



LEEDS
BECKETT
UNIVERSITY

Citation:

Holliday, A and Blannin, AK (2017) Appetite, food intake and gut hormone responses to intense aerobic exercise of different duration. *Journal of Endocrinology*, 235 (3). pp. 193-205. ISSN 1479-6805 DOI: <https://doi.org/10.1530/JOE-16-0570>

Link to Leeds Beckett Repository record:

<https://eprints.leedsbeckett.ac.uk/id/eprint/4468/>

Document Version:

Article (Accepted Version)

The aim of the Leeds Beckett Repository is to provide open access to our research, as required by funder policies and permitted by publishers and copyright law.

The Leeds Beckett repository holds a wide range of publications, each of which has been checked for copyright and the relevant embargo period has been applied by the Research Services team.

We operate on a standard take-down policy. If you are the author or publisher of an output and you would like it removed from the repository, please [contact us](#) and we will investigate on a case-by-case basis.

Each thesis in the repository has been cleared where necessary by the author for third party copyright. If you would like a thesis to be removed from the repository or believe there is an issue with copyright, please contact us on openaccess@leedsbeckett.ac.uk and we will investigate on a case-by-case basis.

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

2 *Dr Adrian Holliday^{1,2}, and Dr. Andrew Blannin¹*

3

4 *¹School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Edgbaston, Birmingham.*

5 *United Kingdom. B15 2TT.*

6 *²Carnegie School of Sport, Leeds Beckett University, Headingley Campus, Leeds. United Kingdom. LS6 3QS.*

7

8 **Corresponding Author:**

9 Dr Adrian Holliday

10 Fairfax G18,

11 Headingley Campus,

12 Leeds Beckett University,

13 Leeds.

14 LS6 3QS

15 a.j.holliday@leedsbeckett.ac.uk

16

17 **Short title:** *Exercise duration effects on appetite measures*

18 **Keywords:** Appetite hormones; eating behaviour; post-exercise nutrition

19

20 **Word count:** 5727

21

22

23

24

25

26

27 **Footnote.** Dr Adrian Holliday's current affiliation: School of Sport, Leeds Beckett University, Headingley

28 Campus, Leeds. LS6 3QS.

29

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⁻²; VO_{2max}=61.6±6.0 mL•kg⁻¹•
35 min⁻¹) 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_{2max}. 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

63

64 **WORD COUNT: 5727**

65

66

67

68

69

70

71

72

73

74

75

76

77

78

79

80

81

82

83

84

85

86

87

88

89

90

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 ≥ 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 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 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⁻¹, 1.4 pg•ml⁻¹ and 1.5 pg•ml⁻¹ 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 (η^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 (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 ± 1397 kJ, 15-MIN = 3474 ± 1233 kJ, 30-MIN = 3636 ± 1254 kJ, 45-MIN = 3769 ± 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 ± 1616 kJ) versus 30-MIN (1039 ± 1520 kJ, $p<0.001$, $d=1.03$, 95% CI=908 –

301 2297 kJ) and 45-MIN (260 ± 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 ± 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 ± 29 $\text{pg} \cdot \text{mL}^{-1}$) versus REST (369 ± 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 ± 42 $\text{pg} \cdot \text{mL}^{-1}$) and 30=MIN (246 ± 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 ± 35 $\text{pg} \cdot \text{mL}^{-1}$ vs. 365 ± 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 ± 33 $\text{pg} \cdot \text{mL}^{-1}$ vs. 396 ± 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 ± 7 $\text{pg} \cdot \text{mL}^{-1}$ vs. 396 ± 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 ± 29 $\text{pg} \cdot \text{mL}^{-1}$) and at $t=20$ (239 ± 35 $\text{pg} \cdot \text{mL}^{-1}$), versus baseline (366 ± 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 ± 11.1 $\text{pg}\cdot\text{mL}^{-1}$) compared with REST (26.5 ± 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 ± 19.2
347 $\text{pg}\cdot\text{mL}^{-1}$) versus 15-MIN (28.4 ± 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 ± 19.9) versus 15-MIN (27.1 ± 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 ± 19.9) versus REST, with the difference approaching
351 significance (vs. 26.7 ± 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 ± 11.1 vs. 25.9 ± 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 ± 19.2 vs. 25.8 ± 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 $\geq 60\%$ $\text{VO}_{2\text{max}}$ 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₍₃₋₃₆₎. However, the abundance of
447 PYY₍₃₋₃₆₎ is greater than that of PYY₍₁₋₃₆₎, meaning that most of the total circulating PYY in plasma is in the
448 form of PYY₍₃₋₃₆₎. Additionally, total PYY and PYY₍₃₋₃₆₎ 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₍₃₋₃₆₎.

