

## **Acute effect of exercise intensity and duration on acylated ghrelin and hunger in men**

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1 **Acute effect of exercise intensity and duration on acylated ghrelin and hunger in men**

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20

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25 **ABSTRACT**

26 Acute exercise transiently suppresses the orexigenic gut hormone acylated ghrelin, but the  
27 extent exercise intensity and duration determine this response is not fully understood. The  
28 effects of manipulating exercise intensity and duration on acylated ghrelin concentrations and  
29 hunger were examined in two experiments. In experiment one, nine healthy males completed  
30 three, 4-hour conditions (control, moderate-intensity running (MOD) and vigorous-intensity  
31 running (VIG)), with an energy expenditure of ~2.5 MJ induced in both MOD (55 min  
32 running at 52% peak oxygen uptake ( $\dot{V}O_{2\text{peak}}$ )) and VIG (36 min running at 75%  $\dot{V}O_{2\text{peak}}$ ). In  
33 experiment two, nine healthy males completed three, 9-hour conditions (control, 45 min  
34 running (EX45) and 90 min running (EX90)). Exercise was performed at 70%  $\dot{V}O_{2\text{peak}}$ . In  
35 both experiments, participants consumed standardised meals, and acylated ghrelin  
36 concentrations and hunger were quantified at predetermined intervals. In experiment one,  
37 delta acylated ghrelin concentrations were lower than control in MOD (ES=0.44,  $P=0.01$ ) and  
38 VIG (ES=0.98,  $P<0.001$ ); VIG was lower than MOD (ES=0.54,  $P=0.003$ ). Hunger ratings  
39 were similar across the conditions ( $P=0.35$ ). In experiment two, delta acylated ghrelin  
40 concentrations were lower than control in EX45 (ES=0.77,  $P<0.001$ ) and EX90 (ES=0.68,  
41  $P<0.001$ ); EX45 and EX90 were similar (ES=0.09,  $P=0.55$ ). Hunger ratings were lower than  
42 control in EX45 (ES=0.20,  $P=0.01$ ) and EX90 (ES=0.27,  $P=0.001$ ); EX45 and EX90 were  
43 similar (ES=0.07,  $P=0.34$ ). Hunger and delta acylated ghrelin concentrations remained  
44 suppressed at 1.5h in EX90 but not EX45. In conclusion, exercise intensity, and to a lesser  
45 extent duration, are determinants of the acylated ghrelin response to acute exercise.

## 46 INTRODUCTION

47 Obesity is characterised by a chronic energy imbalance reflecting a surplus of energy intake  
48 above expenditure, and remains a major global public health and economic burden (Wang *et al.*  
49 *al.* 2011; Ng *et al.* 2014). Recent years have witnessed significant research into the  
50 relationship between exercise, appetite regulation and energy balance (Schubert *et al.* 2014).  
51 Exercise is recommended as a therapeutic weight management strategy because it increases  
52 energy expenditure which contributes to a negative energy balance if unaccompanied by an  
53 increase in energy intake (Donnelly *et al.* 2009). Evidence suggests acute exercise transiently  
54 suppresses feelings of hunger during and shortly after exercise (Broom *et al.* 2007, 2009;  
55 King *et al.* 2010a), which has been termed ‘exercise-induced anorexia’ (King *et al.* 1994).  
56 Furthermore, these responses often coincide with exercise-induced fluctuations in hormones  
57 that regulate energy balance and appetite (Schubert *et al.* 2014).

58 Appetite and energy intake are regulated by the neuroendocrine system, of which gut peptides  
59 play an integral role as episodic signals for hunger and satiety (Karra & Batterham 2010;  
60 Hussain & Bloom 2013). Ghrelin is the only known orexigenic gut peptide, and is  
61 predominantly secreted from the stomach (Karra & Batterham 2010). Ghrelin exists in two  
62 forms – acylated and unacylated – and, although only 10–20% of circulating ghrelin is  
63 acylated, it is believed that this form is solely responsible for appetite stimulation (Ghigo *et al.*  
64 2005). Considering the central role of acylated ghrelin in appetite regulation, it is  
65 unsurprising that the interaction between exercise and acylated ghrelin continues to attract  
66 scientific enquiry.

67 Acute moderate- to high-intensity exercise suppresses acylated ghrelin concentrations (King  
68 *et al.* 2013; Schubert *et al.* 2014). This hormonal alteration appears transient and typically  
69 coincides with a reduction in hunger during and immediately after exercise (Broom *et al.*

70 2007, 2009; King *et al.* 2010a). Exercise intensity has been identified as a potential  
71 determinant modulating the acylated ghrelin response to exercise (Broom *et al.* 2007; King *et*  
72 *al.* 2010a), with suppression occurring after exercise at higher ( $\geq 60\%$  peak oxygen uptake  
73 ( $\dot{V}O_{2\text{peak}}$ )) (Broom *et al.* 2007, 2009; King *et al.* 2010a) but not lower ( $\leq 50\%$   $\dot{V}O_{2\text{peak}}$ ) (Ueda  
74 *et al.* 2009; King *et al.* 2010b) intensities. Studies comparing acute moderate- vs. high-  
75 intensity exercise suggest exercising at a higher intensity may be more potent for suppressing  
76 acylated ghrelin concentrations (Deighton *et al.* 2013; Metcalfe *et al.* 2015). However, the  
77 effect of isoenergetic exercise bouts at different intensities has revealed contrasting findings  
78 (Sim *et al.* 2014; Martins *et al.* 2015; Howe *et al.* 2016); therefore, further research is  
79 required to elucidate the importance of exercise intensity on appetite regulation.

80 Alterations in ghrelin concentrations and hunger perceptions may also be influenced by  
81 manipulations in exercise duration. Erdmann *et al.* (2007) reported that 30, 60 and 120 min of  
82 cycling at 50 W resulted in a similar increase in total ghrelin concentrations (50 to 70  $\text{pg}\cdot\text{mL}^{-1}$ )  
83 during exercise without any changes in hunger. However, the assessment of total ghrelin  
84 may obscure important changes in acylated ghrelin (Hosoda *et al.* 2004), and exercise studies  
85 measuring total ghrelin have yielded equivocal findings (King *et al.* 2013). The effect of  
86 exercise duration on acylated ghrelin concentrations has not yet been examined and may have  
87 important implications regarding the use of exercise as a weight control strategy.

