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1 **TITLE:** Changes in appetite, energy intake, body composition and circulating ghrelin constituents during an
2 incremental trekking ascent to high altitude

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22 **ABSTRACT**

23 Purpose: Circulating acylated ghrelin concentrations are associated with altitude-induced anorexia in laboratory
24 environments, but have never been measured at terrestrial altitude. This study examined time course changes in
25 appetite, energy intake, body composition and ghrelin constituents during a high altitude trek. Methods: Twelve
26 participants (age: 28(4) years, BMI: 23.0(2.1)kg.m²) completed a 14-day trek in the Himalayas. Energy intake,
27 appetite perceptions, body composition, and circulating acylated, des-acylated and total ghrelin concentrations
28 were assessed at baseline (113m; 12 days prior to departure) and at three fixed research camps during the trek
29 (3619m, day seven; 4600m, day 10; 5140m, day 12). Results: Relative to baseline, energy intake was lower at
30 3619m (P=0.038) and 5140m (P=0.016) and tended to be lower at 4600m (P=0.056). Appetite perceptions were
31 lower at 5140m (P=0.027) compared with baseline. Acylated ghrelin concentrations were lower at 3619m
32 (P=0.046) and 4600m (P=0.038), and tended to be lower at 5140m (P=0.070), compared with baseline. Des-
33 acylated ghrelin concentrations did not significantly change during the trek (P=0.177). Total ghrelin
34 concentrations decreased from baseline to 4600m (P=0.045). Skinfold thickness was lower at all points during the
35 trek compared with baseline (P≤0.001) and calf girth decreased incrementally during the trek (P=0.010).
36 Conclusions: Changes in plasma acylated and total ghrelin concentrations may contribute to the suppression of
37 appetite and energy intake at altitude but differences in the time course of these responses suggests that additional
38 factors are also involved. Interventions are required to maintain appetite and energy balance during trekking at
39 terrestrial altitudes.

40 **KEYWORDS:** ghrelin; hypoxia; altitude-induced anorexia; terrestrial altitude

41

42 **Abbreviations**

| | |
|------------------|---|
| AG:DG | Acylated ghrelin to des-acylated ghrelin ratio |
| AMS | Acute mountain sickness |
| ANOVA | Analysis of variance |
| BMI | Body mass index |
| CAS | Composite appetite score |
| GOAT | Ghrelin-O-acyltransferase |
| ISAK | International Society for the Advancement of Kinanthropometry |
| LLS | Lake Louise Score |
| MoDREC | Ministry of Defence Research Ethics Committee |
| RPE | Rating of perceived exertion |
| SD | Standard deviation |
| SE | Standard error |
| SpO ₂ | Arterial oxygen saturations |
| VAS | Visual analogue scales |

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45 INTRODUCTION

46 Acute exposure to hypoxic environments has been demonstrated to suppress appetite and energy intake (Armellini
47 et al. 1997; Matu et al. 2017; Wasse et al. 2012; Westerterp et al. 2000). This effect appears to be maintained
48 during prolonged sojourns to high altitude, which results in significant decreases in body mass, of which greater
49 than 50 % is from fat-free mass (Rose et al. 1988; Sergi et al. 2010). These declines in lean mass will likely lead
50 to a drop in physical capabilities at altitude (Sergi et al. 2010), which can have deleterious implications for
51 individuals ascending to high altitude. A better understanding of the time course of these changes during a trek,
52 as well as the mechanisms involved, is required to develop guidance for those travelling to high altitudes.

53 Over the past twenty years, changes in the circulating concentrations of gastrointestinal hormones and
54 leptin have been implicated as potential mechanisms for the alterations in appetite and energy intake at altitude.
55 However, although several hormones such as pancreatic polypeptide (Riepl et al. 2012), leptin (Sierra-Johnson et
56 al. 2008), glucagon-like-peptide-1 (Snyder et al. 2008) and total ghrelin (Benso et al. 2007; Riepl et al. 2012;
57 Shukla et al. 2005) have been measured in response to terrestrial altitude exposure, the findings remain equivocal.
58 One major limitation of the current research is the measurement of total ghrelin concentrations at altitude, rather
59 than the constituent components of acylated and des-acylated ghrelin which have opposing effects on appetite
60 regulation (Fernandez et al. 2016). The differentiation of ghrelin constituents in response to terrestrial altitude is
61 imperative as acylated ghrelin has been found to be particularly responsive to hypoxic exposure in a laboratory
62 environment with decreases in this hormone correlated with a reduction in appetite (Bailey et al. 2015) and energy
63 intake (Wasse et al. 2012). Furthermore, recent evidence suggests that des-acylated ghrelin may inhibit the
64 orexigenic effects of acylated ghrelin (Fernandez et al. 2016), which further emphasises the need to measure both
65 hormones as well as the ratio between the two (Al Massadi et al. 2014). It seems feasible that the measurement of
66 total ghrelin in previous research (Benso et al. 2007; Debevec et al. 2014; Riepl et al. 2012) may have masked
67 changes in acylated and des-acylated ghrelin, which may explain the lack of association between changes in
68 appetite and circulating ghrelin concentrations at altitude.

69 Although circulating total ghrelin concentrations have been extensively investigated in response to
70 hypoxic exposure (Benso et al. 2007; Debevec et al. 2014; Debevec et al. 2016; Mekjavic et al. 2016; Riepl et al.
71 2012; Shukla et al. 2005), the investigation of acylated ghrelin is currently limited to four studies, all of which
72 lasted for ≤ 7 h and were all conducted in normobaric environments (Bailey et al. 2015; Matu et al. 2017;
73 Morishima and Goto 2016; Wasse et al. 2012). Although laboratory studies of this nature are valuable to gain

74 greater mechanistic understanding, further field studies are required to assess the combined effects of trekking,
75 gradual ascent and other environmental stimuli such as cold exposure which occur during real life ascent to high
76 altitude. The measurement of acylated and des-acylated ghrelin during ascent to terrestrial altitude is vital to
77 understand the changes that occur during a real-world environment and the importance of these changes as a basis
78 for the development of future interventions. The lack of investigation into the constituents of total ghrelin to date
79 is likely due to the complexities of the necessary chemical preparation required to prevent the degradation of the
80 analytes (Hosoda et al. 2004), which is particularly difficult to achieve in an extreme field environment.