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

540 ***FUNDING***

541 Adrian Holliday was funded by a University of Birmingham PhD Studentship

542

543 ***ACKNOWLEDGEMENTS***

544 The authors would like to acknowledge and thank Dr Oliver Wilson for assisting with the drafting of the
545 manuscript.

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

569 ***REFERENCES***

570 Bailey DP, Smith LR, Christmas BC, Taylor L, Stensel DJ, Deighton K, Douglas JA & Kerr CJ 2015 Appetite
571 and gut hormone responses to moderate-intensity continuous exercise versus high-intensity interval exercise, in
572 normoxic and hypoxic conditions. *Appetite* **89** 237-245.
573

574 Bellissimo N, Thomas SG, Goode RC & Anderson GH 2007 Effect of short-duration physical activity and
575 ventilation threshold on subjective appetite and short-term energy intake in boys. *Appetite* **49** 644-651.
576

577 Bland JM & Altman DG 1995 Statistics notes: Calculating correlation coefficients with repeated observations:
578 Part 1—correlation within subjects. *British Medical Journal* **310** 446.
579

580 Broom DR, Batterham RL, King JA & Stensel DJ 2009 Influence of resistance and aerobic exercise on hunger,
581 circulating levels of acylated ghrelin, and peptide YY in healthy males. *American Journal of Physiology-
582 Regulatory Integrative and Comparative Physiology* **296** R29-R35.
583

584 Broom, DR, Miyashita M, Wassw, LM, Pulsford R, King JA, Thackray AE & Stensel DJ 2017 Acute effect of
585 exercise intensity and duration on acylated ghrelin and hunger in men. *Journal of Endocrinology* **232** 411-422.
586

587 Broom DR, Stensel DJ, Bishop NC, Burns SF & Miyashita M 2007 Exercise-induced suppression of acylated
588 ghrelin in humans. *Journal of Applied Physiology* **102** 2165-2171.
589

590 Deighton K, Barry R, Connon CE & Stensel DJ 2013 Appetite, gut hormone and energy intake responses to low
591 volume sprint interval and traditional endurance exercise. *European Journal of Applied Physiology* **113** 1-10.
592

593 Deighton K, Batterham RL & Stensel DJ 2014 Appetite and gut hormone responses to exercise and calorie
594 restriction. The effect of modest energy deficits. *Appetite* **81**(1) 52-59.
595

596 Deighton, K., J. C. Zahra and D. J. Stensel, 2012 Appetite, energy intake and resting metabolic responses to 60
597 min treadmill running performed in a fasted versus a postprandial state. *Appetite*, **58**, 946-954.
598

599 Druce MR, Neary NM, Small CJ, Milton J, Monteiro M, Patterson M, Ghatei MA & Bloom SR 2005
600 Subcutaneous administration of ghrelin stimulates energy intake in healthy lean human volunteers. *International
601 Journal of Obesity and Related Metabolic Disorders* **30** 293-296.
602

603 Erdmann J, Tahbaz R, Lippl F, Wagenpfeil S & Schusdziarra V 2007 Plasma ghrelin levels during exercise --
604 Effects of intensity and duration. *Regulatory Peptides* **143** 127-135.
605

606 Filaire E, Rouveix M, Pannafieux C, Ferrand C. 2007 Eating Attitudes, Perfectionism and Body-esteem of Elite
607 Male Judoists and Cyclists. *Journal of Sports Science & Medicine* **6**(1) 50-7.
608

609 Goodyear LJ, Kahn BB. Exercise, glucose transport, and insulin sensitivity. *Annual Review of Medicine*.
610 1998;49(1):235-61. doi:doi:10.1146/annurev.med.49.1.235
611

612 Hagobian TA, Yamashiro M, Hinkel-Lipsker J, Stredler K, Evero N & Hackney T 2013 Effects of acute exercise
613 on appetite hormones and ad libitum energy intake in men and women. *Applied Physiology Nutrition and
614 Metabolism* **38** 66-72
615

616 Heden TD, Liu Y, Park Y, Dellsperger KC & Kanaley JA 2013 Acute aerobic exercise differentially alters
617 acylated ghrelin and perceived fullness in normal-weight and obese individuals. *Journal of Applied Physiology*
618 **115** 680-687.
619

620 Hill AJ & Blundell JE 1982 Nutrients and behaviour: Research strategies for the investigation of taste
621 characteristics, food preferences, hunger sensations and eating patterns in man. *Journal of Psychiatric Research*
622 **17** 203-212.
623

624 Holliday A & Blannin K 2017 Very low volume sprint interval exercise suppresses subjective appetite, lowers
625 acylated ghrelin, and elevates GLP-1 in overweight individuals: A pilot study. *Nutrients* **9** 362-376
626

627 Howarth KR, Moreau NA, Phillips SM, Gibala MJ. Coingestion of protein with carbohydrate during recovery
628 from endurance exercise stimulates skeletal muscle protein synthesis in humans. *Journal of Applied Physiology*.
629 2009;106(4):1394-402. doi:10.1152/jappphysiol.90333.2008.

