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

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23 **Abstract**

24 Acute exposure to high altitude (>3500m) is associated with marked changes in appetite regulation
25 and substrate oxidation but the effects of lower altitudes are unclear. This study examined appetite,
26 gut hormone, energy intake and substrate oxidation responses to breakfast ingestion and exercise at
27 simulated moderate and severe altitudes compared with sea-level. Twelve healthy males (mean±SD;
28 age 30±9years, body mass index 24.4±2.7kg.m⁻²) completed in a randomised crossover order three,
29 305 minute experimental trials at a simulated altitude of 0m, 2150m (~15.8% O₂) and 4300m (~11.7%
30 O₂) in a normobaric chamber. Participants entered the chamber at 8am following a 12h fast. A
31 standardised breakfast was consumed inside the chamber at 1h. One hour after breakfast, participants
32 performed a 60 minute treadmill walk at 50% of relative $\dot{V}O_{2max}$. An *ad-libitum* buffet meal was
33 consumed 1.5h after exercise. Blood samples were collected prior to altitude exposure and at 60, 135,
34 195, 240 and 285 minutes. No trial based differences were observed in any appetite related measure
35 before exercise. Post-exercise area under the curve values for acylated ghrelin, pancreatic polypeptide
36 and composite appetite score were lower (all P<0.05) at 4300m compared with sea-level and 2150m.
37 There were no differences in glucagon-like peptide-1 between conditions (P=0.895). Mean energy
38 intake was lower at 4300m (3728±3179kJ) compared with sea-level (7358±1789kJ; P=0.007) and
39 2150m (7390±1226kJ; P=0.004). Proportional reliance on carbohydrate as a fuel was higher (P=0.01)
40 before breakfast but lower during (P=0.02) and after exercise (P=0.01) at 4300m compared with sea-
41 level. This study suggests that altitude-induced anorexia and a subsequent reduction in energy intake
42 occurs after exercise during exposure to severe but not moderate simulated altitude. Acylated ghrelin
43 concentrations may contribute to this effect.

44

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

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47 Introduction

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

55 Historically, studies have attributed altitude-induced appetite inhibition to acute mountain
56 sickness (AMS). However, it has been found that appetite remains inhibited once the symptoms of
57 AMS have subsided (54). In an attempt to identify possible mechanisms behind altitude-induced
58 anorexia, studies have investigated changes in the circulating levels of various hormones in response
59 to hypoxia. This includes the measurement of glucagon-like peptide-1 (GLP-1) (37, 51), leptin (37, 50),
60 pancreatic polypeptide (PP) (46) and peptide YY (PYY) (37, 55) with particular recent interest towards
61 acylated ghrelin (2, 39, 55). Wasse et al. (55) found that a seven hour exposure to hypoxia (12.7% FiO₂,
62 ~4000m), commencing with a one hour exercise period, significantly reduced acylated ghrelin
63 concentrations and *ad-libitum* energy intake compared with sea-level. However, reports in the
64 literature present contradictory findings regarding the response of acylated ghrelin to moderate
65 altitude (1500m - 3500m). In this regard, Bailey et al. (2) reported lower acylated ghrelin area under
66 the curve (AUC) concentrations in hypoxia (14.5% FiO₂, ~2980m) than normoxia, whereas Morishima
67 and Goto (39) found no significant effect of a seven hour moderate hypoxic exposure (15% FiO₂,
68 ~2700m) on acylated ghrelin concentrations compared with normoxia. The reasons for this
69 discrepancy are unclear and the lack of energy intake assessment in these studies means that the
70 effects of moderate hypoxia on energy intake remains unknown.

71 In addition to changes in appetite regulation, high altitude exposure also appears to increase
72 the body's reliance on carbohydrate as a fuel for substrate oxidation in comparison with sea-level (8,
73 31, 44). This response is hypothesised to be acutely beneficial, due to the higher yield of ATP per
74 molecule of oxygen with carbohydrate utilisation in comparison with fat (22). However, this oxygen-
75 efficiency theory has been disputed by other studies which show no effect of altitude on substrate
76 oxidation if relative exercise intensities are matched (6, 34). An increased reliance on carbohydrate as
77 a fuel could also lead to a faster depletion of valuable and limited liver and muscle glycogen stores
78 (44), which could have adverse effects at altitude.

