Effects of prolonged hypoxia and bed rest on appetite and appetite-related hormones

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Abstract

Environmental hypoxia and inactivity have both been shown to modulate appetite. To elucidate the independent and combined effects of hypoxia and bed rest-induced inactivity on appetite-related hormones and subjective appetite, eleven healthy, non-obese males underwent three experimental interventions in a cross-over and randomized fashion: 1) Hypoxic confinement combined with daily moderate-intensity exercise (HAMB, FiO2 = 0.141 ± 0.004; PiO2 = 90.0 ± 0.4 mmHg) 2) Bed rest in normoxia (NBR, FiO2 = 0.209; PiO2 = 133.1 ± 0.3 mmHg) and 3) Bed rest in hypoxia (HBR, FiO2 = 0.141 ± 0.004; PiO2 = 90.0 ± 0.4 mmHg). A mixed-meal tolerance test (MTT), followed by an ad libitum meal were performed before (Pre) and after 16-days (Post) of each intervention. Composite satiety scores (CSS) during the MTT were calculated from visual analogue scores, while fasting and postprandial concentrations of total ghrelin, peptide YY (PYY), glucagon-like peptide-1 (GLP-1) and leptin were quantified from arterialized-venous samples. Postprandial CSS were significantly lower at Post compared to Pre in NBR only (P < 0.05) with no differences observed in ad libitum meal intakes. Postprandial concentrations and incremental area under the curve (AUC) for total ghrelin and PYY were unchanged following all interventions. Postprandial GLP-1 concentrations were only reduced at Post following HBR (P < 0.05) with resulting AUC changes being significantly lower compared to HAMB (P < 0.01). Fasting leptin was reduced following HAMB (P < 0.05) with no changes observed following NBR and HBR. These findings suggest that independently, 16-day of simulated altitude exposure (~4000 m) and bed rest-induced inactivity do not significantly alter subjective appetite or ad libitum intakes. The measured appetite-related hormones following both HAMB and HBR point to a situation of hypoxia-induced appetite stimulation, although this did not reflect in higher ad libitum intakes.

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1. Introduction

Appetite reduction and decreased dietary intakes are consistently reported in lowlanders exposed to high altitudes (Benso et al., 2007; Kalson et al., 2010; Westerterp & Kayser, 2006). The so called “altitude anorexia” was initially thought to be a consequence of acute mountain sickness and other altitude-related factors (cold, dehydration, etc.) (Hackett & Roach, 2001). However, accumulating evidence from well-controlled laboratory investigations suggests that hypoxemia per se provokes appetite changes (Bailey et al., 2015; Wasse, Sunderland, King, Batterham, & Stensel, 2012; Westerterp-Plantenga et al., 1999).

While altitude-related appetite modulation is not entirely understood, hypoxia-induced alterations in hormonal appetite regulation might be among the key factors (Kayser & Verges, 2013;
Changes in several appetite-related gut hormones and adipokines were demonstrated as a consequence of terrestrial (Riepl et al., 2012) or simulated altitude (Wasse et al., 2012) exposures (3500–4000 m altitude). While short-term (≤48 hrs) hypoxia seems to blunt appetite via reductions of the appetite-related hormone ghrelin (Bailey et al., 2015; Shukla et al., 2005) and increases in the satiety-related adipokine leptin (Shukla et al., 2005; Snyder, Carr, Deacon, & Johnson, 2008), longer (≥7-day) hypoxic exposures provoked conflicting outcomes (Debevec, Simpson, Macdonald, Eiken, & Mekjavic, 2014b; Shukla et al., 2005). In addition, the role of peptide YY (PYY) (Wasse et al., 2012) and glucagon-like peptide 1 (GLP-1) (Morishima & Goto, 2016; Snyder et al., 2008) on hypoxia-related appetite changes is unresolved (Bailey et al., 2015; Debevec et al., 2014b).

Inactivity has also been shown to affect appetite and nutritional status of humans (Bergouignan et al., 2010; Gretebeck, Schoeller, Gibson, & Lane, 1995; Stein et al., 1999). Significant appetite reductions have been observed following prolonged (Bergouignan et al., 2010; Blanc et al., 1998), but not short-term (Stubbs et al., 2004) inactivity. Whether these changes are associated with altered hormonal markers related to appetite modulation is currently unclear. To-date, studies provided conflicting results for inactivity-induced effects on circulating GLP-1 (Bergouignan et al., 2010; Nielsen et al., 2016) and leptin (Guerra et al., 2014; Kanikowska et al., 2010), whereas no changes were noted in ghrelin (Kanikowska et al., 2010) and PYY (Bergouignan et al., 2010).