630
631 Howe S, Hand T, Larson-Meyer D, Austin K, Alexander B & Manore M 2016 No Effect of Exercise Intensity
632 on Appetite in Highly-Trained Endurance Women. *Nutrients* **8** 223.
633
634 Imbeault P, Saint-Pierre S, Alm-Ras N & Tremblay A 1997 Acute effects of exercise on energy intake and
635 feeding behaviour. *British Journal of Nutrition* **77** 511-521.
636
637 Ivy JL. Glycogen resynthesis after exercise: effect of carbohydrate intake. *International Journal of Sports*
638 *Medicine*. 1998;19 Suppl 2:S142-5.
639
640 Jentjens R, Jeukendrup A. Determinants of Post-Exercise Glycogen Synthesis During Short-Term Recovery.
641 *Sports Medicine*. 2003;33(2):117-44. doi:10.2165/00007256-200333020-00004.
642
643 Jones TE, Basilio JL, Brophy PM, McCammon MR & Hickner RC 2009 Long-term Exercise Training in
644 Overweight Adolescents Improves Plasma Peptide YY and Resistin. *Obesity* **17** 1189-1195.
645
646 Jurimae J, Hofmann P, Jurimae T, Maestu J, Purge P, Wonisch M et al. 2006 Plasma Adiponectin Response to
647 Sculling Exercise at Individual Anaerobic Threshold in College Level Male Rowers. *International Journal of*
648 *Sports Medicine* **27**(04) 272-277.
649
650 Jurimae J & Jurimae T 2005 Leptin responses to short term exercise in college level male rowers. *British*
651 *Journal of Sports Medicine* **39**(1) 6-9. doi:10.1136/bjism.2003.008516.
652
653 Jurimae J, Jurimae T & Purge P 2007 Plasma Ghrelin Is Altered After Maximal Exercise in Elite Male Rowers.
654 *Experimental Biology and Medicine* **232**(7):904-9.
655
656 Jürimäe J, Mäestu J & Jürimäe T 2003 Leptin as a marker of training stress in highly trained male rowers?
657 *European Journal of Applied Physiology*. **90** 533-538. doi:10.1007/s00421-003-0879-2.
658
659 Jurimae J, Ramson R, Maestu J, Purge P, Jurimae T, Arciero PJ et al. 2009 Plasma Visfatin and Ghrelin
660 Response to Prolonged Sculling in Competitive Male Rowers. *Medicine & Science in Sports & Exercise*. **41**(1)
661 137-143 10.1249/MSS.0b013e31818313e6.
662
663 Kawano H, Mineta M, Asaka M, Miyashita M, Numao S, Gando Y, Ando T, Sakamoto S & Higuchi M 2013
664 Effects of different modes of exercise on appetite and appetite-regulating hormones. *Appetite* **66** 26-33.
665
666 King JA, Miyashita M, Wasse LK & Stensel DJ 2010 Influence of prolonged treadmill running on appetite,
667 energy intake and circulating concentrations of acylated ghrelin. *Appetite* **54** 492-498.
668
669 King JA, Wasse LK, Ewens J, Crystallis K, Emmanuel J, Batterham RL & Stensel DJ 2011a Differential
670 Acylated Ghrelin, Peptide YY3-36, Appetite, and Food Intake Responses to Equivalent Energy Deficits Created
671 by Exercise and Food Restriction. *Journal of Clinical Endocrinology & Metabolism* **96** 1114-1121.
672
673 King JA, Wasse LK & Stensel DJ 2011b The Acute Effects of Swimming on Appetite, Food Intake, and Plasma
674 Acylated Ghrelin. *Journal of Obesity* **2011**.
675
676 King NA, Burley VJ & Blundell JE 1994 Exercise induced suppression of appetite: effects on food intake and
677 implications for energy balance. *European Journal of Clinical Nutrition* **48** 715-724
678
679 King NA, Tremblay A & Blundell JE 1997 Effects of exercise on appetite control: implications for energy
680 balance. *Medicine & Science in Sports & Exercise* **29** 1076-1089.
681
682 Kissileff HR, Pi-Sunyer FX, Segal K, Meltzer S & Foelsch PA 1990 Acute effects of exercise on food intake in
683 obese and nonobese women. *American Journal of Clinical Nutrition* **52** 240-245.
684
685 Laan DJ, Leidy HJ, Lim E & Campbell WW 2010 Effects and reproducibility of aerobic and resistance exercise
686 on appetite and energy intake in young, physically active adults. *Applied Physiology, Nutrition, and Metabolism*
687 **35** 842-847.
688

