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deAppetite and gut hormone responses to moderate-intensity continuous exercise versus high-intensity interval exercise, in normoxic and hypoxic conditions

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Abstract

This study investigated the effects of continuous moderate-intensity exercise (MIE) and high-intensity interval exercise (HIIE) in combination with short exposure to hypoxia on appetite and plasma concentrations of acylated ghrelin, peptide YY (PYY), and glucagon-like peptide-1 (GLP-1). Twelve healthy males completed four, 2.6 h trials in a random order: *1*) MIE-normoxia, *2*) MIE-hypoxia, *3*) HIIE-normoxia, and *4*) HIIE-hypoxia. Exercise took place in an environmental chamber. During MIE, participants ran for 50 min at 70% of altitude-specific maximal oxygen uptake ($\dot{V}O_{2max}$) and during HIIE performed 6 x 3 min running at 90% $\dot{V}O_{2max}$ interspersed with 6 x 3 min active 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 hypoxic trials, exercise was performed at a simulated altitude of 2,980 m (14.5% O_2). Exercise was completed after a standardised breakfast. A second meal standardised to 30% of participants' daily energy requirements was provided 45 min after exercise. Appetite was suppressed more in hypoxia than normoxia during exercise, post-exercise, and for the full 2.6 h trial period (linear mixed modelling, $p < 0.05$). Plasma acylated ghrelin concentrations were lower in hypoxia than normoxia post-exercise and for the full 2.6 h trial period ($p < 0.05$). PYY concentrations were higher in HIIE than MIE under hypoxic conditions during exercise ($p = 0.042$). No differences in GLP-1 were observed between conditions ($p > 0.05$). These findings demonstrate that short exposure to hypoxia causes suppressions in appetite and plasma acylated ghrelin concentrations. Furthermore, appetite responses to exercise do not appear to be influenced by exercise modality.

Keywords

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

Highlights

- Effects of exercise modalities and hypoxia on appetite are explored

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

Abbreviations

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

Introduction

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

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

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

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

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

Methods

Participants

Following approval from the University of Bedfordshire ethics review board, 12 physically active (≥ 150 min/wk of moderate-to-vigorous physical activity) and apparently healthy normal-weight men (mean ± SD; age, 21.6 ± 2.0 years; body mass index, 23.5 ± 2.0 kg/m^{-2}) gave written informed consent to participate in the study following a verbal and written explanation of the nature and risks involved. Participants were non-smokers, normotensive, not taking any medications, and had no known history of cardiometabolic disease.

Preliminary tests

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

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

Maximum oxygen uptake

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

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

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

Standardised meals

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

Ratings of perceived appetite and nausea

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

Blood sampling

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

Comment [d1]: Should read: VACUETTE®, Greiner Bio-One, Austria)

Blood biochemistry

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

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

Statistical analysis

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

Linear mixed models were chosen to determine if there were any differences in the dependent variables between the conditions across time. This type of analysis was preferred as it i) allows for missing data, ii) can accurately model different covariate structures for repeated measures data, and iii) can model between-subject variability [47,49]. Area under the curve (AUC) was calculated for all blood metabolite and appetite variables using the trapezoidal method for the total trial period (2.6 h), the period during exercise (0 to 0.8 h), and the post-exercise period (0.8 to 2.6 h). Fixed and random factors for the linear mixed model were fit for each dependent variable and the main effects for *1*) altitude (hypoxia vs. normoxia), and *2*) exercise (HIIE vs. MIE), as well as 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 $\mathrm{Q}-\mathrm{Q}$ 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

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

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

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

Figure 2 about here.

Figure 3 about here.

Gut hormone concentrations

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

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

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

Table 2 about here.

Glucose and insulin concentrations

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

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

Figure 4 about here.

