Influence of rest and exercise at a simulated altitude of 4,000 m on appetite, energy intake, and plasma concentrations of acylated ghrelin and peptide YY

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Wasse IK, Sunderland C, King JA, Batterham RL, Stensel DJ. Influence of rest and exercise at a simulated altitude of 4,000 m on appetite, energy intake, and plasma concentrations of acylated ghrelin and peptide YY. J Appl Physiol 112: 552–559, 2012. First published December 15, 2011; doi:10.1152/japplphysiol.00090.2011.—The reason for high altitude anorexia is unclear but could involve alterations in the appetite hormones ghrelin and peptide YY (PYY). This study examined the effect of resting and exercising in hypoxia (12.7% O2; ~4,000 m) on appetite, energy intake, and plasma concentrations of acylated ghrelin and PYY. Ten healthy males completed four, 7-h trials in an environmental chamber in a random order. The four trials were control-normoxia, control-hypoxia, exercise-normoxia, and exercise-hypoxia. During exercise trials, participants ran for 60 min at 70% of altitude-specific maximal oxygen consumption (VO2max) and then rested. Participants rested throughout control trials. A standardized meal was consumed at 2 h and an ad libitum buffet meal at 5.5 h. Area under the curve values for hunger (assessed using visual analog scales) tended to be lower during hypoxic trials than normoxic trials (repeated-measures ANOVA, P = 0.07). Ad libitum energy intake was lower (P = 0.001) in hypoxia (5,291 ± 2,189 kJ) than normoxia (7,718 ± 2,356 kJ; means ± SD). Mean plasma acylated ghrelin concentrations were lower in hypoxia than normoxia (82 ± 66 vs. 100 ± 69 pg/ml; P = 0.005) while PYY concentrations tended to be higher in normoxia (32 ± 4 vs. 30 ± 3 pmol/l; P = 0.059). Exercise suppressed hunger and acylated ghrelin and increased PYY but did not influence ad libitum energy intake. These findings confirm that hypoxia suppresses hunger and food intake. Further research is required to determine if decreased concentrations of acylated ghrelin orchestrate this suppression.

normoxia; hypoxia; high altitude anorexia; appetite-regulating hormones

LOWLANDERS WHO ARE ACUTELY exposed to high altitude (>2,500 m; Ref. 4) often suffer from a loss of appetite termed “high altitude anorexia.” A loss of body mass, dependent on the duration of exposure and altitude reached (23), is evident after several days or more and appears to be an inevitable consequence of hypobaric hypoxia (7, 18, 36, 50). Energy balance is possibly disturbed at altitude due to an increase in daily energy expenditure through physical exertion, which is not matched by a comparable increase in energy intake due to a reduced desire to eat and/or lack of availability of food (52). Although appetite loss has long been recognized as a side effect of high altitude (33), there is limited empirical evidence available, but recently this has been confirmed in both laboratory (52) and field studies (22). Most studies attribute the observed decrease in energy intake to a loss of appetite associated with acute mountain sickness (AMS), symptoms of which include headache and anorexia. However, loss of appetite cannot be merely a by-product of AMS because anorexia and weight loss still persist when symptoms of AMS have subsided (43).

Energy intakes are reported to decrease by between 17 and 57% at altitudes ranging from 3,600 to 6,000 m (11, 18, 20, 29, 36, 42, 48). It is inconclusive whether food preferences change at altitude. An increased preference for carbohydrate has been reported (7, 33, 36, 52), whereas other studies have shown no change in diet composition (50, 51). A lack of palatable foods available at altitude may explain the reduced energy intake often reported. However, studies conducted in hypobaric chambers where other environmental stressors that could affect appetite can be eliminated (i.e., extreme cold and physical exertion) and a variety of plentiful foods are available demonstrate that individuals lose body mass because of a reduced energy intake in spite of the availability of palatable foods (36, 52). Thus it appears hypoxia per se causes the reduced appetite and energy intake, and although findings are inconclusive, this decrement in energy intake could be related to changes in endocrine factors responsible for the control of appetite.