88 This investigation comprises two experiments which aimed to advance understanding of  
89 appetite and hormonal responses to different acute exercise manipulations. Experiment one  
90 compared the effect of acute isoenergetic moderate- and vigorous-intensity running on  
91 acylated ghrelin concentrations and hunger perceptions. In experiment two, the acylated  
92 ghrelin and hunger responses to single bouts of 45 and 90 min running were examined.

## 93 **METHODS**

### 94 **Participants**

95 This investigation contains two experimental studies that were approved by the University  
96 Ethical Advisory Committee. Two different groups of healthy, recreationally active men  
97 provided their written informed consent to participate in one of the experiments. Information  
98 from a health screen questionnaire revealed that all participants were metabolically healthy,  
99 non-smokers, not taking medication, body mass stable for at least 6 months ( $\pm 2$  kg) and not  
100 currently dieting. Physical and physiological characteristics of participants are presented in  
101 Table 1.

### 102 **Preliminary measures**

103 Participants attended the laboratory for two preliminary visits before the main conditions in  
104 each experiment. During the first visit, anthropometric data (stature, body mass, waist  
105 circumference, skinfold thickness) were collected and participants were familiarised with  
106 exercising on the treadmill (RUNRACE, Techno gym, Gambettola, Italy).

107 During the second visit, participants completed two exercise tests. The first test consisted of a  
108 16-min submaximal incremental running test to determine the relationship between running  
109 speed and oxygen consumption. Participants completed 4×4 min stages with the initial  
110 running speed set between 7–8 km·h<sup>-1</sup> depending on the participant's fitness level, which was  
111 increased by 1–1.5 km·h<sup>-1</sup> at the start of each subsequent stage. Oxygen consumption and  
112 carbon dioxide production were determined from expired air samples collected in the final  
113 minute of each stage along with the participant's rating of perceived exertion (RPE) using  
114 Borg's 6–20 scale (Borg 1973). Heart rate was monitored continuously using short-range  
115 telemetry (Polar A3, Kempele, Finland).

116 After 30-min standardised rest,  $\dot{V}O_{2\text{peak}}$  was measured using an incremental uphill treadmill  
117 protocol at a constant speed (Taylor *et al.* 1955). The initial treadmill gradient was set at  
118 3.5% which was increased by 2.5% every 3 min until volitional exhaustion (Taylor *et al.*  
119 1955). Peak oxygen consumption was determined from an expired air sample collected  
120 during the final minute of the test when participants indicated that they could only continue  
121 for an additional 1 min. Heart rate and RPE were monitored throughout the test as described  
122 previously. Data from the two preliminary exercise tests were used to determine the running  
123 speeds required during the main conditions.

## 124 **Experimental design**

125 In each experiment, participants completed three, 1-day conditions in a random order  
126 separated by at least one week. Participants weighed, recorded and replicated their food  
127 intake in the 24-h before each main condition. Participants abstained from caffeine, alcohol  
128 and strenuous physical activity during the same period. All conditions commenced between  
129 08:00 and 09:00 after an overnight fast of at least 10-h. The study design in both experiments  
130 is presented in Figure 1.

### 131 **Experiment one: exercise intensity**

132 Nine men (20–25 years) completed three, 4-h experimental conditions: control, moderate-  
133 intensity running (MOD) and vigorous-intensity running (VIG). Participants rested in the  
134 laboratory throughout the control condition. The exercise conditions commenced with  
135 participants running on the treadmill at a speed predicted to elicit either 50%  $\dot{V}O_{2\text{peak}}$  (MOD)  
136 or 75%  $\dot{V}O_{2\text{peak}}$  (VIG), which was designed to induce a gross energy expenditure of 2510 kJ.  
137 Expired air samples were collected at regular intervals to calculate the relative exercise  
138 intensity and the treadmill speed was adjusted occasionally to ensure the target intensity was  
139 met. The exercise energy expenditure and substrate oxidation were estimated via indirect

140 calorimetry (Frayn 1983). Heart rate was monitored throughout and RPE was recorded during  
141 the last 10 s of each expired air sampling period. After the exercise bout, participants rested  
142 in the laboratory for the remainder of the condition.

143 A standardised meal prescribed relative to body mass was provided at 3-h and consumed  
144 within 15 min, which consisted of white bread, tuna, mayonnaise, chocolate bar, potato crisps,  
145 apple and orange juice. The standardised meal provided 60 kJ energy, 2.13 g (56% of meal  
146 total energy) carbohydrate, 0.53 g (15%) protein and 0.47 g (29%) fat per kilogram body  
147 mass. Water was provided *ad libitum* throughout each condition.

#### 148 **Experiment two: exercise duration**

149 Nine men (21–28 years) completed three, 9-h experimental conditions: control, 45 min  
150 running (EX45) and 90 min running (EX90). Participants rested in the laboratory throughout  
151 the control condition. During the exercise conditions, participants ran on the treadmill at a  
152 speed predicted to elicit 70%  $\dot{V}O_{2peak}$  for 45 min (EX45) or 90 min (EX90). Expired air  
153 samples were collected at regular intervals to calculate the relative exercise intensity and the  
154 treadmill speed was adjusted occasionally to ensure the target intensity was achieved. The  
155 exercise energy expenditure and substrate oxidation were estimated via indirect calorimetry  
156 (Frayn 1983). Heart rate was monitored throughout and RPE was recorded during the last 10  
157 s of each expired air sampling period. After the exercise bout, participants rested in the  
158 laboratory for the remainder of the condition.

159 Participants consumed identical standardised meals prescribed relative to body mass within  
160 15 min at 2 and 6 h. The meals consisted of white bread, Cheddar cheese, mayonnaise, butter,  
161 potato crisps, milkshake powder and whole milk. The standardised meals provided 46 kJ  
162 energy, 0.95 g (33%) carbohydrate, 0.31 g (11%) protein and 0.69 g (56%) fat per kilogram  
163 body mass. Water was provided *ad libitum* throughout each condition.



164 **Hunger perceptions**

165 Ratings of perceived hunger were assessed at baseline (fasted) and every 30 min during both  
166 experiments using a 100 mm visual analogue scale (Flint *et al.* 2000). An additional  
167 measurement was taken at 45 min in experiment two.

