post-exercise period.

51

52

53 **ABBREVIATIONS**

54 15-MIN, 15-minute exercise condition

55 30-MIN, 30-minute exercise condition

56 45-MIN, 45-minute exercise condition

57 DEBQ, Dutch Eating Behaviour Questionnaire

58 GLP-1, glucagon-like peptide 1

59 PYY, polypeptide tyrosine-tyrosine

60 REI, relative energy intake

61 REST, resting condition

62 VAS, Visual analogue scale

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64 **WORD COUNT: 5727**

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91 **INTRODUCTION**

92 High-intensity aerobic exercise ( $\geq 60\%$   $\text{VO}_{2\text{max}}$ ) commonly elicits a transient suppression of appetite in  
93 lean, recreationally active individuals (Broom *et al.*, 2007, Martins *et al.*, 2007, Broom *et al.*, 2009, Ueda *et al.*,  
94 2009b, Ueda *et al.*, 2009a, King *et al.*, 2010). This phenomenon, termed the “anorexia of exercise” (King *et al.*,  
95 1994), is often coupled with anorexigenic changes in appetite-associated hormones (Schubert *et al.*, 2014).

96 While the exercise intensity dependency of post-exercise appetite suppression appears well  
97 established, the effect of the duration of exercise is yet to be comprehensively investigated. Suppressions in  
98 appetite, accompanied by increases in the plasma concentration of satiety peptides peptide tyrosine-tyrosine  
99 (PYY) and glucagon-like peptide 1 (GLP-1) have been observed with continuous, high-intensity aerobic bouts  
100 of exercise lasting as little as 30 minutes (Ueda *et al.*, 2009a), and with intermittent exercise bouts yielding  
101 energy expenditure values of as little as ~150 kcal (~628 kJ) (Deighton *et al.*, 2013). Conversely, bouts of very  
102 low energy cost (~51 kcal (213kJ)) have elicited increases in subjective appetite (Bellissimo *et al.*, 2007). In  
103 contrast, appetite has been shown to be unaffected after continuous exercise bouts lasting as long as 90 minutes  
104 (King *et al.*, 2011a). When directly comparing exercise of different duration, Erdman *et al.* (Erdmann *et al.*,  
105 2007) observed an increase in total ghrelin with low intensity exercise (cycling at 50W), lasting 30, 60 and 120  
106 minutes, that was not duration dependent. Similarly, Broom and colleagues (Broom *et al.*, 2017) observed a  
107 comparable immediate post-exercise suppression of hunger and acylated ghrelin after 45 minutes and 90  
108 minutes of aerobic exercise at 70%  $\text{VO}_{2\text{max}}$ ; however, the suppression was more enduring after the 90-minute  
109 bout. It remains unknown whether any of the appetite-associated hormones are released in a dose-response  
110 manner to exercise duration or energy cost, or whether there is a duration or energy cost threshold for a  
111 hormonal response.

112 The transient nature of both a suppression of subjective appetite and changes in plasma appetite-  
113 associated hormone concentration means that *ad libitum* food intake can be reduced when administered in close  
114 proximity to the cessation of exercise (~10 minutes (Westerterp-Plantenga *et al.*, 1997); ~15 minutes (Kissileff  
115 *et al.*, 1990); ~30 minutes (Ueda *et al.*, 2009a); ~60 minutes (Ueda *et al.*, 2009b)), but is largely unaffected  
116 when a meal is consumed  $\geq 60$  minutes after exercise (Thompson *et al.*, 1988, King *et al.*, 1997, Martins *et al.*,  
117 2007, King *et al.*, 2010, King *et al.*, 2011b, Schubert *et al.*, 2013).

118 The majority of previous studies have used study populations of recreationally active individuals, and  
119 the study of trained individuals is limited (Howe *et al.*, 2016). Trained endurance athletes regularly complete  
120 very strenuous bouts of exercise that are of high intensity, long duration and continuous in nature. It is yet to be

121 confirmed whether appetite responses to such strenuous bouts in athletic populations is akin to exercise of a less  
122 strenuous nature in untrained individuals. It is possible that a more strenuous bout of exercise may elicit a  
123 greater and more enduring appetite suppression.

124 Any post-exercise appetite suppression could have implications for trained athletes. Post-exercise  
125 nutrition is often considered of crucial importance to optimise recovery and maximise adaptations to training  
126 (Burke, 1997). In addition, many athletes value weight management highly (Filaire *et al.*, 2007, Sundgot-Borgen  
127 *et al.*, 2013), as an increase in body mass can result in an increase in the energy cost of performing.  
128 Nevertheless, few investigations have addressed the effect of exercise on any appetite-related measures in  
129 athletic populations. Both increases (Jurimae *et al.*, 2003, Jurimae *et al.*, 2005, Jurimae *et al.*, 2006, Jürimäe *et*  
130 *al.*, 2007, Jurimae *et al.*, 2009, O'Connor *et al.*, 1995, O'Connor *et al.*, 2006) and decreases (Jurimae *et al.*, 2003,  
131 Jürimäe *et al.*, 2005) in anorexigenic gut hormones with strenuous exercise have been observed, while increases  
132 in the orexigenic hormone ghrelin have also been reported (Jurimae *et al.*, 2007, Jurimae *et al.*, 2009). These  
133 data suggest that changes in the concentration of appetite-associated hormones in response to high-intensity  
134 aerobic exercise may be affected by training status. However, it has yet to be investigated whether this translates  
135 to altered appetite and food intake responses.

136 The purpose of the current study was to address the effect of exercise duration on subjective appetite,  
137 food intake and circulating concentrations of acylated ghrelin, total PYY and total GLP-1 in trained endurance  
138 athletes, utilising high-intensity exercise bouts, akin to the habitual training of endurance athlete, lasting 15, 30  
139 and 45 minutes.

140 It was hypothesised that exercise would elicit a transient suppression of appetite in a dose-response  
141 fashion, with longer duration of exercise resulting in more enduring appetite suppression. It was surmised that  
142 this would be accompanied by anorexigenic changes to appetite-associated hormones. An enduring appetite  
143 suppression with greater exercise load of the 45 minute condition may lead to a lower post-exercise energy  
144 intake.

145

## 146 ***MATERIALS AND METHODS***

### 147 ***Participants***

148 Twelve endurance trained male athletes were recruited for the study (see **Table 1**). Inclusion criteria  
149 were: a minimum total training duration of 6 hours per week, habitual breakfast eaters, self-reported weight  
150 stable for the past 6 months, and aged between 18 and 40 years. Exclusion criteria were: a score of 3.5 or greater

151 for restricted eating on the Dutch Eating Behaviour Questionnaire (DEBQ, (van Strien *et al.*, 1986)); illness  
152 such as upper respiratory tract infections; smoking and the taking of medication likely to affect appetite or  
153 induce weight-loss. Ethical approval was obtained from the Ethics Subcommittee of the School of Sport,  
154 Exercise and Rehabilitation Sciences, University of Birmingham.

155  
156 **(Table 1)**  
157

### 158 *Study design*

159 Using a within-subject, counterbalanced, crossover study design, participants were randomly assigned  
160 to each trial condition: resting (REST), 15 minutes of cycling exercise (15-MIN), 30 minutes of cycling exercise  
161 (30-MIN), and 45 minutes of cycling exercise (45-MIN). Exercise was completed at an intensity of ~80%  
162  $VO_{2max}$ , with measures of subjective appetite and circulating hormone concentrations recorded throughout each  
163 trial.

164

### 165 *Pre-testing*

166 A single session of pre-testing preceded study trials in order to calculate the specific intensity of  
167 exercise for each participant. Participants reported to the Exercise Metabolism Laboratory, in the School of  
168 Sport, Exercise and Rehabilitation Sciences, University of Birmingham after a minimum 2 hour fast and having  
169 refrained from strenuous exercise during the previous 48 hours. The participant information pack was  
170 administered and explained, and the participant was given the opportunity to ask any questions regarding the  
171 study. Informed written consent was obtained before the completion of a health questionnaire and the DEBQ,  
172 which was used to assess the participants' habitual degree of eating restraint. Height and weight were recorded.  
173 An incremental exhaustive exercise test was completed to obtain  $VO_{2max}$ . The exercise test was carried out on an  
174 electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). The test, preceded  
175 by a ten minute warm-up at a self-selected power output, consisted of 3 minute stages, starting at a power output  
176 of 95W and increasing in increments of 35W. Breath-by-breath measures of exhaled gas, averaged every eight  
177 breaths, were recorded using Oxycon Pro (Jaeger, Wuerzburg, Germany) apparatus. Participants were adjudged  
178 to have reached the end of the test when they voluntarily stopped pedalling, if their cadence dropped to <60 rpm  
179 or if  $VO_2$  or heart rate ceased to increase with increasing workload.  $VO_{2max}$  was calculated as the highest mean  
180 value obtained for any 1 minute period. Submaximal  $VO_2$  values were obtained for each stage by disregarding  
181 data from the first 2 minutes of the stage. From the  $VO_{2max}$  value obtained, linear regression was used to

182 calculate the work output (in Watts) which would equate to an exercise intensity of 80%  $\text{VO}_{2\text{max}}$ . This value was  
183 used for each of the three exercise trials.