81 The purpose of this study was to investigate the effects of a high altitude trek to 5300 m on appetite,
82 energy intake and body composition responses in healthy men and women, with a further focus on circulating
83 acylated and des-acylated ghrelin concentrations as mechanistic variables. These data provide novel insights into
84 the time course of changes in appetite, energy intake and body composition during a real-life ascent to high
85 altitude. This study also provides a better understanding of the mechanisms responsible for altitude-induced
86 anorexia, representing the first investigation of acylated ghrelin and des-acylated ghrelin at terrestrial altitude. We
87 hypothesised that exposure to increasingly high altitudes would suppress appetite, circulating acylated ghrelin
88 concentrations and energy intake, which would be associated with a reduction in lean and total body mass.

89

90 METHODS

91 **Participants.** This study was conducted according to the guidelines laid down in the Declaration of
92 Helsinki and all procedures were approved by the Ethics Advisory Committee at Leeds Beckett University and
93 the Ministry of Defence Research Ethics Committee (MoDREC; protocol number 624). Twelve members (nine
94 male, three female) of the British Military volunteered to participate in this study. Informed consent was obtained
95 from all participants included in the study. All participants were non-smokers, had no known disease, allergies or
96 intolerances, and had not been to an altitude over 1000 m for at least 3 months. All participants were physically
97 fit and could run 2.4 km on a treadmill at a 2 % gradient in under 13 minutes 37 seconds in accordance with
98 military requirements. The physical characteristics of participants (mean (SD)) were as follows: age 28 (4) years,
99 body mass 71.3 (10.3) kg, body mass index (BMI) 23.0 (2.1) kg.m⁻².

100 **Study design.** This study represents part of the 'British Services Dhaulagiri Medical Research
101 Expedition' which took place in March – May 2016 (Mellor et al. 2017). In April 2016, participants in the present
102 study travelled from the UK to Nepal and completed a 14-day trek around the Dhaulagiri circuit in the Himalayas.
103 Travel from the UK to Nepal lasted for one day and participants were in Nepal for three days prior to starting the
104 trek. The trek commenced from Darbang (~1100 m), peaked on day 11 at the French Pass (~5300 m) and ended
105 on day 14 at Marpha (~2700 m). Pre-planned rest days were included at fixed camps at 3619 m (Camp 1; day
106 seven), 4600 m (Camp 2; day 10) and 5140 m (Camp 3; day 12). Participants walked a mean distance of 8.2
107 km.day⁻¹ with a mean elevation gain of 471 m.day⁻¹ whilst carrying a day pack weighing ~ 5 kg. Further
108 information about the ascent profile and trek characteristics has been published elsewhere (Mellor et al. 2017).
109 Data collection took place at baseline (113m; 12 days prior to departure from the UK) and at each fixed camp. On
110 the day preceding data collection at camp 1, camp 2 and camp 3 participants walked 4.3 km, 4.3 km and 9.1 km
111 and gained an elevation of 512 m, 528 m and 540 m, respectively. All trekking on these days was completed by
112 5pm. All participants wore the same type of clothing throughout the trek and experienced the same degree of cold
113 exposure. Baseline measurements were collected in the laboratories at Leeds Beckett University and the measures
114 at each camp were collected in a designated research tent. At the time data was collected ambient temperatures in
115 the laboratory and research tents were 19.8 °C, 4.9 °C, 1.2 °C and -6.4 °C at baseline, 3619 m, 4600 m and 5140
116 m, respectively. All participants remained rested on the day of testing. Participants were staggered for the
117 collection of all measurements between 7 am and 10 am, with each participant having their measures taken at a
118 consistent time on all occasions and after an overnight fast of at least 10 h.

119 **Food Provision.** Throughout the trek all food and fluid was available *ad libitum*, and was provided by
120 Nepalese cooks and staff who accompanied the trekking team. Typical foods offered at each meal were as follows:
121 Breakfast – cereals, porridge, omelette, pancakes; Lunch – noodles, meats, soup, beans, vegetables, fruit; Dinner
122 – curry, pasta, pizza, potatoes, dumplings, cheese, vegetables; Snacks – chocolate bars, biscuits, cake, fruit. At all
123 meals participants were given more than one option and thus could decide what they wanted to eat. The mean
124 (SD) macronutrient composition of the food consumed during the trek was 49.0 (6.6) % carbohydrate, 36.3 (6.2)
125 % fat and 14.7 (2.6) % protein, respectively.

126 **Food intake.** Energy intake was assessed at baseline via a 24-hour dietary recall interview by an
127 experienced researcher (Academic Associate of the Sport and Exercise Nutrition Register) using the multiple-pass
128 approach (Guenther et al. 1997). In addition to collecting dietary intake information, this approach was used to
129 demonstrate the level of detail required from the participants when completing a food diary during the trek. All
130 participants completed a food diary on the day preceding each fixed camp and this process was monitored and
131 verified by the same experienced researcher throughout the trek. Although there are acknowledged limitations of
132 self-reported dietary intake methods (Hill and Davies 2001), the oversight of food diaries by the researcher present
133 on the trek ensured accurate completion of all food diaries. Food intake was monitored during the day before each
134 fixed camp to consider acute dietary changes when interpreting the data for fasted appetite ratings and blood
135 samples at the fixed camps. The palatability of each meal consumed was measured using 100 mm visual analogue
136 scales (VAS) with the anchors “the worst taste that I have ever experienced” and “the best taste that I have ever
137 experienced” at each end of the scale. A mean palatability score was calculated for each day to control for any
138 influence of palatability on food consumption.

