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

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

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**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

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

*- INSERT FIGURE 1 NEAR HERE –*

Measurements

Ratings of perceived appetite and symptoms of acute mountain sickness

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

166 ≥6 in the presence of a headache. Appetite perceptions were measured using validated 100 mm visual
167 analogue scales (VAS) (19). Using these scales a composite appetite score (CAS) was calculated using
168 the following formula: composite appetite score = ([hunger + prospective food consumption + (100 –
169 fullness) + (100 – satisfaction)] / 4) (53). A higher value is associated with a greater appetite sensation
170 and subsequently a stronger motivation to eat.

171 Online gas analysis

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

180 Blood sampling

181 Venous blood samples were obtained from a 20-gauge cannula (Introcan Safety; B Braun, Sheffield,
182 UK) which was fitted into an antecubital vein upon arrival to the laboratory. The first blood sample
183 was collected > 10 minutes after the insertion of the cannula because the procedure can stimulate the
184 vagus nerve which can affect measured blood analytes such as ghrelin (10). Participants then entered
185 the chamber and subsequent samples were drawn at 1h, 2h 15 minutes, 3h 15 minutes, 4h and 4h 45
186 minutes. At each time point samples were collected into one five mL and one nine mL pre-cooled EDTA
187 tube (Sarstedt, Leicester, UK). The nine mL tube was used for the determination of plasma
188 concentrations of glucose, insulin, lactate, PP and total GLP-1. The five mL tube was used for the
189 determination of plasma acylated ghrelin concentrations. These tubes were pre-treated on the
190 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 -*

283 Gut hormones concentrations and metabolic variables

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

287 During exercise, AUC for acylated ghrelin was significantly lower at 4300m (48 ± 23 pg·mL$^{-1}$·h$^{-1}$)
288 compared with sea-level (69 ± 27 pg·mL$^{-1}$·h$^{-1}$; P = 0.005; *d* = 0.84) and 2150m (67 ± 31 pg·mL$^{-1}$·h$^{-1}$; P
289 = 0.01; *d* = 0.70). During the post exercise period AUC for acylated ghrelin was significantly lower at
290 4300m (49 ± 31 pg·mL$^{-1}$·h$^{-1}$) compared with sea-level (116 ± 49 pg·mL$^{-1}$·h$^{-1}$; P < 0.001; 1.63) and 2150m
291 (111 ± 62 pg·mL$^{-1}$·h$^{-1}$; P = 0.002; *d* = 1.26) (figure 3a).

292 Similarly to acylated ghrelin, AUC PP values were significantly lower during exercise at 4300m
293 (315 ± 201 pg·mL$^{-1}$·h$^{-1}$) compared with sea-level (473 ± 271 pg·mL$^{-1}$·h$^{-1}$; P = 0.002; *d* = 0.66) and 2150m
294 (446 ± 280 pg·mL$^{-1}$·h$^{-1}$; P = 0.002; *d* = 0.54). During the post exercise period AUC for PP was significantly
295 lower at 4300m (242 ± 160 pg·mL$^{-1}$·h$^{-1}$) compared with sea-level (366 ± 225 pg·mL$^{-1}$·h$^{-1}$; P = 0.001; *d* =
296 0.64) and 2150m (318 ± 203 pg·mL$^{-1}$·h$^{-1}$; P = 0.002; *d* = 0.41) (figure 3b).

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

299

300 *- INSERT FIGURE 3 NEAR HERE –*

301

302 During the exercise period there were no significant differences in AUC insulin concentrations
303 between conditions (P = 0.25). During the post-exercise period AUC insulin concentrations were higher
304 at 4300m (16.2 ± 6.1 µlU·mL$^{-1}$·h$^{-1}$) than at sea-level (10.4 ± 5.4 µlU·mL$^{-1}$·h$^{-1}$; P = 0.02; *d* = 0.99) and
305 2150m (10.7 ± 5.3 µlU·mL$^{-1}$·h$^{-1}$; P = 0.045; *d* = 0.96) (figure 4a).