184

### 185 ***Procedure & protocol***

186 A minimum period of 3 days separated the pre-testing session and the first of four study trials.  
187 Participants were asked to refrain from moderate or high intensity exercise during the 24h prior to each trial. A  
188 food diary was completed for the 24h prior to the first trial, with participants asked to replicate food intake as  
189 closely as possible for the 24h prior to subsequent trials. There was a minimum wash out period of 3 days  
190 between trials, but typically trials were separated by 7 days.

191 Participants arrived at the laboratory at approximately 08:00, after a minimum 10-hour overnight fast.  
192 On arrival, and after voiding, participants were weighed (body mass was recorded at each visit to ensure  
193 participants were weight-stable throughout). A resting blood sample was obtained following the insertion of a  
194 venous cannula into the antecubital vein, prior to the measure of baseline subjective appetite.

195 The exercise bout then commenced. Exercise consisted of cycling on an electromagnetically braked  
196 cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) at a target intensity of 80%  $\text{VO}_{2\text{max}}$ , for a  
197 duration of 15, 30 or 45 minutes. During each exercise bout, exhaled gas samples were obtained intermittently  
198 to monitor  $\text{VO}_2$  and to retrospectively calculate energy expenditure. Initial monitoring during minutes 3 and 4  
199 allowed the ergometer resistance to be adjusted to achieve the target  $\text{VO}_2$ . A blood sample was obtained at the  
200 half way point of the exercise trial. At 5 minute intervals throughout the exercise bout, measures of heart rate  
201 and perceived exertion were obtained. The mean of these values were calculated for the entire bout.

202 Exercise ceased upon reaching the exercise duration target. A blood sample was obtained and measures  
203 of subjective appetite were completed immediately post-exercise. The 60 min rest period then commenced,  
204 during which the participant sat reading or watching television. Subjective appetite measures and blood samples  
205 were collected at 20, 40 and 60 minutes post-exercise. In REST, the participant remained sedentary throughout,  
206 resting for an additional 30 minutes to equate to the median duration of time spent exercising in the three  
207 exercise trials.

208 At 60 minutes post-exercise, the participant consumed an *ad libitum* breakfast meal. This consisted of a  
209 buffet, offering the following food: cornflakes, semi-skimmed milk, sugar, bread, margarine, strawberry jam,  
210 orange juice and apple juice (nutritional information shown in Appendix 1). All food was pre-weighed and  
211 presented in excess. After volitional satiation was reached, the remaining food was weighed and subtracted



212 from the known quantity provided, allowing for determination of consumed food. From this, energy intake was  
213 calculated. As the macronutrient content of each food item was known, absolute (grams) and relative  
214 (percentage of total energy) macronutrient intake was also calculated.

215

### 216 ***Measures***

217 Post-exercise energy intake was assessed using the *ad libitum* breakfast test meal, as described above.  
218 A carbohydrate-rich breakfast meal was selected and participants were screened prior to enrolment in the study  
219 to ensure that they habitually consumed breakfasts of a similar composition. Participants were allocated a  
220 maximum of 15 minutes in which to complete the meal. Subjective appetite was assessed using the 4-question,  
221 150mm, visual analogue scale (VAS) test for subjective appetite, as adapted from Hill & Blundell (Hill &  
222 Blundell, 1982). Measures of “hunger,” “fullness,” “desire to eat,” and “expected food intake” were obtained. A  
223 composite appetite score was calculated to simplify data analysis and presentation (Holliday & Blannin, 2017).  
224 (Composite appetite score = hunger score + desire score + expected intake score + (150-fullness score). The  
225 fullness score was reversed due to its opposing direction to the other three questions).

226

### 227 ***Blood sampling and analysis***

228 All blood samples were immediately transferred to disodium EDTA-treated tubes for analysis of  
229 appetite-associated hormones. For the measure of PYY, GLP-1 and acylated ghrelin concentrations, test tubes  
230 were pre-treated with the protease inhibitors DPP IV inhibitor (Millipore, MA, USA) and 4- (2 – Aminoethyl)  
231 benzenesulfonylfluoride hydrochloride (AEBSF, Alexis Biochemicals, Lausen, Switzerland). Blood was  
232 centrifuged at 3000 RPM and at a temperature of 4°C for 15 minutes to isolate plasma. Plasma was separated  
233 and transferred to micro tubes for later analysis. Two micro tubes were pre-treated with hydrochloric acid (1N,  
234 100microlitres per millilitre of plasma) to further protect acylated ghrelin from degradation. Plasma was stored  
235 at -70°C until hormone assays were conducted. Acylated ghrelin, total PYY and total GLP-1 were measured in  
236 duplicate using ELISA (Human Ghrelin(active) ELISA kit, Millipore, MA, USA; Human PYY(total) ELISA  
237 kit, Millipore, MA, USA; Multi Species GLP-1(total) ELISA kit, MA, USA). The sensitivity of these ELISA  
238 kits were 8 pg•ml<sup>-1</sup>, 1.4 pg•ml<sup>-1</sup> and 1.5 pg•ml<sup>-1</sup> respectively and the coefficient of variation values were 2.36%,  
239 5.26% and 3.28% respectively.

240

### 241 ***Statistical analysis***

242 Data are presented as means  $\pm$  standard deviation (SD) in tables and text and as mean  $\pm$  standard error  
243 of the mean (SEM) in figures. For the determination of differences in energy intake from the test meal between  
244 each exercise condition, a one-way analysis of variance (ANOVA) with repeated measures was conducted. To  
245 compare differences in both subjective appetite and plasma concentration of appetite-associated hormones with  
246 time and between trial conditions, a 2-way factorial ANOVA with repeated measures was conducted. Post-hoc  
247 pairwise comparisons were conducted using the Bonferroni correction for multiple comparisons. For all  
248 analyses of variance, there were no significant between-condition differences at baseline. The Shapiro Wilks test  
249 for normality revealed that data for all outcome measures were normally distributed.

250 To further investigate the relationship between changes in appetite hormones and changes in subjective  
251 appetite with exercise, correlation analysis was conducted for within-subject comparisons. For data of REST,  
252 subjective appetite scores and hormone concentrations immediately prior to the *ad libitum* test meal were  
253 correlated with energy intake using Pearson product moment correlation. Hormone concentration was correlated  
254 with appetite scores immediately prior to the test meal, as well as at t=0. Data of all three exercise trials were  
255 collated and Pearson product moment correlation coefficients with repeated observations were calculated, as  
256 described by Bland & Altman (Bland & Altman, 1995). With this collated data, subjective appetite scores and  
257 hormone concentrations immediately prior to the *ad libitum* test meal were correlated with energy intake.  
258 Hormone concentrations were correlated with subjective appetite scores immediately prior to the test meal. To  
259 assess the relationship between changes in appetite-associated hormones and subjective appetite in the  
260 immediate post exercise period, absolute values at t=0 and change-from-baseline values at t=0 were correlated  
261 for hormone concentration and subjective appetite scores.

262 A statistical significance level of  $p < 0.05$  was used throughout. When significant differences were  
263 observed, effect sizes were calculated. For all analyses of variance (ANOVA), effect size was calculated as partial  
264 eta squared ( $\eta^2_p$ ). For pairwise comparisons of note effect size was calculated as Cohen's d (d) and 95% confidence  
265 intervals (CI) are expressed. All statistical analysis was carried out using the SPSS software programme (SPSS  
266 inc., Chicago, Illinois, USA).

