LUNAR HABITAT SIMULATION: THERMOPHYSIOLOGICAL CHANGES ASSOCIATED WITH HYPOXIC INACTIVITY

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Doctoral Dissertation

SIMULACIJA LUNARNEGA HABITATA: VPLIV HIPOKSIČNE NEAKTIVNOSTI NA TERMOFIZIOLOŠKE ODZIVE

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‘Every day some new fact comes to light – some new obstacle which threatens the gravest obstruction. I suppose this is the reason which makes the game so well worth playing.’’

Robert Falcon Scott

Abstract

Future lunar habitats will be hypobaric and hypoxic in order to minimise the risk of decompression sickness and eliminating the need for lengthy decompression procedures, which are required during the preparation for extra vehicular activity (EVA) on the International Space Station. Consequently, during long-term missions to the Moon, astronauts will be exposed to the reduced gravity on the Moon and hypoxia. The manner in which the microgravity-induced changes in physiological systems are effected by hypoxia are unknown. To address this question, a suitable simulation of a lunar habitat needs to be established, which can then be used in ground-based studies to investigate the effects of the anticipated environment within future lunar habitats on astronauts and cosmonauts. This was the impetus for the present thesis.

A programme of research was initiated to establish a lunar habitat simulation (Part A), which was then used to investigate the effects of a simulated lunar habitat environment on the circadian rhythm of peripheral perfusion and emotional state. These effects were assessed under conditions of no exercise (Part B) and with a daily exercise programme (Part C). The latter mimicked more realistically the type of daily activity that would be performed by astronauts nowadays, since they need to devote several hours each day to exercise countermeasures, implemented to counteract musculoskeletal deconditioning.

In the present thesis, the effects of inactivity and unloading experienced by astronauts in Space were simulated with the bed rest experimental model. Conducting the bed rest studies in a normobaric hypoxic environment simulated the anticipated hypobaric hypoxia in future lunar habitats. The bed rest studies were conducted in accordance with the European Space Agency (ESA) standardised bed rest protocol (Heer et al., 2009).

The thesis comprises the following three parts:

- Part A: The development and maintenance of a hypoxic lunar habitat simulation facility
- Part B: The effects of hypoxic bed rest and confinement on peripheral perfusion and emotional state
- Part C: The effects of hypoxic confinement, with and without exercise, on peripheral perfusion and emotional state

Part A: The development and maintenance of a hypoxic lunar habitat simulation facility

The aim of this section (Part A) was to develop the lunar habitat simulation facility and collect the basic core physiological data (BCD) associated with a 10-day exposure to the

environment of such a habitat. A total of 11 healthy male participants took part in three separate studies: NBR: normoxic bed rest; HBR: Hypoxic bed rest and HAMB: hypoxic ambulatory confinement. The participants were confined for 10 days to all 3 conditions in a randomised order. The HBR trial represented the simulation, or rather the exaggeration, of certain conditions anticipated in future lunar habitats. The remaining two trials were included to be able to assess the separate and combined effects of inactivity/unloading and hypoxia on the various physiological systems. The BCD were recorded according the ESA standards and included: maximal oxygen uptake, maximal voluntary contract of the quadriceps and hamstrings, respiratory function and complete blood count. Additionally a full nutritional analysis was completed.

The BCD collected during the current study in the NBR trial compare well with those reported for previous NBR studies. The establishment of a hypoxic bed rest and confinement facility was successful and the logistics utilised in the current study are suitable for use in further hypoxic confinement or lunar habitat simulation studies.

We observed significant decreases in whole-body mass, secondary to lean-mass loss, during the 10-day confinement in all conditions. Alterations in macronutrient diet composition and other possible nutritional interventions seem warranted for future studies and missions incorporating combined unloading / inactivity and hypoxia.

Part B: The effects of hypoxic bed rest and confinement on peripheral perfusion and emotional state

Part B tested the hypothesis that two nonthermal factors known to induce vasoconstriction during the daytime, namely hypoxia and bed rest, might cause sleep disruption should their vasoconstrictive effect persist during the night. A further objective of this part was to assess the effect of hypoxia and inactivity on emotional stress.

The present results reveal that the daytime vasoconstrictive effects of hypoxia and bed rest do not persist at night. It is likely that hypoxia-induced sleeplessness is induced via a different pathway than through peripheral afferent information. Secondly, the results indicate that both bed rest and hypoxia have a detrimental effect on the positive emotions. Hypoxia combined with bed rest will have an additive effect, leading to a further decrement of positive emotions. Additionally, hypoxia leads to an increase in the participants' negative emotions with increasing time spent in bed rest. However, these differences were not noted in the ambulatory participants.

In conclusion, Part B found that hypoxia and bed rest have a negative effect on daytime peripheral perfusion and on emotional state. A small level of physical activity relieved the increase in negative emotions. In terms of future space travel, these studies are too short in duration to make general recommendations, however, it appears that these two non-thermal factors can be managed within the short term at least.

Part C: The effects of hypoxic confinement with and without exercise on peripheral perfusion and emotional state

During lunar habitation, astronauts will spend a large part of their day confined to the habitat, which will likely be hypoxic. Part C focussed more on the effects of the confinement and hypoxia that these astronauts may face. Additionally, athletes or alpinists utilising hypoxia for performance gains may also be confined to quarters during their sojourn. In this regard, Part C tested the hypothesis, that exercise training compared to a relatively sedentary lifestyle would have beneficial effects on peripheral perfusion and emotional state.

Eight participants took part in 2 hours of exercise per day (LH-TH), while 6 participants were confined to the Planica facility (LH). Both sets of participants were confined in hypoxia at the Planica facility for 24 hours per day for 10 days. We found that there was no difference between the two groups for either daytime or nighttime perfusion of the toes. There was no change in sleep onset times, which may indicate that any change in sleep architecture throughout the night is mediated through central mechanisms rather than peripheral. There was no difference between the two groups in emotional state. However it was found that hypoxia increases the correlations between depression and the other factors of the POMS subscale. This suggests that while a negative mood has not developed, the potential for relatively innocuous events to trigger a more negative emotional state is increased.

Overall conclusions

The use of hypoxia during a bed rest scenario to simulate a lunar habitat was successful. The BCD collected during the normoxic bed rest were in line with the results previously reported for normoxic bed rest studies of similar duration. Hypoxia had the expected effect on the cardiovascular parameters (heart rate, arterial saturation and pressure) and on the haematological variables. Hypoxia did not appear to have any further effect compared to normoxic bed rest on aerobic capacity, muscle strength and size.

There was a significant nocturnal peripheral vasodilatation in all interventions. Sleep onset did not differ between groups (LH-TH and LH) or as a result of hypoxia. Any sleep disturbances or changes to sleep architecture are likely a result of central rather than peripheral mechanisms.

Emotional state may be negatively affected as a result of inactivity and exacerbated by hypoxia. Increasing the level of activity can alleviate some of the negative effects of hypoxia.

Povzetek

Ozračje v bodočih lunarnih habitatih bo hipobarično in hipoksično, kar bo omogočilo krajše in bolj varne priprave za dejavnosti zunaj habitata (angl.: extra-vehicular activity; kratica: EVA). Trenutno, priprava za EVA na Mednarodni vesoljski postaji vključuje več urni protokol dihanja čistega kisika ob počasnem zmanjševanju tlaka v zaščitni obleki. Normoksična breztežnost na Mednarodni vesoljski postaji, oziroma zmanjšana težnosti na Luni, povzroča razbremenitev spodnjih okončin, in posledično atrofijo mišičnoskeletnega, in srčnožilnega sistema, ter zmanjšano aerobno sposobnost astronavtov. Zaradi načrtovanega hipoksičnega okolja v bodočih lunarnih habitatih, je eno od prioritetnih vprašanj kako hipoksija vpliva na procese adaptacije na razbremenitev in neaktivnost. Namen pričujoče naloge je bil ovrednotiti simulacijo bivanja v lunarnem habitatu, in preveriti vpliv hipoksije in razbremenitev na periferno vazomotoriko in psihološko stanje. V pričujoči nalogi so bili učinki neaktivnosti in razbremenitev spodnjih okončin, s katerimi se soočajo astronauti v vesolju, simulirani z eksperimentalnim modelom ležanja v horizontalni legi.

Naloga je razdeljena na tri vsebinske dele.

Del A: Simulacija lunarnega habitat
Cilj prvega sklopa je bil ustvariti simulacijo lunarnega habitata in izmeriti osnovne fiziološke odzive (BCD) na 10-dnevno bivanje v tem simuliranem okolju. Zdravi preiskovanci (N=11) so sodelovali v treh poskusih: a) 10-dnevno ležanje v horizontalnem položaju v normoksičnem okolju (NBR); b)10-dnevno ležanje v horizontalnem položaju v hipoksičnem okolju (HBR); c) 10-dnevno bivanje in normalno gibanje v hipoksičnem okolju (HAMB). Poskus HBR je predstavljal simulacijo učinkov bivanja znotraj lunarnega habitata. Preostala dva poskusa sta bila dodana kot kontrolna poskusa, za lažje vrednotenje učinka hipoksije (primerjava rezultatov HBR in NBR), in neaktivnosti (primerjava rezultatov HBR in HAMB). BCD podatki, izbrani po priporočilu Evropske vesoljske agencije (ESA), so vključevali: maksimalno porabo kisika, mišično moč, pljučno funkcijo, hemogram, in prehrano.

BCD podatki NBR poskusa so primerljivi z objavljenimi rezultati predhodnih tovrstnih raziskav, kar potrujejo uspešnost izvedbe poskusa z ležanjem v horizontalni legi. Simulacija bivanja v lunarnem habitat z eksperimentalni modelom ležanja v hipoksičnem okolju (HBR) je bila uspešna. Prav tako se je logistika simulacije iskazala primerna za prihodnje tovrstne simulacije.

V vseh treh poskusih smo opazili znatno izgubo mišične mase, in posledično tudi

izgubo telesne mase. Zaključili smo, da je za preprečevanje izgube mase v tovrstnih raziskavah potreben zelo strog nadzor nad prehrano in sprememba v sestavi makronutricionistične diete.

Del B: Učinki ležanja in bivanja v hipoksiji na cirkadialni ritem periferne vazomotorike in na psihološko stanje

V tem sklopu je ovrednotena hipotezo, da netermalna faktorja hipoksija in neaktivnost, povzročata periferno vazokonstrikcijo po dnevi. V skladu s teorijo o vplivu termoaferentih dražljajev na spanje, bi prisotnost periferne vazokonstrikcije ponoči vplivalo na montje v spanju. Dodaten namen tega sklopa je bil ovrednotiti učinek hipoksije in neaktivnosti na psihološko stanje.

Pričujoči rezultati kažejo, da vazokonstriktorni vpliv hipoksije in neaktivnosti ni prisoten ponoči. Večerna vazodilatacija je bila enaka v vseh pogojih (NBR, HBR in HAMB), in je nastopila skoraj istočasno s spancem. Motnje spanca v visokogorskem (hipoksičnem) okolju je eden od simptomov višinske bolezni. Glede na to, da hipoksija ne vpliva na nočno vazodilatacijo, in v skladu s teorijo da je le-ta potrebna za spanec, smo zaključili, da periferna vazomotorika ne vpliva na nespečnost v hipoksčnem okolju.

Rezultati psiholoških testov kažejo, da imata ležanje in hipoksija škodljiv učinek na pozitivna čustva. Hipoksija dodatno povzroča povečanje negativnih emocij, ki se med ležanjem potencira. Te spremembe nismo opazili med HAMB poskusom, kar potrjuje da že manjša stopnja fizične aktivnosti zmanjša porast negativnih čustev.

Del C: Učinek aktivnosti med bivanjem v omejenega bivalnem na periferno vazomotoriko in psihološko stanje.

Med bivanjem na Luni se predvideva, da bodo astronavti izvajali dnevno vadbo za preprečevanje mišičnoskeletne atrofije. Del C je ovrednotil hipotezo, da ima vadba pozitivne učinke na periferno vazomotoriko in čustveno stanje. Preiskovanci (N=14) so bili naključno razvrščeni v aktivno (LH-TH, N=8) in neaktivno skupino (LH, N=6). Aktivna skupina je dnevno izvajala dvourno vadbo, medtem ko je neaktivna skupina imela neomejeno gibanje v hipoksičnem okolju, vendar brez dnevne vadbe. Med skupinama ni bilo razlike v cirkadialnem ritmu periferne vazomotorike v prstih nog. Začetna faza spanja se ni razlikovala med skupinama. Psihološko stanje je bilo pri obeh skupinah enako. Rezultati psiholoških testov so pokazali, da hipoksija poveča korelacijo med depresijo in drugimi faktorji POMS (Profile of Mood States) lestvice. Zaključili smo, da čeprav se negativno razpoloženje ni razvilo, se je povečal potencial, ki bi relativno neškodljiv pripetljaj lahko sprožil v negativno čustveno stanje.

Splošni povzetek

Simulacija bivanja v lunarnem habitatu z ležanjem v hipoksičnem ozračju je bila uspešna. Fiziološki odzivi med NBR (BCD) so v skladu z rezultati podobnih raziskav s primerljivim obdobjem trajanja. Hipoksija ni dodatno vplivala na potek mišičnoskeletne in srčnožilne atrofije, ter na izgubo aerobne sposobnosti, opažen med ležanjem v normoksiji. Hipoksija in aktivnost nista vplivala na nočno vazodilatacijo, in na začetno

fazo spanja. Negativna čustva so se povečala med hipksijo, vendar je aktivnost in dnevna vadba negativen učinek hipoksije omilil.

Simulacija lunarnega habitata s hipoksičnim ležanjem je uspešen in relavanten zemeljski eksperimentalni model za prihodnje raziskave.