Effects of combined hypoxia and inactivity on appetite-related hormones have not yet been investigated. Appetite alterations as a consequence of these two factors have important implications for both clinical populations and future space exploration. In particular, numerous hospitalised patients are bedridden for prolonged time-periods and are concomitantly hypoxic due to underlying medical conditions (e.g., pulmonary disease, heart failure). Simultaneous microgravity-related unloading and hypoxia are especially important from a future manned space exploration perspective given that such conditions are envisaged within the planetary habitats (Bodkin, Escalera, & Bocam, 2006). In both settings, understanding of appetite modulation is of critical importance for preservation of optimal nutritional status (Smith & Zwart, 2008) and consequent prevention of cardiovascular and musculoskeletal deconditioning (Pavy-Le Traon, Heer, Narici, Rittweger, & Vernikos, 2007; Puthucheary et al., 2013).

Given the scarcity of prolonged studies investigating hypoxia-induced appetite alternations, we aimed to elucidate the independent and combined effects of 16-day hypoxic exposure and bed rest-induced inactivity on appetite modulation. To this end, we assessed fasting and postprandial appetite-related hormonal markers and subjective appetite responses before and after three experimental interventions: 1) Hypoxia confinement combined with moderate-intensity exercise 2) Bed rest in normoxia and 3) Bed rest in hypoxia. We hypothesized that: i) Hypoxia and inactivity would independently, blunt appetite, reduce ad libitum intake and result in alterations in appetite-related hormonal markers, ii) The addition of hypoxia to inactivity would further reduce subjective appetite and ad libitum intake, and impact on appetite-related hormonal markers.

2. Method

2.1. Study outline

The present study was part of a larger research project (PlanHab – Planetary habitat simulation) investigating the independent and combined effects of exposure to normobaric hypoxia and bed rest-induced inactivity on modulation of a multitude of physiological systems in healthy humans. Detailed methodological outline of the PlanHab project, performed at the Olympic Sport Centre Planica research facility (Ratece, Slovenia), has been reported elsewhere (Debevec et al., 2014a; Simpson, Debevec, Eiken, Mekjavic, & Macdonald, 2016). In summary, the participants underwent three experimental interventions in a cross-over designed and randomized manner: 1) Normobaric hypoxic confinement combined with daily moderate-intensity exercise (HAMB, Inspired O2 fraction (FiO2) = 0.141 ± 0.004; Inspired O2 partial pressure (P(O2)) = 90.0 ± 0.4 mmHg; -4000 m simulated altitude) to assess the effects of hypoxia per se 2) Bed rest in normoxia (NBR, FiO2 = 0.209; P(O2) = 133.1 ± 0.3 mmHg) to investigate the independent effect of bed rest-induced unloading/inactivity and 3) Bed rest in hypoxia (HBR, FiO2 = 0.141 ± 0.004; P(O2) = 90.0 ± 0.4 mmHg) to investigate the combined effects of inactivity and hypoxia. During each intervention the participants underwent their designated condition (HAMB, NBR or HBR) for exactly 21-days. The participants were accommodated in the facility seven days before, to enable participants’ acclimation to the facility and to obtain all baseline measures and four days after each intervention to obtain the post-intervention measurements. The present paper describes a study performed before each intervention (Pre) and on day 17 of each respective intervention (Post) that aimed to assess the independent and combined effects of hypoxia and inactivity on perceived appetite as well as fasting and postprandial levels of selected appetite-related hormonal markers. The participants were under strict dietary and activity control two days prior to the Pre intervention test. A four-month washout period was implemented between the interventions. The participants were not supervised during both washout periods but were requested to maintain comparable habitual activities and diets.

The normobaric hypoxic environment within the designated rooms and common hypoxic area was generated and maintained using a Vacuum Pressure Swing Adsorption system (b-Cat, Tiel, The Netherlands). The ambient air O2 and CO2 fractions were sampled and analysed using calibrated analysers at 15-min intervals. Immediate adjustments were made in the event of a ≥0.5% O2 variation. The average levels of CO2 during the interventions within the hypoxic area were 0.23 ± 0.07% but did not exceed 0.45%. For security purposes the participants had portable ambient O2 concentration analysers (Rae PGM-1100, California, USA) in close proximity throughout hypoxic interventions.

During the HAMB intervention the participants were allowed to move freely in the common hypoxic area and performed two moderate-intensity, exercise sessions (30-min) daily to mimic their habitual activity levels as detailed previously (Debevec et al., 2014a). Participants were confined to strict horizontal bed rest (Pavy-Le Traon et al., 2007) during both NBR and HBR interventions. The study was approved by the National Committee for Medical Ethics at the Ministry of Health of the Republic of Slovenia and registered at ClinicalTrials.gov (NCT02293772). All experimental procedures conformed to the principles of the Declaration of Helsinki.

