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1 **Appetite and gut hormone responses to moderate-intensity continuous exercise versus high-**
2 **intensity interval exercise, in normoxic and hypoxic conditions**

3

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18

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26 **Abstract**

27 This study investigated the effects of continuous moderate-intensity exercise (MIE) and high-
28 intensity interval exercise (HIIE) in combination with short exposure to hypoxia on appetite and
29 plasma concentrations of acylated ghrelin, peptide YY (PYY), and glucagon-like peptide-1 (GLP-1).
30 Twelve healthy males completed four, 2.6 h trials in a random order: 1) MIE-normoxia, 2) MIE-
31 hypoxia, 3) HIIE-normoxia, and 4) HIIE-hypoxia. Exercise took place in an environmental chamber.
32 During MIE, participants ran for 50 min at 70% of altitude-specific maximal oxygen uptake ($\dot{V}O_{2max}$)
33 and during HIIE performed 6 x 3 min running at 90% $\dot{V}O_{2max}$ interspersed with 6 x 3 min active
34 recovery at 50% $\dot{V}O_{2max}$ with a 7 min warm-up and cool-down at 70% $\dot{V}O_{2max}$ (50 min total). In
35 hypoxic trials, exercise was performed at a simulated altitude of 2,980 m (14.5% O₂). Exercise was
36 completed after a standardised breakfast. A second meal standardised to 30% of participants' daily
37 energy requirements was provided 45 min after exercise. Appetite was suppressed more in hypoxia
38 than normoxia during exercise, post-exercise, and for the full 2.6 h trial period (linear mixed
39 modelling, $p < 0.05$). Plasma acylated ghrelin concentrations were lower in hypoxia than normoxia
40 post-exercise and for the full 2.6 h trial period ($p < 0.05$). PYY concentrations were higher in HIIE than
41 MIE under hypoxic conditions during exercise ($p = 0.042$). No differences in GLP-1 were observed
42 between conditions ($p > 0.05$). These findings demonstrate that short exposure to hypoxia causes
43 suppressions in appetite and plasma acylated ghrelin concentrations. Furthermore, appetite
44 responses to exercise do not appear to be influenced by exercise modality.

45

46 **Keywords**

47 Hypoxia; high altitude anorexia; high-intensity exercise; appetite-regulating hormones; acylated
48 ghrelin

49

50 **Highlights**

- 51 • Effects of exercise modalities and hypoxia on appetite are explored

- 52 • Short exposure to hypoxia causes appetite suppressions
- 53 • Appetite responses to exercise are not dependant on exercise modality
- 54 • Suppressed appetite may be explained by decreased circulating acylated ghrelin

55

56 **Abbreviations**

57 PYY, peptide YY; HIIE, high-intensity interval exercise; MIE, moderate-intensity exercise; GLP-1,
58 glucagon-like peptide-1; $\dot{V}O_{2max}$, maximum oxygen uptake; PFC, prospective food consumption;
59 AUC, area under the curve.

60 Introduction

61 The current obesity epidemic is a major concern since excess weight is associated with morbidity
62 and premature mortality [5,8]. Exercise can play an important role in weight management as it may
63 improve the comorbidities of obesity [37] and contribute to a negative energy balance by increasing
64 energy expenditure [9]. Individuals do not tend to compensate for the energy expended during
65 exercise in the immediate hours after by altering food intake and such energy deficits could be
66 important for weight management if repeated over long periods of time [40]. Increasing exercise
67 intensity may increase energy expenditure and evidence suggests high-intensity exercise produces
68 greater short term reductions in appetite compared to moderate-intensity exercise [13,29].

69 One form of exercise training that is receiving more attention in health-enhancing
70 research is high-intensity interval exercise (HIIE), which may reduce cardiometabolic disease risk [28]
71 and promote similar or even superior physiological adaptations compared to traditional endurance-
72 based training [20]. All-out sprint interval exercise may acutely suppress appetite more than
73 continuous moderate-intensity exercise (MIE) [13], but this form of supramaximal exercise may not
74 be safe, tolerable, or practical for many individuals [13,20]. Submaximal HIIE may thus be preferred
75 and recent evidence suggests this form of interval exercise may also acutely suppress appetite and
76 increase the satiating gut hormone, peptide YY (PYY), more than an energy-matched continuous
77 bout of MIE [14]. Bartlett et al. [4] observed higher levels of enjoyment during a high-volume HIIE
78 protocol that involved 3 min intervals at 90% of maximum oxygen uptake ($\dot{V}O_{2max}$) compared to a
79 continuous MIE session matched for average intensity (70% $\dot{V}O_{2max}$). It would be of interest to
80 explore whether this interval exercise protocol suppresses appetite and affects gut hormone
81 concentrations more than continuous MIE.

82 A loss of appetite, termed “high altitude anorexia”, is often apparent when individuals are
83 exposed to high altitude (> 2,500 m) [26]. Reduced energy intake and weight loss are observed in
84 both normobaric and hypobaric hypoxia and studies using hypobaric chambers suggest it is hypoxia,
85 per se, that causes this altitude-related loss of appetite [50]. The role of appetite-regulating

86 hormones in high-altitude anorexia is unclear. The acute and chronic effect of hypoxia on leptin; a
87 hormone released from white adipose tissue that reduces food intake and modulates adiposity; is
88 controversial [12,27,42]. Acute suppression of appetite and acylated ghrelin (the post-translationally
89 modified form of this gut peptide essential for its appetite-stimulatory effects) was observed during
90 7 h exposure to normobaric hypoxia, while PYY tended to be higher than in normoxic conditions
91 [48]. The response of the satiating gut hormone, glucagon-like peptide-1 (GLP-1), to hypoxia has only
92 been investigated in one previous study that showed a trend towards increased concentrations
93 following overnight hypoxic exposure [42]. The effect of short exposure to hypoxia (i.e. ≤ 1 h) on
94 appetite and appetite-related hormones has not been studied, nor has the effect of different
95 exercise modalities performed in hypoxia.

96 This study therefore investigated the effects of continuous MIE versus HIIE in combination
97 with short exposure to hypoxia on appetite and plasma concentrations of acylated ghrelin, PYY, and
98 GLP-1.

