PlanHab Report Summary

Project ID: 284438
Funded under: FP7-SPACE
Country: Slovenia

Final Report Summary - PLANHAB (Planetary Habitat Simulation)

Executive Summary:
4.1.1 Executive summary

The PlanHab project investigated the separate and combined effects of hypoxia and sustained recumbency (bedrest), on human physiological systems. The partial pressure of oxygen in the environmental gas inside future planetary habitats will be lower than in atmospheric air. Prolonged exposure to low gravity will result in deconditioning of vital physiological systems, and may consequently constitute a threat to the health of the astronauts. However, it is unknown how prolonged exposure to both reduced gravity and hypoxia will affect health. Subjects (N=14) participated in three 21-day trials conducted at the Olympic Sport Centre Planica (Rateče, Slovenia): hypoxic bedrest (target simulated altitude 4000 m), normoxic bedrest, and hypoxic ambulation. Bedrest induced the anticipated reductions in muscle and bone mass, which were not modified by hypoxia. Hypoxia appears to counteract the inactivity-induced orthostatic intolerance, but aggravates the bedrest-induced reductions in plasma volume, peak oxygen uptake and increases of negative mood indices. All interventions induced changes in cardiac dimensions and functions attributable to the concomitant reductions in circulating blood volume. Normoxic and hypoxic bedrest reduced exercise endurance, presumably also as a consequence of the hypovolemia. The thickness of the pulmonary diaphragm was unaffected by normoxic and hypoxic bedrest but increased by hypoxic ambulation. Bedrest induced a significant impairment of skeletal muscle oxidative metabolism, both in vivo and in isolated muscle fibres ex vivo. In contrast, hypoxia caused impairment only ex vivo; during exercise with small muscle masses carried out (in normoxia) following hypoxic exposure the increased O2 delivery and [haemoglobin] could compensate, in vivo, the impairment described at the isolated permeabilized fibres level. The superposition of hypoxia does not aggravate the impairment described following bedrest alone. The present project also demonstrated that immobilization and hypoxia do interact with regard to muscle atrophy in the thigh, but not (or not as much) in the calf. Moreover, the study convincingly produced the expected bone losses in five of the six measurement sites analysed in this study. The lack of any consistent findings in bone geometrical measures is likely due to the small magnitude of bone losses. Furthermore the obtained data demonstrated that BR induced alterations in bone formation and bone resorption are largely unaffected by addition of hypoxia. There was, however, a strong effect upon calcium homeostasis, with substantial reductions of urinary excretion of calcium and phosphate. With regards to the immune system the separate and combined effects of hypoxia and bedrest are visible in some conditions of cell stimulations. In particular, hypoxia caused degradation of energy rich nucleotides and the generation of adenosine and inosine and were paralleled by significant time and antigens dependent immune dysregulation. Sixteen days bedrest resulted in an increase in insulin resistance, adverse fasting circulating lipid profile and reduction in postprandial thermogenesis, which were not ameliorated by hypoxia. Increased fat and decreased carbohydrate oxidation was noted in both hypoxic conditions in the fed and fasted state. Postprandial subjective appetite and ad libitum food intake were unaffected by the interventions. Bedrest and/or hypoxia decreased erythrocyte availability of glutathione and all its precursors even though such changes were not related to altered synthetic capacity in red blood cells. No effects of inactivity and hypoxia on autonomic and behavioural thermoregulatory function were noted during rest. Hypoxia caused an increase in central sleep apnea and modified sleep macrostructure leading to sleep disturbance. The new foreground has also implications for society in general, since chronic hypoxia and inactivity constitutes a model of the basic conditions experienced by patients suffering from respiratory
insufficiency restricting them to a physically inactive lifestyle.

Project Context and Objectives:
4.1.2 Project context and Objectives

The PlanHab project was a ground-based space life sciences project investigating the effect of the environmental stressors in future planetary habitats, namely reduced gravity and hypoxia, on astronauts. The simulation of the effect of planetary habitat environments was achieved with the bedrest experimental model, in which subjects were recumbent for 21 days, but also exposed to hypoxia. The hypoxic bedrest experimental model developed within the framework of the PlanHab project, has proven to be a unique experimental model, which evokes the anticipated changes in the structure and function of organ systems also in bedridden and/or inactive patients rendered hypoxic by their illness. As such, the results of the PlanHab project not only provides valuable information regarding the effects of planetary habitats on the health and well-being of astronauts, but also provides further insight into the changes in organ systems of specific patient populations, such as patients with chronic obstructive pulmonary disease (COPD), who are hypoxic and inactive as a result of the illness. Similarly, the results of the PlanHab project will also contribute to our better understanding of the aetiology of the acquired weakness in intensive care unit patients, and thus improvement in recovery of such patients. These important contributions of the PlanHab foreground to Science and Society are detailed in the impact section.

4.1.2.1 Project Context
One problem associated with the design of human habitats for use at the surface of the Moon or Mars concerns the environmental control and life support system, which, for both technical and medical safety reasons, is desired to have a low operating pressure [1]. A low operating pressure in the planetary habitat will reduce the potential risk of decompression sickness during extravehicular activities [2]. To avoid any detrimental effects of the resultant hypoxia amongst the crewmembers, the fraction of oxygen must be increased in the habitat gas mixture. The trade-off for restoring the oxygen partial pressure to normoxic levels by increasing the oxygen fraction of the habitat gas mixture is a markedly increased flammability of the gas. A compromise between the elevated oxygen fraction and the reduced environmental gas pressure has been proposed; the environmental gas pressures and oxygen fractions that have been discussed range from 55 to 57 kPa, and from 30 to 40%, respectively [1].

The aim of the PlanHab project was not to create high-fidelity simulations. This is not possible since all the parameters for future space flight and sojourns on Mars and the Moon have not yet been precisely defined. The justification for lowering the oxygen fraction in the proposed experiments to the extent that the PO2 will be considerably lower than the stipulated minimum value is scientific rather than operational. Thus, it can be assumed that any interactions between reduced gravity and low inspired PO2 as regards physiological responses, will be easier to reveal, if the two stimuli are somewhat exaggerated (i.e. more pronounced unloading and lower oxygen tension) than would be expected in an operational setting. As mentioned earlier, the other rationale for the exaggerated stimuli is that it is premature to try and create a high-fidelity simulation, since too many factors regarding the nature and duration of lunar EVAs are as yet unknown.

Study design
A series of bedrest and hypoxia studies involving human subjects were conducted at the Olympic Sport Centre Planica (Rateče, Slovenia) capable of housing up to 20 subjects simultaneously at any simulated altitude. During the course of the studies, subjects either maintained a horizontal position (bedrest), or were ambulatory, but confined to the facility (ambulation) for 21 days. The studies were conducted using a repeated measures cross-over design, such that each subject participated in three trials: hypoxic bedrest (HBR), normoxic bedrest (NBR), and hypoxic ambulation (HAMB). In between trials, the subjects had a wash-out period, which was approx. 3 times the length of the bedrest or ambulation period (a minimum of 3 months). In the normoxia trial, subjects were exposed to a normoxic environment (21 kPa oxygen) during the bedrest, whereas in the hypoxia trials, subjects were exposed to a hypoxic environment for the duration of the bedrest or confinement. The level of hypoxia was maintained at 12.5 kPa (corresponding to 4000 m above sea level), which is somewhat lower than the anticipated oxygen partial pressure in future planetary habitats.
The justification for lowering the oxygen fraction in the proposed experiments to the extent that the PO2 will be considerably lower than the stipulated minimum value is scientific rather than operational. Thus, it can be assumed that any interactions between reduced gravity and low inspired PO2 as regards physiological responses, will be easier to reveal, if the two stimuli are somewhat exaggerated (i.e. more pronounced unloading and lower oxygen tension) than would be expected in an operational setting. The other rationale for the exaggerated stimuli is that it is premature to try to create a high-fidelity simulation since too many factors regarding the nature and duration of EVAs are as yet unknown.

The project investigated the interactions between hypoxia and unloading as regards several physiological responses. Since levels of unloading and hypoxia were exaggerated compared to those encountered during real-life missions, such interactions may be qualitatively rather than quantitatively relevant from an operational perspective.

Physiological effects of long-term exposure to reduced oxygen and low gravity

Even though it is well known that humans can acclimatize to such levels of hypoxia [3], information is scarce as to how different physiological systems may respond to combined chronic exposure to hypoxia and low gravity force field. Several significant interactions between prolonged hypoxia and low gravity are envisaged. Long-term exposure to microgravity brings about mechanical unloading of weight-bearing bones and postural muscles, as well as physical inactivity, resulting in bone demineralization, muscle atrophy and reduced muscle strength, collectively referred to as musculoskeletal deconditioning [4]. In particular, the weight-bearing bones [5] and the muscles involved in postural control [6] are affected. Microgravity also abolishes hydrostatic pressure gradients acting along blood vessels, resulting in redistribution, and subsequently reduction, of the circulating blood volume [4], as well as reduced cardiac function [7-8] and increased distensibility in dependent blood vessels [9-10]. These adaptations, commonly referred to as cardiovascular deconditioning, manifest themselves upon return to Earth’s gravity force field as reduced aerobic exercise capacity, reduced tolerance to upright posture and to increased gravitoinertial load in the head-to-foot direction [4], the latter constituting a problem during the space shuttle landing phase [11]. It can be assumed that chronic exposure to 0.16 G (Lunar gravity) will induce cardiovascular and musculoskeletal deconditioning, which are qualitatively similar to those brought about by microgravity. From the perspective of physiological adaptation, it is desired that the Lunar/planetary missions be preceded by relevant ground-based simulations.

4.1.2.2 Project Objectives

The objectives of the PlanHab project were to evaluate the effects of hypoxia on the deconditioning induced by bedrest. Studies compared the processes of deconditioning associated with inactivity/unloading in normoxic and hypoxic environments. Since information is scarce concerning interactive effects of such interventions, the study was explorative in the sense that a broad spectrum of physiological functions were investigated. To a large extent basic data were collected, corresponding to that obtained when bedrest experiments are conducted by ESA/NASA (bedrest core data). Since the duration (21 days) of each intervention was identical to that of a medium-term bedrest study according to ESA standards, data from the proposed studies can readily be compared with core data from previous bedrest studies, and hence the added effects of hypoxia should be apparent. In addition, specific experiments were conducted to investigate the combined effects of hypoxia and bedrest on:

- cardiovascular and respiratory functions
- musculoskeletal functions
- haematological and immunological functions
- oxidative stress and thermogenesis
- thermoregulatory functions as well as sleep disorders and altitude sickness

The above overall objectives of the PlanHab study were attained through the successful and timely completion of the following specific scientific objectives defined in six work packages:

WP1: Hypoxic bedrest facility and protocol

The PlanHab project investigated the combined effects of inactivity and hypoxia on cardiovascular, respiratory, musculoskeletal, metabolic, immunologic and thermoregulatory functions, as they relate to life in reduced gravity habitats (space and planetary habitats), and in specific patient populations (i.e. COPD, congestive heart failure, obesity, etc.). All work
packages conducted their experiments on the same subjects participating in a series of experimental trials (bedrest/confinement campaigns). Thus, the objective of work package 1 was to implement the interventions, which were necessary for the simulation of the effects of reduced gravity combined with hypoxia. This WP dealt with the overall performance of the study interventions as well as with the collection of basic core data in conjunction with these interventions, which were used by WPs 2 to 6. The study was conducted using an intra-individual comparison, cross-over design with three 21-day interventions: 1) normoxic horizontal bedrest, 2) hypoxic horizontal bedrest, 3) hypoxic ambulatory confinement. These interventions were preceded by a 1-week baseline period and a 4-month recovery period. This WP dealt with the organisation of the experimental interventions. The tasks within this workpackage enabled the attainment of the following specific objectives:

Objective 1: Prepare hypoxic facility for experimental campaigns.
Objective 2: Establish data storage and management system.
Objective 3: Arrange, and conduct experimental campaigns necessary for WPs 2 to 6.
Objective 4: Collect, analyse and archive the basic core data required by all WPs.

WP2: Effects of hypoxia and bedrest on cardiovascular and musculoskeletal function
This WP investigated the effects of prolonged bedrest and hypoxia, per se and in combination, on cardiovascular and respiratory functions at rest and during physical exercise. The tasks within this workpackage addressed the separate and combined effects of hypoxia and inactivity on:

Objective 1: Systolic and diastolic cardiac function at rest.
Objective 2: Volitional and nonvolitional respiratory muscle strength at rest.
Objective 3: Cardiovascular and respiratory functions during exercise, with special reference to effects on the various levels of the O2 pathway from ambient air to the mitochondria of skeletal muscles.

WP3: Effects of hypoxic inactivity on the musculoskeletal system
This WP assessed the effect of separate and combined effects of inactivity and hypoxia on the musculoskeletal system, specifically on:

Objective 1: Muscle volume, strength and power.
Objective 2: Muscle ultra-structure and metabolic function.
Objective 3: Changes in bone mass and geometry.
Objective 4: Changes in biochemical markers of bone metabolism.

WP4: Monitoring of stress and hypoxia-sensitive immune and haematological changes
This WP focused on stress and hypoxia-sensitive responses during bedrest and/or hypoxia, and their impact on immune function and the oxygen-delivering haematological system, respectively. The objectives of this WP were:

Objective 1: To determine whether a correlation exists between the perceived emotional stress and alterations in cognitive function inherently observed in an environment of confinement/hypoxia/bedrest are related to immune function.
Objective 2: To determine if the endocannabinoid system (ECS) is activated under such conditions and if so, interacts with the other stress responsive systems, including: the autonomic nerve system, (ANS), the glucocorticoid system (GS) and ultimately, if these alterations are associated with markers of immunological changes.
Objective 3: To determine the magnitude to which hypoxia can activate hypoxia-inducible immunosuppressive signalling pathways (HIF, purines), contributing to immune-modulating effects of stress and to what degree these alterations are accompanied by haematological changes (EPO).
Objective 4: To determine non-invasive immune changes via exhaled volatile immunological markers.