168 **Blood sampling**

169 Venous blood samples were collected via a cannula (Venflon, Becton Dickinson, Helinsborg,  
170 Sweden) inserted into an antecubital vein. All samples were collected in the semi-supine  
171 position, except the samples scheduled during exercise, which were taken while participants  
172 straddled the treadmill. Plasma acylated ghrelin concentrations were determined from blood  
173 samples collected into pre-chilled 4.9 mL EDTA monovettes (Sarsedt, Leicester, UK) at 0  
174 (baseline), 0.08, 0.5, 1, 3, 3.5 and 4 h in experiment one and at 0 (baseline), 0.75, 1.5, 2, 3, 6,  
175 7 and 9 h in experiment two. These monovettes contained p-hydroxymercuribenzoic acid  
176 (PHMB) to prevent the degradation of acylated ghrelin by protease. Monovettes were spun at  
177 1,287×g for 10 mins at 4°C (Burkard, Hertfordshire, UK). The plasma supernatant was  
178 aliquoted into a storage tube and 100 µL of 1 M hydrochloric acid was added per millilitre of  
179 plasma (Hosoda *et al.* 2004). Samples were re-centrifuged at 1,287×g for 5 mins at 4°C prior  
180 to storage at -80°C for later analysis.

181 Plasma glucose and insulin concentrations were determined from blood samples collected  
182 into pre-chilled 9 mL EDTA monovettes (Sarsedt, Leicester, UK). Glucose concentrations  
183 were measured at 0 (baseline), 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 h in experiment one and at 0  
184 (baseline), 0.75, 1.5, 2, 2.5, 3, 4, 5, 6, 6.5, 7, 8 and 9 h in experiment 2. Insulin concentrations  
185 were measured at 0 (baseline), 0.5, 1, 2, 3, 3.5 and 4 h in experiment one and at 0 (baseline),  
186 0.75, 1.5, 2, 3, 6, 7 and 9 h in experiment two. Monovettes were centrifuged immediately at

187 1,681×g for 10 mins at 4°C (Burkard, Hertfordshire, UK). The plasma supernatant was  
188 aliquoted into Eppendorf tubes prior to storage at -80°C for subsequent analysis.

189 At each blood sampling point, haemoglobin concentration (via the cyanmethaemoglobin  
190 method) and haematocrit (via microcentrifugation) were determined to estimate acute  
191 changes in plasma volume (Dill & Costill 1974).

## 192 **Biochemical analysis**

193 In both experiments, plasma acylated ghrelin concentrations were determined using a  
194 commercially available enzyme immunoassay (SPI BIO, Montigny le Bretonneaux, France).  
195 Plasma glucose concentrations were determined using an automated centrifugal analyser  
196 (Cobas Mira Plus, Roche, Basel, Switzerland). For experiment one, plasma insulin  
197 concentrations were determined by a solid phase <sup>125</sup>I radioimmunoassay available in a  
198 commercial kit (MP Biomedicals, Orangeburg, NY) using an automated gamma counter  
199 system (Cobra II, Packard Instrument, Downers Grove, IL). For experiment two, plasma  
200 insulin concentrations were quantified using a commercially available enzyme-linked  
201 immunoassay (Merckodia, Uppsala, Sweden). The within-batch coefficient of variation for  
202 acylated ghrelin, glucose and insulin were 7.0%, 1.4% and 8.9%, respectively in experiment  
203 one and 2.2%, 0.6% and 4.7%, respectively in experiment two.

## 204 **Statistical analyses**

205 Data were analysed using IBM Statistics Software for Windows version 21 (IBM Corporation,  
206 New York, USA). Time-averaged area under the curve (AUC) values were calculated using  
207 the trapezoidal rule. Normality of the data was checked using Shapiro-Wilk tests. Normally  
208 distributed data are presented as mean (SD). Data for hunger, glucose and insulin were  
209 natural log transformed prior to analysis. These data are presented as geometric mean (95%

210 confidence interval) and analysis is based on ratios of the geometric means. Acylated ghrelin  
211 concentrations are presented relative to baseline concentrations (i.e., delta) to minimise the  
212 potential influence of day-to-day biological variation in this appetite hormone (Deighton *et al.*  
213 2013).

214 In both experiments, linear mixed models repeated for condition were used to examine  
215 differences in exercise responses, fasting (baseline) concentrations and AUC values.  
216 Differences in metabolite concentrations between conditions over time were examined using  
217 linear mixed models repeated for condition and time. In experiment two, temporal changes in  
218 AUC responses for hunger and acylated ghrelin between experimental conditions were  
219 examined over sub-sections of the 9 h measurement period (0–2 h, 2–6 h, 6–9 h) using  
220 separate linear mixed models with condition as the sole factor. All linear mixed models  
221 included a random effect for each participant. Where significant condition and interaction  
222 effects were found, post-hoc analysis was performed using the Holm-Bonferroni correction  
223 for multiple comparisons (Atkinson 2002). Correction of acylated ghrelin, glucose and  
224 insulin concentrations for changes in plasma volume did not alter the interpretation of the  
225 results; therefore, the unadjusted values are presented for simplicity. Pearson's product  
226 moment correlations were used to examine relationships between variables. Statistical  
227 significance was accepted as  $P < 0.05$ . Absolute standardised effect sizes (ES) are included to  
228 supplement important findings. An ES of 0.2 was considered the minimum important  
229 difference in all outcome measures, 0.5 moderate and 0.8 large (Cohen 1988). Graphical  
230 representations of results are presented as mean (SEM) to avoid distortion of the figures.

## 231 **RESULTS**

### 232 **Experiment one: exercise intensity**

#### 233 *Exercise responses*

234 Exercise responses for MOD and VIG are shown in Table 2. Exercise duration was  
235 significantly shorter, and treadmill speed, heart rate, RPE and oxygen uptake were all greater  
236 in VIG compared with MOD ( $P \leq 0.05$ ). Respiratory exchange ratio was higher in VIG than  
237 MOD ( $P < 0.001$ ), with the relative contributions of carbohydrate and fat to energy provision  
238 higher and lower, respectively, in VIG compared with MOD (both  $P < 0.001$ ). Gross energy  
239 expenditure was not significantly different between the exercise conditions ( $P = 0.38$ ).

#### 240 *Hunger perceptions*

241 Fasting hunger ratings were similar across the conditions at baseline ( $P = 0.50$ ) (Table 3).  
242 Linear mixed models revealed no differences in hunger ratings across the conditions (main  
243 effect condition  $P = 0.35$ ; main effect time  $P < 0.001$ ; condition by time interaction  $P = 0.78$ )  
244 (Figure 2). Hunger total AUC was similar across the conditions ( $P = 0.65$ ) (Table 3).