99

100 **Methods**

101 *Participants*

102 Following approval from the University of Bedfordshire ethics review board, 12 physically active (\geq
103 150 min/wk of moderate-to-vigorous physical activity) and apparently healthy normal-weight men
104 (mean \pm SD; age, 21.6 ± 2.0 years; body mass index, 23.5 ± 2.0 kg/m²) gave written informed
105 consent to participate in the study following a verbal and written explanation of the nature and risks
106 involved. Participants were non-smokers, normotensive, not taking any medications, and had no
107 known history of cardiometabolic disease.

108

109 *Preliminary tests*

110 Participants attended the University of Bedfordshire Sport and Exercise Science laboratories for
111 preliminary tests to attain anthropometric measures (height and body mass) and determine $\dot{V}O_{2max}$.

112 Height was measured to the nearest 0.1 cm using a stadiometer (Horlaint Ltd, Crymych, UK) and
113 body mass to the nearest 0.1 kg using electronic weighing scales (Tanita BWB-800, Tanita Corp.,
114 Tokyo, Japan).

115

116 *Maximum oxygen uptake*

117 $\dot{V}O_{2max}$ was assessed under two blinded conditions: normoxia and hypoxia. Both conditions were
118 generated by a custom built environmental chamber (T.I.S. Services, Hampshire, UK) regulated by a
119 microprocessor control. In addition to the chamber control panel display readings, all environmental
120 conditions were monitored and checked by independent calibrated instruments: temperature and
121 humidity via a Testo 625 hygrometer and oxygen levels via a Kane 250 Gas Meter. Humidity and
122 temperature were controlled at 40% relative humidity and 18°C, respectively. Hypoxic conditions
123 represented a simulated altitude of 2,980 m (14.5% O₂). In both conditions an incremental exercise
124 test was performed on a motorised treadmill (Woodway PPS55 Med-i, GmbH, Germany) with a 0%
125 gradient. Oxygen uptake was measured continuously during exercise using an online gas analysis
126 system (Cortex Metalyzer 3B, GmbH, Germany). The gas analyser used was daily volume- and gas-
127 calibrated and corrected for barometric pressure, temperature, and humidity. Following
128 familiarisation, participants were asked to warm up for 5 min at a velocity they felt they could
129 comfortably maintain for 30 min. The participants then began the test with a 2 min stage at this
130 speed. The speed was then increased by 1 km/h every 2 min until volitional exhaustion. $\dot{V}O_{2max}$ was
131 taken as the highest $\dot{V}O_2$ value averaged over a 10 sec period. Criteria used to confirm a true
132 maximum value included two or more of the following: 1) heart rate within 10 bpm of age predicted
133 maximum, 2) respiratory exchange ratio > 1.15, 3) plateau of $\dot{V}O_2$ despite increasing workload, and
134 4) rating of perceived exertion \geq 18 on the Borg scale [6]. $\dot{V}O_{2max}$ was significantly higher in
135 normoxia compared to hypoxia (56.0 ± 7.8 vs. 44.0 ± 5.8 mL/kg⁻¹/min⁻¹, respectively, $p < 0.001$).

136

137

138 *Main trials*

139 This was a randomised four-way cross-over design study. Participants completed four trials
140 separated by ≥ 7 days: 1) MIE-normoxia, 2) MIE-hypoxia, 3) HIIE-normoxia, and 4) HIIE-hypoxia. The
141 environmental condition of each trial (normoxic versus hypoxic) was single blinded. Fig. 1 shows the
142 trial protocol. Participants weighed and recorded food intake for 24 h before the first main trial and
143 were asked to replicate the quantity and timings of eating prior to each subsequent testing day and
144 to refrain from alcohol and moderate-to-vigorous physical activity during this time.

145

146 Figure 1 about here.

147

148 Participants arrived at the laboratory between 7am and 8am having fasted for a minimum of 9 h
149 overnight and were weighed in light clothing and no footwear. A breakfast meal was then consumed
150 followed by a 1.75 h rest period. Exercise bouts then commenced at 0 h and participants were
151 informed of the exercise session (MIE or HIIE) that they would be performing upon entering the
152 chamber. The environmental condition remained blinded to the participant during all trials. The
153 chamber replicated those conditions outlined above for the normoxic and hypoxic conditions,
154 respectively. Exercise was performed for 50 min in the environmental chamber with participants
155 seated in a normal laboratory testing room for the remainder of each trial. During MIE, participants
156 ran for 50 min at a speed predicted to elicit 70% $\dot{V}O_{2max}$. HIIE consisted of 6 x 3 min bouts at a
157 running velocity corresponding to 90% $\dot{V}O_{2max}$ interspersed with 6 x 3 min bouts of active recovery
158 at a velocity corresponding to 50% $\dot{V}O_{2max}$, and was preceded by a 7 min warm-up and followed by a
159 7 min cool-down at a velocity of 70% $\dot{V}O_{2max}$. This protocol thus consisted of 36 min interval exercise
160 and total exercise duration of 50 min. These protocols were selected based on a comparative study
161 in recreationally active males that reported greater levels of perceived enjoyment following HIIE,
162 similar energy expenditure (811 ± 83 and 832 ± 136 kcal for the HIIE and MIE protocols,
163 respectively), and were matched for an average intensity of 70% $\dot{V}O_{2max}$ [4]. As such, the same

164 duration and mean intensity of exercise was used in both exercise conditions but with alternating
165 high and low intensity bouts in the HIIE trials.

166

167 *Standardised meals*

168 On arrival, a standardised breakfast was provided to each participant following collection of fasted
169 blood samples. The breakfast consisted of cornflakes and semi-skimmed milk and was consumed
170 within 15 min. The macronutrient content of this meal was 78% carbohydrate, 16% protein, and 6%
171 fat. The breakfast provided 20% of the estimated sedentary daily energy needs for each individual
172 (mean energy content 494 ± 27 kcal). Resting daily energy requirements were calculated [33] and
173 this value multiplied by 1.4 to represent a sedentary day. An instant pasta lunch meal was consumed
174 at 1.6 h (i.e. 45 min post-exercise), which provided 30% of the daily energy requirements for each
175 individual (mean energy content 741 ± 40 kcal). Macronutrient content was 74.5% carbohydrate,
176 21% protein, and 4.5% fat. Water was available *ad libitum* throughout trials.