WP5: Hypoxia and physical inactivity regulation of oxidative stress and thermogenesis
This WP described, in healthy volunteers and under controlled nutritional conditions, changes mediated by physical inactivity and reduced environmental oxygen availability on oxidative stress and thermogenesis. In particular the objectives of this WP were to determine:
Objective 1: The impact of oxidative stress measured at whole body level (blood markers) and in muscle biopsies on muscle atrophy and insulin resistance.

Objective 2: Thermogenesis and insulin resistance in adipose tissue.

WP6: Hypoxia-induced thermoregulatory dysfunction, sleep disorders, and ventilator equivalent altitude

This WP assessed autonomic and behavioural thermoregulatory function during, rest, exercise and recovery, before and after hypoxic bedrest, and compared the results to those observed during normoxic bedrest, and hypoxic confinement. The WP also tested the hypothesis that bedrest disrupts sleep patterns due to the reduced perfusion of the lower extremities, and that this is exacerbated due to the vasoconstrictive effect of hypoxia. Finally, this work-package also included altitude tests with alternatively low density and high density breathing gases to determine the ventilatory altitude equivalence of the normobaric hypoxia used in the present study, and to test the hypothesis that the discrepancy noted regarding the equivalent air altitude model is due to the lower gas density at altitude. The specific objectives of this WP were:

Objective 1: Assess the effect of hypoxic bedrest on autonomic and behavioural thermoregulatory function during rest, exercise and sleep.

Objective 2: Evaluate the theory relating sleep quality to thermal afferent information from peripheral regions. In particular, determine the contribution of hypoxia- and bedrest-induced peripheral vasoconstriction, and consequently cooling of the lower limbs, to sleep disturbance.

Objective 3: Evaluate the Equivalent Altitude Theory on the hypoxic ventilatory response.

Project Results:

4.1.3 Main S&T Results

4.1.3.1 Hypoxic bedrest facility and protocol (WP1)

Normoxic bedrest reduced orthostatic tolerance. The bedrest-induced increase in the incidence of orthostatic syncope/imminent syncope agrees well with reports from previous bedrest studies [11]. Novel findings of the present study were that the number of orthostatic synapses/imminent synapses was less and MAP during the orthostatic provocation was higher after NBR than after HBR. These findings suggest that hypoxia counteracts bedrest-induced orthostatic intolerance.

Normoxic bedrest reduced peak power output and peak oxygen uptake. The magnitudes of the reductions were in agreement with, but on the low side of, those reported in the literature (for review see [7]). There are two likely explanations for the relatively modest average NBR-drop in aerobic capacity. Firstly, since aerobic capacity was tested on two occasions post-bedrest, on R1 and on R3, the calculated average NBR-induced drop in aerobic capacity was affected by the partial recovery taking place during the first two days post-bedrest. Secondly, as a group the present subjects were aerobically fit at the outset of the study; bedrest-induced reductions in aerobic capacity is less in unfit than fit subjects [12]. The bedrest-induced decrease in VO2peak was exaggerated by hypoxia, most likely due to a more pronounced reduction in convective O2 transport, as indicated by the lower peak values of cardiac output following the HBR than the NBR.

Bedrest induced a drop in plasma volume, which is in agreement with previous studies [4]. It should be noted that the present plasma-volume estimations should be interpreted with caution, since, particularly in the hypoxic conditions, values obtained during the latter portion of the intervention may have been confounded by neoerythrocytosis. Nevertheless, it appears clear that hypoxia aggravated the bedrest-induced plasma-volume reduction.

Bedrest decreased muscle strength in the postural muscle groups, whereas the other muscle groups were unaffected by this intervention. Likewise, bedrest reduced vertical jump capacity. The findings agree with results from previous bedrest studies. Also the magnitude of the present decrease in knee-extensor strength induced by 3 weeks of bedrest agrees well with results from previous bedrest studies (cf. [6]). Likewise, present findings that hypoxia does not affect the strength of postural leg muscles is in agreement with those reported in the literature [13]. A novel finding of the present study is that hypoxia does not appear to affect decrements in muscle strength induced by prolonged inactivity. Bedrest reduced postural stability, but this effect was not influenced by hypoxia.

In agreement with previous investigations [14] the present normoxic bedrest induced reductions in whole-body mass and fat-free mass. The body mass and composition data suggest that exposure to a hypoxia does not aggravate the reductions in...
whole-body and fat-free mass induced by prolonged bedrest. The HAMB-induced reduction in body mass and modulation of body composition needs to be further investigated but may suggest a role of confinement per se on nutrition. Substantial and consistent increments on negative mood indices were noted only during HBR, suggesting that hypoxia may aggravate any tendencies of negative mood induced by bedrest.

4.1.3.2 Effects of hypoxia and bedrest on cardiovascular and respiratory function (WP2)

Pulmonary function

The exposure to ambulatory hypoxia causes a significant increase in ventilation, which is still present 2-3 days after confinement during spontaneous breathing at rest but not during exercise. This is confirmed by the significant increased thickness of the diaphragm. These results suggest that during exercise the efficiency of oxygen extraction of locomotor muscles remains unaltered.

The exposure to bedrest does not induce any change in the use of the respiratory muscles. This is confirmed by the analysis of breathing pattern at rest (not significantly different than pre-confinement) and by the unaltered diaphragm thickness, suggesting that they have to maintain their vital function of allowing ventilation. Conversely, the increase in ventilation observed during exercise suggests a reduced efficiency of oxygen extraction of locomotor muscles and consequent increased ventilatory needs.

The exposure to combined bedrest and hypoxia induces significant effects on the breathing pattern both during rest and exercise. During combined exposure, the increased ventilation and work of breathing maintain the efficiency of both the diaphragm and the non-diaphragmatic respiratory muscles (confirmed by the increased diaphragm thickness), while bedrest induces significant dysfunction in the locomotor muscles (confirmed by the increased ventilation observed during exercise). The significant changes in the breathing pattern observed during exercise, are presumably the result of the complex set of phenomena including bedrest-induced dysfunction of locomotor muscles, maintenance of respiratory muscle function, redistribution of blood flow among the different skeletal muscles and inefficient ventilation.

Cardiac function

Several of the present findings suggest that all three interventions (NBR, HBR, HAMB) reduced the circulating blood volume, and that this intervention-induced hypovolemia was more pronounced after HBR than after NBR and HAMB. Thus, intervention-induced reductions in end-diastolic volume, stroke volume and ejection fraction were more pronounced and/or more persistent (i.e. present both in normoxia and hypoxia) after HBR than after NBR and HAMB. The notion that all interventions reduced the circulating blood volume and that the reduction was exaggerated after HBR is supported by concomitant assessments of blood volume at rest and cardiac output responses during cycle-ergometry exercise. The majority of the present intervention-induced changes in variables, that under certain circumstances may indicate diastolic or systolic dysfunction, are attributable to the aforementioned hypovolemia.

Exercise cardiovascular function

Cycle exercise endurance capacity was markedly reduced by normoxic bedrest, which is in good agreement with results in previous investigations (for reviews see [7 15]) A main finding of the present study was that, despite that HBR induced a greater reduction in peak oxygen uptake than did NBR, the impairment of maximal exercise performance during a constant-load trial to exhaustion was similar after HBR as after NBR, regardless of whether the test was performed in normoxia or in hypoxia. We have previously shown that the more pronounced drop in peak oxygen uptake following HBR than NBR was most likely due to a lower cardiac output, resulting from a greater degree of hypovolemia induced by HBR. It is noteworthy that during the constant-load tests, which were conducted a few hours after the termination of interventions, and a few hours after the initial incremental-load cycle ergometry trial (afternoon hours), the stroke volume and cardiac output responses did not differ between the two bedrest interventions. This may suggest a rapid partial recovery of the bedrest-induced drops in plasma volume, in particular following HBR, diminishing the initial post-bedrest difference in cardiac output between HBR and NBR.

The level of exercise-induced muscle deoxygenation was similar after HBR as after NBR. It should be noted, however, that the more pronounced drop in peak oxygen uptake and peak power output after the HBR resulted in a higher relative intensity
during the maximal constant-load tests after HBR than after NBR (HBR: ~95%; NBR: ~86%). Despite the higher relative exercise intensity following HBR, the degree of muscle deoxygenation was similar in the post-NBR and post-HBR trials, which might suggest that HBR induced specific muscle adaptations, allowing the subjects to attain equivalent performance times after both bedrest interventions. Contrary to the case following the bedrest interventions, a marked increase in performance time was noted after HAMB. The enhanced endurance time was not due to increased convective O2 transport, given that the cardiac output values at a given submaximal work load, as well during exhaustive exercise, were unaltered by HAMB. Instead it appears that the enhancement in exercise capacity most likely can be ascribed to increased diffusive O2 transport in the exercising muscles, especially in the vastus lateralis, as indicated by the NIRS data.

Regional oxygenation: the effect on skeletal muscle oxidative metabolism

Variables of functional evaluation of oxidative metabolism were determined in vivo, in normoxic conditions, during one-leg knee-extension exercise [16], an exercise paradigm in which the involvement of a limited muscle mass (knee extensors of one leg, about 2.5 kg of muscle; see [17]) does not pose a significant burden on the cardiovascular system. This allows any impairment related to skeletal muscle oxidative metabolism to become fully manifest. It has been shown that during this type of exercise skeletal muscle O2 delivery and VO2, normalized per unit of muscle mass, exceed by a factor of 2.5-3 the values obtained during conventional cycle ergometer exercise [17 18]. Although in the present study we did not determine cardiac output, the observed peak values of heart rate corresponded in all experimental conditions to only about 65% of the maximal “theoretical” values, confirming that the adopted exercise protocol did not represent, also at exhaustion, a maximal “stress” for the cardiovascular function. The main variables evaluating skeletal muscle oxidative function (VO2peak and Δ[deoxy(Hb+Mb)] peak, estimating peak skeletal muscle fractional O2 extraction) were significantly impaired following BR and HBR, whereas they were not affected by hypoxia. Percentage-wise the decreases in VO2peak (-8%) and Δ[deoxy(Hb+Mb)] peak (-12%) observed following NBR and HBR were less pronounced that those obtained by our group (-19% and -30%, respectively, for the two variables) following NBR exposures of longer duration (35 days). This suggests that the impairment of skeletal muscle oxidative function in vivo is greater as a function of NBR exposure, at least up to 35 days. Both VO2 peak and Δ[deoxy(Hb+Mb)] peak were not affected following hypoxia, and the addition of hypoxia to BR (HBR) did not worsen the impairment observed following bedrest alone. This represents one of the major findings of the present study, with obvious implication in terms of exercise tolerance in planetary habitats, as well as in pathological conditions characterized by inactivity and hypoxia. It can be hypothesized that the absence of an impairment in hypoxia, and, presumably as a consequence of this, the absence of a further impairment during HBR compared to NBR, could be attributed, at least in part, to the increased blood [hemoglobin] occurring in hypoxia. In other words, it can be hypothesized that in hypoxia an enhanced O2 delivery could counterbalance, in vivo, the impairment of oxidative function, which was observed at the fibre level. An unchanged VO2max after altitude acclimatization, during small muscle mass exercise, has been described by Calbet et al. [19]. Interestingly, in that study VO2 max determined during large muscle mass exercise decreased after altitude acclimatization, confirming previous results by others (see [20]). Thus, the increased haemoglobin concentration would preserve VO2max in chronic hypoxia only during small muscle mass exercise, in which cardiovascular O2 delivery capacity is less “stressed”.

In the present study we also evaluated, ex vivo, variables of functional evaluation of oxidative metabolism in permeabilized vastus lateralis fibers obtained by biopsy, by utilizing the method of high-resolution respirometry. The main results obtained by these measurements are presented in this report. Maximal ADP-stimulated mitochondrial (state 3) respiration, which can be considered to reflect VO2 max at the fiber level, in conditions of unlimited substrate and O2 availability, was significantly and similarly reduced following all experimental conditions (NBR, HBR, HAMB). Thus, maximal respiratory capacity at the muscle fiber level was affected following NBR and HAMB. Also, for this variable, the impairment observed following NBR was not aggravated in HBR. For other variables related to mitochondrial respiration the picture is less clear. “Leak” respiration (a “dissipation” of the H+ gradient across the inner mitochondrial membrane, not associated with rephosphorylation of ADP) was enhanced following NBR, whereas this effect was reversed, with a trend towards a decrease, following HBR and HAMB. The same pattern was observed for RCR, the variable evaluating the degree of coupling between oxidation and phosphorylation: a decrease (indicating less coupling) following BR, which was reversed following HBR and showed a trend toward being reversed
following HAMB. A decreased maximal respiratory capacity in the presence of an enhanced coupling has been described by Jacobs et al. [21] after altitude exposure. In summary, for variables evaluating leak respiration and the degree of coupling of oxidative phosphorylation an impairment was observed following NBR, whereas no changes or slight improvements were described following HAMB and HBR. As far as the molecular mechanisms responsible for the impairments of oxidative function described at the fibre level are concerned, some hypotheses can be proposed. Skeletal muscle atrophy following disease/microgravity is due to decreased protein synthesis and increased protein degradation, triggered by oxidative stress [22] and/or mitochondrial dysfunction, altered mitochondrial fusion and fission, at least in part regulated by PGC-1α (mainly in slow muscles) [23], or by a down-regulation of pro-fusion proteins, energy stress and induction of atrogens through AMPK activation (mainly in “mixed” muscles) [24].

At high altitude (real or simulated, as in the present study), when maximal aerobic power during large muscle mass exercise becomes limited, alterations in mitochondrial volume and function can be considered part of the acclimatization process [25]. This would be in agreement with the general concept of symmorphosis [26], which postulates that biological systems are inherently economical, and structural parameters are closely matched to functional capacity. In hypoxia the reduction of mitochondrial volume density, the downregulation of electron transport chain complexes (possibly mitigating the increase in reactive oxygen species production) would be regulated by a decrease in PGC-1α [25], and would explain the impaired respiratory capacity described in the present study, as well as in the study by Jacobs et al. [21]. A role for uncoupling protein 3 could be hypothesized. During small muscle mass exercise, this impairment would not translate into a functional impairment in vivo, as observed in the present study as well as in the study by Calbet et al. [19]. Moreover, the results of the present study demonstrate that the impairment in fiber oxidative function occurring in one condition (bedrest) is not aggravated by the superposition of the other (hypoxia).

