Article:
Matu, J, Deighton, K, Ispoglou, T et al. (1 more author) (2017) The effect of moderate versus severe simulated altitude on appetite, gut hormones, energy intake and substrate oxidation in men. Appetite, 113. pp. 284-292. ISSN 0195-6663

https://doi.org/10.1016/j.appet.2017.02.041

Title: The effect of moderate versus severe simulated altitude on appetite, gut hormones, energy intake and substrate oxidation in men

Authors names: Jamie Matu[1], Kevin Deighton[1], Theocharis Ispoglou[1] and Lauren Duckworth[1]

Department and institution: [1]Institute for Sport Physical Activity & Leisure, Leeds Beckett University, Leeds, United Kingdom

Abbreviated title: Appetite, gut hormones and energy intake at altitude

Corresponding author: J. Matu, Carnegie Research Institute Room 104, Institute for Sport Physical Activity & Leisure, Leeds Beckett University, Leeds, LS6 3QS, United Kingdom (email: J.Matu@leedsbeckett.ac.uk)

Emails: Jamie Matu: J.Matu@leedsbeckett.ac.uk; Kevin Deighton: K.Deighton@leedsbeckett.ac.uk; Theocharis Ispoglou: T.Ispoglou@leedsbeckett.ac.uk; Lauren Duckworth: L.Duckworth@leedsbeckett.ac.uk

Telephone number: +44 (0)113 8123603

Abstract

Acute exposure to high altitude (>3500m) is associated with marked changes in appetite regulation and substrate oxidation but the effects of lower altitudes are unclear. This study examined appetite, gut hormone, energy intake and substrate oxidation responses to breakfast ingestion and exercise at simulated moderate and severe altitudes compared with sea-level. Twelve healthy males (mean±SD; age 30±9years, body mass index 24.4±2.7kg.m^{-2}) completed in a randomised crossover order three, 305 minute experimental trials at a simulated altitude of 0m, 2150m (~15.8% O_2) and 4300m (~11.7% O_2) in a normobaric chamber. Participants entered the chamber at 8am following a 12h fast. A standardised breakfast was consumed inside the chamber at 1h. One hour after breakfast, participants performed a 60 minute treadmill walk at 50% of relative $\dot{V}O_{2max}$. An *ad-libitum* buffet meal was consumed 1.5h after exercise. Blood samples were collected prior to altitude exposure and at 60, 135, 195, 240 and 285 minutes. No trial based differences were observed in any appetite related measure before exercise. Post-exercise area under the curve values for acylated ghrelin, pancreatic polypeptide and composite appetite score were lower (all $P<0.05$) at 4300m compared with sea-level and 2150m. There were no differences in glucagon-like peptide-1 between conditions ($P=0.895$). Mean energy intake was lower at 4300m (3728±3179kJ) compared with sea-level (7358±1789kJ; $P=0.007$) and 2150m (7390±1226kJ; $P=0.004$). Proportional reliance on carbohydrate as a fuel was higher ($P=0.01$) before breakfast but lower during ($P=0.02$) and after exercise ($P=0.01$) at 4300m compared with sea-level. This study suggests that altitude-induced anorexia and a subsequent reduction in energy intake occurs after exercise during exposure to severe but not moderate simulated altitude. Acylated ghrelin concentrations may contribute to this effect.

Keywords: hypoxia; altitude-induced anorexia; hunger; acylated ghrelin; carbohydrate utilization

Introduction

An increasing number of people ascend to high altitude each year for recreational and occupational purposes and these sojourns often involve rapid ascents that do not allow time for acclimatisation to the hypoxic environment. High altitude exposure can induce a negative energy balance due to appetite inhibition (2, 37, 55, 56) and elevated basal metabolic rate (57), in combination with the completion of physically demanding activities such as trekking, skiing and climbing. This may have deleterious effects for performance at high altitude due to a loss of body mass (48, 58, 60), and possibly functional capacity (24, 49).

Historically, studies have attributed altitude-induced appetite inhibition to acute mountain sickness (AMS). However, it has been found that appetite remains inhibited once the symptoms of AMS have subsided (54). In an attempt to identify possible mechanisms behind altitude-induced anorexia, studies have investigated changes in the circulating levels of various hormones in response to hypoxia. This includes the measurement of glucagon-like peptide-1 (GLP-1) (37, 51), leptin (37, 50), pancreatic polypeptide (PP) (46) and peptide YY (PYY) (37, 55) with particular recent interest towards acylated ghrelin (2, 39, 55). Wasse et al. (55) found that a seven hour exposure to hypoxia (12.7% FiO_2, ~4000m), commencing with a one hour exercise period, significantly reduced acylated ghrelin concentrations and *ad-libitum* energy intake compared with sea-level. However, reports in the literature present contradictory findings regarding the response of acylated ghrelin to moderate altitude (1500m - 3500m). In this regard, Bailey et al. (2) reported lower acylated ghrelin area under the curve (AUC) concentrations in hypoxia (14.5% FiO_2, ~2980m) than normoxia, whereas Morishima and Goto (39) found no significant effect of a seven hour moderate hypoxic exposure (15% FiO_2, ~2700m) on acylated ghrelin concentrations compared with normoxia. The reasons for this discrepancy are unclear and the lack of energy intake assessment in these studies means that the effects of moderate hypoxia on energy intake remains unknown.

In addition to changes in appetite regulation, high altitude exposure also appears to increase the body's reliance on carbohydrate as a fuel for substrate oxidation in comparison with sea-level (8, 31, 44). This response is hypothesised to be acutely beneficial, due to the higher yield of ATP per molecule of oxygen with carbohydrate utilisation in comparison with fat (22). However, this oxygen-efficiency theory has been disputed by other studies which show no effect of altitude on substrate oxidation if relative exercise intensities are matched (6, 34). An increased reliance on carbohydrate as a fuel could also lead to a faster depletion of valuable and limited liver and muscle glycogen stores (44), which could have adverse effects at altitude.

Currently the effects of varying severities of normobaric hypoxia on appetite, gut hormones, energy intake or substrate oxidation have not been measured within a single study. Subsequently, this experiment investigated the effect of both moderate (2150m) and severe (4300m) simulated altitudes on these variables in comparison with sea-level. The results of this research will help to inform nutritional considerations and practices at both moderate and severe altitude.

Methods

Participants

Twelve healthy male volunteers (age 30 ± 9 years, body mass index 24.4 ± 2.7 $kg.m^{-2}$, body mass 80.5 ± 10.5 kg) provided written informed consent to participate in this study. The study, which received institutional ethics approval, was conducted in accordance with the Declaration of Helsinki. All participants were non-smokers, normotensive, free from food allergies and were not taking any medication. None of the participants had travelled to an altitude >1500m during the previous three months and were all currently residing at an altitude <500m.

Experimental design

Participants were required to make a total of seven visits to the laboratory. The first visit involved screening, anthropometry, verbal familiarisation with testing procedures, a food preferences assessment and a sickle cell trait test. Sickle cell trait was an exclusion criteria due to complications that may occur at altitude, for example splenic infarction (21). Further exclusion criteria included diabetes and thyroid disorders.

Over the second, third and fourth visits the participants completed three exercise capacity tests (one at each altitude of 0m, 2150m and 4300m) in order to calculate workloads relative to each altitude for the main experimental trials. These preliminary visits were separated by ≥48h and conducted in a single-blind randomised fashion using a Latin Square design. Over the fifth, sixth and seventh visits the participants completed three 305 minute experimental trials (one at each altitude of 0m, 2150m, and 4300m). These visits were separated by ≥7days and were randomised independently from the maximal exercise tests, also using a single-blind Latin Square design. On the morning of each testing day the following equation was used to calculate and set target FiO_2: FiO_2 = *PiO2 divided by* (P_B *- 47);* where P_B is barometric pressure in mmHg and 47mmHg is the vapour

pressure of water at 37°C (9, 18). Simulated PiO_2 was 149mmHg at sea-level (FiO_2 ~20.9%), 113mmHg at 2150m (FiO_2 ~15.8%) and 83mmHg at 4300m (FiO_2 ~11.7%).