#### 245 *Acylated ghrelin, glucose and insulin concentrations*

246 Boxplot analysis of acylated ghrelin total AUC values identified one participant as an outlier  
247 (Field 2009). This participant exhibited a mean acylated ghrelin concentration 21 times  
248 greater than the mean SD of the remaining participants (range: 74–1489  $\text{pg} \cdot \text{mL}^{-1}$ ). Therefore,  
249 this participant was removed and results are presented for eight participants. Fasting acylated  
250 ghrelin concentrations were similar across the conditions at baseline ( $P = 0.57$ ) (Table 3).  
251 Linear mixed models for delta acylated ghrelin revealed a significant main effect of condition  
252 ( $P < 0.001$ ), time ( $P < 0.001$ ) and condition by time interaction ( $P = 0.03$ ) (Figure 2). Post-hoc  
253 analysis of between-condition differences revealed delta acylated ghrelin concentrations were

254 lower than control in MOD (ES=0.44,  $P=0.01$ ) and VIG (ES=0.98,  $P<0.001$ ); VIG was lower  
255 than MOD (ES=0.54,  $P=0.003$ ). Post-hoc analysis of the condition by time interaction  
256 revealed the delta acylated ghrelin concentration was lower than control in VIG at 0.5 h  
257 (ES=5.49,  $P=0.005$ ) and 1 h (ES=2.46,  $P=0.02$ ); VIG was lower than MOD at 0.5 h (ES=4.68,  
258  $P=0.02$ ). Delta total AUC for acylated ghrelin was lower in VIG compared with control  
259 (ES=2.45,  $P=0.01$ ) (Table 3).

260 Fasting glucose concentrations were similar across the conditions at baseline ( $P=0.63$ ) (Table  
261 3). Linear mixed models for glucose identified a main effect of condition ( $P=0.02$ ) and time  
262 ( $P<0.001$ ), but not a condition by time interaction ( $P=0.46$ ) (Figure 2). Post-hoc analysis of  
263 between-condition differences revealed mean VIG glucose concentration was 6% and 5%  
264 higher than control (ES=0.31,  $P=0.02$ ) and MOD (ES=0.27,  $P=0.04$ ), respectively; CON and  
265 MOD were similar (1%; ES=0.04,  $P=0.73$ ). The VIG glucose AUC was meaningfully, albeit  
266 not significantly, higher than control (6%; ES=0.62,  $P=0.09$ ) and MOD (6%; ES=0.58,  
267  $P=0.09$ ); CON and MOD were not different (0%; ES=0.05,  $P=0.86$ ) (Table 3).

268 Fasting insulin concentrations were similar across the conditions at baseline ( $P=0.19$ ) (Table  
269 3). No differences in insulin concentrations were seen across the conditions (main effect  
270 condition  $P=0.28$ ; main effect time  $P<0.001$ ; condition by time interaction  $P=0.26$ ) (Figure 2).  
271 Insulin total AUC was similar across the conditions ( $P=0.95$ ) (Table 3).

## 272 *Correlations*

273 There were no significant correlations between delta acylated ghrelin concentrations and  
274 changes in hunger, glucose or insulin values.

## 275 **Experiment two: exercise duration**

### 276 *Exercise responses*

277 Exercise responses for EX45 and EX90 are displayed in Table 2. The only significant  
278 difference was the anticipated increase in gross energy expenditure for EX90 compared with  
279 EX45 ( $P<0.001$ ).

### 280 *Hunger perceptions*

281 Fasting hunger ratings were similar across the conditions at baseline ( $P=0.73$ ) (Table 4).  
282 Linear mixed models for hunger revealed a significant main effect of condition ( $P=0.001$ ),  
283 time ( $P<0.001$ ) and condition by time interaction ( $P<0.001$ ) (Figure 3). Post-hoc analysis of  
284 between-condition differences revealed hunger perceptions were 15% and 20% lower than  
285 control in EX45 (ES=0.20,  $P=0.01$ ) and EX90 (ES=0.27,  $P=0.001$ ), respectively; EX45 and  
286 EX90 were similar (-6%; ES=0.07,  $P=0.34$ ). Post-hoc analysis of the condition by time  
287 interaction revealed hunger perceptions were lower than control in EX45 at 0.5, 0.75 and 1 h  
288 (all ES $\geq$ 1.71,  $P\leq$ 0.05); EX90 was lower than control at 0.5, 0.75, 1, 1.5 and 2 h (all ES $\geq$ 1.30,  
289  $P\leq$ 0.05). The hunger total AUC was 14% and 18% lower than control in EX45 (ES=0.36,  
290  $P=0.07$ ) and EX90 (ES=0.48,  $P=0.02$ ), respectively; EX45 and EX90 were similar (-5%;  
291 ES=0.13,  $P=0.42$ ) (Table 4). Specifically, hunger AUC was lower than control between 0–2 h  
292 in EX45 (43%; ES=1.96,  $P=0.001$ ) and EX90 (54%; ES=2.77,  $P<0.001$ ); EX90 was  
293 meaningfully, albeit not significantly, lower than EX45 (20%; ES=0.81,  $P=0.08$ ).

### 294 *Acylated ghrelin, glucose and insulin concentrations*

295 Fasting acylated ghrelin concentrations were similar across the conditions at baseline ( $P=0.88$ )  
296 (Table 4). Linear mixed models for delta acylated ghrelin identified a significant main effect  
297 for condition ( $P<0.001$ ) and time ( $P<0.001$ ), but not a condition by time interaction ( $P=0.47$ )

298 (Figure 3). Post-hoc analysis of between-condition differences revealed delta acylated ghrelin  
299 concentrations were lower than control in EX45 (ES=0.77,  $P<0.001$ ) and EX90 (ES=0.68,  
300  $P<0.001$ ); EX45 and EX90 were similar (ES=0.09,  $P=0.55$ ). The delta total AUC for acylated  
301 ghrelin was lower than control in EX45 (ES=0.99,  $P=0.03$ ) and EX90 (ES=0.81,  $P=0.07$ ),  
302 respectively; EX45 and EX90 were similar (ES=0.18,  $P=0.68$ ) (Table 4). Specifically, EX45  
303 was lower than control between 0–2 h (ES=1.93,  $P<0.001$ ) and 2–6 h (ES=1.05,  $P=0.05$ );  
304 EX90 was lower than control between 0–2 h (ES=2.16,  $P<0.001$ ) and 2–6 h (ES=0.83,  
305  $P=0.18$ ).