Abbreviations

°C	=	Degree centigrade
AMS	=	Acute mountain sickness
ANOVA	=	Analysis of variance
AP	=	Arterial pressure
Avg	=	Average (mean)
BCD	=	Basic Core Data
BR	=	Bed rest
BF	=	Body fat
BMI	=	Body mass index
BPM	=	Beats per minute
CBC	=	Complete blood count
CO_2	=	Carbon Dioxide
COPD	=	Chronic Obstructive Pulmonary Disease
CSA	=	Muscle cross sectional area
ΔTc-t	=	Calf - toe temperature gradient
ΔTfo-fi	=	Forearm – fingertip temperature gradient
ΔTp-d	=	Proximal to distal skin temperature gradient
DAP	=	Diastolic arterial pressure
Day	=	Daytime
D1	=	Day 1
D2	=	Day 2
D3	=	Day 3
D5	=	Day 5
D7	=	Day 7
D10	=	Day 10
D11	=	Day 11
EAA	=	Equivalent Air Altitude
EPO	=	Erythropoietin
ESA	=	European Space Agency
EVA	=	Extra-vehicular activity
FEV1	=	Forced expiratory volume in the first second
F_IO_2	=	Fraction of inspired oxygen
FO_2	=	Fraction of oxygen
FVC	=	Forced vital capacity
HAMB	=	Hypoxic ambulatory confinement
HBR	=	Hypoxic bed rest
HECS	=	Hypoxic Confinement and Exercise

HH	=	Hypobaric Hypoxia
HL	=	Heat loss
HP	=	Heat production
HR	=	Heart rate
HRex	=	Exercising heart rate
HRrest	=	Resting heart rate
HRmax	=	Maximal hear rate
HUT	=	Head-up tilt
ISS	=	International Space Station
LH-TH	=	Live-High Train-High
LH	=	Live-High
LLMS	=	Lake Louis Mountain Sickness
LunHab	=	Lunar Habitat Simulation
m	=	Metres
min	=	Minute
MSR	=	Modular Signal Recorder
MVC	=	Maximum voluntary contraction
MVV	=	Maximum voluntary ventilation
N2	=	Night 2
N9	=	Night 9
NA	=	Negative affect
NASA	=	National Aeronautics and Space Administration
NBR	=	Normoxic bed rest
NH	=	Normobaric Hypoxia
Ni	=	Night
O_2	=	Oxygen
OPKP	=	Open Platform for Clinical Nutrition
PA	=	Positive affect
PAL	=	Physical activity levels
PANAS	=	Positive Affect – Negative Affect Schedule
P_B	=	Atmospheric pressure
Pcomp	=	Last completed workload
PEF	=	Peak expiratory flow
PH_2O	=	Saturation pressure for water vapour at 37 °C
P_IO_2	=	Inspired partial pressure of oxygen
PlanHab	=	Planetary Habitat Simulation
Pmax	=	Maximal power
POAH	=	Pre Optic Anterior Hypothalamus
POMS	=	Profile of Mood State
POST	=	Post confinement/intervention
PPO	=	Peak power output
pQCT	=	Peripheral quantitative computer tomography
PRE	=	Baseline, prior to confinement / intervention
RBC	=	Red blood cell count
RER	=	Respiratory exchange ratio

RMANOVA	=	Multivariate analysis of variance with repeated measure
ROI	=	Regions of Interest
s	=	Seconds
SAP	=	Systolic arterial pressure
S_aO_2	=	Capillary oxyhaemoglobin saturation
SCN	=	Suprachiasmatic Nuclei
SD	=	Standard deviation
SOL	=	Sleep onset latency
SV	=	Shared variance
SVC	=	Slow vital capacity
t	=	Time
T1	=	Time point 1 (11:00 to 13:00)
T2	=	Time point 2 (16:00 to 19:00)
T3	=	Time point 3 (20:00 to 23:00)
Taural	=	Aural temperature
Tb	=	Body core temperature
Tex	=	Exercise duration
TMD	=	Total mood disturbance score
Trans	=	Transition period
TRIMP	=	Training impulse
Tsk	=	Skin temperature
TST	=	Total sleep time
VO_{2max}	=	Maximal oxygen uptake
VPSA	=	Vacuum Pressure Swing Adsorption
W	=	Watt
WASO	=	Number of awakenings after sleep onset
Wcompl	=	The last completed workload

1 Introduction

In more recent years, the aims of manned space travel have become more focused on long duration missions to the Moon and Mars. There are many reasons for this, including advancing human knowledge of not only our solar system but also of the universe. These Space activities will benefit all scientific fields. Particularly Space life sciences will provide benefits to target populations on Earth. It is envisioned that long-term missions to the Moon and Mars will require permanent habitats in order to provide such missions with the appropriate support. One important consideration for a planetary habitat is the atmospheric pressure and oxygen content. It has been suggested (Bodkin et al., 2006; Norcross et al., 2013), that to overcome some technical and structural difficulties involved in maintaining a planetary habitat a low atmospheric pressure is desired. Furthermore, astronauts will be required to conduct extra vehicular activities (EVA) much more frequently on the Moon and Mars than is currently required on the International Space Station (ISS). During the preparation for EVAs, the risk of decompression sickness becomes an important consideration, because astronauts are required to move from the normal ambient pressure within the spacecraft to the lower ambient pressure utilised within the spacesuit during EVAs. During the preparation for EVAs, astronauts prebreathe oxygen and are exposed to a slow decompression profile, both procedures implemented to eliminate the dissolved inert gas nitrogen from the body, and thus minimise the risk of it forming a gas phase during the decompression procedure. The EVA preparation protocol normally requires several hours. Therefore, a second benefit to a lower cabin / habitat pressure would be a reduced risk of decompression sickness associated with the more frequent EVAs anticipated during missions on the Moon and Mars. However, as a consequence of reducing the atmospheric pressure, the partial pressure of oxygen is also reduced. This means that, astronauts inhabiting such structures would be exposed not only to a reduced gravitational force, but also to a hypobaric hypoxic environment (Bodkin et al., 2006; Norcross et al., 2013). It is therefore important to develop a ground-based lunar Habitat Simulation Facility to simulate every aspect of future lunar missions in order to minimise not only life support and technical difficulties, but also to gain a complete understanding of the combined effects of long-term exposure to reduced gravity and hypoxia.

To investigate the effect of the inactivity and unloading of the weight-bearing bones and postural muscles as experienced in weightlessness on physiological systems, ground-based studies use the bed rest experimental model. Namely, the changes observed in the musculoskeletal and cardiovascular systems during a Space mission of certain duration, is similar to the changes observed in participants confined to a horizontal bed rest for the same duration. Astronauts on the Moon would not be exposed to a weightless environment, since the gravity on the Moon is 1/6 the gravity on Earth.

The focus of the present thesis is the establishment of an experimental model in which certain conditions anticipated in lunar habitats are simulated or rather exaggerated (see chapter 2) and the effect of a 10-day exposure to such a habitat on circadian rhythm of peripheral perfusion and emotional status is examined.

1.1 Nutrition

While often overlooked in everyday life, correct nutrition and efficient dietary support are among the most critical features for maintaining astronaut health and wellbeing. Furthermore, nutrition plays a vital role in upholding astronauts' ability to carry out operational duties during space exploration (Lane and Feeback, 2002; Smith and Zwart, 2008; Smith et al., 2005). Mechanical loading is the keystone in triggering muscle and bone growth with physical activity as the protagonist (Tagliaferri et al., 2015). During space flight or planetary habitation, the gravitationally induced mechanical loading is critically reduced compared to on earth. The resulting effect leaves bone and muscle open to wasting and deconditioning. While the dietary intake in bed rest studies varies in the macronutrient content and calorie intake (Inniss et al., 2009; Krebs et al., 1990; Smith et al., 2001), a decrease in body mass, related to a decrease in muscle mass (Blanc et al., 1998; Trappe, 2009) is commonly observed. In order to combat this body mass loss in bed rest and in space, exercise countermeasures have been investigated, which has lead to an increase in the volume, intensity and diversity of exercise employed on the ISS. Nutritional countermeasures have been explored and are continuing to be. If an inadequate diet is presented, then a reduction of protein synthesis usually seen in flight and in bed rest may be exacerbated (Stein et al., 1999). Conversely, Paddon-Jones et al., (2004) found that ingesting amino acids maintained leg muscle mass following 28 days of bed rest. Furthermore, Smith et al., (2012) found that combining exercise and nutritional countermeasures was the most affective technique in preventing bone loss during 4 to 6 months of microgravity. Lee et al., (2014) found that a protein supplement during 60 days of bed rest did not prevent a decrease in muscle performance or lean mass of the leg. While the results are equivocal in terms of body mass, muscle and bone loss prevention, the benefits of nutritional support in terms of health and well-being are essential.

There is direct evidence to suggest that dietary inadequacies can affect the microvasculature, skin blood flow and arterial pressure. During physiological hyperinsulinemia, insulin mediated vasodilatation of skin blood can occur (Serné et al., 2002). Circulating free fatty acids can also modulate vascular function (de Jongh et al., 2004). Furthermore, postprandial thermogenesis can increase following a high protein versus a high carbohydrate diet (Johnston et al., 2002). Following postprandial thermogenesis, to maintain core body temperature (Tb) within the thermoneutral and thermal comfort zone, heat production must decrease and heat loss increase. Under constant routine conditions, the circadian rhythm of Tb can quickly absorb the changes in temperature associated with food intake to maintain Tb rhythm (Krauchi and Wirz-

Justice, 1994).

1.2 Circadian rhythm of perfusion and its role in sleep

It appears that most species sleep or rest during their Tb trough (Dijk and Czeisler, 1995; Krauchi, 2007). This concept has lead some to consider that there is a link between thermoregulation and sleep. There is however, a more specific view that peripheral vasodilatation is the key event in the management of sleep onset (Krauchi and Wirz-Justice, 1994, 2001; Raymann et al., 2008). Krauchi and colleagues have shown a functional link between temperature regulation, specifically heat loss, through the periphery and sleep propensity (Krauchi et al., 1999, 2000; Krauchi and Wirz-Justice, 2001). Raymann et al., (2008) found that through mild manipulation of peripheral skin temperature one could enhance the depth of sleep. If it is possible to enhance sleep through thermal manipulation of the skin, then is it not plausible that decreasing the skin temperature will lead to a disruption in sleep?

The suprachiasmatic nuclei (SCN) and the preoptic-anterior hypothalamus (POAH) are primary pacemakers and regulators of mammalian circadian rhythm, (Kubota et al., 2002; Van Someren, 2004). The pineal gland can function to reset the circadian clock located in the SCN. In doing so it allows humans to keep track of seasonal or yearly occurrences and in more recent times allows for global travellers to overcome 'jet-lag' (Mortola, 2007). (Van Someren, 2004), proposed that skin temperature - indicative of skin blood flow - is the source of a signal directed towards a sub population of thermosensitive neurons in the POAH. The function of these thermosensitive neurons is to initiate sleep.

Krauchi et al., (2000), Krauchi and Wirz-Justice, (2001) and in a review, Krauchi, (2007) came to the conclusion that warm feet, a consequence of peripheral vasodilation, contribute to the onset of sleep. They also found that the greater the peripheral vasodilatation, the more likely one is to have a good quality of sleep including less waking after sleep onset.

Switching off the lights is generally considered to be “implicit permission” to fall asleep, (Krauchi et al., 2000), therefore, a strict light-dark cycle maybe vital in maintaining the circadian rhythm during confinement.

1.3 The effect of in / activity on peripheral perfusion

Physical activity and exercise leads to an increase in cutaneous blood flow through most likely a decrease in vasoconstrictor activity and an increase in active vasodilatation. The results of exercise training therefore improve circulation to the periphery, which in turn can increase the capacity for heat less (Johnson, 1998). It appears that the opposite is true when physical activity and exercise are removed. Greenleaf, (1997) found that following

14 days of bed rest, participants had impaired thermoregulatory function and a reduced skin blood flow in the forearm. Crandall et al., (1994) also found that 15 days of bed rest reduced thermoregulatory function and impaired skin blood flow during passive heating. Additionally, Lee et al., (2002) found impaired thermoregulatory function following 13 days of bed rest. They suggest that this is a result of adaptations to skin blood flow and sweating rate, in particular, a delayed onset of an increased in skin blood flow during exercise. Finally in a preliminary report for only two crewmembers, following 115 days of space flight, Fortney et al., (1998), found that heat loss responses were compromised. It appears then that there is a strong temporal relationship between peripheral perfusion and inactivity, bed rest and space flight (Golja et al., 2002).

1.4 The effect of hypoxia on peripheral perfusion

At altitudes above 2500m, the occurrence of acute mountain sickness (AMS) is possible in the majority of individuals (Bartsch and Saltin, 2008). Insomnia and poor sleep are among the main symptoms of the AMS syndrome. One of the signs of AMS, a change in the sleep architecture is attributed to the effect of hypoxia on central nervous mechanisms. According to the theory of Krauchi (Krauchi et al., 1999, 2000; Krauchi and Wirz-Justice, 2001), sleep is initiated through increased vasodilatation of the periphery, specifically the toe. It may hold true then, that sleep at altitude is affected as a result of a change in peripheral perfusion.

Exposure to hypoxia has an effect on vasoaction through increased sympathetic drive and through local humoral systems. This can result in conflicting signals to peripheral vasculature whereby during exposure to hypoxia sympathetic vasoconstriction maybe masked by hypoxic vasodilatation in the forearm or calf (Weisbrod et al., 2001). Heffernan and Muller, (2014) found that hypoxia stimulates the carotid bodies, which in turn increases sympathetic outflow and that there are competing signals of vasodilatation and vasoconstriction (Weisbrod et al., 2001). Additionally, Heffernan and Muller, (2014) demonstrated that while breathing a hypoxic gas mixture, vasomotor tone in the muscles and in the skin was under differential control. Pollock et al., (2014) corroborate these findings. Interestingly, following a very acute bout of hypoxia (5 minutes), Simmons et al., (2007a) demonstrated cutaneous vasodilatation in their participants. However, when the exposure time to hypoxia increased (45 minutes) and was combined with cold exposure, cutaneous vasoconstriction was present (Simmons et al., 2011). While Sagawa et al., (1986) also found a decrease in the perfusion of the fingers during hypoxia, their participants were exposed to heat as opposed to the cold (Simmons et al., 2011). From the literature, it is unclear exactly what changes in skin blood flow to expect following a bed rest / confinement and hypoxic study. There appears to be regional variations in perfusion and differences dependant on exposure duration and height.

Systemic hypoxaemia is common in patients suffering from certain cardiovascular and pulmonary diseases such as Chronic Obstructive Pulmonary Disease (COPD). Many of

these patients live a sedentary lifestyle as a result of their condition. Thus, it may be presumed that healthy individuals in a hypoxic environment under an unloading or inactive protocol would closely mimic these patient populations. Furthermore, there is currently a growing pandemic of obese and overweight individuals. There is evidence that individuals' who travel to high altitude areas commonly lose weight, which may be due to a number of reasons including reduced appetite and altered metabolism.

1.5 Ambient Pressure

The Equivalent Air Altitude Theory (EAA) states that the physiological responses to any given combination of barometric pressure (P_B) and fraction of inspired oxygen (F_IO_2) will be the same provided that they result in the same pressure of inspired oxygen (P_IO_2) (Conkin and Wessel, 2008)(eg. hypobaric (HH) versus normobaric hypoxia (NH)). There is some speculation that the EAA theory does not hold true (Conkin and Wessel, 2008; Loeppky et al., 2005). However, we do not know what effect, if any, there may be on the peripheral perfusion as a result of the decrease in pressure in Hypobaric Hypoxia. Indeed, some indirect evidence suggests that the barometric pressure may modify some signs and symptoms of acute mountain sickness (Fulco et al., 2011) and other respiratory-dependent physiological variables (Beidleman et al., 2014; Savourey et al., 2003). Saugy et al., (2014) found that 3km performance was significantly improved 21days following an 18-day Live-High Train-Low study in HH compared to the same protocol in NH. It is noteworthy that throughout the literature the average hypoxic dose received by experimental participants in NH (8 to 12 hours per day) is lower than that in HH (16 to 18 hours per day) and duration spent at altitude is also different: NH (11 to 21 days); HH (13 to 28 days) (Saugy et al., 2014). As a result, it may be difficult to draw exact comparisons from these different exposures from the literature presently. It remains to be proven if, or to what extent, hypobaric or normobaric hypoxic environments produce physiologically relevant differences, with particular reference to peripheral perfusion.