Exercise Capacity Tests

Participants completed an exercise capacity test on a treadmill (Woodway PPS 55; Waukesha, WI) which included both a submaximal and maximal phase. The incremental submaximal phase consisted of four, 4 minute stages in which the participant walked carrying a 10 kg backpack at a 10% gradient. This exercise modality was chosen to mimic the demands of high altitude activities. The speed of the treadmill was increased by 1 $km \cdot h^{-1}$ each stage and the starting speeds were 3 $km \cdot h^{-1}$, 2 $km \cdot h^{-1}$ and 1 $km \cdot h^{-1}$ for 0m, 2150m and 4300m, respectively. Lower starting speeds were employed in hypoxia based on the knowledge of a reduced aerobic capacity at altitude and the need for all participants to elicit 50% of $\dot{V}O_{2max}$ within the 16 minute test. On completion of the submaximal phase participants were allowed 5 minutes of recovery before commencing the maximal phase. Prior to this phase the participants removed the backpack and the treadmill was set at 1% gradient (30). The participants then ran at a constant speed, which was dependent upon fitness and altitude, aiming for a rating of perceived exertion (RPE) of 12. The gradient of the treadmill was then increased by 1% per minute until volitional exhaustion. All subjects were deemed to reach $\dot{V}O_{2max}$ as they all expressed >2 of the following criteria: a plateau in $\dot{V}O_2$ in the final exercise stage, respiratory exchange ratio ≥ 1.15, heart rate within 10 $b \cdot min^{-1}$ of age predicted maximum (220-age), rating of perceived exertion ≥ 19 and/or blood lactate ≥ 8mM (27). Expired gas was collected using an online gas analyser (Metalyzer 3B R3; Leipzig, Germany) throughout both phases of this test to allow regression analysis between oxygen consumption and walking speed. This allowed for the calculation of a speed that would elicit 50% of relative $\dot{V}O_{2max}$ whilst walking on a treadmill and carrying a 10 kg backpack at 10% gradient.

Experimental trials

Participants recorded their food intake for the 24h prior to the first experimental trial; the quantity and timing of this intake was then repeated before each subsequent trial. Alcohol, caffeine and

142 strenuous exercise were not permitted during this period. Participants consumed a standardised
143 evening meal (1037kcal, 57% carbohydrate, 28% fat, 15% protein) between 7pm and 8pm on the day
144 before each trial. This meal was consumed to minimise the possibility of a 'second-meal' effect
145 confounding glycemic control or any other measured variables (52, 59) and included: fusilli pasta,
146 pasta sauce, cheddar cheese, milk, and jelly beans. After a 12h overnight fast participants arrived at
147 the laboratory and entered the chamber at 8am (figure 1). At 1h participants were allowed 15 minutes
148 to consume a standardised breakfast (322kcal, 72% carbohydrate, 17% fat, 11% protein). This meal
149 included rolled oats, semi-skimmed milk and orange juice, and was selected because it is typical of the
150 type of breakfast consumed in the UK (45). Participants remained rested (working, reading or watching
151 DVDs) throughout trials, with the exclusion of the exercise period. At 2h 15 minutes a 60 minute
152 treadmill walk at 50% of altitude specific $\dot{V}O_{2max}$ was completed at a 10% gradient and carrying a 10 kg
153 backpack. Throughout the trials heart rate and arterial oxygen saturations (SpO_2) were monitored
154 every 15 minutes via a fingertip pulse oximeter (Nellcor™ PM10N; Medtronic, Minneapolis, MN).
155 Rating of perceived exertion was measured at 15 minute intervals throughout exercise (5). Water was
156 allowed *ad-libitum* throughout all trials.

157

158 *- INSERT FIGURE 1 NEAR HERE –*

159

160 Measurements

161 Ratings of perceived appetite and symptoms of acute mountain sickness

162 Ratings of perceived appetite and AMS scores were taken at baseline and throughout each
163 experimental trial at 30 minute intervals with the exclusion of the 15 minute interval for the
164 standardised breakfast (figure 1). AMS was assessed using the Lake Louise AMS (LLAMS) score (47);
165 mild AMS was defined as LLAMS of ≥3 in the presence of a headache and severe AMS was defined as

≥6 in the presence of a headache. Appetite perceptions were measured using validated 100 mm visual analogue scales (VAS) (19). Using these scales a composite appetite score (CAS) was calculated using the following formula: composite appetite score = ([hunger + prospective food consumption + (100 – fullness) + (100 – satisfaction)] / 4) (53). A higher value is associated with a greater appetite sensation and subsequently a stronger motivation to eat.

Online gas analysis

Online gas analysis was conducted for two 10 minute periods before breakfast, two 10 minute periods after breakfast and before exercise, throughout exercise, and two 10 minute periods after exercise (figure 1). The facemask was fitted five minutes before each 10 minute collection period whilst the participant was seated. A seated position was deemed appropriate as previous research has found no significant differences in energy expenditure between seated and supine positions (38). The respiratory exchange ratio was determined from $\dot{V}O_2$ and $\dot{V}CO_2$ measurements and substrate oxidation was estimated using equations for both resting (20) and exercise (29) periods. Substrate oxidation rates were then used to estimate energy expenditure at rest and during exercise.

Blood sampling

Venous blood samples were obtained from a 20-gauge cannula (Introcan Safety; B Braun, Sheffield, UK) which was fitted into an antecubital vein upon arrival to the laboratory. The first blood sample was collected > 10 minutes after the insertion of the cannula because the procedure can stimulate the vagus nerve which can affect measured blood analytes such as ghrelin (10). Participants then entered the chamber and subsequent samples were drawn at 1h, 2h 15 minutes, 3h 15 minutes, 4h and 4h 45 minutes. At each time point samples were collected into one five mL and one nine mL pre-cooled EDTA tube (Sarstedt, Leicester, UK). The nine mL tube was used for the determination of plasma concentrations of glucose, insulin, lactate, PP and total GLP-1. The five mL tube was used for the determination of plasma acylated ghrelin concentrations. These tubes were pre-treated on the morning of testing, to minimise the degradation of acylated ghrelin, with 50µl of a solution containing

p-hydroxymercuribenzoic acid, potassium phosphate buffer and sodium hydroxide (25). Both tubes were spun at 1500 x *g* for 10 minutes in a centrifuge (CompactStar CS4, VWR) immediately after being filled with venous blood. Plasma from the nine mL tube was dispensed into five Eppendorf tubes and one mL of plasma from the five mL tube was mixed with 100µl of 1M hydrochloric acid. This solution was then spun at 1500 x *g* for five minutes before the supernatant was transferred into a separate Eppendorf tube. Eppendorf tubes were immediately frozen at -20°C before being transferred to -80°C and stored until analysis.

With each venous sample, 10 µL and ~45 µL of whole blood was collected into a microcuvette and a heparinised micro haematocrit tube, respectively, for the measurement of haemoglobin and haematocrit concentrations. This data was used to estimate plasma volume changes over time (15). To control for postural changes in plasma volume all blood samples were collected whilst the participant was seated (17).

Blood analyses

Commercially available enzyme immunoassays were used to determine plasma concentrations of acylated ghrelin (SPI BIO, Montigny Le Bretonneux, France), GLP-1 (EMD Millipore, Darmstadt, Germany), PP (EMD Millipore, Darmstadt, Germany) and insulin (IBL, Hamburg, Germany). To eliminate interassay variation, all samples from each participant were analysed on the same plate. Glucose and lactate were measured photometrically with reagents from Instrumentation Laboratory (Lexington, MA) and Randox Laboratories (Crumlin, UK), respectively. The within batch coefficients of variation were as follows: acylated ghrelin 3.3%, GLP-1 5.1%, insulin, 5.6%, PP 3.9%, lactate 1.5% and glucose 1.8%.

Ad-libitum meal

A cold *ad-libitum* buffet meal was administered at 4h 45 minutes in which the participants were given 20 minute access for food consumption. The meal was identically presented between trials and

consisted of: three types of cereal, semi-skimmed milk, orange juice, white bread, brown bread, cheese, ham, tuna, bananas, apples, oranges, crisps, butter, margarine, mayonnaise, cereal bars, chocolate bars, cookies, muffins and chocolate rolls (13). The buffet was presented identically in each trial and food was provided in excess of expected consumption. Participants were informed to 'eat until comfortably full' and that additional quantities of each food item was available if desired. Meals were consumed behind a privacy screen to minimise social influence on food intake. Energy intake was calculated by weighing the food before and after consumption (to the nearest 0.1g), and with reference to the manufacturers tables of nutritional information.