The role that episodic appetite-regulating hormones play in high altitude anorexia is not well established. Levels of total ghrelin, an appetite-stimulating hormone, decrease at high altitude in the short term (<2 days; Ref. 39) but are unchanged in the long term (7 days to 7 wk) (5, 39). No studies have examined the effect of altitude upon acylated ghrelin, the posttranslationally modified form of this peptide essential for its appetite-stimulatory effects (8). Similarly, no studies have investigated the effect of altitude on the anorectic gut hormone peptide YY (PYY). The present study sought to examine the role, if any, that these appetite-regulating hormones play in the pathogenesis of high altitude anorexia.

To isolate the effects of normobaric hypoxia from other confounding factors present in real altitude situations, this study was undertaken in an environmental chamber where temperature, humidity, and physical activity levels could be controlled. Participants were exposed to normobaric hypoxia designed to mimic an altitude of 4,000 m. Both a resting control trial and an exercise trial were undertaken in normoxia and hypoxia to determine whether exercise in hypoxia would exacerbate any effects of hypoxia on appetite, energy intake, and the appetite-regulating hormones acylated ghrelin and PYY. Exercise was undertaken at the same relative exercise intensity in normoxia and hypoxia. Compared with normoxia, it was expected that hypoxia would suppress appetite and that energy intake at an ad libitum buffet meal would be decreased.
We hypothesized that this would be mediated by an alteration in concentrations of acylated ghrelin and PYY.

METHODS

Participants. Ten healthy, young (age 24 ± 3 yr; means ± SD), normal-weight (body mass index: 24.8 ± 2.4 kg/m²) male volunteers gave their written informed consent to participate in this study. The study was reviewed and approved by both the Nottingham Trent University and Loughborough University Ethical Advisory Committees. The participants were all recreationally active individuals recruited from the student and staff populations at Nottingham Trent University and Loughborough University. Participants were non-smokers, normotensive, not taking any medication, free from allergies to foods, and resided at an elevation no more than 150 m above sea level with no history of travel to altitude 3 mo before commencing the study.

Experimental design. Before the main trials, participants were required to attend two preliminary sessions. During the first, anthropometric measurements (height, body mass, and skinfold thickness) were made and participants were familiarized with the laboratory equipment and treadmill protocols. Participants completed questionnaires about their current and past health status, habitual physical activity levels, dietary habits, and food preferences. Participants then completed a submaximal incremental treadmill running test and a maximum oxygen uptake treadmill running test on a motorized treadmill (Woodway ELG 55; Weil am Rhein, Germany). The second preliminary trial was undertaken in an environmental chamber (De- troleum, Gwent, UK). The trials were as follows: a resting control trial in normoxia, an exercise trial in normoxia, a resting control trial in hypoxia, and an exercise trial in hypoxia. Participants were exposed to normobaric hypoxia as in the preliminary trial. Oxygen levels within the chamber were set at 12.7% O₂ (simulating an altitude of ~4,000 m) and the same submaximal and maximal treadmill running tests as before.

The four main trials were carried out in a randomized four-way crossover design with each trial separated by ≥7 days. All trials were completed in an environmental chamber (Design Environmental, Gwent, UK). The trials were as follows: a resting control trial in normoxia, an exercise trial in normoxia, a resting control trial in hypoxia, and an exercise trial in hypoxia. Participants were exposed to normobaric hypoxia as in the preliminary trial. Oxygen levels within the chamber were closely monitored throughout trials with a gas analyzer and adjusted if necessary. Exercise trials commenced with a 60-min treadmill run followed by 6 h of rest, and control trials involved 7 h of rest. Relative humidity was set at 50% and temperature at 23°C with the exception of the 60-min exercise period where for participant comfort temperature was set at 18°C and increased on cessation of exercise.

Anthropometry. Body mass was measured to the nearest 0.01 kg using a balance beam scale (Avery Industrial, Leicester, UK), and height was measured to the nearest 0.1 cm using a stadiometer (Seca). Percent body fat was estimated (40) from skinfold measurements (14).