689 Larson-Meyer D, Palm S, Bansal A, Austin KJ, Hart AM & Alexander BM 2012 Influence of Running and
690 Walking on Hormonal Regulators of Appetite in Women. *Journal of Obesity* **2012** 15.
691
692 Lluch A, King NA & Blundell JE 2000 No energy compensation at the meal following exercise in dietary
693 restrained and unrestrained women. *British Journal of Nutrition* **84** 219-225.
694
695 Martins C, Kulseng B, King NA, Holst JJ & Blundell JE 2010 The Effects of Exercise-Induced Weight Loss on
696 Appetite-Related Peptides and Motivation to Eat. *Journal of Clinical Endocrinology & Metabolism* **95** 1609-
697 1616.
698
699 Martins C, Morgan LM, Bloom SR & Robertson MD 2007 Effects of exercise on gut peptides, energy intake
700 and appetite. *Journal of Endocrinology* **193** 251-258.
701
702 Martins C, Stensvold D, Finlayson G, Holst J, Wisloff U, Kulseng B, Morgan L & King NA 2015 Effect of
703 moderate- and high-intensity acute exercise on appetite in obese individuals. *Medicine & Science in Sports &*
704 *Exercise* **47** 40-48.
705
706 O'Connor AM, Johnston CF, Buchanan KD, Boreham C, Trinick TR & Riddoch CJ 1995 Circulating
707 gastrointestinal hormone changes in marathon running. *International Journal of Sports Medicine* **16**(5) 283-287.
708
709 O'Connor AM, Pola S, Ward BM, Fillmore D, Buchanan KD & Kirwan JP 2006 The gastroenteroinsular
710 response to glucose ingestion during postexercise recovery. *American Journal of Physiology - Endocrinology*
711 *and Metabolism* **290**(6) E1155-61. doi:10.1152/ajpendo.00500.2005.
712
713 Phillips SM 2006 Dietary protein for athletes: from requirements to metabolic advantage. *Applied Physiology,*
714 *Nutrition, and Metabolism* **31**(6) 647-654. doi:10.1139/h06-035.
715
716 Phillips SM & Van Loon LJC 2011 Dietary protein for athletes: From requirements to optimum adaptation.
717 *Journal of Sports Sciences* **29**(sup1) S29-S38. doi:10.1080/02640414.2011.619204.
718
719 Pomerleau M, Imbeault P, Parker T & Doucet E 2004 Effects of exercise intensity on food intake and appetite in
720 women. *American Journal of Clinical Nutrition* **80** 1230-1236.
721
722 Rocha J, Paxman J, Dalton C, Winter E & Broom D 2013 Effects of an acute bout of aerobic exercise on
723 immediate and subsequent three-day food intake and energy expenditure in active and inactive men. *Appetite* **71**
724 369-378.
725
726 Roth CL, Enriori PJ, Harz K, Woelfle J, Cowley MA & Reinehr T 2005 Peptide YY Is a Regulator of Energy
727 Homeostasis in Obese Children before and after Weight Loss. *Journal of Clinical Endocrinology and*
728 *Metabolism* **90** 6386-6391.
729
730 Russel RR, Willis KS, Ravussin E & Larson-Meyer ED 2009 Effects of endurance running and dietary fat on
731 circulating ghrelin and peptide YY. *Journal of Sports Science & Medicine* **8** 574-583.
732
733 Schubert MM, Desbrow B, Sabapathy S & Leveritt M 2013 Acute exercise and subsequent energy intake. A
734 meta-analysis. *Appetite* **63** 92-104.
735
736 Schubert MM, Sabapathy S, Leveritt M & Desbrow B 2014 Acute Exercise and Hormones Related to Appetite
737 Regulation: A Meta-Analysis. *Sports Medicine* **44** 387-403.
738
739 Sundgot-Borgen J, Meyer NL, Lohman TG, Ackland TR, Maughan RJ, Stewart AD et al. How to minimise the
740 health risks to athletes who compete in weight-sensitive sports review and position statement on behalf of the
741 Ad Hoc Research Working Group on Body Composition, Health and Performance, under the auspices of the
742 IOC Medical Commission. *British Journal of Sports Medicine*. 2013;47(16):1012-22. doi:10.1136/bjsports-
743 2013-092966.
744
745 Thackray A, Deighton K, King JA & Stensel DJ, 2016 Exercise, Appetite and Weight Control: Are There
746 Differences between Men and Women? *Nutrients* **8** 583.
747

748 Thompson DA, Wolfe LA & Eikelboom R 1988 Acute effects of exercise intensity on appetite in young men.
749 *Medicine & Science in Sports & Exercise* **20** 222-227.
750

751 Tipton KD, Ferrando AA, Phillips SM, Doyle D, Wolfe RR 1999 Postexercise net protein synthesis in human
752 muscle from orally administered amino acids. *American Journal of Physiology - Endocrinology and Metabolism*
753 **276**(4):E628-E34.
754

