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1 **Title:** A high fat breakfast attenuates the suppression of appetite and acylated ghrelin during exercise at
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7 **Running heading:** Changes in appetite after a high fat and a high carbohydrate breakfast at simulated altitude

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15

16 **Abstract**

17 High-altitude exposure induces a negative energy balance by increasing resting energy expenditure and decreasing
18 energy intake. This diminished energy intake is likely caused by altitude-induced anorexia and can have
19 detrimental effects for those travelling to high-altitude. We aimed to investigate whether altering the
20 macronutrient composition of breakfast could attenuate altitude-induced anorexia and augment energy intake at
21 high-altitude. Twelve healthy men (aged 26 (8) years, body mass index 23.9 (2.7) kg·m⁻²) completed two, 305
22 minute experimental trials at 4300m simulated altitude (~11.7% O₂). After an overnight fast, participants entered
23 a normobaric hypoxic chamber and rested for one hour, before receiving either a high fat (HF; 60% fat, 25%
24 carbohydrate) or an isocaloric high carbohydrate (HC; 60% carbohydrate, 25% fat) breakfast. One hour after
25 breakfast, participants performed 60 minutes of treadmill walking at 50% of relative $\dot{V}O_{2max}$. An *ad-libitum* buffet
26 meal was consumed 1h 30 minutes after exercise. Appetite perceptions, blood samples and substrate oxidation
27 rates were measured throughout. A significantly higher area under the curve for composite appetite score was
28 observed during exercise in HF (40 (12) mm·h⁻¹) compared with HC (30 (17) mm·h⁻¹, P=0.036). During exercise,
29 lower insulin concentrations (P=0.013) and elevated acylated ghrelin concentrations (P=0.048) were observed in
30 HF compared with HC. After exercise there was no significant difference in composite appetite score (P=0.356),
31 acylated ghrelin (P=0.229) or insulin (P=0.513) between conditions. Energy intake at the buffet did not
32 significantly differ between conditions (P=0.384). A HF breakfast attenuated appetite suppression during exercise
33 at 4300m simulated altitude, however *ad-libitum* energy intake did not increase.

34 **Key words:** Hypoxia; Medium-chain fatty acids; Energy balance; Altitude-induced anorexia

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49 **1 Introduction**

50 Appetite suppression has previously been observed during acute exposure to both simulated [1-3] and terrestrial
51 altitude [4]. This effect appears to be maintained during chronic altitude exposures [5, 6] which is associated with
52 significant decreases in energy intake [7, 8], body mass [7-9] and physical performance at altitude [9, 10].
53 Additionally, resting energy expenditure is suggested to be elevated at altitude [1, 11], which may further stimulate
54 a negative energy balance. Maintaining energy balance is therefore vital for individuals ascending to altitude to
55 maintain body mass and physical capabilities.

56 Previous research at sea level has identified that acute dietary interventions can alter postprandial gut
57 hormone responses [12, 13] and subsequent energy intake [14, 15]. It is well established that protein is the most
58 satiating macronutrient [16, 17]. Contrastingly, high fat meals have been found to produce the smallest magnitude
59 of postprandial acylated ghrelin suppression and the highest appetite scores, compared with other macronutrients
60 [12, 13, 18, 19]. Ghrelin is a 28 amino acid peptide which is post-translationally modified at its serine 3 residue
61 with medium-chain fatty acids (MCFAs), catalysed by the enzyme Ghrelin-O-Acyl-Transferase (GOAT) [20, 21].
62 This acylation of ghrelin is necessary for it to bind with the growth hormone secretagogue receptor-1a (GHS-R1a)
63 and exert its orexigenic effects [22]. Furthermore, des-acylated ghrelin has been found to inhibit the orexigenic
64 effects of acylated ghrelin, independently of the GHS-R1a [23]. A growing body of evidence suggests that
65 ingested MCFAs are directly utilised in the acylation of ghrelin, increasing circulating concentrations of acylated
66 ghrelin [24, 25]. This effect can increase appetite and has been found to promote a positive energy balance,
67 preventing weight loss in cachectic patients [26]. In addition, compared with other macronutrients, high fat meals
68 may result in a decreased energy expenditure due to their lower thermic effect [27].

69 Several circulating hormones have been implicated in the development of altitude-induced anorexia,
70 including glucagon-like peptide-1, peptide YY and pancreatic polypeptide. However, recent studies have
71 identified acylated ghrelin as the strongest mediator of this response based on concomitant decreases of appetite
72 and circulating acylated ghrelin concentrations at altitude [1, 2, 28]. It seems plausible that the ingestion of a high
73 fat meal rich in MCFAs may increase circulating acylated ghrelin concentrations, elevate subjective appetite
74 ratings, augment energy intake and decrease energy expenditure. A combination of these factors over a prolonged
75 period would be beneficial in a high altitude environment by helping to maintain energy balance and body mass.