Submaximal and maximal exercise tests. Participants completed a 16-min submaximal treadmill running test as described previously (10). This test was continuous in nature and involved four, 4-min stages. Treadmill running speed was increased at the start of each new stage, and expired air was collected into Douglas bags during the final minute of each 4-min stage so that the relationship between running speed and oxygen consumption could be determined. After time was allowed for recovery, participants then completed a maximum oxygen uptake test using a protocol where participants ran at a constant velocity with treadmill grade increased by 1% every minute until volitional fatigue (21). Expired air was collected during the final minute of the test when participants indicated that they could continue running for only 1 more minute. Heart rate, ratings of perceived exertion (6), and capillary blood oxygen saturation were monitored throughout the test and in the final minute during expired air collection. At the end of each test, the expired air samples were analyzed for oxygen consumption and carbon dioxide production. Once maximum oxygen consumption (ml·kg⁻¹·min⁻¹) was calculated, this value was used together with data from the submaximal running test to calculate the running speed required to elicit 70% of maximum oxygen uptake. This running speed was used in the main trials.

Main trials. Participants weighed and recorded their food intake for 24 h before the first main trial; they then replicated the quantity and timing of this before each subsequent trial. During this time, participants refrained from vigorous physical activity and alcohol consumption. Participants arrived at the laboratory after a 10-h overnight fast and were weighed wearing light clothing and no footwear. Upon entry to the chamber on exercise trials, participants ran for 60 min at a speed predicted to elicit 70% of altitude-specific maximal oxygen consumption (Vo₂max) during the exercise trials. Expired air samples were collected for 60 s every 15 min during exercise, and running speed was adjusted if necessary to keep oxygen consumption as close to the value for 70% of Vo₂max as possible. Values for energy expenditure were estimated from oxygen consumption and carbon dioxide production using indirect calorimetry (16). During exercise, heart rate was measured by short range telemetry (Polar Electro FS1; Kempele, Finland), oxygen saturation was monitored by fingertip pulse oximetry (Nonin 3100 Series; Nonin Medical, Plymouth, MA), and ratings of perceived exertion were recorded. After the treadmill run, participants rested (working, reading, or watching DVDs) in the chamber for the remaining 6 h. During the normoxic and hypoxic control trials the protocol was identical to that of the exercise trials, except participants rested (working, reading, or watching DVDs) in the chamber for the entire 7-h trial duration. Expired air samples were collected during the first 60 min of the control trials to enable estimation of resting metabolic rate.

Standardized and ad libitum buffet meals. A standardized meal was provided for participants at 2 h, and participants were instructed to consume the meal within 15 min. The meal consisted of white bread, ham, a chocolate bar, a banana, and plain salted crisps. The macronutrient content of the meal was as follows: 1.62 g carbohydrate, 0.3 g fat, 0.21 g protein, and 42 kJ per kg body mass. This provided 65% of the calories as carbohydrate, 27% as fat, and 8% as protein. The meal was prescribed according to each participant’s body weight on the first trial, and the quantity was replicated for subsequent trials.

A cold buffet meal was provided at 5.5 h, and participants had 30 min access to this where they could eat ad libitum. The buffet was set up identically before each meal with food being presented in excess of expected consumption. The items available were as follows: semi-skimmed milk, three varieties of cereal, cereal bars, white bread, brown bread, ham, cheddar cheese, tuna, mayonnaise, butter, margarine, cookies, chocolate rolls, apples, oranges, and bananas. Participants were told to eat until satisfied and that additional food was available if desired. Meals were consumed in isolation so that social influence did not affect food selection. To enable calculation of energy intake, foods were covertly weighed before being given to participants and again when the meal was finished. Energy and macronutrient intake were estimated using food tables. Participants were unaware that their energy intake was being monitored but were debriefed upon completion of all the trials.