139 **Appetite.** Appetite perceptions were measured after an overnight fast at baseline and upon waking at
140 each fixed camp using validated 100 mm VAS for hunger, satisfaction, fullness and prospective food consumption
141 (PFC) (Flint et al. 2000). Using these scales, a composite appetite score (CAS) was calculated using the following
142 formula: composite appetite score = ([hunger + prospective food consumption + (100 – fullness) + (100 –
143 satisfaction)] / 4) (Stubbs et al. 2000). A higher value is associated with a greater appetite sensation and
144 subsequently a stronger motivation to eat. In addition, the extent to which participants desired sweet, salty,
145 savoury, and fatty foods was assessed using VAS anchored at each end with “yes, very much” and “no, not at all”.

146 **Acute Mountain Sickness, oxygen saturation and rating of perceived exertion.** Acute mountain
147 sickness (AMS) was assessed every morning and evening using the Lake Louise AMS (LLS) score (Roach et al.

148 1993); mild AMS was defined as LLS of ≥ 3 in the presence of a headache and severe AMS was defined as LLS
149 of ≥ 6 in the presence of a headache. Arterial oxygen saturations (SpO_2) were measured via a fingertip pulse
150 oximeter (Nellcor™ PM10N; Medtronic, Minneapolis, MN) every morning and evening while the participants
151 were resting in a seated position. Rating of perceived exertion (RPE) (Borg 1982) was recorded as the hardest
152 exertion experienced during the day of trekking preceding each fixed camp, as used previously in similar
153 environments (Mellor et al. 2014).

154 **Body composition.** Participants were weighed at baseline and each fixed camp in a fasted state whilst
155 wearing minimal clothing and no footwear. A portable multicomponent force plate (Kistler, Switzerland) was
156 used and was stabilised on the mountain using specialised levelling feet (JVD Design & Automation Ltd, Leeds,
157 UK). Skinfolds of the triceps, subscapular, biceps, iliac crest, supraspinale, abdominal, front thigh and medial calf
158 were measured using calibrated Harpenden callipers (John Bull, British Indicators, West Sussex, UK) to the
159 nearest 0.1 mm. The sum of skinfolds was calculated by the addition of each of the eight skinfold values in mm.
160 Girth measurements of the waist and calf, as well as the upper arm in a relaxed and flexed state, were obtained
161 using a steel anthropometric tape (Lufkin W606PM, Cooper Hand Tools, Tyne & Wear, UK) to the nearest 1 mm.
162 All anthropometric assessments were conducted by one researcher who was trained by an individual accredited
163 by the International Society for the Advancement of Kinanthropometry (ISAK). All measures were conducted in
164 duplicate and in accordance with ISAK guidelines on the right side of the body. The coefficient of variation for
165 skinfolds and girths was 2.2% and 0.4%, respectively.

166 **Blood sampling.** Venous blood samples were obtained from an antecubital vein via venepuncture using
167 a 21-gauge butterfly needle (Safety-Lok™; BD, Oxford, UK). Samples were collected at baseline and at all
168 research camps with participants in a fasted state. One 4.9 mL pre-cooled EDTA monovette (Sarstedt, Leicester,
169 UK) was used to obtain samples for the determination of plasma acylated and des-acylated ghrelin concentrations.
170 Monovettes were pre-treated on the morning of testing, to prevent the degradation of acylated ghrelin, with 50 μ l
171 of a solution containing p-hydroxymercuribenzoic acid, potassium phosphate buffer and sodium hydroxide
172 (Hosoda et al. 2004). Immediately after filling, the tube was spun at 1500 x g for 10 minutes in a centrifuge
173 (CompactStar CS4, VWR). Subsequently 1 mL of plasma was mixed with 100 μ l of 1M hydrochloric acid. This
174 solution was then immediately frozen at either -20°C in a freezer (for baseline measurements) or within a dry
175 shipper containing liquid nitrogen at <-80°C (at each fixed camp) before being transferred to a -80°C freezer at
176 the university and stored until analysis. Plasma volume changes as a result of altitude exposure were not assessed
177 during the trek because it is the absolute plasma hormonal concentrations that would determine the body's

178 response at that specific time (Kargotich et al. 1998). However, to prevent any extraneous influences from postural
179 changes, all blood samples were collected after the participant had been seated for at least 5 minutes (Fawcett and
180 Wynn 1960).

181 **Blood analyses.** Commercially available enzyme immunoassays were used to determine plasma
182 concentrations of acylated and des-acylated ghrelin (SPI BIO, Montigny Le Bretonneux, France). To eliminate
183 interassay variation, all samples from each participant were analysed on the same plate. In addition, all samples
184 were analysed in duplicate and on the same day. The within batch coefficients of variation were 2.5% for acylated
185 ghrelin and 2.4% for des-acylated ghrelin. Total ghrelin was computed via the addition of acylated and des-
186 acylated ghrelin concentrations. The ratio between acylated ghrelin and des-acylated ghrelin concentrations
187 (AG:DG) was calculated as acylated ghrelin divided by des-acylated ghrelin, as previously described (Delhanty
188 et al. 2015).