2.2. Participants

Participants’ selection procedure was based on the bed rest protocol recommendations of the European Space Agency (Standardization of bed rest study conditions 1.5, August 2009). In addition to the general inclusion/exclusion criteria outlined in the above-mentioned document, the potential participants were selected among near sea-level residents (≤500 m). Vegetarians, vegans and those exposed to altitudes ≥2000 m within the last two
months were also ineligible. Finally, fourteen healthy, non-obese, male participants were recruited and provided written and oral informed consent before the onset of the study. Three participants withdrew from the study after completing two interventions and have been excluded from the analyses. Accordingly, eleven, moderately active participants with a mean age of 27 ± 6 y, body height of 180 ± 3 cm, body mass of 77 ± 12 kg, body fat of 21 ± 5%, body mass index of 23.7 ± 3.0 kg m⁻² and maximal oxygen uptake of 44.3 ± 6.1 ml kg⁻¹ min⁻¹ completed all three interventions and only their data are reported in the present paper.

2.3. Diet

Detailed dietary methodology and nutritional assessment has been reported previously (Debevec et al., 2014a). In short, the participants were provided with a standardised and individually tailored diet throughout all three interventions (five meals per day), starting with their arrival to the facility seven day prior to the start of each respective intervention. A 14-day meal plan, comprising common Slovenian foods, was provided and repeated during all interventions. Importantly, the same daily menu was employed during each intervention to ensure that the participants consumed identical meals on the same days prior to each assessment. The targeted macronutrient diet composition, expressed as percentage of total dietary energy intake was ~30% fat, ~54% carbohydrates and ~16% protein. Prior to each intervention, individualized energy requirements were calculated using the modified Harris-Benedict resting metabolic rate equation (Hasson, Howe, Jones, & Freedson, 2011). Physical activity factors of 1.4 for the HAMB and 1.2 for the NBR and HBR were used to account for the different activity levels. The food was served in weighed portions and participants were encouraged to consume all of the provided food. The actual daily intakes for each individual were recorded and analysed using an on-line nutritional analysis system OPKP (Open Platform for Clinical Nutrition, Jozef Stefan Institute, Ljubljana, Slovenia).

2.4. Experimental protocol

As noted earlier, the protocol was performed in the morning at the same time-of-day before (Pre) and following 16 days (Post) of each respective intervention. The protocol comprised of a mixed meal tolerance test (MTT) followed immediately by an ad libitum meal. The participants received the same meals during the ante- cedent day with the last meal being provided exactly 12 h prior to the start of protocol. While at Pre, the protocols were all performed in ambient (normoxic; FIO2 = 0.209) conditions, the protocols at Post, during both HAMB and HBR, were performed in the normobaric hypoxic conditions (FIO2 = 0.141 ± 0.004). During the NBR and HBR interventions, the participants were transferred to the laboratory on a gurney to maintain supine position at all times. Participants’ whole body mass and body composition were assessed before and after each intervention using a gurney incorporating calibrated load cells (Sigma 6C, Libels ELSI, Celje, Slovenia) and dual-energy X-ray absorptiometer (DXA; Discovery W – QDR series, Hologic, Bedford USA), respectively.

2.5. Mixed meal tolerance test

Upon arrival at the laboratory, the overnight-fasted participants rested supine with their hand positioned in a heated hand-warming unit (University of Nottingham, Nottingham, UK), which maintained an air temperature of –55 °C. An intravenous cannula (20G Venflon, Becton Dickinson Infusion Therapy, Helsingborg, Sweden) was inserted retrograde into a dorsal hand vein for arterialized-venous blood sampling. The cannula patency was maintained using a slow-running 0.9% saline infusion (Baxter Healthcare, Thetford, U.K.) throughout the protocol. Following fasting samplings collection, the participants consumed a standardised mixed-nutrient milkshake meal (5 ml kg⁻¹; Ensure Plus, Abbott nutrition laboratories Ltd., Maidenhead, U.K.) with an energy value of 1.5 kcal ml⁻¹ and a macronutrient composition of 57% of total energy provided by Carbohydrates, 28% by Fat and 15% by Protein. Blood samples and perceived appetite sensations were collected periodically over the following 120-min postprandial period.