Ratings of perceived appetite and symptoms of AMS. Subjective feelings of hunger, fullness, satisfaction, and prospective food consumption (PFC; an assessment of how much participants think they can eat, ranging from “nothing at all” to “a lot”) were reported on 100-mm visual analog scales (15) at baseline and every 30 min thereafter. Heart rate and oxygen saturation were also recorded at these times. Symptoms of AMS were assessed every hour from baseline using the Lake Louise Consensus AMS scoring system (35). However, question 5 of this questionnaire was omitted because it was not relevant in this setting as it concerns the difficulty an individual had in sleeping the night before while at altitude. This shortened version of the questionnaire correlates highly with other AMS scoring systems as well as a clinical AMS assessment (37). Total scores of ≥3, which include a headache, are suggestive of AMS.
Blood sampling. During the main trials, venous blood samples were collected from a cannula (Venflon; Becton-Dickinson, Helsingborg, Sweden) inserted into an antecubital vein upon arrival at the laboratory. The first blood sample (baseline, 0 h) was obtained immediately after cannulation and before entering the chamber, and subsequent samples were collected at 0.5, 1, 2, 3, 4, 5.5, 6.5, and 7 h within the chamber. Samples were collected into 5-ml precooled EDTA tubes (Sarstedt, Leicester, UK) for the determination of plasma glucose and total PYY concentrations. These samples were centrifuged at 3,500 rpm for 10 min in a refrigerated centrifuge (Fisher Scientific accuSpin 1R; Thermo Fisher Scientific). After this, the plasma was dispensed into separate Eppendorf tubes that were stored overnight at −20°C before being transferred to −80°C until analysis. Additional samples were collected for determination of plasma acylated ghrelin concentrations. These samples were collected into 5-ml precooled EDTA tubes that were pretreated with 50 μl of a solution containing p-hydroxymercuribenzoic acid, PBS, and sodium hydroxide. After centrifugation at 3,500 rpm for 10 min, 2 ml of plasma were dispensed into a plain tube and 200 μl of 1 M hydrochloric acid (HCl) was added. This sample was centrifuged for 5 min at 3,500 rpm before being transferred into Eppendorf tubes for storage as described above. Blood samples were collected with the participant in a supine position with the exception of the 0.5-h sample during the exercise trials, which was taken with the participant standing on the treadmill. After each sample was taken, the cannula was flushed with saline (0.9% wt/vol sodium chloride; Baxter Healthcare, Norfolk, UK). Before the next sample collection, this saline waste was drawn off into a syringe and discarded. From each sample, duplicate 20-μl blood samples were collected into micropipettes for determination of Hb concentration and triplicate blood samples were collected into 20-μl hiraparinized microhaematocrit tubes for determination of Hct to enable an estimation of plasma volume changes (13).

Blood biochemistry. An ELISA was used to determine concentrations of plasma acylated ghrelin (SPI BIO, Montigny le Bretonneux, France) and total PYY (Millipore, Watford, UK). Plasma samples were analyzed for glucose concentrations via enzymatic, colorimetric methods using reagents from ABX Diagnostics (Montpellier, France) with the use of a Pentra 400 automated analyzer (Horiba ABX Diagnostics). To eliminate interassay variation, all samples from the same participant were analyzed in the same run. The within batch coefficients of variation were as follows: acylated ghrelin 5.1% (at a mean concentration of 83 pg/ml), total PYY 4.8% (at a mean concentration of 30 pmol/l), and glucose 0.6% (at a mean concentration of 5.1 mmol/l).

Statistical analysis. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software (v17.0 for Windows; SPSS, Chicago, IL). Three-factor repeated-measures ANOVA with Bonferroni post hoc tests was used to examine differences between trials for appetite perceptions, acylated ghrelin, glucose, and PYY with the three factors being 1) hypoxia vs. normoxia, 2) exercise vs. control, and 3) time (serial measurements over 7 h). Area under the curve (AUC) values were calculated for appetite ratings, acylated ghrelin, PYY, and glucose using the trapezoidal rule by dividing the AUC up into quadrilaterals with one pair of parallel sides and summing the areas of each quadrilateral. Repeated-measures ANOVA was used to assess differences in AUC values between trials, as well as differences between fasting measures of appetite, acylated ghrelin, glucose, and PYY and differences in energy and macronutrient intake. The Pearson product moment correlation coefficient was used to examine relationships between variables. Statistical significance was accepted at the 5% level. When plasma volume changes were adjusted for, statistical findings were not altered; hence, unadjusted values are reported. Results are reported as means ± SD in the text and Tables 1 and 2 and means ± SE in Figs. 1–3.

RESULTS

Exercise responses. Maximum oxygen uptake was significantly lower in hypoxia than normoxia (56.9 ± 6.5 vs. 37.5 ± 4.0 ml·kg⁻¹·min⁻¹, respectively; P < 0.001). Participants ran at 71.2 ± 5.7% of normoxic VO₂max in the normoxic exercise trial and 74.1 ± 5.1% of hypoxic VO₂max in the hypoxic exercise trial (P = 0.279). Gross energy expenditure was 3,663 ± 469 kJ in the normoxic exercise trial and 2,647 ± 427 kJ in the hypoxic exercise trial (P < 0.001). In the normoxic exercise trial, 38 ± 19% of the energy was derived from fat and 62 ± 19% of the energy was derived from carbohydrate. In the hypoxic exercise trial, 36 ± 13% of the energy was derived from fat and 64 ± 13% of the energy was derived from carbohydrate.