189 **Statistical analysis.** Data are expressed as mean (SD) in text and tables and mean (SE) in figures to
190 avoid distortion of the graphs. Diet records were inputted into Nutritics dietary analysis software (v1.8 for
191 Windows; Nutritics, Dublin) to assess energy intake. All data were analysed using IBM SPSS statistics (v22.0 for
192 Windows; SPSS, Chicago, IL). One way repeated measures analysis of variance (ANOVA) was used to assess
193 altitude-based differences in SpO₂, AMS scores, RPE scores, body composition measures, appetite perceptions,
194 energy intake, fluid intake and plasma ghrelin concentrations. Significant effects were further explored using
195 Student's paired *t* tests. Effect sizes are presented as Cohen's *d* and interpreted as ≤ 0.2 trivial, > 0.2 small, > 0.6
196 moderate, > 1.2 large, > 2 very large and > 4 extremely large (Hopkins 2004). The Pearson product moment
197 correlation coefficient was used to investigate relationships between variables at each altitude. The exclusion of
198 participants reporting AMS did not alter the interpretation of the findings; subsequently all participants were
199 included in the data analysis. Based on evidence that males and females exhibit similar appetite, energy intake
200 and gut hormone responses to exercise- and diet-induced energy deficits (Alajmi et al. 2016), data from both
201 genders were combined for analyses. The sample size used within this study was deemed sufficient to detect a
202 significant difference in energy intake between altitudes. The anticipated effect size for a difference in energy
203 intake was based on a similar previous study which investigated energy intake in individuals climbing at
204 approximately 4500 m for 16 days (Armellini et al. 1997). Based on the effect size and an alpha value of 5 %, a
205 sample size of 12 participants would generate a power >95 %. Calculations were performed using G*power (v3
206 for Windows; Düsseldorf) (Faul et al. 2007).

207 **RESULTS**

208 Measurements of SpO₂, AMS, RPE, body composition, fluid intake and energy intake were successfully obtained
209 from all 12 participants. One male participant withdrew consent for blood sampling at the research camps during
210 the trek and one male participant did not complete the appetite perception measurements during the trek.
211 Therefore, data is presented for 11 participants for plasma hormone concentrations and appetite perceptions.

212

213 **Oxygen saturations, acute mountain sickness and rating of perceived exertion.** One way ANOVA
214 revealed a main effect of altitude for SpO₂ ($P < 0.001$). Post-hoc analysis demonstrated lower SpO₂ at each fixed
215 camp compared with the previous location (baseline: 98.4 (0.9) %; 3619 m: 92.4 (2.5) %, $P < 0.001$, $d = 3.19$;
216 4600 m: 83.5 (4.1) %, $P < 0.001$, $d = 2.62$; 5140 m: 79.8 (5.6) %, $P = 0.007$, $d = 0.75$). A positive diagnosis of
217 mild AMS was reported in 50% of participants at some point during the trek. The first incidence of AMS occurred
218 on the eighth day of the trek (the day after the first rest day; 4072 m). Incidence of AMS at the four fixed locations
219 was as follows: baseline: zero participants; 3619 m: zero participants; 4600 m: two participants; and 5140 m: two
220 participants. One way ANOVA revealed a significant effect of altitude for RPE ($P < 0.001$). Relative to 3619 m
221 (11.8 (1.5)), RPE was significantly higher at 4600 m (13.3 (1.5), $P = 0.009$, $d = 1.01$) and relative to 4600 m RPE
222 was significantly higher at 5140 m (16.5 (2.5), $P = 0.003$, $d = 1.58$).

223

224 **Energy and fluid intake.** One way ANOVA revealed a significant effect of altitude for energy intake
225 ($P = 0.015$). Relative to baseline, energy intake was significantly lower at 3619 m ($P = 0.038$, $d = 1.05$) and 5140
226 m ($P = 0.016$, $d = 1.00$) and tended to be lower at 4600 m ($P = 0.056$, $d = 0.82$). There were no differences observed
227 between research camps during the trek (all $P \geq 0.333$, $d \leq 0.22$) (Figure 1a).

228 One way ANOVA revealed a main effect of altitude for fluid intake ($P = 0.029$). Relative to baseline
229 (2769 (1156) mL.day⁻¹) fluid intake was significantly higher at 3619 m (4438 (1847) mL.day⁻¹, $P = 0.008$, $d =$
230 1.08) and 4600 m (4236 (2120) mL.day⁻¹, $P = 0.027$, $d = 0.86$), but not significantly higher at 5140 m (3645 (2026)
231 mL.day⁻¹, $P = 0.126$, $d = 0.53$). There were no differences observed between camps (all $P \geq 0.266$, $d \leq 0.29$).

232 One way ANOVA revealed a main effect of altitude for the daily palatability of food consumed ($P =$
233 0.018). Relative to baseline, palatability was significantly higher at 3619 m ($P = 0.030$, $d = 1.17$). However,
234 palatability was not different at 4600 m ($P = 0.147$, $d = 0.66$) or 5140 m ($P = 0.509$, $d = 0.32$) compared with

235 baseline. Palatability was significantly lower at 5140 m compared with 3619 m ($P = 0.020$, $d = 0.97$) and 4600 m
236 ($P = 0.013$, $d = 0.71$) (Figure 1b).

237 One way ANOVA revealed a main effect of altitude on the desire to eat salty ($P = 0.025$) and savoury (P
238 < 0.001) foods, but not sweet ($P = 0.604$) or fatty ($P = 0.354$) foods. Relative to baseline (67 (12) mm), the desire
239 to eat salty foods was significantly lower at 3619 m (41 (23) mm, $P = 0.018$, $d = 1.42$) and 5140 m (39 (27) mm,
240 $P = 0.024$, $d = 1.38$), and also tended to be lower at 4600 m (45 (27) mm, $P = 0.066$, $d = 1.09$). There were no
241 differences observed between camps (all $P \geq 0.159$, $d \leq 0.22$). The desire to eat savoury foods was significantly
242 increased at 3619 m (70 (13) mm, $P < 0.001$, $d = 2.41$), 4600 m (67 (15) mm, $P < 0.001$, $d = 2.03$) and 5140 m
243 (53 (19) mm, $P = 0.011$, $d = 1.08$) compared with baseline (33 (18) mm). In addition, the desire to eat savoury
244 foods reduced significantly from 4600 m to 5140 m ($P = 0.026$, $d = 0.79$) with no difference observed between
245 3619 m and 4600 m ($P = 0.516$, $d = 0.23$).