Statistical analysis

Data are expressed as mean ± standard deviation (SD) in text and tables and mean ± standard error (SE) in figures. All data were analysed using IBM SPSS statistics (v22.0 for Windows; SPSS, Chicago, IL). The trapezoid method was used to calculate AUC for appetite perceptions and hormone concentrations. The four defined AUC periods were: pre-prandial (the 1h before breakfast), post-prandial (the 1h after breakfast, exercise (the 1h exercise period) and post-exercise (the 90 minutes post-exercise). Repeated measures ANOVA was used to assess trial-based differences in appetite perceptions, AMS scores, heart rate, SpO_2, hormone concentrations and energy intake. Where significant main effects of trial were found, post-hoc analysis was performed using Holm-Bonferroni correction for multiple comparisons. Effect sizes are presented as Cohen's *d* and interpreted as ≤ 0.2 trivial, > 0.2 small, > 0.6 moderate, > 1.2 large, > 2 very large and > 4 extremely large (23). The Pearson product moment correlation coefficient was used to investigate relationships between SpO_2, gut hormone concentrations, appetite perceptions and energy intakes. When plasma volume shifts were accounted for, interpretation of all blood analyte results was unaltered and thus the original data is presented. The sample size used within this study was deemed sufficient to detect a significant difference in energy intake between conditions. The anticipated effect size for a difference in energy intake was based on a similar previous study (55). Based on the effect size and an alpha value of 5%,

a sample size of 12 participants would generate a power >95%. Calculations were performed using G*power (16).

Results

Exercise responses.

Maximal oxygen uptake was significantly reduced at 2150m (48.2 ± 6.5 mL·kg·min^{-1}; P < 0.001; *d* = 1.04) and 4300m (37.7 ± 4.9 mL·kg·min^{-1}; P < 0.001; *d* = 2.83) compared with sea-level (55.6 ± 7.5 mL·kg·min^{-1}). This elicited walking speeds of 4.4 ± 0.4 km·h^{-1} (46.4 ± 4.0% $\dot{V}O_{2max}$), 3.6 ±0.4 km·h^{-1} (47.1 ± 4.7% $\dot{V}O_{2max}$) and 2.5 ± 0.4 km·h^{-1} (47.8 ± 4.3% $\dot{V}O_{2max}$) for the sea-level, 2150m and 4300m conditions, respectively. Mean RPE values were not different between sea-level (12.1 ± 1.5) and 2150m (12.0 ± 1.7; P = 0.437; *d* = 0.08), however were significantly higher at 4300m (14.0 ± 2.9) than at sea-level (P < 0.001; *d* = 0.82) and 2150m (P < 0.001; *d* = 0.85).

Appetite perceptions

At baseline, during the pre-prandial period and during the post-prandial period there were no significant differences in any appetite perceptions between conditions (all P > 0.066; *d < 0.4*). One-way ANOVA revealed a significant difference between conditions for composite appetite score during the exercise (P = 0.03) and the post-exercise (P < 0.001) periods. Post-hoc analysis revealed that, during exercise, AUC for CAS was significantly lower at 4300m (33 ± 17 mm·h^{-1}) compared with 2150m (44 ± 19mm·h^{-1}; P = 0.024; *d* = 0.65) and tended to be lower at 4300m compared with sea-level (42 ± 14 mm·h^{-1}; P = 0.10; *d* = 0.61). In the post-exercise period, AUC for CAS was significantly lower at 4300m (40 ± 19 mm·h^{-1}) compared with sea-level (55 ± 15 mm·h^{-1}; P = 0.004; *d* = 0.90) and 2150m (60 ± 14mm·h^{-1}; P < 0.001; *d* = 1.23) (figure 2).

- INSERT FIGURE 2 NEAR HERE -

Gut hormones concentrations and metabolic variables

There were no baseline differences between trials for the concentrations of any analyte (all $P > 0.152$). Further, (with the exclusion of lactate) there were no differences between trials for any analyte concentrations during the pre-prandial period or the post-prandial period (all $P > 0.206$).

During exercise, AUC for acylated ghrelin was significantly lower at 4300m (48 ± 23 $pg \cdot mL^{-1} \cdot h^{-1}$) compared with sea-level (69 ± 27 $pg \cdot mL^{-1} \cdot h^{-1}$; $P = 0.005$; $d = 0.84$) and 2150m (67 ± 31 $pg \cdot mL^{-1} \cdot h^{-1}$; $P = 0.01$; $d = 0.70$). During the post exercise period AUC for acylated ghrelin was significantly lower at 4300m (49 ± 31 $pg \cdot mL^{-1} \cdot h^{-1}$) compared with sea-level (116 ± 49 $pg \cdot mL^{-1} \cdot h^{-1}$; $P < 0.001$; 1.63) and 2150m (111 ± 62 $pg \cdot mL^{-1} \cdot h^{-1}$; $P = 0.002$; $d = 1.26$) (figure 3a).

Similarly to acylated ghrelin, AUC PP values were significantly lower during exercise at 4300m (315 ± 201 $pg \cdot mL^{-1} \cdot h^{-1}$) compared with sea-level (473 ± 271 $pg \cdot mL^{-1} \cdot h^{-1}$; $P = 0.002$; $d = 0.66$) and 2150m (446 ± 280 $pg \cdot mL^{-1} \cdot h^{-1}$; $P = 0.002$; $d = 0.54$). During the post exercise period AUC for PP was significantly lower at 4300m (242 ± 160 $pg \cdot mL^{-1} \cdot h^{-1}$) compared with sea-level (366 ± 225 $pg \cdot mL^{-1} \cdot h^{-1}$; $P = 0.001$; $d = 0.64$) and 2150m (318 ± 203 $pg \cdot mL^{-1} \cdot h^{-1}$; $P = 0.002$; $d = 0.41$) (figure 3b).

There were no differences in any AUC period for GLP-1 concentrations between conditions (all $P > 0.834$) (figure 3c).

- INSERT FIGURE 3 NEAR HERE –

During the exercise period there were no significant differences in AUC insulin concentrations between conditions ($P = 0.25$). During the post-exercise period AUC insulin concentrations were higher at 4300m (16.2 ± 6.1 $\mu IU \cdot mL^{-1} \cdot h^{-1}$) than at sea-level (10.4 ± 5.4 $\mu IU \cdot mL^{-1} \cdot h^{-1}$; $P = 0.02$; $d = 0.99$) and 2150m (10.7 ± 5.3 $\mu IU \cdot mL^{-1} \cdot h^{-1}$; $P = 0.045$; $d = 0.96$) (figure 4a).

During the exercise and post exercise period blood glucose concentrations were higher at 4300m compared with 2150m (exercise: P = 0.05; *d* = 1.04; post exercise: P = 0.036; *d* = 0.92). There were no other differences in any AUC period between conditions for blood glucose concentrations (all P > 0.20) (figure 4b).

During all four AUC periods lactate was significantly higher at 4300m compared with sea-level and 2150m (all P < 0.05). Entire trial AUC lactate concentrations were significantly higher at 4300m (1.39 ± 0.18 mmol·L·h^{-1}) compared with sea-level (0.90 ± 0.25 mmol·L·h^{-1}; P < 0.001; *d* = 2.21) and 2150m (1.03 ± 0.21 mmol·L·h^{-1}; P < 0.001; *d* = 1.82), with a trend for higher lactate concentrations at 2150m in comparison to sea-level (P = 0.07; *d* = 0.53) (figure 4c).

- INSERT FIGURE 4 NEAR HERE –

Energy intake

Mean energy intake at the *ad-libitum* meal was significantly lower at 4300m (3728 ± 3179 kJ) compared with sea-level (7358 ± 1789 kJ; P = 0.007; *d* = 1.41) and 2150m (7390 ± 1226 kJ; P = 0.004; *d* = 1.52). The absolute amount of carbohydrate, fat and protein consumed (g) were all significantly lower at 4300m than at sea-level and 2150m (all P < 0.019), however the relative proportion of these macronutrients to the total energy intake (%) did not differ significantly between conditions (all P > 0.061). A moderate effect size suggested an increased proportion of carbohydrate intake at 4300m compared with sea-level (P = 0.075; *d* = 0.85) and 2150m (P = 0.061; *d* = 0.86), however these differences were not significant (table 1).

- INSERT TABLE 1 NEAR HERE –

Substrate oxidation and energy expenditure

During the pre-prandial period absolute and relative carbohydrate oxidation was significantly higher at 4300m compared with sea-level (absolute: $P < 0.001$; $d = 1.2$; relative: $P = 0.01$; $d = 0.76$) and 2150m (absolute: $P < 0.001$; $d = 1.02$; relative: $P = 0.01$; $d = 0.69$). In the same period absolute carbohydrate oxidation was significantly higher at 2150m compared with sea-level ($P = 0.048$; $d = 0.46$). This was reversed during the exercise period in which absolute carbohydrate oxidation was significantly lower at 4300m compared with sea-level (absolute: $P < 0.001$; $d = 1.92$) and 2150m (absolute: $P = 0.01$; $d = 0.87$). In the same period absolute carbohydrate oxidation was significantly lower at 2150m compared with sea-level ($P = 0.005$; $d = 1.10$). In the post-exercise period absolute fat oxidation was significantly higher at 4300m compared with sea-level ($P = <0.001$; $d = 0.98$) and 2150m ($P = 0.025$; $d = 0.59$) (table 2). In the same period absolute fat oxidation was significantly higher at 2150m compared with sea-level ($P = 0.003$; $d = 0.50$).