267 An a priori power calculation was conducted using data from an unpublished study conducted within our  
268 laboratory (effect size  $\eta^2_p = 0.291$  from a repeated measures factorial ANOVA model. A. Holliday & A.K.  
269 Blannin). Attributing subjective appetite as the primary outcome measure, and using an alpha level of 0.05 and a  
270 statistical power of 0.8, the calculation yielded a required sample size of 12 participants. This sample is powered

271 to detect a medium effect ( $f=0.62$  in the power calculation) which, based on the aforementioned unpublished data,  
272 represents minimum difference of 12% in subjective appetite.

273

## 274 **RESULTS**

### 275 ***Exercise trials***

276 The characteristics of the four trial conditions are shown in **Table 2**. Absolute and relative intensity  
277 ( $\text{VO}_2$  and  $\% \text{VO}_{2\text{max}}$ ) did not differ between exercise trials. There was, however, a trend for a main effect of trial  
278 for mean power output ( $p=0.052$ ). Rating of perceived exertion was significantly lower for 15-MIN than both  
279 30-MIN and 45-MIN, with no difference between 30-MIN and 45-MIN. The energy expenditure of the exercise  
280 bouts differed significantly between the three exercise conditions ( $p<0.001$ ), resulting in significant differences  
281 in total energy expenditure of the entire trial period between the four conditions ( $p<0.001$ ).

282

283 **(Table 2)**

284

### 285 ***Subjective Appetite***

#### 286 **VAS**

287 Appetite profiles obtained using the VAS technique are shown in **Figure 1** for each condition. There  
288 was no condition x time interaction ( $p=0.083$ ,  $\eta^2_p=0.163$ ), nor condition main effect ( $p=0.244$ ,  $\eta^2_p=0.119$ ). There  
289 was a significant main effect for time ( $p<0.001$ ,  $\eta^2_p=0.779$ ), which showed that appetite rose from the cessation  
290 of exercise ( $t=0$ ) until the test meal ( $t=60$ ), before falling after feeding.

291

292 **(Figure 1)**

293

### 294 ***Food intake at the ad libitum test meal***

295 The mean energy intake values for each of the four trial conditions are shown in **Figure 2a**. There was  
296 no condition effect for energy intake ( $p=0.223$ ,  $\eta^2_p=0.130$ ), suggesting that intakes were similar (REST =  
297  $3268\pm 1397$  kJ, 15-MIN =  $3474\pm 1233$  kJ, 30-MIN =  $3636\pm 1254$  kJ, 45-MIN =  $3769\pm 1591$  kJ). When  
298 accounting for the energy expenditure of exercise and assessing relative energy intake (REI, intake–  
299 expenditure), there was a significant main effect for condition ( $p=0.003$ ,  $\eta^2_p=0.573$ , **Figure 2b**). REI was  
300 significantly greater in REST ( $2641\pm 1616$  kJ) versus 30-MIN ( $1039\pm 1520$  kJ,  $p<0.001$ ,  $d=1.03$ , 95% CI=908 –

301 2297 kJ) and 45-MIN ( $260\pm 1731$  kJ,  $p=0.001$ ,  $d=1.42$ , 95% CI= $1113 - 3648$  kJ), while REI in 45-MIN was also  
302 significantly lower than 15-MIN ( $2699\pm 1239$  kJ,  $p=0.039$ ,  $d=1.62$ , 95% CI= $-4761 - -117$  kJ). There were no  
303 significant differences in macronutrient content of the food consumed between the four conditions (**Table 3**).

304

305 (**Figure 2**)

306

307 (**Table 3**)

308

309 *Plasma appetite-associated hormone concentrations*

310

311 *Acylated ghrelin*

312 There was a significant condition x time interaction for acylated ghrelin concentration ( $p=0.011$ ,  
313  $\eta^2_p=0.285$ . **Figure 3**). Post-hoc analysis of between-condition comparisons showed that, immediately post-  
314 exercise ( $t=0$ ), acylated ghrelin was significantly lower in 45-MIN ( $198\pm 29$   $\text{pg}\cdot\text{mL}^{-1}$ ) versus REST ( $369\pm 48$   
315  $\text{pg}\cdot\text{mL}^{-1}$ ,  $p=0.009$ ,  $d=1.307$ , 95% CI= $-286 - -40$   $\text{pg}\cdot\text{mL}^{-1}$ ). Concentrations were also lower than REST in 15-  
316 MIN ( $273\pm 42$   $\text{pg}\cdot\text{mL}^{-1}$ ) and 30=MIN ( $246\pm 33$   $\text{pg}\cdot\text{mL}^{-1}$ ) immediately post-exercise, with these differences  
317 approaching statistical significance ( $p=0.077$ ,  $d=0.910$ , 95% CI= $-193 - 8$   $\text{pg}\cdot\text{mL}^{-1}$  and  $p=0.055$ ,  $d=0.971$ , 95%  
318 CI= $-239 - 2$   $\text{pg}\cdot\text{mL}^{-1}$ , respectively). The difference in acylated ghrelin concentration between 45-MIN and 30-  
319 MIN also approached significance ( $p=0.057$ ,  $d=0.963$ , 95% CI= $-89 - 1$   $\text{pg}\cdot\text{mL}^{-1}$ ). The difference in plasma  
320 ghrelin concentration between 45-MIN and REST remained significant at  $t=20$  ( $239\pm 35$   $\text{pg}\cdot\text{mL}^{-1}$  vs.  $365\pm 47$   
321  $\text{pg}\cdot\text{mL}^{-1}$ ,  $p=0.023$ ,  $d=1.096$ , 95% CI= $-203 - -14$   $\text{pg}\cdot\text{mL}^{-1}$ ). There were no significant differences between  
322 conditions at  $t=40$  onwards.

323 Within-condition comparisons showed that acylated ghrelin concentration did not change, relative to  
324 baseline, in REST or 15-MIN. In the 30-MIN condition acylated ghrelin decreased during exercise, with a trend  
325 for lower concentration immediately post-exercise versus baseline ( $246\pm 33$   $\text{pg}\cdot\text{mL}^{-1}$  vs.  $396\pm 48$   $\text{pg}\cdot\text{mL}^{-1}$ ,  
326  $p=0.098$ ,  $d=1.093$ , 95% CI= $-305 - 16$   $\text{pg}\cdot\text{mL}^{-1}$ ). This difference approached statistical significance at  $t=20$   
327 ( $249\pm 7$   $\text{pg}\cdot\text{mL}^{-1}$  vs.  $396\pm 8$   $\text{pg}\cdot\text{mL}^{-1}$ ,  $p=0.05$ ,  $d=1.216$ , 95% CI= $-283 - 0$   $\text{pg}\cdot\text{mL}^{-1}$ ). Mean acylated ghrelin  
328 concentration decreased to the greatest extent in the 45-MIN trial, with concentrations significantly lower  
329 immediately post-exercise ( $198\pm 29$   $\text{pg}\cdot\text{mL}^{-1}$ ) and at  $t=20$  ( $239\pm 35$   $\text{pg}\cdot\text{mL}^{-1}$ ), versus baseline ( $366\pm 47$   $\text{pg}\cdot\text{mL}^{-1}$ ,  
330  $p=0.038$ ,  $d=1.269$ , 95% CI= $-312 - -7$   $\text{pg}\cdot\text{mL}^{-1}$  and  $p=0.025$ ,  $d=1.346$ , 95% CI= $-207 - -10$   $\text{pg}\cdot\text{mL}^{-1}$  respectively).

331

332 **(Figure 3)**

333

334 **PYY**

335 There was no significant condition x time interaction for PYY concentration ( $p=0.472$ ,  $\eta^2_p=0.080$ ), nor  
336 was there a significant main effect for condition ( $p=0.691$ ,  $\eta^2_p=0.252$ ) (**Figure 4**). A significant time main effect  
337 ( $p<0.001$ ,  $\eta^2_p=0.522$ ) demonstrated a decrease in PYY concentration during the post-exercise period, compared  
338 with baseline, until the test meal ( $t=60$ ).