246

247 **Appetite perceptions.** One way ANOVA revealed a main effect of altitude for CAS ($P = 0.005$). Post-
248 hoc analysis revealed that CAS was significantly lower at 5140 m compared with baseline ($P = 0.027$, $d = 1.07$),
249 3619 m ($P = 0.005$, $d = 1.19$) and 4600 m ($P = 0.05$, $d = 0.69$). No other differences were observed between
250 altitudes (all $P \geq 0.116$, $d \leq 0.48$) (Figure 1c).

251

252 **Plasma acylated and des-acylated ghrelin concentrations.** One way ANOVA revealed a significant
253 effect of altitude for plasma acylated ghrelin concentrations ($P = 0.048$; Figure 2a), plasma total ghrelin
254 concentrations ($P = 0.047$; Figure 2d) and the AG:DG ratio ($P = 0.046$; Figure 2c). A main effect of altitude was
255 not detected for plasma des-acylated ghrelin concentrations ($P = 0.177$; Figure 2b).

256 Relative to baseline, plasma acylated ghrelin concentrations were significantly lower at 3619 m ($P =$
257 0.046 , $d = 0.25$) and 4600 m ($P = 0.038$, $d = 0.29$), and tended to be lower at 5140 m ($P = 0.070$, $d = 0.28$). There
258 were no differences observed between camps (all $P \geq 0.512$, $d \leq 0.04$; Figure 2a). Plasma AG:DG ratio decreased
259 significantly from baseline to 3619 m ($P = 0.034$, $d = 0.37$), and tended to be lower than baseline at 4600 m ($P =$
260 0.069 , $d = 0.23$) and 5140 m ($P = 0.070$, $d = 0.25$). There were no differences observed between camps (all $P \geq$
261 0.362 , $d \leq 0.15$) (Figure 2c). Plasma total ghrelin concentrations decreased significantly from baseline to 4600 m

262 (P = 0.045, *d* = 0.36), however no other significant differences were observed between altitudes (all P ≥ 0.111, *d*
263 ≤ 0.31) (Figure 2d).

264

265 **Body composition.** One way ANOVA revealed a significant effect of altitude for body mass (P < 0.001),
266 sum of skinfolds (P < 0.001), calf girth (P = 0.010), waist girth (P = 0.016) and relaxed arm girth (P = 0.029), with
267 no significant differences observed for flexed arm girth (P = 0.173) (Table 1).

268 Body mass increased from baseline to 3619 m (P = 0.002, *d* = 0.18), decreased between 3619 m and
269 4600 m (P < 0.001, *d* = 0.22) and did not change between 4600 m and 5140 m (P = 0.415, *d* = 0.03). Sum of
270 skinfolds was lower at 3619 m (P = 0.001, *d* = 0.30), 4600 m (P < 0.001, *d* = 0.34) and 5140 m (P = 0.001, *d* =
271 0.24) compared with baseline. There were no significant differences observed between each of the camps during
272 the trek (all P ≥ 0.116, *d* ≤ 0.09).

273 Calf girth did not differ significantly between baseline and 3619 m (P = 0.127, *d* = 0.30), however was
274 significantly decreased at 4600 m (P = 0.039, *d* = 0.44) and 5140 m (P = 0.008, *d* = 0.60) compared with baseline.
275 Calf girth was also significantly lower at 4600 m compared with 3619 m (P = 0.031, *d* = 0.14), and tended to be
276 lower at 5140 m compared with 4600 m (P = 0.069, *d* = 0.14). Waist girth did not differ between baseline and any
277 of the three camps (all P ≥ 0.122, *d* ≤ 0.15), however was significantly lower at 5140 m than 3619 m (P < 0.001,
278 *d* = 0.29) and 4600 m (P = 0.04, *d* = 0.13). Relaxed arm girth was significantly lower at 3619 m (P = 0.022, *d* =
279 0.30) and 4600 m (P = 0.047, *d* = 0.23) and tended to be lower at 5140 m (P = 0.073, *d* = 0.20) compared with
280 baseline. There was a significant increase in relaxed arm girth between 3619 m and 5140 m (P = 0.047, *d* = 0.10),
281 with no other differences observed between camps (all P ≥ 0.191, *d* ≤ 0.07).

282

283 **Correlations.** There were no correlations observed at any altitude between energy intake and CAS (all r
284 ≤ 0.311, P ≥ 0.352). At 3619 m CAS tended to be associated with plasma acylated ghrelin (r = 0.603, P = 0.065)
285 and total ghrelin (r = 0.626, P = 0.053) concentrations. Additionally, at 3619 m energy intake was significantly
286 correlated with des-acylated ghrelin concentrations (r = 0.686, P = 0.029). At 4600 m CAS was significantly
287 correlated with acylated ghrelin concentrations (r = 0.633, P = 0.049) and the AG:DG ratio (r = 0.667, P = 0.035).
288 There were no other significant correlations observed between any variable, at any altitude (all r ≤ 0.511, P ≥
289 0.108).

290

291 **DISCUSSION**

292 This study presents an assessment of the changes in appetite perceptions, energy intake, body composition, and
293 ghrelin constituents throughout a trek to high terrestrial altitude. The findings demonstrate a reduction in energy
294 intake and skinfold thickness during the trek, with a progressive reduction in appetite at increasing altitudes. This
295 study provides the first investigation of acylated ghrelin and des-acylated ghrelin concentrations at terrestrial
296 altitude and demonstrates a suppression of acylated- but not des-acylated ghrelin during the trek. These findings
297 highlight the importance of measuring ghrelin constituents in addition to total ghrelin concentrations as small
298 fluctuations in des-acylated ghrelin may mask changes in acylated ghrelin if only total ghrelin were to be
299 measured. This phenomenon would have occurred at 3619 m in the present study as observed by a significant
300 decrease in acylated ghrelin levels in the absence of any significant changes in des-acylated and total ghrelin
301 concentrations. The findings from this study also demonstrate the need for interventions to maintain appetite
302 during exposure to terrestrial altitudes, particularly above 4600 m.