Appetite perceptions. There was a main effect of time (P < 0.001) for each of the appetite perceptions assessed (hunger, satisfaction, fullness, and PFC; Fig. 1). Compared with normoxia, ratings of hunger and PFC were lower in hypoxia although these trial effects did not reach significance (both P < 0.062). Conversely, ratings of fullness and satisfaction tended to be higher in hypoxia than normoxia although again these trial effects did not reach significance (both P < 0.086). Altitude × time interaction effects were observed for satisfaction, fullness, and PFC (all P < 0.05), and exercise × time interaction effects were observed for hunger, satisfaction, and fullness (all P < 0.05), indicating differences in responses between trials over time (Fig. 1). For the total 7-h trial duration, AUC values for hunger were 20% lower in hypoxia than normoxia (hypoxia: 265 ± 130 mm·7 h and normoxia 333 ± 97 mm·7 h; P = 0.071). Similarly, AUC values for PFC were lower in hypoxia (334 ± 93 mm·7 h) than normoxia (400 ± 93 mm·7 h). Conversely, values for satisfaction and fullness were higher in hypoxia (satisfaction: 362 ± 102 mm·7 h and fullness: 359 ± 98 mm·7 h) than normoxia (satisfaction: 310 ± 102 mm·7 h and fullness: 297 ± 98 mm·7 h), but none of these values differed significantly.

Energy and macronutrient intake. Energy intake at the buffet meals (Table 1) was lower in hypoxia than normoxia (hypoxia: 5,291 ± 2,189 kJ and normoxia: 7,718 ± 2,356 kJ; P = 0.001) but was similar between the control and exercise trials (6,516 ± 2,270 and 6,493 ± 2,275 kJ, respectively; P = 0.950). After the energy that was expended during the exercise bouts was accounted for, relative energy intake during the whole trial (standardized meal + buffet meal) was lower during hypoxia than normoxia (7,344 ± 2,336 vs. 9,189 ± 2,386 kJ, respectively; P = 0.004) and also during the exercise trials compared with the control trials (exercise: 6,878 ± 2,283 and control 9,657 ± 2,439 kJ; P < 0.001).

When expressed as a percentage of overall energy intake, macronutrient composition at the buffet meal differed between trials with a greater proportion of fat consumed during exercise than control (40 ± 7 vs. 38 ± 6%, respectively; P = 0.006), which appeared to be at the expense of protein where the proportion of protein was higher during the control trials than the exercise trials (15 ± 3 vs. 13 ± 3%, respectively; P = 0.031). Hypoxia did not influence macronutrient preference (Table 1).

Plasma acylated ghrelin. Fasting plasma acylated ghrelin concentration did not differ between trials at baseline (P = 0.219). There was a main effect of hypoxia (P = 0.005), exercise
(P = 0.001), and time (P < 0.001) and an exercise × time interaction (P < 0.05) for plasma acylated ghrelin (Fig. 2). Post hoc tests revealed mean plasma acylated ghrelin concentrations were lower in hypoxia than normoxia (82 ± 66 vs. 100 ± 69 pg/ml, respectively; P = 0.005) and lower during exercise trials than resting trials (86 ± 65 vs. 97 ± 70 pg/ml, respectively; P = 0.001). Acylated ghrelin AUC values (Table 2) were 18% lower in hypoxia than normoxia (573 ± 486 vs. 700 ± 464 pg/ml·7 h, respectively; P < 0.05) and 10% lower during exercise trials than control trials (602 ± 459 vs. 671 ± 491 pg/ml·7 h, respectively; P = 0.001).

*PYY.* Fasting plasma total PYY concentration did not differ at baseline between trials (P = 0.526). There was a main effect of exercise (P = 0.044), time (P < 0.001), and an altitude × time interaction (P = 0.016) for plasma total PYY (Fig. 3).