1.6 The role of psychological stress

Successful human adaptation to adverse environmental conditions such as heat, cold, and high altitude constitutes an area of important research interest. Understanding the psychobiological mechanisms of successful adaptation to adverse environments provides important information regarding human function and needs. The deleterious effects that hypoxia may have on athletes has not undergone equal scrutiny. The hypophagia and hypodipsia associated with altitude exposure will certainly affect the nutritional and hydration state of the athlete or astronaut in that environment and may consequently negatively affect performance. Similarly, hypoxia-induced central sleep apnea will affect the sleep architecture and may also have a negative influence on human performance.

Altitude exposure is linked to alterations in an individual's psychological mood, behaviour and cognitive function. Exposure to high altitude with a consequent reduction

of oxygen supply to the central nervous system is associated with a variety of neuropsychological impairments (Davidson, 2001). The symptomology noted during exposure to hypoxic environments are an impairment of coordination, vision, cognitive function, alertness, vigor, whereas in contrast, there is an increase in apathy, anxiety, negative mood, fatigue, confusion, hostility, and generally an increase of a person's negative psychological characteristics (Bahrke and Shukitt-Hale, 1993; Banderet and Burse, 1991; Missoum et al., 1992). Studies have confirmed that the changes observed in several physiological systems over a given period of time in participants rendered inactive and confined to a horizontal position (bed rest), are similar in nature and magnitude to those observed in astronauts during a sojourn in space of equal length (Pavy-Le Traon et al., 2007b).

Stress is a state of mental or emotional tension resulting from an adverse or demanding situation. The results of such a situation can manifest in a heightened state of arousal or more negatively with an increase of anxiety or fear. Typical stressors are external, usually environmental (cold, hypoxia, bed rest, space flight), internal and psychological (relationships or social encounters). The physiological response to stressors is an increase in heart rate and cardiac output and a re-distribution in blood volume including an increase in peripheral resistance. Specifically, the stress response results in an increased norepinephrine and sympathetic activity and an increased circulating cortisol level (Kvetnansky et al., 2013). Prolonged increases in cortisol level can lead to an increase in bone loss and muscle wastage (Pavy-Le Traon et al., 2007b).

During endeavours, such as space flight or attempting to summit a high altitude peak, there is undoubtedly a heightened level of stress related to the potential success of the mission and the ever-present threat of a fatal accident. Sustained levels of stress cause increases in cortisol and can lead to impaired cognitive function, which could be detrimental to mission success. The anticipated thermoneutral environment within future lunar habitats will be hypobaric and hypoxic, thus the combined stressors of remoteness of lunar environment and hypoxia will certainly impose some strain on the psychological state of the astronauts. During a ground-based simulated lunar habitat or hypoxic confinement strategy, the stress level of the participants is likely to be far lower than that experienced by astronauts. Indeed, results from bed rest studies have shown that cortisol levels remain stable during 14 days (Ferrando et al., 1996), 17 days (Millet et al., 2001) or 20 days (Ishizaki et al., 1994) of bed rest. Custaud et al., (2003) found that during a 17-day bed rest, cortisol levels remained the same during the day but where dampened at night. Ishizaki et al., (1997) and (Ishizaki et al., 2002) found that cortisol increased concomitantly with depression after 20 days of bed rest, however with modifications to the social interaction of participants, both mood and cortisol were unchanged after 20 days of bed rest (Ishizaki et al., 2004). Thus, socialisation is an important factor in such group dynamics (Ishizaki et al., 2004); whilst it can have beneficial effects, it must also be considered that the social interaction and information provided by others in the social group can result in an increase in the stress response (Gallagher et al., 2014), as noted in the results of Ishizaki et al., (2004).

The sympathetic nervous system regulates the cardiovascular response to stress and there are varying results based on the length of time of the stressor and also differential responses in different body regions. Krogstad et al., (1995) found that in response to a stress test (mental arithmetic), the fingertip would vasoconstrict in warm participants (finger tip temperature 33 ± 2 °C) and vasodilatate in cold participants. Additionally, Kistler et al., (1998) found that while watching the murder scene in a Hitchcock movie, sympathetic vasoconstriction resulted in a decreased finger temperature.

The regulation of the peripheral circulation is influenced by thermal and non-thermal factors. As noted by Krogstad et al., (1995), the manner in which a non-thermal factor influences the peripheral circulation may also be dependent on the thermal status of the body: mental arithmetic caused vasodilation in cold subjects and vasoconstriction in warm subjects. Whether the presence of a stressor would also influence the circadian rhythm of perfusion is unknown. Namely, the nocturnal drop in core temperature suggested to be a consequence of the peripheral vasodilatation that is initiated just prior to the onset of sleep (Krauchi et al., 1999, 2000). Whether a stressor that is likely to influence peripheral perfusion, particularly enhancing vasoconstriction, will modify the nocturnal vasodilatory response and thus potentially affect sleep remains unresolved. Certainly bed rest/inactivity, hypoxia and emotional state are factors that influence peripheral perfusion.

Thus, in the scenario of a lunar habitat, the likely main stressors that could be simulated in a ground-based experiment and those that are likely to influence the peripheral circulation are: confinement, reduced gravity (simulated with the bed rest experimental (analogue) and hypoxia. Since the peripheral circulation contributes to the regulation of the temperature of the core region and has been demonstrated to have a role in sleep onset, it is important in such ground-based simulations also monitor the effect of any stressors introduced in the simulation on the circadian rhythm of peripheral circulation. lunar habitat simulations should therefore, at a minimum, assess the effect of hypoxia and emotional state.

Thesis Layout

Future missions to the Moon will require permanent planetary habitats in order to provide appropriate life and mission support. It is therefore important to develop ground-based planetary habitat simulation facilities to simulate every aspect of future travel in order to minimise not only life support and technical difficulties, but also to gain a complete understanding of the effects of long term exposure to reduced gravity and hypoxia. The Olympic Sport Centre Planica (Rateče, Slovenia) was developed in order to carry out hypoxic exposures. However the Planica centre is ideally designed for use as a ground-based planetary habitat simulation facility.

The concept of the Planica facility or others would be to investigate the combined effects of hypoxia and bed rest, a simulation of reduced gravity/unloading and inactivity, on certain physiological systems. Furthermore, the facility could be utilised to investigate the effectiveness of potential countermeasures to protect against the deconditioning associated with bed rest. Essentially, a successful space flight, or interplanetary mission is based on the successful regulation/management of the habitat, be that the ISS, space ship or planetary habitat. In all cases, it is essential to understand the complex interaction of the many physiological, psychological and environmental factors.

The purpose of this thesis is to address some of the primary concerns for future planetary exploration, including but not limited to the deconditioning of the cardiovascular and thermoregulatory systems and psychological state induced by hypoxia and inactivity/unloading. Within the framework of this dissertation a series of studies were completed addressing these concerns. The results of these studies are organised in three sections:

- Part A: The development and maintenance of a hypoxic lunar habitat simulation facility
- Part B: The effects of hypoxic bed rest and confinement on peripheral perfusion and emotional state
- Part C: The effects of hypoxic confinement with and without exercise on peripheral perfusion and emotional state

Part A: The development and maintenance of a hypoxic lunar habitat simulation facility

The aim of this section was to establish an appropriate experimental design for future lunar habitat simulation studies. The establishment of such a facility must first follow the

European Space Agency (ESA) guidelines for the operational procedures and data collection during bed rest studies (Heer et al., 2009). The bed rest protocol should be adapted to include a hypoxic stimulus to mimic proposed environmental conditions within future habitats. Thus, the aim of this section was to collect the recommended basic physiological data outlined in the ESA standardisation protocols. This basic physiological data is referred to as the basic core data (BCD). The BCD is utilised as a comparative set of data, to allow for a comparison across all bed rest studies. The BCD provides a quality assurance; provided that the BCD in our current study is inline with the BCD of previous studies reported in the literature, then it can be assumed that the quality of our study is acceptable.

Additionally, the primary aim of this section was to establish a standard operating procedure for Hypoxic experimental protocols, specifically a logistical model that could be transferred to other experiments and utilised for differing durations of bed rest or hypoxic confinement. This aim has been successfully achieved.

Chapter 3 presents the BCD results along with a detailed methodology of the hypoxic bed rest and confinement study.

Chapter 4 details the nutritional aspect of bed rest and confinement study and presents the changes observed in overall and regional body composition.

Part B: The effects of hypoxic bed rest and confinement on peripheral perfusion and emotional state.

Part B of this dissertation deals with the effects of hypoxic bed rest and confinement on psychological strain and on the circadian rhythm of peripheral perfusion. Whereas Chapters 3 and 4 of Part A provide the basic information and outline of the bed rest and confinement experimental model, Chapters 5 and 6 in Part B deal with the separate and combined effects of hypoxia and bed rest on peripheral perfusion and emotional status.

Chapter 5 presents the effects of inactivity and hypoxia on peripheral perfusion, monitored throughout the day and night prior to and at regular intervals during the bed rest and hypoxic exposures (McDonnell et al., 2014a). It tests the hypothesis that sleep onset coincides with the onset of peripheral vasodilatation during both normoxic and hypoxic exposures.

Chapter 6 examines the effect of hypoxic bed rest and confinement on psychological strain (Stavrou et al., 2015). It is assumed that psychological strain is also reflected in peripheral perfusion, specifically inducing a decrease in peripheral perfusion. Whereas chapter 5 focussed solely on the effect of the environmental stressors on peripheral perfusion, Chapter 6 thus complements Chapter 5, as it presents the potential effect of

hypoxia and bed rest on psychological strain.

Part C: The effects of hypoxic confinement with and without exercise on peripheral perfusion and emotional state.

Parts A and B focus primarily on bed rest and confinement. These sections simulate the conditions with no exercise countermeasures. The present practice on the International Space Station (ISS) is for astronauts to conduct daily aerobic and resistance training to prevent cardiovascular and musculoskeletal deconditioning. Thus, perhaps a more suitable normoxic bed rest protocol mimicking the conditions on the ISS should include exercise. Furthermore, hypoxic confinement is routinely utilised by athletes and alpinists and thus exploring a hypoxic confinement scenario combined with exercise may illuminate other physiological changes not previously noted exclusively in bed rest.

Therefore, Chapters 7 and 8 in Part C examine the effects of hypoxic confinement with and without exercise on the same experimental factors (psychological strain and the circadian rhythm of peripheral perfusion) as Part B.

Chapter 7 presents the combined effects of confinement, hypoxia and either activity or inactivity on peripheral perfusion.

Chapter 8 deals with the indices of psychological strain during hypoxic confinement with or without physical activity.

Null-Hypotheses

Each of the studies introduced above tested a series of hypotheses. These are outlined below:

Chapter 3: Establishing a Lunar Habitat Simulation

$H_0$1: Normoxic bed rest will not result in the deconditioning of the musculoskeletal and cardiovascular system.
$H_0$2: Hypoxic bed rest will not result in a further degradation of the musculoskeletal and cardiovascular system than normoxic bed rest alone.

Chapter 4: Whole Body and Regional Body Composition Changes Following 10-day Hypoxic Confinement and Unloading / Inactivity

$H_0$3: Normoxic bed rest will result in no change to the regional or whole body composition
$H_0$4: Targeted energy intake levels and macronutrient composition will be sufficient to

maintain regional or whole body composition
H_05: The addition of a hypoxic exposure to bed rest will not induce any reduction in whole body mass compared to bed rest only

Chapter 5: The Circadian Rhythm of Peripheral Perfusion During a 10-day Hypoxic Confinement and Bed Rest

H_06: Inactivity/unloading induced vasoconstriction will be equal during the day and night.
H_07: Hypoxia will not result in an increase of peripheral vasoconstriction.
H_08: The synergistic effect of combining hypoxia with inactivity/unloading will not induce a greater vasoconstriction than either factor alone.

Chapter 6: Examining The Effect of Hypoxic Bed Rest and Confinement on Psychological Strain

H_09: Hypoxia will not have a detrimental effect on the participants' psychological profile (an increase of negative and a decrease of the positive psychological characteristics).
H_010: Inactivity/unloading will not have a detrimental effect on the participants' psychological profile.
H_011: Exercise will have no effect on the negative psychological profile.

Chapter 7: The Effect of a Live-High Train-High Regimen on Peripheral Perfusion

H_012: Endurance exercise at altitude will not cause any change in daytime hypoxia-induced vasoconstriction.

Chapter 8: The Effect of a Live-High Train-High Regimen on Emotional State

H_013: Compared to sedentary controls, increased endurance exercise will not counteract the detrimental effect hypoxia has on emotional state.

Part A: The Development and Maintenance of a Hypoxic Lunar Habitat Simulation Facility

2 Establishing a Lunar Habitat Simulation: Hypoxic Bed Rest and Confinement Paradigm

2.1 Introduction

To minimise the risk of decompression sickness during preparation for extravehicular activities, it is envisaged that lunar habitats will have operating pressures ranging from 55.2 to 57.2 kPa, and oxygen concentrations ranging from 30 to 35% (Bodkin et al., 2006) resulting in a partial pressure of oxygen within the habitat as low as 16 kPa. Although humans can acclimatise to such levels of hypoxia (West, 2012), the effect of the combined chronic exposure to hypobaric hypoxia and low gravity force field on physiological systems is unknown.

To date, studies investigating the effect of inactivity and unloading of the weight-bearing limbs, as experienced by astronauts in Space, on the structure and function of various physiological systems, have done so by using the bed rest experimental model. This protocol requires subjects to remain in a horizontal position for various durations, depending on the length of the space mission being investigated. With regards to the deconditioning of the musculoskeletal and cardiorespiratory systems, changes observed as a consequence of a bed rest of certain duration, will mimic the changes observed in astronauts exposed to a weightless environment for an equal duration. Thus the bed rest experimental model has been used extensively to investigate not only the effects of weightlessness on physiological systems in ground-based studies, but also to assess the efficacy of measures to counter the microgravity-induced changes. Each bed rest study, regardless of its scientific aims, must collect a set of physiological data that is used to compare with data from other studies. These “basic core data” are used as a quality assurance, ensuring that the bed rest study yielded the anticipated changes in this set of basic core data. To further ensure that bed rest studies are conducted in a similar manner, ESA has established guidelines on the manner in which a bed rest study should be conducted (Heer et al., 2009) and also standard operating procedures on how the measurements of the basic core data should be conducted (MEDES-IMP). These guidelines have been used for all bed rest studies supported by ESA and the National Aeronautics and Space Administration (NASA), which to date have all been conducted in a normoxic environment.