306 During the exercise and post exercise period blood glucose concentrations were higher at
307 4300m compared with 2150m (exercise: P = 0.05; *d* = 1.04; post exercise: P = 0.036; *d* = 0.92). There
308 were no other differences in any AUC period between conditions for blood glucose concentrations (all
309 P > 0.20) (figure 4b).

310 During all four AUC periods lactate was significantly higher at 4300m compared with sea-level
311 and 2150m (all P < 0.05). Entire trial AUC lactate concentrations were significantly higher at 4300m
312 (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
313 2150m (1.03 ± 0.21 mmol·L·h$^{-1}$; P < 0.001; *d* = 1.82), with a trend for higher lactate concentrations at
314 2150m in comparison to sea-level (P = 0.07; *d* = 0.53) (figure 4c).

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316 *- INSERT FIGURE 4 NEAR HERE –*

317

318 Energy intake

319 Mean energy intake at the *ad-libitum* meal was significantly lower at 4300m (3728 ± 3179 kJ)
320 compared with sea-level (7358 ± 1789 kJ; P = 0.007; *d* = 1.41) and 2150m (7390 ± 1226 kJ; P = 0.004;
321 *d* = 1.52). The absolute amount of carbohydrate, fat and protein consumed (g) were all significantly
322 lower at 4300m than at sea-level and 2150m (all P < 0.019), however the relative proportion of these
323 macronutrients to the total energy intake (%) did not differ significantly between conditions (all P >
324 0.061). A moderate effect size suggested an increased proportion of carbohydrate intake at 4300m
325 compared with sea-level (P = 0.075; *d* = 0.85) and 2150m (P = 0.061; *d* = 0.86), however these
326 differences were not significant (table 1).

327

328 *- INSERT TABLE 1 NEAR HERE –*

329

330 Substrate oxidation and energy expenditure

331 During the pre-prandial period absolute and relative carbohydrate oxidation was significantly higher
332 at 4300m compared with sea-level (absolute: P < 0.001; *d* = 1.2; relative: P = 0.01; *d* = 0.76) and 2150m
333 (absolute: P < 0.001; *d* = 1.02; relative: P = 0.01; *d* = 0.69). In the same period absolute carbohydrate
334 oxidation was significantly higher at 2150m compared with sea-level (P = 0.048; *d* = 0.46). This was
335 reversed during the exercise period in which absolute carbohydrate oxidation was significantly lower
336 at 4300m compared with sea-level (absolute: P < 0.001; *d* = 1.92) and 2150m (absolute: P = 0.01; *d* =
337 0.87). In the same period absolute carbohydrate oxidation was significantly lower at 2150m compared
338 with sea-level (P = 0.005; *d* = 1.10). In the post-exercise period absolute fat oxidation was significantly
339 higher at 4300m compared with sea-level (P = <0.001; *d* = 0.98) and 2150m (P = 0.025; *d* = 0.59) (table
340 2). In the same period absolute fat oxidation was significantly higher at 2150m compared with sea-
341 level (P = 0.003; *d* = 0.50).

342

343 *- INSERT TABLE 2 NEAR HERE –*

344

345 Entire trial energy expenditure was significantly higher during the sea-level trial (4379 ± 415
346 kJ) than during the 4300m trial (4008 ± 429 kJ; P = 0.045; *d* = 0.88) but not different to the 2150m trial
347 (4162 ± 424; P = 0.158; *d* = 0.52). There were no differences between the 2150m condition and the
348 4300m condition (P = 0.282; *d* = 0.36). Resting energy expenditure was significantly higher during the
349 4300m trial (2242 ± 269 KJ) than during the sea-level trial (1826 ± 230 kJ; P = <0.001; *d* = 1.66) and the
350 2150m trial (1924 ± 217 kJ; P = 0.007; *d* = 1.30). There were no differences between sea-level and the
351 2150m condition (P = 0.08; *d* = 0.44). Exercise energy expenditure was significantly higher at sea-level
352 (2552 ± 262 KJ) compared with 2150m (2238 ± 300 kJ; P = 0.004; *d* = 1.11) and 4300m (1766 ± 281 kJ;
353 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 $P \geq 0.359$).

