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PlanHab study: assessment of psycho-neuroendocrine function in male subjects during 21 d of normobaric hypoxia and bed rest

C. Strewe\textsuperscript{a}, R. Zeller\textsuperscript{a}, M. Feuerecker\textsuperscript{a}, M. Hoenl\textsuperscript{a}, I. Kumpreja\textsuperscript{b, a}, A. Crispin\textsuperscript{c}, B. Johannes\textsuperscript{d}, T. Debevec\textsuperscript{b}, I. Mekjavic\textsuperscript{b}, G. Schelling\textsuperscript{a} and A. Chouker\textsuperscript{a}

\textsuperscript{a}Department of Anaesthesiology, Klinikum Großhadern, University of Munich, Stress and Immunology Lab, Munich, Germany; \textsuperscript{b}Department of Automation, Biocybernetics and Robotics, Jozef Stefan Institute, Ljubljana, Slovenia; \textsuperscript{c}Department of Biometry and Epidemiology, Klinikum Großhadern, University of Munich, Munich, Germany; \textsuperscript{d}Department of Space Physiology, Institute of Aerospace Medicine, German Aerospace Center (DLR), Cologne, Germany

ABSTRACT
Immobilization and hypoxemia are conditions often seen in patients suffering from severe heart insufficiency or primary pulmonary diseases (e.g. fibrosis, emphysema). In future planned long-duration and exploration class space missions (including habitats on the moon and Mars), healthy individuals will encounter such a combination of reduced physical activity and oxygen tension by way of technical reasons and the reduced gravitational forces. These overall unconventional extraterrestrial conditions can result in yet unknown consequences for the regulation of stress-permissive, psycho-neuroendocrine responses, which warrant appropriate measures in order to mitigate foreseeable risks. The Planetary Habitat Simulation Study (PlanHab) investigated these two space-related conditions: bed rest as model of reduced gravity and normobaric hypoxia, with the aim of examining their influence on psycho-neuroendocrine responses. We hypothesized that both conditions independently increase measures of psychological stress and enhance neuroendocrine markers of stress, and that these effects would be exacerbated by combined treatment. The cross-over study composed of three interventions (NBR, normobaric hypoxic horizontal bed rest; HRR, normobaric hypoxic horizontal bed rest; HAMB, normobaric hypoxic ambulatory confinement) with 14 male subjects during three sequential campaigns separated by 4 months. The psychological state was determined through three questionnaires and principal neuroendocrine responses were evaluated by measuring cortisol in saliva, catecholamine in urine, and endocannabinoids in blood. The results revealed no effects after 3 weeks of normobaric hypoxia on psycho-neuroendocrine responses. Conversely, bed rest induced neuroendocrine alterations that were not influenced by hypoxia.

Introduction
In the hospital setting, hypoxic states and immobilization represent regularly encountered conditions affecting health. However, healthy individuals can be exposed to acute or chronic hypoxia either through sport activities or living at high altitudes, respectively. Such hypoxic stress has been shown to have various effects on sleep, neuroendocrine pathways, respiratory, and psychological functions (Richalet, 2016; Saugy et al., 2016; Zaeh et al., 2016). Persistent immobilization, in turn, influences bone metabolism, cardiovascular functioning, and muscle composition and thus represents a stressful pathophysiological state (Arentson-Lantz et al., 2016; Yang et al., 2014).

In space research, the approved study concept of bed rest (Smith et al., 2014; Westby et al., 2016) is an accepted method used in examining effects of immobilization by suitably mimicking a microgravity state (Barratt & Pool, 2008). The study conditions can be expanded from orbital (e.g. the International Space Station) to lunar or Mars exploration class missions where future manned habitat atmospheres will likely be hypoxic in order to reduce the biological effects of radiation, the risk of fire, and to simplify extravehicular activities. Therefore, both immobilization and hypoxia represent physical stressors of scientific relevance.