76 The purpose of this study was to compare the effects of a high fat breakfast rich in MCFAs and a high
77 carbohydrate breakfast on appetite, gut hormones, energy intake and substrate oxidation. This study represents
78 the first investigation of an intervention attempting to attenuate reductions in appetite at altitude.

79

80

81 **2 Methods**

82 2.1 Participants

83 Twelve healthy men (age 26 (8) years, body mass index 23.9 (2.7) kg·m⁻², body mass 77 (8.1) kg) volunteered to
84 participate in this study. Informed consent was obtained from all individual participants included in the study. All
85 participants were non-smokers, non-diabetic, normotensive, free from food allergies and were not taking any
86 regular medication. None of the participants had travelled to an altitude >1500m for the previous three months
87 and were currently residing at an altitude of <500m. All study protocols received institutional ethics approval and
88 were performed in accordance with the Declaration of Helsinki.

89

90 2.2 Experimental Design

91 Participants attended the laboratory on four separate occasions. The first visit included screening, anthropometry,
92 a food preferences assessment and a test for sickle cell trait. Sickle cell trait was an exclusion criteria due to
93 complications that may occur in hypoxia, for example splenic infarction [29]. During the second visit participants
94 completed an incremental exercise test at 4300m simulated altitude, to determine a relative treadmill walking
95 speed for subsequent experimental trials, as previously described [1]. In a randomised and counter-balanced
96 fashion, two experimental trials were conducted ≥ 48 h following the incremental exercise test and were separated
97 by ≥ 7 days. Participants were not informed by the researcher which breakfast they were provided with before or
98 during each trial. During the experimental trials participants consumed either a high fat (HF) or a high
99 carbohydrate (HC) breakfast. Target FiO₂ was adjusted on the morning of each testing day using the following
100 equation: $FiO_2 = PiO_2 / (PB - 47)$; where PB is barometric pressure in mmHg and 47 mmHg is the vapour pressure
101 of water at 37 °C [30, 31]. Simulated PiO₂ was 83 mmHg (FiO₂ ~11.7%). Temperature and humidity were
102 maintained at 20 °C and 50% for all tests, respectively.

103

104 2.3 Experimental trials

105 Participants recorded their dietary intake on the day prior to their first experimental trial and repeated the quantity
106 and timing of this intake before their second trial. On the day preceding each experimental trial participants also
107 refrained from alcohol, caffeine and strenuous exercise, and consumed a standardised evening meal (4338 kJ,
108 57% carbohydrate, 28% fat, 15% protein) between 7pm and 8pm. This meal contained: fusilli pasta, pasta sauce,
109 cheddar cheese, milk, and jelly beans and was consumed to minimise the possibility of a 'second meal effect'
110 confounding the outcome measures [32, 33]. Compliance to pre-experimental controls was verbally confirmed
111 with each participant on the morning of each trial. On the day of testing, participants arrived at the laboratory and
112 entered the hypoxic chamber at 8am. At 1h participants were given 15 minutes to consume either a HF (60% fat,
113 25% carbohydrate, 15% protein) or a HC (60% carbohydrate, 25% fat, 15% protein) breakfast (Table 1). Both
114 breakfasts consisted of cooked porridge served in an oversized bowl with a separate drink of 216mL. Participants
115 remained rested (e.g. reading or watching DVDs; material strongly related to the aims of the study was not
116 permitted) throughout both trials with the exclusion of the exercise period. At 2h 15 minutes a 1h exercise protocol
117 was completed in which participants walked on a treadmill at 50% of relative $\dot{V}O_{2max}$ at a 10% gradient whilst

118 carrying a 10kg backpack, to mimic the demands of high altitude trekking [34]. Participants then remained rested
119 until the end of the trial at 5h 05 minutes. Rating of perceived exertion (RPE) was measured at 15 minute intervals
120 during exercise [35]. Acute mountain sickness (AMS) was measured every 30 minutes via the Lake Louise Score
121 (LLS) [36] and used to diagnose mild AMS (LLS ≥ 3 in the presence of a headache) and severe AMS (LLS ≥ 6 in
122 the presence of a headache).

123

124 2.4 Appetite and palatability perceptions

125 Subjective appetite perceptions were measured at baseline and every 30 minutes throughout each experimental
126 trial, with the exclusion of the 15 minute interval for breakfast consumption. During exercise, subjective appetite
127 perceptions were measured whilst maintaining walking on the treadmill. Validated visual analogue scales (VAS)
128 were used to assess appetite and palatability perceptions [37]. A composite appetite score (CAS) was calculated
129 after inverting the values for fullness and satisfaction [38]. A higher CAS is associated with greater appetite
130 sensations and thus a stronger motivation to eat.