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## 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

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

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

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## 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**

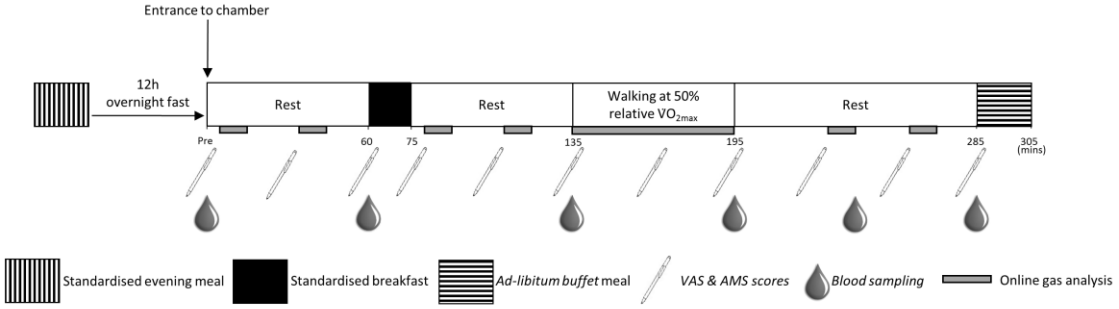


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**Figure 2**

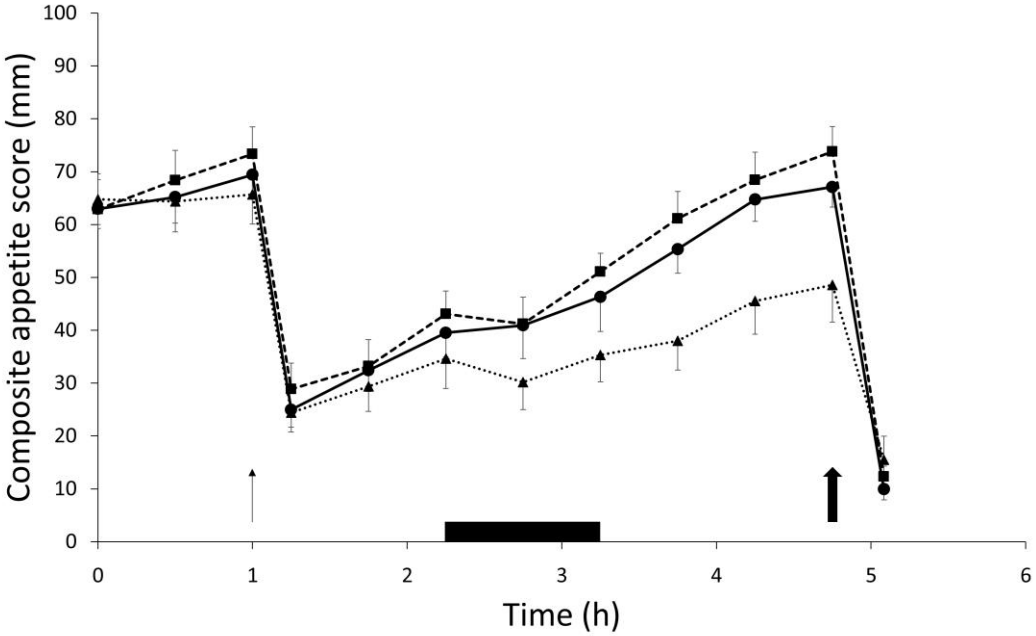
Composite appetite score (mm)
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
Time (h)