Bedrest induced a significant impairment of skeletal muscle oxidative metabolism, both in vivo and in isolated muscle fibers ex vivo. Hypoxia, on the other hand, caused an impairment only ex vivo; during exercise with small muscle masses carried out (in normoxia) following hypoxic exposure the increased O2 delivery and [haemoglobin] could compensate, in vivo, the impairment described at the isolated permeabilized fibers level. The superposition of hypoxia does not aggravate the impairment of skeletal muscle oxidative metabolism described following bedrest alone.

4.1.3.3 Effects of hypoxic inactivity on the musculoskeletal system (WP3)

Muscle size and radiological density
The present study has demonstrated BR-induced losses in muscle size that are compatible with the existing literature [14 27 28]. Hypoxia did not augment the BR-related muscle atrophy in the calf. However, it clearly did so in the thigh. A possible explanation for the discrepancy between both sites departs from the fact that fluid shifts from peripheral to central body segments are much more pronounced from the shank than from the thigh, and that therefore, much of the variation in thigh muscle size is not related to atrophy. Both sites clearly exhibited atrophy during the 21-day hypoxic ambulatory confinement. Although there is a possibility that the atrophy was an effect of the confinement factor, the fact that habitual physical activity levels were maintained by subjects during this period speaks for hypoxia as the effective factor behind the observed changes. Muscle radiological density (MRD), by and large, behaved as expected with an increase during early bedrest and a decrease during early recovery. The increase in MRD during hypoxic ambulatory conditions, which was observed both in the thigh as well as in the calf musculature can well be explained by increases in haemoglobin levels, as the Fe contained in the haemoglobin is an important contributor to MRD readings. In conclusion, immobilization and hypoxia do interact with regard to muscle atrophy in the thigh, but not (or not as much) in the calf. This observation underlines the importance of carefully selecting atmospheric conditions and countermeasure exercises for future space exploration missions.

Muscle ultrastructure and function
The only significant findings in relation to muscle ultrastructure and function were (i) baseline-differences before the bedrest campaigns that were observed both for type-2a fibres and for type-2x fibres, and (ii) a decrease in type-1 fibre content by
hypoxic ambulatory conditions. It is somewhat surprising to see that type-1 fibre content of the muscle was decreased under hypoxic ambulatory conditions, but not as a result of either of the two bedrest conditions. It should also be noted that several trends were observed that failed to reach conventional level of significance, but which still could contain meaningful information. As such, trends indicated a decrease by 3.9% (SE 2.1%) in type-1 fibre content (P = 0.071) and an increase by 5.4% (SE 2.8%) of type-2x fibres (P = 0.072). Moreover, trends indicated a decrease in capillarisation (P = 0.067) as an effect of BR, plus a sparing effect via hypoxia during the BR (P = 0.096). Likewise, a trend indicated an augmenting interaction of BR and hypoxia for cytochrome C content. All of these trends observed were in line with existing literature and/or expectations. It is concluded, that the sample size was probably too small to demonstrate the expected effects with the technical approach chosen. Also, as indicated by the second of the significant findings (ii), it seems that there have been some baseline-differences within this cross over-designed study, which could allude to seasonal effects or to carry-over effects. In future studies, it therefore should be considered how such seasonal and carry-over effects can be better avoided, or whether it would be even better to shift from a cross over-designed study to a parallel-group design.

Bone mass and geometry
Based on results of previous studies it was anticipated that 21 days of immobilization is comparatively short to observe changes in bone mass and geometry [29]. Accordingly, the observed bone losses were of moderate magnitude only, not exceeding 1.5%. They were, notably, consistent with the existing literature as per their magnitude [29 30] as well as for the fact that bone losses were larger at R+14 than at the end of bedrest [29-32]. It is difficult to speculate, however, the meaning of the increases at 66% tibia during bedrest. As with any measurement, the possibility of the baseline measurements (BDC-6 and BR2) being off-set by random variation, cannot be excluded. However, inspection of the individual data showed no outliers. In consideration of the facts that (i) the 66% tibia site is associated with the greatest muscle cross-section, that (ii) there had been significant decreases in muscle cross section by 8.9% on day BR10 and by 14.0% on day BR21, and that (iii) reduced muscle bulk must be expected to produce seemingly greater bone mineral content (BMC) values due to the beam hardening effect [33] suggests the possibility that the seemingly paradoxical findings of increased total bone mineral content (BMC) could be due to masking of real bone losses by beam hardening-induced effect of BMC readings.

With regards to bone geometry, this study has failed to produce any consistent effects. It seems that the bone losses elicited were not of sufficient magnitude to allow for geometrical analyses. Loss of viable data from the 93% tibia and 98% tibia sites is likely related to the facts that: (i) there is more freedom in the knee than in the ankle, that (ii) it is therefore more difficult to obtain reproducible results at the proximal tibia sites. DLR data from previous studies suggest that reproducibility is indeed somewhat poorer for proximal tibia measurements than for distal tibia measurements.

Finally, and most importantly hypoxia had no impact upon bed-rest induced changes. It has to be considered, however, that the study had relatively few subjects, and that bedrest-induced bone losses were small (< 1.5%) in relation to measurement reproducibility. A sensitivity analysis with the results obtained for the 4% tibia site yields that the power of this study was sufficient to detect differences of 41% between hypoxic and normoxic bedrest.

In summary, the study convincingly produced the expected bone losses in five of the six measurement sites that could be analysed in this study. The lack of any consistent findings in bone geometrical measures is likely due to the small magnitude of bone losses. Thus, to improve our understanding of bone geometrical adaptations, either greater bone losses must be studied, or more sensitive technological approaches should be applied. The lacking evidence for modulatory effects of hypoxia on bedrest-induced bone losses is only seemingly reassuring, since the sensitivity analysis for the 4% tibia data shows that differences as large as 40% between conditions could have gone undiscovered. To know effects of this magnitude would surely be important before deciding upon hypoxic or normoxic ambient conditions in long-term space missions.

Bone markers
The most important finding of this study is the non-significance of the BR*Hypoxia interactions for urinary NTX excretion and for P1NP serum levels. Published research demonstrates that these two biochemical markers give an accurate account of bone resorption and bone formation, respectively. It may be concluded that adding hypoxia to BR has no major effect upon BR-induced alterations in bone metabolism. Hypoxic conditions also did not evoke changes in P1NP serum levels, and the trend for urinary NTX was mainly driven by isolated reductions by approximately 200 nmol/day on the 1st and 4th days of the
intervention phase. Therefore hypoxia did not have any substantial effects upon bone formation or bone resorption, neither in BR nor in ambulatory conditions.

By contrast, hypoxia had a very pronounced impact upon calcium homeostasis. Excretion of calcium and of phosphate was reduced in the first half of the BR phase, and serum level of PTH was increased in the second half of the BR phase by addition of hypoxia. Of note, the timing and magnitude of hypoxia effects upon these three variables was closely matched for HAMB and for HBR, thus suggestive of a superposition of hypoxic effects on BR effects. Serum levels of both phosphate and of calcium increased during BR and also during HAMB, but there was, however, no significant HBR interaction for either of the two. Whilst increased serum levels of calcium and phosphate are likely driven by removal of bone apatite, increases during hypoxia are likely caused by hyperventilation-related alkalinisation of blood and increased binding of calcium to protein fix charges. The lack of HBR interaction alludes to the likelihood that PTH-induced regulation of calcium and phosphate by the kidney, as well as endogenous buffers for these solutes, will have limited excessive serum fluctuations by the combined effects of BR and hypoxia.

The study has also yielded a number of unexpected results. Firstly, it is rather surprising to see that there was no increase in urinary excretion during the first 24 hours of BR, as one normally sees a fluid loss of 800 to 1000 mL during this period [34]. Likewise, it is unexpected to see a decrease in urinary excretion by approximately 200 nmol/day during the first 24 hours of BR, and an increase by exactly the same amount on the subsequent day. When combining these numbers, there was no alteration in NTX excretion during the first 48 hour interval, and one might speculate whether the initial fluctuation in NTX excretion is a pharmacokinetic effect of fluid redistributions, rather than an effect upon bone resorption. The expected increase in bone resorption-driven NTX excretion might perhaps be seen in the non-significant elevation by ~90 nmol/day on day BR03 (P = 0.23) but the increase becomes significant only on day BR04, and it peaks on day BR10 only. Finally, it is noteworthy to see that bAP, which has long be regarded as a surrogate marker of bone formation increases initially during BR, when P1NP, admittedly with some delay, shows a decrease. Clearly, therefore, these two biochemical bone markers bear diverging information.

The present study has clearly demonstrated that BR induced alterations in bone formation and bone resorption are largely unaffected by addition of hypoxia. There was, however, a strong effect upon calcium homeostasis, with substantial reductions of urinary excretion of calcium and phosphate. As these two compounds play a major role in the formation renal concrements, the present study provides evidence for a mitigation of renal stone risk in BR [35] and astronauts. The benefit of this may, however, be limited, as the effect was most pronounced in the initial half of the BR, and it was not observed in the second half. Finally, the study also demonstrates that hypoxia-induced perturbation of calcium metabolism leaves bone resorption responses unaffected. This adds further evidence to the understanding that BR induced bone losses are mainly driven by the mechanical environment, and are quite independent of hormonal or endocrine changes.

4.1.3.4 Monitoring stress and hypoxia-sensitive immune and haematological changes (WP4)

Emotional state

The main finding of the present study is the significant change in negative mood in the HBR condition, compared to the HAMB and NBR conditions. This supports the notion that hypoxia exacerbates negative emotions experienced during bedrest. Participants did not exhibit any such psychological changes during the HAMB condition, suggesting that ambulation/activity might counteract the effects of hypoxia. Finally, noteworthy is the significant variation in the intra- and inter-individual psychological responses in the hypoxic and normoxic bedrest environments.

The observed differences in the psychological state between the NBR and HBR conditions can be attributed to the systemic hypoxia induced by hypoxic environmental conditions. In contrast to previous bedrest studies, in which the subjects were confined to their rooms, in the present study the subjects were given the opportunity to spend time in a common area, whilst maintaining the prescribed horizontal position. In addition, the ambulatory subjects were confined to the hypoxic facility at the same time as the hypoxic bedrest subjects, and often spent time with the subjects in their rooms. These factors provided much more social interaction among the subjects then reported for previous bedrest studies. Accounting for the activity within space habitats, this type of interaction certainly better mimics the interaction observed among astronauts on space missions. In the bedrest condition a significant increase appeared in tension on D14 compared to the pre-measurement, while a
decrease was noted just after the end of the BR condition in the post-measures. Although non-significant changes appeared in the remaining psychological factors it is important to note that depression increased on D14 and D21, decreasing after the end of the bedrest exposure. On the other hand, non-significant changes were revealed in participants’ positive psychological state (positive affect, vigor), which is not in accordance with our research findings [36]. There are two principal differences between the present study and our previous study [36]: 1) the duration of the present study (21-d) was double the duration of the previous one (10-d), and 2) as noted above, much more emphasis was placed on the social interaction between subjects, and between subjects and staff in the present study, than in our previous study. The bedrest-induced increase in negative emotions can be ascribed to the strict confinement and restriction of body movements, as well as to the isolation away from familiar environments, such as a reduction or a lack of social interaction [37 38].

Although most of the studies seem to support a detrimental effect of bedrest [36 39 40], others found an improvement during bedrest [41 42], or no significant change [43]. Such equivocal findings might be due to methodological issues, such as duration of bedrest exposure, research instruments and methodologies applied. On the other hand, it might be due to participants’ emotional characteristics, such as the manner in which they individually respond and adapt to adverse environments, the management of negative emotions, or the isolation from familiar environments (e.g. family, friends). Analysis of individual psychological adaptations to adverse environments may assist in the process of selecting crew members for space missions [37]. In addition to the above, understanding the effects of reducing physical activity below that of a sedentary lifestyle has implications for individuals with severely restricted physical activity [44 45].

The results show that HBR intervention elicited the most negative emotional state compared to the NBR and HAMB conditions. HBR participants exhibited higher negative affect, depression, fatigue, and tension compared to the participants of the NBR and the HAMB conditions. In contrast, no significant effect was noted in positive emotions such as positive affect and vigor, which is in accordance with previous research findings [36].

In summary, based on the current and previous results [36] of the joint effect of bedrest and hypoxia on psychological indices, it seems that confinement, rather than bedrest per se, has a detrimental effect on mood, as hypoxia augments the negative psychological responses mainly in the inactive, bedrest participants. This augmented negative mood is not evident in the hypoxic ambulatory participants, as has also been previously reported [36].

The comparison of the emotional responses between HBR and HAMB indicated that the participants in the HBR condition revealed their most negative psychological profile on D14 and D21, as their psychological profile continuously deteriorated. Specifically, the participants in the HBR compared to those of the HAMB condition indicated a more pronounced negative mood, consisting of higher tension, depression, confusion, and fatigue. It seems that the regular daily physical activity (two 30-min low-intensity exercise sessions) or other daily activities performed in the HAMB condition reduced the psychological impairment noted in the HBR condition. This is supported by recent research findings underlying exercise benefits for depression treatment [46 47]. Yeung’s review [48] of eighty-one studies regarding the influence of exercise on mood and mental health, noted that the majority (85%) of the studies supported an improvement on mood and this benefit seems dependent on the duration and intensity of the exercise [49 50]. Hötting and Röder [51], Gordon, Rykhlevskaia, Brumback, Lee, Elavsky, Konopack, McAuley, Kramer et al. [52] and Weinstein, Voss, Prahash, Chaddock, Szabo, White et al. [53] supported the important role of cardiorespiratory fitness and physical exercise in the facilitation of neuroplasticity and greater prefrontal cortex volume, and the improvement of cognitive functioning. Cognitive functioning seems to have a mediating role in subjects’ experience of negative emotions providing a better adaptation to stressful and adverse environments [54].