- INSERT TABLE 2 NEAR HERE –

Entire trial energy expenditure was significantly higher during the sea-level trial (4379 ± 415 kJ) than during the 4300m trial (4008 ± 429 kJ; $P = 0.045$; $d = 0.88$) but not different to the 2150m trial (4162 ± 424; $P = 0.158$; $d = 0.52$). There were no differences between the 2150m condition and the 4300m condition ($P = 0.282$; $d = 0.36$). Resting energy expenditure was significantly higher during the 4300m trial (2242 ± 269 KJ) than during the sea-level trial (1826 ± 230 kJ; $P = <0.001$; $d = 1.66$) and the 2150m trial (1924 ± 217 kJ; $P = 0.007$; $d = 1.30$). There were no differences between sea-level and the 2150m condition ($P = 0.08$; $d = 0.44$). Exercise energy expenditure was significantly higher at sea-level (2552 ± 262 KJ) compared with 2150m (2238 ± 300 kJ; $P = 0.004$; $d = 1.11$) and 4300m (1766 ± 281 kJ; $P <0.001$; $d = 2.89$).

354 Oxygen saturations and acute mountain sickness

355 Mean SpO_2 was significantly lower at 4300m (resting: 74.4 ± 5.3 %, exercise: 61.9 ± 4.2 %) than at
356 2150m (resting: 92.9 ± 2.3 %; P < 0.001; *d* = 4.53, exercise: 87.1 ± 3.5 %; P<0.001; *d* = 6.52) which was
357 significantly lower than at sea level (resting: 97.8 ± 1.1 %; P < 0.001; *d* = 2.72, exercise: 95.9 ± 1.2 %; P
358 < 0.001; *d* = 3.36). Mild AMS did not manifest in any participant during the sea-level or 2150m trials
359 but was present for 10 out of 12 participants at 4300m. Severe AMS was present in 6 out of the 12
360 participants at 4300m. Mean LLAMS score across the entire trial was significantly higher at 4300m
361 (2.33 ± 1.65 AU) than at sea-level (0.16 ± 0.4 AU; P = 0.002; *d* = 1.81) and 2150m (0.13 ± 0.19 AU; P =
362 0.001; *d* = 1.87), with no difference between sea-level and 2150 (P = 0.78; *d* = 0.10).

363

364 Correlations

365 Pooled post-exercise AUC acylated ghrelin concentrations tended to be correlated with pre-buffet
366 hunger (r = 0.326; P = 0.052) and were significantly correlated with energy intake (r = 0.467; P = 0.004).
367 When all data was pooled SpO_2 was significantly correlated with acylated ghrelin concentrations (r =
368 0.323; P < 0.001). Alternatively, PP and GLP-1 concentrations were not significantly correlated with
369 SpO_2, CAS or energy intake (all $r \leq 0.157$; all $P \geq 0.359$).

370

371

372

373

374

375

376

Discussion

This study investigated the effects of moderate and severe simulated altitude on appetite perceptions, gut hormone concentrations, energy intake and substrate oxidation in comparison with normoxia. The primary findings of this investigation are that, in the absence of cold and other stressors, exercise during exposure to severe but not moderate simulated altitude significantly reduced subjective appetite perceptions, acylated ghrelin concentrations and energy intake. Additionally the proportion of carbohydrate oxidation was significantly higher at severe altitude in the pre-prandial phase, however, this pattern was reversed during and after exercise as fat oxidation was proportionally higher at severe altitude compared with normoxia.

The results of the present study demonstrate that energy intake was inhibited by 49% at severe altitude in comparison with sea-level but that inhibition did not occur at moderate altitude. Similarly, composite appetite score was inhibited at severe but not at moderate altitude following exercise. During exercise at 4300m, appetite was significantly inhibited compared with 2150m but only tended to be inhibited compared with sea-level. However, a moderate effect size (d = 0.61) was observed between 4300m and sea-level and no differences were observed between sea level and 2150m. We speculate that this tendency may have become a statistically significant difference if a larger sample size, higher intensity exercise or longer duration of exercise was utilised. In accordance with previous findings (54, 55), the current study provides support for the notion that AMS may contribute to, but is not the sole cause of, altitude-induced appetite inhibition. In this regard, appetite and energy intake were both lower in all twelve participants at 4300m compared with sea-level, whereas only ten of these individuals experienced mild AMS at some point during the trial.

In accordance with the observed appetite responses, acylated ghrelin was significantly inhibited following exercise in severe, but not moderate, altitude in comparison with sea-level. The present findings suggest that hypoxic exercise may have caused this effect rather than hypoxia *per se*, given the lack of response in the pre- and post-prandial periods. These findings concur with others who

found that acylated ghrelin concentrations were reduced following exercise during 7h exposure to 12.7% O_2 (3) and not reduced during 7h resting exposure to 15% O_2 (39). Conversely, one study has found acylated ghrelin inhibition at a moderate altitude (14.5% O_2), however the duration of hypoxic exposure was only 50 minutes (2). It is plausible that the exercise bout in the study of Bailey et al. (2) contributed to the inhibition of acylated ghrelin and appetite. This is further supported by Wasse et al. (55) who found that appetite, energy intake and acylated ghrelin concentrations were lower during an exercise trial in hypoxia compared with hypoxia without exercise. The dose of both hypoxia and exercise appear to substantially influence appetite responses, with higher altitude exerting larger inhibition. However, based on the findings of the present study this dose-response relationship does not appear to be linear. Awareness of appetite inhibition and the need for nutritional strategies appears crucial for those exercising at severe but not moderate altitudes.

It must be noted that in the present study participants were exposed to hypoxia for just 5h, and such short exposures are rare in real-life scenarios. There is potential that hypoxia may influence appetite differently during longer-term exposures, likely due to some acclimatising effects. Following prolonged periods (≥10 days) of normobaric hypoxic exposure (~13.9% O_2) three studies have found no reductions in appetite perceptions or total ghrelin concentrations compared with sea level (11, 12, 37). Chronic investigations at terrestrial altitude, which have found a reduction in appetite (4, 56), have employed altitudes >5000m. It seems plausible that altitudes >5000m may be required to inhibit appetite with chronic hypoxic exposure and that acute exposure produce a greater magnitude of appetite inhibition than chronic exposures at lower altitudes due to a lack of acclimatisation.

The data presented suggests that changes in circulating acylated ghrelin concentrations may contribute to altitude related appetite inhibition. It seems logical that with a significant reduction in acylated ghrelin at severe altitude, and other research showing total ghrelin to be unchanged (4, 11), that it is the acylation of ghrelin being affected rather than secretion. Ghrelin is post-translationally modified and this acylation of the hydroxyl group of the serine 3 (Kojima et al., 1999) occurs mostly

with octanoic acid (C8:0) and less commonly by decanoic acid (C10:0) or decenoic acid (C10:1) (26). Ghrelin O-acyltransferase (GOAT) is the essential gastric enzyme involved in the acylation of ghrelin with a medium chain fatty acid (MCFA), however this condensation reaction is not directly reliant on molecular oxygen. We can only speculate that the activity of GOAT or the availability of MCFAs as a substrate may be affected by hypoxia and thus reducing concentrations of acylated ghrelin. It would be beneficial for future studies to investigate methods of maintaining endogenous acylated ghrelin concentrations at altitude to further elucidate the role of this peptide in appetite inhibition at altitude. In rats, MCFAs have been found to be rate limiting in the acylation of ghrelin (33) and supplementation can increase concentrations of acylated ghrelin (41, 42), however this has not been investigated in humans.

The current study observed significantly lower circulating concentrations of the anorectic gut hormone PP at 4300m compared with sea-level, which conflicts with the observed appetite inhibition at this altitude and suggests that PP does not play a role in altitude-induced anorexia. This substantiates the findings of the only previous investigation to investigate PP at altitude, which found that PP was significantly reduced after 26h exposure to hypobaric hypoxia simulating 3454m (46). Similarly the lack of response in GLP-1 between conditions concurs with previous work showing that circulating concentrations of GLP-1 do not change in response to hypoxia and are therefore unlikely to mediate changes in appetite at altitude (39, 51).