**Figure 3**

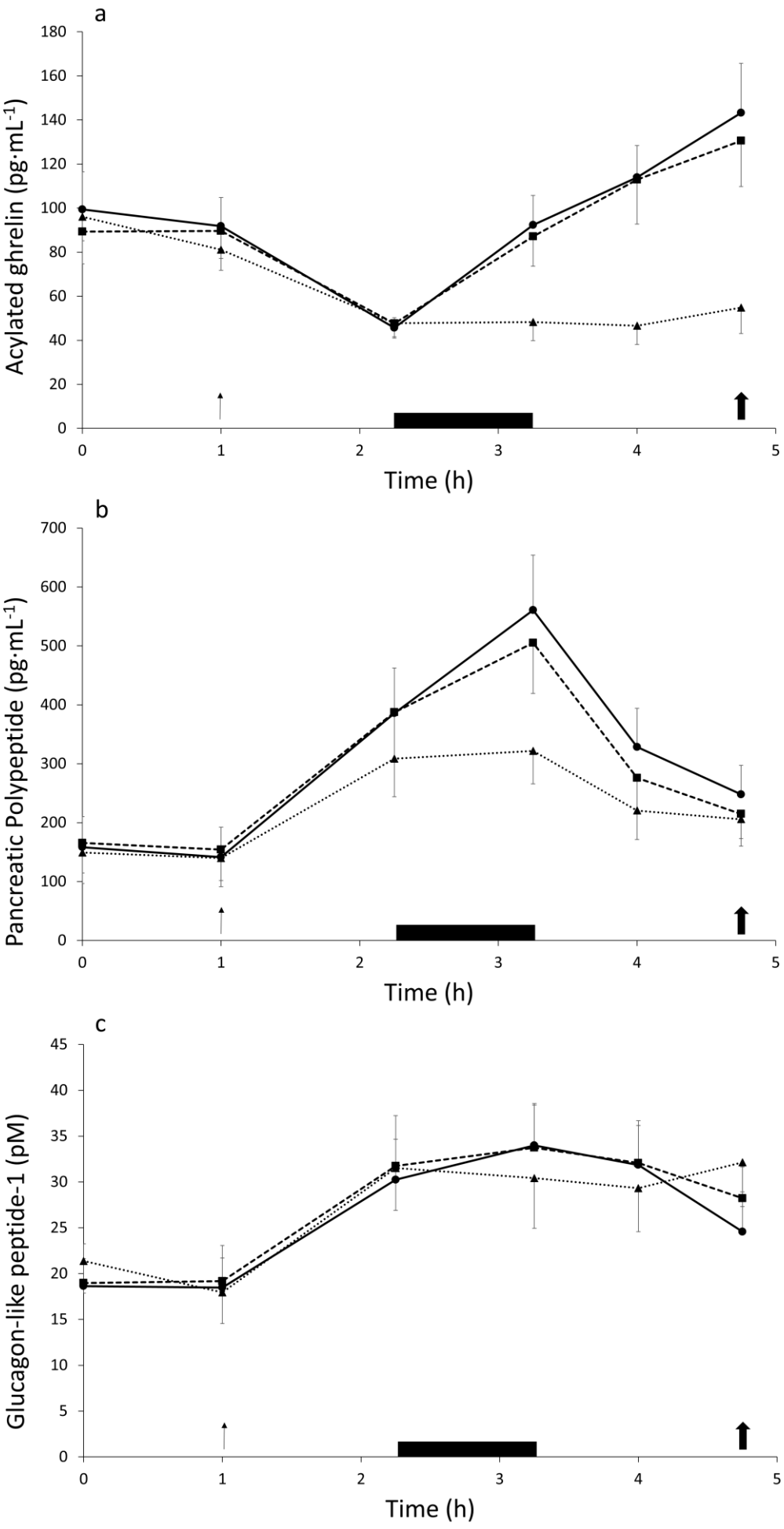
a
Acylated ghrelin (pg·mL-1)
180
160
140
120
100
80
60
40
20
0
0
1
2
3
4
5
Time (h)
b
Pancreatic Polypeptide (pg·mL-1)
700
600
500
400
300
200
100
0
0
1
2
3
4
5
Time (h)
c
Glucagon-like peptide-1 (pM)
45
40
35
30
25
20
15
10
5
0
0
1
2
3
4
5
Time (h)