177

178 *Ratings of perceived appetite and nausea*

179 During each trial subjective feelings of hunger (“How hungry do you feel”), satisfaction (“How
180 satisfied do you feel”), fullness (“How full do you feel”), and prospective food consumption (PFC;
181 “How much do you think you can eat”) were reported on paper using a validated 100-mm visual
182 analogue scale (VAS) [19]. Appetite perceptions were measured at baseline (-2 h), immediately after
183 breakfast (-1.75 h), immediately before exercise (0 h), mid-exercise (0.4 h), immediately post-
184 exercise (0.8 h), immediately before lunch (1.6 h), immediately post-lunch (1.8 h), and 30 and 60 min
185 (2.1 and 2.6 h, respectively) following the first mouthful of the lunch meal. A subjective rating of
186 nausea (“Not at all nauseous” to “Very nauseous”) was also taken at each of these time points using
187 a 100-mm VAS scale. An overall appetite rating was calculated as the mean value of the four
188 appetite perceptions after inverting the values for satisfaction and fullness [43].

189

190 *Blood sampling*

191 During each main trial, blood samples were collected via venepuncture (VACUETTE®, Greiner Bio-
192 One, Austria) from an antecubital vein whilst participants were in a semi-supine position. A fasting
193 venous sample was taken upon arrival at the laboratory followed by samples immediately before
194 exercise (0 h), immediately post-exercise (0.8 h), immediately before lunch (1.6 h), and 30 and 60
195 min (2.1 and 2.6 h, respectively) following the first mouthful of the lunch meal. Samples were
196 collected into two pre-cooled 4.9-mL EDTA vacuettes (Horltaim Ltd, Crymych, UK). One vacuette was
197 immediately centrifuged at 1,500 x g for 10 min at a temperature of 4°C (Heraeus Multifuge X3R,
198 Thermo Scientific, Loughborough, UK). The plasma supernatant was then dispensed into separate 2-
199 mL cryovials and stored at -80°C until later analysis of glucose, insulin, total PYY, and total GLP-1
200 concentrations. From each sample, duplicate 20-µL blood samples were collected into heparinised
201 microhaematocrit tubes for determination of haematocrit and a 10-µL sample into a microcuvette
202 for determination of haemoglobin concentration to enable an estimation of plasma volume changes
203 [16]. To prevent the degradation of acylated ghrelin, a 50-µL solution containing potassium
204 phosphate buffer, p-hydroxymercuribenzoic acid, and sodium hydroxide was added to one 4.9-mL
205 EDTA vacuette, which was then centrifuged at 1,500 x g for 10 min at 4°C. The plasma supernatant
206 was then dispensed into a storage tube and 100-µL of 1 M hydrochloric acid was added per mL of
207 plasma to preserve acylated ghrelin [24]. Thereafter, samples were spun at 1500 x g for 5 min at 4°C
208 prior to storage in 2-mL cryovials at -80°C until analysis.

209

210 *Blood biochemistry*

211 Commercially available enzyme immunoassays were used to determine plasma concentrations of
212 acylated ghrelin (SPI BIO, Montigny le Bretonneux, France), total PYY (Millipore, Watford, UK), total
213 GLP-1 (Millipore, Watford, UK) and insulin (Merckodia, Uppsala, Sweden). Plasma glucose
214 concentrations were determined by enzymatic, colorimetric methods using a bench top analyser
215 (Pentra 400, HORIBA ABX Diagnostics, Montpellier, France). To eliminate interassay variation,

216 samples from each participant were analysed in the same run. The within batch coefficients of
217 variation for the assays were as follows: acylated ghrelin, 4.5%; total PYY, 5.5%; GLP-1, 4.4%; insulin,
218 2.9%; glucose, 0.8%.

219

220 *Statistical analysis*

221 Analyses were completed using the statistical software package IBM SPSS Statistics version 19.0
222 (SPSS Inc., Chicago, IL, USA) and SigmaPlot version 12.3 (Systat Software Inc., CA, USA). Data are
223 presented as mean (SE) in tables, text and figures. Correction of blood parameters for changes in
224 plasma volume did not alter the interpretation of the results; therefore, for simplicity, the
225 unadjusted values are presented. Standard graphical methods were preferred over null hypothesis
226 significance testing to check statistical assumptions [22]. Prior to any inferential statistical analyses
227 descriptive statistics tables were generated to check the central tendency (mean, median) and
228 dispersion (standard deviation, minimum, maximum) of the data. Second, quantile-quantile (Q – Q)
229 plots were used to check the normality assumption of the results obtained for each of the conditions
230 across all trial periods. Where normality was deemed plausible, central tendency and dispersion
231 were reported as the mean and standard error. The two-tailed alpha level for significance testing
232 was set as $p < 0.05$.

233 Linear mixed models were chosen to determine if there were any differences in the
234 dependent variables between the conditions across time. This type of analysis was preferred as it i)
235 allows for missing data, ii) can accurately model different covariate structures for repeated
236 measures data, and iii) can model between-subject variability [47,49]. Area under the curve (AUC)
237 was calculated for all blood metabolite and appetite variables using the trapezoidal method for the
238 total trial period (2.6 h), the period during exercise (0 to 0.8 h), and the post-exercise period (0.8 to
239 2.6 h). Fixed and random factors for the linear mixed model were fit for each dependent variable
240 and the main effects for 1) altitude (hypoxia vs. normoxia), and 2) exercise (HIIE vs. MIE), as well as
241 interactions (altitude x exercise), were analysed by plotting the mean values. Step down Hommel

242 [23] adjusted post-hoc pair wise comparisons were calculated if a significant main effect and/or
243 interaction effect was present. Analysis of serial measurements was also conducted using linear
244 mixed models, for the main effects of 1) altitude (hypoxia vs. normoxia), 2) exercise (HIIE vs. MIE),
245 and 3) time (serial measurements over 2.6 h), as well as interactions (condition x time). The most
246 appropriate model was chosen using the smallest Hurvich and Tsai's criterion (AICC) in accordance
247 with the principal of parsimony. Second, normality and homogeneity of variance of the residuals
248 were checked using Q – Q plots and scatter plots, respectively, and deemed plausible in each
249 instance. Pearson correlation was used to explore within-subject relationships between AUC values
250 for appetite perceptions and gut hormones concentrations for combined hypoxic trials, normoxic
251 trials, HIIE trials, MIE trials, and all trials combined for the 2.6 h trial period.