In modulating stress responses as well as the attempt to maintain homeostasis under stress, hormonal systems such as the hypothalamic–pituitary–adrenocortical axis (HPA) and the catecholaminergic system play an important role (Herman et al., 2003; Tsigos & Chrousos, 2002). Additionally, the endocannabinoid system (ECS), an evolutionarily old but well-conserved part of the nervous system, regulates central and peripheral physiological functions such as memory and the vegetative state (Atsak et al., 2015). Its endogenous agents, i.e. anandamide or 2-arachidonoylglycerol (2-AG), signal through the G-protein-coupled receptor (CB1, CB2) pathway. The ECS is activated by both psychological and physical insults (Feuerecker et al., 2012; Hauer et al., 2014).
The interactions between both systems, the ECS and the HPA, are not completely understood. Previous studies reported increased glucocorticoid concentrations following activation with suppression of the ECS (Hill et al., 2009; Romero et al., 2002). An activated ECS, in turn, reduces HPA activity after stress exposure and is regulated by the HPA related feedback mechanisms to restore its baseline activity levels (Wang et al., 2012). Roberts et al. (2014) demonstrated a time-dependent biphasic interaction of both systems with an early suppression and a late increase of HPA activity through the ECS. Furthermore, compensation for ECS signaling suppression seems to display sexual dimorphism in mice yielding different coping strategies in females and males, illustrating the systems’ complex and cross-linked nature.

Space flight studies have already demonstrated psychological impairment caused by isolation and confinement (Ekuszian, 1999). Therefore, immobilization and hypoxia may also entail relevant psychological alterations, changes seen in modified functions and interactions of the different physiological systems.

As a model for future extraterrestrial habitat design under reduced gravity, the present study hypothesizes that hypoxia and bed rest can increase psycho-neuroendocrine responses with compounding effects.

Material and methods

Study design and participants

The Planetary Habitat Simulation Study (PlanHab Study) was carried out at the Olympic Sport Centre Planica (Ratece, Slovenia), situated at an altitude of 940 m from sea level. The study was approved by the Committee for Medical Ethics at the Ministry for Health of the Republic of Slovenia, respected the Declaration of Helsinki and the bed rest protocols were conducted in accordance with the criteria provided by the European Space Agency (ESA, Standardization of Bed Rest Study Conditions (Version 1.5) (ESTEC contract number 20187/06/NL/VJ) 2009). These guidelines were applied during the selection of participants. Additionally, individuals with recent exposition (< 2 months) to altitudes above 2000 m and individuals that normally reside at altitudes higher than 500 m were excluded. From the initial pool of 65 healthy male applicants, 14 were included in the trial. All subjects gave informed consent prior to enrolling in the study. Given a cross-over study design, the 14 participants (age 26.4 ± 5.2 years); body mass 75.9 ± 10.6 kg; height 1.80 ± 0.05 m; BMI 23.5 ± 2.8 kg/m²) were exposed consecutively to all three protocols during a total of three campaigns being performed between October 2012 and October 2013: normobaric normoxic horizontal bed rest (NBR: FiO₂ = 0.2099%; PiO₂ = 133.1 ± 3 mmHg), normobaric hypoxic horizontal bed rest (HBR: FiO₂ = 0.141 ± 0.004%; PiO₂ = 90.0 ± 0.4 mmHg; equivalent to ~4000 m) and normobaric hypoxic ambulatory confinement (HAM: FiO₂ = 0.141 ± 0.004%; PiO₂ = 90.0 ± 0.4 mmHg). Three participants dropped out before the last campaign due to personal reasons.

To ensure safety during hypoxic conditions, the participants were provided with portable O₂ gas analyzers (RAE PGM-1100, San Jose, CA) with an alarm triggered at a pre-set O₂ level of 13.5%. Details on establishing and maintaining the designated hypoxic conditions have been reported previously (Debevec et al., 2014).

General environmental conditions in the facility were controlled and remained stable during all three campaigns (ambient temperature: 24.4 ± 0.7 °C; relative humidity: 53.5 ± 5.4%; ambient pressure: 684 ± 4 mmHg).

Moreover, the participants were subjected to a standardized diet that was strictly applied in all three phases of each campaign in order to better evaluate individual body composition, energy expenditure, and water balance. They were served five meals a day each day at the same time (breakfast 8:00 am; snack 11:00 am; lunch 1:00 pm; snack 4:00 pm; dinner 7:00 pm).