131

132 2.5 Ad-libitum meal

133 At 4h 45 minutes participants were given 20 minute access to a cold *ad-libitum* meal that was presented identically
134 between trials, and consisted of: three types of cereal, semi-skimmed milk, orange juice, white bread, brown bread,
135 cheese, ham, tuna, bananas, apples, oranges, crisps, butter, margarine, mayonnaise, cereal bars, chocolate bars,
136 cookies, muffins and chocolate rolls. All foods were provided in excess of expected consumption and participants
137 were informed to 'eat until comfortably full'. Participants were also made aware that additional quantities of each
138 food item were available if desired. Meals were consumed in isolation, inside the hypoxic chamber and behind a
139 privacy screen to minimise any effects of social influence on food intake. Energy intake was calculated by
140 weighing foods before and after consumption (to the nearest 0.1g), and with reference to the manufacturers
141 nutritional information. This *ad-libitum* meal allowed for macronutrient preferences to be assessed.

142

143 2.6 Online gas analysis

144 Expired gas analysis was conducted throughout trials using an online gas analyser (Metalyser® 3B, Cortex,
145 Leipzig, Germany), as previously described [1]. Substrate oxidation was estimated using equations for rest [39]
146 and exercise [40]. Substrate oxidation rates were then used to estimate energy expenditure at rest and during
147 exercise.

148

149 2.7 Blood sampling

150 All venous blood samples were obtained from an antecubital vein using a 20-gauge cannula (Introcan Safety; B
151 Braun, Sheffield, UK). The first blood sample was collected >10 minutes after the cannulation procedure to
152 minimise any effect of vagus nerve stimulation on measured blood analytes [41]. Further samples were drawn at

153 1h, 2h 15 minutes, 3h 15 minutes, 4h and 4h 45 minutes. From each venous sample a microcuvette and a
154 heparinised micro haematocrit tube were used to collect 10 μ L and \sim 45 μ L of whole blood, respectively, for the
155 measurement of haemoglobin and haematocrit concentrations. These data were used to estimate plasma volume
156 changes over time [42]. To minimise the effect of any postural changes in plasma volume all blood samples were
157 collected whilst the participant was seated [43]. At each time point samples were collected into one 4.9 mL and
158 one 9 mL pre-cooled EDTA monovette (Sarstedt, Leicester, UK). The 9 mL tube was used for the determination
159 of plasma concentrations of insulin, glucose, lactate, non-esterified fatty acids (NEFA) and triglycerides. The 4.9
160 mL tube was used for the determination of plasma concentrations of acylated and des-acylated ghrelin. To prevent
161 the degradation of acylated ghrelin, 4.9 mL tubes were pre-treated on the morning of each experimental trial, as
162 previously described [1, 44]. Immediately after filling, both tubes were spun at 1500 x *g* for 10 minutes in a
163 centrifuge (CompactStar CS4, VWR). Plasma from the 9 mL tube was transferred into cryovials and 1 mL of the
164 plasma from the 4.9 mL tube was mixed with 100 μ L of 1M hydrochloric acid. This solution was then spun at
165 1500 x *g* for five minutes before the supernatant was then transferred into cryovials. All cryovials were then
166 immediately frozen at -20 °C before being transferred to -80°C and stored until analysis.

167

168 2.8 Blood Analysis

169 Commercially available enzyme-linked immunosorbent assays were used to determine plasma concentrations of
170 acylated ghrelin (SPI BIO, Montigny Le Bretonneux, France), des-acylated ghrelin (SPI BIO, Montigny Le
171 Bretonneux, France) and insulin (IBL, Hamburg, Germany). To eliminate inter-assay variation, all samples from
172 each participant were assayed on the same plate. Photometric analysis was utilised to measure glucose, lactate,
173 NEFA and triglycerides using reagents from Instrumentation Laboratory (Lexington, MA), Randox Laboratories
174 (Crumlin, UK), Wako Chemicals (Dusseldorf, Germany) and Instrumentation Laboratory (Lexington, MA),
175 respectively. The within batch coefficients of variation were as follows: acylated ghrelin 2.4%, des-acylated
176 ghrelin 4.1%, insulin 5.7%, glucose 3.2%, lactate, 2.8%, NEFA 2.8% and triglycerides 3.7%. Total ghrelin was
177 computed via the addition of acylated and des-acylated ghrelin concentrations.