755 Ueda S.-y, Yoshikawa T, Katsura Y, Usui T & Fujimoto S 2009a Comparable effects of moderate intensity
756 exercise on changes in anorectic gut hormone levels and energy intake to high intensity exercise. *Journal of*
757 *Endocrinology* **203** 357-364.
758

759 Ueda S.-y, Yoshikawa T, Katsura Y, Usui T, Nakao H & Fujimoto S 2009b Changes in gut hormone levels and
760 negative energy balance during aerobic exercise in obese young males. *Journal of Endocrinology* **201** 151-159.
761

762 Unick JL, Otto AD, Goodpaster BH, Helsel DL, Pellegrini CA & Jakicic JM 2010 Acute effect of walking on
763 energy intake in overweight/obese women. *Appetite* **55** 413-419.
764

765 van Strien T, Frijters JER, Bergers GPA & Defares PB 1986 The Dutch Eating Behavior Questionnaire (DEBQ)
766 for assessment of restrained, emotional, and external eating behavior. *International Journal of Eating Disorders*
767 **5** 295-315.
768

769 Verdich C, Flint A, Gutzwiller J-P, Naslund E, Beglinger C, Hellstrom PM, Long SJ, Morgan LM, Holst JJ &
770 Astrup A 2001 A Meta-Analysis of the Effect of Glucagon-Like Peptide-1 (7-36) Amide on Ad Libitum Energy
771 Intake in Humans. *Journal of Clinical Endocrinology and Metabolism* **86** 4382-4389.
772

773 Wasse LK, Sunderland C, King JA, Miyashita M & Stensel DJ 2013 The influence of vigorous running and
774 cycling exercise on hunger perceptions and plasma acylated ghrelin concentrations in lean young men. *Applied*
775 *Physiology, Nutrition & Metabolism* **38** 106.
776

777 Westerterp-Plantenga MS, Verwegen CR, Ijedema MJ, Wijckmans NE & Saris WH 1997 Acute effects of
778 exercise or sauna on appetite in obese and nonobese men. *Physiology & Behavior* **62** 1345-1354.

779 Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA & Bloom SR 2001
780 Ghrelin enhances appetite and increases food intake in humans. *Journal of Clinical Endocrinology and*
781 *Metabolism* **86**(12) 5992-

782

783

784

785

786

787

788

789

790

791

792

793

794
795
796

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

		797
		798
		799
Age (years)	21 \pm 2	800
		801
Height (cm)	179.3 \pm 7.2	802
		803
Weight (kg)	67.3 \pm 5.2	804
		805
BMI (kg•m⁻²)	21.0 \pm 1.6	806
		807
VO_{2max} (mL•kg⁻¹•min⁻¹)	61.6 \pm 6.0	808
		809
W_{max} (Watts)	309 \pm 45	810
		811
DEBQ score for restraint	1.9 \pm 0.4	812
		813

814 VO_{2max} = maximal aerobic capacity; RPE = rating of perceived exertion; W_{max} = maximal work load.

815

816

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

818

	REST	15 min	30 min	45 min
VO₂ (mL•min⁻¹)	341 \pm 33*	3150 \pm 368	3180 \pm 405	3138 \pm 416
% VO_{2max}	6 \pm 4*	76 \pm 8	77 \pm 8	76 \pm 8
Power output (W)	-	218 \pm 30†	207 \pm 30	207 \pm 33
% W_{max}	-	70 \pm 4†	66 \pm 6	66 \pm 4
Heart rate (beats min⁻¹)	-	153 \pm 13	156 \pm 15	157 \pm 14
% HR_{max}	-	84 \pm 5	84 \pm 5	86 \pm 3
RPE	-	13 \pm 1#	14 \pm 1	15 \pm 2
Energy Expenditure of bout (kJ)	156 \pm 95*	989 \pm 111*	1987 \pm 252*	2929 \pm 381*
EE of trial (bout + rec. period. kJ)	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_{2max} = maximal aerobic capacity; RPE = rating of perceived exertion; W_{max} = maximal work load.