252 Based on previous data from Deighton et al. [13], a sample size of 12 participants was
253 determined as sufficient to detect a 10% difference in appetite perceptions during the post-exercise
254 period. This calculation was performed using G*power with an alpha value of 5% and a power of
255 80% [18].

256

257 **Results**

258 Table 1 about here

259

260 *Appetite perceptions*

261 There were no significant differences in any fasting appetite perception between trials ($p > 0.05$).

262 Table 1 shows AUC values for each appetite perception for the combined hypoxia and normoxia

263 trials, and for the combined HIIE and MIE trials. Compared with normoxia, hunger AUC was

264 significantly lower during exercise (0 to 0.8 h; $p < 0.001$), post-exercise (0.8 to 2.6 h; $p = 0.003$), and

265 for the total 2.6 h trial period (0 to 2.6 h; $p < 0.001$) in hypoxia. Satisfaction AUC was significantly

266 higher during exercise ($p = 0.010$), post-exercise ($p < 0.001$), and for the total 2.6 h trial period ($p <$

267 0.001) in hypoxia compared to normoxia. The analysis of serial measurements confirmed the

268 findings of the AUC analysis by demonstrating a main effect of altitude for hunger ($p = 0.049$) and
269 satisfaction ($p = 0.025$), respectively.

270 Fullness AUC was significantly higher post-exercise ($p = 0.030$) and for the total 2.6 h trial
271 period ($p = 0.016$) in hypoxia compared with normoxia, and this difference was approaching
272 significance for the exercise time period ($p = 0.056$). The main effect of altitude in the serial
273 measurements analysis for fullness was approaching significance ($p = 0.061$). AUC values for PFC
274 were significantly lower in hypoxia compared with normoxia during exercise ($p < 0.001$), post-
275 exercise ($p = 0.002$), and for the full trial period ($p < 0.001$). Overall appetite AUC was also
276 significantly lower during exercise ($p < 0.001$) and for the full 2.6 h trial period ($p = 0.001$) in hypoxia
277 compared with normoxia, and was approaching significance for the post-exercise period ($p = 0.051$).
278 These findings were confirmed in the serial measurements analysis with a main effect of altitude on
279 PFC ($p = 0.014$) and overall appetite ($p = <0.001$). There were no significant differences for any
280 appetite perception between HIIE and MIE conditions. Perceived appetite responses over time for
281 each trial are shown in Fig. 2.

282 Feelings of nausea did not differ significantly between hypoxic and normoxic trials or
283 between HIIE and MIE trials in the exercise, post-exercise, or full 2.6 h trial periods ($p > 0.05$). There
284 were also no altitude x exercise interaction effects for any trial time period ($p > 0.05$). Differences in
285 appetite perceptions between trials were thus unlikely due to nausea sensations.

286

287 Figure 2 about here.

288

289 Figure 3 about here.

290

291 *Gut hormone concentrations*

292 Fasting plasma acylated ghrelin ($p = 0.402$), PYY ($p = 0.959$), and GLP-1 concentrations ($p = 0.815$) did
293 not differ at baseline between the trials. Table 2 shows AUC values for gut hormone concentrations

294 for the combined hypoxia and normoxia trials, and for the combined HIIE and MIE trials. Compared
295 with normoxia, acylated ghrelin AUC was significantly lower in hypoxia during the post-exercise ($p =$
296 0.020) and total 2.6 h ($p = 0.035$) time periods. Acylated ghrelin AUC did not differ significantly
297 between HIIE and MIE for any time period. Analysis of serial measurements revealed that the main
298 effect of altitude for acylated ghrelin was approaching significance ($p = 0.065$). There were no
299 significant interaction effects for altitude x exercise for acylated ghrelin in any of the analyses.

300 There were no significant main effects between altitude or exercise conditions for PYY AUC.
301 However, there was a significant altitude x exercise interaction effect for PYY AUC in the exercise
302 time period ($p = 0.042$) with concentrations being significantly higher in HIIE than MIE (115 ± 17 and
303 $98 \pm 12 \text{ pg/mL}^{-1}/0.83 \text{ h}^{-1}$, respectively) under hypoxic conditions ($p = 0.042$). The altitude x exercise
304 interaction effect for PYY AUC was also approaching significance for the total 2.6 h time period ($p =$
305 0.076). The analysis of serial measurements confirmed the findings of the AUC analysis by
306 demonstrating a significant altitude x exercise interaction effect ($p = 0.015$) with PYY concentrations
307 being significantly higher in HIIE than MIE (128 ± 12 and $120 \pm 12 \text{ pg/mL}$, respectively) under hypoxic
308 conditions ($p = 0.048$) in addition to revealing significantly higher values in hypoxia than normoxia
309 (128 ± 12 and $120 \pm 12 \text{ pg/mL}$, respectively) during HIIE ($p = 0.027$). There were no main or
310 significant interaction effects for altitude or exercise conditions for GLP-1 concentrations. Gut
311 hormone concentrations over time for each trial are shown in Fig. 3.

312

313 Table 2 about here.

314

315 *Glucose and insulin concentrations*

316 Plasma glucose and insulin AUC values for the combined hypoxia and normoxia trials, and combined
317 HIIE and MIE trials, can be seen in Table 2. Fasting plasma glucose ($p = 0.402$) and insulin ($p = 0.895$)
318 concentrations did not differ at baseline between the trials. Glucose AUC was significantly lower in
319 hypoxia than normoxia during the post-exercise period ($p = 0.024$) and this was approaching

320 significance for the total 2.6 h trial period ($p = 0.051$). Glucose AUC post-exercise was lower in MIE
321 than HIIE and this was approaching significance ($p = 0.076$). Analysis of serial measurements
322 demonstrated a main effect of altitude and exercise with glucose concentrations being lower in
323 hypoxia than normoxia ($p = 0.041$) and lower in MIE than HIIE ($p = 0.034$). Insulin AUC was lower in
324 hypoxia than normoxia during exercise and the total 2.6 h trial period and this was approaching
325 significance ($p = 0.073$ and $p = 0.067$, respectively). There were no significant main effects for insulin
326 in the serial measurements analysis. Plasma glucose and insulin concentrations over time for each
327 trial are shown in Fig. 4.