We conclude that hypoxia enhances the negative psychological characteristics in inactive, bedrest participants, but not in active and ambulatory subjects. Combined with the results of our previous study [36], the present results demonstrate that the hypoxic effect on mood is transient, and is only present during the hypoxic stimulus; it is not sustained once the hypoxic stimulus is removed, nor does it appear to be dependent on the duration of the hypoxic inactivity. The results confirm that a small volume of daily physical activity is sufficient to negate the negative emotional responses induced by a hypoxic environment, and it is germane to both astronauts and athletes to further investigate the potential effect of exercise to improve the psychological responses within a hypoxic environment. This study also provides an impetus for further research into the manner in which increased activity may improve the quality of life of patients rendered hypoxic and inactive by a respiratory or cardiovascular disease, not only by preventing degenerative processes, but by improving their psychological profile.
Effect of bedrest on haematology

Adapting to hypoxic and unnatural conditions, e.g. of immobilisation is not without any repercussions and is accompanied by adverse physiological and psychological effects as on the haematological system. To our knowledge, the steps involved in the adaptation process to such chronic stress are gradual and the biological system either builds up resistance to the stress conditions and maintains a healthy physiological and psychological equilibrium, or succumbs to the stress, resulting in disequilibrium. Beyond emotional reactions and adaptations as described above, the action of hypoxia, its metabolic and hormonal consequences as triggered by its mediators on the one hand, and the regulation of an orchestrated, pathogen/antigen targeted immune response on the other hand have to be elucidated since. These interactions have reached a broader attention in the field of clinical medicine as well as space exploration. In general, stressful conditions of psychological or physical nature can activate and/or paralyze specific immune responses and this was shown in manifold field and epidemiologic studies on Earth (reviewed in [55]) or in Space or Space analogues [as reviewed in “Stress challenges and immunity in Space” [56]).

Firstly, the study demonstrated that the condition of hypoxia leads to significant alterations especially impacting on the red blood cell line. We detected an increase of reticulocytes, the precursor cells of erythrocytes paralleled by an enhanced increase of erythropoietin under hypoxic conditions. Furthermore, the state of hypoxia induced a significant thrombocytosis. Thus, the numeric increase of blood cells lead to a hemoconcentration with probable impact on fluidity and velocity of the blood stream and possible consequences on other physiological functions such as e.g. cardiovascular ones that are to be closer investigated in cooperation with the other workpackages. Concerning the white blood cell line hypoxic bedrest showed a tendency of leuko- and granulocytosis when compared to normoxic bedrest [57]. Secondly, the immune analyses focused on determining the quantitative and qualitative immune changes regarding the immune cells’ activity and their reaction to stimulation by different antigens in vitro. Data collection was performed before, during and after the interventional periods. Blood was drawn and incubated for 48h with various bacterial and fungal antigens followed by an analysis of a battery of cytokines levels, including interleukin 2 and interferon-γ. As a control, blood was incubated without stimulation (resting condition). While the robust in-vitro DTH test as developed by us [58] showed very low cytokine levels under resting conditions and these remained unaffected throughout the entire observation period, irrespectively of the study arm, blood cells responded in a differential way when subjected to a simulated infection (antigen/stimulus). Depending on the stimulus hypoxia, a biphasic response pattern was induced with an early suppression and subsequent activation. During all conditions, the stimulating effects on immune responses tended to increase after 2 weeks of exposure, while the resting state of the immune answers was not affected. The further understanding of the complex cellular and receptor-dependent actions is under current consideration.

Endocannabinoid, glucocorticoid and cathecolamine concentrations

Since stressful conditions of psychological or physical nature can activate and/or paralyze specific immune responses [59-61], it has also been described that space flight associated stressful situations can result in an autonomous/sympathetic (ANS) and/or glucocorticoid (GC)-mediated immune modulation [62], which was associated e.g. with immune alterations resulting in the reactivation of herpes virus [63 64]. Other current investigations confirm the stress permissive role of the catecholaminergic and glucocorticoid system as well as of neural pathways involved in the control of the host’s immune response [65 66]. Additionally, the endocannabinoid (EC) system has been shown to be activated under the conditions of stress [67] and to modulate a variety of immune cell functions in humans and animals, including B, and T (T-helper) cell development and chemotaxis. It hence appears that the “immunocannabinoid” system is involved in regulating the brain-immune axis [68 69], which exerts an important pathophysiological control of inflammation on a cellular level. Especially the endocannabinoid system (ECS) has important defending functions in inflammation and the pronounced ability to limit inflammatory damages through various and partially interacting mechanisms which include effects on neuronal and immune cells. Because of its central and peripheral stress response system with its main ligands 2-arachidonylglycerol (2-AG) and arachidonoylethanolamide (anandamide) it was of our special interest. However, neither 2-AG nor anandamide were effected neither by bedrest nor by hypoxia and both agents showed no significant changes in the different conditions which might indicate also a lower stress level as in other more highly stressed individuals [70]. Glucocorticoid concentrations measured
twice a day (morning and evening) showed only little variations with slightly higher values in the evening. Comparable moderate changes were observed for the two catecholamines norepinephrine and epinephrine, respectively showing relatively low to normal catecholamine excretion with slightly elevated noradrenalin levels under hypoxic bedrest conditions. This altogether indicates that the stress hormones likely do not interfere to a major degree with the immune changes observed.

Hypoxia sensitive purines (e.g. adenosine, inosine) and erythropoietin concentration (EPO)

Of high interest is that the immune system is likely to be affected by conditions of hypoxia by the metabolic stress-response system that is based on the production of the purine nucleoside adenosine (purinergic system, PS) and its binding to four different adenosine (A1, A2A, A2B and A3) receptor sites all known to be involved in the regulation of immune cell effector functions [56 71 72]. The hypoxia-enhanced adenosinergic signalling on T cells induces strong immunomodulatory properties (and also important for virus clearance). In these regulatory mechanisms, oxygen deprivation and extracellular adenosine accumulation serve as "reporters", while A2A adenosine receptors serve as "sensors" of excessive tissue damage [72 73]. The A2A receptor-triggered generation of intracellular cAMP then changes immune cells´ activation status in a delayed negative feedback manner [72 74] while recent data show a contradictory action by the adenosine A2B receptor [75]. But not only the ligands´ action at the four adenosine receptors plays an important role, but also the formation of adenosine under the conditions of hypoxia through the actions of ATP degrading enzymes as well as the catabolism of adenosine through the adenosine deaminase. They are important tools to (re-) calibrate purinergic immune answers and to control the duration and magnitude of adenosin´s action. Moreover, it is not only acting locally, but is considered recently to be linked to the nervous systems´ control [76].

In the course of the PlanHab interventional period, significantly higher adenosine levels were detectable when hypoxia occurred under the conditions of bedrest. Hereby, an endogenous immunomodulatory effect of adenosine was displayed also in the immune answers. However, it was not possible to address all the cell type specific expression of the four adenosine receptors and the ATP, ADP, AMP degrading enzyme CD39 and CD73 [77], due to blood restrictions. However, the adenosine degrading enzyme adenosine deaminase (ADA) is targeted as key of the purinergic system to “switch” from immunosuppressive and pro-inflammatory states.

Erythropoietin is increased as well as expected due to the hypoxic stressor but shows a different pattern of slope from adenosine. This is in line with the current research addressing a potential inverse relation of adenosine and EPO to control cell proliferation and hence oxygen supply to the organs which can be seen here in the PlanHab condition for the first time as well.

Determination of non-invasive immune changes via exhaled markers at selective time points

During the study we assessed a broad assortment of biological markers and bio products in exhaled air and carried out online and/or offline measurements of markers in exhaled air using gas analysers based on mass spectrometry. The molecular analyses of exhaled breath offer a novel and non-invasive method to diagnose a variety of organ dysfunction including lung pathologies and metabolic changes. Functions include e.g. i) the end-tidal oxygen and carbon dioxide concentrations in the exhaled air and ii) the changes in immune activity and oxidative stress as reflected by changes in nitric oxide, propionic acid, isoprene, pentane and malondialdehyde concentrations [78]. The characterization of biomarkers in exhaled breath is the result of research activities during recent years which have identified a number of important biochemical pathways which generate bio products that are released from tissue into the gas phase. Breath air samples were taken according to the protocol at seven different time points over the whole study period. All samples were kept frozen until measurements in our premises. The analysis of the acquired data set revealed complete sets of vials, technically successful analyses of all the samples by the mass spectrometer. Likely due to an un-expected malfunction of the rubber lid of the vials, the collected breath air was partly “contaminated” by CO2 that passively entered the vials from dry ice as used during storage and transportation. Since CO2 is needed to calibrate the quality of the exhaled air sample, those samples will be excluded from the analyses which will show odd high values. A final assessment is under way since a compensation algorithm needs to be established to correct for the higher values.
4.1.3.5 Hypoxia and physical inactivity regulation of oxidative stress and thermogenesis (WP5)

Effects of bedrest and/or hypoxia on insulin sensitivity.

Exercise training in hypoxia has been shown to reduce fasting and postprandial insulin concentration, reduce insulin resistance [79] and improve glucose tolerance [80] more effectively than training in normoxia. In the current study, fasting insulin sensitivity was improved by 17 days confinement in a normobaric, hypoxic environment with daily exercise; the exercise regime being designed to maintain the participants’ habitual daily energy expenditure, so that de-training did not occur over the confinement period. However, participants did not undergo a similar ‘ambulatory’ intervention in normoxia, so although the improvement in fasting insulin sensitivity may have been due to the hypoxic environment, it is also possible that the daily exercise prescribed during HAMB was greater than the individuals were doing in their daily life, such that the improved insulin sensitivity was simply due to increased physical activity. The latter having been demonstrated to improve insulin sensitivity and glucose regulation in numerous studies [81]. There was some suggestion in the current study that insulin sensitivity was negatively affected by bedrest alone (NBR), which is supported by findings from other bedrest studies and investigations in astronauts following periods in microgravity, a condition where there is unloading of the muscles [82 83]. Tobin et al. [83] suggest that the impaired glucose regulation observed in astronauts is the result of decreased insulin secretion. However, this did not seem to be the case in the current study, as normoxic bedrest resulted in a weak trend for circulating serum insulin to be increased; a finding reported previously after a period of bedrest [84]. Interestingly, this tendency for insulin to increase, and proxy measures of fasting insulin sensitivity to decrease with enforced inactivity, was not observed in the HBR intervention. Intermittent hypoxia has been shown to increase whole body glucose uptake (WBGU), and in vitro investigations suggest that this is due to an increase in insulin independent glucose uptake, as a result of up-regulation of GLUT1 gene transcription by hypoxia-inducible factor-1 [85 86]. If occurring in participants in the current study, then an increase in glucose transport via GLUT1, may well act as a counterbalance to the decrements in insulin-mediated glucose uptake seen in skeletal muscle during inactivity, and could explain the absence of bedrest induced changes to insulin sensitivity seen in the HBR intervention. In summary, an index of fasting insulin sensitivity was improved by 17 days confinement in a normobaric, hypoxic environment with daily exercise, but was negatively affected by 17 days of bedrest in a normobaric, normoxic environment. Hypoxia may ameliorate this reduction in fasting insulin sensitivity induced by bedrest.

Effects of bedrest and/or hypoxia on glutathione status

Glutathione status was affected by both physical inactivity and hypoxia, as demonstrated by the decrease in red blood cell concentrations of glutathione and all its precursors (cysteine, glycine and glutamate). However no changes in glutamyl cysteine ligase, the enzyme involved in the limiting step reaction of glutathione synthesis, had been observed in our study suggesting that changes in enzyme activity are not primarily involved in changes in total glutathione. The amino acid 5-oxoproline is an intermediate of the gamma-glutamyl cycle and has been proposed as marker of glutathione status and turnover in cell systems and in animal models [87]. In preliminary work we demonstrated that plasma 5-oxoproline level is a marker of in vivo glutathione synthesis and turnover in humans. In the present study we did not observe changes in glutathione synthesis, as demonstrated by 5-oxoproline data. These data lead us to hypothesize that the decrease in total glutathione could be associated to changes in the utilization of this antioxidant.

Effects of bedrest and/or hypoxia on muscle gene expression

As assessed in muscle samples, expression of genes involved in controlling muscle size was not affected by physical inactivity nor by hypoxia suggesting that muscle atrophy that had been demonstrated to occur is not related to changes expression of genes related to protein synthesis and/or breakdown, but can be related to other mechanisms potentially related to changes in anabolic sensitivity and/or changes in amino acid availability in muscles. Moreover, neither hypoxia nor bedrest affected the adaptation in energy balance and metabolism and only bedrest demonstrated to have negative effects on mitochondrial dynamic, known to be related to several metabolic impairments.

Effects of bedrest and/or hypoxia on metabolic markers assessed in erythrocyte fatty acid membrane.
Membrane fatty acid composition has been demonstrated to be strongly affected by bedrest and to allow the determination of reliable indexes of metabolic changes [88]. In a previous study [88] we have demonstrated that Δ-5 desaturase activity, measured in red blood cell membranes, can be considered a reliable and more sensitive marker of insulin resistance in bed ridden healthy young subjects. The insulin resistance developed following physical inactivity can directly affect the fatty acids membrane composition mainly through changes in fatty acid desaturase activities. Evidence indicates, in fact, that the activities of Δ-5, Δ-6 and Δ-9 desaturases are affected by insulin action [89]. Δ-5 desaturase is an enzyme that converts dihomo-γ-linoleic acid (20:3 n6 Poly Unsaturated FA, PUFA) to arachidonic acid (20:4 n-6 PUFA), a FA with pro-inflammatory function, and eicosatetraenoic acid (20:4 n-3 PUFA) to eicosapentaenoic acid (20:5 n-3 PUFA), a FA with anti-inflammatory function. Interestingly, in the present study, Δ5 desaturase index decreased only due to hypoxia, whereas it increased due to bedrest, both in normoxic and hypoxic condition. More interestingly, these data are in agreement with results obtained by traditional methods (HOMA and QUICKI reported above). Finally, total amount of anti-inflammatory compounds significantly increased after all conditions, whereas the total amount of pro-inflammatory compounds significantly decreased after all conditions. Consequently, indices of systemic inflammation during bedrest associated with normoxia or hypoxia decreased, potentially suggesting the development of an adaptive anti-inflammatory profile after 3 weeks of physical inactivity and/or hypoxia.