178

179 2.9 Statistical analysis

180 Data are expressed as mean (SD) in text and tables and mean (SE) in figures to avoid distortion of the graphs. All
181 data were analysed using IBM SPSS statistics (ν 22.0 for Windows; SPSS, Chicago, IL). Area under the curve
182 (AUC) was calculated using the trapezoid method for appetite perceptions and blood parameters. The four AUC
183 periods were defined as: pre-prandial (the 1h before breakfast), postprandial (the 1h after breakfast), exercise (the
184 1h exercise period), and post-exercise (the 1hr 30 minutes post-exercise). Paired t-tests were used to assess
185 differences in RPE, palatability ratings and energy intake. Two-way repeated measures analysis of variance
186 (ANOVA) was used to assess condition, time, and condition*time based differences in AUC values for appetite
187 perceptions, LLS, blood analyte concentrations, substrate oxidation and energy expenditure. Where significant
188 effects were found, post-hoc analysis was performed using paired *t* tests. . Analysis of covariance (ANCOVA)
189 was performed on appetite perceptions, blood analyte concentrations and energy intake using LLS as a covariate.
190 The interpretation of the findings was unchanged when accounting for LLS as a covariate, and thus the original

191 data are presented. Effect sizes are presented as Cohen's d and interpreted as ≤ 0.2 trivial, >0.2 small, >0.6
192 moderate, >1.2 large, >2 very large and >4 extremely large [45]. Interpretation of all blood analytes was
193 unchanged when plasma volume changes were accounted for, thus the original data is presented. The sample size
194 used was deemed sufficient to detect significant differences in CAS, acylated ghrelin and energy intake between
195 conditions. Based on effect sizes calculated from previous work in our laboratory[1], and an alpha value of 5%, a
196 sample size of 12 participants would generate a power $>95\%$ for these three variables. Calculations were
197 performed using G*power [46].

198 **3 Results**

199 3.1 Exercise responses and acute mountain sickness

200 Maximal oxygen uptake at 4300m was 39.2 (5.1) mL·kg·min⁻¹ and walking speed was 2.8 (0.6) km·h⁻¹ during the
201 experimental trials. There was no difference in RPE between the HF (12 (2)) and the HC (12 (2), $P = 0.467$, $d =$
202 0.08) conditions during exercise. Mild AMS manifested in four and six participants in the HF and HC conditions,
203 respectively. Severe AMS occurred in one and two participants in the HF and HC conditions, respectively. Two-
204 way repeated measures ANOVA revealed a significant effect of time ($P = 0.009$) on LLS, however no effect of
205 condition ($P = 0.313$) or condition*time ($P = 0.318$) was observed. Mean LLS across the entire trial was 1 (2)
206 during the HF and 1 (1) during in HC condition.

207 3.2 Appetite and palatability perceptions

208 Two-way repeated measures ANOVA revealed a significant effect of time ($P < 0.001$) and condition*time ($P =$
209 0.026), but not condition ($P = 0.223$) on CAS. Post-hoc analysis revealed at baseline and during the pre-prandial
210 period there were no significant differences in CAS between the HF and HC conditions (all $P \geq 0.218$, all $d \leq$
211 0.31). During exercise AUC for CAS was significantly higher in HF (40 (12) mm·h⁻¹) compared with HC (30 (17)
212 mm·h⁻¹, $P = 0.036$, $d = 0.63$). During the post-exercise period there was no significant difference in AUC for CAS
213 between conditions ($P = 0.356$, $d = 0.26$) (Figure 1). Two-way ANOVA revealed no significant effects of
214 condition or condition*time for hunger ($P \geq 0.163$), satisfaction ($P \geq 0.288$) or fullness ($P \geq 0.102$). A significant
215 effect of condition*time was observed for prospective food consumption ($P = 0.001$). Post-hoc analysis revealed
216 significantly higher prospective food consumption in HF, compared with HC, during the post-prandial ($P = 0.019$,
217 $d = 0.92$) and exercise ($P = 0.016$, $d = 0.88$) periods. There was no difference observed for appeal ($P = 0.319$, $d =$
218 0.29), smell ($P = 0.507$, $d = 0.19$), taste ($P = 0.843$, $d = 0.06$), aftertaste ($P = 0.208$, $d = 0.33$) and palatability (P
219 = 0.768, $d = 0.09$) of the breakfast between conditions (Supplementary Table 1).

220

221 3.3 Energy intake

222 Mean energy intake at the *ad-libitum* meal was not different after the HF breakfast (5589 (2076) kJ) compared
223 with the HC breakfast (6086 (2235) kJ, $P = 0.384$, $d = 0.23$). In addition, there were no differences in the absolute
224 or relative consumption of carbohydrate (both $P \geq 0.731$, $d \leq 0.08$), fat (both $P \geq 0.348$, $d \leq 0.27$) or protein (both
225 $P \geq 0.260$, $d \leq 0.31$) (Supplementary Table 2).