303 In the present study energy intake was reduced by 27 % (9326 kJ) at 3619 m, 22 % (9886 kJ) at 4600 m,
304 and 27 % (9238 kJ) at 5140 m compared with baseline, which substantiates previous findings at similar altitudes.
305 Armellini et al. (1997) observed a 29 % decrease in energy intake in individuals climbing at approximately 4500
306 m for 16 days, whilst Aeberli et al. (2013) demonstrated a 32 % reduction in energy intake two days after rapid
307 ascent to 4559 m. One study however, found that energy intake, as well as fat and muscle mass, could be
308 maintained up to an altitude of 5050 m when a wide choice of palatable foods were available in a comfortable
309 setting (Kayser et al. 1993). It may therefore be argued that the reduction in energy intake in the present study
310 was caused by a lack of food availability and reduced palatability of food whilst trekking in a foreign country.
311 However, at 3619 m, energy intake was significantly suppressed compared with baseline while food was widely
312 available and the mean palatability of the food consumed was significantly higher than baseline. These findings
313 agree with those of Rose et al. (1988) who found a significant reduction in *ad-libitum* energy intake during a
314 simulated ascent of Mount Everest, despite a variety of palatable foods being available. Despite the reduction in
315 energy intake, fasting appetite perceptions were similar between baseline and 3619 m, which suggests a greater
316 satiating effect of the energy consumed. This response was maintained at 4600 m but appetite perceptions
317 decreased significantly at 5140 m despite consistent food intake. Observations during the trek suggested that
318 participants were consciously trying to maintain energy intakes throughout the trek in an attempt to maintain

319 physical performance. This would support the observation that food intake was similar between the three camps
320 but that appetite perceptions and the desire for foods decreased with increasing altitude. This mismatch between
321 appetite perceptions and energy intake is further supported by the lack of correlation between the two variables at
322 each altitude. The reduced palatability of the foods consumed at 5140 m also supports a reduction in appetite, and
323 occurred despite the same ad libitum food provision throughout the trek. This effect accords with the findings
324 from previous animal studies which suggest that hypoxia degrades the taste of food (Ettinger and Staddon 1982).
325 Considering the significant suppression of appetite at 5140 m, it is unclear whether food intakes could continue
326 to be maintained over a more prolonged period and targeted interventions to better maintain appetite above 4600
327 m may be beneficial.

328 From a mechanistic perspective, trekking to high altitude induced a suppression of acylated but not des-
329 acylated ghrelin concentrations, which resulted in a suppression of total ghrelin levels and the AG:DG ratio.
330 However at 5140 m, the only altitude in which CAS was significantly suppressed, no correlations were observed
331 between any blood marker and CAS or energy intake. These findings suggest that appetite regulation during high-
332 altitude trekking may be influenced by other hormonal (e.g. leptin, glucagon-like peptide-1 and peptide YY
333 (Debevec 2017)) and non-hormonal (e.g. taste degradation (Ettinger and Staddon 1982) potentially altering food
334 reward (Berthoud 2006)) factors. Appetite regulation is a complex multifaceted system which involves the
335 integration of a wide range of neuroendocrine and psychological factors (Murphy and Bloom 2006). Subsequently,
336 appetite suppression at altitude is unlikely to be solely explained by the measurement of a single hormone.
337 However, a better understanding of the neuroendocrine responses to high altitude trekking could be beneficial in
338 the design of interventions to minimise appetite suppression at altitude.

339 The reductions in fasted acylated and total ghrelin concentrations, the day after significantly reduced
340 energy intakes compared with baseline measurements, are particularly interesting considering the evidence that
341 ghrelin levels and appetite perceptions increase in response to reduced food intake at sea level (Alajmi et al. 2016).
342 Furthermore, acylated ghrelin levels remained depressed during the trek despite reductions in body mass between
343 3619 m and 4600 m and the established inverse relationship between body mass and ghrelin concentrations at sea
344 level (Chen et al. 2009; Shiiya et al. 2002). These observations suggest that the reductions in acylated and total
345 ghrelin during this study were genuine effects of high altitude exposure rather than being secondary to any changes
346 in food intake or body composition. Although these changes in ghrelin were small, they appear to be
347 physiologically relevant as changes of this magnitude have previously been associated with reductions in appetite
348 and energy intake in a laboratory environment (Bailey et al. 2015; Wasse et al. 2012). It would be beneficial for

349 future research to attempt to increase circulating plasma acylated ghrelin concentrations at altitude, in order to
350 quantify these effects on appetite responses. Potential methods of accomplishing this include ghrelin infusion
351 (Druce et al. 2005) or dietary interventions to manipulate ghrelin constituents (e.g. increased medium chain
352 triglyceride intake as a substrate for ghrelin acylation (Kawai et al. 2017; Nishi et al. 2005)).

353 The reasons for the observed suppression of acylated ghrelin at altitude are unclear. However,
354 considering the lack of change in total ghrelin levels at 3619 m, it seems plausible that the post-translational
355 acylation of ghrelin may have been inhibited during the early stages of the trek due to inhibited ghrelin-O-
356 acyltransferase (GOAT) activity or reduced availability of medium chain fatty acids as the substrate for acylation
357 (Nishi et al. 2005). Alternatively, the reduction in both acylated ghrelin and total ghrelin at 4600 m suggests
358 inhibited secretion of ghrelin from the P/D1 cells of the stomach (Kojima et al. 1999). A reduction in gut blood
359 flow at altitude (Loshbaugh et al. 2006) has also been proposed to reduce ghrelin concentrations (Wasse et al.
360 2012), however this concept has been disputed (Kalson et al. 2010; Mekjavic et al. 2016). The depression of
361 acylated ghrelin levels prior to the depression of total ghrelin levels at the subsequent research camp suggests that
362 acylated ghrelin may be a more sensitive measure of altered appetite signalling at altitude. Furthermore, the
363 AG:DG ratio of >1 in the present study supports recent data that acylated ghrelin constitutes a much larger
364 proportion of total ghrelin than previously thought (Delhanty et al. 2015) and demonstrates that preservation of
365 this peptide can be achieved during field research in extreme environments.