**Figure 4**

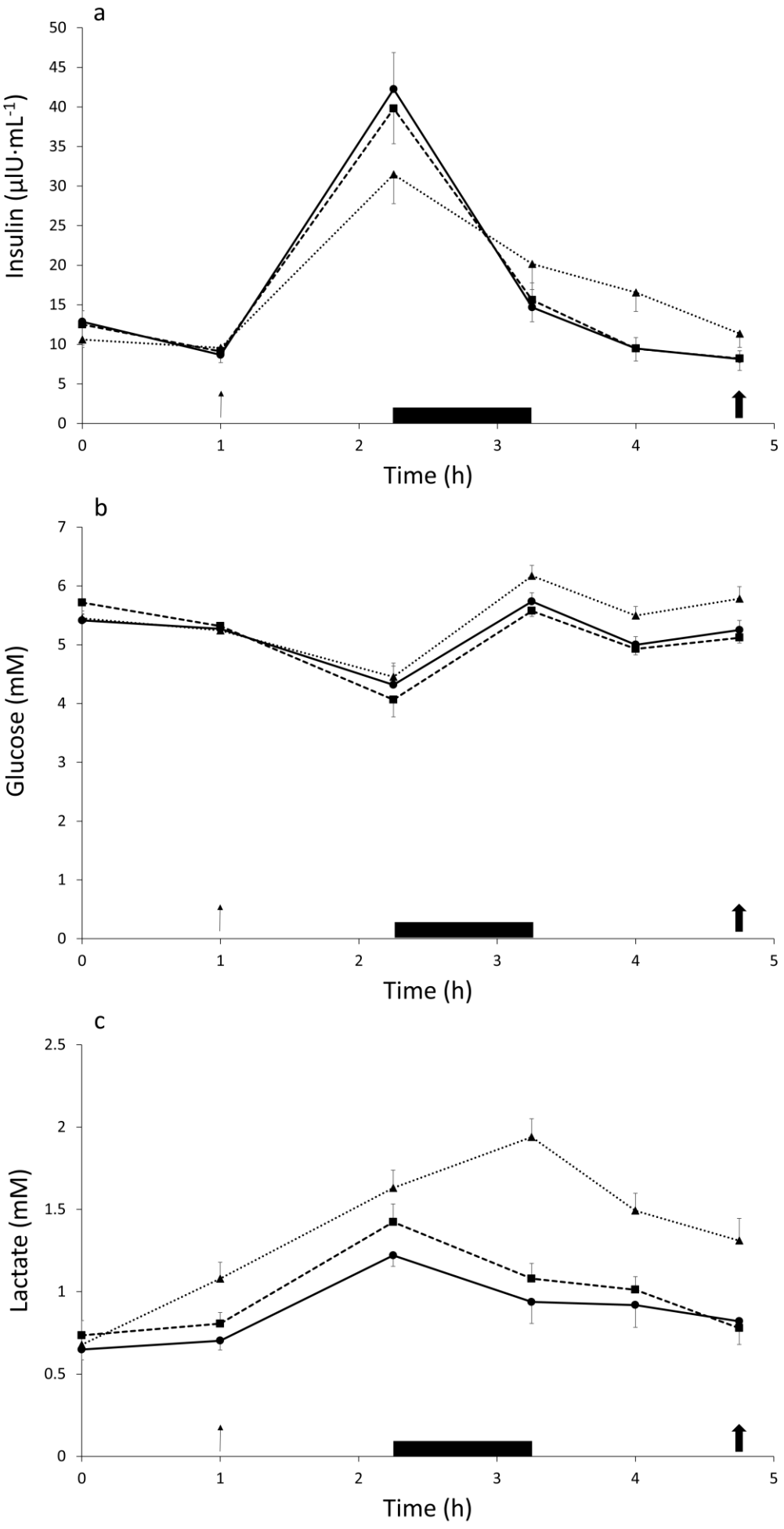

**Table 1.** Macronutrient intakes at the *ad-libitum* buffet meal for the three conditions

| | 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).