226

227 3.4 Substrate oxidation and energy expenditure

228 Two-way repeated measures ANOVA revealed a significant effect of time, condition and condition*time for
229 relative (all $P \leq 0.038$) and absolute (all $P \leq 0.003$) carbohydrate oxidation. Post-hoc analysis revealed that during
230 the pre-prandial period, there were no significant differences in relative or absolute carbohydrate and fat oxidation
231 between conditions (all $P \geq 0.105$, all $d \leq 0.17$). During the postprandial period, exercise and the post-exercise
232 period both relative (all $P \leq 0.012$, $d \geq 0.75$) and absolute (all $P \leq 0.009$, all $d \geq 0.70$) carbohydrate oxidation were
233 significantly higher in HC, compared with HF. A significant effect of time ($P < 0.001$) and condition*time ($P =$

234 0.003) was observed for absolute fat oxidation, however no effect of condition was revealed ($P = 0.111$). Post-
235 hoc analysis revealed absolute fat oxidation was not significantly different between conditions in the postprandial
236 or the post-exercise period (both $P \geq 0.133$, $d \leq 0.26$). However, during exercise absolute fat oxidation was
237 significantly higher after the HF breakfast compared with the HC breakfast ($P = 0.014$, $d = 0.76$) (Table 2).

238 Two-way repeated measures ANOVA revealed a significant effect of time ($P < 0.001$) on energy
239 expenditure but not condition*time ($P = 0.617$). There was however a tendency towards an effect of condition (P
240 $= 0.060$) on energy expenditure, with lower values observed for total energy expenditure across the entire trial in
241 HF (3635 (561) kJ) compared with HC (3848 (491) kJ).

242

243 3.5 Blood parameters

244 There were no differences between trials for the concentrations of any blood analyte at baseline (all $P \geq 0.137$, all
245 $d \leq 0.18$).

246 There was a significant main effect of time ($P = 0.029$) and condition*time ($P = 0.002$), but not condition
247 ($P = 0.100$) on acylated ghrelin concentrations. Post-hoc analysis revealed that during the postprandial period
248 AUC for acylated ghrelin tended to be higher after the HF breakfast compared with the HC breakfast ($P = 0.069$,
249 $d = 0.15$) (Figure 2A). During exercise AUC for acylated ghrelin was significantly higher after the HF breakfast
250 (151.9 (180.2) $\text{pg}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$) compared with the HC breakfast (100.6 (106.1) $\text{pg}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$, $P = 0.048$, $d = 0.35$).
251 During the post-exercise period, AUC for acylated ghrelin was not significantly different between conditions (P
252 $= 0.153$, $d = 0.14$). There was no effect of time ($P = 0.857$), condition ($P = 0.219$) or condition*time ($P = 0.605$)
253 for des-acylated ghrelin concentrations (Figure 2B). Furthermore, there was a tendency for a main effect of
254 condition ($P = 0.052$), time ($P = 0.079$) and condition*time ($P = 0.089$) for total ghrelin concentrations (Figure
255 2C).

256 There was a main effect of time ($P = 0.005$) and condition*time ($P = 0.039$), but not condition ($P = 0.494$)
257 for glucose concentrations. Post-hoc analysis revealed that glucose concentrations tended to be higher and were
258 significantly higher during the postprandial ($P = 0.094$, $d = 0.25$) and exercise ($P = 0.033$, $d = 0.37$) periods in HC
259 compared with HF. There was no difference in glucose concentrations between conditions in the post-exercise
260 period ($P = 0.199$, $d = 0.32$) (Figure 3A). There was a main effect of time ($P < 0.001$) and condition*time ($P <$
261 0.001), and a tendency for condition ($P = 0.053$) for insulin concentrations. Post-hoc analysis revealed that during
262 the postprandial period AUC for insulin was significantly lower in HF (24.9 (16.1) $\mu\text{U}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$) compared with
263 HC (34.3 (14.2) $\mu\text{U}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$, $P = 0.003$, $d = 0.62$). During exercise AUC for insulin was also lower in HF (27.0
264 (15.9) $\mu\text{U}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$) compared with HC (39.5 (15.0) $\mu\text{U}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$, $P = 0.013$, $d = 0.81$). There was no difference
265 in AUC for insulin between conditions in the post-exercise period ($P = 0.513$, $d = 0.11$) (Figure 3B).