366 In accordance with previous research, considerable changes in body composition were observed during
367 the trek. This includes a mean reduction in body mass of 2.3 kg in the three days between 3619 m and 4600 m,
368 which was associated with significant decreases in calf girth despite very high levels of physical activity (mean
369 distance walked: 8.8 km.day⁻¹, mean elevation gain: 491 m.day⁻¹). It seems likely that the reductions in body mass
370 were not only caused by decreases in energy intake, but also by increases in energy expenditure due to the high
371 altitude environment and high physical activity levels. Such decreases in body and muscle mass have been
372 observed in previous altitude research (Benso et al. 2007; Rose et al. 1988; Shukla et al. 2005; Westerterp et al.
373 2000) and it is likely that these losses would impair physical performance in these environments (Sergi et al.
374 2010). The further reductions in calf girth between 3619 m and 5140 m also aligns with previous research (Rose
375 et al. 1988) but the reasons for this response are unknown. We speculate that an increase in protein degradation
376 may have occurred due to increasing altitude exposure (Holm et al. 2010). However, contrary to these changes in
377 calf girth, an increase in relaxed arm girth was observed between 3619 m and 5140 m. Although this increase was
378 statistically significant, the absolute increase of 0.3 mm seems trivial. This may represent a compensatory response

379 to the significant reduction in arm girth between baseline and 3619 m which is most likely due to atrophy caused
380 by reduced activity of the arms during final preparations and low altitude trekking. Despite these insights into
381 changes in body composition, it must be acknowledged that the baseline measures were collected 12 days before
382 departure from the UK, which may have confounded comparisons between baseline values and those obtained
383 during the trek due to changes in body composition during the final preparations for the expedition. Potential
384 increases in lean body mass from final preparations and reductions in body fat would support the observed increase
385 in body mass at the first fixed camp and reduced skinfold values at all camps relative to baseline. At sea level, an
386 increase in body mass would usually result in a reduction in acylated ghrelin concentrations (Chen et al. 2009),
387 which accords with our findings at the first fixed camp. However, in the present study body mass then significantly
388 reduced at the second and third camps, without a subsequent increase in ghrelin. This suggests that changes in
389 ghrelin were unlikely to be the result of fluctuations in body mass during the trek and were more strongly mediated
390 by high altitude exposure.

391 Although the findings of the present study provide novel information regarding the appetite and
392 metabolic responses during an incremental trekking ascent to high altitude, some notable limitations must be
393 acknowledged. First, the current study design did not include a control group to separate the effects of trekking
394 and high altitude exposure. Therefore it is not possible to conclude that the obtained results are a consequence of
395 hypobaric hypoxia *per se*, but are a result of high altitude trekking which combines various environmental and
396 psychological factors as well as demanding physical exercise. An example of this being that cold exposure may
397 interfere with energy balance, potentially by increasing non-shivering thermogenesis (van der Lans et al. 2013).
398 Although this limits interpretation of the influence of each of these factors individually, the study design allowed
399 us to investigate the effects of a real world gradual ascent to high altitude in order to understand the practical
400 implications for high altitude trekking. Second, it was not possible to standardise the trekking distance on the day
401 preceding each fixed camp due to the extreme terrain and environment. The greater trekking distance performed
402 on the day prior to the final research camp in combination with higher altitude exposure resulted in markedly
403 higher RPE scores at 5140 m. However, although exercise-induced anorexia occurs acutely in response to
404 strenuous exercise, tightly controlled laboratory studies suggest that this does not affect appetite perceptions and
405 ghrelin concentrations during the next day (King et al. 2015). Therefore the suppression of appetite at 5140 m is
406 unlikely to be due to greater exertion during trekking on the previous day. Furthermore, increased energy
407 expenditure from the greater trekking distance and the continued suppression of energy intake suggests that
408 participants would have been in their greatest energy deficit at the final research camp. This would be expected

409 to increase appetite under sea level conditions (Alajmi et al. 2016) and provides further support for a genuine
410 altitude-mediated suppression of appetite. Third, the current study did not assess hydration status during the trek,
411 therefore it is possible that the observed reduction in body mass could be partly attributed to dehydration.
412 However, it should be noted that reductions in skinfold and girth measurements were observed during the trek
413 which suggests that the changes in body mass were at least partly due to genuine reductions in fat mass and muscle
414 mass. Although the estimation of body composition using skinfold and girth measurements contains limitations,
415 this was deemed to be the most practical and achievable method of assessment considering the extreme research
416 environment encountered in the present study. Additionally, mean fluid intake on the day before each fixed camp
417 was $>3.6 \text{ L}\cdot\text{day}^{-1}$ which makes it unlikely that the participants experienced severe dehydration. It is not expected
418 that the higher fluid intake during the trek, compared with baseline, would have influenced the measured ghrelin
419 constituents given that gastric distension from water ingestion does not appear to influence plasma ghrelin
420 concentrations (Tschop et al. 2000).

421 In conclusion, this study represents the first investigation of circulating ghrelin constituents in response
422 to terrestrial altitude exposure and provides a time course of the changes in appetite, energy intake and body
423 composition during gradual trekking ascent to high altitude. These findings demonstrate consistently reduced
424 energy intake during high altitude exposure and an incremental reduction in appetite perceptions with increasing
425 altitude. These changes were associated with reductions in circulating concentrations of acylated and total ghrelin
426 during the trek but differences in the time course of these responses suggests that additional factors are also
427 involved.. A negative energy balance during the trek caused reductions in body mass and lower body muscle mass
428 which may have negative consequences for physical performance. Future investigations are required to develop
429 nutritional and/or physiological interventions to maintain appetite, energy intake and muscle mass at altitude.