2.6. Blood sample processing and analysis

Two five ml baseline blood samples were obtained before the meal ingestion (~15 and ~5 min). The postprandial samples (3 ml) were thereafter collected at 20-min intervals throughout the MTT (20, 40, 60, 80, 100, and 120 min). The plasma concentrations of total ghrelin, PYY and GLP-1, were determined at all sampling points, while the concentrations of serum leptin and adiponectin were determined from the fasting samples only. The blood samples for total ghrelin and PYY determination were collected into a pre-cooled EDTA tube (Vacutainer K2E, Becton Dickinson, Plymouth, U.K.) containing 50 μl of aprotinin (Nordic Group, Paris, France). A pre-cooled EDTA tube containing 50 μl of dipeptidyl peptidase-4 (Millipore, Bilerica, USA) was used for GLP-1 sample collection. The samples were immediately centrifuged (3500 rpm; 10-min @ 4 °C; Centric 200R, Tehtnica, Zelezniki, Slovenia). The remaining two ml of blood, obtained during both baseline samplings, were collected into a separate tube (Vacutainer SST II Advance tube, Becton Dickinson, Plymouth, U.K.) for serum leptin and adiponectin determination and was centrifuged following 15-min clotting period at room temperature. The obtained plasma and serum were pipetted into microtubes (Microtube Cap, Sarstedt, Nümbrecht, Deutschland) and frozen at ~80 °C immediately after the MTT for subsequent analysis. The analysis of all samples was performed in duplicate using the methods outlined below. The concentrations of total ghrelin, PYY, leptin and adiponectin were determined using appropriate radio-immunoassays (EMD Millipore, Billerica, U.S.). The total GLP-1 concentrations were determined using sandwich ELISA (EMD Millipore, Billerica, U.S.), comprising amide acid GLP-1 (7–36 and 7–37). The inter and intra-assay CVs for total ghrelin, PYY, GLP-1, leptin and adiponectin were 9.1%, 6.7%, 4.9%, 1.0%, 8.6% and 4.1%, 6.3%, 6.2%, 4.6%, 7.4%, respectively.

2.7. Perceived appetite evaluation

Perceived appetite sensations (‘how full do you feel’, ‘how strong is your desire to eat’, ‘how hungry do you feel’ and ‘how much food do you think you could eat’) were assessed using a custom-designed visual analogue score (VAS) application on an iPad tablet (Apple, Cupertino, USA) (Stubbs et al., 2000). Handheld electronic devices for VAS collection have previously been validated (Gibbons, Caudwell, Finlayson, King, & Blundell, 2011). The VAS scale was a 100 mm digital line, anchored on the left with ‘sensation not felt at all’ and on the right with ‘sensation felt the greatest’. The participants placed a line according to their perceived feeling at that particular point in time. The VAS were assessed before and at 30-min intervals throughout the MTT. The composite satiety score (CSS) was subsequently calculated from the individual appetite scores using the following equation:

\[
CSS = \frac{(Fullness + (100 – Desire to eat) + (100 – Hunger) + (100 – Prospective food consumption))}{4}
\]
A higher CSS score indicated greater satiety and a lower score indicated the converse.

2.8. Ad libitum meal

The participants were given an ad libitum pasta-based test meal immediately following cessation of the MTT. The meal was prepared in the metabolic kitchen of the facility under standardised conditions and comprised cooked dried white pasta (Penne Rigate, Barilla, Parma, Italy), tomato and basil sauce (Barilla, Parma, Italy), olive oil (Classico, Nonini, Spoleto, Italy) and grated mozzarella cheese (Mu-Cuisine, Ljubljanske milekarne, Ljubljana, Slovenia). The average energy value of the pasta-meal was 1.3 kcal g⁻¹ with 37% of total energy derived from fat, 48% from carbohydrate, and 16% from protein. The participants were told to eat until they felt comfortably full and were provided with water ad libitum throughout the meal. The pasta-meal plate was re-filled before it became empty in order to encourage the participants to eat until fullness and to avoid the cue for cessation of eating being an empty plate. To limit social influence, the meal was always consumed in isolation within the laboratory with the same researcher providing additional food during all test meal sessions.

2.9. Data analysis and statistics

Data analysis was performed using statistical package SPSS (version 21.0). The data were coded before processing and checked for normality of distribution using the criteria of skewness and kurtosis Z-score between −1.96 and 1.96. Two parametric or non-parametric data points were compared using paired students t-test or Wilcoxon signed ranks, respectively. One-way ANOVA analysis was used to compare variables at a single time point across the three interventions and to analyse the changes in postprandial responses compared to fasting values within interventions at each visit. Postprandial profiles of variables within the three interventions (Pre vs. Post) and the differences between the interventions in changes of postprandial variable profiles (Δ Pre-Post) were examined using 2-way repeated measures ANOVA. Two-way ANOVA analysis was also employed for the post-hoc comparisons of the NBR and HBR interventions to elucidate the independent effects of hypoxia during bed rest (NBR vs. HBR) and of the HAMB and HBR to determine the effect of activity levels during hypoxic exposures. Violation of the assumption of sphericity was assessed using Mauchly's test, and where appropriate the Greenhouse-Geisser statistic was used to determine significance. Incremental area under the postprandial curve (AUC) during MTT was calculated for CSS, total ghrelin, PYY and GLP-1, using the conventional trap-ezoid rule. The amount of food eaten by each individual during the ad libitum meal was correlated to the CSS values obtained at the end of the respective MTT. Pearson's correlation coefficient was employed to analyse the bivariate correlations between select variables. Relationships were considered significant at P < 0.05. Based on previous reports (Bailey et al., 2015; Wasse et al., 2012) we estimated that a sample size of eleven is sufficient to obtain power > 0.80 for the assessment of ad libitum intakes and perceived appetite. An α priori power calculation, using our previous data set (Debevec et al., 2014b) for the main hormonal outcome (total ghrelin) with an alpha value of 0.05, indicated that a sample size of ≥ 9 participants was required to obtain ≥ 0.80 power for the assessment of the effects of hypoxia or activity per se. Data in the text and Tables are displayed as means ± standard deviations, while the data in the Figures are displayed as means ± standard error of the mean.