The notion that altitude exposure may induce an increase in carbohydrate oxidation (8, 31, 44) compared with sea-level is supported by the current findings in the pre-prandial state. On the contrary, during the exercise and post-exercise periods, relative carbohydrate oxidation was significantly lower at 4300m compared with sea-level. In addition absolute and relative fat oxidation was significantly higher at 2150m compared to sea-level in the post-prandial period. These findings contradict the ‘oxygen-efficiency theory’ and support the perspective that the body needs to meet a metabolic compromise between the efficiency of oxidising carbohydrate and the need to conserve

452 valuable and limited glycogen stores (36). This study also observed significantly higher lactate
453 concentrations at 4300m, compared with sea-level and 2150m, which suggests a higher contribution
454 of anaerobic glycolysis to ATP production. At 4300m the lower SpO_2 may cause pyruvate, the end
455 product of glycolysis, to be shunted towards lactate production and away from oxidative metabolism
456 (40). Hypoxia has been found to deactivate pyruvate dehydrogenase (PDH) which may explain the
457 inability for pyruvate to convert into acetyl-coA for oxidation, thus increasing lactate concentrations
458 in hypoxic conditions (32, 43). In hypoxic muscle fibres it appears that the fatty acid-activated
459 transcription factor peroxisome proliferator-activated receptor (PPARα) can be upregulated which
460 may deactivate PDH thus promoting anaerobic glycolysis (28). This PPARα activation would also lead
461 to an increase in fatty acid oxidation. These mechanisms support our findings that the percentage of
462 the energy yield from fat oxidation was significantly higher at 4300m compared with sea-level during
463 the latter stages of the trial.

464 Despite the novel findings observed in the present study, some notable limitations must be
465 acknowledged. Firstly, during the sea-level condition energy expenditure was found to be higher due
466 to the higher absolute exercise intensity. It may therefore be expected that energy compensation may
467 be higher in this condition, which was observed in the present study. However, previous literature
468 suggests that acute bouts of exercise do not typically stimulate compensatory increases in appetite
469 and energy intake on the day of exercise (14). Furthermore, at 4300m the energy expenditure of the
470 trial was only 88.6 kcal lower than sea-level, which is unlikely to cause the 867 kcal deficit observed at
471 the buffet meal. This severe inhibition of energy intake would have a significant impact on body
472 composition if it persisted for several days/weeks. However, due to the acute nature of the present
473 study we cannot speculate that body composition, and thus functional capacity, would be affected in
474 the long term as there may be some compensation for the energy deficit in subsequent meals/days.
475 Further, subjects in the present study were healthy young males and thus caution should be applied
476 when applying the results to other populations. It has been suggested that females possess higher
477 plasma total ghrelin concentrations (35) and show differing substrate oxidation profiles at altitude

478 when compared to their male counterparts (7); although recent evidence suggests that males and
479 females exhibit similar appetite, energy intake and gut hormone responses to exercise- and diet-
480 induced energy deficits (1).

481 In conclusion, exercise during acute exposure to a simulated severe altitude (4300m; FiO_2
482 ~11.7%) inhibits appetite, acylated ghrelin concentration and energy intake in comparison with sea-
483 level, but exercise during exposure to simulated moderate altitude (2150m, FiO_2 ~15.8%) does not
484 influence these variables compared with sea-level. In addition, exposure to severe altitude
485 significantly increased the proportion of carbohydrate oxidation in the first hour compared with sea-
486 level. This pattern was then reversed as the proportion of fat oxidation was significantly higher in the
487 postprandial period. These data suggest that individuals exercising at severe altitude should be aware
488 of the risk for potential reductions in appetite but that this is unlikely to occur at moderate altitudes.
489 Based on the findings of the present study, it would be beneficial for future research to establish the
490 effects of acclimatisation on appetite responses to severe altitude and to identify methods of
491 minimising altitude-induced anorexia.

492

493

494

495

496

497

498

499

500

Acknowledgements

We would like to thank all of the volunteers who participated in this study. We would also like to thank Joe Sails, Fraser Chadwick, Aston Quinney, Milton Holt and Rachael Bradley for help with the data collection.

Disclosures

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. **Alajmi N, Deighton K, King JA, Reischak-Oliveira A, Wasse LK, Jones J, Batterham RL, and Stensel DJ**. Appetite and Energy Intake Responses to Acute Energy Deficits in Females versus Males. *Medicine and science in sports and exercise* 48: 412-420, 2016.

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

3. **Barnett BP, Hwang Y, Taylor MS, Kirchner H, Pfluger PT, Bernard V, Lin YY, Bowers EM, Mukherjee C, Song WJ, Longo PA, Leahy DJ, Hussain MA, Tschop MH, Boeke JD, and Cole PA**. Glucose and weight control in mice with a designed ghrelin O-acyltransferase inhibitor. *Science* 330: 1689-1692, 2010.

4. **Benso A, Broglio F, Aimaretti G, Lucatello B, Lanfranco F, Ghigo E, and Grottoli S**. Endocrine and metabolic responses to extreme altitude and physical exercise in climbers. *European journal of endocrinology* 157: 733-740, 2007.

5. **Borg GA**. Psychophysical bases of perceived exertion. *Medicine and science in sports and exercise* 14: 377-381, 1982.

6. **Bouissou P, Guezennec CY, Defer G, and Pesquies P**. Oxygen consumption, lactate accumulation, and sympathetic response during prolonged exercise under hypoxia. *International journal of sports medicine* 8: 266-269, 1987.

7. **Braun B, Mawson JT, Muza SR, Dominick SB, Brooks GA, Horning MA, Rock PB, Moore LG, Mazzeo RS, Ezeji-Okoye SC, and Butterfield GE**. Women at altitude: carbohydrate utilization during exercise at 4,300 m. *Journal of applied physiology* 88: 246-256, 2000.

8. **Brooks GA, Butterfield GE, Wolfe RR, Groves BM, Mazzeo RS, Sutton JR, Wolfel EE, and Reeves JT**. Increased dependence on blood glucose after acclimatization to 4,300 m. *Journal of applied physiology* 70: 919-927, 1991.

9. **Conkin J**. PH2O and simulated hypobaric hypoxia. *Aviation, space, and environmental medicine* 82: 1157-1158, 2011.

10. **Date Y**. The Vagus Nerve and Ghrelin Function. In: *Central Functions of the Ghrelin Receptor*, edited by Portelli J, and Smolders I. New York, NY: Springer New York, 2014, p. 53-61.

11. **Debevec T, Simpson EJ, Macdonald IA, Eiken O, and Mekjavic IB**. Exercise training during normobaric hypoxic confinement does not alter hormonal appetite regulation. *PloS one* 9: e98874, 2014.

12. **Debevec T, Simpson EJ, Mekjavic IB, Eiken O, and Macdonald IA**. Effects of prolonged hypoxia and bed rest on appetite and appetite-related hormones. *Appetite* 107: 28-37, 2016.

13. **Deighton K, Barry R, Connon CE, and Stensel DJ**. Appetite, gut hormone and energy intake responses to low volume sprint interval and traditional endurance exercise. *European journal of applied physiology* 113: 1147-1156, 2013.

14. **Deighton K, and Stensel DJ**. Creating an acute energy deficit without stimulating compensatory increases in appetite: is there an optimal exercise protocol? *The Proceedings of the Nutrition Society* 73: 352-358, 2014.

15. **Dill DB, and Costill DL**. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of applied physiology* 37: 247-248, 1974.

16. **Faul F, Erdfelder E, Lang AG, and Buchner A**. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior research methods* 39: 175-191, 2007.

17. **Fawcett JK, and Wynn V**. Effects of posture on plasma volume and some blood constituents. *Journal of clinical pathology* 13: 304-310, 1960.

18. **Fenn WO, Rahn H, and Otis AB**. A theoretical study of the composition of the alveolar air at altitude. *The American journal of physiology* 146: 637-653, 1946.

19. **Flint A, Raben A, Blundell JE, and Astrup A**. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International journal of obesity and related metabolic disorders* 24: 38-48, 2000.

20. **Frayn KN**. Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of applied physiology: respiratory, environmental and exercise physiology* 55: 628-634, 1983.

21. **Goodman J, Hassell K, Irwin D, Witkowski EH, and Nuss R**. The splenic syndrome in individuals with sickle cell trait. *High altitude medicine & biology* 15: 468-471, 2014.

22. **Hochachka PW, Stanley C, Matheson GO, McKenzie DC, Allen PS, and Parkhouse WS**. Metabolic and work efficiencies during exercise in Andean natives. *Journal of applied physiology* 70: 1720-1730, 1991.