328

329 Figure 4 about here.

330

331 *Correlations between appetite perceptions and appetite-regulating hormones*

332 Within-subject AUC correlations for the full 2.6 h trial period for all trials combined revealed a
333 significant negative relationship between plasma acylated ghrelin and satisfaction ($r = -0.403$, $p =$
334 0.005) and fullness ($r = -0.497$, $p < 0.000$), and a significant positive relationship with PFC ($r = 0.456$, p
335 $= 0.001$) and overall appetite ($r = 0.428$, $p = 0.003$). Acylated ghrelin was also significantly negatively
336 related with fullness in the HIIE trials combined for the 2.6 h trial period ($r = -0.593$, $p = 0.042$). No
337 significant correlations between plasma PYY and GLP-1 with appetite perceptions were observed in
338 the analyses.

339

340 **Discussion**

341 This study investigated the effects of HIIE versus continuous MIE exercise combined with short
342 exposure to hypoxia on appetite and gut hormone concentrations. Our novel data suggest that
343 appetite perceptions and plasma acylated ghrelin may be suppressed in response to as little as 50
344 min normobaric hypoxic exposure whilst performing exercise. Acute suppressions in the active form
345 of ghrelin were observed previously during 7 h exposure to a simulated altitude of 4,000 m [48] and

346 these data suggest that this response in acylated ghrelin in the absence of cold and other stressors
347 may be implicated in high altitude anorexia. The effect of hypoxia on ghrelin is in its early stages of
348 research and the mechanisms responsible for hypoxia-induced suppressions of this hormone are
349 thus unclear. Ghrelin is predominantly derived from the stomach [2] and crosses the blood-brain
350 barrier to exert its appetite-stimulating effects in the food-regulating centre of the hypothalamus
351 [3]. Ghrelin secreted from the stomach passes through the liver from the portal vein into the
352 peripheral circulation [21]. Decreased oxygen saturation in hypoxia may result in compensatory
353 reductions in splanchnic blood flow in an attempt to maintain oxygen delivery elsewhere in the body
354 [52]. Given that the liver may be involved in the acylation of ghrelin [21], reduced blood flow to this
355 organ could explain hypoxia-induced reductions in circulating concentrations of ghrelin in its
356 acylated form. One study also observed reduced blood flow to the superior mesenteric artery, which
357 supplies the intestine, in a fasted and postprandial state following 2 h exposure to a simulated
358 altitude of 4,800 m [31], which might suggest impaired gut blood flow as a mechanistic explanation
359 for high altitude anorexia. However, similar postprandial increases in arterial and venous blood flow
360 in the gut at sea level and high altitude have been observed after a 3 day exposure to hypobaric
361 hypoxia [25]. Appetite was also suppressed in the study by Kalson et al. [25], thus suggesting that
362 high altitude anorexia after several days was not due to impaired gut blood flow. It is possible that
363 changes in gut blood flow occur in response to acute hypoxia and contribute to suppressed acylated
364 ghrelin concentrations and high altitude anorexia, while, in the longer term, different mechanisms
365 are responsible [48].

366 It has been suggested that the postprandial suppression of ghrelin may be in part glucose-
367 induced [36] and previous research that exposed participants to 7 h hypoxia observed higher glucose
368 and suppressed acylated ghrelin concentrations in hypoxia than normoxia [48]. However, glucose
369 concentrations in the current study were suppressed in the hypoxic trials and this was concomitant
370 with suppressed acylated ghrelin concentrations and another study found hyperglycaemia of 11
371 mmol.L⁻¹ did not affect ghrelin concentrations [39]. Other research has suggested that insulin is an

372 important physiological and dynamic modulator of ghrelin [36,38], although insulin did not differ
373 between hypoxia and normoxia conditions in the current study. These data suggest that the array of
374 other hormones released after eating may be involved in the observed postprandial ghrelin response
375 in hypoxia [30].

376 GLP-1 concentrations were unaffected by short exposure to hypoxia combined with exercise.
377 To the authors' knowledge, only one previous study has investigated the response of GLP-1 to
378 hypoxia [42]. In that study, fasting concentrations of GLP-1 did not differ compared to normoxia
379 following overnight exposure to a simulated altitude of 4,100 m, while there was a tendency for GLP-
380 1 to be higher 40 min postmeal. This might suggest that hypoxia does not influence GLP-1 in the
381 absence of feeding. Research into the effects of hypoxia on PYY is also limited, although Wasse et al
382 [48] observed a tendency for higher total PYY concentrations in normoxia compared to 7 h hypoxic
383 exposure. However, the current study observed higher total PYY concentrations in trials where HIIE
384 was performed in hypoxia compared to when HIIE was performed in normoxia. However, these
385 differences in PYY concentrations were not accompanied by changes in perceived appetite and more
386 research is needed to establish if PYY is important in high altitude anorexia. A limitation of these
387 studies, though, is that total PYY was measured and not concentrations of PYY₃₋₃₆, which is the form
388 of PYY that is more potent in suppressing hunger [11]. However, total PYY and PYY₃₋₃₆ are highly
389 correlated [44] and changes in total PYY are thus likely to reflect changes in PYY₃₋₃₆.