3. Results

3.1. Dietary intakes and general adaptation

As reported previously (Debevec et al., 2014a), the targeted daily energy intakes were 2558 ± 226 kcal for the HAMB and 2139 ± 193 kcal for the NBR and HBR interventions. The actual intakes were lower than targeted in all interventions (HAMB: −14%; NBR: −5%; HBR: −6%; P < 0.01). Changes in body mass and body composition (Debevec et al., 2014a) as well as insulin sensitivity and circulating lipid data (Simpson et al., 2016) from the PlanHab project have been published previously. Briefly, whole body mass decreased following all interventions (HAMB Pre: 76.6 ± 14.5 kg, Post: 73.4 ± 13.3 kg; NBR Pre: 74.9 ± 10.9 kg, Post: 72.8 ± 9.9 kg; HBR Pre: 76.1 ± 10.0 kg, Post: 72.5 ± 10.9 kg; P < 0.01) with no within-intervention differences. The whole body mass reduction was a consequence of a reduction in fat free mass (HAMB = −5%; NBR = −4%; HBR = −5%; P < 0.01) with no differences observed in fat mass following the interventions (P = 0.19).

In regards to the general adaptation, the morning fasting heart rate responses were significantly higher during both hypoxic interventions (HAMB: 68 ± 3; HBR: 72 ± 2 beats min⁻¹) as compared to NBR (60 ± 2; P < 0.05). In contrast, capillary oxyhemoglobin saturation was, significantly lower (P < 0.01) during both the HAMB (88 ± 1%) and HBR (88 ± 2%) than during the NBR (97 ± 2%). Acute mountain sickness was observed during the first two days in three participants in the HAMB and five participants in the HBR intervention.

3.2. Perceived appetite

As noted in Fig. 1, no differences in fasting CSS were observed between the three interventions both at Pre and at Post (P = 0.19). A trend for postprandial satiety to be maintained for longer before the NBR, as opposed to HBR, was noted (P = 0.07). No differences were observed in the postprandial CSS values between the three interventions at Post (P = 0.94). A significant decrease in postprandial CSS was noted at Post compared to Pre in the NBR only (P < 0.05; Fig. 1). Changes in postprandial CSS AUC were comparable between interventions (P = 0.14; Fig. 5A).

3.3. Ad libitum meal consumption

Intakes during the ad libitum meal are shown in Table 1. No differences in intakes between the three interventions were observed both at Pre and at Post (P = 0.60). Also, no differences were noted between Pre and Post intakes both between (P = 0.59) and within the interventions (P = 0.53). The ad libitum meal intakes were significantly related to the pooled CSS obtained before and after each intervention (r = 0.27; P < 0.01).

3.4. Total ghrelin

Fasting total ghrelin concentrations were comparable before all interventions (P = 0.42; Table 2). At Pre, the consumption of the MTT meal resulted in a reduction of total ghrelin, with the median nadir time of 100-min for all interventions (range 60–120 min; Fig. 2). The mean nadir for HAMB, NBR and HBR was 609 ± 178 pg ml⁻¹, 542 ± 170 pg ml⁻¹, 538 ± 150 pg ml⁻¹, respectively with no differences between the interventions (P = 0.12). Significantly higher fasting total ghrelin values were observed at Post following both hypoxic interventions (P < 0.05; Table 2) with no changes following the NBR (P = 0.55). Postprandial total ghrelin values were significantly higher at Post compared to Pre in HBR (P < 0.05; Fig. 2), with a similar trend observed in HAMB.
Values are mean ± SD.

**Table 2**
Fasting and change (Δ) values of select hormonal appetite markers obtained before (Pre) and following 16 days (Post) of hypoxic confinement combined with daily moderate-intensity exercise (HAMB); bed rest in normoxia (NBR) and bed rest in hypoxia (HBR) interventions.