823

824

825

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

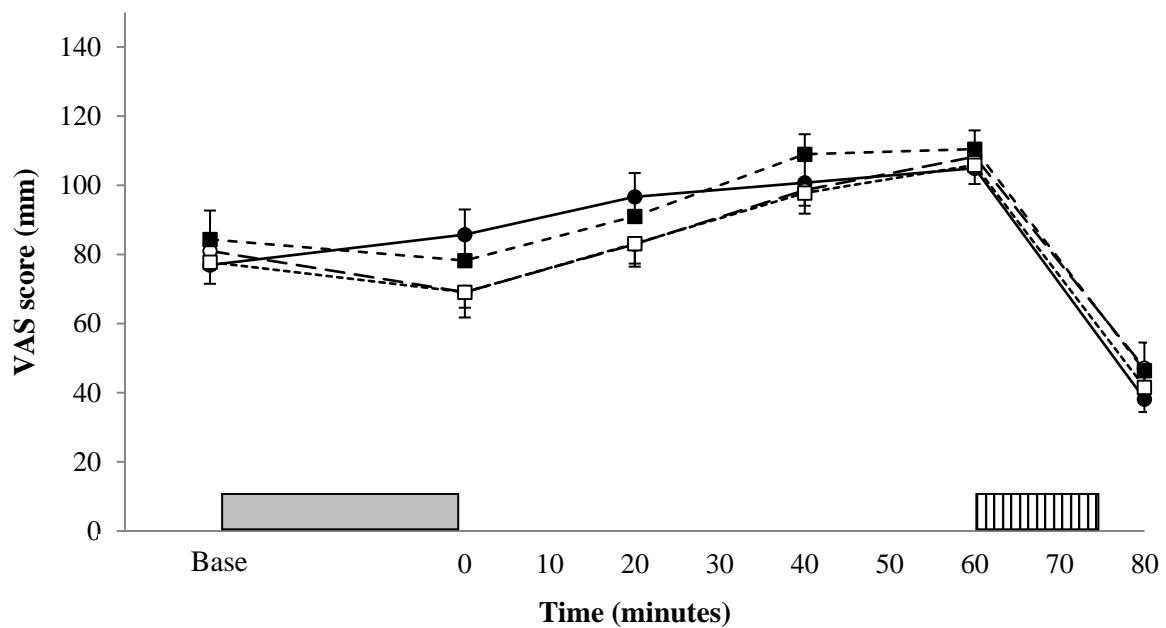
	REST	15-MIN	30-MIN	45-MIN
Weight consumed (grams)	735 \pm 331	793 \pm 281	836 \pm 262	822 \pm 264
Carbohydrate (g)	148 \pm 64	157 \pm 55	167 \pm 55	165 \pm 57
% energy CHO	76.3 \pm 6.7	77.2 \pm 5.7	77.5 \pm 5.7	75.4 \pm 9.3
Fat (g)	11.1 \pm 6.6	11.3 \pm 6.4	11.5 \pm 6.4	17.2 \pm 21.2
% energy fat	13.4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