**Table 1. Macronutrient and energy intake values at the buffet meal for each of the four trials**

<table>
<thead>
<tr>
<th></th>
<th>Carbohydrate, g (%)</th>
<th>Fat, g (%)</th>
<th>Protein, g (%)</th>
<th>Energy Intake, kJ (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxic control</td>
<td>215 ± 82 (47 ± 7)</td>
<td>74 ± 21 (37 ± 7)</td>
<td>70 ± 17 (16 ± 3)</td>
<td>7,535 ± 2,112 (1,800 ± 505)</td>
</tr>
<tr>
<td>Normoxic exercise</td>
<td>207 ± 67 (45 ± 8)</td>
<td>90 ± 37 (41 ± 7)</td>
<td>66 ± 24 (14 ± 3)</td>
<td>7,909 ± 2,599 (1,889 ± 621)</td>
</tr>
<tr>
<td>Hypoxic control</td>
<td>151 ± 59 (47 ± 6)</td>
<td>59 ± 32 (39 ± 6)</td>
<td>48 ± 23 (14 ± 4)</td>
<td>5,504 ± 2,427 (1,315 ± 580)</td>
</tr>
<tr>
<td>Hypoxic exercise</td>
<td>144 ± 55 (49 ± 6)</td>
<td>55 ± 25 (39 ± 6)</td>
<td>39 ± 15 (12 ± 4)</td>
<td>5,084 ± 1,952 (1,215 ± 466)</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 10. NB: mean energy intake was significantly lower in hypoxia vs. normoxia trials: 5,291 ± 2,189 vs. 7,718 ± 2,356 kJ (P = 0.001). Mean percent fat intake significantly higher during exercise vs. control trials: 40 ± 7 vs. 38 ± 6% (P = 0.006). Mean percent protein intake significantly higher in control vs. exercise trials: 15 ± 3 vs. 13 ± 3% (P = 0.031). There are no significant differences in percent intake of carbohydrate, fat, or protein between hypoxic and normoxic trials.

Post hoc tests showed that mean total PYY concentrations were higher in the exercise trials than the control trials (32 ± 4 vs. 30 ± 4 pmol/l, respectively; P = 0.044) and higher in normoxia (32 ± 4 pmol/l) than hypoxia (30 ± 3 pmol/l), although this latter difference did not quite reach statistical significance (P = 0.006), exercise (P = 0.001), and time (P < 0.001) and an exercise × time interaction (P < 0.05).

Fig. 1. Changes in appetite perceptions assessed using VAS during the normoxic control trial (●), normoxic exercise trial (▲), hypoxic control trial (□), and hypoxic exercise trial (●). Values are means ± SE; n = 10. Some error bars have been omitted for clarity. Black rectangle indicates treadmill running, thin downward arrow indicates standardized meal consumption, and bold downward arrow indicates buffet meal consumption. Repeated-measures ANOVA revealed a main effect of altitude (P < 0.001), exercise (P < 0.001), and time (P < 0.016) for each appetite perception, an altitude × time interaction for satisfaction, fullness and prospective food consumption (PFC; all P < 0.05) and an exercise × time interaction for hunger, satisfaction, and fullness (all P < 0.05).

Fig. 2. Plasma acylated ghrelin concentrations during the normoxic control trial (●), normoxic exercise trial (▲), hypoxic control trial (□), and hypoxic exercise trial (●). Values are means ± SE; n = 10. Some error bars have been omitted for clarity (trial means ± SE values for omitted trials are as follows: normoxic exercise: 92 ± 18 pmol/l and hypoxic control: 85 ± 19 pg/ml). Black rectangle indicates treadmill running, thin downward arrow indicates standardized meal consumption, and bold downward arrow indicates buffet meal consumption. Repeated-measures ANOVA revealed a main effect of altitude (P = 0.005), exercise (P = 0.001), and time (P < 0.001) and an exercise × time interaction (P < 0.05).
Correlations between appetite-regulating hormones and other variables. Energy intake at the buffet meal was positively correlated with premeal hunger scores in all trials, correlation coefficients ranged from 0.670 to 0.770 \((P < 0.05)\). Analysis of the normoxic trials combined revealed that the percent increase in hunger \((4–5.5 \text{ h})\) before the buffet tended to be associated with the percent increase in acylated ghrelin \((4–5.5 \text{ h}; r = 0.386; P = 0.093)\); however, no relationship was found in the hypoxic trials. Conversely, there was a significant positive relationship between the percent increase in acylated ghrelin before the buffet meal \((4–5.5 \text{ h})\) and ad libitum energy intake \((r = 0.521; P = 0.018)\) in the hypoxic trials but not in the normoxic trials. Greater energy intakes were also significantly associated with larger percent decreases in acylated ghrelin in the hypoxic trials \((r = -0.589; P = 0.006)\) but not in the normoxic trials \((r = -0.146; P = 0.316). PYY was not correlated with hunger at any times nor with subsequent energy intake at the buffet meal. PYY concentrations were significantly correlated with satisfaction, fullness, and PFC at some time points on some trials, but there was not a consistent pattern. Finally, we performed correlations between AUC values for AMS scores and appetite perceptions to assess whether symptoms of AMS might be influencing appetite but no significant relationships were observed.