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAMB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ghrelin (pg mL⁻¹)</td>
<td>778 ± 289</td>
<td>854 ± 296*</td>
<td>76 ± 102</td>
</tr>
<tr>
<td>GLP-1 (pmol L⁻¹)</td>
<td>2.14 ± 1.15</td>
<td>1.69 ± 0.51</td>
<td>-0.45 ± 1.09</td>
</tr>
<tr>
<td>Leptin (µg L⁻¹)</td>
<td>4.49 ± 2.98c</td>
<td>3.45 ± 2.89h</td>
<td>-1.04 ± 1.40</td>
</tr>
<tr>
<td>Adiponectin (µg mL⁻¹)</td>
<td>6.84 ± 2.51</td>
<td>5.41 ± 1.77a</td>
<td>-1.43 ± 1.40</td>
</tr>
<tr>
<td>NBR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ghrelin (pg mL⁻¹)</td>
<td>783 ± 258</td>
<td>796 ± 246</td>
<td>+13 ± 82</td>
</tr>
<tr>
<td>GLP-1 (pmol L⁻¹)</td>
<td>103 ± 36b</td>
<td>114 ± 42</td>
<td>+11 ± 30</td>
</tr>
<tr>
<td>Leptin (µg L⁻¹)</td>
<td>2.92 ± 1.54</td>
<td>3.26 ± 1.90</td>
<td>+0.35 ± 0.72</td>
</tr>
<tr>
<td>Adiponectin (µg mL⁻¹)</td>
<td>6.38 ± 2.08</td>
<td>5.02 ± 1.63</td>
<td>-1.36 ± 0.81</td>
</tr>
<tr>
<td>HBR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ghrelin (pg mL⁻¹)</td>
<td>761 ± 241</td>
<td>851 ± 254*</td>
<td>+91 ± 87</td>
</tr>
<tr>
<td>GLP-1 (pmol L⁻¹)</td>
<td>129 ± 46</td>
<td>135 ± 41</td>
<td>+6.3 ± 24</td>
</tr>
<tr>
<td>Leptin (µg L⁻¹)</td>
<td>5.19 ± 4.32c</td>
<td>4.43 ± 3.69</td>
<td>-0.76 ± 1.38</td>
</tr>
<tr>
<td>Adiponectin (µg mL⁻¹)</td>
<td>6.73 ± 1.98</td>
<td>4.99 ± 1.60</td>
<td>-1.74 ± 1.09</td>
</tr>
</tbody>
</table>

Values are mean ± SD. PYY, peptide YY; GLP-1, glucagon-like peptide-1. For Δ changes, + denotes increase and − denotes a decrease from Pre.

Denotes significant differences compared to Pre values (P < 0.05).

Denotes significant differences compared to corresponding HBR value (P < 0.05).

Denotes significant differences compared to corresponding NBR value (P < 0.05).

### 3.5. Peptide YY

Fasting PYY concentrations were significantly higher at Pre in the HBR as compared to both HAMB and NBR (P < 0.01; Table 2). Consumption of the MTT meal at Pre resulted in a rise in PYY values with a median peak time of 40-min (range 20–120 min) and a mean value of 137 ± 39.6 pg mL⁻¹, 149 ± 55.4 pg mL⁻¹ and 187 ± 60.3 pg mL⁻¹ for the HAMB, NBR and HBR, respectively (Fig. 3). The mean peak PYY value was significantly lower before NBR (P < 0.05; Fig. 3), and tended to be lower before HAMB (P = 0.07; Fig. 3), than before HBR (Fig. 3). Compared to Pre the PYY fasting values as well as postprandial peak and time to peak were not significantly different following all interventions (Table 2 & Fig. 3). No differences were also observed in the postprandial PYY profile (Δ Pre-Post; P = 0.29) and AUC (P = 0.15; Fig. 5C) changes between the three interventions.

### 3.6. Glucagon-like peptide-1

Fasting GLP-1 concentrations were comparable before the interventions (P = 0.28; Table 2). The MTT meal consumption induced a rapid rise in plasma GLP-1 with a median time of 20 min, to a mean peak of 7.02 ± 1.25 pmol L⁻¹, 9.92 ± 10.86 pmol L⁻¹ and 10.70 ± 6.16 pmol L⁻¹ before the HAMB, NBR and HBR intervention, respectively (Fig. 4). No differences were noted between these peak values (P = 0.43). While no differences in fasting GLP-1 values were noted at Post compared to Pre, the postprandial values were significantly lower following HBR (P < 0.05; Fig. 4) with a trend for a reduction observed following the NBR (P = 0.07; Fig. 4). No significant differences in the postprandial GLP-1 response, from that seen at Pre, were noted following HAMB (P = 0.13). The change in GLP-1 postprandial incremental AUC was significantly lower following the HBR than the HAMB, indicating lower postprandial GLP-1 response (P < 0.01; Fig. 5D). Similarly, a significantly greater change in the postprandial GLP-1 profile (Δ Pre-Post) was observed in HBR compared to HAMB.
3.7. Leptin

Fasting leptin concentrations were significantly lower before the NBR compared to both HAMB and HBR \( (P < 0.05; \text{Table 2}) \). Leptin was significantly reduced at Post, compared to Pre following the HAMB \( (P < 0.05) \), with no changes observed following the NBR \( (P = 0.14) \) and HBR \( (P = 0.11) \) interventions. When the (Pre-Post) changes in fasting leptin were compared between the two bed rest interventions, a significant effect of hypoxia was noted \( (P < 0.05; \text{Table 2}) \). No correlations for the pooled or within intervention changes in fasting leptin and changes in whole body fat mass were noted in the present study \( (P > 0.1) \).