339

340 **(Figure 4)**

341

342 **GLP-1**

343 There was a significant time x condition interaction for GLP-1 plasma concentration ( $p<0.001$   
344  $\eta^2_p=0.433$ ; **Figure 5**). Post-hoc analysis of between-condition comparisons showed that, at  $t=0$ , there was a trend  
345 for a greater GLP-1 concentration in the 30-MIN trial ( $33.4\pm 11.1$   $\text{pg}\cdot\text{mL}^{-1}$ ) compared with REST ( $26.5\pm 10.0$   
346  $\text{pg}\cdot\text{mL}^{-1}$ ,  $p=0.093$ ,  $d=0.878$ , 95% CI= $-0.9 - 14.7$   $\text{pg}\cdot\text{mL}^{-1}$ ) and a greater concentration in 45-MIN ( $38.5\pm 19.2$   
347  $\text{pg}\cdot\text{mL}^{-1}$ ) versus 15-MIN ( $28.4\pm 13.8$   $\text{pg}\cdot\text{mL}^{-1}$ ,  $p=0.076$ ,  $d=0.912$ , 95% CI= $-0.7 - 18.2$   $\text{pg}\cdot\text{mL}^{-1}$ ). At  $t=20$ , the  
348 trend for a greater concentration in 30-MIN versus REST was maintained, while plasma GLP-1 concentration  
349 was significantly greater in 45-MIN ( $40.6\pm 19.9$ ) versus 15-MIN ( $27.1\pm 13.8$   $\text{pg}\cdot\text{mL}^{-1}$ ,  $p=0.024$ ,  $d=1.121$ , 95%  
350 CI= $1.4 - 22.7$   $\text{pg}\cdot\text{mL}^{-1}$ ) and greater in 45-MIN ( $40.6\pm 19.9$ ) versus REST, with the difference approaching  
351 significance (vs.  $26.7\pm 10.1$   $\text{pg}\cdot\text{mL}^{-1}$ ,  $p=0.080$ ,  $d=0.905$ , 95% CI= $-1.1 - 25.9$   $\text{pg}\cdot\text{mL}^{-1}$ ). This elevated  
352 concentration in 45-MIN was significantly greater than both REST and 15-MIN at  $t=40$  ( $p=0.035$  and  $p=0.047$   
353 respectively) and  $t=60$  ( $p=0.012$  and  $p=0.040$  respectively), remaining higher than REST immediately after the  
354 test meal ( $p=0.035$ ). Plasma GLP-1 concentration was significantly higher in 30-MIN, compared with REST at  
355  $t=60$  ( $p=0.032$ ).

356 Post-hoc analysis of within-condition differences showed that, in 30-MIN, plasma GLP-1 increased  
357 above baseline concentration at  $t=0$  ( $33.4\pm 11.1$  vs.  $25.9\pm 9.0$   $\text{pg}\cdot\text{mL}^{-1}$ ,  $p=0.018$ ,  $d=1.418$ , 95% CI= $1.1 - 14.0$   
358  $\text{pg}\cdot\text{mL}^{-1}$ ), remaining elevated until  $t=40$ . In 45-MIN, GLP-1 concentration increased significantly above  
359 baseline at  $t=0$  ( $38.5\pm 19.2$  vs.  $25.8\pm 12.4$   $\text{pg}\cdot\text{mL}^{-1}$ ,  $p=0.043$ ,  $d=1.243$ , 95% CI= $0.2 - 22.4$   $\text{pg}\cdot\text{mL}^{-1}$ ) and stayed  
360 elevated for the remainder of the trial period.

361

362 **(Figure 5)**

363

364 ***Relationship between hormones, subjective appetite and food intake.***

365 In REST, there were no significant correlations, or trends for correlations, between hormone  
366 concentration and subjective appetite scores, either at t=0 or immediately prior to the test meal (all  $r < |0.5|$ ,  
367  $p > 0.1$ ). In addition neither VAS score nor concentrations of acylated ghrelin, PYY or GLP-1 were significantly  
368 correlated with energy intake ( $r < |0.5|$ ,  $p > 0.1$ ).

369 After exercise, there was a trend for a strong positive correlation between acylated ghrelin  
370 concentration and subjective appetite score at t=0 ( $r = 0.665$ ,  $p = 0.087$ ). However, there were no other significant  
371 relationships, or trends for relationships, between hormone concentration and subjective appetite scores, either  
372 at t=0, as change-from-baseline at t=0, or immediately prior to the test meal (all  $r < |0.6|$ ,  $p > 0.1$ ). PYY  
373 concentration immediately prior to the test meal showed a moderate, negative correlation with energy intake  
374 ( $r = -0.484$ ,  $p = 0.019$ ) and with relative energy intake ( $r = -0.417$ ,  $p = 0.048$ ). GLP concentration immediately prior  
375 to the test meal showed a moderate, negative correlation with relative energy intake ( $r = -0.599$ ,  $p = 0.002$ ), but  
376 was not significantly associated with absolute energy intake. Acylated ghrelin concentration was associated with  
377 neither absolute nor relative energy intake.

378

## 379 ***DISCUSSION***

380 The aim of the current study was to assess the effect of the duration of high-intensity aerobic exercise  
381 on subjective appetite, food intake and appetite-regulating hormones in highly-trained male endurance athletes.  
382 Subjective appetite was not significantly suppressed post-exercise in any of the three exercise conditions, with  
383 only modest, non-significant reductions of ~10-15% in appetite scores from baseline, and scores in none of the  
384 three exercise conditions differed significantly from the resting condition scores at any point in the trial period.  
385 This was despite significant responses of both acylated ghrelin and GLP-1 towards a more anorexigenic state.

386 Exercise at an intensity  $\geq 60\%$   $VO_{2max}$  often elicits a transient suppression of appetite in untrained, lean  
387 individuals (Martins *et al.*, 2007, King *et al.*, 2010, Laan *et al.*, 2010; Deighton *et al.*, 2013; Kawano *et al.*,  
388 2013). To the knowledge of the authors, the exercise of the present study is the highest intensity bout of  
389 continuous exercise utilised in research of this nature. Yet, no suppression of appetite was observed. It is  
390 possible that this lack of a commonly-observed appetite suppression is due to a difference in responses to

391 exercise between athletic and non-athletic populations. Though equivocal, studies conducted in recreationally  
392 active young males with mean  $\text{VO}_{2\text{max}}$  values of  $\sim 55\text{-}57 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ , utilising prolonged bouts of continuous  
393 exercise at  $\sim 65\text{-}70\%$   $\text{VO}_{2\text{max}}$  have elicited modest, non-significant appetite suppression similar to those of the  
394 present study (King *et al.*, 2011; Wasse *et al.*, 2013; Deighton *et al.*, 2014). These data, allied with the findings  
395 of the current study, suggest that appetite response to high-intensity aerobic exercise may be somewhat different  
396 for individuals of differing training/activity status or fitness. However, in contrast to the findings of the current  
397 study, Howe and colleagues (Howe *et al.*, 2016) did observe a suppression of appetite after both moderate-  
398 (60%  $\text{VO}_{2\text{max}}$ ) and high-intensity (80%  $\text{VO}_{2\text{max}}$ ) in trained females. The differing responses could be due to the  
399 different sex of participants (although there is currently minimal evidence to suggest sex differences in acute  
400 appetite responses to exercise in non-athletes (Thackray *et al.*, 2016)). A limitation of the present study is the  
401 lack of a female study cohort for the direct investigation of sex differences. Nonetheless, the present findings  
402 suggest that regular endurance exercise training may blunt the appetite suppression response typically observed  
403 in those less familiar with such exercise.

404 Aligned with the absence of a post-exercise appetite suppression, there was no significant difference in  
405 food intake at the *ad libitum* test meal, administered 60 minutes after the cessation of exercise. No difference in  
406 food intake post-exercise is a commonly observed finding, especially when the test meal is consumed 60  
407 minutes post-exercise (Thompson *et al.*, 1988, King *et al.*, 1997, King *et al.*, 2010, King *et al.*, 2011b, Schubert  
408 *et al.*, 2013). The absence of a reduction in food intake after exercise would indicate, in contrast to the  
409 hypothesis, that the prolonged bout of continuous, high-intensity exercise of the present study was not sufficient  
410 to induce an appetite suppression that was sufficiently enduring to influence food intake 60 minutes post-  
411 exercise. It is acknowledged that a limitation of the present study is the acute measure of food intake. There is  
412 evidence that compensatory increases in food intake can occur in the hours and days after exercise, and that this  
413 response may differ depending on physical activity status (Rocha *et al.*, 2013). Therefore, monitoring food  
414 intake over the remainder of the trial day and perhaps over the following 48 hours would have proved insightful.