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

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94 **Methods**

95 Participants

96 Twelve healthy male volunteers (age 30 ± 9 years, body mass index 24.4 ± 2.7 kg.m⁻², body mass 80.5
97 ± 10.5 kg) provided written informed consent to participate in this study. The study, which received
98 institutional ethics approval, was conducted in accordance with the Declaration of Helsinki. All
99 participants were non-smokers, normotensive, free from food allergies and were not taking any
100 medication. None of the participants had travelled to an altitude >1500 m during the previous three
101 months and were all currently residing at an altitude <500 m.

102 Experimental design

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

108 Over the second, third and fourth visits the participants completed three exercise capacity
109 tests (one at each altitude of 0m, 2150m and 4300m) in order to calculate workloads relative to each
110 altitude for the main experimental trials. These preliminary visits were separated by ≥ 48 h and
111 conducted in a single-blind randomised fashion using a Latin Square design. Over the fifth, sixth and
112 seventh visits the participants completed three 305 minute experimental trials (one at each altitude
113 of 0m, 2150m, and 4300m). These visits were separated by ≥ 7 days and were randomised
114 independently from the maximal exercise tests, also using a single-blind Latin Square design. On the
115 morning of each testing day the following equation was used to calculate and set target FiO_2 : $FiO_2 =$
116 PiO_2 divided by $(P_B - 47)$; where P_B is barometric pressure in mmHg and 47mmHg is the vapour

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

119 Exercise Capacity Tests

120 Participants completed an exercise capacity test on a treadmill (Woodway PPS 55; Waukesha, WI)
121 which included both a submaximal and maximal phase. The incremental submaximal phase consisted
122 of four, 4 minute stages in which the participant walked carrying a 10 kg backpack at a 10% gradient.
123 This exercise modality was chosen to mimic the demands of high altitude activities. The speed of the
124 treadmill was increased by $1 \text{ km}\cdot\text{h}^{-1}$ each stage and the starting speeds were $3 \text{ km}\cdot\text{h}^{-1}$, $2 \text{ km}\cdot\text{h}^{-1}$ and 1
125 $\text{km}\cdot\text{h}^{-1}$ for 0m, 2150m and 4300m, respectively. Lower starting speeds were employed in hypoxia
126 based on the knowledge of a reduced aerobic capacity at altitude and the need for all participants to
127 elicit 50% of $\dot{V}O_{2\text{max}}$ within the 16 minute test. On completion of the submaximal phase participants
128 were allowed 5 minutes of recovery before commencing the maximal phase. Prior to this phase the
129 participants removed the backpack and the treadmill was set at 1% gradient (30). The participants
130 then ran at a constant speed, which was dependent upon fitness and altitude, aiming for a rating of
131 perceived exertion (RPE) of 12. The gradient of the treadmill was then increased by 1% per minute
132 until volitional exhaustion. All subjects were deemed to reach $\dot{V}O_{2\text{max}}$ as they all expressed >2 of the
133 following criteria: a plateau in $\dot{V}O_2$ in the final exercise stage, respiratory exchange ratio ≥ 1.15 , heart
134 rate within $10 \text{ b}\cdot\text{min}^{-1}$ of age predicted maximum ($220 - \text{age}$), rating of perceived exertion ≥ 19 and/or
135 blood lactate $\geq 8 \text{ mM}$ (27). Expired gas was collected using an online gas analyser (Metalyzer 3B R3;
136 Leipzig, Germany) throughout both phases of this test to allow regression analysis between oxygen
137 consumption and walking speed. This allowed for the calculation of a speed that would elicit 50% of
138 relative $\dot{V}O_{2\text{max}}$ whilst walking on a treadmill and carrying a 10 kg backpack at 10% gradient.

139 Experimental trials

140 Participants recorded their food intake for the 24h prior to the first experimental trial; the quantity
141 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 (SpO₂) 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 -

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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

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 = $([\text{hunger} + \text{prospective food consumption} + (100 -$
169 $\text{fullness}) + (100 - \text{satisfaction})] / 4)$ (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 50 μ L of a solution containing

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

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

203 Blood analyses

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

212 Ad-libitum meal

213 A cold *ad-libitum* buffet meal was administered at 4h 45 minutes in which the participants were given
214 20 minute access for food consumption. The meal was identically presented between trials and