829

830

831

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.
 837

838

839

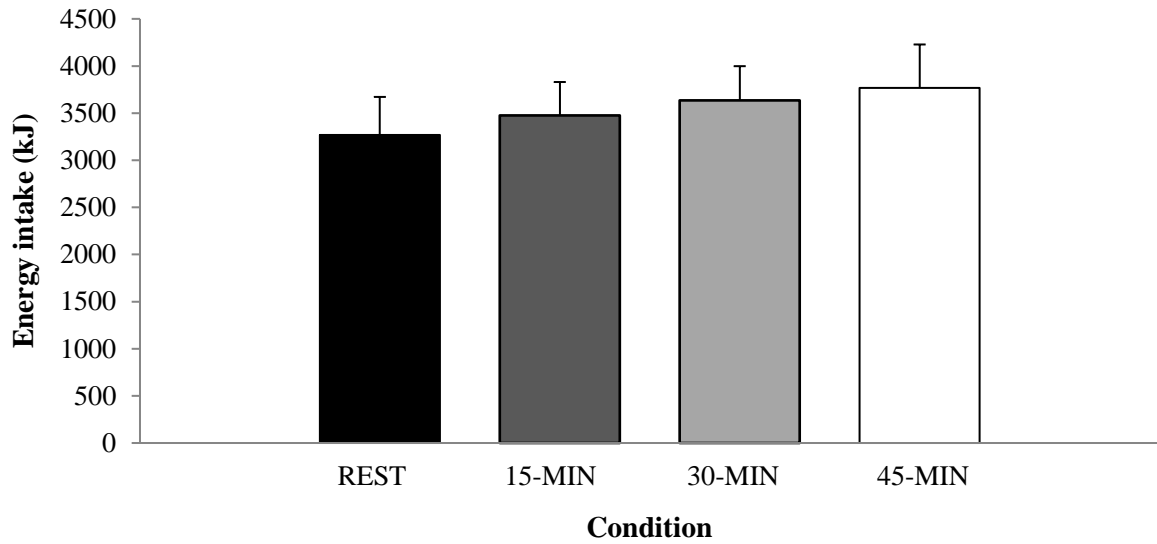
840

841

842 **Figure 2**

843

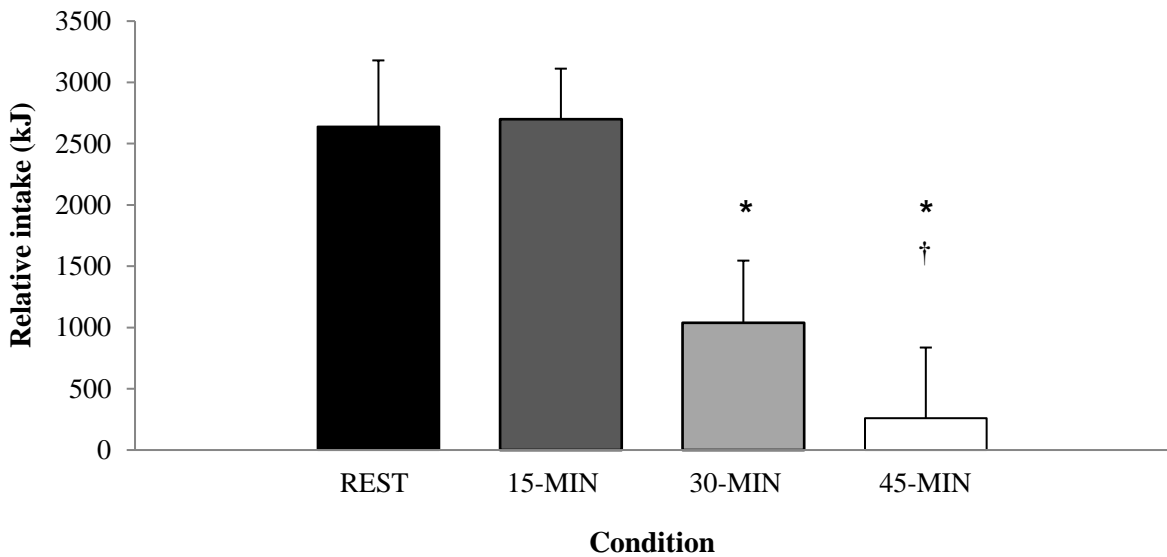
844 (a)



845

846

847 (b)



848

849

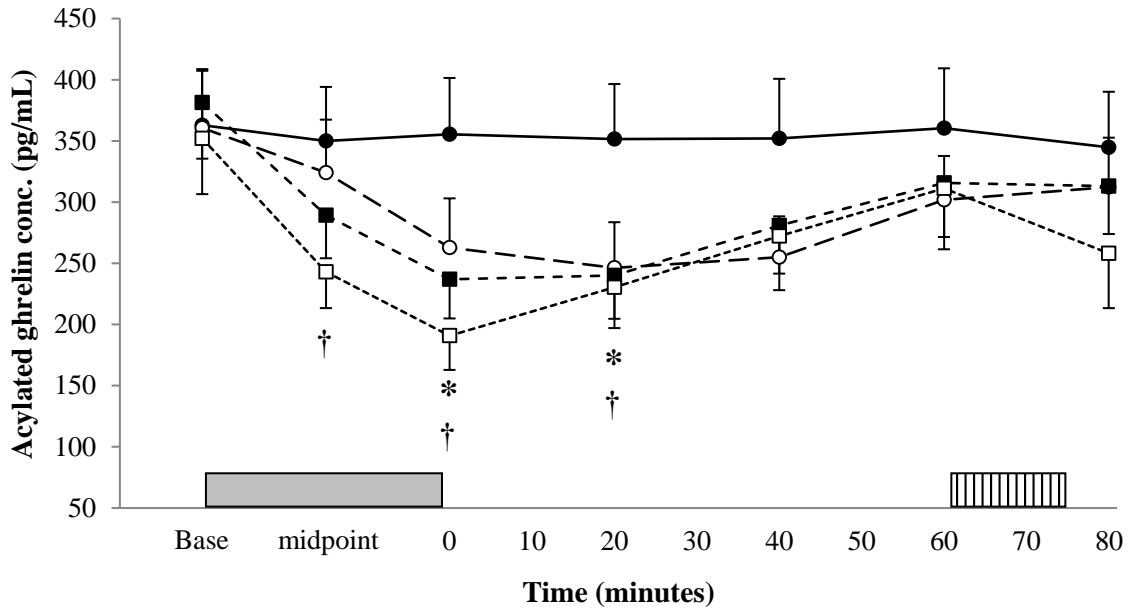
850 **Figure 2.** Energy intake (a) and relative energy intake (b) at the *ad libitum* breakfast test meal for REST, 15-
851 MIN, 30-MIN and 45-MIN. Values are mean \pm SEM. * significantly lower than REST. † significantly lower
852 than 15-MIN.