415 Relative energy intake did differ between trial conditions. This has often been observed in the absence  
416 of a lower post-exercise absolute energy intake (Imbeault *et al.*, 1997, Lluch *et al.*, 2000, King *et al.*, 2010,  
417 Unick *et al.*, 2010), or even after absolute energy intake is greater post-exercise (Pomerleau *et al.*, 2004, Martins  
418 *et al.*, 2007). As such, it would appear there was a lack of an immediate compensatory increase in drive to eat  
419 after prolonged strenuous exercise and extensive energy expenditure, and that such exercise can elicit short-term  
420 energy deficit, compared with rest. However, the monitoring of food intake, physical activity and metabolic rate

421 over the course of the whole day and beyond would be required to determine likely effects of the exercise bout,  
422 and subsequent appetite response, on energy balance.

423 The post-exercise period is important for athletes with regard to nutrition for recovery and adaptation to  
424 training. Post-exercise carbohydrate intake is valued by many endurance athletes, with exercise-induced GLUT-  
425 4 translocation leading to an increased potential for glucose uptake and glycogen resynthesis after exercise (see  
426 Ivy, 1998, Goodyear *et al.*, 1998, Jentjens & Jeukendrup, 2003). In addition, amino acid delivery and a positive  
427 energy balance stimulate net muscle protein synthesis after resistance- (Tipton *et al.*, 1999) and endurance-type  
428 exercise (Howarth *et al.*, 2009), meaning that the ingestion of protein in close proximity to exercise is often  
429 desired for optimal rates of muscle protein synthesis and subsequent adaptation (Phillips, 2006, Phillips & Van  
430 Loon, 2011). Therefore, a suppression of appetite post-exercise may be detrimental for recovery and adaptation,  
431 should it impact upon nutrition. The findings of the present study would suggest that this was not the case, even  
432 after a strenuous bout of 45 minutes of cycling at 76%  $VO_{2max}$ .

433 A lack of significant exercise-induced suppression of appetite in the present study was allied with no  
434 significant change in plasma concentration of the satiety peptide PYY with exercise. While PYY concentration  
435 has commonly been observed to be responsive to high-intensity aerobic exercise (Martins *et al.*, 2007, Broom *et al.*  
436 *et al.*, 2009, Russel *et al.*, 2009, Ueda *et al.*, 2009b, Ueda *et al.*, 2009a, Larson-Meyer *et al.*, 2012), this was not  
437 the case in the present study. It is possible that this is due to the study population; well-trained athletes, familiar  
438 with exercising at such a high-intensity may be resistant to exercise-induced alterations in PYY secretion.  
439 Chronic exercise training has been postulated to sensitise satiety peptides to food intake, with greater late post-  
440 prandial period concentrations of PYY and GLP-1 with food intake after exercise training (Martins *et al.*, 2010).  
441 In addition, exercise training (Jones *et al.*, 2009) and exercise-induced weight-loss (Roth *et al.*, 2005) have been  
442 shown to increase fasting PYY concentrations. These may be mechanisms by which regular physical activity  
443 assists with tighter regulation of energy balance, through limiting over-eating. Similarly, the blunting of an  
444 exercise-induced increase in PYY with regular exercise may regulate energy balance through the avoidance of  
445 appetite suppression and increased energy deficit post-exercise. A limitation of the present study is that  
446 concentrations of total PYY were measured, rather than the active form PYY<sub>(3-36)</sub>. However, the abundance of  
447 PYY<sub>(3-36)</sub> is greater than that of PYY<sub>(1-36)</sub>, meaning that most of the total circulating PYY in plasma is in the  
448 form of PYY<sub>(3-36)</sub>. Additionally, total PYY and PYY<sub>(3-36)</sub> have been shown to respond similarly to food intake  
449 (Pfluger *et al.*, 2007), and have demonstrated similar responses to exercise of a similar nature (Deighton *et al.*,  
450 2013a and 2013b). Hence, responses of total PYY to exercise are likely to reflect those of PYY<sub>(3-36)</sub>.



451           Despite no significant suppression of appetite, there was a clear response of GLP-1 during and after  
452 exercise. Concentrations rose with exercise in the 30-MIN (29%) and 45-MIN (49%) conditions, with levels  
453 remaining elevated during the 60 minute recovery period. This finding is in agreement with previous studies in  
454 obese (Ueda *et al.*, 2009b) and healthy-weight (Martins *et al.*, 2007, Ueda *et al.*, 2009a) individuals, after  
455 exercise of an intensity lower than that of the current study, lasting 30-60 minutes. No such response was  
456 observed in the 15-MIN condition however, with the GLP-1 profile closely resembling that of REST. This is in  
457 contrast to previous studies that have observed a suppression of GLP-1 with very low volume, high-intensity  
458 interval exercise in recreationally active (Bailey *et al.*, 2015) and overweight (Martins *et al.*, 2015) individuals.  
459 These data would suggest that just 15 minutes of high-intensity cycling at 76%  $\text{VO}_{2\text{max}}$  was an insufficient  
460 stimulus to cause any exercise-induced increase in plasma GLP-1 in trained endurance athletes, and that the  
461 contrast with observations from maximal, or near maximal, high-intensity interval exercise studies could be due  
462 to the differing intensity of exercise or differing study populations. These data would also suggest that GLP-1  
463 concentration during high-intensity aerobic exercise exhibits some duration or energy expenditure dependency,  
464 possibly with a threshold duration for its secretion.

465           Acylated ghrelin also proved responsive to exercise. The plasma concentration declined with exercise  
466 in all three exercise conditions, with the greatest decrease seen after 45 minutes of cycling. This suppression of  
467 acylated ghrelin was transient, with concentrations not significantly different to baseline by 40 minutes post-  
468 exercise, even in 45-MIN; neither was there a significant difference between any exercise condition and REST  
469 at this time point. This was despite acylated ghrelin concentration being 28%, 20% and 23% lower than REST  
470 in the 15-MIN, 30-MIN and 45-MIN conditions, respectively. As with the finding of Broom *et al* (2017), this  
471 present study would indicate that acylated ghrelin responses to exercise may be duration-dependent. However,  
472 while the findings of Broom *et al.* (2017) suggest duration-dependent differences in the longevity of the  
473 suppression, the present findings suggest difference in the magnitude of the suppression. Data of the present  
474 study would suggest that, with high-intensity aerobic exercise, plasma acylated ghrelin concentration begins to  
475 decline in the very early stages of exercise and continues to decline as the bout continues. While this would  
476 suggest a physiological mechanism by which the duration of exercise is an important regulatory factor in post-  
477 exercise appetite suppression, the absence of a significant suppression of appetite (either compared with baseline  
478 or with REST) dispels this theory somewhat and also questions the role of acylated ghrelin as a regulator of  
479 appetite in the post-exercise state.

480           It would appear there are some inconsistencies in the hormonal response and appetite in the present  
481 study. Firstly, changes in acylated ghrelin and GLP-1 in favour of an anorexigenic state were not observed with  
482 PYY. It has generally been observed that alterations in appetite-associated hormones occur concurrently,  
483 especially with regards to satiety peptides (Broom *et al.*, 2009, King *et al.*, 2011a). Differential responses in  
484 PYY and GLP-1 have, however, been observed following 30 minutes of cycling at 50%  $VO_{2max}$  and 75%  
485  $VO_{2max}$  (Ueda *et al.*, 2009a). It was found that PYY secretion appeared to be exercise intensity-dependent, with  
486 concentration elevated to a greater extent after exercise at 70%  $VO_{2max}$ , compared with after exercise at 50%  
487  $VO_{2max}$ . In contrast, GLP-1 concentration increased similarly in both exercise trials. The authors suggest that  
488 their data would advocate specific exercise responses in plasma kinetics of PYY and GLP-1. The data of the  
489 present study would support the notion of a specific response, but contrasts the findings somewhat, with GLP-1,  
490 but not PYY, suggested to change in a duration- or energy expenditure-dependant manner. Further, if an  
491 increase in plasma PYY is exercise intensity-dependent, then it may be the case that athletes possess a blunted  
492 response, or have elevated their threshold intensity for PYY release.

493           Secondly, the anorexigenic stimulus of an increase in GLP-1 concentration and a decrease in acylated  
494 ghrelin was not reflected by a suppression of subjective appetite or reduced absolute food intake. Both total  
495 (Wren *et al.*, 2001) and acylated ghrelin (Druce *et al.*, 2005), have been shown to be potent appetite regulators  
496 when administered pharmaceutically in the resting state. However, some studies infused non-physiological  
497 concentrations (Wren *et al.*, 2001), while lower concentration infusion has yielded conflicting effects on food  
498 intake on overweight and lean individuals (Druce *et al.*, 2005). Studies investigating the effect of GLP-1  
499 administration, at a physiological concentration, on food intake are equivocal (Verdich *et al.*, 2001). In the  
500 present study, exercise-induced alterations that would be expected to favour an anorexigenic state did not lead to  
501 a suppression of subjective appetite in the post-exercise period.