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

223 Statistical analysis

224 Data are expressed as mean \pm standard deviation (SD) in text and tables and mean \pm standard error
225 (SE) in figures. All data were analysed using IBM SPSS statistics (v22.0 for Windows; SPSS, Chicago, IL).
226 The trapezoid method was used to calculate AUC for appetite perceptions and hormone
227 concentrations. The four defined AUC periods were: pre-prandial (the 1h before breakfast), post-
228 prandial (the 1h after breakfast, exercise (the 1h exercise period) and post-exercise (the 90 minutes
229 post-exercise). Repeated measures ANOVA was used to assess trial-based differences in appetite
230 perceptions, AMS scores, heart rate, SpO₂, hormone concentrations and energy intake. Where
231 significant main effects of trial were found, post-hoc analysis was performed using Holm-Bonferroni
232 correction for multiple comparisons. Effect sizes are presented as Cohen's *d* and interpreted as ≤ 0.2
233 trivial, > 0.2 small, > 0.6 moderate, > 1.2 large, > 2 very large and > 4 extremely large (23). The Pearson
234 product moment correlation coefficient was used to investigate relationships between SpO₂, gut
235 hormone concentrations, appetite perceptions and energy intakes. When plasma volume shifts were
236 accounted for, interpretation of all blood analyte results was unaltered and thus the original data is
237 presented. The sample size used within this study was deemed sufficient to detect a significant
238 difference in energy intake between conditions. The anticipated effect size for a difference in energy
239 intake was based on a similar previous study (55). Based on the effect size and an alpha value of 5%,

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

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260 **Results**

261 Exercise responses.

262 Maximal oxygen uptake was significantly reduced at 2150m ($48.2 \pm 6.5 \text{ mL}\cdot\text{kg}\cdot\text{min}^{-1}$; $P < 0.001$; $d =$
263 1.04) and 4300m ($37.7 \pm 4.9 \text{ mL}\cdot\text{kg}\cdot\text{min}^{-1}$; $P < 0.001$; $d = 2.83$) compared with sea-level (55.6 ± 7.5
264 $\text{mL}\cdot\text{kg}\cdot\text{min}^{-1}$). This elicited walking speeds of $4.4 \pm 0.4 \text{ km}\cdot\text{h}^{-1}$ ($46.4 \pm 4.0\% \dot{V}O_{2\text{max}}$), $3.6 \pm 0.4 \text{ km}\cdot\text{h}^{-1}$ (47.1
265 $\pm 4.7\% \dot{V}O_{2\text{max}}$) and $2.5 \pm 0.4 \text{ km}\cdot\text{h}^{-1}$ ($47.8 \pm 4.3\% \dot{V}O_{2\text{max}}$) for the sea-level, 2150m and 4300m
266 conditions, respectively. Mean RPE values were not different between sea-level (12.1 ± 1.5) and
267 2150m (12.0 ± 1.7 ; $P = 0.437$; $d = 0.08$), however were significantly higher at 4300m (14.0 ± 2.9) than
268 at sea-level ($P < 0.001$; $d = 0.82$) and 2150m ($P < 0.001$; $d = 0.85$).

269

270 Appetite perceptions

271 At baseline, during the pre-prandial period and during the post-prandial period there were no
272 significant differences in any appetite perceptions between conditions (all $P > 0.066$; $d < 0.4$). One-
273 way ANOVA revealed a significant difference between conditions for composite appetite score during
274 the exercise ($P = 0.03$) and the post-exercise ($P < 0.001$) periods. Post-hoc analysis revealed that,
275 during exercise, AUC for CAS was significantly lower at 4300m ($33 \pm 17 \text{ mm}\cdot\text{h}^{-1}$) compared with 2150m
276 ($44 \pm 19 \text{ mm}\cdot\text{h}^{-1}$; $P = 0.024$; $d = 0.65$) and tended to be lower at 4300m compared with sea-level ($42 \pm$
277 $14 \text{ mm}\cdot\text{h}^{-1}$; $P = 0.10$; $d = 0.61$). In the post-exercise period, AUC for CAS was significantly lower at
278 4300m ($40 \pm 19 \text{ mm}\cdot\text{h}^{-1}$) compared with sea-level ($55 \pm 15 \text{ mm}\cdot\text{h}^{-1}$; $P = 0.004$; $d = 0.90$) and 2150m (60
279 $\pm 14 \text{ mm}\cdot\text{h}^{-1}$; $P < 0.001$; $d = 1.23$) (figure 2).