**DISCUSSION**

This study investigated the effects of rest and exercise in hypoxia on appetite, energy intake and concentrations of the gut hormones acylated ghrelin and total PYY. The main findings are 1) energy intake is suppressed in normobaric hypoxia, 2) acylated ghrelin concentrations are suppressed in normobaric hypoxia, and 3) total PYY has a tendency to be suppressed in normobaric hypoxia. Thus short-term exposure to hypoxia, in the absence of cold and other stressors, suppresses plasma acylated ghrelin concentrations, and this suppression may be implicated in high altitude anorexia.

We can only speculate about the mechanisms underlying the suppression of plasma acylated ghrelin in hypoxia. For ghrelin to bind to its receptor and exert its appetite-stimulating effects, it must be acylated by an eight carbon fatty acid (octanoate) at its serine 3 residue by the enzyme ghrelin O-acyl transferase (GOAT) \((19, 54)\). Kirchner et al. \((25)\) posited that ghrelin...
Acetylation and the secretion of acylated ghrelin are likely two different processes. It is possible that hypoxia might interfere with the ghrelin/GOAT system in some way, causing an impairment of the acylation process by GOAT, an inhibition of GOAT secretion, or an altered secretion pattern of ghrelin after its enzyme-requiring modification.

Ghrelin is secreted primarily from the stomach into the bloodstream (1) and can reach the food-regulating centers of the hypothalamus by passing through the blood brain barrier (3). Ghrelin produced from the stomach will pass through the liver from the portal vein where it will then reach the peripheral circulation. Data from a study by Goodyear et al. (17) tentatively suggest that the liver may be involved in the acylation of ghrelin. Wolff (53) suggests that the splanchnic area may be vulnerable to the needs of blood flow elsewhere in the body, and thus the decrease in oxygen saturation in hypoxia may result in blood “stolen” from the splanchnic area so that oxygen delivery can be maintained elsewhere. Impaired gut blood flow has been proposed as a mechanism behind high altitude anorexia. Research has shown that 2 h at a simulated altitude of 4,800 m significantly reduced superior mesenteric artery blood flow before and after food ingestion (28). However, a recent study by Kalson et al. (22) employing a similar protocol shows that increases in arterial and venous blood flow in the gut after a food challenge are similar at sea level and after a 3-day exposure to hypobaric hypoxia. In the study by Kalson et al. (22), all participants experienced hunger loss with hypobaric hypoxia, suggesting that impaired gut blood flow was not responsible for high altitude anorexia after several days as had initially been proposed. However, the suppression of acylated ghrelin we observed in hypoxia over 7 h could be involved in high altitude anorexia, and this might be linked to the observation of altered gut blood flow in response to acute hypoxia (28). In the longer term, different mechanisms may exist because Kalson et al. (22) observed no differences in blood flow in the gut after a 3-day exposure to hypoxia compared with sea level.

Ghrelin concentrations increase preprandially and decrease postprandially (12, 44). The mechanisms by which ghrelin is suppressed postprandially are unclear, although a glucose induced suppression has been proposed (31). However, one study (38) has shown that hyperglycaemia of 11 mmol/l does not suppress plasma ghrelin concentrations. In the present study, glucose concentrations were elevated and acylated ghrelin concentrations were suppressed in the hypoxic trials compared with the normoxic trials. The observed elevation in plasma glucose in the hypoxic trials, although significant, was only 0.2 mmol/l, which is likely insufficient to suppress plasma ghrelin concentrations. In the postprandial period there are many other hormones released in response to nutrient ingestion and these may be involved in the postmeal ghrelin response at altitude (26).