390 There is convincing evidence that exercise at $\geq 60\% \dot{V}O_{2max}$ causes acute suppressions in
391 appetite [15]. Given the recent rise in popularity of HIIE in the media and scientific literature, several
392 recent studies have compared appetite responses of this mode of exercise to traditional moderate-
393 intensity endurance-based exercise [1,13,14,32,41]. The current study did not observe suppressed
394 appetite in response to submaximal HIIE compared to continuous MIE, which has similarly been
395 reported in studies using overweight and obese participants [32,41]. Alkahtani et al [1] also observed
396 no differences in appetite perceptions following HIIE compared with moderate-intensity interval
397 exercise in overweight and obese males. However, the current data is not in agreement with

398 previous research in healthy males that did observe suppressed appetite in HIIE compared with
399 continuous MIE [14,51]. One study in healthy males reported increased appetite sensations
400 following HIIE [13], but this exercise protocol was supramaximal and might suggest there is an
401 exercise intensity threshold above which appetite is increased post-exercise. However, another
402 study employing a supramaximal HIIE protocol did not observe any differences in appetite
403 perceptions compared with submaximal HIIE or continuous MIE [41] and this theory thus requires
404 further investigation. Nonetheless, an important observation in the literature that the current study
405 supports is that traditional endurance based exercise does not elicit reduced appetite compared to
406 submaximal HIIE [15].

407 There were no differences in appetite perceptions, acylated ghrelin, or GLP-1
408 concentrations between HIIE and MIE for any trial period. However, total PYY concentrations during
409 exercise were higher in HIIE than MIE when exercising under hypoxic conditions. Although research
410 exploring the effects of HIIE on appetite-regulating hormones is limited, higher mean plasma PYY₃₋₃₆
411 concentrations were recently reported following submaximal HIIE than continuous MIE [14]. Greater
412 increases in PYY₃₋₃₆ concentrations were also observed following 30 min of high intensity continuous
413 exercise than 30 min continuous MIE [45], although these exercise sessions were not matched for
414 energy expenditure. It is thus possible that the kinetics of PYY in blood might differ in response to
415 different modes and intensities of exercise. The reason for PYY response to exercise is not well
416 understood but it is known that gut hormones interact with one another and with glucose
417 metabolism and these may be important mechanistic factors [35].

418 The current study found no difference in acylated ghrelin concentrations between HIIE
419 and MIE. Previous research also demonstrated no difference in acylated ghrelin following
420 submaximal HIIE compared with continuous MIE exercise in overweight men [41]. However, another
421 study in overweight and obese participants reported decreased acylated ghrelin and increased GLP-1
422 concentrations following both HIIE and continuous MIE, while no differences were observed for
423 PYY₃₋₃₆ [32]. Different responses to HIIE versus MIE between studies may be attributable to

424 variations in protocols employed, such as exercise intensity and duration, and the participants
425 studied. It is also important to note that it is difficult to make direct comparisons between total PYY
426 measured in the current study with PYY₃₋₃₆ responses in other investigations as the conversion rate
427 between these two forms of this hormone is unknown. Based on data from the current study, it is
428 not possible to advise which mode of exercise (HIIE or MIE) individuals should engage in under
429 hypoxic or normoxic trials to elicit preferable appetite responses.

430 Responses in appetite perceptions to exercise and/or hypoxia are not always concomitant
431 with changes in appetite-regulating hormone concentrations, and vice versa [7,13,14,32,41,48]. In
432 the current study, appetite perceptions and acylated ghrelin concentrations were suppressed in the
433 hypoxic compared with normoxic trials. Wasse et al [48] also observed suppressed appetite
434 perceptions and acylated ghrelin following hypoxia. In other studies, appetite was suppressed
435 following high-intensity exercise without changes in appetite-regulating hormone concentrations [7],
436 while on the contrary, gut hormone concentrations have been affected without associated changes
437 in appetite perceptions [14,32]. This emphasises the complex nature of appetite regulation that
438 comprises a range of both neuroendocrine and psychological factors [17,34,41] and responses
439 observed may be dependent on the nature of exposure to exercise (e.g. intensity, mode, duration)
440 and/or hypoxia.

441 The current study presents both strengths and limitations. The main strength is the
442 crossover design and the measurement of an array of appetite-related variables (subjective feelings
443 and plasma levels of several appetite-related hormones). The findings of the current study are
444 limited by the population sample as participants were all healthy young males. Although previous
445 research suggests similar appetite responses in lean and overweight individuals [46], further studies
446 in overweight and obese individuals are warranted to inform the design of effective weight
447 management interventions. Although the HIIE and MIE trials in the current study were matched for
448 average intensity (70% $\dot{V}O_{2max}$) based on data from $\dot{V}O_{2max}$ testing, this was not confirmed during
449 the trials as a measure of oxygen consumption was not taken. Another limitation is that it could not

450 be determined whether the observed responses in appetite and acylated ghrelin result in reduced
451 energy intake as participants were provided standardised meals throughout the study. However, the
452 purpose of a fixed-size meal was to distinguish the effects of food intake and of exercise and altitude
453 conditions on objective and subjective measures of appetite. Furthermore, carbohydrate and
454 protein content of a breakfast meal could alter ventilatory and metabolic responses to exercise in
455 hypoxia [10]. Since the breakfast meal in the current study is high in carbohydrate and low in protein
456 the findings may be limited to high-carbohydrate breakfasts only. The breakfast and lunch meals
457 provided were also relatively low in fat compared to realistic conditions and this limits application of
458 the findings to meals with higher fat content. The absence of a control condition for hypoxia and
459 exercise conditions is also a limitation, but this would have meant a total of six trials per participant,
460 which we believe would have been too substantial. Although symptoms of nausea were assessed,
461 other symptoms of acute mountain sickness (AMS) such as headache, fatigue, and dizziness were
462 not. Although Wasse et al [48] reported no significant correlations between AMS scores and appetite
463 perceptions during rest and exercise, it is possible symptoms other than nausea could have
464 influenced appetite perceptions in the current study. Lastly, it could not be determined if hypoxia or
465 exercise affected water intake, or whether water intake was related to appetite perceptions or gut
466 hormone concentrations, as no measure was taken.

467 In conclusion, short exposure to normobaric hypoxia whilst performing exercise causes
468 suppressions in appetite and circulating plasma acylated ghrelin concentrations. Furthermore,
469 appetite responses to exercise do not appear to be influenced by exercise modality (interval versus
470 continuous). Further research is needed to establish the chronic effects of hypoxia on appetite
471 regulation and whether there are differences in appetite following repeated bouts of HIIE versus
472 continuous MIE.