23. **Hopkins WG**. How to interpret changes in an athletic performance test. *Sportscience* 8: 1–7, 2004.

24. **Hoppeler H, and Vogt M**. Muscle tissue adaptations to hypoxia. *The Journal of experimental biology* 204: 3133-3139, 2001.

25. **Hosoda H, Doi K, Nagaya N, Okumura H, Nakagawa E, Enomoto M, Ono F, and Kangawa K**. Optimum collection and storage conditions for ghrelin measurements: octanoyl modification of ghrelin is rapidly hydrolyzed to desacyl ghrelin in blood samples. *Clinical chemistry* 50: 1077-1080, 2004.

26. **Hosoda H, Kojima M, Mizushima T, Shimizu S, and Kangawa K**. Structural divergence of human ghrelin. Identification of multiple ghrelin-derived molecules produced by post-translational processing. *The Journal of biological chemistry* 278: 64-70, 2003.

27. **Howley ET, Bassett DR, Jr., and Welch HG**. Criteria for maximal oxygen uptake: review and commentary. *Medicine and science in sports and exercise* 27: 1292-1301, 1995.

28. **Huss JM, Levy FH, and Kelly DP**. Hypoxia inhibits the peroxisome proliferator-activated receptor alpha/retinoid X receptor gene regulatory pathway in cardiac myocytes: a mechanism for

O2-dependent modulation of mitochondrial fatty acid oxidation. *The Journal of biological chemistry* 276: 27605-27612, 2001.

29. **Jeukendrup AE, and Wallis GA**. Measurement of substrate oxidation during exercise by means of gas exchange measurements. *International journal of sports medicine* 26 Suppl 1: S28-37, 2005.

30. **Jones AM, and Doust JH**. A 1% treadmill grade most accurately reflects the energetic cost of outdoor running. *Journal of sports sciences* 14: 321-327, 1996.

31. **Katayama K, Goto K, Ishida K, and Ogita F**. Substrate utilization during exercise and recovery at moderate altitude. *Metabolism: clinical and experimental* 59: 959-966, 2010.

32. **Kim JW, Tchernyshyov I, Semenza GL, and Dang CV**. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell metabolism* 3: 177-185, 2006.

33. **Kirchner H, Gutierrez JA, Solenberg PJ, Pfluger PT, Czyzyk TA, Willency JA, Schurmann A, Joost HG, Jandacek R, Hale JE, Heiman ML, and Tschöp MH**. GOAT links dietary lipids with the endocrine control of energy balance. *Nature medicine* 15: 741-745, 2009.

34. **Lundby C, and Van Hall G**. Substrate utilization in sea level residents during exercise in acute hypoxia and after 4 weeks of acclimatization to 4100 m. *Acta physiologica Scandinavica* 176: 195-201, 2002.

35. **Makovey J, Naganathan V, Seibel M, and Sambrook P**. Gender differences in plasma ghrelin and its relations to body composition and bone - an opposite-sex twin study. *Clinical endocrinology* 66: 530-537, 2007.

36. **McClelland GB, Hochachka PW, and Weber JM**. Carbohydrate utilization during exercise after high-altitude acclimation: A new perspective. *Proceedings of the National Academy of Sciences of the United States of America* 95: 10288-10293, 1998.

37. **Mekjavic IB, Amon M, Kölegård R, Kounalakis SN, Simpson L, Eiken O, Keramidas ME, and Macdonald IA**. The effect of normobaric hypoxic confinement on metabolism, gut hormones and body composition. *Frontiers in Physiology* 7: 2016.

38. **Miles-Chan JL, Sarafian D, Montani JP, Schutz Y, and Dulloo AG**. Sitting comfortably versus lying down: is there really a difference in energy expenditure? *Clinical nutrition* 33: 175-178, 2014.

39. **Morishima T, and Goto K**. Ghrelin, GLP-1, and leptin responses during exposure to moderate hypoxia. *Applied physiology, nutrition, and metabolism* 41: 375-381, 2016.

40. **Murray AJ**. Metabolic adaptation of skeletal muscle to high altitude hypoxia: how new technologies could resolve the controversies. *Genome Medicine* 1: 117, 2009.

41. **Nishi Y, Hiejima H, Hosoda H, Kaiya H, Mori K, Fukue Y, Yanase T, Nawata H, Kangawa K, and Kojima M**. Ingested medium-chain fatty acids are directly utilized for the acyl modification of ghrelin. *Endocrinology* 146: 2255-2264, 2005.

42. **Nishi Y, Mifune H, and Kojima M**. Chapter Nineteen - Ghrelin Acylation by Ingestion of Medium-Chain Fatty Acids. In: *Methods in Enzymology*, edited by Masayasu K, and Kenji KAcademic Press, 2012, p. 303-315.

43. **Papandreou I, Cairns RA, Fontana L, Lim AL, and Denko NC**. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell metabolism* 3: 187-197, 2006.

44. **Peronnet F, Massicotte D, Folch N, Melin B, Koulmann N, Jimenez C, Bourdon L, Launay JC, and Savourey G**. Substrate utilization during prolonged exercise with ingestion of (13)C-glucose in acute hypobaric hypoxia (4,300 m). *European journal of applied physiology* 97: 527-534, 2006.

45. **Reeves S, Halsey LG, McMeel Y, and Huber JW**. Breakfast habits, beliefs and measures of health and wellbeing in a nationally representative UK sample. *Appetite* 60: 51-57, 2013.

46. **Riepl RL, Fischer R, Hautmann H, Hartmann G, Muller TD, Tschop M, Toepfer M, and Otto B**. Influence of acute exposure to high altitude on basal and postprandial plasma levels of gastroenteropancreatic peptides. *PloS one* 7: e44445, 2012.

47. **Roach RC, Bärtsch P, Oelz O, and Hackett PH**. The Lake Louise acute mountain sickness scoring system. . In: *Hypoxia and Molecular Medicine*, edited by Sutton JR, Houston CS, and Goates G. Burlington, VT: Queens City 1993, p. 272-274.

48. **Rose MS, Houston CS, Fulco CS, Coates G, Sutton JR, and Cymerman A**. Operation Everest. II: Nutrition and body composition. *Journal of applied physiology* 65: 2545-2551, 1988.

49. **Sergi G, Imoscopi A, Sarti S, Perissinotto E, Coin A, Inelmen EM, Zambon S, Busetto L, Seresin C, and Manzato E**. Changes in total body and limb composition and muscle strength after a 6-8 weeks sojourn at extreme altitude (5000-8000 m). *The Journal of sports medicine and physical fitness* 50: 450-455, 2010.

50. **Sierra-Johnson J, Romero-Corral A, Somers VK, and Johnson BD**. Last word on viewpoint: effect of altitude on leptin levels, does it go up or down? *Journal of applied physiology* 105: 1691, 2008.

51. **Snyder EM, Carr RD, Deacon CF, and Johnson BD**. Overnight hypoxic exposure and glucagon-like peptide-1 and leptin levels in humans. *Applied physiology, nutrition, and metabolism* 33: 929-935, 2008.

52. **Stevenson E, Williams C, Nute M, Swaile P, and Tsui M**. The effect of the glycemic index of an evening meal on the metabolic responses to a standard high glycemic index breakfast and subsequent exercise in men. *International journal of sport nutrition and exercise metabolism* 15: 308-322, 2005.

53. **Stubbs RJ, Hughes DA, Johnstone AM, Rowley E, Reid C, Elia M, Stratton R, Delargy H, King N, and Blundell JE**. The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. *The British journal of nutrition* 84: 405-415, 2000.

54. **Tschop M, and Morrison KM**. Weight loss at high altitude. *Advances in experimental medicine and biology* 502: 237-247, 2001.

55. **Wasse LK, Sunderland C, King JA, Batterham RL, and 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. *Journal of applied physiology* 112: 552-559, 2012.

56. **Westerterp-Plantenga MS, Westerterp KR, Rubbens M, Verwegen CR, Richelet JP, and Gardette B**. Appetite at "high altitude" [Operation Everest III (Comex-'97)]: a simulated ascent of Mount Everest. *Journal of applied physiology* 87: 391-399, 1999.

57. **Westerterp KR, Kayser B, Wouters L, Le Trong JL, and Richalet JP**. Energy balance at high altitude of 6,542 m. *Journal of applied physiology* 77: 862-866, 1994.

58. **Westerterp KR, Meijer EP, Rubbens M, Robach P, and Richalet JP**. Operation Everest III: energy and water balance. *European journal of physiology* 439: 483-488, 2000.