3.8. Adiponectin

Fasting adiponectin values were comparable before all three interventions \( (P = 0.643; \text{Table 2}) \). A significant reduction in adiponectin at Post compared to Pre was noted following all three interventions \( (P < 0.01) \). No differences between the interventions were noted in the (Pre-Post) fasting adiponectin changes \( (P = 0.67; \text{Table 2}) \).

4. Discussion

This study investigated the independent and combined effects of hypoxia and bed rest-induced inactivity on appetite and select appetite-related hormones. In contrast to the initial hypothesis, hypoxia per se did not significantly alter subjective appetite or ad libitum intakes. Interestingly, the increased fasting total ghrelin and decreased leptin concentration following HAMB even point to a situation of hormonal appetite stimulation. We did not observe any independent effects of bed rest-induced inactivity on appetite or appetite-related hormonal markers. While the combined exposure to hypoxia and inactivity did not alter subjective appetite measures it, similar to hypoxia alone, provoked changes in the measured...
hormonal markers that could lead to appetite stimulation (i.e. increased fasting and postprandial total ghrelin and decreased postprandial GLP-1).

While previous studies demonstrated reduced appetite and dietary intakes in response to acute (Bailey et al., 2015; Kalson et al., 2010; Wasse et al., 2012) and prolonged (Benso et al., 2007; Westerterp-Plantenga et al., 1999) hypoxia, no changes in subjective appetite and ad libitum meal intakes were noted in the present study following 16-days of normobaric hypoxic exposure. Although somewhat surprising, these data corroborate our previous investigations reporting unaltered appetite following 10-day hypoxic exposure at the same (simulated) altitude (Debevec et al., 2014b; Mekjavic et al., 2016). The employed intensity/dose of hypoxia might provide a potential explanation for the ambiguous outcomes. In particular, all long-term investigations reporting blunted appetite (Benso et al., 2007; Westerterp-Plantenga et al., 1999) and consequent weight-loss (Reynolds et al., 1999; Westerterp, Kayser, Wouters, Le Trong, & Richalet, 1994) have utilized high/extreme...
Modulation of the satiety-signalling peptide PYY, by both hypoxia and inactivity, is not well characterised. While a tendency for suppressed circulating PYY upon acute hypoxia has been shown (Wasse et al., 2012), no changes were observed as a result of 30 and 60 days of bed rest (Bergouignan et al., 2010). In the current study, no significant changes in fasting and postprandial PYY values were noted as a result of any intervention. The data from HAMB and HBR interventions, along with the lack of PYY changes observed in other acute (Bailey et al., 2015) and long-term (Debevec et al., 2014b) studies, suggest that PYY does not play a role in hypoxia-induced appetite modulation. The lack of PYY changes during the NBR corroborates previous findings from long-term bed rest investigations (Bergouignan et al., 2010) and collectively suggests that PYY might not be significantly affected by unloading/inactivity and/or does not modulate inactivity-related appetite alterations. It is of note however, that higher baseline PYY concentrations were observed before the HBR compared to both, HAMB and NBR interventions. Although we are unable to fully explain these observations, it seems likely that external factors such as changes in diet and/or activity of participants prior to entering each campaign might have influenced the PYY and, as noted later, also the leptin baseline values. Although we aimed to minimize these influences by randomized assignments to interventions and two-day activity as well as dietary control before each intervention, these differences need to be considered when interpreting the observed differences.

GLP-1 is thought to promote satiety through several mechanisms, including slowing gastric emptying and enhancing insulin biosynthesis and secretion (Baggio & Drucker, 2007). Although the effect of hypoxia on GLP-1 has been investigated (Bailey et al., 2015; Morishima & Goto, 2016; Snyder et al., 2008), its role and contribution to the previously reported, complex altitude-related appetite reduction is unclear. The data from the HABM intervention confirm previous observations showing a lack of change in fasting and postprandial circulating GLP-1 in response to both acute (Bailey et al., 2015; Snyder et al., 2008), and prolonged (Debevec et al., 2014b) hypoxia. Interestingly, postprandial GLP-1 levels were significantly reduced, or tended to be reduced following HBR and NBR, respectively. It therefore seems that the bed rest-induced inactivity per se provoked these changes. While the underlying mechanisms are unclear, inactivity-induced insulin resistance, that was noted in the present study (Simpson et al., 2016) following both NBR and HBR, could be a potential factor given that insulin resistance seems to be associated with a blunted postprandial GLP-1 response (Rask et al., 2001).