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281

- INSERT FIGURE 2 NEAR HERE -

282

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

292 Similarly to acylated ghrelin, AUC PP values were significantly lower during exercise at 4300m
293 ($315 \pm 201 \text{ pg}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$) compared with sea-level ($473 \pm 271 \text{ pg}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$; $P = 0.002$; $d = 0.66$) and 2150m
294 ($446 \pm 280 \text{ pg}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$; $P = 0.002$; $d = 0.54$). During the post exercise period AUC for PP was significantly
295 lower at 4300m ($242 \pm 160 \text{ pg}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$) compared with sea-level ($366 \pm 225 \text{ pg}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$; $P = 0.001$; $d =$
296 0.64) and 2150m ($318 \pm 203 \text{ pg}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$; $P = 0.002$; $d = 0.41$) (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 ($16.2 \pm 6.1 \text{ }\mu\text{U}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$) than at sea-level ($10.4 \pm 5.4 \text{ }\mu\text{U}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$; $P = 0.02$; $d = 0.99$) and
305 2150m ($10.7 \pm 5.3 \text{ }\mu\text{U}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$; $P = 0.045$; $d = 0.96$) (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 ($1.39 \pm 0.18 \text{ mmol}\cdot\text{L}\cdot\text{h}^{-1}$) compared with sea-level ($0.90 \pm 0.25 \text{ mmol}\cdot\text{L}\cdot\text{h}^{-1}$; $P < 0.001$; $d = 2.21$) and
313 2150m ($1.03 \pm 0.21 \text{ mmol}\cdot\text{L}\cdot\text{h}^{-1}$; $P < 0.001$; $d = 1.82$), with a trend for higher lactate concentrations at
314 2150m in comparison to sea-level ($P = 0.07$; $d = 0.53$) (figure 4c).

315

316 - INSERT FIGURE 4 NEAR HERE -

317

318 Energy intake

319 Mean energy intake at the *ad-libitum* meal was significantly lower at 4300m ($3728 \pm 3179 \text{ kJ}$)
320 compared with sea-level ($7358 \pm 1789 \text{ kJ}$; $P = 0.007$; $d = 1.41$) and 2150m ($7390 \pm 1226 \text{ kJ}$; $P = 0.004$;
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 SpO₂ 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).

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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 SpO₂ 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 SpO₂, CAS or energy intake (all r ≤ 0.157; all P ≥ 0.359).

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377 **Discussion**

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

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

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

402 found that acylated ghrelin concentrations were reduced following exercise during 7h exposure to
403 12.7% O₂ (3) and not reduced during 7h resting exposure to 15% O₂ (39). Conversely, one study has
404 found acylated ghrelin inhibition at a moderate altitude (14.5% O₂), however the duration of hypoxic
405 exposure was only 50 minutes (2). It is plausible that the exercise bout in the study of Bailey et al. (2)
406 contributed to the inhibition of acylated ghrelin and appetite. This is further supported by Wasse et
407 al. (55) who found that appetite, energy intake and acylated ghrelin concentrations were lower during
408 an exercise trial in hypoxia compared with hypoxia without exercise. The dose of both hypoxia and
409 exercise appear to substantially influence appetite responses, with higher altitude exerting larger
410 inhibition. However, based on the findings of the present study this dose-response relationship does
411 not appear to be linear. Awareness of appetite inhibition and the need for nutritional strategies
412 appears crucial for those exercising at severe but not moderate altitudes.

413 It must be noted that in the present study participants were exposed to hypoxia for just 5h, and
414 such short exposures are rare in real-life scenarios. There is potential that hypoxia may influence
415 appetite differently during longer-term exposures, likely due to some acclimatising effects. Following
416 prolonged periods (≥10 days) of normobaric hypoxic exposure (~13.9% O₂) three studies have found
417 no reductions in appetite perceptions or total ghrelin concentrations compared with sea level (11, 12,
418 37). Chronic investigations at terrestrial altitude, which have found a reduction in appetite (4, 56),
419 have employed altitudes >5000m. It seems plausible that altitudes >5000m may be required to inhibit
420 appetite with chronic hypoxic exposure and that acute exposure produce a greater magnitude of
421 appetite inhibition than chronic exposures at lower altitudes due to a lack of acclimatisation.

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

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

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

445 The notion that altitude exposure may induce an increase in carbohydrate oxidation (8, 31, 44)
446 compared with sea-level is supported by the current findings in the pre-prandial state. On the
447 contrary, during the exercise and post-exercise periods, relative carbohydrate oxidation was
448 significantly lower at 4300m compared with sea-level. In addition absolute and relative fat oxidation
449 was significantly higher at 2150m compared to sea-level in the post-prandial period. These findings
450 contradict the 'oxygen-efficiency theory' and support the perspective that the body needs to meet a
451 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 SpO₂ 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 (PPAR α) can be upregulated which
460 may deactivate PDH thus promoting anaerobic glycolysis (28). This PPAR α 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 $\sim 11.7\%$) inhibits appetite, acylated ghrelin concentration and energy intake in comparison with sea-
483 level, but exercise during exposure to simulated moderate altitude (2150m, $\text{FiO}_2 \sim 15.8\%$) 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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505

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756 **Figure legends**

757 **Figure 1.** Experimental trial schematic.