502           Assessment of the relationships for within-subject changes in appetite, hormone concentration and  
503 energy showed little consistent association between concentration of hormones and subjective appetite, both at  
504 rest and post-exercise. There was a trend for a strong correlation between acylated ghrelin and VAS score  
505 immediately post-exercise, which does suggest that immediate post-exercise appetite responses may be  
506 mediated by changes in acylated ghrelin. However, this association was not statistically significant and was not  
507 evident at other post-exercise measures. Neither PYY nor GLP-1 were associated with subjective appetite at any  
508 time. However, PYY concentration immediately prior to the test meal was inversely related to energy intake,  
509 and both PYY and GLP-1 concentration were inversely related to relative energy intake. Such inconsistencies

510 are not uncommon (Broom *et al.*, 2007, Broom *et al.*, 2009) and there is evidence that in the post-exercise  
511 period, there is blunting to hormonal regulators of appetite. In a study by Heden *et al.* (Heden *et al.*, 2013),  
512 acylated ghrelin and subjective appetite responded differently with exercise in healthy-weight and obese  
513 individuals, and Deighton *et al.* (Deighton *et al.*, 2013) observed contrasting positive and negative correlations  
514 between acylated ghrelin and subjective appetite in the period after endurance and sprint-interval exercise,  
515 respectively, in healthy-weight males. Further, previous studies have also shown weak (Broom *et al.*, 2009;  
516 Hagobian *et al.*, 2013; Wasse *et al.*, 2013; Beaulieu *et al.*, 2014) or inconsistent (Ueda *et al.*, 2009a; Deighton *et*  
517 *al.*, 2014; Bailey *et al.*, 2015) relationships between hormone concentration and both subjective appetite and  
518 food intake, yet the relevance of such findings are largely overlooked. These data question the commonly-  
519 accepted importance of exercise-induced changes in appetite-associated hormones for appetite regulation and  
520 acute absolute energy intake. Although, the data of the present study suggests that the satiety peptides PYY and  
521 GLP-1 may influence relative energy intake. As such, it is possible that the role of these hormones is to defend  
522 against overeating and a compensation for energy expenditure, as opposed to suppressing food intake *per se*.  
523 Further investigation is required to clarify the regulatory role of these hormones, at physiological  
524 concentrations, in appetite and food intake responses, especially in the post-exercise period.

525         In conclusion, neither 15, 30 nor 45 minutes of cycling at 76%  $\text{VO}_{2\text{max}}$  significantly suppressed  
526 subjective appetite in male highly-trained endurance athletes. Acute absolute food intake was unaffected by  
527 exercise, although with no compensatory increase in energy intake, exercise of 30 minutes and 45 minutes in  
528 duration induced an acute energy deficit, compared with remaining rested. The lack of observed appetite  
529 suppression was despite a transient suppression of acylated ghrelin and a sustained increase in GLP-1, with  
530 some evidence that the concentration of these hormones change in an exercise-duration-dependent manner.  
531 These findings suggest that those accustomed to high-intensity aerobic exercise may exhibit a blunted response  
532 to exercise-induced appetite suppression, or a dissociation of appetite perception and hormonal signals post-  
533 exercise. The role of appetite-associated hormones in regulating post-exercise appetite, food intake and acute  
534 energy balance warrants further investigation.

535

536

#### 537 ***DECLARATION OF INTERESTS***

538 The authors have no competing interests to declare.

539

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546

547 ***COMPLIANCE WITH ETHICAL STANDARDS***

548 ***ETHICAL APPROVAL***

549 Ethical approval was obtained from the Ethics Subcommittee of the School of Sport, Exercise and  
550 Rehabilitation Sciences at the University of Birmingham. Ethical Review Number – ERN\_09-996. All research  
551 was performed in accordance with the 1964 Declaration of Helsinki.

552

553 ***ETHICS, CONSENT AND PERMISSION***

554 Informed written consent was obtained from each participant after both written and verbal information about the  
555 study was provided. This consent included permission to publish research data.

556

557 ***CONSENT TO PUBLISH***

558 Informed written consent was obtained from each participant after both written and verbal information about the  
559 study was provided. This consent included permission to publish research data. No personal information of any  
560 participant is included in the manuscript.

561

562 ***AVAILABILITY OF DATA***

563 The raw data is available as a supplementary article to the manuscript.

564

565 ***AUTHOR CONTRIBUTIONS***

566 AH and AB conceived the study question and study design; AH completed the data collection and data analysis;  
567 AH wrote the manuscript; AB assisted with the drafting of the manuscript.

568

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**Table 1.** Participant characteristics. Values are mean  $\pm$  SD.

|  |                 | 797 |
|--|-----------------|-----|
|  |                 | 798 |
|  |                 | 799 |
| <b>Age (years)</b>   | 21 $\pm$ 2      | 800 |
|  |                 | 801 |
| <b>Height (cm)</b>   | 179.3 $\pm$ 7.2 | 802 |
|  |                 | 803 |
| <b>Weight (kg)</b>   | 67.3 $\pm$ 5.2  | 804 |
|  |                 | 805 |
| <b>BMI (kg•m<sup>-2</sup>)</b>                                 | 21.0 $\pm$ 1.6  | 806 |
|  |                 | 807 |
| <b>VO<sub>2max</sub> (mL•kg<sup>-1</sup>•min<sup>-1</sup>)</b> | 61.6 $\pm$ 6.0  | 808 |
|  |                 | 809 |
| <b>W<sub>max</sub> (Watts)</b>                                 | 309 $\pm$ 45    | 810 |
|  |                 | 811 |
| <b>DEBQ score for restraint</b>                                | 1.9 $\pm$ 0.4   | 812 |
|  |                 | 813 |

814 VO<sub>2max</sub> = maximal aerobic capacity; RPE = rating of perceived exertion; W<sub>max</sub> = maximal work load.

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816

817 **Table 2.** Characteristics of exercise. Values are mean  $\pm$  SD.

818

|   | <b>REST</b>   | <b>15 min</b>   | <b>30 min</b>   | <b>45 min</b>   |
|---|---------------|-----------------|-----------------|-----------------|
| <b>VO<sub>2</sub> (mL•min<sup>-1</sup>)</b> | 341 $\pm$ 33* | 3150 $\pm$ 368  | 3180 $\pm$ 405  | 3138 $\pm$ 416  |
| <b>% VO<sub>2max</sub></b>                  | 6 $\pm$ 4*    | 76 $\pm$ 8      | 77 $\pm$ 8      | 76 $\pm$ 8      |
| <b>Power output (W)</b>                     | -             | 218 $\pm$ 30†   | 207 $\pm$ 30    | 207 $\pm$ 33    |
| <b>% W<sub>max</sub></b>                    | -             | 70 $\pm$ 4†     | 66 $\pm$ 6      | 66 $\pm$ 4      |
| <b>Heart rate (beats min<sup>-1</sup>)</b>  | -             | 153 $\pm$ 13    | 156 $\pm$ 15    | 157 $\pm$ 14    |
| <b>% HR<sub>max</sub></b>                   | -             | 84 $\pm$ 5      | 84 $\pm$ 5      | 86 $\pm$ 3      |
| <b>RPE</b>                                  | -             | 13 $\pm$ 1#     | 14 $\pm$ 1      | 15 $\pm$ 2      |
| <b>Energy Expenditure of bout (kJ)</b>      | 156 $\pm$ 95* | 989 $\pm$ 111*  | 1987 $\pm$ 252* | 2929 $\pm$ 381* |
| <b>EE of trial (bout + rec. period. kJ)</b> | 623 $\pm$ 52* | 1420 $\pm$ 110* | 2516 $\pm$ 157* | 3414 $\pm$ 228* |

819

820 \* significantly different to all other conditions, p < 0.001. † significantly different to 45 min, p < 0.05. #  
821 significantly different to 30 min and 45 min, p < 0.05.

822 VO<sub>2max</sub> = maximal aerobic capacity; RPE = rating of perceived exertion; W<sub>max</sub> = maximal work load.