The need to now investigate the effects of the anticipated environment in future lunar and Mars habitats, poses new challenges. Firstly, the gravity on the Moon is a sixth of that on Earth. Simulation of the effect of this gravity vector on the cardiovascular system

would require subjects to maintain a 9° head-up tilt. In contrast to simulations of the effect of the (zero) gravity on the International Space Station, which require subjects to be in a 6° head-down bed rest for 24 hr/day, simulations of the lunar environment would require a 9° head-up position during the daytime hours and a horizontal position during the night. Secondly, since the anticipated environments will be hypobaric and hypoxic, the simulations of the lunar habitat should ideally expose the subjects to hypobaric hypoxia, while maintaining a daytime 9° head-up tilt (HUT) position and a nighttime horizontal position. This type of precise simulation is termed a "high-fidelity" simulation. At present, the precise conditions within future habitats are, as yet, unresolved and thus a high-fidelity simulation is not warranted. For this reason, ESA established a Topical team on "Lunar Habitat Simulation", to recommend a research strategy focussing on the effect of future lunar habitats on astronauts. The conclusion of this Topical team was that research into this problem was warranted, but that the experiments need not be limited by the proposed conditions within these habitats. Indeed, it concluded that the bed rest and hypoxia conditions could even be exaggerated to assess the effects of hypoxia and bed rest on physiological systems. The elucidation of this issue required the establishment of an appropriate paradigm, and also the preparation of a facility in which to conduct the simulations. This was the principle aim of the study described in this Chapter. The goal was also to compare the basic core data with existing data from bed rest studies of similar length to ensure that the bed rest was conducted according to the ESA guidelines.

The study was therefore designed to investigate the separate and combined effects of hypoxia and inactivity/unloading on physiological systems. The former was investigated by confining participants to a normobaric hypoxic environment, and the latter was investigated using the inactivity/unloading (bed rest) experimental model, whereby the participants are requested to remain in the horizontal position throughout the period of the intervention. The combined effect of activity/inactivity and hypoxia was investigated by confining the participants to a normobaric hypoxic environment, where the participants performed regular physical activity. An important aim of the study was to compare the obtained basic core data, with that obtained in similar normoxic studies supported by either ESA and/or NASA.

2.2 Methods

The aim of the study was to establish the logistics of conducting long-term confinement and bed rest studies. These confinement studies were performed at the Olympic Sport Centre Planica (Rateče, Slovenia) situated at 940m above sea level.

The study was a cross-over repeated measures design, such that subjects participated in three 10-day interventions in a randomised order:

- NBR: normoxic bed rest trial
- HBR: hypoxic bed rest trial (target simulated altitude equivalent to ~4,000m)
- HAMB: hypoxic ambulatory confinement (~4,000m).

The design of the experimental facility at the Olympic Sport Centre, Planica, allowed all three conditions to be conducted simultaneously. Thus, in the first experimental campaign approximately one third of the participants were in the NBR condition, a third in the HBR and a third in the HAMB conditions. In the second and third campaigns the participants participated in the remaining conditions. The ascent to simulated altitude in the HBR and HAMB trials followed the standard mountaineering guidelines and therefore was achieved over a three-day period. The target simulated altitudes were therefore: Day 1: 3,000m, Day 2: 3,400m and Day 3 and thereafter: 4,000m (Bartsch and Saltin, 2008). The participants were confined to a thermoneutral environment where environmental conditions were kept constant throughout the three study campaigns. Please refer to Table 3.1 for an overview of the experimental design.

Table 2.1: *Experimental design*. HAMB: Hypoxic ambulatory confinement; NBR: Normoxic bed rest; HBR: Hypoxic bed rest. Each participant would enter into a condition in Experimental Campaign 1, following across the table they would then enter the corresponding recovery period followed by Experimental campaign 2 and so forth.

Participants	Experimental Campaign 1	Recovery Period 1	Experimental Campaign 2	Recovery Period 2	Experimental Campaign 3	Recovery Period 3
1 2 3 4	HAMB (4,000m, Ambulatory)		NBR		HBR	
5 6 7 8	HBR (4,000m, Bed Rest)		HAMB		NBR	
9 10 11 12	NBR (940m, Bed Rest)		HBR		HAMB	

2.3 Hypoxic gas generation

The study was conducted in the hypoxic facility at the Olympic Sport Centre Planica (Rateče, Slovenia). This facility comprises one entire floor of the Centre with 10 double rooms and a common living area. In addition, a laboratory can also be rendered hypoxic. The oxygen content in this facility can be reduced, thus establishing a normobaric hypoxic environment. Simulated altitudes are maintained with a Vacuum Pressure Swing Adsorption (VPSA) system (b-Cat, Tiel, The Netherlands). The VPSA system generates and delivers the oxygen-depleted gas mixture to the designated rooms/areas. The VPSA system passes ambient air through a canister containing a proprietary oxygen adsorber, which removes more than 90% of the oxygen from the air. At regular intervals, a specific volume of the hypoxic gas is then delivered to the hypoxic rooms. Once the adsorber is saturated and can no longer provide an adequate hypoxic gas mixture, the adsorption is switched to another canister. The saturated adsorber in the initial canister is regenerated,

such that a vacuum is applied to the canister, enabling the adsorbed oxygen to be removed from the canister and exhausted into the environment. The initial adsorber is thus ready and is activated once the second adsorber is saturated. In this manner, the delivery of oxygen-depleted gas to the hypoxic rooms continues unhindered. The control unit of the VPSA system can maintain the O_2 concentration in each room separately. Air in each hypoxic room is sampled at intervals of 15 minutes and analysed for O_2 and carbon dioxide (CO_2) content. In the event that the O_2 concentration is too high compared to the required value, the VPSA system continues to deliver O_2 depleted gas to the room. If the O_2 concentration is too low, O_2 delivery is discontinued. Should the O_2 concentration not reach the desired level by the next air-sampling and analysis period, a ventilator is activated drawing air from the external environment into the room. Failure of the system to maintain the oxygen concentration at the desired level, or a drop in oxygen concentration that exceeds a pre-set limit activates an audible alarm, alerting the occupants and research staff to the low O_2 levels. Finally, all measurements of O_2 and CO_2 from the active rooms are stored on a secure server for later analysis.

2.4 Bed rest / confinement

The participants arrived at the Planica facility 4 days prior to the start of the intervention (NBR, HBR or HAMB). The participants also remained in the facility four days after the completion of the intervention. As the participants took part in all 3 interventions, a recovery period was required between experimental campaigns to ensure recovery of the cardiovascular and musculoskeletal systems, specifically that the functional values returned to baseline values. This recovery period was set as a minimum of 2.5 times the duration of the intervention, which was 25 days. During the interventions, the participants were confined to the Planica facility 24 hours per day for 10 days. Each intervention commenced at 09:00 on day 1 (D1) and the participants exited the confinement area at 09:00 on Day 11, so that the cumulative exposure for each individual was exactly 240 hours. During the 4 days prior to and after the experimental campaign, the participants where permitted to leave the hypoxic or bed rest area for supervised walks or social time when the schedule allowed.

During the bed rest (HBR and NBR) interventions participants maintained a horizontal position at all times, including during their hygiene routine (urination, defecation and shower). A bespoke bed was utilised for the hygiene routine; the bed was fitted with water resistant padding and a drain. The participants were allocated one pillow for head support and were allowed to support themselves on an elbow during meal times. They were requested to refrain from stretching and from initiating static muscle contractions during the bed rest intervention.

During all interventions the participants were instructed to sleep between 23:00 and 07:00. After being woken at 07:00, the participants' body weight was measured and time for breakfast and their daily hygiene was allocated. Daily measurements of heart rate

(HR), arterial pressure (AP), arterial oxygen saturation (S_aO_2) and body temperature (Tb) were recorded at 16:00. Furthermore, this time point was chosen as it coincided with the beginning of the afternoon shift for the staff nurses, thus affording the incoming nurses an opportunity to monitor the participants' well-being.

During the HBR and NBR trials participants were requested to maintain a horizontal position at all times. They were allowed one pillow to prop their head up. The participants were asked to refrain from stretching and performing any static muscular contractions, particularly of the muscles of the lower limbs, while in bed.

In the HAMB intervention participants were also confined to the Planica facility for 10 days but were ambulatory. In addition, they were requested to complete a daily regimen of exercise designed to mimic their normal everyday activity at home.

2.5 Hypoxic safety procedures

All participants in the HBR and HAMB interventions were issued a personal clip-on O_2 analyser (Rae PGM-1100, California, USA) throughout the hypoxic exposure. An audible alarm would be activated in the event that the oxygen level decreased below a pre-set level (FO_2 = 0.12). In the event that a participant presented with an adverse reaction to altitude, the provision was made for the project physician to assess their AMS symptoms. If the symptoms continued or deteriorated, the project physician would make a decision whether to maintain the current altitude, to descend, or ultimately to exclude the participant from the study. If the decision was to descend, the participant could be moved to a separate room (on the same floor) where the altitude level could be regulated individually with the VPSA system, thereby allowing continued involvement in the study at a safer lower altitude until the AMS symptoms subsided. In order to monitor the participants' well-being during all interventions, HR and S_aO_2 responses were monitored at regular intervals daily using personal finger oximetry devices (3100 WristOx, Nonin Medicals, Minnesota, USA).

2.6 Lake Louise Mountain Sickness score (LLMS)

According to Basnyat and Murdoch, (2003), approximately 50% of individuals travelling to high altitude will be become ill as a result of maladaptation to hypoxia. The presence of such an adverse effect of hypoxia may have negative consequences on participants' psychological status. Furthermore, due to the possibility that acute mountain sickness (AMS) might affect peripheral perfusion (Loeppky et al., 2003), its presence was assessed with the Lake Louis Mountain Sickness (LLMS) questionnaire (Roach et al., 1993). The LLMS questionnaire was administered and completed each evening between 20:00 and 21:00 during both studies to monitor for signs and symptoms of acute mountain sickness (AMS). The LLMS questionnaire is self-reported and involves the participants rating their

own perception of five symptoms (please see appendix 11.3). The participants were asked to rate each of the following on a scale from 0 to 3: headache, gastrointestinal symptoms, fatigue and/or weakness, dizziness or light-headedness and difficulty sleeping. A diagnosis of AMS is assigned, if an individual has ascended to altitude within the last four days with the presence of a headache, plus one other symptom, and a total score of 3 or more. A total score of 6 or more represents severe AMS. Individuals presenting with AMS did not ascend further and if symptoms deteriorated an immediate descent was initiated, as described above.

2.7 Medical supervision of the participants

2.7.1 Medical staff

The project physicians carried out the final medical examinations of the participants during the recruitment phase of the project. Following on from that, their responsibilities included supervision of any medical procedures (i.e. muscle and/or fat biopsies) and maintaining the general medical care of the participants with a particular focus on AMS.

2.7.2 Nurse and assistants

The participants while bedridden are extremely limited in their ability to do every day tasks for themselves. In order to cater to their needs and provide the required level of dignity, it is essential to put in place a good quality team of nurses and project assistants. The general duties of nurses and assistants were to provide any support required of them to the participants, including transporting them from their beds to the hygiene facility (shower and lavatory), overseeing minor medical issues (headaches, general malaise, rashes, venipuncture and biopsy care) and socialising with the participants. Please see appendix 11.2 for full list of duties.

2.7.3 Video surveillance

In order to provide a safe environment for the participants during the bed rest and hypoxic trials, twenty-four hour video surveillance was maintained. Additionally, participants were provided with a “walkie-talkie”, which allowed them to call the nurse/assistant on duty in the case of an emergency. Furthermore, the video surveillance footage could be retrospectively viewed if there were any suspicions that a participant was not adhering to the strict bed rest protocol.

2.7.4 Physiotherapist

During the initial days of bed rest, there is a period of relaxation and tightening of the

muscles of the legs, hips and back. As the participants were restricted from stretching and exercising while in bed, a physiotherapist provided regular passive stretching and gentle massage to alleviate any pain and discomfort reported by the participants.

2.7.5 Nutritionist

Nutritional support is essential for maintaining the health status of the participants during a bed rest or confinement study. Additionally, in order to limit any confounding factors that may influence the repeatability of the results (Pre testing to Post testing), a standardised diet is vital. In order to achieve this, a nutritionist was involved in the development of the standardised study diet.

2.8 Nutrition

For a detailed analysis of the nutritional aspect of the Lunar Habitat Simulation experiment, please see Chapter 3. Briefly, while it is known that individually microgravity and hypoxia can significantly alter nutritional status and body composition (Boyer and Blume, 1984; Zwart et al., 2009b) there is a paucity of data regarding their combined effect (Stevens et al., 1966). Proper nutrition and efficient dietary support are among the most critical features for maintaining health and well-being, as well as the operational abilities of humans during space sojourns (Lane and Feeback, 2002; Smith and Zwart, 2008; Smith et al., 2005). Environmental hypoxia also alters metabolism and consequently modulates body mass and composition (Boyer and Blume, 1984). Following long-term hypoxic exposures of different durations, body mass is decreased (Quintero et al., 2010) secondary to reductions in fat and muscle mass (Kayser et al., 1993; Westerterp et al., 1994; Westerterp-Plantenga et al., 1999).

2.8.1 Diet planning and monitoring

The menus were constructed using the web-based application Open Platform for Clinical Nutrition (OPKP; www.opkp.si), developed by a research group at the Jozef Stefan Institute (Korošec et al., 2013). A 10-day menu was prepared before the start of the study, and repeated during each campaign to ensure that the participants consumed identical food on each given study day. The individual daily targeted energy intakes were calculated using the Mifflin equation (Mifflin et al., 1990) and multiplied by Physical Activity levels (PAL) 1.4 for the HAMB and PAL 1.2 for the HBR and NBR campaigns to account for different activity levels. All five daily meals (breakfast, morning snack, lunch, afternoon snack & dinner (please see appendix 11.10) were prepared by the kitchen staff of the Planica facility, where the components of each meal were weighed to an accuracy ± 1 g. The meals were served at the same time of the day throughout the confinement period. The participants were instructed to consume all the food provided. In case of any leftovers, the unconsumed food was weighed and the measured amount

deducted from the initial weight, thus providing a net value of the participants' actual food intake. The energy, macronutrient and fluid intake analysis was performed subsequently using the OPKP for each individual participant.

2.9 Physical activity and exercise monitoring

The ambulatory participants (HAMB) were allowed to move freely within the confinement area and were encouraged to be active, so as to mimic as much as possible their normal daily activities. To ensure that they did indeed maintain a normal level of activity in the limited space available, the HAMB participants engaged in two 30-minute bouts of aerobic exercise (stepping at a rate of 100 steps per minute on a 30-cm step) once in the morning and again in the afternoon. All exercise sessions were performed in a designated training room under similar environmental conditions as to those during the normobaric hypoxic confinement. Participants' HR and S_aO_2 were monitored throughout the exercise session using the finger oximetry device (3100 WristOx, Nonin Medicals, Minnesota, USA), respectively.