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

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

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

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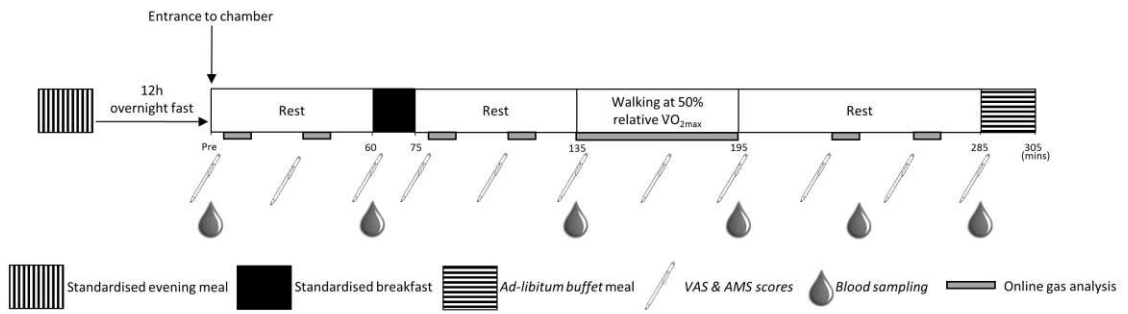
769 **Table legends**

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

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

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

779 **Figure 1**



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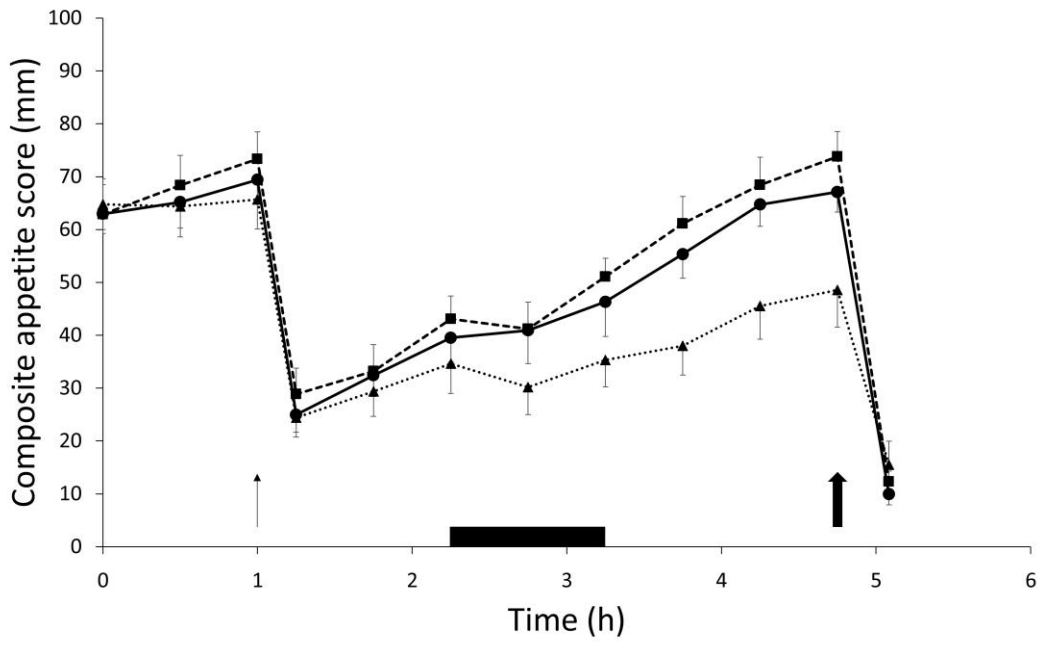