Correlations between appetite perceptions and appetite-regulating hormones

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

Discussion

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

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

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

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

GLP-1 concentrations were unaffected by short exposure to hypoxia combined with exercise. To the authors' knowledge, only one previous study has investigated the response of GLP-1 to hypoxia [42]. In that study, fasting concentrations of GLP-1 did not differ compared to normoxia following overnight exposure to a simulated altitude of 4,100 m, while there was a tendency for GLP-1 to be higher 40 min postmeal. This might suggest that hypoxia does not influence GLP-1 in the absence of feeding. Research into the effects of hypoxia on PYY is also limited, although Wasse et al [48] observed a tendency for higher total PYY concentrations in normoxia compared to 7 h hypoxic exposure. However, the current study observed higher total PYY concentrations in trials where HIIE was performed in hypoxia compared to when HIIE was performed in normoxia. However, these differences in PYY concentrations were not accompanied by changes in perceived appetite and more research is needed to establish if PYY is important in high altitude anorexia. A limitation of these studies, though, is that total PYY was measured and not concentrations of PYY_{3-36}, which is the form of PYY that is more potent in suppressing hunger [11]. However, total PYY and PYY_{3-36} are highly correlated [44] and changes in total PYY are thus likely to reflect changes in PYY_{3-36}.

There is convincing evidence that exercise at ≥ 60% $\dot{V}O_{2max}$ causes acute suppressions in appetite [15]. Given the recent rise in popularity of HIIE in the media and scientific literature, several recent studies have compared appetite responses of this mode of exercise to traditional moderate-intensity endurance-based exercise [1,13,14,32,41]. The current study did not observe suppressed appetite in response to submaximal HIIE compared to continuous MIE, which has similarly been reported in studies using overweight and obese participants [32,41]. Alkahtani et al [1] also observed no differences in appetite perceptions following HIIE compared with moderate-intensity interval 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 PYY_{3-36}
411 concentrations were recently reported following submaximal HIIE than continuous MIE [14]. Greater
412 increases in PYY_{3-36} 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 PYY_{3-36} [32]. Different responses to HIIE versus MIE between studies may be attributable to

variations in protocols employed, such as exercise intensity and duration, and the participants studied. It is also important to note that it is difficult to make direct comparisons between total PYY measured in the current study with PYY_{3-36} responses in other investigations as the conversion rate between these two forms of this hormone is unknown. Based on data from the current study, it is not possible to advise which mode of exercise (HIIE or MIE) individuals should engage in under hypoxic or normoxic trials to elicit preferable appetite responses.

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

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

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

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

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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 ± 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