853

854

855

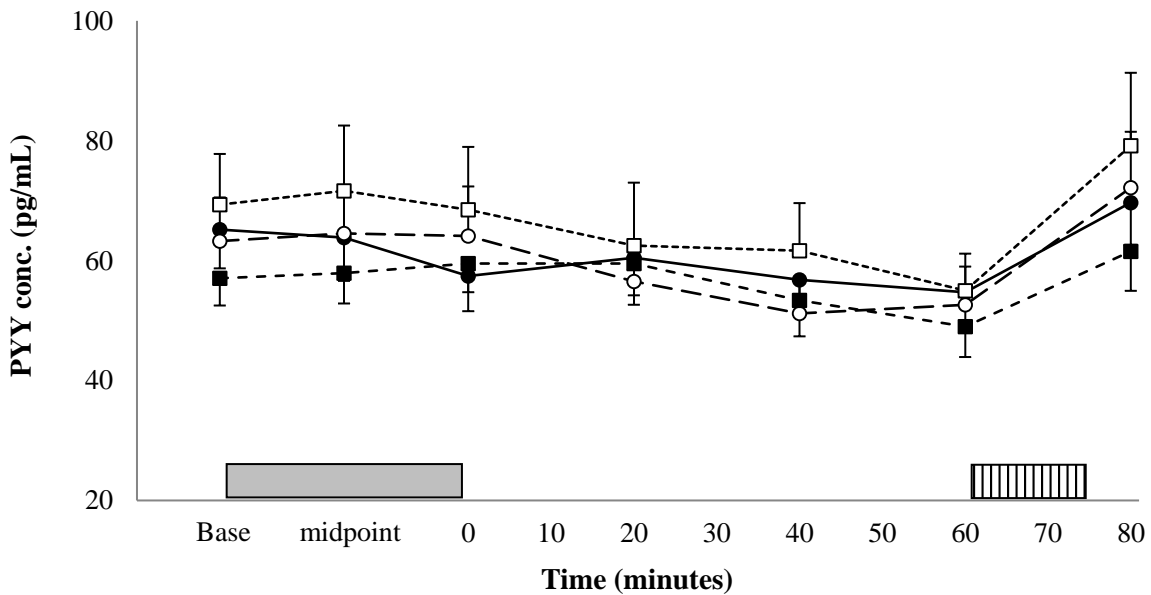
856 **Figure 3**



857

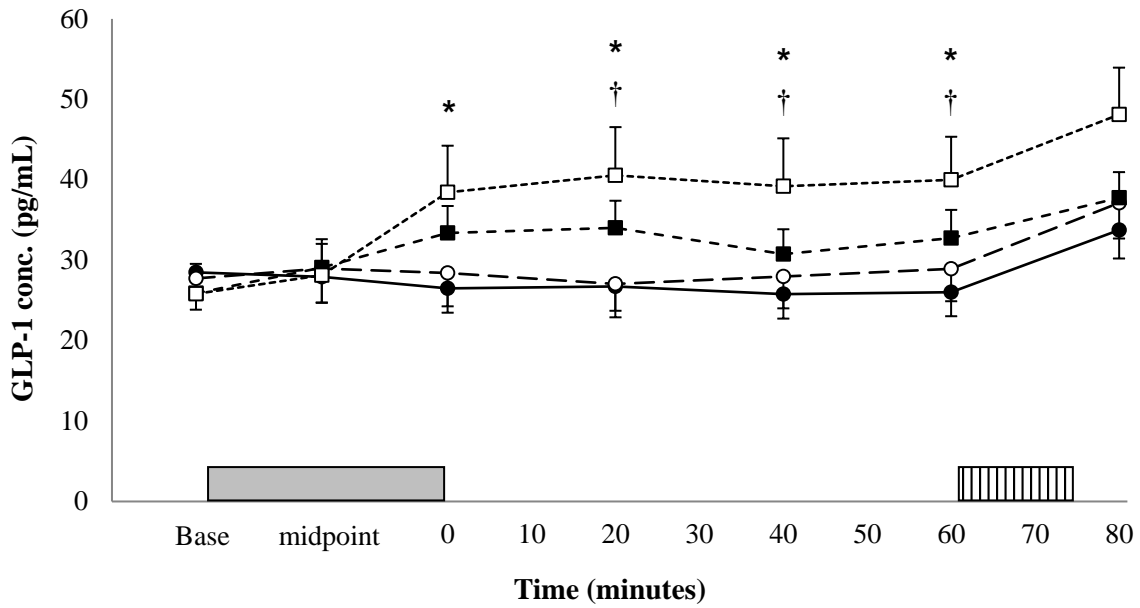
858 **Figure 3.** Mean plasma concentration of acylated ghrelin. ●, solid line = REST; ○, large dash = 15-MIN; ■, 859
 860 medium dash = 30-MIN; □, small dash = 45-MIN. Hollow rectangle = exercise; filled, black rectangle = *ad libitum* breakfast meal. Values are mean ± SEM. * significant within-condition,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

876

877

878

879

880

881

882

883