To ensure that the participants were performing the same level of relative exercise intensity a training impulse (TRIMP: TR = training, IMP = impulse) score was calculated for each exercise session according to the formula (Banister et al., 1975):

Equation 1: Training Impulse (TRIMP)

$$TRIMP = tex * (HRex – HRrest / HRmax – HRrest)$$

where,

- tex = exercise duration
- HRex = exercising heart rate
- HRrest = resting heart rate
- HRmax = maximal hear rate

2.10 Participant recruitment procedures

Male participants were recruited through local advertisement. The respondents to the advertisement initially made contact through email where any further information the potential participants required was provided. Subsequent to this preliminary briefing, all interested persons were invited to attend a group presentation, providing detailed information regarding the experimental design and procedures. Following the presentation, those individuals still interested in taking part were invited to attend an interview with a panel of investigators. Participants were given an extensive briefing regarding the study protocol. The possible risks involved were also explained prior to inclusion in the study. Participant recruitment was based on the European Space Agency

recommendation regarding the standardisation of bed rest study conditions. Exclusion criteria included a medical history of respiratory, haematological or cardiovascular illness, recent altitude exposures (< 2 months), participation in dietary programs (< 6 months) a history of mental illness or depression or the use of drugs or medications. All participants gave their written informed consent to participate in the studies, which were approved by the National Committee for Medical Ethics at the Ministry of Health of the Republic of Slovenia (see Appendix 12.6) and performed according to the guidelines of the Declaration of Helsinki. All participants were lowland residents (<500m) and were recreationally active, healthy males.

2.10.1 Recruitment strategy

Advertisements for participation in the PlanHab study were placed with the Student Services in Slovenia. Advertisements were also placed on some web portals.

Within a number of weeks of placing the advertisements, over 100 phone calls and emails were received from individuals requesting more information. Those who were still interested in participating following the telephone conversation were forwarded more detailed information regarding the project and were also informed of the details of the upcoming presentations providing further details of what to expect as a project participant. During this period participants interested in taking part in the experiments were requested to attend presentations, which were arranged in Ljubljana (Jozef Stefan Institute), Maribor (University of Maribor) and Koper (University of Primorska). Following these presentations, individual interviews were arranged for those who still retained an interest in participating in the study.

2.10.2 Interviews

Of the original applicants, approximately 100 were invited to attend a panel interview at the Jozef Stefan Institute. The panel comprised a minimum of three researchers with experience conducting bed rest and hypoxic studies. One panel member presented the questions, which were identical for each participant. Once the participant responded to the question, the panel may have continued a further discussion on the topic of the question. During the interviews, the panel members graded each response on a 5-point scale (1-unsatisfactory, 2-satisfactory, 3-good, 4-very good, 5-excellent). Upon completion of the interview, the grades were summed. In addition, the panel conducted an extensive discussion on the participants' responses and suitability to enter the study. A threshold of 12 points (total from the three panel members) was established for acceptance into the study. Participants not achieving this threshold value were not invited for further testing. Those that attained a total value of 12 or more points were scheduled for a medical examination at the University Clinical Centre, where they provided blood samples for a variety of tests. Once these were completed, participants were invited to attend a familiarization weekend at the Olympic Sport Centre, Planica. During this familiarization

weekend they socialised with the remaining candidates and the IJS research staff. In order to familiarise with the testing protocols, they conducted tests of aerobic capacity, pulmonary function and also underwent whole body composition assessment.

2.10.3 Medical examinations

Despite the several examinations conducted at the University Clinical Centre Ljubljana, the final medical examination took place at the Planica facility. Participation in the study was subject to the project physicians' approval. In addition, the inclusion/exclusion criteria recommended by the ESA guidelines (Heer et al., 2009) were used in the final participant selection process. The following is the list of inclusion/exclusion criteria as stipulated in the ESA guidelines. Although, some of the criteria are irrelevant for the current studies, as they pertain to female participants, it is the complete ESA approved list.

2.10.4 Inclusion criteria

- Physically and mentally healthy participants
- Age range 20 – 45years
- Body mass index 20 - 26kg/m^2
- 158 - 190cm
- Volunteers that are able and declare their willingness to participate in the entire study
- Willing to be assigned randomly either to the treatment or the control group
- Successfully pass the psychological and medical screening
- Signed informed consent
- Social insurance

2.10.5 Exclusion criteria

- Medication required that may interfere with the interpretation of the results
- Bone mineral density (measured by DEXA) more than 1.5 standard deviation ≤ tscore
- Using hormonal contraceptives (less than 2 months before)
- Recent sub-standard nutritional status
- Menopause
- Family history of thrombosis or positive response in thrombosis screening procedure
- Biochemical analysis of the following parameter: (ATIII, High sensitive C-Reactive
- Protein, S-Akt., F-V-Leiden, Protrombinmut, Lupus-PTT, Factor II)
- History of: thyroid dysfunction, renal stones, diabetes, allergies, hypertension,

- Hypocalcaemia, uric acidaemia, lipidaemia or hyperhomocystinaemia
- Gastro-oesophageal reflux disease or renal function disorder, Hiatus hernia
- History of mental illness
- Smoker within six months prior to the start of the study
- Abuse of drugs, medicine or alcohol
- Participation in another study up to two months before study onset
- Cannot clear a criminal background check
- No signed consent form before the onset of the experiment
- Blood donors in the past three months before the onset of the experiment
- One year after pregnancy, breast feeding, abortion (not relevant for this study since it was limited to males only)
- Vegetarian and Vegans
- Migraines
- History of orthostatic intolerance
- History of vestibular disorders
- Claustrophobia
- Use of an intra uterine device
- Metallic implants, osteosynthesis material
- Incarcerated persons
- Chronic back pain

2.10.6 Haematological examination

Screening Process

All potential participants that attained 12 points during the interview were invited to the haematological laboratory at the University Medical Centre Ljubljana (Slovenia). The participants were fasted prior to the blood sampling. A 10 mL blood sample was taken from an antecubital vein. A 4 mL blood sample was immediately analysed for complete blood count (including differential blood count) using an automated laser-based haematology analyser (ADVIA 2120, Siemens, München, Germany). The remaining venous blood was centrifuged (10-min at 3,500 rpm; 4° C), stored in five 400-µL aliquots and frozen at -80°C for subsequent analysis for coagulation factors and erythropoietin. The measured markers were selected based on the ESA guidelines for participant recruitment. The tests performed on the blood sample included complete blood count (CBC) with differential, test of liver function, drug screen, lipid panel, vitamin and mineral status and thrombosis sensibility screening.

Experimental Process

Baseline measurements of erythropoietin (EPO, $mU.mL^{-1}$) and red blood cell count (RBC, $10^{12}/L$) were obtained at the Planica facility prior to entry into the experimental intervention. Post-intervention measurements of EPO and RBC were sampled in the morning on D 11 just prior to the participants exiting the intervention. All blood samples were taken from the antecubital vein in the morning, ~ 07:00, after an overnight fast and prior to the participants rising from bed. EPO samples were collected in EDTA

vacutainers, whereupon the blood was immediately centrifuged and the plasma was frozen at −80°C for subsequent analysis. The concentration of EPO was determined via sandwich enzyme-linked immunoassay (Quantikine IVD EPO ELISA; R&D Systems, Minneapolis, MN, USA). Optical density was quantified on a microplate reader Quant (Bio-Tek Instruments, Winooski, VT, USA) set at 450nm and corrected at 600nm. All samples were assayed in duplicate. The coefficient of variation of the analysis was 5%. A 2ml whole blood sample was immediately transferred to a nearby medical institution for analysis of the complete blood count. RBC count was obtained with an automated laser-based haematology analyser (Advia 120; Siemens, Munich, Germany). The apparatus was calibrated before each measurement. All samples were assayed in duplicate.

2.10.7 Aerobic capacity

To determine the participants' baseline aerobic capacity, a criterion graded exercise test to voluntary exhaustion on an electromagnetically controlled cycle-ergometer Ergo Bike Premium (Daum electronics, Fürth, Germany) was performed. Resting and exercise cardiorespiratory responses were measured using a Quark CPET metabolic cart (Cosmed, Rome, Italy). During the test the participants breathed through an oro-nasal mask (Vmask, Hans Rudolph, Shawnee, USA), connected to a two-way valve. The turbine flow meter and gas analyser were calibrated before each test using a 3L syringe and two different gas mixtures, respectively. The testing protocol commenced with a 5-min resting period, followed by a 3-min warm up at a work rate of 60 W. Thereafter the workload was increased each minute by 30W. The participants were required to maintain a cadence of 60rpm throughout the whole test and were given verbal encouragement. The test was terminated when the participant was unable to maintain the assigned cadence. In addition, a plateau in oxygen uptake and a respiratory exchange ratio (RER) > 1.1 were used to confirm the attainment of the maximal oxygen consumption (VO_{2max}). VO_{2max} was defined as the highest 60s average of oxygen uptake (VO_2) over the course of the test. Heart rate was continuously monitored throughout the tests using a short range telemetry heart rate monitor (Vantage NVTM, Polar Electro Oy, Kempele, Finland). Peak power output (PPO) was calculated using the following equation:

Equation 2: Peak Power Output calculation

$$PPO = Wcompl + (t/60 \times 30)$$

where,

- PPO = peak power output
- Wcompl = the last completed workload
- t = the number of seconds during final uncompleted workload.

2.10.8 Pulmonary function assessment

Pulmonary function was assessed to exclude any problems related to pulmonary system including asthma. Forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), slow vital capacity (SVC), peak expiratory flow (PEF) and maximum voluntary ventilation (MVV) were measured using a pneumotachograph (Cardiovit AT-2plus, Schiller, Baar, Switzerland). The spirometer was calibrated with a 3L syringe before each test. Tests were repeated three times and the highest of the three acceptable values obtained was used for the subsequent analysis.

2.11 Experimental scheduling

The experimental schedule is vital to the success of the project and to the quality of the results. In large research projects of this nature, there are usually several different research laboratories collaborating and vying to have access to the participants. Each of the experiments that take place before, during and after the bed rest or confinement study will have a lasting effect and may potentially influence the following experiment. Most haematological parameters require the participant to be fasted and therefore take place first thing in the morning. However, it is impossible to carry out a maximal oxygen uptake test immediately prior to another maximal exercise test, as the participant needs a recovery period. Furthermore, a number of tests use the same equipment and would therefore be unavailable to the other tests throughout the day. Scheduling conflicts of such nature can lead to poor experimental results. The experimental schedule designed for the present study is in Appendix 12.8.

In addition to the basic core data (BCD), pre- and post-intervention periods also included measurements performed within the framework of sub-projects, which will be reported elsewhere. The schedule of all measurements, taken for the purposes of the BCD and sub-projects is presented in appendix 12.9.

2.12 Basic core physiological data

The BCD are a set of measurements recommended by the ESA to be recorded in the pre- and post-intervention period and are considered quality control measurements. In this manner, the BCD of any bed rest study can be compared with the BCD of previous studies archived by ESA and NASA. This archive contains the effect of bed rest of different durations on the specific functions of the cardiorespiratory, musculoskeletal, neurohumoral systems and on body composition. All bed rest studies archived to date by both ESA and NASA, have been conducted in normobaric normoxia. Information like that depicted in Figure 3.1 can be compiled from the archived BCD providing an evidential basis for changes expected during bed rest/space flight.

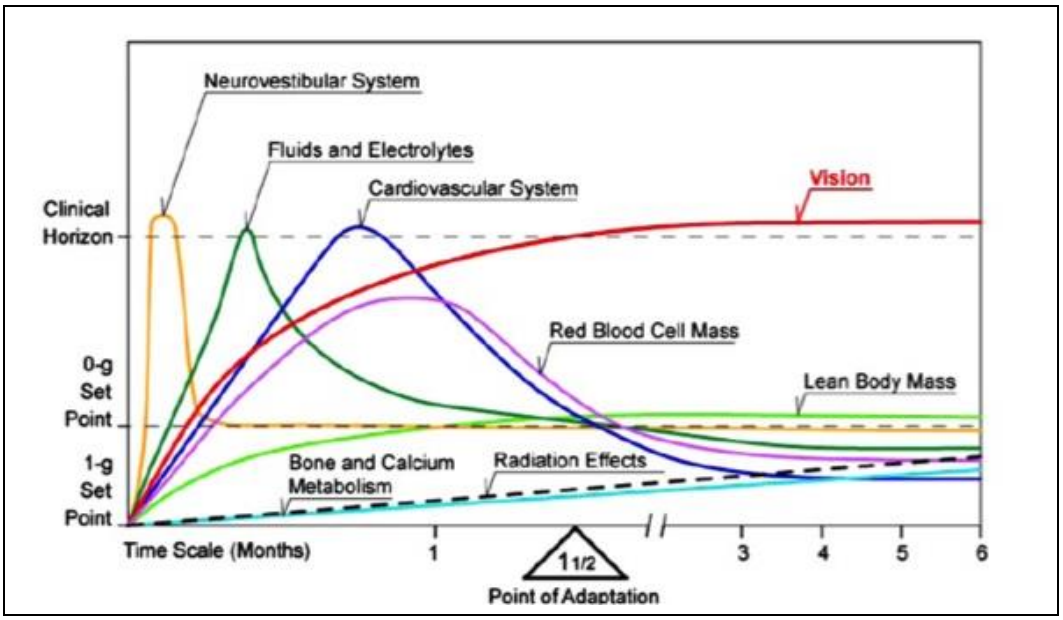


Figure 2.1: Space Adaptation Kinetics. This figure depicts the standard time course of several physiological systems adapting from Earth (1-g Set Point) to Space (0-g Set Point) and whether or not these changes or adaptations are of clinical significance. Figure adapted from Nicogossian and Parker, (1982)

2.12.1 Anthropometry

Before and after each intervention participants' body composition was determined with Dual Energy X-ray Absorptiometry (Hologic, Discovery ODR Series, Discovery W, Bedford, Massachusetts, USA). Body scans were conducted according to the standard operating procedures outlined in the ESA guidelines (Heer et al., 2009). Participant height was measured to the nearest 0.1cm using a stadiometer (Seca 703, Seca Hamburg, Germany) and weight was measured to the nearest 0.1kg (Soehnle, Nassau, Germany). These data were measured in the morning during the data collection phase prior to each intervention and immediately after the orthostatic tolerance test in the morning on the first day after each intervention.

2.12.2 Maximal aerobic capacity (VO_{2max})

Maximal aerobic capacity was determined with an incremental-load test to exhaustion on a cycle ergometer (Ergo Bike Premium, Daum electronics, Fürth, Germany). The test comprised a 3-min resting period while seated on the ergometer, followed by the exercise period, during which the load was incremented by 30 Watts every minute, increasing from a 60W warmup. During the rest and exercise periods, participants breathed through an oro-nasal mask (Hans Rudolf, Shawnee, Kansas, USA). The fraction of O_2 and CO_2 in the inspired and expired volumes was monitored by a metabolic analyser (COSMED, Rome, Italy), thus provided on-line breath-by-breath measurement of oxygen uptake (VO_2, L.min-^{1}) and carbon dioxide production (VCO_2, L.min^{-1}). In the pre- and post-

intervention periods, the participants performed two tests of maximal aerobic capacity separated by two days. In one test the participant inspired room air and in the other test, a hypoxic gas mixture was inspired (14.4% O_2 / 85.6% N_2).