306 Fasting glucose concentrations were similar across the conditions at baseline ( $P=0.98$ ) (Table  
307 4). Linear mixed models for glucose identified a significant main effect for condition  
308 ( $P<0.001$ ), time ( $P<0.001$ ) and condition by time interaction ( $P<0.001$ ) (Figure 3). Post-hoc  
309 analysis of between-condition differences revealed mean glucose concentrations were 5%  
310 higher than control in EX45 (ES=0.40,  $P=0.001$ ) and EX90 (ES=0.40,  $P=0.001$ ); EX45 and  
311 EX90 were similar (0%; ES=0.00,  $P=0.97$ ). Post-hoc analysis of the condition by time  
312 interaction revealed the glucose concentration was higher than control in EX45 at 0.75 h  
313 (26%; ES=4.17,  $P=0.01$ ). Linear mixed models identified a trend for differences in glucose  
314 total AUC across the conditions ( $P=0.06$ ), but post-hoc analysis revealed no significant  
315 between-condition differences after Holm-Bonferroni correction ( $P\geq 0.09$ ) (Table 4).

316 Fasting insulin concentrations were similar across the conditions at baseline ( $P=0.74$ ) (Table  
317 4). Linear mixed models for insulin revealed a significant main effect for condition ( $P=0.03$ )  
318 and time ( $P<0.001$ ), but not a condition by time interaction ( $P=0.18$ ) (Figure 3). Post-hoc  
319 analysis of between-condition differences revealed mean insulin concentrations were 20%  
320 and 25% lower in EX90 than control (ES=0.22,  $P=0.08$ ) and EX45 (ES=0.27,  $P=0.03$ ),  
321 respectively; CON and EX45 were similar (6%; ES=0.05,  $P=0.61$ ). Insulin total AUC was  
322 not significantly different across the conditions ( $P=0.81$ ) (Table 4).

323 *Correlations*

324 There were no significant correlations between delta acylated ghrelin concentrations and  
325 changes in hunger, glucose or insulin values for any time period.

326 **DISCUSSION**

327 The purpose of the present experiments was to elucidate the effect of exercise intensity and  
328 duration on acylated ghrelin concentrations and hunger perceptions. The primary findings are  
329 that isoenergetic vigorous-intensity running transiently suppressed acylated ghrelin  
330 concentrations to a greater extent than moderate-intensity running, but was not accompanied  
331 by a change in hunger. Furthermore, acylated ghrelin concentrations and hunger were  
332 suppressed to a similar extent during 45 and 90 min treadmill running, but the effect appears  
333 prolonged when the exercise duration is extended.

334 Research has demonstrated that acute exercise suppresses acylated ghrelin concentrations,  
335 with perturbations returning to control values within 30 min after exercise (King *et al.* 2013;  
336 Schubert *et al.* 2014). Experiment one extends these findings by demonstrating that acylated  
337 ghrelin concentrations were reduced to a greater extent during vigorous-intensity running  
338 than moderate-intensity running, despite a similar exercise-induced energy expenditure. This  
339 is consistent with previous research identifying exercise intensity as an important determinant  
340 of the acylated ghrelin response to acute exercise, with suppression occurring at intensities  
341  $\geq 60\%$   $\dot{V}O_{2peak}$  typically (Broom *et al.* 2007, 2009; Ueda *et al.* 2009; King *et al.* 2010a,  
342 2010b). The importance of exercise intensity is highlighted further by studies reporting that  
343 sprint interval exercise suppresses acylated ghrelin to a greater extent than moderate-intensity  
344 exercise (Deighton *et al.* 2013; Metcalfe *et al.* 2015). However, studies directly comparing  
345 isoenergetic bouts of moderate- and vigorous-to-high-intensity exercise have reported  
346 contrasting findings, with one study reporting greater suppression of acylated ghrelin at the



347 higher exercise intensity (akin to experiment one) (Sim *et al.* 2014), whilst others  
348 demonstrate a similar level of suppression independent of exercise intensity (Martins *et al.*  
349 2015; Howe *et al.* 2016). The discrepancy in findings is likely related to key variations in the  
350 protocols adopted including differences in the participant groups, exercise energy expenditure,  
351 completion of exercise in the fasted or postprandial state and timing of meal intake.  
352 Differences in meal size and macronutrient composition, and methods utilised to quantify  
353 acylated ghrelin are likely to further confound the interpretation of these findings. Additional  
354 work is clearly required to elucidate the impact of exercise intensity on acylated ghrelin.

355 Surprisingly, despite the decrease in acylated ghrelin during vigorous-intensity exercise,  
356 hunger did not differ significantly between conditions. Although this contrasts previous  
357 studies reporting simultaneous reductions in acylated ghrelin and hunger in response to  
358 exercise (Broom *et al.* 2007, 2009; King *et al.* 2010a), exercise-induced changes in acylated  
359 ghrelin and hunger do not always occur in parallel (Deighton *et al.* 2013; Sim *et al.* 2014;  
360 Martins *et al.* 2015). This apparent disassociation highlights the complex nature of appetite  
361 regulation, which involves the interaction of many physiological and psychological factors  
362 (Hussain & Bloom 2013).

363 In accordance with previous studies, experiment two demonstrated a reduction in acylated  
364 ghrelin concentrations and hunger in both exercise conditions (Broom *et al.* 2007, 2009; King  
365 *et al.* 2010a). Although the hunger and acylated ghrelin responses were not statistically  
366 different between the two exercise interventions, the values remained suppressed at 1.5 h in  
367 the 90 min, but not 45 min, exercise bout (Figure 3). This suggests that increasing the  
368 exercise duration may extend the exercise-induced suppression in hunger and acylated  
369 ghrelin concentrations. Although this is the first study to investigate the effect of exercise  
370 duration on acylated ghrelin concentrations, Erdmann *et al.* (2007) reported no differences in  
371 total ghrelin concentrations in response to 30, 60 and 120 min cycling. However, acylated

372 ghrelin is the form of ghrelin thought to be solely responsible for appetite stimulation (Ghigo  
373 *et al.* 2005), and may be obscured when total ghrelin is measured (Hosoda *et al.* 2004).  
374 Furthermore, research examining the effect of acute exercise on total ghrelin concentrations  
375 has yielded equivocal findings with evidence of acute increases, decreases and no change  
376 (King *et al.* 2013).