Each campaign lasted 21 d under applied study conditions (NBR, HBR, and HAM) preceded by a period of 7 d for baseline data collection (BDC) and followed by a 4-day recovery period during which post-intervention measurements were obtained (R). In each experimental campaign, two subjects began the designated intervention each day, and in the same order in all interventions. The experimental campaigns were separated by a 4-month wash-out period.

Blood and saliva collections to measure the endocannabinoid and cortisol concentrations as well as the paper-form questionnaires were carried out 2 d before the beginning of the intervention period (baseline data collection, BDC), at days 2, 5, 14, and 21 during the confinement and 2 and/or 4 d after the end of confinement (R2/R4). The time points for urine sampling differed only slightly due to organizational reasons with the baseline data collection at day 1 before the condition (BDC) and day 4 during the condition. R4 was not quantifiable due to the early departure of subjects at the end of each campaign.

Bed rest and hypoxic ambulatory restrictions

During the three campaigns, two participants were assigned to share one room with two single beds. Bed rest conditions (NBR and HBR) implicated that all daily routines (e.g. showering, toilet, leisure activities) had to be performed in the horizontal position and physical activity was prohibited except for changing of the position in bed from lateral to supine or prone, and vice versa. Continuous video surveillance was used to ensure strict compliance with these regulations. Additionally, passive stretching by a physiotherapist and mild medication (paracetamol) against bed rest induced neck- or backache was provided on demand.

Participants subjected to HAMB could carry out their normal daily routines and move around in the confined and restricted hypoxic area (110 m²). They participated in daily exercise sessions (30 min twice a day, in the morning and afternoon) performing either stepping, cycling, or dancing as physical activity similar to their habitual level.

In all three conditions, the circadian rhythm was standardized by a clock-regulated wake-up/sleep cycle (wake-up 7:00 am; lights off 11:00 pm). Taking naps during day hours was prohibited.
**Psychological response**

The quantification of each individual’s stress level and the intensity of perceived anxiety was collected by using three different questionnaires completed at several time points throughout the observation period.

1. **KAB (short questionnaire on current stress)** evaluates the stress level by benchmarking opposing adjectives indicating stress or ease on a Likert scale (Mueller & Basler, 1993). It was surveyed twice a day, in the morning and evening.

2. **Spielberger State and Trait Anxiety Inventory (STAI)** differentiates between the perceived anxiety in a specific situation (state anxiety) and the anxiety belonging to one’s character (trait anxiety) (Spielberger et al., 1983). Two parts with 20 questions each rate the answers on a 4-point scale with a global score ranging from 40 to 160.

3. **Post-traumatic symptom scale 10 (PTSS-10)** is a two part test investigating feelings associated with anxiety and depression (e.g. nightmares, pain). Part A asks for their existence in the last month and comprises of yes or no answers. Part B grades 10 negative feelings in the past few days on a 7-point scale leading to a minimum score of 10 up to a maximum of 70 points in total. Both parts were answered at time points BDC and R4 to cover the baseline and the interventional period.

**Neuroendocrine response**

**Cortisol**

The collection of saliva samples twice a day, in the morning (7 am) and evening (8 pm) enabled monitoring of the diurnal rhythm of cortisol. The morning collection took place immediately after the subjects awakened from an overnight fast. The evening collection was carried out before dinner. Subjects chewed on a cotton swab for 45 s that was subsequently stored in a SALIVETTE tube (Sarstedt, Nümbrecht, Germany) and frozen at -20°C. An automated immunoassay system based on the principle of electrochemiluminescence (Elecsys Cortisol, Roche Diagnostics, Mannheim, Germany) quantified cortisol levels (reference values: morning <0.87 μg/dl; evening <0.35 μg/dl; the intra-assay CV ranged from 1.5 to 5.4% for concentrations from 0.007 to 0.972 μg/dl; and the inter-assay CV ranged from 1.9 to 10.1% for concentrations from 0.013 to 1.17 μg/dl according to the reference ranges of the manufacturer).