Postprandial insulin sensitivity
Classically, as insulin sensitivity falls, circulating insulin concentration increases to maintain normal glucose tolerance, and this appears to be due to both increased secretion, and reduced clearance of insulin [90]. C-peptide and insulin are secreted in equimolar concentrations from pancreatic β cells, although insulin has a shorter half-life in the body, primarily due to first-pass hepatic extraction and a peripheral clearance rate that exceeds that of c-peptide [91]. C-peptide appearance in the circulation thus provides a useful indication of insulin secretion, albeit semi-quantitative [92]. As insulin resistance develops, circulating insulin ceases to be able to control blood glucose concentration, and blood glucose rises. In the current study, postprandial glucose, insulin and c-peptide concentration were increased at V2 in NBR, suggesting that insulin sensitivity was lower after 16 days supine bedrest in normoxia. Indeed, the index of fasting insulin sensitivity (QUICKI) was also reduced in this intervention, and this observation of lower glucose tolerance and development of insulin resistance is corroborated by other bedrest studies and investigations of crew members following different periods of space travel [93 94]. Tobin et al. suggested that the impaired glucose regulation observed in astronauts was the result of decreased insulin secretion. However, this did not seem to be the case in the present NBR condition, as circulating serum insulin increased from pre-intervention; a finding reported previously after periods of bedrest [84]. Interestingly, 16 days of bedrest in hypoxia also resulted in higher postprandial plasma glucose, but this was not accompanied by a rise (as seen in NBR), or fall (as reported in astronauts) in circulating insulin. However, although no change to circulating insulin was observed, a similar c-peptide response to that reported for NBR occurred, suggesting that a stimulus for insulin secretion was indeed evident in HBR, but that increased clearance of insulin was occurring in the hypoxic bedrest condition. This was reflected in an increase in the c-peptide to insulin ratio, both when fasted and over the 120 min postprandial period, whereas at V2 in the NBR condition, insulin clearance did not appear to be affected. Although there are limitations with c-peptide:insulin as an index of insulin clearance, especially in non-steady state situations, it is noteworthy that a postprandial elevation in this ratio, of similar magnitude to that induced by HBR, was also observed at day 17 of the HAMB intervention, in the absence of significant changes to circulating c-peptide levels. These observations are not unprecedented, as it has been suggested that those living at altitude may display higher hepatic insulin clearance than those residing at sea-level [95].

In the normobaric, hypoxic environment with daily exercise (HAMB), the exercise regimen was designed to maintain the participants’ habitual daily energy expenditure, so that de-training did not occur over the confinement period. This intervention appeared to conserve the individuals’ postprandial insulin sensitivity, as neither insulin nor glucose measures in the fed state changed significantly after the 16 days. Indeed, there was some suggestion that fasting insulin sensitivity may have been improved. Those living at high altitude have been shown to have lower blood glucose compared to age and weight matched lowlanders [96], and these observations have been attributed to an increase in glycolysis and augmentation of insulin-independent glucose uptake, both mediated through the effects of hypoxia-inducible factor-1α [86 97]. In the current study, fasting glucose was significantly lower after 16 days in hypoxia (HAMB), giving credence to the notion of increased
insulin-independent glucose uptake, and was accompanied by a trend for lactate to be higher, the latter perhaps indicating that glycolysis was also increased. However, in the insulin-stimulated state, blood glucose concentration in HAMB did not differ from the pre-intervention value, and similar to in other reports, postprandial glucose and lactate profiles did not provide convincing evidence that postprandial glycolysis was significantly increased in hypoxia [98]. In contrast, in the bedrest conditions, where reduced insulin-stimulated uptake may be occurring, postprandial lactate concentration in HBR was greater than in NBR, although the addition of hypoxia did not appear to diminish the elevation in blood glucose concentration induced by bedrest in NBR in the fed or fasted state, or to affect the rate of appearance of 13CO2 in the breath from breakdown of ingested 13C-glucose.

Adiponectin is a hormone produced by adipose tissue, that plays a role in glucose and lipid regulation [99]. It is negatively correlated with body fat mass, and is reduced in insulin resistance states and type 2 diabetes [100]. Few studies have been carried out to investigate changes to circulating levels of this hormone in relation to altitude, hypoxia and inactivity / bedrest, and as a result changes in adiponectin are poorly characterised. In the current study, there was a significant fall in this variable irrespective of intervention. Alterations in adiponectin concentration have been related to changes in adiposity. However, total body fat in this study did not change significantly over the interventions, and actually a small numerical fall in fat mass was noted. Therefore, adiposity per se cannot adequately explain the findings. It is possible that in the bedrest conditions this reduction in circulating adiponectin was due to (or was involved in the development of) an increase in insulin resistance. Indeed, in female patients who had been on long term bedrest, low circulating adiponectin levels were reported, although most of these patients were not ambulatory due to a cerebral vascular accident, a condition associated with hypoadiponectinemia [101]. However, reduced adiponectin levels were also observed in the non-bedrest condition (Hamb), where changes to insulin sensitivity did not appear to be occurring. In vitro, studies have indicated that in hypoxic conditions, production of adiponectin (both at the level of transcription and protein synthesis) is reduced in adipocytes [102], which may help to explain the lower circulating levels in Hamb. However, contrary to this in vitro work, an increase in adiponectin has been observed in mountaineers following 9 days at altitude, although this was accompanied by a significant reduction in body fat mass, which could have confounded the findings [103].

In summary, an index of fasting insulin sensitivity was improved by 16 days confinement in a normobaric, hypoxic environment with daily exercise, but this and postprandial insulin sensitivity was negatively affected by 16 days of bedrest in a normobaric, normoxic environment. Hypoxia per se may increase insulin clearance, although this study cannot indicate where the site of this action may lie.

Appetite

Although subjective appetite assessments using visual analogue scales (VAS) have been shown to have poor reproducibility [104], a relationship between satiety (as assessed by VAS) and subsequent food intake has been demonstrated [105]. Indeed, in the current study, there was a significant correlation between CSS and subsequent food intake at each visit. At altitude, reduction in appetite and dietary energy intake has been recorded [106]. However, acute mountain sickness is thought to play a large part in this phenomenon [107], with changes to perceived palatability of food and an uncoupling of ‘hunger’ and ‘desire to eat’ also occurring during hypobaric hypoxia [108]. In the current study, changes to subjective appetite, and intake at the ad libitum meal, were not observed as a result of 17 days confinement in normobaric hypoxia (Hamb), which corroborates findings from a previous study investigating the effect on appetite of 10-days in a similar environment [109]. This lack of an effect on appetite may be due to an absence of symptoms of acute mountain sickness, or reflect differences between normobaric hypoxia and hypobaric hypoxia as a stimulus for appetite changes. During sojourns in conditions of microgravity, and periods of bedrest with head-down tilt, reduced dietary energy intake has consistently been reported [110 111]. This is coupled with a reduction in appetite and a shift in dietary preference to high carbohydrate foods. In the current study, 17 days of supine bedrest (NBR and HBR) did not appear to affect the participants’ appetite or food intake at the ad libitum meal, and as the meals provided to participants were standardised, they did not have the ability to change their macronutrient intake to any great extent. With regards to the ad libitum meal, there may have been an issue with the palatability of the pasta dish used, or the participants appeared to exhibit a degree of restrained eating during this test, such that this assessment of appetite may not have been sensitive enough in this cohort to detect changes. Individuals were asked to eat until ‘full’, which would expect to trigger a satiety score close to the maximum score of 100. However, less than half of
the occasions resulted in a satiety score of >85 at the end of eating and on ~15% of occasions, eating terminated with a CSS of <75.

Leptin is a hormone primarily secreted from adipose tissue, although production also occurs in a range of other tissues including gastric cells in the fundus region of the stomach. It acts as a central satiety signal, and circulating levels are closely associated with body fat mass [112 113]. Hypoxia has been shown to cause an increase in leptin levels [114-116], an elevation which persisted after 7 days at altitude [114]. However, the response of leptin to hypoxia has been widely debated and reports in the literature present contradictory findings in regards to leptin responses to this stimulus [117 118], perhaps due to the different experimental paradigms (hypobaric vs. normobaric hypoxia, rest vs. exercise and different hypoxic doses) used. Similarly, in bedrest conditions, leptin has been shown to increase, or stay the same [119-121]. In the current study, fasting leptin did not change significantly with bedrest (NBR), although a reduction in this variable, as a result of hypoxia, was observed. These findings are contrary to what has previously been described and it is possible that small alterations in fat mass may have influenced these outcomes. It is the intention that these data will be further investigated in conjunction with basic core data collected during PlanHab to address this hypothesis.

Peptide YY (PYY) is a short chain peptide released by L-cells in the gut in response to the presence of food, and appears to act as a central satiety signal, with peripheral PYY infusions reducing volitional food intake in humans [122]. Although the effect of altitude on PYY has not been well characterised, acute normobaric hypoxia appeared to reduce fasting and postprandial circulating PYY, both at rest and after a single bout of exercise [123]. However, in the current study, there were no statistical changes in circulating PYY, when fasted, or in the postprandial period, as a result of any of the interventions. Other studies investigating the effect of longer term hypoxic exposure on appetite, have also failed to detect changes in PYY, thus implying that PYY might not be involved in hypoxia induced appetite modulation [109]. Similarly, in the current cohort, no change to PYY appeared to occur as a result of bedrest, a finding confirming that reported in women after 30 and 60 days of bedrest [124], suggesting that PYY is also unaffected by periods of inactivity.

Glucagon-like peptide-1 (GLP-1) is secreted, in response to meal ingestion, from intestinal endocrine L-cells, located mainly in the distal ileum and colon [125]. It is associated with promoting satiety through several mechanisms, including slowing gastric emptying. It also enhances insulin secretion and biosynthesis in the pancreas [125]. Postprandial concentration of GLP-1 is reduced in obese and type 2 diabetic individuals, which is proposed to be due to reductions in GLP-1 secretion [125], whilst increases in fasting plasma GLP-1 have been reported in females following 60 days bedrest [120]. In contrast to the Bergouignan study, 17-days bedrest in our cohort did not result in an increase in fasting GLP-1, although the intervention was much shorter than the former protocol, which may explain why no changes were observed [120]. However, postprandial GLP-1 response in the current study was decreased as a result of 17 days bedrest (significantly in HBR and a trend for this to occur in NBR). To our knowledge, the effect of bedrest on postprandial GLP-1 responses has not previously been examined, so it is not possible to compare our results with similar protocols. However, the attenuated postprandial GLP-1 secretion seen in obese individuals has been proposed to be due to increases in circulating free fatty acids [126], although this does not seem to be pertinent in our cohort. To date, little attention has been given to the effect of hypoxia on GLP-1 [115]. Hence, its role and contribution to the complex altitude-related appetite reduction is currently unclear. In response to acute hypoxia [115], and 10 days of hypoxic confinement [109], fasting and postprandial circulating GLP-1 concentration did not change. With regards to the effect of hypoxia alone on GLP-1, our findings confirm those from other studies, in that no change to fasting or postprandial GLP-1 was detected in HAMB.

Ghrelin is an appetite-stimulating hormone, and its infusion (both peripherally and directly into the cerebral ventricles of rodents), results in increased energy intake and body weight gain [127]. It is secreted by cells primarily found in the stomach, and release of this hormone is suppressed by feeding. Ghrelin is the only appetite-stimulating gut peptide proposed to play a role in altitude-related appetite modulation. Acute hypoxia has been shown to reduce circulating levels of this hormone, both in the fasted [114] and fed state [128 129], although fasting ghrelin concentration is purported to return to baseline concentrations after 7 and 49 days of continuous exposure [114 117]. However, reports in the literature have also reported no change to ghrelin as a result of hypoxia [106 109], or bedrest [130], the latter being supported by our findings, as ghrelin did not change in NBR. However, in the current study, contrary to other reports, fasting ghrelin was significantly higher or showed a strong trend to be higher, in the hypoxic conditions, with the suppression of ghrelin as a result of feeding also being attenuated. However, these differences in circulating ghrelin did not appear to affect subjective appetite or energy intake at...
the ad libitum meal in the current study. Indeed, the role of ghrelin in hypoxic anorexia is ambiguous, with conditions such as exercise having been found to suppress postprandial acylated ghrelin in normoxia, without a corresponding reduction in appetite [123]. The reason ghrelin increased in HAMB and HBR is difficult to explain. Although macronutrient composition of the diet have been shown to influence ghrelin secretion, with carbohydrate in single test meal studies inducing the greatest postprandial suppression in ghrelin [131 132], this is unlikely to be a pertinent confounder in the current study, as individuals consumed the same antecedent diet prior to the nutrition study at each intervention. Circulating ghrelin is also thought to be affected by serum insulin concentration, as inverse associations between the two have been reported, and decreased ghrelin concentration has been observed during insulin infusions [131 133]. However, in our cohort, fasting and postprandial circulating insulin did not change in Hamb, or HBR. In summary, although a trend for reduced postprandial GLP-1 was observed in NBR, this intervention did not result in changes to appetite related hormones, or subjective appetite. In HBR, the circulating hormone results point towards a situation of appetite stimulation (decreased fasting leptin and postprandial GLP-1, and increased fasting and postprandial ghrelin). However, this was not reflected in any changes to measures of appetite made in the current study. Finally, hypoxia alone (HAMB) resulted in a decrease in leptin and an increase in GLP-1 concentrations, but these have been shown to cause opposite effects on appetite, and no changes to measures of appetite were observed.