2.12.3 Muscle strength (MVC)

Maximum voluntary contraction (MVC) was assessed bilaterally in the quadriceps and hamstring muscle groups, the knee extensors and flexors. MVC was determined utilising an isometric dynamometer (Biodex Medical Systems, System Pro 4, Shirley, New York, USA). The dynamometer was calibrated daily. Prior to each test, the participant was strapped tightly to the chair and the centre of rotation for the knee joint was aligned with the axis of rotation of the dynamometer. The angle of the knee joint was set to and tested at 60°. In order to test MVC, participants were required to give maximal effort during 5 contractions of each muscle group (flexion, extension). The protocol used was, 5 second isometric contraction of the agonist followed by a 5 second recovery, then 5 second isometric contraction of the antagonist followed by a 5 second recovery. The pattern was repeated until 5 contractions of each were completed.

2.12.4 Muscle cross sectional area (CSA)

Peripheral quantitative computer tomography (pQCT: XCT3000, Stratec Medizintechnik GmbH, Pforzheim, Germany) was used to measure muscle cross sectional area (mm2). pQCT scans were completed three days prior to entering each intervention (Pre) and on the first day after exiting each intervention (Post). Participants were positioned in a supine position with their right leg fully stretched and positioned within the device. The foot was fixed to a custom plastic holder and secured with a belt, while the thigh was fixed with a supporting holder provided by the manufacturer. The positioning and stability of the participant is integral to recording accurate and reliable scans, furthermore, no movement was tolerated during scanning.

Muscle CSA was determined at 66% of the tibial length measured from the medial tuberosity and at 33% of the femur length from the distal part of lateral condyle. The chosen area of measurement was based on ESA protocol and the literature, (Rittweger et al., 2005). In order to accurately scan at 33% of the femur length, the initial scan completed was the scout view. The scout view is performed in the coronal plane and is used to determine the anatomical reference line at 0% of the tibia and femur length. This position was for the tibia distal articular surface and for the femur the intercondylar eminence. Scans were processed with the computer software provided by the manufacturer (Stratec Medizintechnik software)

2.12.5 Statistical analysis

A comparison of the results of the NBR and HBR trials would reveal whether hypoxia exacerbates, suppresses or has no effect on the inactivity / unloading-induced physiological responses. Comparison of the results of the HBR and HAMB trials would further reveal the effect of activity on these physiological responses.

The average HR, AP and S_aO_2 responses, the LLMS score and Taural data were all analysed using a repeated measures ANOVA (Condition (HAMB, HBR, NBR) X time (D1, D2, D3...D10). A repeated measures ANOVA was utilised in most tests (the cardiovascular, musculoskeletal and the facility ambient data) as the data was comprised of 3 conditions with multiple measures while all subjects completed each of the 3 conditions. Values are reported as arithmetic mean ± standard deviation unless otherwise indicated. The alpha level of significance was defined *a priori* at 5 %. All statistical analyses were performed using Statistica 5.0 (StatSoft, Inc., Tulsa, Oklahoma, USA).

2.13 Results

Eleven male participants successfully completed the three 10-day confinements. Their physical characteristics are presented in Table 3.2.

Table 2.2: *Participants' Physical Characteristics.* Table presents each participants' physical characteristics. These data are relevant for Chapters 4, 5 and 6. Data presented as means ± standard deviation.

Participant	Age (yr)	Height (m)	Weight (kg)	BMI[1] ($kg.m^{-2}$)	BF[2] (%)
1	26	1.89	86.8	24.1	24.1
2	22	1.86	97.1	28.0	31.7
3	24	1.69	65.9	22.8	22.5
4	23	1.72	58.2	19.7	18.2
5	22	1.75	74.7	24.2	24.6
6	23	1.89	71.5	19.9	23.2
7	28	1.82	66.9	20.0	13.9
8	24	1.80	58.9	18.1	17.8
9	27	1.83	66.9	19.9	17.5
10	21	1.73	67.1	22.4	19.2
11	25	1.76	84.0	27.0	26.0
Average	24.1	179.9	72.5	22.4	21.7
SD	2.2	6.9	12.2	3.2	4.9

[1]Body mass index; [2]Body fat.

2.13.1 Ambient conditions

The three interventions were conducted over a period of 7 months, with the first taking place in March 2011 and the last finishing in September 2011. As the first intervention took place in Spring, the central heating in the Planica facility was turned on. Consequently, the ambient atmosphere of the first intervention was significantly warmer ($p < 0.01$) and dryer ($p < 0.01$) compared to the second and third interventions. The temperature during the first, second and third interventions were 23.4 ± 0.4, 21.8 ± 0.3 and 21.7 ± 0.2°C, respectively. The relative humidity during the first, second and third interventions were 28.2 ± 1.5, 49.4 ± 2 and 45.7 ± 1%, respectively. The average ambient pressure at the Planica facility remained stable throughout the interventions at 684 ± 1mmHg.

The F_IO_2 in the hypoxic trials was maintained stable at 0.1629 ± 0.0005 on Day 1 (simulated equivalent altitude of 2,990m), 0.1549 ± 0.0005 on Day 2 (simulated equivalent altitude of 3,380m) and 0.144 ± 0.0002 (simulated equivalent altitude of 3,881m) throughout the remaining period of the hypoxic interventions. The equivalent altitudes were derived from the data of Gallagher and Hackett, (2004), please see appendix 11.7. The partial pressure of oxygen (P_IO_2) was calculated according to the following formula:

Equation 3: Pressure of Inspired Oxygen

$$P_IO_2 = (P_B - PH_2O) * F_IO_2$$

where,

- P_B = the atmospheric pressure
- PH_2O = the saturation pressure for water vapour at 37 °C
- F_IO_2 = the inspired fraction of oxygen

The P_IO_2 in the HAMB and HBR trials was 103mmHg on Day 1, 98mmHg on Day 2 and 92mmHg for the remaining days. The P_IO_2 in the NBR intervention was 133 ± 1mmHg.

2.13.2 Aerobic Capacity

There was a trend for both VO_{2max} and PPO to decline during bed rest (HBR and NBR) however, these differences were not statistically significant. Please see Table 3.3 for absolute and relative change in VO_2max and PPO during all 3 interventions.

Table 2.3: *Aerobic capacity.* Maximal aerobic capacity (VO_{2max}) and peak power output (PPO) are presented for each intervention (Pre and Post). Data presented as means ± standard deviation.

	HBR		NBR		HAMB	
	Pre	Post	Pre	Post	Pre	Post
VO2 ($ml.kg^{-1}.min^{-1}$)	43.35 ± 6.36	40.34 ± 5.58	42.90 ± 4.35	40.90 ± 4.18	42.05 ± 3.42	42.57 ± 4. 00
VO2 (% Change)		- 7.0%		- 4.7%		+1.2%
PPO (W)	276 ± 51	254 ± 55	276 ± 44	266 ± 48	273 ± 45	278 ± 48
PPO (% Change)		- 8.0%		- 3.8%		+ 1.7%

2.13.3 The aerobic physical activity of the HAMB group

There were no differences in any of the variables measured during the morning or afternoon exercise bouts. The heart rates during the morning and afternoon sessions were 115.9 ± 3.3 and 112.5 ± 2.7bpm, respectively, which represented an average of 64 ± 5% of the HR attained during the hypoxic maximal oxygen capacity test. The S_aO_2 values were 88.3 ± 0.4 and 88.2 ± 0.4% during the morning and afternoon exercise bouts, respectively. The TRIMP values were 2.2 ± 0.1 and 2.1 ± 0.1 during the morning and afternoon exercise bouts, respectively.

2.13.4 Arterial saturation, heart rate and aural temperature

Arterial saturation was significantly lower in both hypoxic trials (HBR, HAMB) on all days during the intervention compared to the normoxic trial (NBR), $p < 0.05$ (Figure 3.2). Heart rate significantly rose from day 1 to day 10 in all 3 conditions, $p < 0.05$ (Figure 3.2). Taural did not change as a result of any intervention (Figure 3.2). Systolic arterial pressure significantly increased across both the HBR and HAMB trials, such that D10 was different to D1, $p < 0.05$. Diastolic arterial pressure increased only in the HAMB significantly by D10 and was significantly higher than that of the NBR trial (Figure 3.3). For further details please refer to the results section of Chapter 4 and 5.

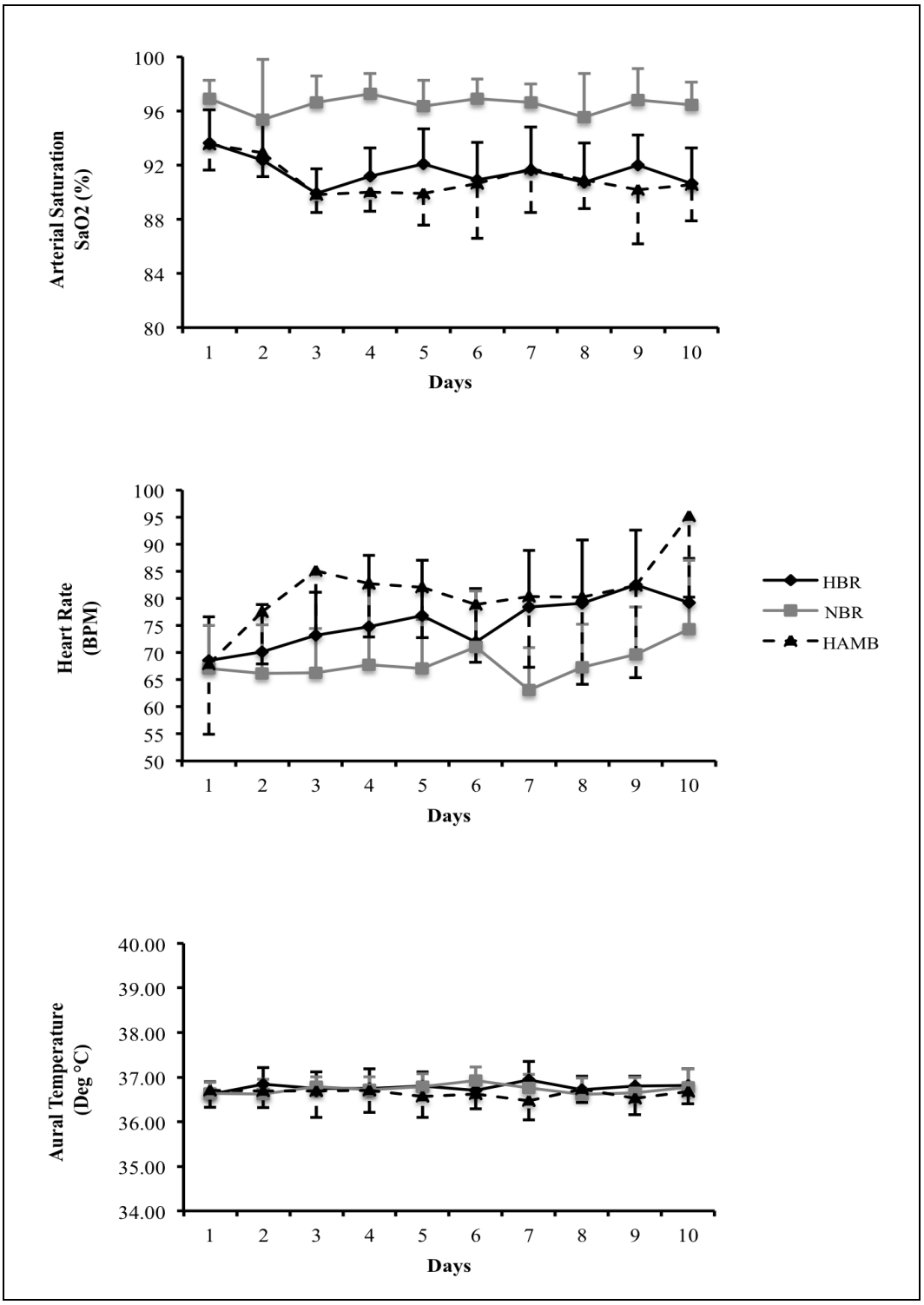


Figure 2.2: *S_aO_2, HR and Taural.* Capillary oxyhaemoglobin saturation, heart rate and aural temperature are presented for all 10-days of each of the 3 interventions (HBR: Hypoxic Bed Rest, black solid line; NBR: Normoxic Best Rest, grey solid line; HAMB; Hypoxic Ambulatory, black dashed line). Data presented as means ± standard deviation.

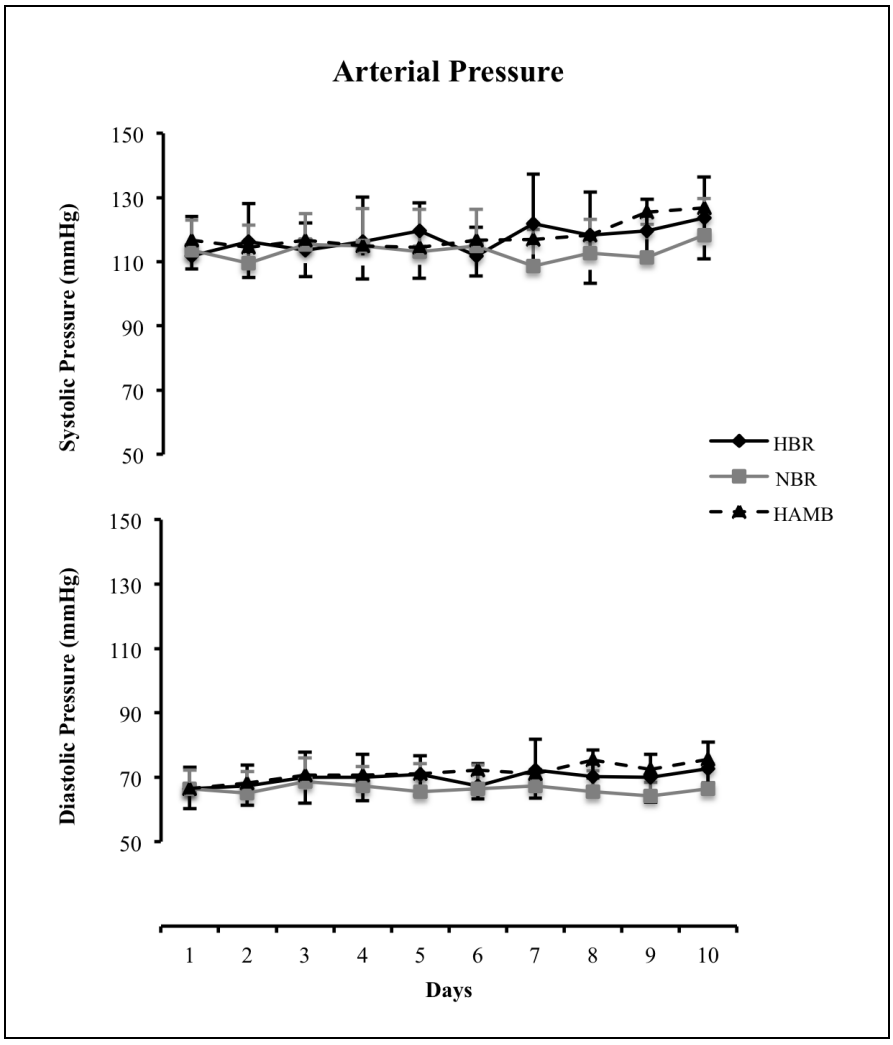


Figure 2.3: *Arterial Pressure.* Systolic and Diastolic arterial pressure are presented for all 10-days of each of the 3 interventions (HBR: Hypoxic Bed Rest, black solid line; NBR: Normoxic Best Rest, grey solid line; HAMB; Hypoxic Ambulatory, black dashed line). Data presented as means ± standard deviation.