**Catecholamines**

Urine was gathered twice a day at 12 h intervals (daytime: 7:00 am to 7:00 pm; nighttime: 7:00 pm to 7:00 am). The volume was measured, a 10 ml sample extracted and immediately frozen at -20°C. From this sample, the catecholamine norepinephrine was analyzed with high-performance liquid chromatography (Chromosystem, Martinsried, Germany). Finally, the absolute mass of norepinephrine was calculated with respect to urine volumes (reference values: norepinephrine <100 μg/24h; intra-assay CV <2.7%; inter-assay CV <4.1% according to the reference ranges of the manufacturer and to values surveyed by the laboratory of clinical chemistry, Ludwig-Maximilians-University, Munich, Germany).

**Endocannabinoids (ECs)**

Sample measurements of EC concentrations of arachidonylethanolamide (AEA), 2-arachidonoylglycerol (2-AG), palmitoylethanolamin (PEA), oleoylethanolamide (OEA), and stearoylethanolamide (SEA) were taken from lithium-heparinized whole blood drawn from fasting subjects in supine position. Samples were collected according to standard operating procedures, collection times were strictly adhered to (7 am as for morning saliva and nighttime urine collection) and samples were immediately frozen on-site at -80°C. Prior to the final analysis, whole blood samples were thawed, mixed, and vortexed with 20 μl of internal standard (stable isotope-labeled ECs 2-AG-d5, PEA-d4, NADA-d8, AEA-d4, AG-d8 (Cayman Europe, Tallinn, Estonia)) as well as 1 ml of methyl tertiary-butyl ether (MTBE, Sigma-Aldrich, Milano, Italy), and centrifuged at 13,000 x g for 5 min. The clear supernatant was transferred into a clean 1 ml polypropylene tube (Sarstedt, Nümbrecht, Germany) and evaporated in the Speed Dry/Speed Vac centrifuge (Christ, Osterode am Harz, Germany) for 30 min at room temperature until drying of the organic phase. Dried organic phases were reconstituted in 80 μl of acetonitrile plus 80 μl R1 buffer (5 mM ammonium formate (NH4OFe) added to water with 15% methanol) and vortexed for 30 s. After centrifugation at 13,000 x g for 5 min, 110 μl of clear solution was transferred to HPLC/MS-MS suitable tubes made of polypropylene and frozen at -80°C. Subsequent analysis and quantification of the ECs were carried out by the HPLC Tandem Mass Spectrometry Technique as described previously (Hauer et al., 2013) at the Institute of Doping Analysis und Sports Biochemistry, Kreischa/Dresden, Germany.

**Statistics**

Data were analyzed and plotted with SPSS 23.0 (IBM, Armonk, NY) and Sigma Plot 12.5 (Systat Software Inc., San Jose, CA). Outcome variables were tested for deviations from the normal distribution using Kolmogorov–Smirnov tests followed by a Box–Cox Transformation where deviations were present. Statistical inferences regarding the effects of different conditions and time points during the same campaign were imputed through mixed linear models (LME). We included fixed effects for campaign, sequence of campaigns, potential carry-over from the previous campaign, condition, time within the respective campaign, and the interaction of condition and time. Random effects were included for subject and carry-over using a covariance matrix with variance components structure. We regarded p values <.05 as statistically significant.

**Results**

**Psychological responses**

**KAB (short questionnaire on current stress)**

The KAB generally displayed relative low-stress levels with scores below 3 in all groups in the morning and evening.
The indicated values remained consistently low over the observation period with minor differences between morning and evening values. The HBR group showed a tendency towards elevated stress levels, which was more pronounced in the evening than in the morning (Figure 1).

Spielberger State and Trait Anxiety Inventory (STAI)

The results from the STAI were similar to those based on the KAB. Threshold values indicating psychic disequilibrium and anxiety (state anxiety mean total: 36.83 ± SD 9.82; trait anxiety men total: 34.45 ± SD 8.83 (Laux et al., 1981)) were not exceeded (data for trait anxiety not shown). However, values tended to be higher, albeit not significantly, in both bed rest groups compared to the HAMB trial (Table 1).

Post-traumatic symptom scale 10 (PTSS-10)

Both parts of the test demonstrated no significant increase in negative feelings between the three trials. At R4, the stress levels showed a slight increase in all groups but with a statistically significant difference only for part B in the HBR trial (t (36) = 2.2, p = .033, Cohen’s d = 0.588) (Table 2).