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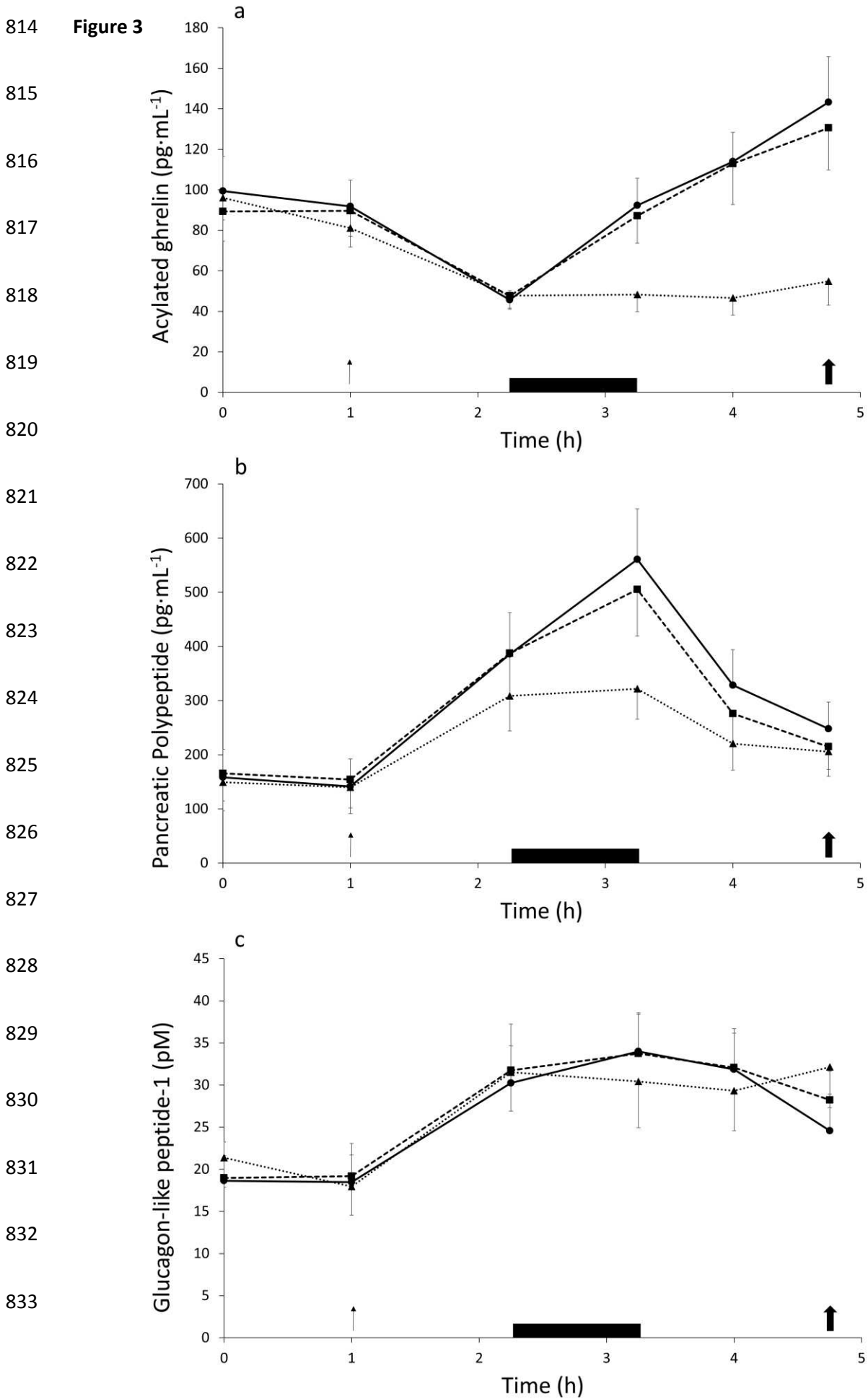
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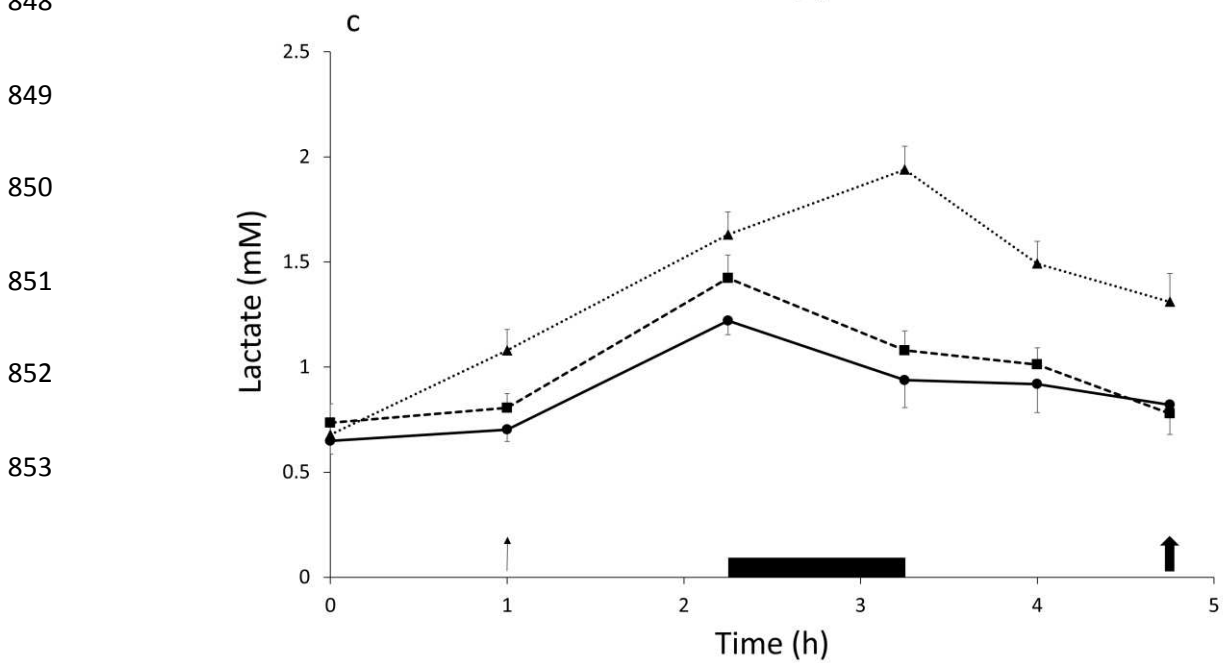
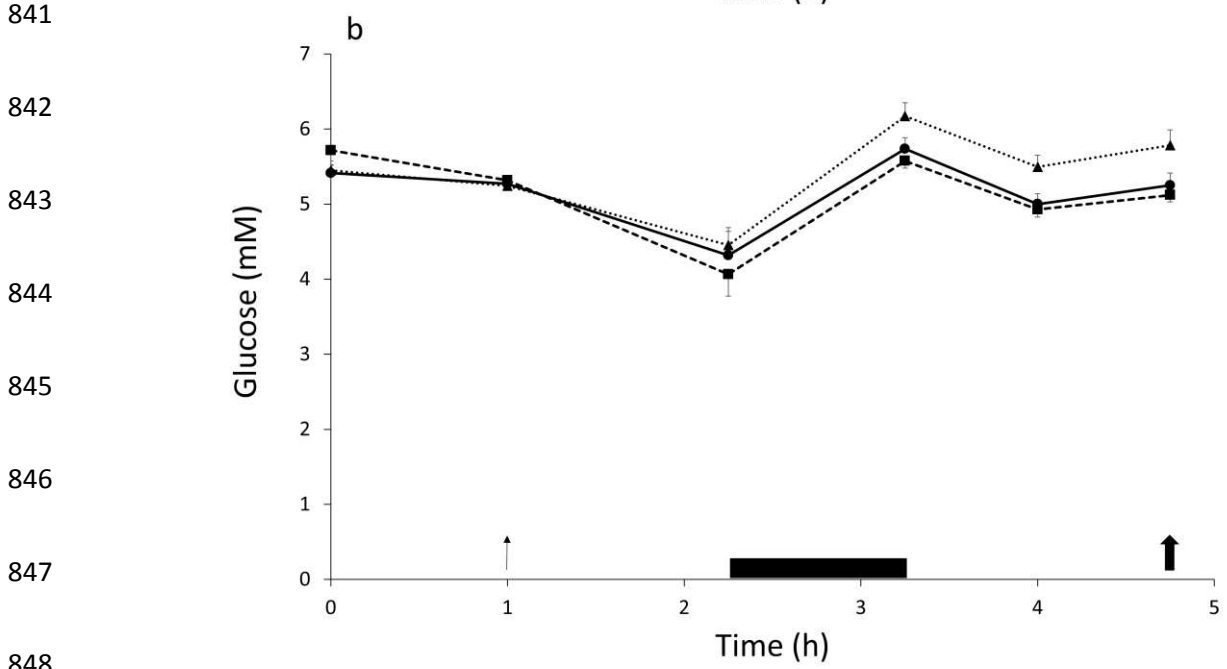
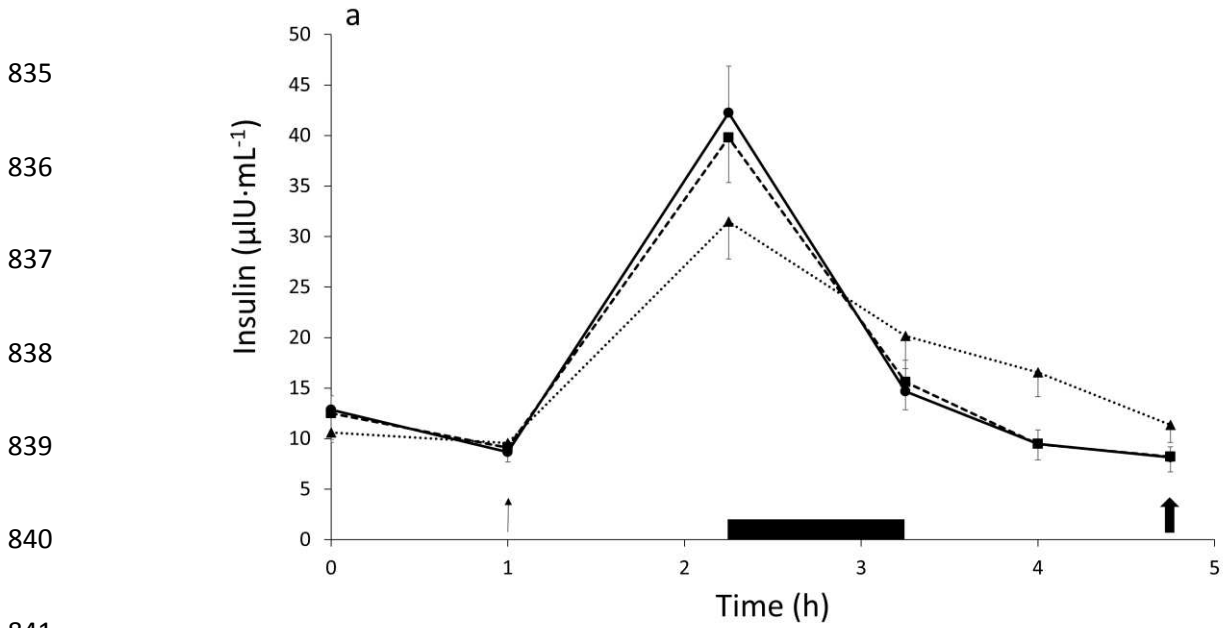
794 **Figure 2**