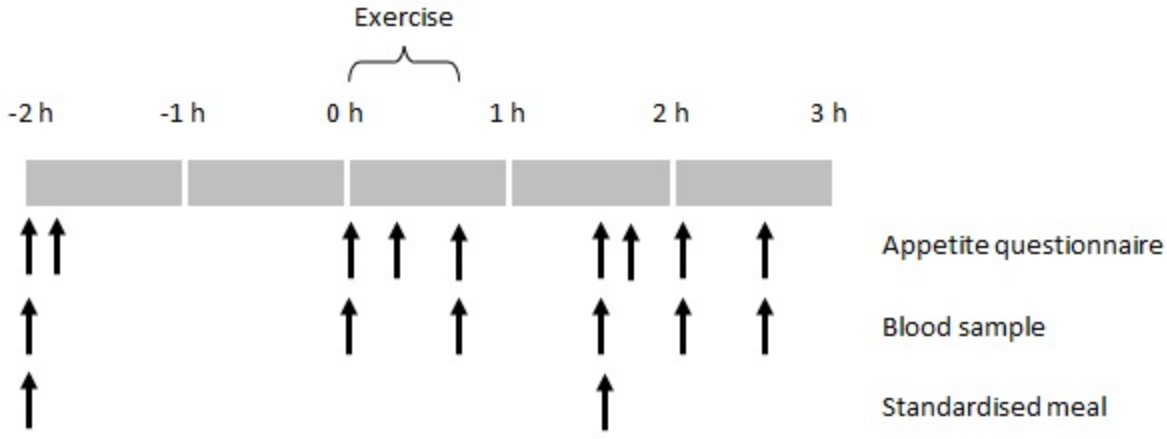
621 Figure 3

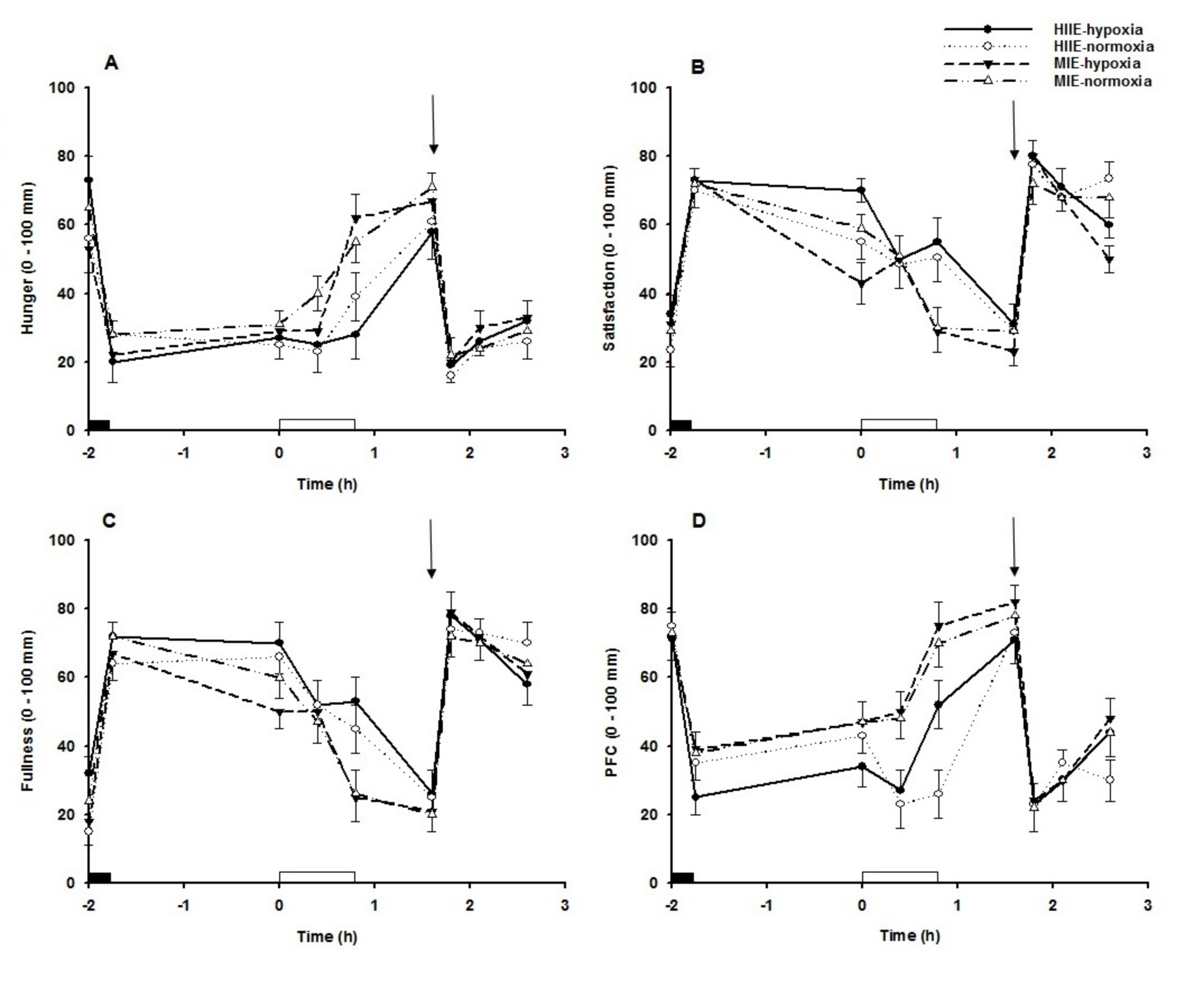
622 Fig. 3. Changes in plasma concentrations of (A) acylated ghrelin, (B) total PYY, and (C) GLP-1 during
623 moderate-intensity exercise (MIE)-normoxia, MIE-hypoxia, high-intensity interval exercise (HIIE)-
624 normoxia, and HIIE-hypoxia. Values are means ± SE; *n* = 12. Some error bars have been omitted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