377 Similar to experiment one, there was a divergence in the acylated ghrelin and hunger  
378 responses to acute exercise, further highlighting the complexity of appetite regulation.  
379 Although simultaneous reductions in acylated ghrelin and hunger were seen during exercise,  
380 hunger ratings returned to similar values between conditions at 2.5 h, but acylated ghrelin  
381 remained suppressed in the exercise conditions after meal consumption. The reason for this  
382 disparity is unclear but the findings of the present experiments contribute to the debate  
383 concerning the importance of reductions in acylated ghrelin as a potential determinant of  
384 hunger.

385 The physiological significance of transient reductions in acylated ghrelin during and after  
386 exercise is not fully understood. The divergence between acylated ghrelin and hunger  
387 demonstrated in the present experiments and previous studies (Deighton *et al.* 2013; Sim *et al.*  
388 2014; Martins *et al.* 2015) challenges the role acylated ghrelin plays in mediating appetite  
389 responses to exercise. Furthermore, although the implementation of standardised meals in the  
390 present experiments precluded the assessment of energy intake, the consensus of evidence  
391 suggests that acute aerobic exercise does not stimulate compensatory increases in appetite  
392 and energy intake on the same day (Deighton & Stensel 2014). This may point to the  
393 existence of alternative compensatory mechanisms, for example, reductions in unstructured  
394 physical activity (i.e., non-exercise activity thermogenesis) and/or increased sedentary  
395 behaviours on the day of exercise, but further work is required to support this. Nevertheless,  
396 acylated ghrelin is the only gut peptide known to stimulate appetite and energy intake, with

397 circulating concentrations increasing preprandially and decreasing postprandially on a meal-  
398 to-meal basis (Cummings *et al.* 2004). Consequently, this temporal pattern of fluctuation in  
399 acylated ghrelin is indicative of an important role in coordinating meal initiation and/or  
400 termination (Cummings *et al.* 2004; Kara & Batterham 2010).

401 The mechanisms underpinning the transient exercise-induced suppression of acylated ghrelin  
402 are unclear but are likely to reflect processes interfering with the synthesis and/or secretion of  
403 acylated ghrelin into the circulation. A recent review suggests the redistribution of blood flow  
404 from splanchnic areas to active skeletal muscle may be particularly pertinent for suppressing  
405 ghrelin, and appear dependent on the exercise intensity (Hazell *et al.* 2016). Exercise-induced  
406 changes in glucose and insulin concentrations have also been implicated mechanistically  
407 (Hazell *et al.* 2016), with elevations associated with decreased ghrelin concentrations  
408 (Flanagan *et al.* 2003; Cummings & Overduin 2007; Iwakura *et al.* 2015). The elevation in  
409 glucose concentration during vigorous-intensity exercise in experiment one and both exercise  
410 conditions in experiment two coincided with the reduction in acylated ghrelin concentrations.  
411 However, insulin concentrations were reduced in the 90 min exercise condition, and previous  
412 exercise studies provide conflicting findings by reporting no effect of glucose and insulin on  
413 acylated ghrelin concentrations (Broom *et al.* 2007, 2009; King *et al.* 2010a). Further  
414 research is required to develop a mechanistic understanding of the exercise-induced  
415 suppression of acylated ghrelin.

416 One limitation of the present experiments represents the measurement of a single appetite-  
417 regulating hormone. Despite the unique role of acylated ghrelin as the only appetite-  
418 stimulating gut hormone, it is only one component of the appetite-regulating neuroendocrine  
419 system. Therefore, it may be prudent for future studies to investigate anorexigenic hormones  
420 (e.g., peptide-YY, glucagon-like peptide-1, pancreatic polypeptide and cholecystokinin) to  
421 provide a broader scientific understanding of the role exercise intensity and duration play in

422 modulating appetite regulation. Secondly, appetite perceptions were limited to the assessment  
423 of hunger; however, utilising multiple scales (e.g., satisfaction, fullness and prospective food  
424 consumption) may provide a more holistic insight into appetite perceptions (Blundell *et al.*  
425 2010). Finally, we recruited a small group of healthy and recreationally active men to both  
426 experiments, which may limit applications to other population groups and the ability to detect  
427 meaningful associations between variables. Additional research is needed in overweight and  
428 obese populations who are most likely to benefit from weight management strategies. Despite  
429 these limitations, our findings provide important insight into the role that exercise intensity  
430 and duration play in modulating hormonal and hunger responses to exercise.

431 In conclusion, the present experiments demonstrate that exercise intensity, and to a lesser  
432 extent duration, are determinants of the acylated ghrelin response to exercise. Acylated  
433 ghrelin is transiently suppressed after a bout of exercise, an effect that appears greater when  
434 exercise is performed at a higher intensity. Increasing the exercise duration may prolong the  
435 transient suppression in hunger and acylated ghrelin, but the disassociation between hunger  
436 and acylated ghrelin responses requires further investigation. Future research is warranted to  
437 examine these responses chronically and in overweight/obese populations for whom exercise  
438 may be a therapeutic strategy for weight management.

#### 439 **Declaration of interest**

440 The authors declare no conflict of interest.

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544 **Figure legends**

545 **Figure 1** Schematic representation of the study protocol in experiment one and two.

546 **Figure 2** Perceptions of hunger (A), and concentrations of plasma delta acylated ghrelin  
547 (B), insulin (C) and glucose (D) in the control (■), moderate-intensity exercise  
548 (●) and vigorous-intensity exercise (△) conditions. Values are mean (SEM),  
549  $n = 9$  for hunger, insulin and glucose and  $n = 8$  for acylated ghrelin. Black  
550 rectangle indicates moderate-intensity exercise, grey rectangle indicates  
551 vigorous-intensity exercise and open rectangle indicates consumption of the  
552 standardised meal.

553 **Figure 3** Perceptions of hunger (A), and concentrations of plasma delta acylated ghrelin  
554 (B), insulin (C) and glucose (D) in the control (■), 45 min exercise (●) and 90  
555 min exercise (△) conditions. Values are mean (SEM),  $n = 9$  for hunger,  
556 acylated ghrelin, insulin and glucose. Black rectangle indicates 90 min  
557 exercise, grey rectangle indicates 45 min exercise and open rectangles  
558 indicates consumption of the standardised meals.