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826 **Table 3.** Summary of food intake at the *ad libitum* test meal for each of the four conditions. Values  
 827 are mean  $\pm$  SD.  
 828

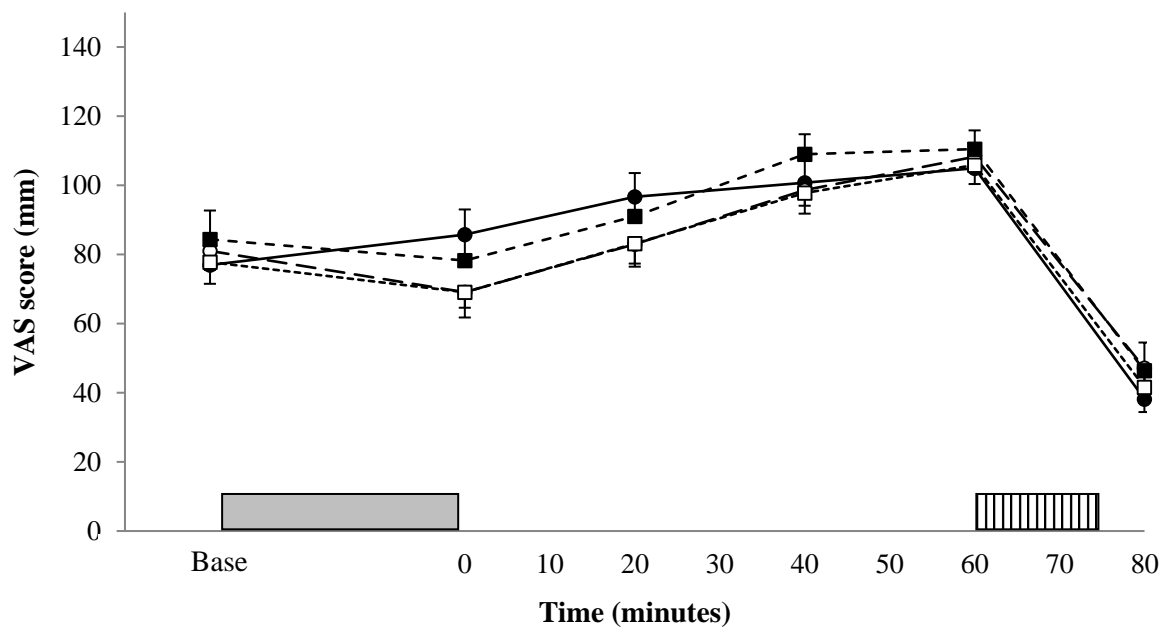
|                                | <b>REST</b>    | <b>15-MIN</b>  | <b>30-MIN</b>  | <b>45-MIN</b>   |
|--------------------------------|----------------|----------------|----------------|-----------------|
| <b>Weight consumed (grams)</b> | 735 $\pm$ 331  | 793 $\pm$ 281  | 836 $\pm$ 262  | 822 $\pm$ 264   |
| <b>Carbohydrate (g)</b>        | 148 $\pm$ 64   | 157 $\pm$ 55   | 167 $\pm$ 55   | 165 $\pm$ 57    |
| <b>% energy CHO</b>            | 76.3 $\pm$ 6.7 | 77.2 $\pm$ 5.7 | 77.5 $\pm$ 5.7 | 75.4 $\pm$ 9.3  |
| <b>Fat (g)</b>                 | 11.1 $\pm$ 6.6 | 11.3 $\pm$ 6.4 | 11.5 $\pm$ 6.4 | 17.2 $\pm$ 21.2 |
| <b>% energy fat</b>            | 13.4 $\pm$ 5.8 | 12.9 $\pm$ 4.8 | 12.5 $\pm$ 4.7 | 15.3 $\pm$ 10.6 |
| <b>Protein (g)</b>             | 20.6 $\pm$ 9.4 | 20.9 $\pm$ 8.2 | 22.3 $\pm$ 9.2 | 20.3 $\pm$ 8.3  |
| <b>% energy protein</b>        | 10.3 $\pm$ 2.2 | 9.9 $\pm$ 2.0  | 10.0 $\pm$ 2.1 | 9.3 $\pm$ 2.3   |

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832 **Figure 1**



833

834 **Figure 1.** Mean appetite scores, as measured using VAS. ●, solid line = REST; ○, large dash = 15-MIN; ■,  
 835 medium dash = 30-MIN; □, small dash = 45-MIN. Hollow rectangle = exercise; filled, black rectangle = *ad*  
 836 *libitum* breakfast meal. Values are mean  $\pm$  SEM.  
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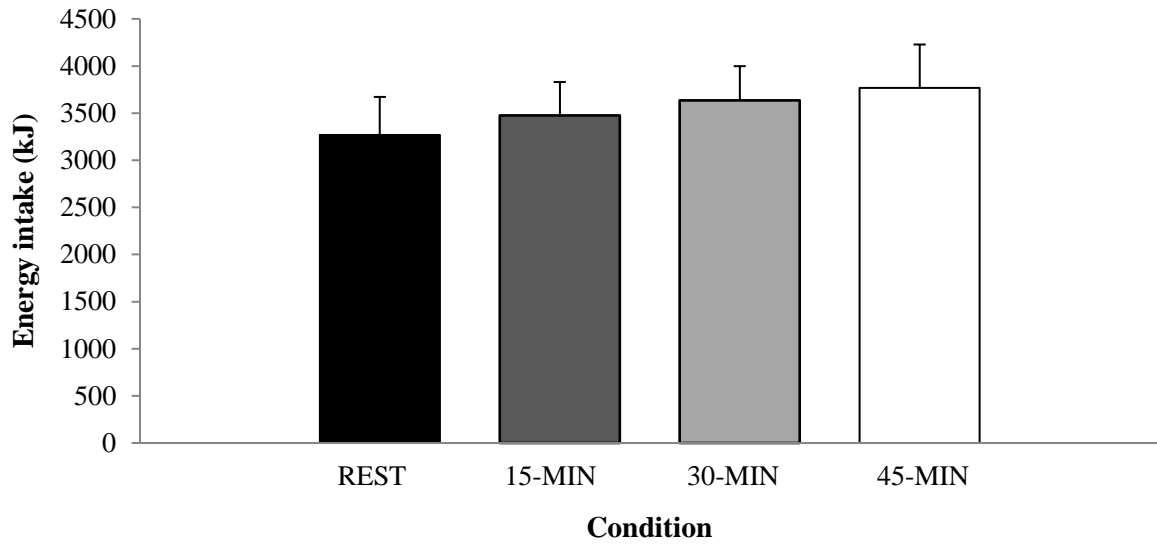
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842 **Figure 2**

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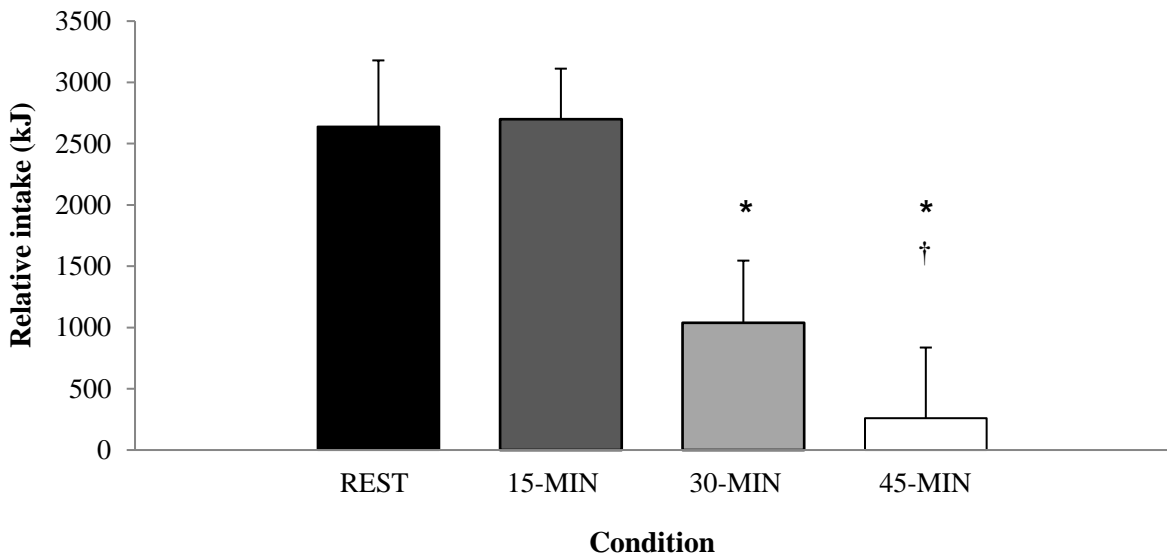
844 (a)