Figure 1. KAB (short paper questionnaire on current stress to quantify an individual’s stress level); A/B: morning/evening measurement; data are means ± SEM; units are points; HBR: hypoxic bed rest (n = 13–14); NBR: normoxic bed rest (n = 13); HAMB: hypoxic ambulation (n = 12); BDC: Baseline Data Collection; R2/4 = 2/4 d after the end of condition.

### Table 1. State and Trait Anxiety Inventory (STAI).

<table>
<thead>
<tr>
<th>Time points</th>
<th>STAI state anxiety</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBR</td>
</tr>
<tr>
<td>BDC</td>
<td>33.07 ± 9.23</td>
</tr>
<tr>
<td>2</td>
<td>36.36 ± 12.45</td>
</tr>
<tr>
<td>5</td>
<td>35.50 ± 9.01</td>
</tr>
<tr>
<td>14</td>
<td>36.21 ± 10.53</td>
</tr>
<tr>
<td>21</td>
<td>36.21 ± 11.74</td>
</tr>
<tr>
<td>R2</td>
<td>35.64 ± 11.24</td>
</tr>
<tr>
<td>R4</td>
<td>30.36 ± 6.21</td>
</tr>
</tbody>
</table>

Data are means ± SD; units are points; HBR: hypoxic bed rest (n = 14); NBR: normoxic bed rest (n = 13); HAMB: hypoxic ambulation (n = 12); BDC: Baseline Data Collection; R2/4 = 2/4 d after the end of condition.

### Neuroendocrine response

#### Cortisol

Cortisol concentrations in the morning and evening did not exceed the reference interval (see Cortisol section). There was neither a statistical significance between the three groups nor between the levels in the pre, per-, and post-intervention periods (Figure 2(A,B)).

#### Catecholamines

Daytime norepinephrine concentrations varied only slightly between the HBR and NBR groups but were consistently lower than in the HAMB group with significant differences during confinement – most prominent at day 14 (HAMB to NBR day 14: t (151.381) = 4.471, p < .001, Cohen’s d = 0.894). In the HBR and NBR campaigns, norepinephrine concentrations decreased significantly from BDC to day 14 (HBR p < .001; NBR p = .049) but subsequently showed a significant increase in HBR and NBR at R2 compared with day 14 (HBR p < .001; NBR p < .001) and in NBR also compared with day 21 (p = .039). The nighttime norepinephrine concentrations showed similar patterns with a generally lower catecholamine secretion. A significant difference with lower values in the bed rest groups was detected at day 14 (HAMB to NBR t (150.383) = 4.243, p < .001, Cohen’s d = 0.832; HAMB to NBR t (149.709) = 4.471, p < .001, Cohen’s d = 0.894). From BDC to day 14, norepinephrine concentrations decreased significantly in both bed rest groups (HBR p < .001; NBR p = .001) and subsequently increased at R2 compared with day 14 (HBR p < .001; NBR p < .001) (Figure 2(C,D)).

### Endocannabinoids

There was no significant increase in endocannabinoid levels during the interventional period irrespective of the trial or between the three trials (Table 3).
Table 2. Post-traumatic symptom scale (PTSS-10) Part A/B.

<table>
<thead>
<tr>
<th>Time points</th>
<th>Pain</th>
<th>Troubles of breathing</th>
<th>Nightmares</th>
<th>Anxiety/panic</th>
<th>Pain</th>
<th>Troubles of breathing</th>
<th>Nightmares</th>
<th>Anxiety/panic</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDC</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>R4</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>NBR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAMB</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>BDC</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R4</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PTSS-10 Part B

| HBR         |      |                        |            |               | NBR         |      |                        |            |               | HAMB         |      |                        |            |               |
| BDC         | 15.50 ± 3.82 |      |                        |            |               | 14.77 ± 4.57 |      |                        |            |               | 15.33 ± 2.74 |      |                        |            |               |
| R4          | 18.71 ± 7.62 |      |                        |            |               | 16.54 ± 7.40 |      |                        |            |               | 16.17 ± 4.91 |      |                        |            |               |

Data are means ± SD; units are points; HBR: hypoxic bed rest (n = 14); NBR: normoxic bed rest (n = 13); HAMB: hypoxic ambulation (n = 12); BDC: Baseline Data Collection; R4 = 4 d after the end of condition. *Significant difference between R4 and BDC in HBR (p < .05).