266 There was a main effect of time ($P < 0.001$), condition ($P = 0.002$) and condition*time ($P = 0.005$) for
267 lactate concentrations. Post-hoc analysis revealed that lactate concentrations were significantly lower in the
268 postprandial, exercise and post-exercise periods (all $P \leq 0.011$, all $d \geq 0.62$) in HF compared with HC (Figure 3C).
269 There was a main effect of condition*time ($P = 0.002$), a tendency for condition ($P = 0.086$) and no effect of time
270 ($P = 0.235$) on NEFA concentrations. Post-hoc analysis revealed that during the postprandial period there was no

271 difference between conditions for concentrations of NEFA ($P = 0.553$, $d = 0.20$). However during the exercise
272 and post-exercise periods, concentrations of NEFA were significantly higher in HF compared with HC (both $P \leq$
273 0.047 , $d \geq 0.97$) (Figure 4A). There was a main effect of time ($P < 0.001$) and condition*time ($P = 0.015$), and a
274 trend for an effect of condition ($P = 0.052$) on triglyceride concentrations. Post-hoc analysis revealed that during
275 the postprandial period there was a tendency for higher triglycerides concentrations in HF compared with HC (P
276 $= 0.078$, $d = 0.73$). During the exercise and post-exercise periods triglyceride concentrations were significantly
277 higher in HF compared with HC (both $P \leq 0.049$, $d \geq 0.81$) (Figure 4B).

278

279

280 4 Discussion

281 This study investigated the effects of a HF and a HC breakfast on changes in appetite perceptions, gut hormones,
282 energy intake and substrate oxidation during a 5h exposure to 4300m simulated altitude. The primary finding of
283 this investigation is that consumption of a HF breakfast, compared with a HC breakfast, resulted in significantly
284 higher CAS and acylated ghrelin concentrations during a subsequent exercise bout. However, this effect was
285 transient and thus did not alter *ad-libitum* energy intake 1hr 30 minutes after exercise. In addition, absolute and
286 relative carbohydrate oxidation was significantly lower in all periods after the HF breakfast compared with the
287 HC breakfast. This effect produced a trend for a lower total energy expenditure in HF compared with HC.

288 Although AUC for CAS was 31% higher and AUC for acylated ghrelin was 51% higher during exercise
289 in HF compared with HC, no differences were observed in subsequent energy intake. This was likely due to the
290 1hr 30 minute time period between exercise and *ad-libitum* feeding, in which appetite perceptions and acylated
291 ghrelin concentrations converged between conditions. It seems feasible that a difference in energy intake may
292 have been observed if *ad-libitum* feeding was administered immediately after exercise and future research is
293 warranted in this respect. This future research could hold ecological validity, as trekking at terrestrial altitude is
294 often followed immediately by a meal, e.g. a stop for lunch. Furthermore, the increased appetite in the present
295 study during exercise in HF may have increased *ad-libitum* feeding during exercise, had foods been made
296 available. This could increase energy intake at terrestrial altitude as snacks are usually available during trekking.
297 The appetite responses in the present study corroborate the findings of similar investigations at sea level.
298 Monteleone, Bencivenga [47] observed that a 77% carbohydrate meal suppressed hunger to a significantly greater
299 extent than a 75% fat meal. Furthermore, previous data has suggested that a high carbohydrate meal induces a
300 greater decrease in postprandial ghrelin concentrations than an isocaloric high fat meal [12, 18, 47]. Previous
301 research shows that this larger postprandial decrease in appetite and plasma ghrelin concentrations, begins to
302 manifest approximately 60 minutes after food ingestion [12, 18, 47], which coincided with the start of exercise in
303 the present study. Therefore, it is possible that the observed differences in appetite and plasma acylated ghrelin
304 concentrations during exercise may be attributable to nutrient transit through the gastrointestinal tract over time,
305 rather than being induced by exercise.

306 The findings of the present study suggest a possible role of insulin in postprandial appetite suppression
307 at altitude. Insulin has been shown to suppress appetite and food intake via signalling in the hypothalamus [48]
308 and it seems feasible that the larger insulin response following the HC breakfast in the present study may have
309 contributed to the significantly lower appetite perceptions compared with HF. In addition, the higher postprandial
310 insulin concentration in the HC condition could have influenced appetite indirectly by contributing to the larger
311 suppression of acylated ghrelin, compared with the HF condition [49-51]. Without dietary intervention, acylated
312 ghrelin appears to be more strongly associated with altitude-induced anorexia than circulating insulin
313 concentrations [1]. Subsequently it seems reasonable to suggest that changes in CAS in the present study may
314 have been mediated predominantly by the acylated ghrelin responses, as opposed to changes in insulin
315 concentrations. This is the first study to directly influence acylated ghrelin concentrations at simulated altitude
316 via dietary intervention, and this elevation of acylated ghrelin concentrations during exercise resulted in a
317 simultaneous elevation of CAS. This strengthens the concept of a causal relationship between circulating acylated
318 ghrelin concentrations and subjective appetite responses at altitude. This speculation is supported at sea level as

319 studies have found ghrelin infusion to decrease satiety [52], increase hunger [53-55] and increase energy intake
320 [55, 56] in a variety of populations.