**Table 1** Physical and physiological characteristics in experiments one and two.

<b>Characteristic</b>	<b>Experiment one (<i>n</i> = 9)</b>	<b>Experiment two (<i>n</i> = 9)</b>
Age (years)	21.4 (1.7)	23.2 (2.1)
Body mass (kg)	78.3 (11.0)	72.0 (5.6)
Stature (m)	1.79 (0.07)	1.78 (0.05)
Body mass index (kg·m <sup>-2</sup> )	24.5 (2.4)	22.7 (1.5)
Sum of skinfolds (mm)	33.1 (5.7)	26.1 (4.5)
Percent body fat (%)	15.3 (2.7)	12.0 (2.3)
Waist circumference (cm)	77.7 (5.7)	76.7 (2.1)
Peak oxygen uptake (mL·kg <sup>-1</sup> ·min <sup>-1</sup> )	58 (6)	63 (6)

Values are mean (SD)

**Table 2** Responses to treadmill running in experiments one and two.

	Experiment one: exercise intensity			Experiment two: exercise duration		
	Moderate-intensity	Vigorous-intensity	Effect size	45 min	90 min	Effect size
Exercise time (min)	55 (7)	36 (5)	2.97 <sup>a</sup>	45 (0)	90 (0)	-
Treadmill speed (km·h <sup>-1</sup> )	7.5 (0.8)	11.0 (1.5)	2.84 <sup>a</sup>	10.6 (1.4)	10.4 (1.3)	0.09
Heart rate (beats·min <sup>-1</sup> )	136 (15)	163 (19)	1.57 <sup>a</sup>	169 (11)	169 (12)	0.00
Rating of perceived exertion	12 (1)	14 (2)	1.49 <sup>a</sup>	13 (1)	14 (1)	0.59
Oxygen uptake (L·min <sup>-1</sup> )	2.37 (0.35)	3.41 (0.40)	2.74 <sup>a</sup>	3.19 (0.36)	3.17 (0.34)	0.06
Percent peak oxygen uptake (%)	52 (3)	75 (4)	6.32 <sup>a</sup>	70 (2)	70 (2)	0.20
Respiratory exchange ratio	0.90 (0.03)	0.96 (0.04)	1.95 <sup>a</sup>	0.93 (0.05)	0.89 (0.11)	0.52
Fat oxidation (%)	32 (9)	11 (10)	2.26 <sup>a</sup>	24 (10)	33 (27)	0.44
Carbohydrate oxidation (%)	68 (9)	89 (10)	2.26 <sup>a</sup>	76 (10)	67 (27)	0.44
Gross energy expenditure (kJ)	2580 (152)	2504 (165)	0.48	2918 (329)	5949 (653)	5.86 <sup>b</sup>

Values are mean (SD)

<sup>a</sup> Significant difference between moderate-intensity exercise and vigorous-intensity exercise ( $P < 0.05$ )

<sup>b</sup> Significant difference between 45 min exercise and 90 min exercise ( $P < 0.05$ )

**Table 3** Fasting and time-averaged total area under the concentration versus time curve in the control, moderate-intensity exercise and vigorous-intensity exercise conditions in experiment one.

	Control	Moderate-intensity exercise	Vigorous-intensity exercise	Main effect condition <i>P</i>
<b>Hunger</b>				
Fasting (mm)	33 (18 to 60)	30 (16 to 55)	25 (14 to 46)	0.50
TAUC (mm)	53 (44 to 65)	49 (41 to 60)	51 (42 to 61)	0.65
<b>Acylated ghrelin</b>				
Fasting (pg·mL <sup>-1</sup> )	67.2 (31.4)	68.1 (25.9)	78.9 (42.0)	0.57
Delta TAUC (pg·mL <sup>-1</sup> )	2.29 (8.21)	-6.83 (11.76)	-17.78 (19.16)	0.01 <sup>a</sup>
<b>Glucose</b>				
Fasting (mmol·L <sup>-1</sup> )	5.21 (4.68 to 5.80)	5.44 (4.89 to 6.07)	5.52 (4.96 to 6.15)	0.63
TAUC (mmol·L <sup>-1</sup> )	5.20 (4.87 to 5.54)	5.22 (4.89 to 5.57)	5.52 (5.17 to 5.89)	0.06
<b>Insulin</b>				
Fasting (pmol·L <sup>-1</sup> )	137 (107 to 175)	175 (137 to 224)	168 (131 to 215)	0.19
TAUC (pmol·L <sup>-1</sup> )	297 (238 to 371)	292 (234 to 365)	302 (242 to 377)	0.95

Values for acylated ghrelin are mean (SD) for  $n = 8$ . Values for hunger, glucose and insulin are geometric mean (95% confidence interval) for  $n = 9$ , and statistical analyses are based on natural log transformed data. TAUC, time-averaged total area under the concentration versus time curve.

<sup>a</sup> Significant difference between vigorous-intensity exercise and control conditions (linear mixed model  $P < 0.05$  after Holm-Bonferroni correction)

**Table 4** Fasting and time-averaged total area under the concentration versus time curve in the control, 45 min exercise and 90 min exercise conditions in experiment two.