Although the majority of studies suggest that hypoxia augments adipose-derived leptin concentrations (Shukla et al., 2005; Snyder et al., 2008; Tsipop, Stradburger, Hartmann, Biollaz, & Bartsch, 1998), which are thought to influence satiety signalling, this is still debated and some studies report contradictory findings (Benso et al., 2007; Debevec et al., 2014b; Sierra-Johnson, Romero-Corral, Somers, & Johnson, 2008). The response of leptin to bed rest-induced inactivity is also unclear, with some studies showing increased or unchanged levels (Bergouignan et al., 2010; Guerra et al., 2014; Zwart et al., 2009). The fasting leptin concentration did not change significantly in response to bed rest (NBR and HBR) in the present study, while a reduction as a result of hypoxia per se was observed. This hypoxia-induced leptin reduction is difficult to interpret and contrary to previous reports (Shukla et al., 2005; Snyder et al., 2008; Tsipop, 1998). Since circulating leptin is closely associated with body fat mass (Shimizu et al., 1997), we also scrutinized potential relations between changes in fat mass and leptin levels, but did not observe any correlations between the two variables. These, relatively surprising results might have been influenced by the leptin differences observed before interventions.
In particular, fasting leptin levels were significantly lower before NBR compared to both hypoxic interventions (HAMB & HBR). Again, we are unable to explain these baseline differences, but these do not seem to arise from differences in participants’ body composition (i.e. altered body fat levels), as these were comparable. Regardless, these differences need to be taken into account when interpreting the above outcomes.

Given the purported adiponectin effect on appetite modulation (Steinberg & Kemp, 2007) we included this marker in the analysis and noted a significant fall in this variable irrespective of intervention. Similarly to leptin, alterations in adiponectin concentration have been related to changes in adiposity. However, given that no correlation was observed between body fat changes and adiponectin, changes in adiposity per se cannot adequately explain the findings. Potential explanations for the bed rest-induced reduction could again include worsened insulin resistance (Blaslov, Bulum, Zidar, & Duvnjak, 2013). Interestingly, reduced adiponectin levels were also observed following HAMB, where insulin sensitivity changes were not observed (Simpson et al., 2016). Although, in contrast to our findings, increases in adiponectin have previously been reported following prolonged altitude exposure (Smith et al., 2011), the outcomes of that particular study might be confounded by changes in body fat mass. The fact that adiponectin production in humans is reduced in adipocytes under hypoxic conditions (Chen et al., 2006), might at least partly explain the reductions observed in HAMB.

As mentioned earlier, the obtained data not only have implications for future manned space exploration but are also highly pertinent to bedridden clinical populations rendered inactive and hypoxic by their underlying condition. Especially, given the potential detrimental effects of hypoxia or inactivity-induced cachexia on optimal nutritional status, as well as bone (Pavy-Le Traon et al., 2007) and muscle (Puthucheary et al., 2013) wasting. The strictly controlled environmental conditions, (in)activity levels, dietary intakes as well as monitoring of body composition (Debevec et al., 2014a) and insulin sensitivity (Simpson et al., 2016) are among the key methodological strengths of the present study. Especially given that all these factors might have importantly influenced/confounded the appetite-related outcomes of the previous, particularly long-term (field-based) studies. Nevertheless, there are a few limitations we would like to address. Firstly, confinement per se might have influenced the appetite-related outcomes given that participants were kept within the facility throughout the study and confinement/isolation-induced changes in nutritional status have previously been reported (Custaud et al., 2004). Secondly, the fact that the study was performed in normobaric rather than hypobaric hypoxia needs to be taken into account (Millet, Fais, Pialoux, Mounier, & Brugniaux, 2012), even though no appetite-related effects of pressure per se have to-date been reported. Moreover, the employed hypoxic dose (~4000 m) might have been insufficient to exert important reductions in appetite, as higher altitude threshold (~5000 m) for perturbed energy balance (Westerterp & Kayser, 2006) was suggested previously. It is also of note that total ghrelin and total PYY concentrations were determined in the present study albeit their “active” forms (i.e. acylated ghrelin and PYY3-36) have been shown to primarily underlie appetite-related effects of both hormones (Broglio et al., 2004). Finally, the use of single food item (i.e. pasta) instead of a buffet meal as well as the lack of assessment of the meal palatability, limits the strength of the ad libitum meal appetite assessment. Taken together, and given the complex appetite and food intake regulation (Hussain & Bloom, 2013), future well-controlled studies investigating acute and long-term dose-response effects of both hypoxia and inactivity on appetite modulation are thus warranted in healthy individuals and clinical cohorts.

In summary, the results of the present study suggest that 16-day exposure to ~4000 m simulated altitude and/or bed rest-induced inactivity does not significantly alter subjective appetite or ad libitum intakes. Paradoxically, the measured appetite-related hormonal markers following both hypoxia alone and hypoxia combined with bed rest-induced inactivity point to a situation of hypoxia-induced appetite stimulation. However, given that these changes were not reflect in ad libitum intake or subjective appetite changes, the observed hormonal changes might not have been the crucial factor in appetite modulation under these experimental conditions.

Conflict of interest
None.

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Disclosures
The authors have no conflicts of interest to declare.

Author contributions

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