Figure 2. Cortisol in saliva; norepinephrine in urine; A/B: cortisol morning/evening (HBR: n = 14; NBR: n = 12–13; HAMB: n = 12); C/D: norepinephrine day/night (HBR: n = 10–14; NBR: n = 8–13; HAMB: n = 12); data are means ± SEM; units are µg/dl (cortisol) or µg/12 h (norepinephrine); HBR: hypoxic bed rest; NBR: normoxic bed rest; HAMB: hypoxic ambulation; BDC: Baseline Data Collection; R2 = 2 d after the end of condition; norepinephrine daytime: # significant difference between HAMB and NBR; * significant difference between HAMB and HBR; µ significant difference BDC to day 14 in HBR/NBR; + significant difference R2 to day 14 in HBR/NBR; ~ significant difference R2 to day 21 in NBR; norepinephrine nighttime: # significant difference between HAMB and NBR; * significant difference between HAMB and HBR; + significant difference R2/BDC to day 14 in HBR/NBR.
Discussion

The present study demonstrates that 3 weeks of normobaric hypoxia evoke no significant stress response, neither on the psychological nor on hormone levels. However, bed rest, an independent factor to hypoxia, induces a significantly different stress response, with lower norepinephrine concentrations during confinement (day 14) compared with HAMB. Furthermore, in both bed rest groups, norepinephrine concentrations significantly decreased from BDC to day 14 and increased again at R2.

We aimed to assess whether hypoxia alone, or in combination with bed rest, induces psycho-neuroendocrine responses, since it is well established that hypoxia affects physiological responses and neuropsychological capacities (Barcroft et al., 1923; de Aquino Lemos et al., 2012). We anticipated a hypoxia-triggered exacerbated stress response with higher levels of psychological stress and a subsequently increased neuroendocrine response, with both supposedly already increased by immobilization alone. It is well documented that hypoxia leads to specific reactions of different neuroendocrine systems essential to the maintenance of the HPA axis with subsequent negative effects on the fetus/neonates (Newby et al., 2015) demonstrating a dysregulation of the HPA axis and the ECS are carried out in animal models aimed to define the threshold level of hypoxia (King et al., 2015; Smith et al., 2015). The applied hypoxic levels ranged from 18 to 3% \( \text{FiO}_2 \) and most of the effects were recorded at levels of \( \text{FiO}_2 \leq 12\% \). In contrast to previous studies, the aim of this trial was not to define threshold values in humans but to identify the consequences of a fixed and likely applicable \( \text{O}_2 \) content for manned space habitats. We focused on identifying the consequences of normobaric hypoxia at 14% and its impact on human physiology.

Previous studies carried out with human subjects surveyed physiological reactions predominantly under the influence of hypobaric hypoxia (Feuerecker et al., 2014) or normobaric/hyperbaric hyperoxia (Mazdeh et al., 2015; Strewe et al., 2015). Millet et al. (2012) argue that hypobaric compared to normobaric hypoxia induces different physiological responses concerning factors such as ventilation (Savourey et al., 2003) or fluid balance (Loeppky et al., 2005) whereas Mounier et al. (Mounier & Brugniaux, 2012) refuted this hypothesis. With no clear answer to the question to date and a lack of comparable data, exploration of these results to healthy humans or to a clinical setting (immobilized patients with (normobaric) hypoxemic diseases (i.e. emphysema, fibrosis)) would not be prudent.

The PlanHab project has enabled us to investigate healthy humans precisely in such conditions for the first time: under normobaric hypoxia and concomitant immobilization with highly standardized conditions for 3 weeks.