Fasting lipids

Cholesterol homeostasis has been shown to be affected by many factors, including dietary intake, stress and physical activity. In the current study, the meal pattern and composition was standardised to control for these potential confounders, and macronutrient composition of the diet was the same across all interventions (data presented previously in deliverable D5.29). Dyslipidaemia is associated with an increased risk of cardiovascular disease, and a beneficial lipid profile has been characterised as; total cholesterol <5mmol/l, LDL <3mmol/l, HDL >1.2mmol/l and a total cholesterol to HDL ratio of <4.5. Before the interventions, only one participant had a total cholesterol and LDL outside of the healthy range. However, elevation of these variables in this individual was mild and his HDL and total:HDL ratio was within the healthy range. Reduced total cholesterol and LDL have been observed following 17 days at high altitude [134], whilst HDL concentration has been shown to increase [135 136], decrease [103], or stay the same [134] in response to moderate or high altitude. In the HAMB condition, we too saw a reduction in total and LDL cholesterol, and also a reduction in HDL. However, the latter finding may have simply been a facet of the overall reduction in circulating lipids in this condition, as the ratio of total cholesterol to HDL did not change after 16 days of HAMB. In HBR, a reduction in total and HDL cholesterol was again seen. However, contrary to HAMB, a significant fall in LDL was not realised, resulting in a significant decrease in the proportion of total cholesterol that was HDL. After 21 days of bedrest (with head-down tilt), total cholesterol has been shown to decrease [121], with HDL also falling in response to short [137] and medium term bedrest [138]. Although this pattern of lipid modification was observed in HBR, in NBR a significant fall in LDL was not detected, but the reduction in HDL did manifest. As a consequence, in each bedrest condition an increase in the proportion of total cholesterol that was LDL, and reduction in that which was HDL occurred, and this more negative lipid profile is often associated with insulin resistance and cardiovascular disease [139]. Indeed, although the numbers were low, the bedrest intervention resulted in an increase in the number of individuals with a Total:HDL ratio in the range associated with increased long term risk of developing cardiovascular disease [139] and might have implications for future planetary habitation.

In summary, 16 days of bedrest promoted a more negative fasting lipid profile, whereas the converse occurred in hypoxia, and these observations may be associated with changes to markers of insulin sensitivity.

Body composition modulation under hypoxia and bedrest

Both unloading [140 141] and hypoxia [142 143] can alter body mass and composition. In the present study the participants’ body mass was significantly reduced following all three experimental campaigns with similar temporal changes. Thus, no specific effects of unloading or hypoxia per se could be identified based on the body mass outcomes. Under appropriate nutritional support, unloading results in muscle volume reductions [14], whereas hypoxia seems to affect both muscle and fat tissue [144]. However, when comparing both the whole and regional body composition results following the NBR and HBR confinements no additive effect of hypoxia could be elucidated. This is also confirmed by the unchanged
android to gynoid ratio indicating the lack of any specific regional fat tissue re-distribution. The only difference between the two bedrest confinements was that the FFM remained reduced at Rec only following the NBR. The faster regain of the FFM following the HBR campaign might suggest that hypoxia-induced water shifts between the extracellular and intracellular compartments [145] have contributed to the observed FFM changes after hypoxic confinements (a similar trend was noted for HAMB). However, given that no significant difference in water balance were observed between the campaigns, these observations are most likely the result of different dietary patterns and/or unsupervised activity during the recovery period. We also could not elucidate any specific effect of hypoxia during unloading when analysing individual responses. While correlation between initial body mass and FFM and subsequent changes in these two parameters was noted in the NBR, no correlations in body composition measures were observed following the HAMB and HBR.

The magnitude of the FFM reduction observed following the NBR campaign is in line with that previously observed following medium-term bedrest studies; i.e. a ~10 % reduction in the lower limb postural muscles [14]. Profound reduction of SpO2 throughout the HAMB and HBR confinements confirms the hypoxic nature of these interventions. However, sustained systemic hypoxia did not significantly affect body mass or composition. This, rather surprising finding, contradicts numerous studies indicating that exposure to altitude/hypoxia per se will reduce muscle and fat tissue mass [146 147]. Although other environmental factors than hypoxia could have confounded the initial field studies, the controlled laboratory trials confirmed that hypoxia per se may alter body mass and composition [148]. A viable explanation for the lack of any discernable effect of hypoxia in this study could be the dose of hypoxia. Indeed, high/extreme altitude (> 5000 m) sojourns have been utilized in the majority of studies reporting hypoxia-induced body mass reductions in healthy individuals [146-148]. On the other hand, reductions in body mass following lower altitude sojourns or simulated altitudes have predominantly been observed in overweight/obese populations [149 150]. As noted earlier by Westerterp and Kayser [151] it seems that, at least in healthy humans, hypoxia significantly effects body mass and composition, secondary to perturbed energy balance beyond a threshold altitude of ~5000 m. Nevertheless, our findings indicate that a sustained hypoxic stimulus equivalent to ~4000 m altitude, more severe than that envisaged within the Moon and Mars habitats, does not significantly affect body mass modulation during unloading.

Surprisingly, FFM reductions following the HAMB were similar to those observed during the NBR and HBR. Furthermore, significant reduction in the upper arm FFM was only observed following the HAMB. While the latter observation might not be of significant physiological relevance, the HAMB did induce significant reduction of whole-body FFM. The potential factors underlying these FFM reductions include insufficient energy and macronutrient intakes, water content shifts between extracellular and intracellular compartments, reduced physical activity levels and effects of confinement per se. In addition, exercise-related protein synthesis suppression during chronic [152] and acute [153] hypoxic exposure might have played a role in the FFM modulation following the HAMB. However, since the exercise sessions were performed at a low intensity the contribution of this mechanism would be limited.

Energy expenditure and appetite sensations

No differences in REE observed during the NBR campaign is in line with previous reports showing unaltered or slightly reduced REE during unloading [154]. Interestingly, the REE was also unchanged during both hypoxic campaigns. This is in contrast with our hypothesis and previous reports showing increased REE during exposures to environmental hypoxia in humans [149 155 156]. This increase in basal metabolic rate has been attributed to the altitude-induced beta-sympathetic activation [157]. On the other hand, the studies by Nair et al. [158] and Westerterp et al. [159] demonstrated decreased REE as a result of hypobaric hypoxia. The discrepancies between the study outcomes might result from different experimental settings (field vs. laboratory), environmental factors (cold, confinement) and different activity levels. The only significant difference observed during the indirect calorimetry measurements were the increased E values during both HAMB and HBR, reflecting the well established augmented hypoxic ventilatory response.

Similarly to REE, the measured appetite markers did not significantly differ between groups and/or between different time periods. This is surprising given the fact that the majority of previous studies reported significant decreases in appetite as a result of hypoxia per se [142 148]. It could be argued that the simulated altitude (~4000 m) in this study was insufficient to significantly alter appetite. However a recent study demonstrated that even relatively short (7-hrs) normobaric hypoxic exposures to (~4000 m) suppress hunger and dietary intake [128]. The fact that the perceived appetite sensations were
unchanged and the energy intakes were reduced suggest, that the participants were eating according to their feelings.

Nevertheless, during the HAMB campaign, hypoxia might have modulated their appetite/satiety, since the intakes were reduced significantly more than during both bedrest campaigns. Confinement per se could have additionally influenced the appetite outcomes, and thereby mask the effects of hypoxia per se, since monotony secondary to confinement has previously been implicated in appetite and intake alterations [160].

Energy and macronutrient intakes
The purpose of the standardized diet was to minimize the potential confounding effects of different energy and macronutrient intakes. The actual energy intakes of the participants were insufficient to prevent a significant body mass decrease in all three experimental campaigns. Interestingly, the participants in all campaigns maintained their fat mass even though their energy intakes were considerably lower than targeted. The Harris-Benedict equation for calculating basal metabolic rate is often employed for determining energy demands during simulated microgravity and seems to accurately reflect the energy requirements during such conditions [161]. It is of note, that the calculated REE were lower (~15%) than those determined by indirect calorimetry. However, even though the calculation-based targeted intakes might have been slightly underestimated, the participants in all campaigns consumed significantly less food than they were provided with.

The macronutrient diet composition used in this study was similar to that of other recent studies scrutinizing microgravity effects on nutritional status. These studies mostly employ ratios of 55% for carbohydrate, 30% for fat and 15% for protein intakes, which is in line with the present study. This is important since alterations of the macronutrient composition can significantly affect the body composition modulation. The protein intake might be particularly important during unloading periods as it has been documented that a higher protein intake can lessen the inactivity-induced reduction of muscle protein synthesis and consequently reduce muscle mass wasting [162]. Nevertheless, in our previous study [163] a higher protein intake (20% of total intake) did not prevent FFM reductions following both normoxic and hypoxic bedrest. Furthermore, no significant correlation was noted between the participants’ protein intakes and subsequent changes and lean mass following all campaigns. The potential contribution of the high sodium intake related exacerbation of inactivity-induced muscle loss [164] was minimized in the present study as the sodium intakes were well below the targeted levels (< 3500 mg day⁻¹). The used PAL factors of 1.4 and 1.2 for the HAMB and both bedrest campaigns, respectively are in line with other studies investigating the effects of simulated microgravity on healthy humans [165-167]. While the results of the NBR and HBR campaigns do not indicate that a PAL higher than 1.2 should be employed when unloading is combined with hypoxia the 1.4 PAL used for the HAMB might have been insufficient to maintain energy balance and consequently body composition. As reviewed by Melzer at al. [168], reducing physical activity below 1.8 PAL does not allow for compensatory appetite and energy intake adaptation. Although the participants performed two low-intensity exercise sessions daily to simulate their daily physical activity throughout the HAMB campaign their activity levels might have been below their habitual ones. The low estimated PAL (1.3) values during the HAMB campaign (obtained from the energy balance data) support this notion and also suggest that the participants were not underfed. These data collectively indicate that, besides hypoxia, reduced activity during the HAMB contributed to the observed FFM reduction.

Water balance
Hypoxia-induced water retention, closely related to AMS [169], can alter water balance especially during the initial acclimatization period [145]. Similarly to hypoxia, microgravity has been shown to influence water balance [170]. To date only two studies investigated the combined effects of hypoxia and unloading [163 171]. The first study by Loeppky et al. [171] reported greater alterations in balance during 7-day hypoxic than normoxic bedrest. In particular, the urinary output was comparable during the first few days but was significantly higher during the latter stage of the experiment in the HBR group only [171]. In contrast, our recent study [163] noted decreased urinary output during the latter stages of both 10-day hypoxic ambulatory and hypoxic bedrest conditions. This finding could be explained by the reported reduction of urinary output following altitude acclimatization [172]. Although both above mentioned studies reported significant changes in water balance the data from this study do not suggest any major alteration in water balance. The water intakes were comparable between and throughout the campaigns. Given that altitude-related water retention is tightly associated to AMS [169] the low incidence and severity of AMS in the present study, might at least partly, explain the lack of significant changes observed as a result of
hypoxic exposure. Furthermore, the fact that the water balance was not assessed on a daily basis could have masked potential differences.

In conclusion, our data suggest that exposure to simulated altitude of ~4000 m does not aggravate the whole body mass and fat free mass reductions during a 21-day simulated microgravity. The hypoxic dose used in the present study also does not seem to exert any significant effect on resting energy expenditure, perceived appetite or water balance. Finally, higher levels of energy intakes, than those targeted in the present study are not warranted when unloading is combined with hypoxic exposures up to simulated altitudes of 4000 m. The observed reductions in body mass and body composition modulation following hypoxic ambulation suggest potential role of confinement in such experimental settings. This study provides novel insight into the combined effects of unloading and continuous hypoxia on body composition modulation in healthy humans.

4.1.3.6 Hypoxia-induced thermoregulatory dysfunction, sleep disorders, and ventilatory equivalent altitude (WP6)

Behavioural thermoregulation

During all three interventions (NBR, HBR and HAMB) we determined the lower (Tlow) and upper (Thigh) limits of the thermal comfort zone (TCZ), with the difference between these temperatures reflecting the magnitude of TCZ. No statistically significant differences were found between the interventions, or between different measurement days (D-5, D10 and D20) within each intervention.

The TCZ was significantly wider during the second than the first half of the trial in NBR10, and it exhibited a similar tendency (p=0.05) in the NBR20 trial. It can be hypothesised that this pattern, with similar lower limits and higher upper limits of regulated temperatures, was due to greater cutaneous vasoconstrictor response in the legs during the NBR10 than the baseline measurements. Since subjects were not wearing socks during the experiments, they might have been experiencing cold feet, especially towards the end of the experiment and hence might have started up-regulating the temperature in the WPS.

Although a 35-day normoxic bedrest has been reported to affect not only autonomic temperature regulation during immersion in 28°C water, but also thermal perception with subjects perceiving given combinations of skin and core temperatures as warmer and thermally less uncomfortable after than before the bedrest [173], the study of Yogev et al. [174], reported no effect of a 21-day horizontal bedrest under normoxic conditions, on the thermal comfort zone. Although cold sensitivity increased, the change did not seem to be of sufficient magnitude to cause any alteration in behavioural thermoregulatory responses [174].

Hypoxia has also been reported to alter autonomic thermoregulatory responses, such as cutaneous vasoconstriction [175 176], and to decrease sensitivity to cold [177], hence potentially changing the perception of thermal comfort. It has been suggested that a normobaric hypoxic exposure induces slowing of neural activity in the sensor-to-effector pathway, but does not affect the threshold for cutaneous detection of either warmth or cold [178]. However, the results reported by Golja and Mekjavic [179] and Golja et al. [180] indicate that behavioural temperature regulation does not appear to be compromised by moderate hypoxia. Present results from the HAMB condition, confirm previous findings [179 180], and extend them to suggest that neither acute nor prolonged exposure to moderate hypoxia affects behavioural thermoregulation. Moreover, previous findings that neither hypoxia nor inactivity/unloading per se alter behavioural thermoregulatory responses [174 179 180] are confirmed by present results, and extended to include that the combination of hypoxia and inactivity/unloading does not affect behavioural thermoregulation. No statistically significant differences were found when observing the separate and combined effects of normobaric hypoxia and/or inactivity/unloading. Even though not significant, there was a difference in the lower limits of the thermal comfort zone between conditions, with Tlow higher during HAMB (D10 and D20), and lower during HBR and NBR (D10 and D20). Since the upper limits of the TCZ did not differ as much as the lower limits, the latter contributed to a widening, albeit not significant, of TCZ in the HBR and NBR conditions.