2.13.5 Muscle strength and CSA

Muscle strength was measured using isometric MVC at the knee. As a result of bed rest, knee flexion MVC decrease 8.92 ± 16.27% (NBR) and 7.49 ± 19.74% during bed rest with a hypoxic exposure (HBR). Following the HAMB trial, knee flexion MVC changed -2.73 ± 12.78% from baseline. Knee extension showed significant differences between then the NBR and HAMB conditions, $p < 0.05$. NBR and HBR did not differ. NBR knee extension MVC decreased 14.51 ± 11.01%, while MVC decreased 7.83 ± 10.26% and 0.2 ± 9.05% for the HBR and HAMB conditions, respectively, Figure 3.4. The muscle CSA decreased in both the HBR and NBR trials at 66% of the tibial length, $p < 0.05$. The calf CSA decreased 7.2 ± 2.9% in the HBR trial and 7.4 ± 2.1% in the NBR trial. There was no change measured during the HAMB intervention (-1.1 ± 2.6%). The same pattern was recorded at 33% of the femur length, however the decrease was not of statistical

significance. Thigh CSA decreased 4.7 ± 6% in the HBR intervention and 3.6 ± 3.2% after the NBR intervention. However, both decreases in thigh CSA were significantly larger than that noted in the HAMB trial (0 ± 4.5%), $p < 0.05$. Please see Fig 3.5 for more detail.

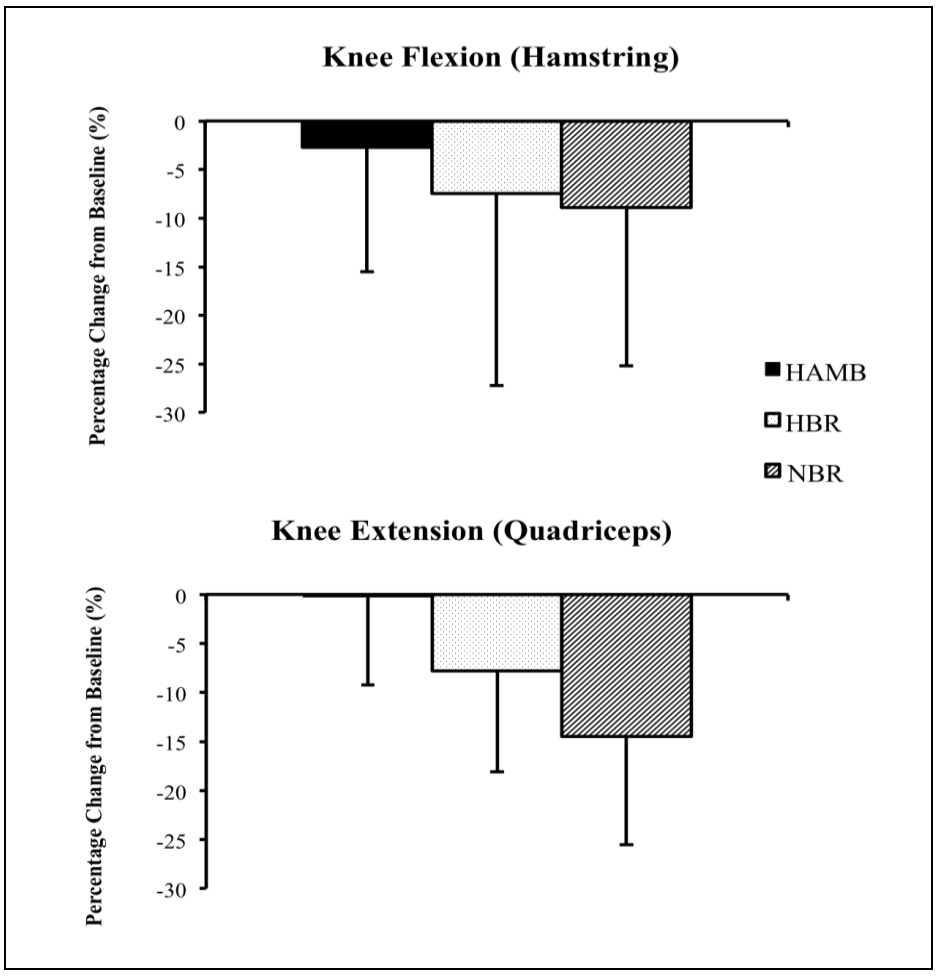


Figure 2.4: *Muscle MVC*. Knee flexion is presented in the upper panel and knee extension in the lower. The information in both of the graphs represents the change in maximal voluntary contraction from Pre to Post intervention. (HBR: Hypoxic Bed Rest, grey; NBR: Normoxic Best Rest, grey stripe; HAMB; Hypoxic Ambulatory, black). Data presented as means ± standard deviation.

2.13.6 Haematology

As a result of the continuous 10-day (240-hr) exposure to hypoxia, there was a significant ($p < 0.01$) increase in erythropoietin concentration. The delta values during the HAMB (3.87 ± 3.45mU.mL^{-1}) and HBR (3.16 ± 2.86mU.mL^{-1}) interventions were significantly different to the NBR (-0.09 ± 0.75mU.mL^{-1}) intervention. Additionally, there was a significant increase in the red blood cell content ($p < 0.05$) on D10 in the HBR (delta = 0.7 ± 0.38) intervention compared to NBR (delta = 0.28 ± 0.23 10^{12}/L). RBC content did increase during the HAMB (delta = 0.59 ± 0.29 10^{12}/L) trial but was not significantly different compared to NBR ($p = 0.059$). There were no statistically significant differences

between the HBR and HAMB conditions. Please see Table 3.4 for absolute values of haematological parameters.

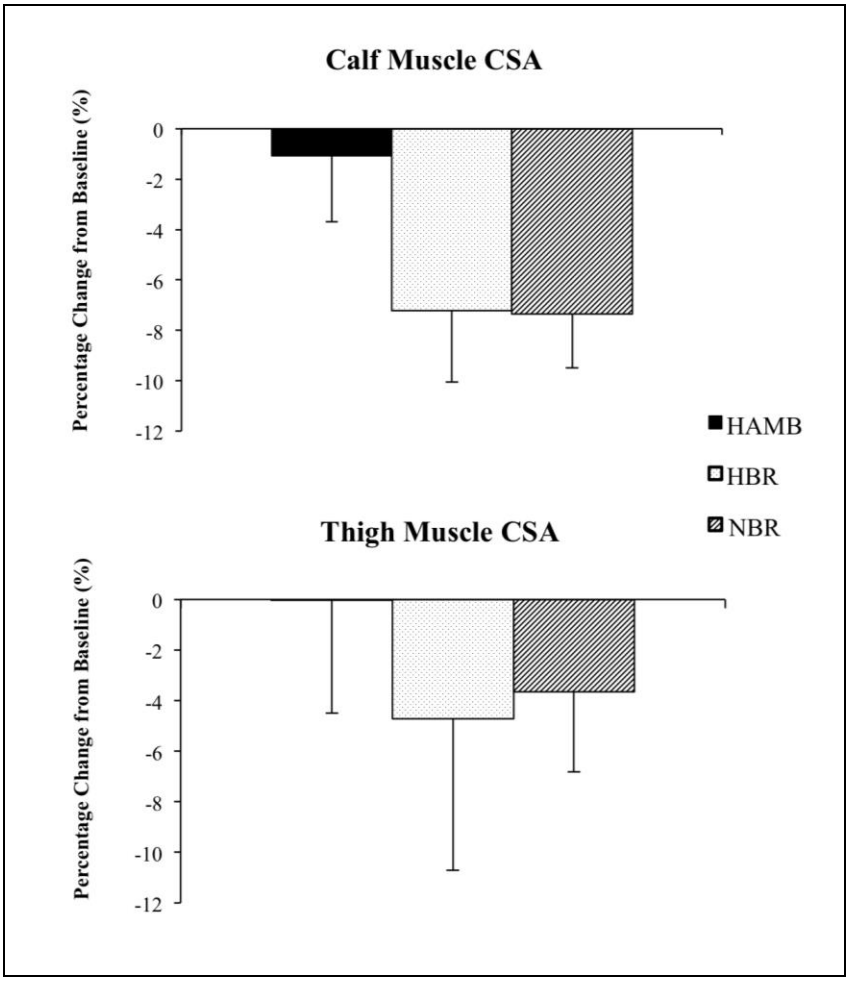


Figure 2.5: *Muscle CSA*. Muscle cross sectional area calculated from pQCT scans at 66% tibial length and 33% femoral length are presented for each of the 3 conditions (HBR: Hypoxic Bed Rest, grey; NBR: Normoxic Best Rest, grey stripe; HAMB; Hypoxic Ambulatory, black). Graphs represent a change in CSA from Pre to Post intervention. Data are presented as means ± standard deviation.

Table 2.4: *Haematology.* Absolute values for haematological parameters recorded before (Pre) and after (Post) each intervention (HAMB: Hypoxic Ambulatory; HBR: Hypoxic Bed Rest and NBR: Normoxic Bed Rest. Participants numbers for each parameter are: EPO = 9; RBC = 6; Haemoglobin = 6; Haematocrit = 6. Data are presented as mean (Avg) ± standard deviation (SD).

		Erythropoietin (mU/mL)		RBC (10^{-12}/L)		Haemoglobin (g/L)		Haematocrit (%)	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post
HAMB	Avg	5.61	11.35	5.06	5.70	155.43	173.67	0.46	0.51
	SD	1.77	4.75	0.32	0.51	11.07	12.01	0.03	0.04
HBR	Avg	7.55	10.64	5.05	5.72	154.64	173.86	0.46	0.51
	SD	2.42	3.82	0.43	0.46	11.86	6.47	0.03	0.03
NBR	Avg	7.49	7.34	5.03	5.24	154.27	160.14	0.45	0.47
	SD	1.75	2.32	0.32	0.32	7.31	8.07	0.02	0.02

2.14 Discussion

While space flight imposes a number of environmental and psychological stressors on astronauts (confinement, altered light / dark cycle, noise level, life support systems), gravity plays a significant role in any physiological alterations as a result of space flight. The bed rest analogue is the most frequently used ground-based experimental model to investigate the physiological changes associated with exposure to microgravity in a normobaric normoxic environment. The main finding from the present study is that the normoxic bed rest trial yielded the anticipated level of deconditioning and that hypoxia did not modify the process of deconditioning observed in NBR during such a short-term bed rest.

2.14.1 Basic core physiological data

The bed rest protocol was sufficient to induce reductions in muscular strength and volume in accordance with the literature. The participants complained of some muscular stiffness and soreness within the first few days of bed rest, however this was alleviated through passive physiotherapy and time within the condition.

2.14.2 Muscle MVC and CSA

Space flight and bed rest have been associated with the atrophy and deterioration of skeletal muscle (Edgerton and Roy, 1994). Muscle atrophy of the lower extremity and a deterioration in muscle function is a well-known consequence of space flight (Rittweger et al., 2007; Rittweger et al., 2005). Following spaceflight, bed rest and lower limb immobilisation, the lower limbs demonstrate significant functional and structural adaptations. These adaptations may be attributed to the muscular unloading caused by unloading / inactivity (Berg et al., 2007a). Furthermore, while in the current study we

measured solely muscular force, it is evident that mechanisms other than muscular atrophy, i.e. reduced neuronal drive and/or altered skeletal muscle contractile machinery are responsible for a large portion of the decrement in maximal voluntary force generation (Alkner and Tesch, 2004; Berg et al., 1997).

Atrophy of the weight bearing muscles leads to decreases in the CSA of approximately 2-3% per week during the first months of unloading, (Berg et al., 2007a). Cephalad fluid shift may play a role in the decrease in CSA within the first 2 to 5 days. Within just a few days of disuse, muscle atrophy sets in and after 7 days of bed rest a 3% decrease in thigh muscle volume is observable (Ferrando et al., 1995). Furthermore, it appears that the loss of contractile power and strength that occur with unloading / inactivity are consistently greater than the decreases noted in muscle size (de Boer et al., 2007).

The present findings of a loss in CSA (Thigh CSA; HBR: -4.7 ± 6%, NBR: -3.6 ± 3.2%; Calf: HBR: -7.2 ± 2.9%, NBR: -7.4 ± 2.1%) correspond well to previously reported data (Berg et al., 2007b; de Boer et al., 2007; Pavy-Le Traon et al., 2007a). While we observed no difference in the change in MVC of the hamstring, we did find that the MVC after the NBR trial was different to the HAMB for the quadriceps. However, 7% decrease found during the NBR is in line with the literature. Additionally, HBR was not different from NBR. The hamstring as opposed to the quadriceps is not a postural muscle which is likely a factor in the differences noted.

A 10 day bed rest study is considered to be a short term bed rest by ESA and therefore, while hypoxia does not appear to negatively effect muscular strength, it is indeed too short a period of time to conclude any significance that may be relevant to future studies of a longer duration.

2.14.3 Aerobic capacity

A reduction in maximal aerobic capacity has been documented following head down bed rest (Convertino, 1997; Greenleaf et al., 1989). Greenleaf et al., (1989) and Greenleaf, (1997) reported a reduction of up to 31 after 30 days of bed rest. Trappe et al., (2006) report a 10% decrease in VO_{2max} after 17 days space flight and 7% after 17 days bed rest (Lee et al., 2010). Convertino, (1997) reported during the first 30 days of bed rest, aerobic capacity decreases 0.8 to 0.9%/day. The current study found that while not significant, there was a decrease in the VO_{2max} and PPO by 7 and 8% in the HBR trial, respectively and 3 and 4% in the NBR trail, respectively. The decrease in performance is generally associated with hypovolemia and a reduced venous pressure and a reduction in cardiac output. Furthermore, a decrease in muscular strength and contractile power may also play a role in the reduced VO_{2max}. Figure 3.6 is a graph adapted from Capelli et al., (2006) highlighting the decrease in aerobic capacity as the time in bed rest increases. When are data are plotted against this graph, the results from the current study are very much in line with previously reported results. Additionally, hypoxia did not have an additive

degenerative effect on VO_{2max} or PPO compared to NBR.