473

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- 609

610 Figure 1

611 Fig. 1. Schematic representation of the study protocol.

612

613 Figure 2

614 Fig. 2. Changes in perceptions of (A) hunger, (B) satisfaction, (C) fullness, and (D) prospective food
615 consumption during moderate-intensity exercise (MIE)-normoxia, MIE-hypoxia, high-intensity
616 interval exercise (HIIE)-normoxia, and HIIE-hypoxia. Values are means \pm SE; $n = 12$. Some error bars
617 have been omitted for clarity. *Black rectangle* indicates standardised breakfast, *open rectangle*
618 indicates treadmill exercise and hypoxia (or normoxia), *downward arrow* indicates standardised
619 lunch meal.

620

621 Figure 3

622 Fig. 3. Changes in plasma concentrations of (A) acylated ghrelin, (B) total PYY, and (C) GLP-1 during
623 moderate-intensity exercise (MIE)-normoxia, MIE-hypoxia, high-intensity interval exercise (HIIE)-
624 normoxia, and HIIE-hypoxia. Values are means \pm SE; $n = 12$. Some error bars have been omitted for
625 clarity. *Black rectangle* indicates standardised breakfast, *open rectangle* indicates treadmill exercise
626 and hypoxia (or normoxia), *downward arrow* indicates standardised lunch meal.

627

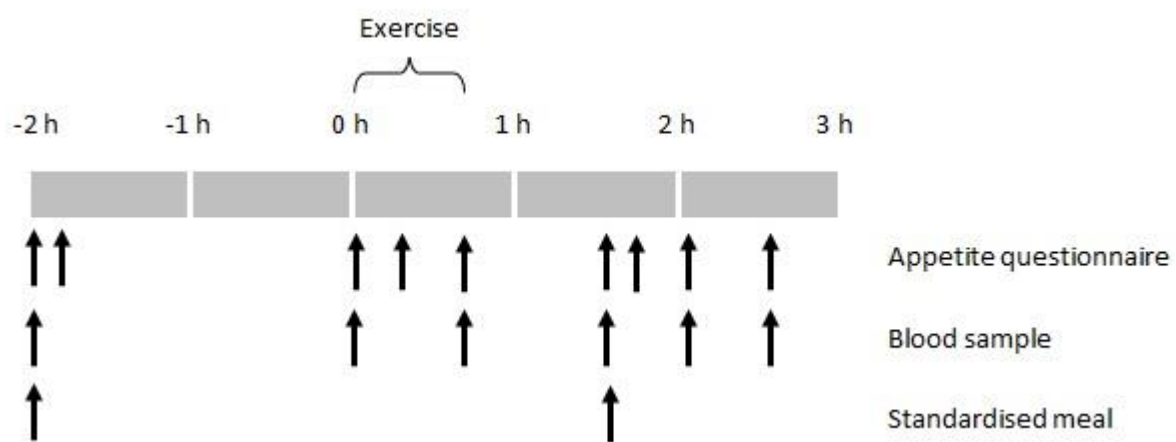
628 Figure 4

629 Fig. 4. Changes in plasma concentrations of (A) glucose and (B) insulin during moderate-intensity
630 exercise (MIE)-normoxia, MIE-hypoxia, high-intensity interval exercise (HIIE)-normoxia, and HIIE-
631 hypoxia. Values are means \pm SE; $n = 12$. Some error bars have been omitted for clarity. *Black*
632 *rectangle* indicates standardised breakfast, *open rectangle* indicates treadmill exercise and hypoxia
633 (or normoxia), *downward arrow* indicates standardised lunch meal.

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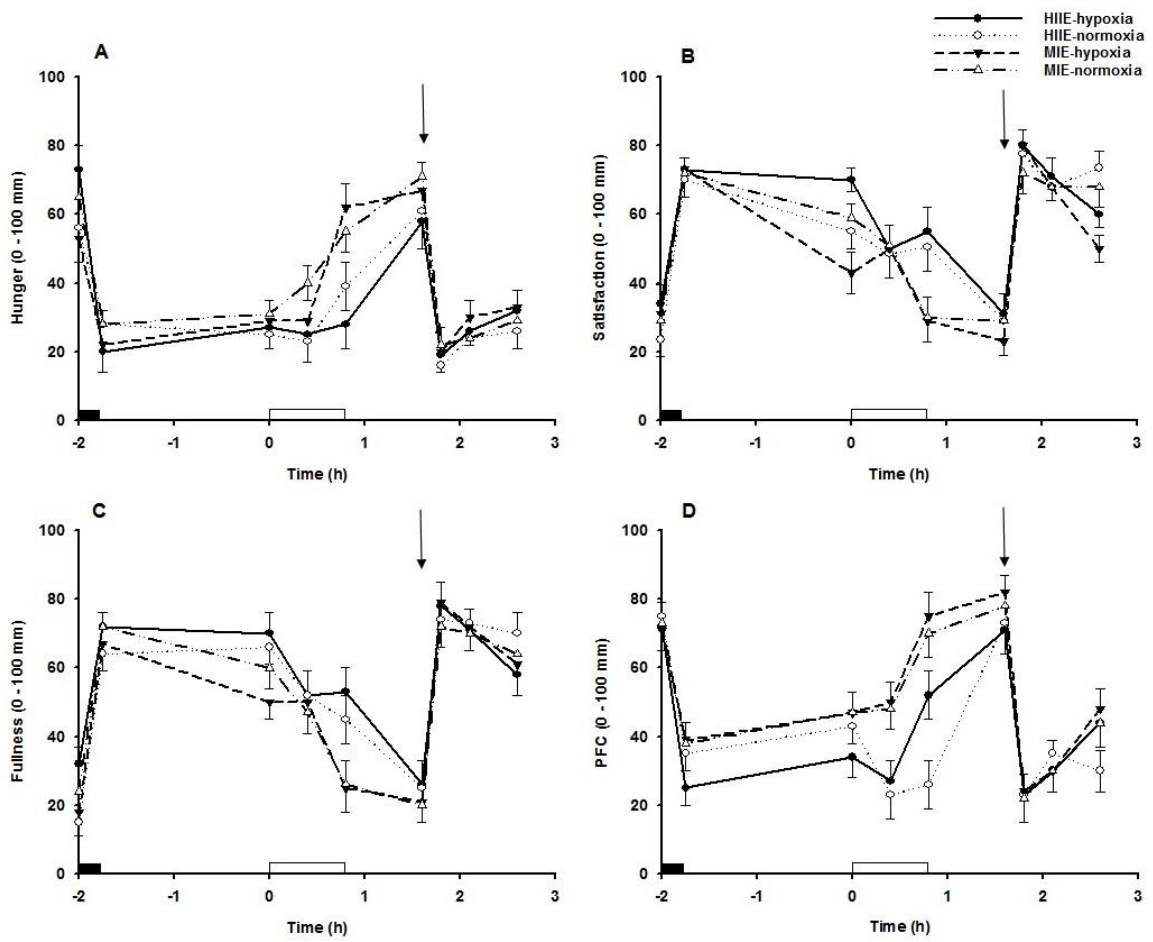
635 **Figure 1**