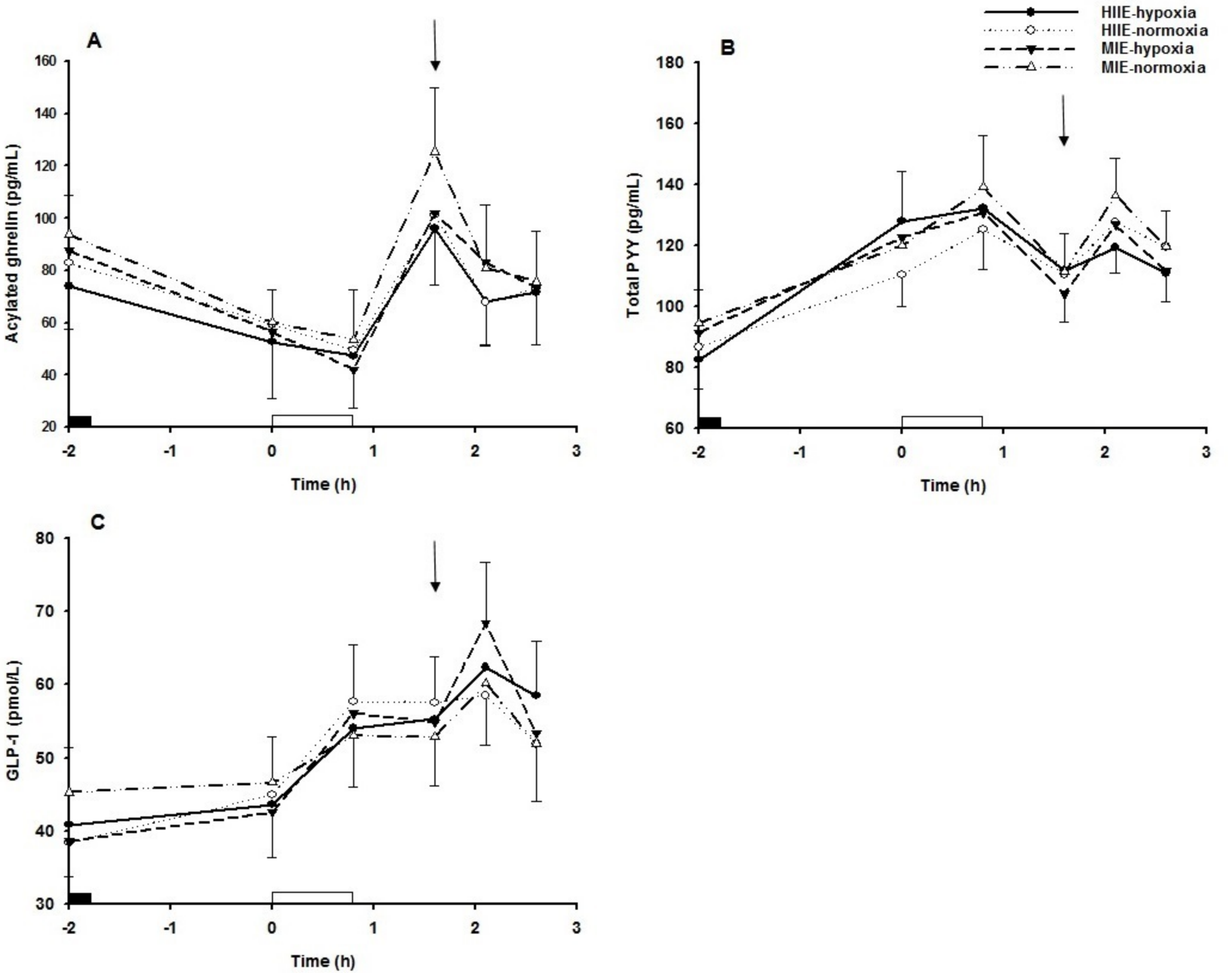
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628 Figure 4

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 ± 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.