The results of the present study do not align with previous findings (Keramidas et al., 2016; Stavrou et al., 2015), which may partly be explained by different methodologies. Specifically, the duration of exposure to the stressors and the experimental set-up as well as questionnaire methods differed. In contrast to our results, a former study of hypoxic bed rest of only 10 d demonstrated negative effects on mood with amelioration when omitting immobilization (Stavrou et al., 2015). Thus, an acute, short-term exposure of 10 d seems to generate a negative psychic response whereas a subacute influence of 3 weeks as in our actual study evokes an inert response. This may reflect a state of adaptation and habituation over time. Hence, two questions arise: Which time frame represents a chronic, long-term exposure? Would this adaptation process sustain chronic exposure or negatively exaggerate the stressors’ impact, subsequently leading to negative physiological reactions?

Another recent report also stated negative emotions under severe hypoxia (Keramidas et al., 2016) but the experimental set-up differed from that of our study. The results were obtained following incremental exercise until exhaustion, thus preventing a direct comparison.

Feuerecker et al. (2012) found that the activation of the ECS through exercise was further enhanced by hypoxia,
whereas hypoxia alone had no effects on the ECS. The ECS constitutes a part of the nervous and endocrine system that strongly interacts with responses of the HPA axis (Evanston et al., 2010). Previous research in different domains clearly showed that impairment of the ECS is regularly associated with anxiety or depression (Hauer et al., 2014). Furthermore, alterations of psycho-neuroendocrine responses have been demonstrated in several normoxic head down tilt bed rest studies of varying duration from 20 to 120 d (Chouker et al., 2001; Ishizaki et al., 2002). However, other such studies have reported no alterations of mood (DeRoshia & Greenleaf, 1993; Shehab et al., 1998), which illustrates the diversity of the findings. Concerning our hypothesis with respect to time-dependent psycho-neuroendocrine reactions, these studies emphasize once again that a definitive statement about the time line of an acute, subacute, or chronic exposure to stressors cannot be made without further studies. Whether this is due to the heterogeneity of the stressors or if the exposure period of 120 d is insufficient to trigger chronic responses, many questions remain open and must be elucidated by further research.

Our finding of significantly lower urinary norepinephrine levels during bed rest compared with the ambulatory group, independent of hypoxia, was in accordance with previous studies proclaiming an inhibition of sympathoadrenal responses during bed rest (Goldstein et al., 1995; Sigaudo et al., 1998). Additionally, Greenleaf et al. stated that the physical training status of the subject was relevant to the degree of sympathetic inhibition (Kaciuba-Uscilko et al., 2003; Smorawinski et al., 2000). In addition, we observed a significant increase in norepinephrine concentrations at the end of the bed rest period which can be attributed to orthostatic regulation with increased sympathetic activity in compensating for cardiovascular deconditioning during immobilization (Sigaudo et al., 1998; Whitson et al., 1995). Successive regulation depends also on baroreceptor sensitivity, which appears to be regularly reduced after bed rest (Eckberg & Fritsch, 1992). These regulatory mechanisms were not altered by hypoxic conditions and seem to be independent from psychological influences.

Limitations
The installation of a fourth group – normobaric normoxic ambulatory confinement (NAMB) – in this cross-over trial would have involved an additional campaign, which was not feasible due to financial and organizational restrictions. The interpretation of the data is, therefore, limited as a control group for HAMB is missing. In addition, the study could not be conducted in a blinded manner due to logistical and safety reasons which might have had an influence on the psychological data acquisition via questionnaires. The present study examined only normo- and not hypobaric hypoxia. In the light of missing studies under hypobaric hypoxic conditions, the results should be translated carefully. This can be of importance as future habitats are indeed hypobaric and hypoxic. Finally, the expenses and organization associated with such studies limit the number of subjects and thus statistical power.

Conclusion
This study showed that 3 weeks of normobaric hypoxia do not affect the psycho-neuroendocrine stress axis and that hypoxia does not alter neuroendocrine responses to immobilization. The clinical states of immobilization/low activity and hypoxemia (e.g. lung emphysema) seem to induce physiological alterations that are associated with the illness itself rather than with disease symptoms such as hypoxia and/or immobilization.

For planning space exploration class missions, this study shows that future experimental protocols should aim to emulate mission specific conditions as accurately as possible given its complexity.

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Disclosure statement
The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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