59. **Wolever TM, Jenkins DJ, Ocana AM, Rao VA, and Collier GR**. Second-meal effect: low-glycemic-index foods eaten at dinner improve subsequent breakfast glycemic response. *The American journal of clinical nutrition* 48: 1041-1047, 1988.

60. **Zaccagni L, Barbieri D, Cogo A, and Gualdi-Russo E**. Anthropometric and body composition changes during expeditions at high altitude. *High altitude medicine & biology* 15: 176-182, 2014.

Figure legends

Figure 1. Experimental trial schematic.

Figure 2. Composite appetite scores during sea-level (●), 2150m (■) and 4300m (▲) trials. Values are mean ± SE; n = 12. Thin upward arrow represents breakfast and thick upward arrow represents *ad-libitum* meal. Black rectangle represents exercise.

Figure 3. Plasma acylated ghrelin (a), pancreatic polypeptide (b) and glucagon-like peptide-1 (c) concentrations during sea-level (●), 2150m (■) and 4300m (▲) trials. Values are mean ± SE; n = 12. Thin upward arrow represents breakfast and thick upward arrow represents *ad-libitum* meal. Black rectangle represents exercise.

Figure 4. Plasma insulin (a), glucose (b) and lactate (c) concentrations during sea-level (●), 2150m (■) and 4300m (▲) trials. Values are mean ± SE; n = 12. Thin upward arrow represents breakfast and thick upward arrow represents *ad-libitum* meal. Black rectangle represents exercise.

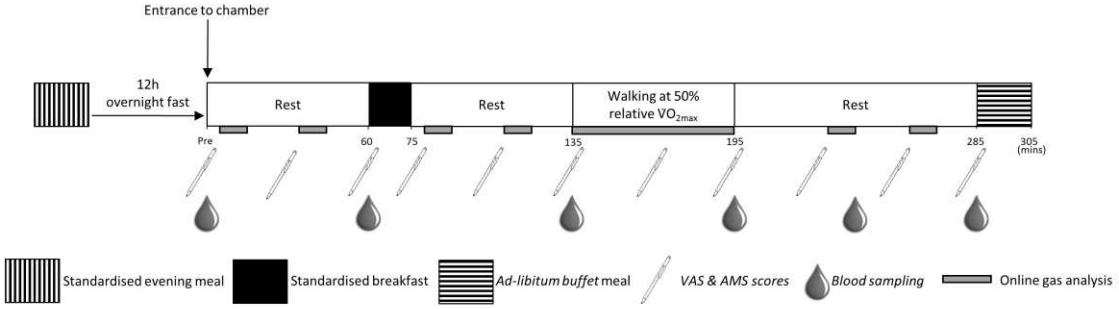
Table legends

Table 1. Macronutrient intakes at the *ad-libitum* buffet meal for the sea-level, 2150m and 4300m trials Values are mean ± SD, N = 12. * Significant difference between sea-level and 2150m. † Significant difference between sea-level and 4300m. # Significant difference between 2150m and 4300m (One way ANOVA; $P < 0.05$ after Holm-Bonferroni adjustment).

Table 2. Area under the curve carbohydrate and fat oxidation for the sea-level, 2150m and 4300m trials

Values are mean ± SD, N = 12. % is percentage of energy yield. * Significant difference between sea-level and 2150m. † Significant difference between sea-level and 4300m. # Significant difference between 2150m and 4300m (One way ANOVA; $P < 0.05$ after Holm-Bonferroni adjustment).

779 **Figure 1**



780

781

782

783

784

785

786

787

788

789

790

791

792

793

Figure 2

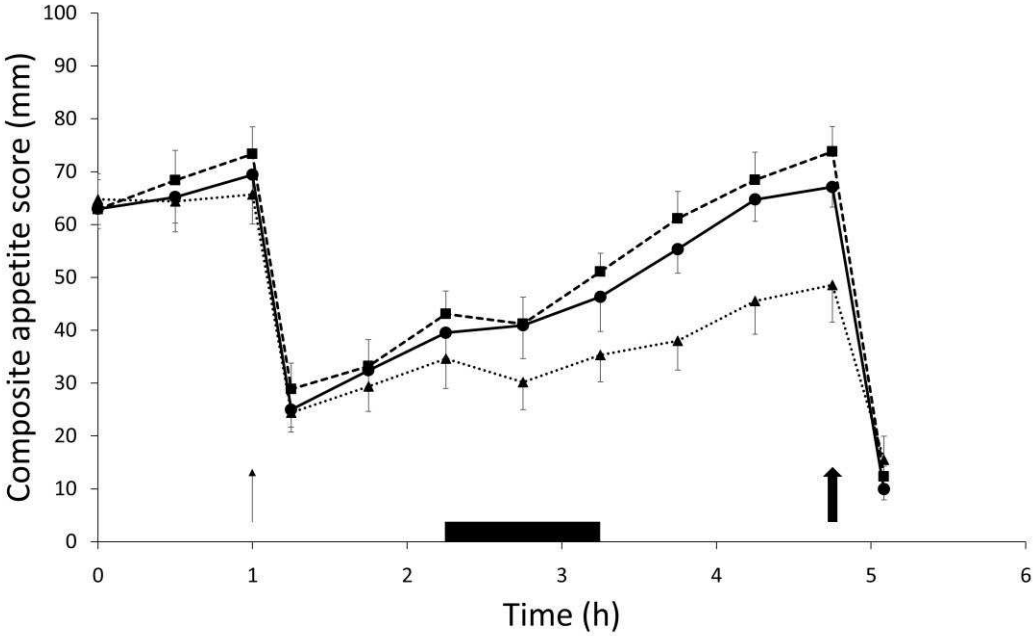

Composite appetite score (mm)
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
Time (h)