Significantly higher Tre was measured during baseline HAMB, than after 10 and 20 days in the HAMB condition. No other significant differences in Tre and Tsk were observed within or between experimental conditions. There was no difference between or during each of the interventions as regards the ΔTforearm-fingertip and ΔTcalf-toe, reflecting no inter-condition difference in skin perfusion, with the levels of the ΔTforearm-fingertip and ΔTcalf-toe indicating a slight cutaneous vasoconstriction in all three conditions. That neither bedrest, hypoxia, nor the combination of these interventions substantially
increased proximal-to-distal skin temperature gradient is somewhat surprising considering that previous studies indicate that prolonged exposure to both hypoxia and sustained bedrest induce cutaneous vasoconstriction. Golja et al. [175] observed a progressive decrease in subjects’ skin temperature during 35 days of horizontal bedrest, with changes particularly obvious in distal body regions, and with the most pronounced temperature decrements observed at the tips of the toes, suggesting that cutaneous vasomotor tone increased gradually during the course of the bedrest. During sustained hypoxic exposure, cutaneous vasoconstriction was shown to increase from day 1 to day 10, especially in lower extremities [176]. Yogev et al. [174], on the other hand, observed no significant difference in ΔTforearm-fingertip and ΔTcalf-toe between days 1 and 22 of bedrest, a finding which is in agreement with the observations in the present study when comparing days 10 and 20 with baseline values within all three experimental conditions.

Autonomic temperature regulation

Similar to observations of exercise thermoregulatory responses reported following 35-day normoxic bedrest [173], we did not observe any alterations in sweating but a higher vasodilation and a lower skin temperature during the submaximal exercise on a cycle ergometer after normoxic bedrest. However, hypoxic bedrest resulted in exacerbation of the skin temperature decline in parallel with the abolished vasodilation during exercise. However, in contrast to this previous study [173], we did not observe any effect of bedrest conducted either under normoxic or hypoxic conditions, on the immersion thermoregulatory responses.

The thermoregulatory study was conducted on the 2nd day of recovery, with subjects performing maximal and submaximal exercise tests within the framework of other studies in the PlanHab project. Whereas it is quite likely that the changes in plasma volume associated with prolonged bedrest will affect the heat loss responses, the changes in plasma volume were certainly redressed after more than 24 hrs of normal upright activity, interspersed with exercise trials. Consequently any alterations in heat loss responses as a result of bedrest-induced changes in plasma volume would be minimal on the second recovery day. Since the purpose of the pre-immersion submaximal exercise protocol was to elevate core temperature and initiate the heat loss responses, the exercise in the pre- and post-intervention trials was conducted at the same absolute work intensity. Whereas in the HAMB condition the relative intensity of both pre- and post-trials was similar, the relative work intensity was higher in the post-intervention trials of the NBR and HBR conditions, due to muscular and cardiorespiratory deconditioning.

The exercise-induced elevation in core temperature was similar in NBR and HAMB but the Tsk and vasoconstriction were lower in the former condition as a result of bedrest, finding which agrees with previous studies [173, 181]. Despite the higher relative work intensity in NBR, the sweating remained unaffected in opposite to what was expected, since the higher relative work intensity (at least as a result of imposed hypoxia or ischaemia) it is known that exacerbates sweating [182]. In other words, at a given core and skin temperature, an augmented sweating and vasodilatory responses would be anticipated during an exercise inducing a higher relative, but same absolute, work rate. The results can be explained by the effect of bedrest on Tsk and vasodilation. Namely, the lower resting and exercise Tsk and the higher vasodilatory response as bedrest adaptations, resulted in unaltered sweating despite the differences in relative exercise intensity. In BRH condition the Tsk was even lower and the vasodilatory response induced by bedrest was restored. These two responses, which can be considered as an effect of prolonged hypoxia exposure, which was additive to bedrest, resulted in lower heat loss and higher Tre after the HBR. As in the previous 35-d bedrest [173] we did not observe any affect on the shivering response. This confirms our previous observation [183] that the muscles of the lower extremities do not contribute significantly to the shivering thermogenesis. Namely, despite the significant loss of muscle mass in the lower limbs, immersion in 15°C water induced the same oxygen uptake response suggesting that it may be attributed to the muscles in the upper torso, and that shivering is not of any significance in the muscles of the lower limbs.

It is important to emphasise that the present study did not investigate the effect of the conditions within a planetary habitat on thermoregulatory responses, but instead focussed on how long sojourns in such conditions would influence the thermoregulatory system. In other words, the study investigated whether thermoregulatory function of astronauts would be compromised on return to Earth. The results of this study clearly demonstrate that 21-day missions in hypoxic environments with reduced gravity will impair the capacity of the thermoregulatory system during exercise but not during immersion. This does not preclude the possibility that alterations in thermoregulatory function are evident during exposure to such
environments. This will need to be addressed in future studies.

The protocol implemented in the present study examines the regulation of body temperature in three scenarios: rest, exercise (endogenous heat production) and exposure to a high heat loss environment (immersion). It may therefore be concluded that under these three scenarios, mimicking the range of activities of astronauts on return to Earth, prior 21-d exposure to the conditions anticipated within future planetary habitats compromise heat loss via vasodilatation, and heat flux but not via sweating and have no major impact on heat preservation (vasoconstriction) and heat production (shivering) responses.

Breathing stability and sleep macrostructure
The primary finding of this study is that breathing stability is worsened after 21-d in NBR, and that the breathing instability experienced during initial exposure to normobaric hypoxia remains poor over 21-d exposure, whether people remain recumbent or ambulatory. There is a direct relationship between breathing instability and mean night SpO2 when otherwise healthy adults are confined to bed, echoing published data reporting ventilatory drive changes in patients [184 185], and lower hypoxic chemoresponsiveness in susceptible individuals at altitude [186].

The initial increase in ventilation to hypoxia (i.e. hypoxic ventilatory response; HVR) is extremely variable [187]. Individuals with blunted HVR will consequently have lower alveolar PO2 and thus, a greater stimulus to HPV at a given altitude [188]. Some studies have stratified test populations into “susceptible” and “resistant” phenotypes when characterising one’s predisposition to suffering acute mountain sickness (AMS) and/or high-altitude pulmonary edema (HAPE) [186 189]. In one investigation, AMS susceptible patients’ night SpO2 concentrations were ~5% lower than their non-susceptible counterparts, yet these patients experienced significantly fewer AHI events per hour (18 versus 33 events per hour; p=0.038) [186]. The present study observed a highly individualized response in breathing stability, especially after 21-d in bedrest, such that those who experienced the greatest change in breathing stability from Night 1 to Night 21 also maintained or increased night SpO2 saturations. Greater oscillations in breathing stability may serve to circulate more overall blood flow to cerebral tissues, affecting ventilatory drive, and further optimising night mean SpO2 values [190 191] compared to those who have more stable breathing patterns. These findings are in-line with research conducted on otherwise normal, healthy subjects who develop periodic breathing at altitude, where one usually finds that mean SpO2 are higher than in individuals who do not develop periodic breathing [191]. Of note, duty ratios are calculated based on the number of apneas and hyperpneas experienced. Thus, although we observed a significant decline in breathing stability in the NBR trial, these data are based on far fewer events per hour compared to hypoxic trials (either bedrest or ambulatory). Thus, the significant decrease in duty ratio and increased total cycle length observed in NBR may be more heavily weighted on less disturbances overall. These initial relationships between breathing stability, ventilatory drive and prolonged bedrest must be confirmed with future research.

Finally, respiration can vary markedly throughout each sleep stage, independent of environmental conditions. Sensitivity to high CO2 and low oxygen is lowest in REM versus NREM sleep [192 193]. Transitioning from wakefulness to stage one light sleep is characterised by significant decreases in minute ventilation, and generally attributed to variations in respiratory rate and tidal volume [194]. Thus, as NREM sleep progresses, hypoventilation, and a 3-7 mm Hg increase in arterial PCO2, occurs as a result of: moderated central respiratory drive, upper respiratory muscle relaxation, and increased airway resistance [194]. Minute ventilation also decreases across N2 and SWS sleep. Although the alterations in sleep architecture observed in the present study may be, predominantly, an after-effect of the large periodic breathing experienced from the continuous normobaric hypoxic stimulus, it is useful to note that spending more time in light sleep may affect arterial PCO2 pressure in its own right. These data have significant implications to clinical populations who are bedridden, military personnel stationed at altitude, and humans on prolonged space expeditions in microgravity, or ground-based analogue environments.

In conclusion, breathing stability is worsened after bedrest, throughout hypoxic exposure, and when bedrest and hypoxic stimuli are combined. Sleep architecture is significantly affected in all test conditions, with greater time spent in light sleep consistent in each condition.

Effect of gas density on ventilation and arterial oxygen saturation during normobaric and hypobaric hypoxia
The results of the present study did not show any effects either gas density or of ambient pressure on the ventilatory pattern or on oxygenation.

Since the publication of the review by Conkin and Wessel 3rd [195] there has been a lively debate regarding the effect of
pressure per se on hypoxia and the possible difference between normobaric and hypobaric hypoxia [196]. Several new studies have been published in the intervening years, and the present results are in contrast to some, for instance those of Biedelman et al. [197] who showed larger decrement on cycling performance caused by hypobaric hypoxia than normobaric hypoxia. On the other hand the present results are consistent with the results of Richard et al. [198] who did not show any difference between normobaric and hypobaric hypoxia on cardiorespiratory functions.

It has been pointed out that the different effects of normobaric and hypobaric hypoxia may not be evident until several hours or even days after the start of the exposure [199 200]. Loeppky et al. [201] showed a change in ventilation first after three hours of hypobaric exposure. However, others have shown a difference between hypobaric and normobaric ventilation and oxygen saturation already after 30 - 45 min hypoxic exposure [202]. It has been suggested that one mechanism that could cause a difference between normobaric and hypobaric hypoxia is the second gas effect caused by outflow of nitrogen during hypobaric exposure [203]. During exposure to air at low ambient pressure there is an imbalance between inhaled and stored nitrogen in the body, and there is a nitrogen wash-out. However, the nitrogen flow will inevitably be larger during the early phase of the exposure and it is difficult to see why this effect would result in the difference between normo- and hypobaric exposures being evident first after several hours.

It is more likely that a delay in the difference between normo- and hypobaric hypoxia would be due to a difference in gas density causing a different work of breathing. Though, the absolute difference in gas density is small it is not inconceivable that a lower cost of breathing could over hours produce a difference in ventilatory pattern (see [201] for further discussion on this subject). This will have to be tested in future experiments.

Two further aspects of these tests must be noted. The first is the fact that similar ambient partial pressures of oxygen at different altitudes will not cause the same oxygen partial pressure in the lungs, even given the same ventilation, oxygen consumption and carbon dioxide production. This is due to the fact that the inhaled gas is heated and saturated with water. The partial pressure of water vapour is 6.2 kPa at 37°C and is independent of ambient pressure. The effect on the partial pressures of the other gases in the lung will be larger the lower the total pressure. It has been pointed out that one has to take the water vapour into account when simulating hypobaric hypoxia at normal ambient pressure [195], but unfortunately it appears that this well known fact is not always adhered to even in scientific publications. In some of the papers cited here the authors have either not totally compensated for the water vapour or stated that the comparisons are made with similar oxygen partial pressures in the ambient gas, something that will result in a lower oxygen partial pressure in the altitude condition.

In conclusion, neither gas density nor ambient pressure affected acute (< 1 hour) ventilatory response to hypoxia. Over this period and at these oxygen partial pressures the equivalent air model is consistent with the data.

Potential Impact:

4.2 Impact and Dissemination

4.2.1 Impact

Stakeholders: The PlanHab results are of importance to the space agencies, particularly ESA and NASA, preparing missions to the Moon and Mars. It is now accepted that the environments within future Lunar and Mars habitats will be hypobaric and hypoxic, thus knowledge regarding the combined effect of partial pressure of oxygen and reduced gravity on physiological systems is essential. The knowledge gained from the PlanHab project will contribute to the maintenance of health and well-being of astronauts on future Lunar and Mars missions.

Science: The results obtained during the PlanHab project help us to better understand the combined effects of simulated microgravity and hypoxia on the different physiological systems. For example, the immune system displays these effects by inducing an enhanced shedding of L-selectin under bed rest conditions that are mitigated by hypoxia. In combination with the absence of an inflammatory state or clinical illness of the subjects, the reasons for this activation and the possible mitigation via hypoxia are linked to arterial wall shear stress and fluid shift, a condition often occurring in the intensive care treatments.

Furthermore, the stated immune-modulatory effect of the purinergic system could be demonstrated as significantly higher
Adenosine levels were measured under hypoxic bed rest conditions and their immune impact was displayed. Since adenosine has been shown to be strongly immune-modulatory and suppressive for distinct key responses in experimental conditions, PlanHab results for the first time show such effects under high fidelity study conditions. Antagonizing the adenosine-hypoxia immune-regulatory pathway can help to treat / counterbalance these effects as anticipated in patients suffering from hypoxia and/or immobilization. In summary, the PlanHab results on immunology may help to improve the understanding of the human physiological functioning during pathological states such as COPD, patients in septic states and under long-lasting intensive care.

In addition, the combined effects of inactivity and hypoxia on sleep architecture and respiration during sleep in humans were unknown. In the last decade there has been an increase in sleep-breathing disorders, which represent an important risk factor for cardiovascular disorders, such as myocardial infarction, stroke, and sudden death during sleep. Results of the study are important for understanding pathophysiology of sleep-breathing and cardiovascular disorders, and represent the opportunity for new therapeutic approaches.

In regards to the muscle function, the PlanHab project results suggest that the superposition of hypoxia to prolonged bed rest did not aggravate the bed rest-induced impairment of skeletal muscle oxidative function in vivo and mitochondrial respiration of isolated muscle fibers. These results substantially support the critical role of physical inactivity in the development of muscle metabolic dysfunction, which represents a hallmark sign of many disease states and a key predictor of exercise intolerance. Overall these findings can be of interest for a better understanding of the physiological adaptations to planetary habitats, as well as the pathophysiology of chronic diseases/conditions (pulmonary, cardiovascular and metabolic diseases, ageing), which expose patients to a combination of muscle deconditioning by physical inactivity and cellular hypoxia.

The Planhab has been eye-opening for the study of interactions between respiration and mechano-adaptation of bone to disuse. Results suggest that hypoxia-induced hyperventilation (i.e. reduction of arterial CO2-concentrations in the blood) can suppress regulatory responses of DKK1 and Sclerostin. It could be postulated from the observed responses, that elevated CO2-concentrations predispose to exaggerated bone losses in immobilization, in space, and also in COPD. Future studies should therefore explore the relationship between CO2 and bone’s mechano-adaptive responses.