430

431

432 **CONFLICT OF INTEREST**

433 No conflicts of interest, financial or otherwise, are declared by the author(s). The content of this manuscript is
434 solely the responsibility of the authors and does not necessarily represent the official views of the Defence Medical
435 Services.

436

437 **ETHICAL APPROVAL**

438 All procedures performed in this study were in accordance with the ethical standards of the institutional and
439 Ministry of Defence research ethics committees and with the 1964 Helsinki declaration and its later amendments
440 or comparable ethical standards.

441

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625 **Figure 1** Energy intake (a), palatability score (b) and composite appetite score (c) at baseline, 3619 m, 4600 m
626 and 5140 m. *significant difference from baseline. ‡significant difference between 4600 m and 5140 m.
627 §significant difference between 3619 m and 5140 m (One way ANOVA; $P < 0.05$ after post-hoc analyses). Values
628 are mean (SE), $N = 12$ for energy intake and palatability, $N = 11$ for composite appetite score

629

630

631 **Figure 2** Acylated ghrelin (a), des-acylated ghrelin (b), AG:DG ratio (c) and total ghrelin (d) concentrations at
632 baseline, 3619 m, 4600 m and 5140 m. *significant difference from baseline (One way ANOVA; $P < 0.05$ after
633 post-hoc analyses). Values are mean (SE), $N = 11$

634

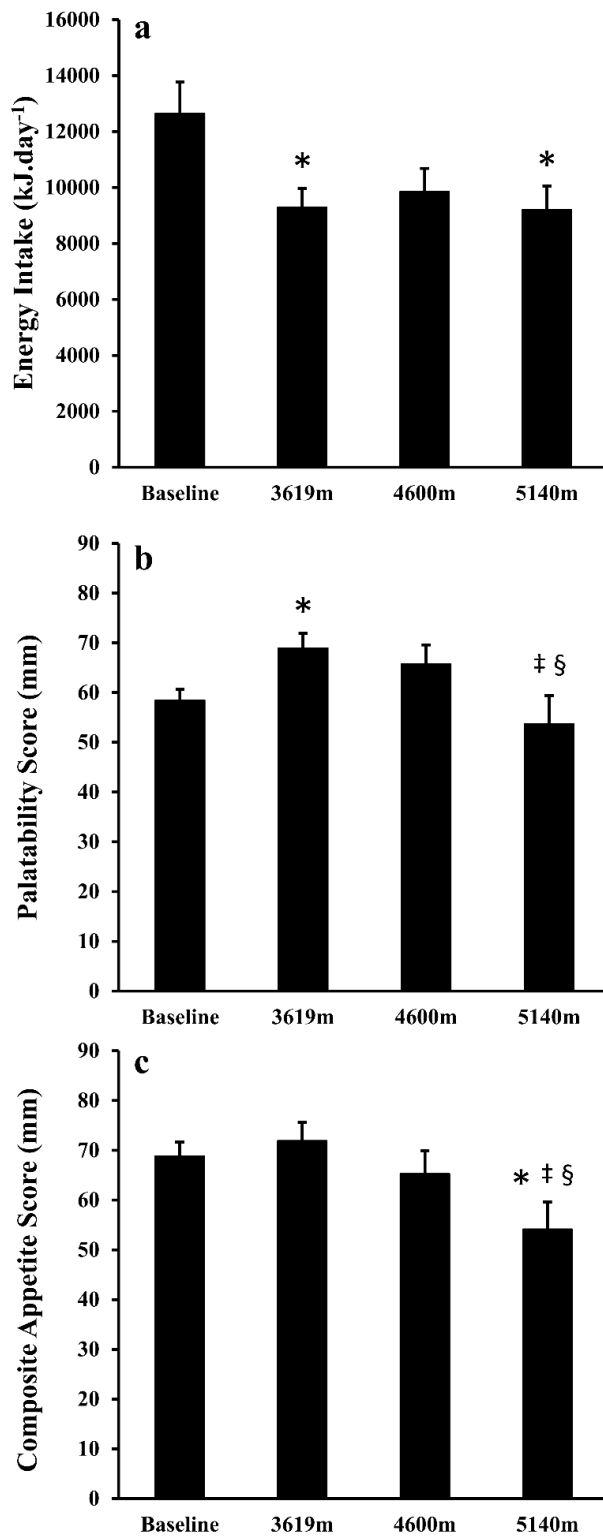
635 **Table 1** Body composition measurements at baseline, 3619 m, 4600 m and 5140 m.

| | Baseline | 3619 m | 4600 m | 5140 m |
|-------------------------|-------------|--------------|--------------------------|--------------------------|
| Body mass (kg) | 71.3 (10.3) | 73.1 (10.2)* | 70.8 (10.7) [†] | 71.1 (10.0) [§] |
| Sum of 8 Skinfolts (mm) | 81.2 (23.7) | 74.2 (22.3)* | 73.5 (21.7)* | 75.5 (23.9)* |
| Calf girth (cm) | 38.1 (1.9) | 37.5 (2.1) | 37.2 (2.2)* [†] | 36.9 (2.1)* [§] |
| Waist girth (cm) | 77.5 (6.6) | 78.2 (5.5) | 77.3 (5.5) | 76.6 (5.4) ^{‡§} |
| Relaxed arm girth (cm) | 29.5 (3.0) | 28.6 (3.0)* | 28.8 (3.1)* | 28.9 (2.9) [§] |
| Flexed arm girth (cm) | 30.6 (3.2) | 30.2 (3.2) | 30.1 (3.2) | 30.1 (3.1) |

636 Values are mean (SD), N = 12. *significant difference from baseline. [†]significant difference between 3619 m and
637 4600 m. [‡]significant difference between 4600 m and 5140 m. [§]significant difference between 3619 m and 5140 m
638 (One way ANOVA; P < 0.05 after post-hoc analyses)

639

640 Figure 1



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