321 The high-fat breakfast provided within the current study was rich in coconut oil, a foodstuff known for
322 high concentrations of MCFAs [57]. It seems plausible that the significantly higher acylated ghrelin
323 concentrations after the HF breakfast may be due to an increased availability of MCFAs as a substrate for GOAT
324 [24], which is supported by the elevated NEFA and triglyceride concentrations in the present study. This
325 speculation is corroborated by evidence that ingested MCFAs are directly utilised in the acyl modification of
326 ghrelin in rodents [24]. Substantiating this, others have found MCFA ingestion increases circulating acylated
327 ghrelin concentrations in ruminants [58], piglets [59] and cachectic patients [26]. Additionally, following a meal,
328 neural signals are produced from the gastrointestinal tract which represent direct post-ingestive satiety signals,
329 outlined in the satiety cascade [60]. It may be possible that circulating glucose elicits more potent satiating neural
330 signals compared with circulating free fatty acids at altitude.

331 Entire trial energy expenditure tended to be lower after the HF breakfast compared with the HC breakfast.
332 This phenomenon would be beneficial in minimising an altitude-induced negative energy balance, potentially
333 aiding the maintenance of body composition at altitude, were it to persist. The thermic effect of food is reported
334 to be 0–3% and 5–10% of the caloric content of the fat and carbohydrate administered [27], supporting the lower
335 energy expenditure after the HF breakfast, compared with the HC breakfast. Interestingly we found that, whilst
336 resting, absolute fat oxidation rate remained unchanged between conditions and absolute carbohydrate oxidation
337 was lower after the HF breakfast, explaining the lower energy expenditure in the HF condition. The higher
338 absolute carbohydrate oxidation observed after the HC breakfast aligns with the simultaneously higher lactate
339 concentrations, which substantiates previous data at sea level [61]. This is likely the result of an increased
340 glycolytic flux in the HC condition producing a higher rate of lactate production, and thus plasma lactate
341 concentrations. Recent research has demonstrated an increased reliance on fat oxidation during acute exposure to
342 altitude compared with a matched sea level condition in fed individuals at rest [1] and during exercise [1, 62]. The
343 current study demonstrated that, although reliance on fat as a substrate was already high due to acute hypoxic
344 exposure, feeding with a HF breakfast further increased relative reliance on fat up to 74.6 (12.5)% in the
345 postprandial stage.

346 Despite the novel findings observed in the present study, some notable limitations must be
347 acknowledged. Firstly, the hypoxic exposure was relatively short and it is possible that acylated ghrelin and CAS
348 may respond differently over a longer period of time. It seems feasible that the consumption of additional high fat
349 meals during more prolonged exposure may produce additional smaller magnitudes of postprandial acylated
350 ghrelin suppression compared with high carbohydrate meals and this area warrants further research. In addition,
351 tightly controlled laboratory studies of this nature are valuable to gain mechanistic understanding and provide a
352 proof of concept but these findings require application in further field studies to assess the effects of high fat
353 feeding when combined with the effects of trekking, gradual ascent and other environmental stimuli (e.g. cold
354 exposure) which occur during real life ascent to high-altitude. It is not possible to attribute the findings of the
355 present study to high fat feeding, or MCFAs *per se*. In order to make this distinction a control high-fat breakfast
356 would be necessary containing minimal amounts of MCFAs, this is a potential area for further research.
357 Additionally, without a sea level control group in the present study it is not possible to state that an altitude related

358 suppression of appetite occurred. However, in our laboratory we have previously observed significant altitude-
359 induced appetite, acylated ghrelin and energy intake suppression using an extremely similar population and
360 protocol at the same altitude [1]. Therefore it seems likely that participants were suffering appetite suppression as
361 a result of altitude exposure. Finally, human energy balance is regulated by a complex multifaceted system.
362 Although the current study shows an augmentation of appetite after consuming a high fat breakfast, it is overly
363 simplistic to attribute all of the findings to a single gut hormone. Further research is needed to provide a full
364 mechanistic explanation of these findings. In conclusion, the consumption of a high fat breakfast at 4300m
365 simulated altitude attenuated the suppression of CAS and acylated ghrelin during subsequent exercise. However,
366 this was transient and had no effect on energy intake at an *ad-libitum* meal provided 1hr 30 minutes after exercise.
367 In addition, high fat feeding resulted in a lower energy expenditure during the five hour trial in comparison with
368 high carbohydrate feeding. It seems plausible that a combination of these factors would help to maintain energy
369 balance at altitude, however further research would be beneficial to establish whether energy intake can be
370 augmented at altitude.