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847 (b)



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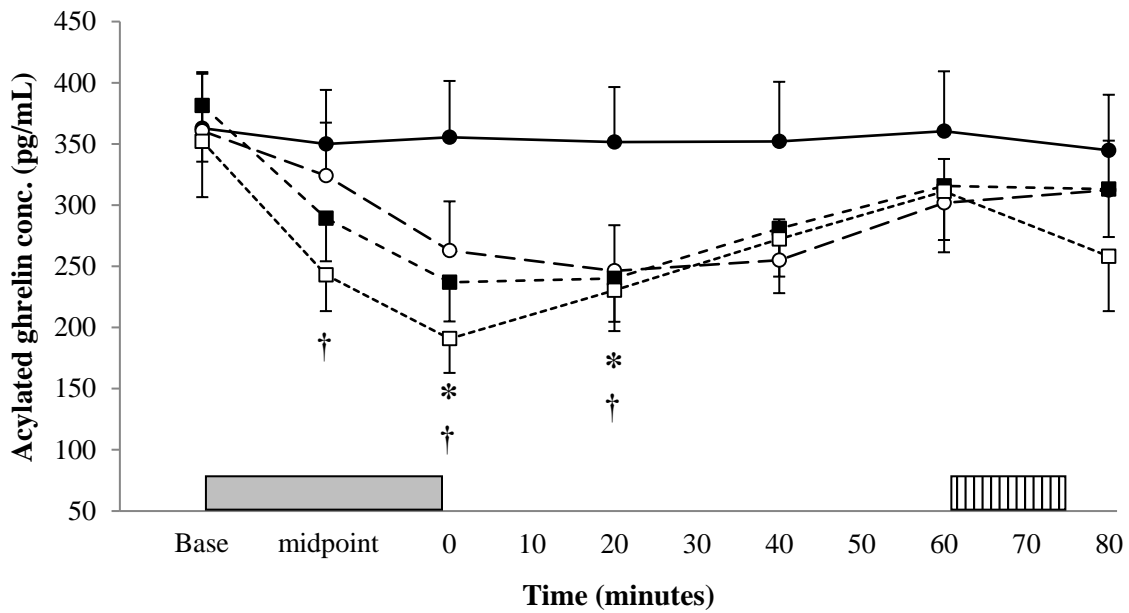
850 **Figure 2.** Energy intake (a) and relative energy intake (b) at the *ad libitum* breakfast test meal for REST, 15-  
851 MIN, 30-MIN and 45-MIN. Values are mean  $\pm$  SEM. \* significantly lower than REST. † significantly lower  
852 than 15-MIN.

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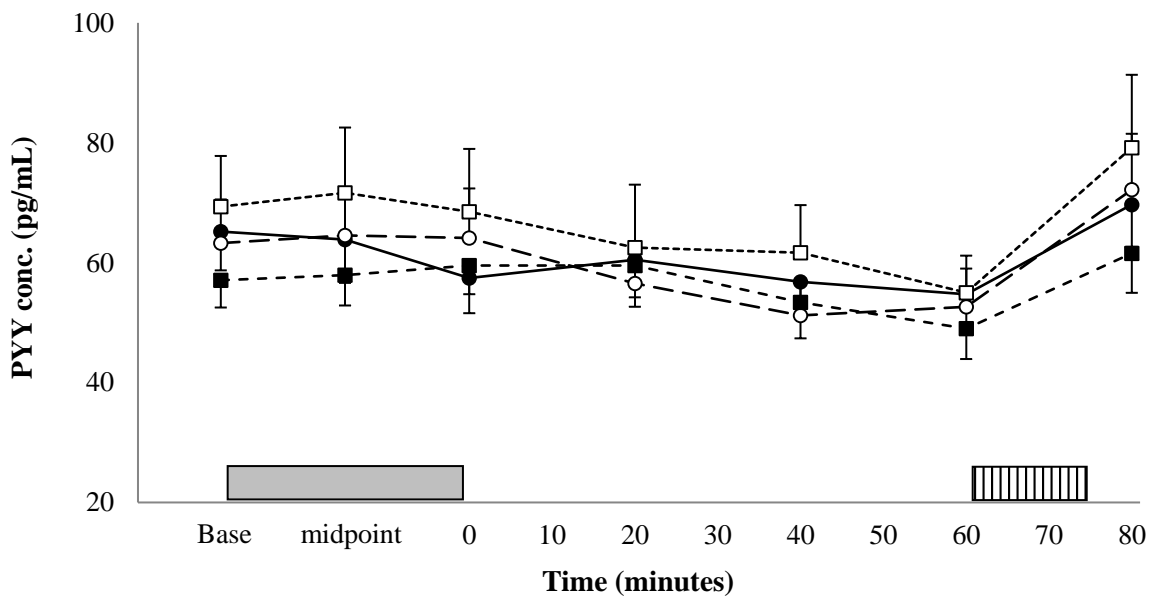
856 **Figure 3**



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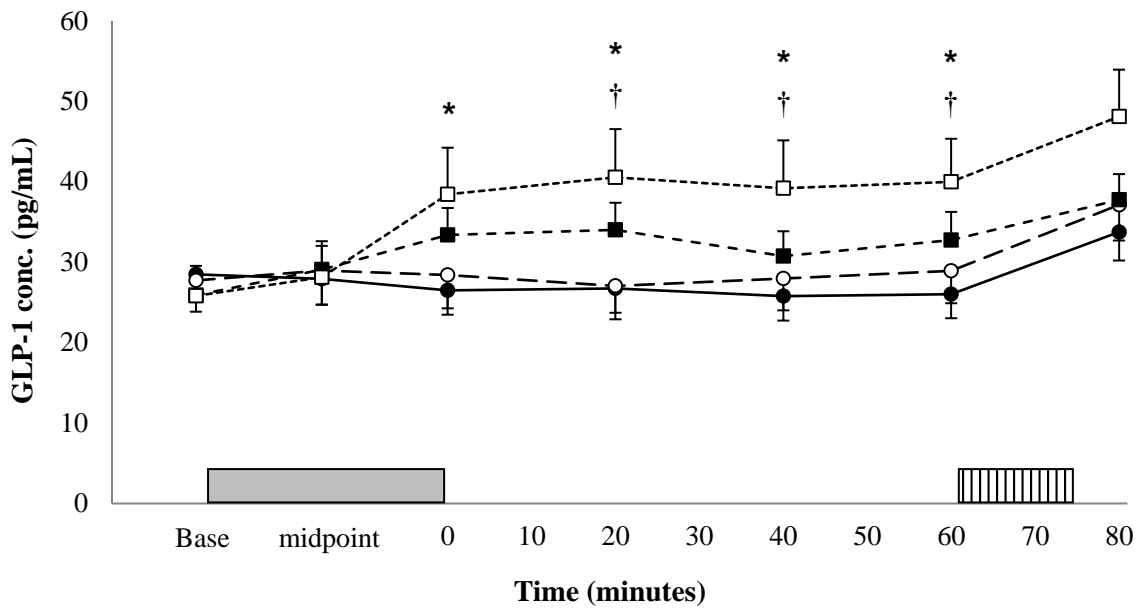
858 **Figure 3.** Mean plasma concentration of acylated ghrelin. ●, solid line = REST; ○, large dash = 15-MIN; ■, 859  
 860 medium dash = 30-MIN; □, small dash = 45-MIN. Hollow rectangle = exercise; filled, black rectangle = *ad libitum* breakfast meal. Values are mean ± SEM. \* significant within-condition, lower than baseline. † 861  
 862 significant between-condition effect, 45-MIN lower than REST. 863  
 864

865 **Figure 4**



866 **Figure 4.** Mean plasma concentration of PYY. ●, solid line = REST; ○, large dash = 15-MIN; ■, medium dash 867  
 868 = 30-MIN; □, small dash = 45-MIN. Hollow rectangle = exercise; filled, black rectangle = *ad libitum* breakfast meal. Values are mean ± SEM. 869

870 **Figure 5**



871

872 **Figure 5.** Mean plasma concentration of GLP-1. ●, solid line = REST; ○, large dash = 15-MIN; ■, medium dash  
873 = 30-MIN; □, small dash = 45-MIN. Hollow rectangle = exercise; filled, black rectangle = *ad libitum* breakfast  
874 meal. Values are mean ± SEM. \* significant within-condition effect, vs. baseline. † significant between-  
875 condition effect.

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