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641 **Figure 2**

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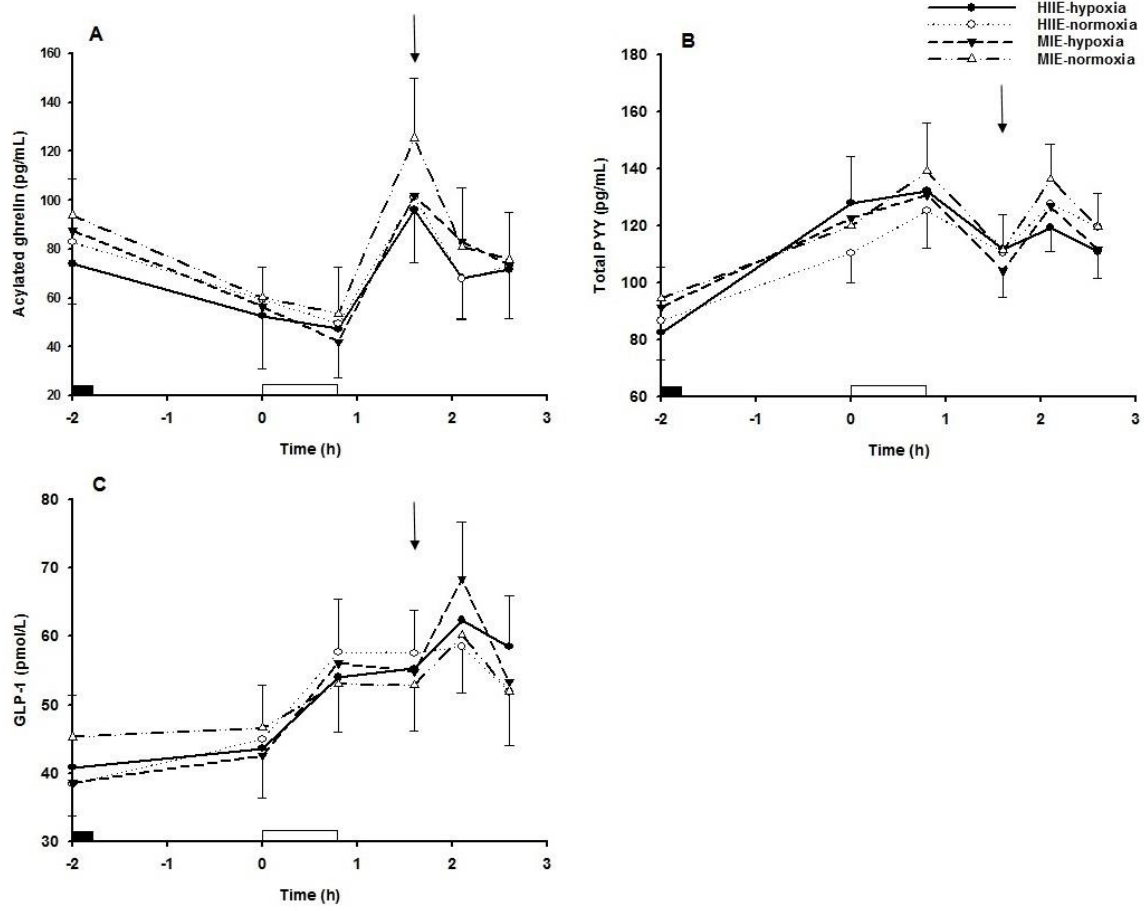


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645 **Figure 3**

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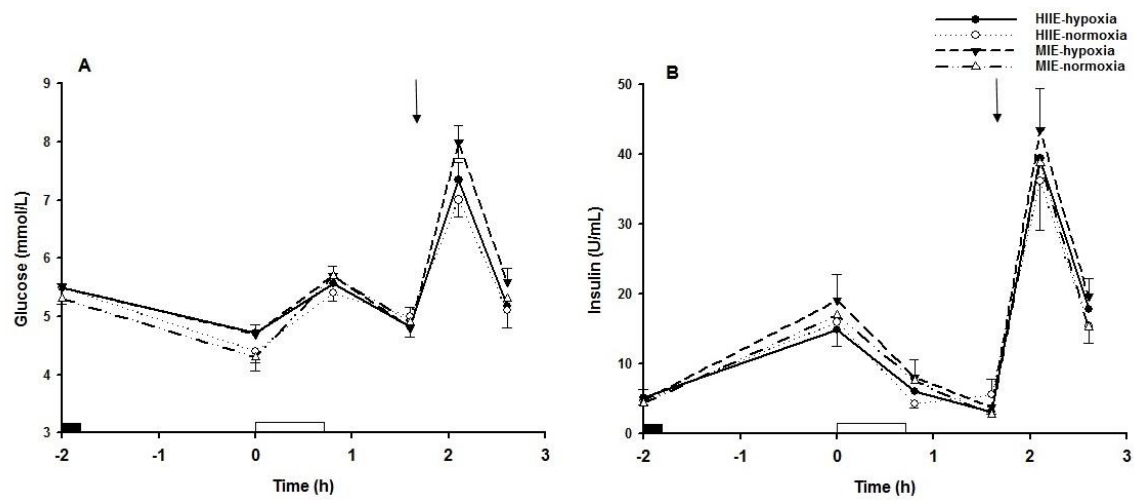


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649 **Figure 4**

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651