371

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379

380 **Table 1.** Characteristics of the breakfasts

Meal	Porridge ingredients	Drink	Total energy and macronutrient composition
High fat breakfast	43 g rolled oats (598 kJ, 26 g carbohydrate, 3 g fat, 4 g protein)	216 mL whole milk (597 kJ, 10 g carbohydrate, 9 g fat, 7 g protein)	2927 kJ, 47 g (25%) carbohydrate, 47 g (60%) fat, 25 g (15%) protein
	233 mL whole milk (645 kJ, 10 g carbohydrate, 9 g fat, 8 g protein)		
	26 g coconut oil (961 kJ, 1 g carbohydrate, 25 g fat, 0 g protein)		
	7 g unflavoured whey (125 kJ, 0 g carbohydrate, 1 g fat, 6 g protein)		
High carbohydrate breakfast	81 g rolled oats (1122 kJ, 49 g carbohydrate, 6 g fat, 8 g protein)	216 mL orange juice (354 kJ, 19 g carbohydrate, 1 g fat, 1 g protein)	2917 kJ, 112 g (60%) carbohydrate, 19 g (25%) fat, 26 g (15%) protein
	437 mL semi-skimmed milk (868 kJ, 20 g carbohydrate, 8 g fat, 15 g protein)		
	25 g maltodextrin (369 kJ, 24 g carbohydrate, 0 g fat, 0 g protein)		
	10 mL double cream (186 kJ, 0 g carbohydrate, 5 g fat, 0 g protein)		
	1 g unflavoured whey (17 kJ, 0 g carbohydrate, 0 g fat, 1 g protein)		

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390 **Table 2.** Absolute and relative contributions of carbohydrate and fat oxidation in the high fat and high carbohydrate trials.

	Pre-prandial		Postprandial		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 [%]
High fat	0.19 (0.08) [48.6 (13.4)]	0.09 (0.03) [51.4 (13.4)]	0.17 (0.07)* [34.9 ± (10.1)]*	0.13 (0.05) [65.1 (10.1)]*	0.69 (0.19)* [39.7 (10.2)]*	0.45 (0.08)* [60.3 (10.2)]*	0.12 (0.06)* [25.4 (12.5)]*	0.16 (0.05) [74.6 (12.5)]*
High carbohydrate	0.21 (0.11) [48.9 (17.1)]	0.10 (0.04) [51.1 (17.1)]	0.25 (0.11) [46.6 (15.9)]	0.12 (0.05) [53.4 (15.9)]	0.84 (0.24) [47.5 (10.7)]	0.40 (0.07) [52.5 (10.7)]	0.17 (0.10) [33.6 (18.4)]	0.15 (0.06) [66.4 (18.4)]

391 Values are mean (SD), N = 12. % is percentage of energy yield. * Significantly (P < 0.05) different to high carbohydrate condition.

392

393 **Supplementary Table 1.** Palatability perceptions after the high fat and high carbohydrate breakfasts

	Appeal, mm	Smell, mm	Taste, mm	Aftertaste, mm	Palatability, mm
High fat	32 (17)	31 (16)	36 (25)	47 (26)	35 (23)
High carbohydrate	38 (22)	35 (17)	34 (22)	56 (26)	37 (20)

394 Values are mean (SD), N = 12.

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396

397 **Supplementary Table 2.** Macronutrient intakes at the *ad-libitum* meal in the high fat and high carbohydrate
398 conditions.

	Carbohydrate, g [%]	Fat, g [%]	Protein, g [%]
High fat	155 (57) [48 (11)]	59 (29) [39 (11)]	42 (19) [13 (2)]
High carbohydrate	160 (62) [47 (15)]	68 (34) [40 (14)]	48 (20) [13 (3)]

399 Values are mean (SD), N = 12.

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605 **Figure Legends**

606 **Figure 1.** Composite appetite score in the high fat (solid line) and high carbohydrate (dashed line) trials
607 expressed as mean (SE), N = 12. Thin upward arrow represents breakfast and thick upward arrow represents *ad-*
608 *libitum* meal. Black rectangle represents exercise.

609 **Figure 2.** Acylated ghrelin (A), des-acylated ghrelin (B) and total ghrelin (C) concentrations in the high fat
610 (solid line) and high carbohydrate (dashed line) trials expressed as mean (SE), N = 12. Thin upward arrow
611 represents breakfast and thick upward arrow represents *ad-libitum* meal. Black rectangle represents exercise.

612 **Figure 3.** Glucose (A), insulin (B) and lactate (C) concentrations in the high fat (solid line) and high
613 carbohydrate (dashed line) trials expressed as mean (SE), N = 12. Thin upward arrow represents breakfast and
614 thick upward arrow represents *ad-libitum* meal. Black rectangle represents exercise.

615 **Figure 4.** Non-esterified fatty acids (A) and triglycerides (B) concentrations in the high fat (solid line) and high
616 carbohydrate (dashed line) trials expressed as mean (SE), N = 12. Thin upward arrow represents breakfast and
617 thick upward arrow represents *ad-libitum* meal. Black rectangle represents exercise.