814 **Figure 3**



834 **Figure 4**



854 **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)

855 Values are mean ± SD, N = 12. * Significant difference between sea-level and 2150m.
 856 † Significant difference between sea-level and 4300m. # Significant difference between
 857 2150m and 4300m (One way ANOVA; P < 0.05 after Holm-Bonferroni adjustment).

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864 **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 (42.1 ± 14.0)	0.10 ± 0.04 (57.9 ± 14.0)	0.28 ± 0.07 (59.1 ± 15.2)	0.09 ± 0.04 (40.9 ± 15.2)	1.56 ± 0.35 (62.8 ± 13.3)	0.41 ± 0.18 (37.2 ± 13.3)	0.21 ± 0.08 (46.4 ± 16.2)	0.11 ± 0.04 (53.6 ± 16.2)
2,150m	0.18 ± 0.06* (42.1 ± 18.9)	0.11 ± 0.05 (57.9 ± 18.9)	0.28 ± 0.09 (57.2 ± 17.6)	0.09 ± 0.04 (42.8 ± 17.6)	1.18 ± 0.34* (55.1 ± 18.9)	0.44 ± 0.21 (44.9 ± 18.9)	0.16 ± 0.08 (36.2 ± 18.6*)	0.13 ± 0.04* (63.8 ± 18.6*)
4,300m	0.29 ± 0.14†# (56.8 ± 23.4†#)	0.09 ± 0.05 (43.2 ± 23.4†#)	0.30 ± 0.14 (52.4 ± 22.7)	0.12 ± 0.05† (47.6 ± 22.7)	0.87 ± 0.37†# (50.8 ± 19.8†)	0.38 ± 0.19 (49.2 ± 19.8†)	0.19 ± 0.16 (34.8 ± 22.6†)	0.16 ± 0.06†# (65.2 ± 22.6†)

865 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
 866 difference between 2150m and 4300m (One way ANOVA; P < 0.05 after Holm-Bonferroni adjustment).

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