Finally, the observation in PlanHab that hypoxia may offset some of the changes in insulin sensitivity seen with prolonged bedrest and may also alter the clearance of insulin from the circulation, provides a basis for future studies to identify the molecular basis of these observations. This in turn could lead to the identification of future pharmacological approaches to improve insulin sensitivity and maintain better metabolic control in these chronic conditions.

Society: Knowledge concerning the combined effects of hypoxia and unloading/inactivity is envisaged to also have important implications for society in general. Thus, a growing number of individuals suffer from chronic hypoxia due to respiratory insufficiency, such as advanced chronic obstructive pulmonary disease. Due to their limited physical capacity, these individuals are commonly restricted to an inactive life style, and hence may also suffer from inactivity-related diseases/conditions such as skeletal muscle atrophy, bone demineralization, diabetes, immune dysfunctions etc. To understand the pathophysiology of such conditions, it is warranted to, in a controlled manner, investigate the combined effects of hypoxia and inactivity/unloading. Moreover, the data obtained on skeletal muscle response to hypoxia and bed rest could contribute to optimization of clinical rehabilitation and nutritional interventions in COPD patients.

The combined effect of inactivity and hypoxia on i) sleep architecture and respiration during sleep ii) iii), cardiorespiratory function at rest and exercise, iv) energy balance and metabolism, v) musculoskeletal function, vi) immune responses vii) thermoregulatory function and circadian rhythm in humans is not known. For instance, in the last decade there has been an explosion of sleep-breathing disorders, which represent an important risk factor for cardiovascular disorders, such as myocardial infarction, stroke, and sudden death during sleep. In this respect, the results of the study are important for understanding pathophysiology of sleep-breathing and cardiovascular disorders, musculoskeletal, metabolic and immune
disorders and represent the opportunity for new preventive or therapeutic approaches.

Industry: The success of the PlanHab project can, in part, be attributed to the contribution of SMEs in the development of hardware and software for the various studies conducted within the framework of the PlanHab project. The coordinator hosted visits of several SMEs at the Planica facility, and made presentations regarding opportunities for SMEs in the Space industry, especially the Space Life Sciences field. The coordinator organized a one-day meeting at the European Centre in Ljubljana for SMEs interested in participating future Space projects. Following the keynote address by the past director of Life Sciences at ESA, participating SMEs presented their technological innovations, which could be of interest to ESA. The SMEs attending the meeting created a strong lobby group in support of Slovenia becoming a regular member of ESA.

Sleep
The PlanHab project has enabled the development of new software for clinical analysis of periodic breathing during sleep. The analytical approach has been described in the publication:

Medical data storage (cloud computing)
SME Iskratel (Slovenia) was interested in the manner in which to collect, store and archive medical information. For this purpose they equipped all PlanHab subjects with their own personal tablet computer (iPad, Apple, Cupertino, CA, USA). The applications to monitor and store physiological data, including results of questionnaires were established by SME Pilon (Slovenia). During the study, Iskratel studied the manner in which medical data measured was stored and used. This enabled them to assess the possibility of creating a marketable cloud data storage facility for hospitals.

Health monitoring
SME Pilon (Slovenia), together with the makers of the iBody App for the iOS (Germany), developed a package that was used by the medical staff to record the morning values of heart rate, blood pressure (systolic and diastolic), tissue oxygen saturation, and tympanic temperature. This information was entered into each subject’s iPad, and transferred to the PlanHab server. The information was available on a password-protected site to all partners.

SME Libela Elsi (Slovenia) developed a gurney incorporating load sensors and a blue tooth connection. This gurney was used to weigh the subjects on a daily basis. Each subject’s bar code was first read by a bar code reader, and the information of the subject’s weight then transferred to an application on their iPad, and also to a central server. The information was available on a password-protected site to all partners at all times.

Application for administering psychological questionnaires
SME Pilon (Slovenia) developed a protocol for the iPads to present a series of questionnaires at designated times to the subjects. Each subject was required to complete the questionnaires as soon as possible. The results were transferred to the PlanHab servers for later analysis.

Determination of thermal comfort (Slovene patent granted)
Urša Ciuha and principal investigator Igor Mekjavic received a Slovene patent for the water-perfused suit used in the PlanHab study to determine the thermal comfort zone before and after the three 21-day interventions. The water-perfused suit is described in the published paper:

Nutrition
The web-based application Open Platform for Clinical Nutrition (OPKP, www.opkp.si) was used in the PlanHab study to monitor
the energy intake of the subjects. Considering the entire duration of the subjects’ stay in Planica (7 days prior to the start of the intervention, 21 days of the intervention and 5 days following the intervention), and the fact that they each had 5 meals per day (breakfast, morning snack, lunch, afternoon snack, dinner), this amounted to a total of 5,940 meals for energy analysed during the PlanHab study. The OPKP application was modified by Pilon for the PlanHab project. In addition, SME Libela Elsi designed a weighing scale, which allowed the sequential weighing of the components of a meal to be recorded. The process was as follows:

• For the three experimental campaigns, a rotating 14-day menu was established together with the cooks at the Olympic Sport Hotel Planica. This menu comprised of 5 meals per day, as described in the publication:

• The nutritional intake of each subjects was calculated as described in the publication:

• Each meal was prepared separately for each subject. Using a bar-code reader, a subject’s bar code would be scanned. The software application determined the amount of each of the meal components the subject should received.

• A multi-compartment tray was placed on a precision Libela Elsi scale, which was connected to an iMac computer (Apple, Cupertino, California, USA). The programme linked the 5-function buttons on the scale controller to the components of the meal. Thus, by depressing one function key, the programme would provide the user with the amount of the food item (i.e. soup) that had to be placed in one compartment of the tray. Since the software was hardware connected to the scale, it would provide the user with on-line feedback as to the amount of the food item that was still required. This procedure was then repeated for the remaining food items (i.e. steak, vegetables, salad, desert, etc.) of that meal. Once the meal was prepared in the insulated tray, it was delivered to the subject.

• Upon completion of each meal, the individual compartments of the tray were again weighed by the nutritional hardware/software, to measure the amount of the food items that were not consumed. In this manner the software was able to compute the energy intake based only on the amount of food items ingested.

• Data obtained on the response of the glutathione system to hypoxia and bed rest could also be relevant for the development of new antioxidant nutraceuticals.

4.2.2 Main dissemination activities

Scientific: The majority of the results of the PlanHab project were disseminated to a broader scientific, international audience. The research results were presented to experts at three major conferences in the last two years where several presentations / sessions were granted to the PlanHab project:

- at the Annual Scientific Meetings of the International Society for Gravitational Physiology (ISGP) in 2014 (Waterloo, Canada)
- at the Annual Scientific Meetings of the International Society for Gravitational Physiology (ISGP) in 2015 (Ljubljana, Slovenia), and
- at the Humans in Space Symposium in 2015 (Prague, Czech Republic).

In addition, researchers from the consortium were invited to give presentations on the topic of the PlanHab project at various
occasions, e.g. also at the Annual Meeting of the International Society for Environmental Ergonomics in New Zealand (2013).

Hosting the ISGP meeting 2015 in Ljubljana was also one of the major goals defined in the PlanHab project. The meeting was attended by representatives of all space agencies. A highlight of the meeting was an excursion to the Olympic Sport Centre Planica, where the delegates were given a tour of the facility and briefed regarding the logistics of conducting hypoxic bedrest studies at the facility.

The preliminary and final results regarding sleep architecture and central sleep apnea were presented at the annual meetings of the European sleep research society (ESRS) in Paris (France, 2012) and Tallin (Estonia, 2014), and at the annual meeting of the World Association of Sleep Medicine in Valencia (Spain, 2013). Abstracts of the results presented at the ESRE meetings were published in the Journal of Sleep Research.

Media: The PlanHab project received a tremendous amount of media coverage (newspapers, magazines, radio, television), and these are itemized in the Dissemination Table.

• Space Expo (EC initiative):
Space Expo is an initiative of the European Commission and is intended to inform EU citizens of EU achievements in Space research. The following is a description of this event available at the website indicated below:

“Over 500,000 European citizens have now visited the European Space Expo, as it continues its tour of major European cities. The free exhibition highlights the many ways in which the EU space programme helps EU citizens 'on the ground' every day. The Expo presents key information on the European space programmes - from satellite navigation (Galileo and EGNOS) to Earth observation (Copernicus) in an engaging and entertaining way. Highlights include the 'OmniGlobe' - an interactive hologram of the earth's atmosphere, an impressive model of the ‘Galileo' satellite and lots more...

The aim of the Expo is to show citizens how European space policy and space-based technologies benefit our everyday lives on earth and also of course, their importance for the European economy and job creation. Completely FREE to the general public, record attendances have greeted the Expo throughout its tour.”


The scientific representative (Igor Mekjavic) of the Coordinator (IJJS) was a guest speaker at Space Expo. His talk titled: “What it would be like to live on Mars? Simulating Lunar and Martian habitats” is shown on the schedule of Space Expo at the following website:


• Noč raziskovalcev (transl.: “Night of researchers”, EU initiative)
The Night of Researchers (directly translated from the Slovene) has now been held twice in Slovenia, and the Planica facility has been included in this initiative each time. This event is described on the website as follows:

“In the framework of the Night of Researchers 2015 we will present the initiative of the European Union for research and innovation at the following participating sites:

Ljubljana: Novi trg, Šentvid High School, Park “Ljubljansko Barje”
Novo mesto: Faculty for Information Studies, Faculty for Health Sciences, College for Technology and Systems, Faculty for Business and Administration
Planica: Center for planetary biomedical research Planica

At the above events you will learn more about the work of the European Commission, and its priorities. “

The “Night of researchers” initiative is funded by the European Commission's Research and Innovation Framework Programme
H2020 (2014-2020) by the Marie Skłodowska-Curie actions (Directorate-General for Education and Culture) Grant Agreement No. 633162.

The website for the Night of researchers is:

http://www.zaznanost.si/?page_id=359

The Centre for biomedical planetary research Planica is described on the web page http://www.zaznanost.si/?page_id=2904 as follows:

“The Centre for biomedical planetary research Planica is located at the Olympic Sport Center Planica and is managed by the Jozef Stefan Institute, which is the largest and most successful research Institute in Slovenia. The aim of the Planica Centre is to conduct leading-edge science in the field of Planetary habitat simulation. The Centre allows the simulation of the effects of weightlessness and of high altitude on physiological systems. As a result of collaborative studies with international partners, the European Space Agency (ESA), and the National Aerospace Agency (USA), Planica is developing into one of the leading centres for space life sciences and ergonomics.

• Znanost na cesti (transl.: “Coffee break with Science”; supported by the Slovene Research Agency)

An initiative supported by the Slovene Research Agency is Science on the Street. It is organized as an evening presentation at the Grand Hotel Union Coffee Room. In addition to a slide/powerpoint presentation for the general public, the invited speaker is also interviewed during his presentation by an invited member of the media. Information regarding this initiative is provided on their webpage:

(http://www.znanostnacesti.si)

PlanHab principal investigator Igor Mekjavic was invited to give a presentation on November 4th, 2015, titled: Acclimatising for Mars. The moderator for this event is Mrs. Renata Dancinger of the National Television of Slovenia.

• Visit of scientists attending the Annual Meeting of the International Society for Gravitational Physiology in Ljubljana

The Annual Meeting of the International Society for Gravitational Physiology was held in Ljubljana from June 7 to 12, 2015. An excursion was organized for the last day of the conference (June 12). All delegates were driven to the Olympic Sport Centre Planica and given a tour of the facility. Since the PlanHab study was completed, several mock-up stations were arranged so that the delegates could appreciate the logistics of conducting hypoxic bed rest studies at this facility. Feedback from all the delegates regarding the visit, and the facility itself, was very positive.

• Space school

Within the framework of a 1-week Nature School organized by the Ministry of Education at the Olympic Sport Centre, principal investigator Mekjavic organized presentations and demonstrations on the topic of Space Sciences. On three afternoons, participants were educated regarding Space Life Sciences, Human-Robot teaming in Space, and Rocket Propulsion.

Academia: A postgraduate course curriculum on Space Life Sciences was established. The proposed course curriculum was presented to the Dean of the International Postgraduate School Jozef Stefan, and the course is now being prepared for inclusion in the 2016 academic calendar.

The course provides fundamental knowledge regarding the physiology and medicine of living and working in a reduced gravity environment. It will review the advances in Space Life Sciences that have made it possible for humans to live and work on the International Space Station, and will examine the problems that will need to be solved for future missions to Mars, and for the colonisation of the Moon. The one-week, 40-hour course will be conducted at the Olympic Sport Centre Planica (Rateče, Slovenia). Participants will be required to live at the Olympic Sport Centre Planica during this time.

Lecture topics include:

• Manned Space Flight (Historical perspectives)
• Space Environment (Orbital flight & planetary environments)
• Spaceflight Systems and Procedures
- Space vehicles
- Spacecraft atmospheres and life support
- Extravehicular activities
  • Physiological Adaptation to Space Flight
  - Overall physiological responses to space flight
  - Neurovestibular system
  - Neurophysiology and performance
  - Cardiopulmonary system
  - Ophthalmology
  - Lean body mass and energy metabolism
  - Bone and mineral metabolism
  - Blood, fluid and electrolytes
  - Simulations and analogues of weightlessness
  • Health Maintenance of Crewmembers
  - Selection of astronauts and space personnel
  - Biomedical training of space crews
  - Ground-based medical programmes
  - Countermeasures to space deconditioning
  • Medical Problems of Space Flight
  - Toxic hazards
  - Radiation exposure issues
  - Medical care and health maintenance in flight

In addition to the above lectures, the course will also incorporate the following laboratory demonstrations:
• Orthostatic tolerance test
• Aerobic performance
• Body composition (using Dual Energy X-ray absorption)
• Nutrition (using the IJS-developed web-based App Open Platform for Clinical Nutrition)
• Energy metabolism
• Muscle strength (using isokinetic dynamometry)
• Posturography (including jump test)
• Cognitive performance
• Polysomnography
• Thermal balance
• Psychological questionnaires

List of Websites:
4.3 Address of project public website and relevant contact details

4.3.1 Address of public website:
www.planhab.com

4.3.2 Relevant contact details (Principal investigators in alphabetical order):

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Last updated on 2016-02-17
Retrieved on 2019-08-06

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