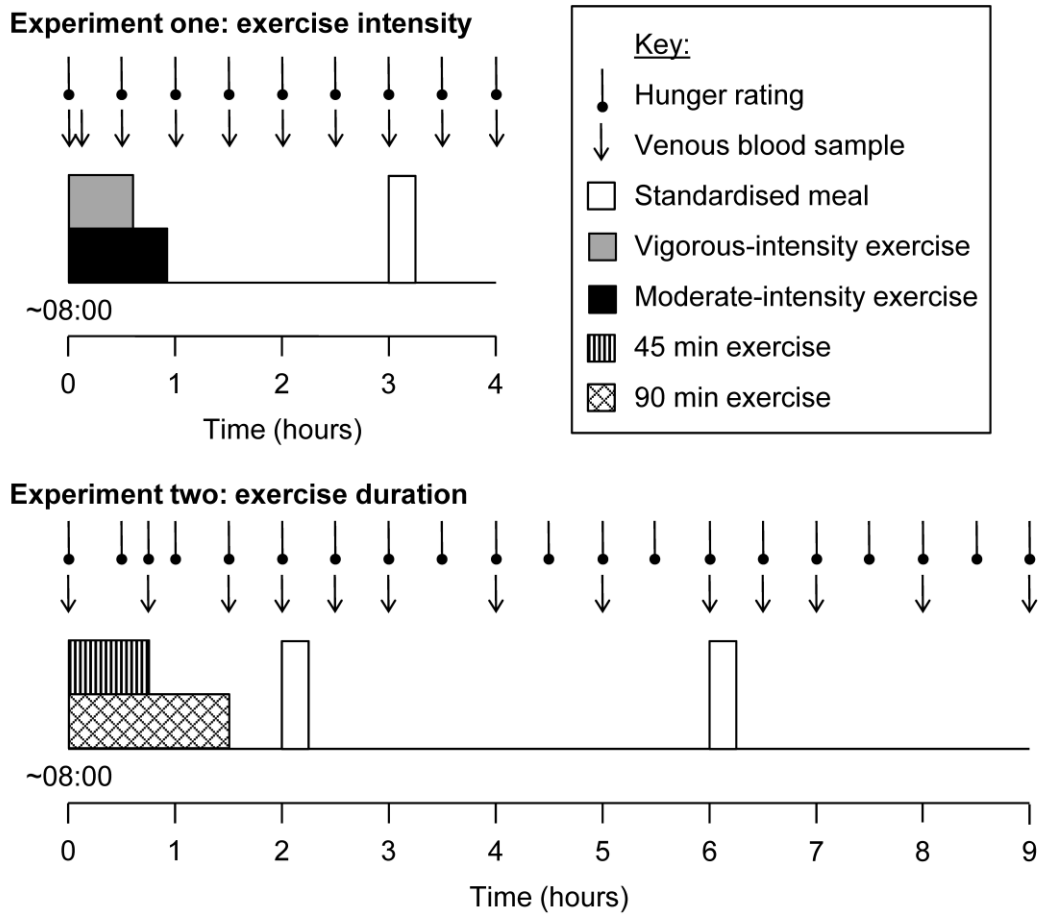
	Control	45 min exercise	90 min exercise	Main effect condition <i>P</i>
<b>Hunger</b>				
Fasting (mm)	45 (30 to 68)	47 (31 to 72)	43 (28 to 65)	0.73
TAUC (mm)	37 (27 to 49)	31 (23 to 42)	30 (22 to 40)	0.02 <sup>a</sup>
<b>Acylated ghrelin</b>				
Fasting (pg·mL <sup>-1</sup> )	159 (140)	163 (140)	153 (128)	0.88
Delta TAUC (pg·mL <sup>-1</sup> )	-7.44 (48.30)	-55.20 (77.34)	-46.56 (53.75)	0.07
<b>Glucose</b>				
Fasting (mmol·L <sup>-1</sup> )	5.04 (4.78 to 5.32)	5.06 (4.80 to 5.34)	5.04 (4.78 to 5.32)	0.98
TAUC (mmol·L <sup>-1</sup> )	5.05 (4.88 to 5.23)	5.32 (5.14 to 5.50)	5.35 (5.17 to 5.54)	0.06
<b>Insulin</b>				
Fasting (pmol·L <sup>-1</sup> )	21.3 (12.1 to 37.7)	19.6 (11.1 to 34.6)	17.1 (9.7 to 30.2)	0.74
TAUC (pmol·L <sup>-1</sup> )	67.8 (47.7 to 96.5)	68.2 (47.9 to 97.1)	61.5 (43.2 to 87.5)	0.81

Values for acylated ghrelin are mean (SD) for  $n = 9$ . Values for hunger, glucose and insulin are geometric mean (95% confidence interval) for  $n = 9$ , and statistical analyses are based on natural log transformed data. TAUC, time-averaged total area under the concentration versus time curve.

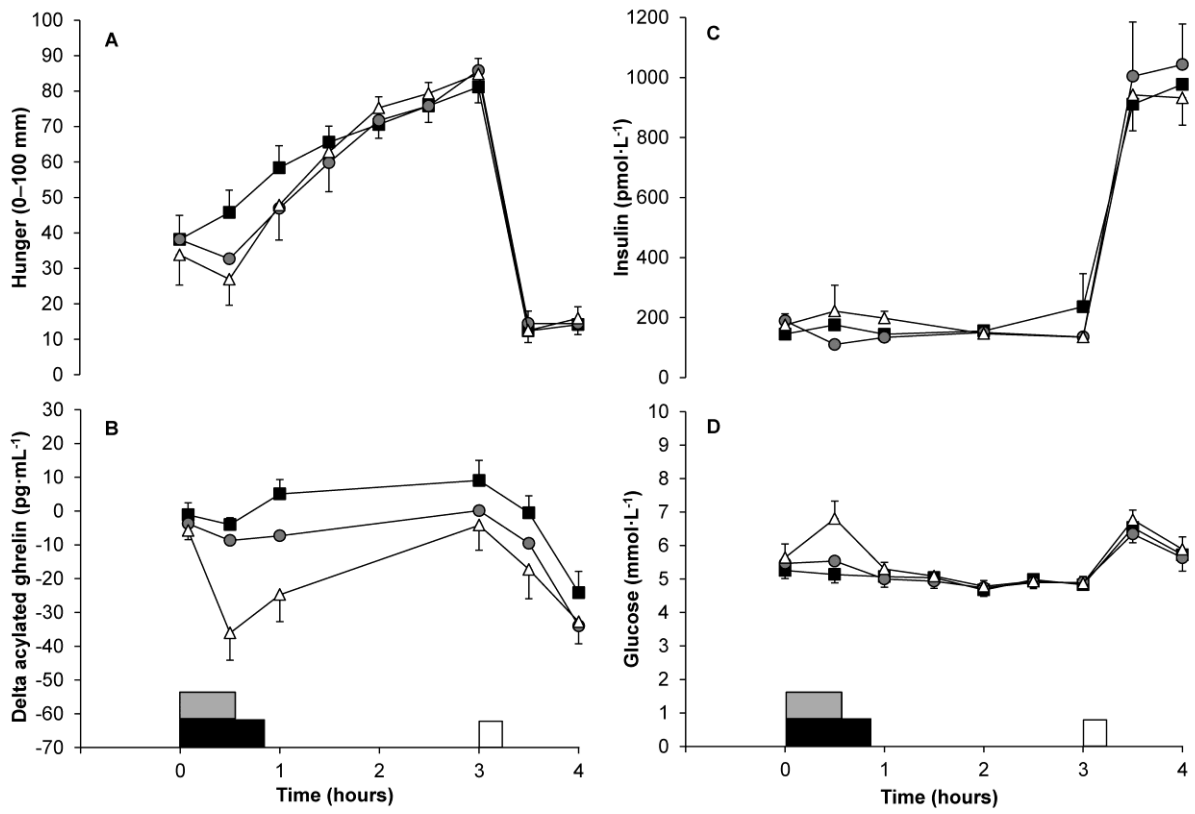
<sup>a</sup> Significant difference between 90 min exercise and control conditions (linear mixed model  $P < 0.05$  after Holm-Bonferroni correction)



**Figure 1**



**Figure 2**



**Figure 3**