Figure 3

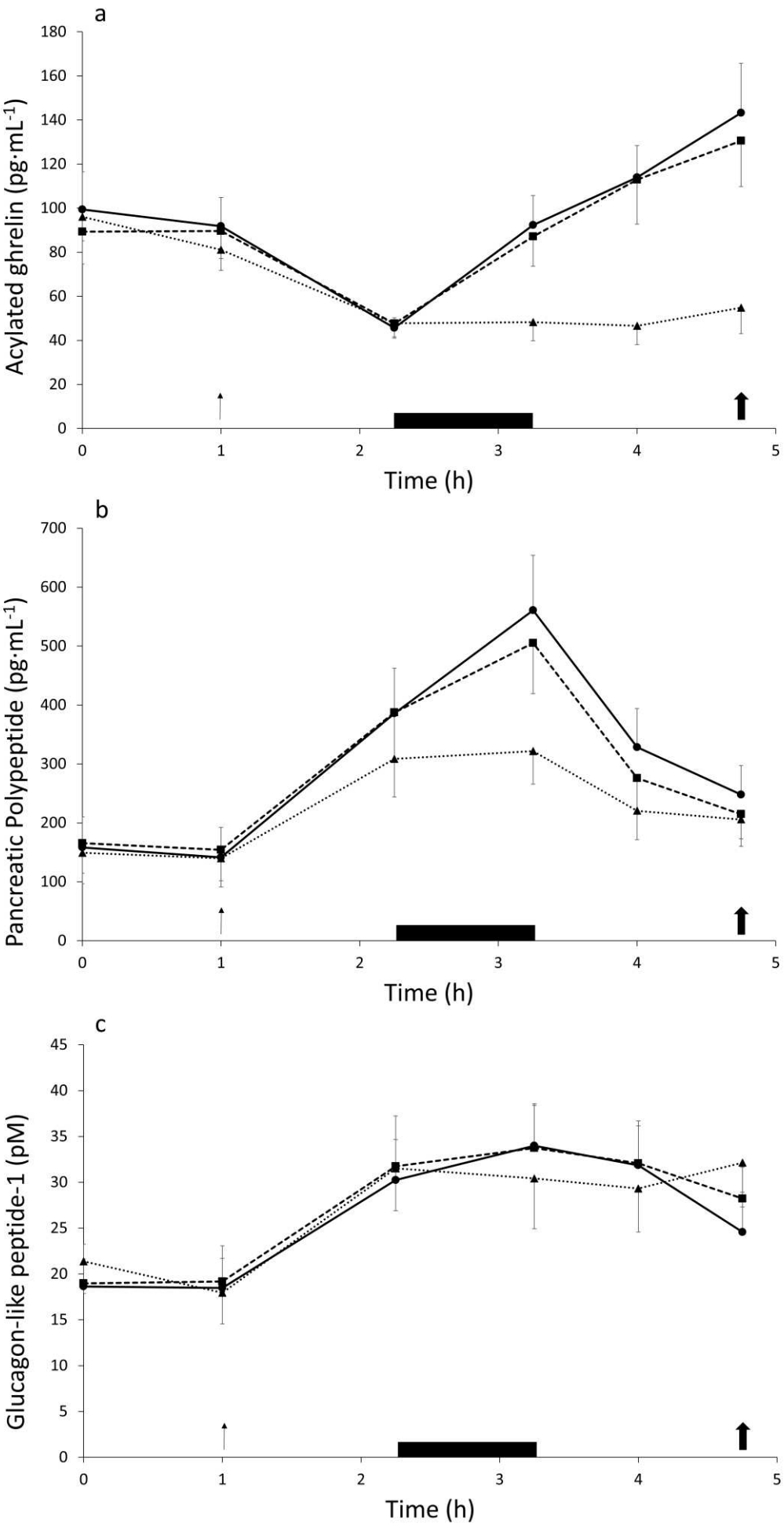

a
Acylated ghrelin (pg·mL-1)
180
160
140
120
100
80
60
40
20
0
0
1
2
3
4
5
Time (h)
b
Pancreatic Polypeptide (pg·mL-1)
700
600
500
400
300
200
100
0
0
1
2
3
4
5
Time (h)
c
Glucagon-like peptide-1 (pM)
45
40
35
30
25
20
15
10
5
0
0
1
2
3
4
5
Time (h)

Figure 4

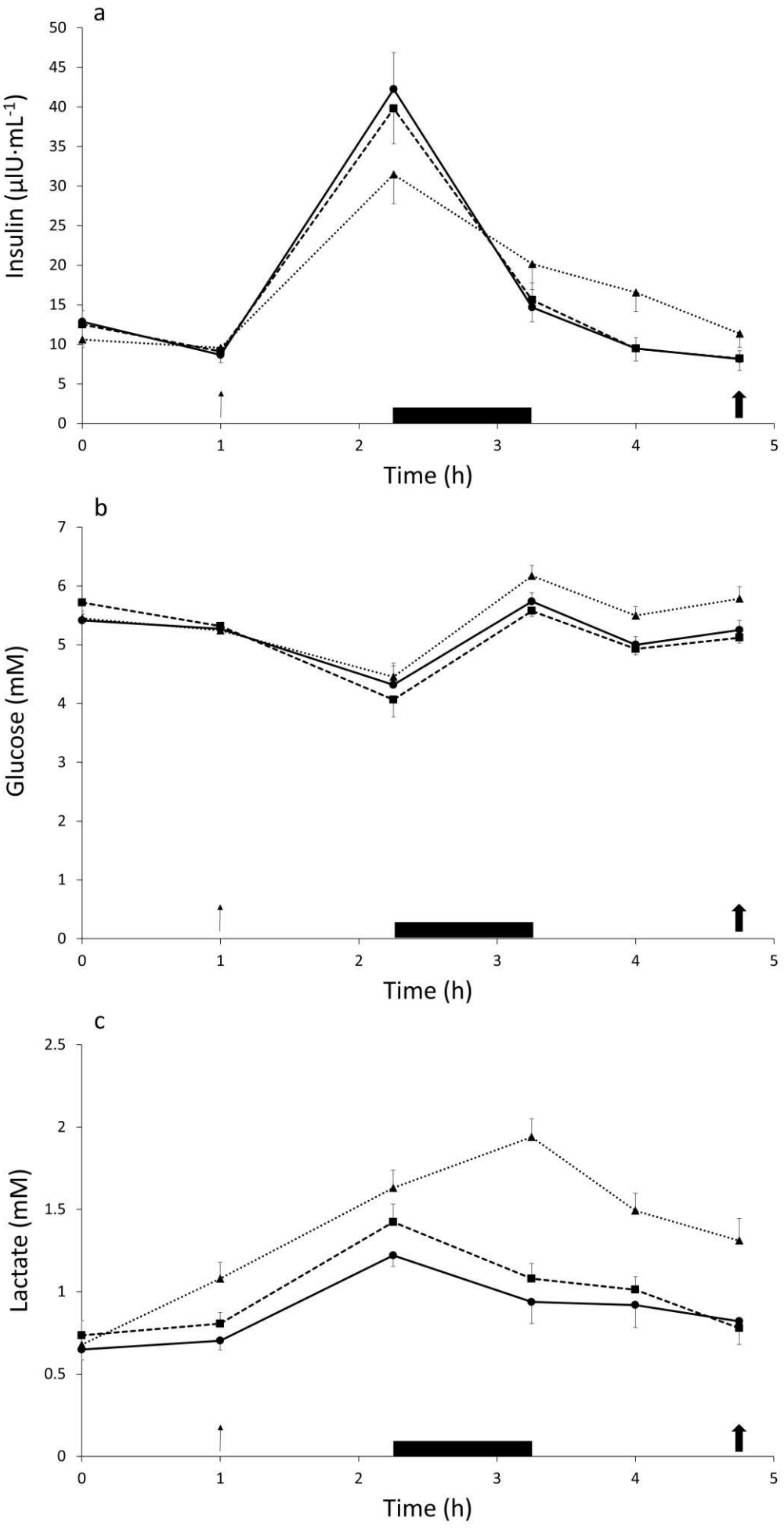


Table 1. Macronutrient intakes at the *ad-libitum* buffet meal for the three conditions

	Carbohydrate, g (%)	Fat, g (%)	Protein, g (%)
0m	174 ± 46 (39 ± 6)	87 ± 28 (46 ± 7)	64 ± 26 (15 ± 4)
2,150m	175 ± 37 (39 ± 5)	90 ± 20 (48 ± 6)	58 ± 15 (14 ± 3)
4,300m	97 ± 77 †# (51 ± 19)	43 ± 46 †# (38 ± 17)	27 ± 24 †# (11 ± 4)

Values are mean ± SD, N = 12. * Significant difference between sea-level and 2150m. † Significant difference between sea-level and 4300m. # Significant difference between 2150m and 4300m (One way ANOVA; $P < 0.05$ after Holm-Bonferroni adjustment).

Table 2. Area under the curve carbohydrate and fat oxidation for the three conditions

	Pre-prandial		Post-prandial		Exercise		Post-exercise	
	Carbohydrate oxidation, $g.min^{-1}$ (%)	Fat oxidation, $g.min^{-1}$ (%)	Carbohydrate oxidation, $g.min^{-1}$ (%)	Fat oxidation, $g.min^{-1}$ (%)	Carbohydrate oxidation, $g.min^{-1}$ (%)	Fat oxidation, $g.min^{-1}$ (%)	Carbohydrate oxidation, $g.min^{-1}$ (%)	Fat oxidation, $g.min^{-1}$ (%)
0m	0.16 ± 0.07	0.10 ± 0.04	0.28 ± 0.07	0.09 ± 0.04	1.56 ± 0.35	0.41 ± 0.18	0.21 ± 0.08	0.11 ± 0.04
	(42.1 ± 14.0)	(57.9 ± 14.0)	(59.1 ± 15.2)	(40.9 ± 15.2)	(62.8 ± 13.3)	(37.2 ± 13.3)	(46.4 ± 16.2)	(53.6 ± 16.2)
2,150m	0.18 ± 0.06*	0.11 ± 0.05	0.28 ± 0.09	0.09 ± 0.04	1.18 ± 0.34*	0.44 ± 0.21	0.16 ± 0.08	0.13 ± 0.04*
	(42.1 ± 18.9)	(57.9 ± 18.9)	(57.2 ± 17.6)	(42.8 ± 17.6)	(55.1 ± 18.9)	(44.9 ± 18.9)	(36.2 ± 18.6*)	(63.8 ± 18.6*)
4,300m	0.29 ± 0.14†#	0.09± 0.05	0.30 ± 0.14	0.12 ± 0.05†	0.87 ± 0.37†#	0.38 ± 0.19	0.19 ± 0.16	0.16 ± 0.06†#
	(56.8 ± 23.4†#)	(43.2 ± 23.4†#)	(52.4 ± 22.7)	(47.6 ± 22.7)	(50.8 ± 19.8†)	(49.2 ± 19.8†)	(34.8 ± 22.6†)	(65.2 ± 22.6†)

Values are mean ± SD, N = 12. % is percentage of energy yield. * Significant difference between sea-level and 2150m. † Significant difference between sea-level and 4300m. # Significant difference between 2150m and 4300m (One way ANOVA; $P < 0.05$ after Holm-Bonferroni adjustment).