Exercise
-2 h
-1 h
0 h
1 h
2 h
3 h
Appetite questionnaire
Blood sample
Standardised meal


HIIE-hypoxia
HIIE-normoxia
MIE-hypoxia
MIE-normoxia
A
B
C
D
Hunger (0 - 100 mm)
Satisfaction (0 - 100 mm)
Fullness (0 - 100 mm)
PFC (0 - 100 mm)
Time (h)
0
20
40
60
80
100
-2
-1
0
1
2
3


A
Acylated ghrelin (pg/mL)
160
140
120
100
80
60
40
20
-2
-1
0
1
2
3
Time (h)
B
Total PYY (pg/mL)
180
160
140
120
100
80
60
Time (h)
HIIE-hypoxia
HIIE-normoxia
MIE-hypoxia
MIE-normoxia
C
GLP-1 (pmol/L)
80
70
60
50
40
30
Time (h)

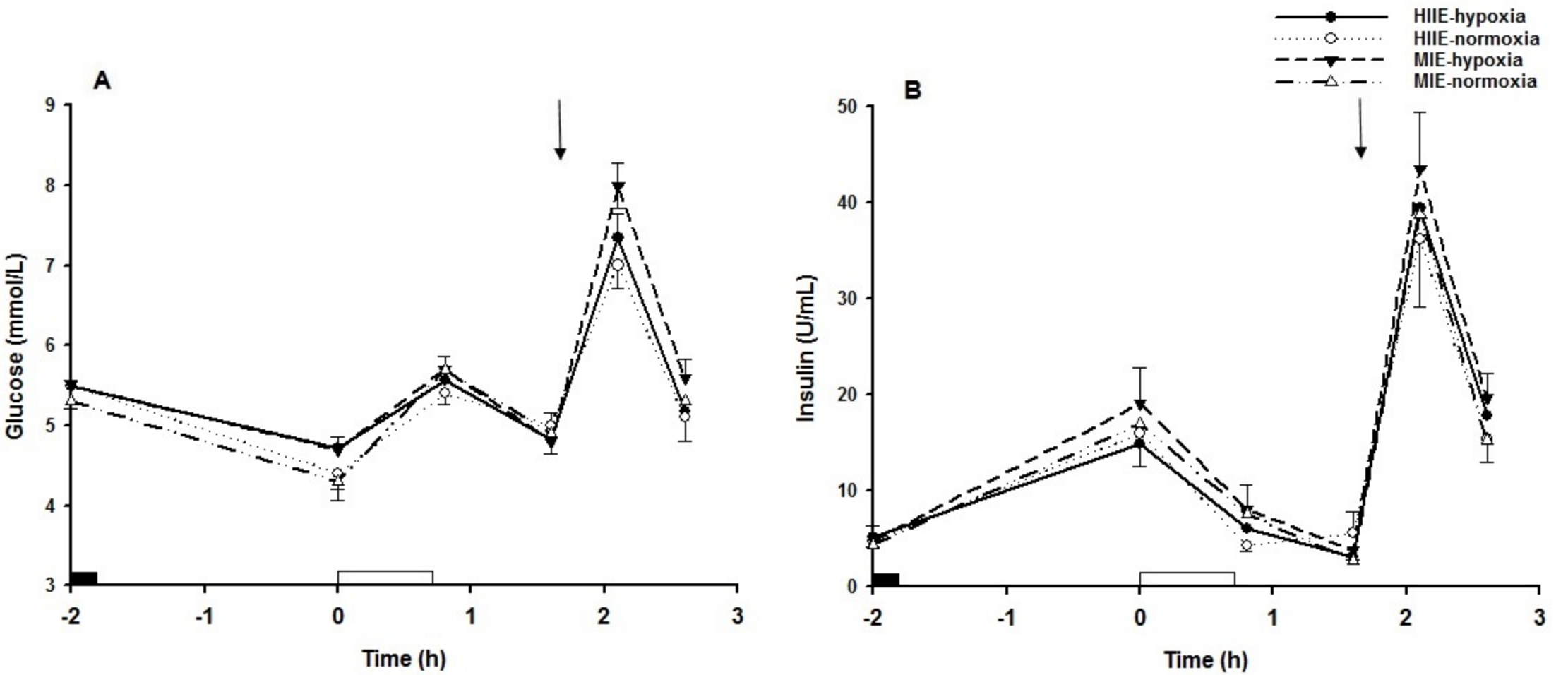

A
B
HIIE-hypoxia
HIIE-normoxia
MIE-hypoxia
MIE-normoxia
Glucose (mmol/L)
Insulin (U/mL)
Time (h)
Time (h)
9
8
7
6
5
4
3
50
40
30
20
10
0
-2
-1
0
1
2
3