The effect of altitude on PYY levels has not been examined. In the present study, concentrations of total PYY tended to be higher in normoxia than in hypoxia, suggesting that PYY does not play a role in high altitude anorexia. The reason for the suppression of total PYY in hypoxia is unknown but might be related to changes in concentrations of other appetite-regulatory peptides that we did not measure, such as cholecystokinin (CCK) or glucagon-like peptide-1 (GLP-1). Acute exercise in normoxia has been shown to increase concentrations of the satiety peptide CCK (2). However, the same response did not occur in acute normobaric hypoxia, with concentrations being suppressed compared with a resting trial (2). Thus the tendency for lower levels of PYY during hypoxia in the present study might be related to the previously observed decrease in CCK during acute exercise in hypoxia. PYY release is also inhibited by GLP-1 (32); hence, alterations in concentrations of GLP-1 are another possible mediator of the PYY response to hypoxia. Research is limited with only one study investigating the effect of hypoxia on GLP-1 concentrations. This study (41) demonstrated a tendency for a meal-induced increase in GLP-1 concentrations after 17 h of exposure to hypoxia simulating an altitude of ~4,100 m. However, before the meal GLP-1 concentrations were unchanged after hypoxic exposure compared with sea level, suggesting GLP-1 is not affected by hypoxia in the absence of feeding. Finally, the findings from the present study that total PYY concentrations are higher in the exercise trials than in the resting trials confirms previous findings that an acute bout of aerobic exercise increases PYY concentrations, this holds true in both the fed (30, 47) and fasted states (9). A limitation of the present study is that total PYY concentrations were measured rather than PYY3–36 concentrations. However, total PYY and PYY3–36 are highly positively correlated (46) and hence changes in total PYY are likely to reflect changes in PYY3–36.

In the present study, spontaneous energy intake at a buffet meal, in the presence of ad libitum palatable food, was decreased by ~31% in hypoxia. This response confirms previous findings (36, 52) from studies using hypobaric chambers and supports the notion that hypoxia alone is a sufficient stimulus for reducing energy intake. When decreased energy intake at altitude persists over time, body mass loss is inevitable (7, 18, 36, 52). Given the acute nature of this study, it is not possible to know whether this decreased energy intake would persist over time and translate into a loss of body mass. The simulated altitude participants were exposed to in the present study is low enough for total acclimatization to occur after several days (34, 49). It is therefore probable that the decrease in energy intake we observed in hypoxia would disappear over time unless a higher altitude was simulated. Also, meals were prescribed at set times in the present study so it is unclear when individuals would have initiated feeding. At least one previous study (52) has reported an increase in meal frequency and reduction in meal size at altitude.

Given the persistence of high altitude anorexia over time, in future, studies should examine the effects of chronic hypoxia on plasma acylated ghrelin, desacyl ghrelin, and PYY3–36 responses. The participants in the present study were young healthy males; whether these findings extend to other groups including females, older individuals, and overweight and obese individuals in whom circulating ghrelin levels are already decreased (45) is unclear. Hypoxia exposure has been postulated as a potential therapy for obesity (34). In the absence of acclimatization, our findings lend support to this idea and exercise in hypoxia may be an effective weight loss strategy. Limited data are available regarding the effects of hypoxia on energy intake and weight loss in obese individuals, but recently it has been demonstrated that 1 wk of moderate hypoxic exposure (2,650 m) in obese participants results in a significant, albeit modest, sustained weight loss (27). Hence, the combined effect of exercise and hypoxia on weight loss in overweight and obese individuals clearly warrants attention.

In conclusion, acute exposure to normobaric hypoxia causes a reduction in spontaneous energy intake that may be related to
suppressed plasma acylated ghrelin concentrations. Exercise also suppresses plasma acylated ghrelin concentration but does not appear to exacerbate the suppression observed in hypoxia. It has been suggested that studying the physiological effects of hypoxia in healthy individuals voluntarily exposing themselves to hypoxia may enhance understanding of the effects of hypoxia in clinical disease (24). Hence, the present findings may be relevant not just for mountaineers and climbers who sojourn to high altitude for recreational purposes and suffer from high altitude anorexia but potentially also for those suffering from disordered eating due to illness and disease.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