Table 1: Area under the curve values for appetite perceptions

	During exercise (0 to 0.8 h)	Post-exercise (0.8 to 2.6 h)	Total trial (0 to 2.6 h)
Hunger			
Hypoxia	25 (4)	67 (6)	91 (9)
Normoxia	32 (4)*	77 (6)*	109 (9)*
HIIE	29 (4)	74 (6)	102 (9)
MIE	28 (4)	71 (6)	99 (9)
Satisfaction			
Hypoxia	47 (4)	96 (6)	143 (9)
Normoxia	39 (4)*	83 (6)*	122 (9)*
HIIE	43 (4)	89 (6)	132 (9)
MIE	43 (4)	90 (6)	133 (9)
Fullness			
Hypoxia	46 (4)	91 (6)	136 (9)
Normoxia	40 (4)	83 (6)*	122 (9)*
HIIE	42 (4)	87 (6)	129 (9)
MIE	42 (4)	87 (6)	129 (9)
PFC			
Hypoxia	32 (4)	82 (7)	115 (10)
Normoxia	42 (4)*	95 (7)*	137 (10)*
HIIE	37 (4)	89 (7)	126 (10)
MIE	38 (4)	88 (7)	126 (10)
Overall appetite			
Hypoxia	33 (3)	78 (5)	111 (8)
Normoxia	40 (3)*	86 (5)	127 (8)*
HIIE	37 (3)	81 (5)	118 (8)
MIE	37 (3)	83 (5)	120 (8)

Values are mean (SE), $N = 12$

HIIE high-intensity interval exercise; *MIE* moderate-intensity exercise; *PFC* prospective food consumption

*significant difference hypoxia vs. normoxia ($p < 0.05$)

Table 2: Area under the curve values for gut hormone, insulin, and glucose concentrations

	During exercise (0 to 0.8 h)	Post-exercise (0.8 to 2.6 h)	Total trial (0 to 2.6 h)
Acylated ghrelin (pg/mL)			
Hypoxia	41 (12)	129 (20)	171 (45)
Normoxia	43 (13)	148 (35)*	191 (52)*
HIIE	40 (13)	133 (32)	173 (49)
MIE	45 (12)	144 (32)	189 (48)
Peptide YY (pg/mL)			
Hypoxia	106 (14)	215 (23)	321 (30)
Normoxia	106 (16)	213 (21)	319 (30)
HIIE	110 (17)	212 (20)	322 (29)
MIE	103 (14)	215 (25)	318 (31)
GLP-1 (pmol/L)			
Hypoxia	42 (6)	102 (12)	145 (18)
Normoxia	41 (6)	100 (12)	141 (18)
HIIE	42 (6)	104 (13)	145 (18)
MIE	42 (5)	98 (11)	140 (17)
Glucose (mmol/L)			
Hypoxia	4.2 (0.1)	10.1 (0.2)	14.3 (0.3)
Normoxia	4.3 (0.1)	10.5 (0.2)*	14.7 (0.3)
HIIE	4.3 (0.1)	10.4 (0.2)	14.6 (0.3)
MIE	4.2 (0.2)	10.1 (0.2)	14.4 (0.3)
Insulin (μU/mL)			
Hypoxia	9 (1)	27 (5)	35 (5)
Normoxia	11 (2)	30 (4)	41 (5)
HIIE	10 (2)	30 (5)	40 (5)
MIE	9 (2)	27 (5)	36 (5)

Values are mean (SE), $N = 12$

HIIE high-intensity interval exercise; *MIE* moderate-intensity exercise; *GLP-1* glucagon-like peptide-1

*significant difference hypoxia vs. normoxia ($p < 0.05$)

Altitude x exercise interaction effect for PYY during exercise ($p = 0.042$)