The ability of an astronaut to complete physical activity of either a repetitive nature or long duration is quintessential to space flight. Particularly during emergency, the need to act quickly and with maximum effort may be imperative. Furthermore, the future demands placed on astronauts of explorative Planetary missions may include habitat constructions, the demands of which are currently unknown. The maintenance therefore of aerobic capacity is vital.

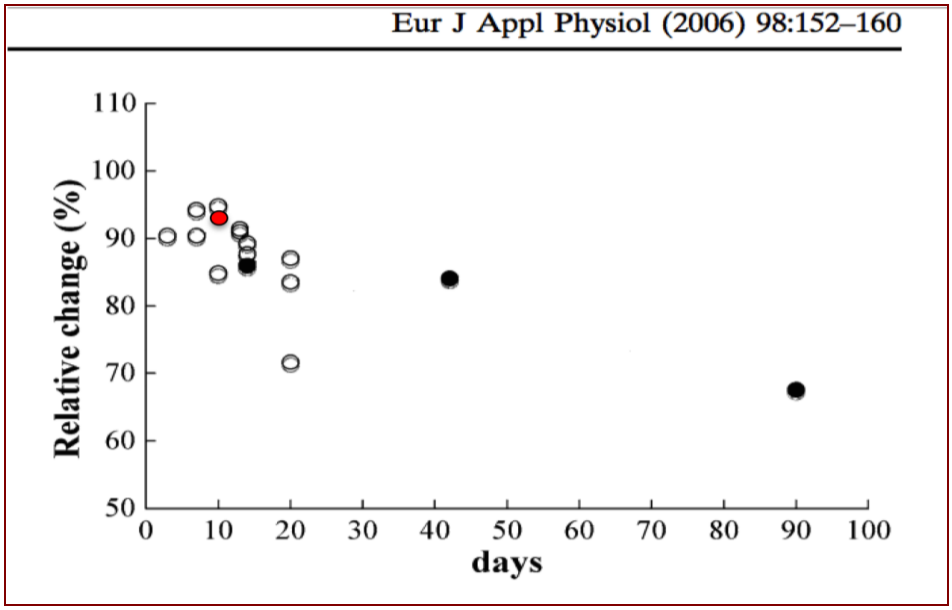


Figure 2.6: *VO_{2max} Bed Rest.* This figure is adapted from Capelli et al., (2006). The information presented represents the average decline in VO_{2max} from pre bed rest to post. Capelli et al., (2006) compiled the data from a number of studies. The red dot represents the decline in VO_{2max} measured in the current NBR study (7%).

Most studies to date have conducted bed rest studies without exercise to investigate the effect of inactivity and unloading. Exercise was introduced in bed rest studies to investigate the benefits of different countermeasures. The inactivity / unloading model of bed rest may mimic the inactivity/unloading experience by astronauts over 30 years ago. Currently, the astronauts have a daily programme of aerobic and resistive exercise, thus complete inactive bed rest does not reflect their current activity on the International Space Station. In fact, astronaut Suni Williams completed a marathon on board the ISS, while her colleague Karen Nyberg completed the Boston marathon in April 2006. Williams was an official entrant in the Boston marathon at the time. Additionally, astronaut Chris Hadfield anecdotally reported returning to earth in 2014 having gained muscle mass due to the volume of exercise he carried out during his stay on the ISS. Perhaps bed rest protocols and procedures should be updated now and in the future to include more activity to provide a bed rest analogue that conforms more to the current space flight exercise regimen. Additionally, the volume of exercise astronauts undertake in space is not well documented in a manner that allows good comparisons between astronauts and

bed rest participants. Therefore, it would be prudent to have one number that allows comparisons. For this purpose we chose to use the TRIMP score. Thus providing a single number that identifies the level or volume of exercise. However it is unclear whether a TRIMP value in the supine position is comparable to that in the upright or seated on a cycle ergometer.

2.14.4 Haematology

A shortcoming of the present study was that we did not measure blood volume. Thus, we can only speculate whether the increase in RBC content observed in HBR reflected a hypoxia-induced augmented reduction in plasma volume or was due to hypoxia-induced erythropoiesis or decreased erythrocyte lysis (West et al., 2012); or was caused by a combination of these mechanisms. Considering the relatively short duration of the present BR and the fact that neocytolysis has mainly been observed during long-term BR interventions (De Santo et al., 2005) it appears unlikely that neocytolysis played a major role in the HBR-induced increase in RBC. Additionally, the number of participants whose blood was analysed is small, N = 6 in some cases. The statistical power may be lacking in certain cases. However, where the numbers increased to 9 or 10, the statistical significance was detectable.

2.14.5 Hypoxia

The development and maintenance of the hypoxic level within the Planica facility worked well. The use of a stepwise protocol on the ascent to 4,000m while it may have provided protection from AMS for the participants, it did cause logistical issues, particularly related to generating and maintaining a hypoxic environment in the common areas. The design of the VPSA system provides a hypoxic gas mixture to the designating sleeping rooms only. As a result, the doors to each room must be kept open to allow the hypoxic gas to permeate to the common areas. Therefore, future studies confining large numbers of participants to the Planica facility should utilise an immediate ascent to altitude and avoid multiple days of several altitude settings.

2.14.6 Medical supervision of the participants

The participants provided vital feedback after each experimental campaign, which allowed us to developed more suitable guidelines for the staff and assistants to provide adequate care. Please see appendix 11.2 for details of nurse duties.

2.14.7 Nutrition

The nutrition and diet are vital to the success of human space travel and as such play an important role in ground based research. Thus, the nutritional information recorded during

the Lunar Habitat Simulation is given due consideration and the results are presented in Chapter 4.

2.14.8 Participant recruitment

The recruitment strategy proved to be very beneficial in terms of participant selection. The familiarisation weekend proved to be an extremely beneficial exercise. Participants were provided with presentations made by the researchers, outlining the study aims and detailing the protocol of each experiment that would be conducted. In addition, they were introduced to the hypoxic facility and the experimental laboratories at the Olympic Sport Centre, Planica. The questions raised generated lengthy discussions, which dispelled many of the major concerns the participants may have had. By conducting some of the inclusion tests at the Olympic Sport Centre, Planica, the participants were also acquainted with the logistics of conducting human experiments at the facility. During each of the tests, the responsible scientist guided participants through the procedure. Since most of the tests were also performed during the interventions, the time spent explaining the procedures during the familiarisation period was saved during the actual interventions. During the experimental interventions, the participants were already aware of the procedures and only needed reminding of the procedure. They were also exposed to a room set at the level of hypoxia the study would be conducted in. In this way, participants were able to go into the room for a time and experience what it would be like to live and work in that environment during the real testing.

The familiarisation weekend allowed the researchers to make a much better judgement regarding the suitability of the short-listed participants. Some participants, with excellent physical characteristics, were deemed to have an inappropriate attitude to participate in such a long-term, intensive study, whilst in contrast, other individuals, who may have been slightly below the threshold on an inclusion criteria, proved to be the spirit of the group. Certainly, the group dynamics observed during the familiarisation weekend proved to be quite insightful when establishing the final group of participants.

2.14.9 Scheduling of the studies

The schedule generally worked well, the research studies were all performed at the same or similar time of day Pre and Post intervention. The participants, as a result of the schedule had a good volume of free time, which allowed them to recover appropriately for the next experimental test. However, it is impossible to eliminate all confounding effects from all experiments as the volume of testing in any bed rest study is simply too great. It is important that this is taken into account when discussing individual experiments. For further details (Pavy-Le Traon et al., 2007b) provide a further examples of logistical considerations. It was found that the staff required more time in the mornings to weight all participants and carry out the required venipunctures. The schedule was therefore immediately updated to identify specific individuals and provide them with

priority over the others, for example, A participant who is required to attend an experiment at 07:30, needs time to eat breakfast, have daily cardiovascular parameters measured and blood drawn, they would have priority for these measures over another participants who was not due for experimentation until later in the day.

2.15 Conclusions

The BCD collected during the current study in the NBR trial compares well to the literature. Additionally, we found that hypoxia did not exacerbate the NBR condition and therefore no differences between NBR and HBR were found. The establishment of a hypoxic bed rest and confinement facility was successful and the logistics utilised in the current study are suitable to use in further hypoxic confinement or lunar habitat simulation studies. Of note, we found that the recruitment process is vital to obtaining dedicated participants. The inclusion/exclusion criteria should also require a section on the sensitivity to hypoxia and AMS. AMS has a high degree of variability within and between participants. Given the debilitating affects of AMS and the individual variation of symptoms, Astronauts should become acclimatised to hypoxic gradients even though the expected level of hypoxia in future planetary habitats will be lower than that used within the current thesis.

3 Whole body and Regional Body Composition Changes Following 10-day Hypoxic Confinement and Unloading / Inactivity

3.1 Introduction

Future missions to the Moon and Mars, although initially exploratory in nature, will undoubtedly assess the possibility of a permanent presence there. Proper nutrition and efficient dietary support are among the most critical features for maintaining health and wellbeing, as well as operational abilities of humans during space operations (Lane and Feeback, 2002; Smith and Zwart, 2008; Smith et al., 2005). To minimize the risk of decompression sickness and fire, it is anticipated that future lunar and Mars habitats will be hypobaric and hypoxic, with ambient pressure maintained at around 413mmHg and partial pressure of O_2 (P_IO_2) ranging from 100 to 115mmHg, corresponding to simulated altitude exposure ~ 3,400 to 3,800m (Bodkin et al., 2006). In addition to hypobaric hypoxia, inhabitants will be exposed to only a fraction of Earth's gravity; $1/6^{th}$ and $1/3^{th}$ Earth's gravity on the Moon and Mars, respectively. Due to the reduced gravitational force, the weight-bearing bones and muscles will be unloaded, and combined with the relative confinement during such missions; the astronauts will have a substantially reduced level of activity. It is therefore important to enhance our understanding of the combined effects of hypoxia and inactivity/unloading on nutritional status and body composition, so that appropriate nutritional strategies and recommendations for future explorations may be developed.

It is well documented that microgravity *per se*, as experienced during space flight, affects nutritional status and body composition of astronauts (Smith and Zwart, 2008; Smith et al., 2005; Zwart et al., 2009b). Terrestrial studies simulating the effects of microgravity in Space, by rendering participants inactive and unloading their weight-bearing limbs using the bed rest experimental model, have observed similar changes in body composition and nutritional status (Smith and Zwart, 2008; Smith et al., 2005). Hormonal status alterations (Blanc et al., 2000) along with changes in appetite and energy intake (Blanc et al., 1998) are among the main observations. Significant decreases in the whole body mass as well as changes in the regional body composition, mostly within the lower limbs, have been reported previously (Blanc et al., 1998; Inniss et al., 2009; Krebs et al., 1990; LeBlanc et al., 1992). As nutritional responses to weightlessness and bed rest have been shown to be qualitatively similar (Zwart et al., 2009b), the application of the bed rest model seems suitable for investigating the effects of microgravity on nutritional status, dietary intake and body composition (Smith et al., 2001).

Environmental hypoxia also alters metabolism, and consequently modulates body mass and composition (Boyer and Blume, 1984). Following long-term hypoxic exposures of different durations, body mass is decreased (Quintero et al., 2010) secondary to reductions in fat and muscle mass (Kayser et al., 1993; Westerterp et al., 1994; Westerterp-Plantenga et al., 1999). However, the underlying mechanisms of hypoxia-induced reductions in body weight are not completely understood. Appetite reduction (i.e. altitude anorexia), secondary to hypoxia-induced increases in satiety-signalling peptides leptin (Tschop and Morrison, 2001) and cholecystokinin (Lieverse et al., 1994) along with accompanying hunger-stimulating hormone ghrelin reduction (Shukla et al., 2005) seems to be one of the key underlying mechanisms. Additionally, altitude-related changes in gastro-intestinal function (Dinmore et al., 1994) and increased resting energy expenditure (Reynolds et al., 1999) might also play an important role in the aetiology of weight loss at altitude.

Although it is known that individually microgravity and hypoxia significantly alter nutritional status and body composition (Boyer and Blume, 1984; Zwart et al., 2009b), there is a paucity of data regarding their combined effect (Loeppky et al., 1993a; Stevens, 1966). The planned environmental conditions in future planetary habitats merit further investigations, with particular emphasis on the evaluation of the current guidelines regarding energy and nutrient intake levels.

We also reasoned that in addition to providing further evidence regarding the effects of hypoxia and inactivity on nutritional status and energy requirements of astronauts in planetary habitats, the experimental protocol, exposing healthy young human participants to hypoxic bed rest, might provide some insight into the changes observed in patient populations rendered hypoxic and inactive by their clinical pathologies, for instance, patients suffering from chronic obstructive pulmonary disease, peripheral vascular diseases and/or obesity.

In order to examine the separate and combined effects of hypoxia and inactivity / unloading we conducted a repeated measures cross-over design study, in which on separate occasions participants were exposed in a randomised order to 10 days of normoxic bed rest (NBR), hypoxic bed rest (HBR) and hypoxia with ambulation (HAMB). A comparison of the results of the NBR and HBR trials would reveal the effect of hypoxia on inactive participants, whereas the comparison of the results from the HBR and HAMB would provide information regarding the effect of activity on participants exposed to hypoxia. In this manner, we specifically examined the combined and separate effects of a 10-day continuous simulated microgravity (horizontal bed rest) and normobaric hypoxia (simulated altitude of ~ 4,000m) on regional and whole body mass and composition. Secondly, we assessed whether the targeted energy intake levels and the selected macronutrient composition used in this study would be sufficient to maintain stable body mass and composition during such exposures. We hypothesized that the addition of hypoxic exposure to bed rest (HBR) would either induce greater reduction or

smaller increase in whole body mass compared to bed rest only (NBR).

3.2 Materials and methods

Details regarding the basic participant recruitment and their physical characteristics are provided in chapter 3. The participants' information is presented in Table 3.2. Furthermore, please refer to chapter 3 for the detailed outline of the bed rest protocol.

Testing periods were performed during four days before (Pre) and four days after (Post) each intervention. The participants were placed on a prescribed diet throughout their accommodation at the Olympic Sport Centre, Planica including during the Pre and Post testing periods. The participants were always accommodated in rooms with two single beds (i.e. two participants per room).

3.2.1 Diet planning and monitoring

The menus were constructed using the web-based application Open Platform for Clinical Nutrition (OPKP; www.opkp.si), developed by a research group at the Jozef Stefan Institute (JSI, Ljubljana, Slovenia) (Korošec et al., 2013). A 10-day menu was prepared before the start of the study, and repeated during each campaign to ensure that the participants consumed identical food on each given study day. The individual daily targeted energy intakes were calculated using the Mifflin equation (Mifflin et al., 1990) and multiplied by Physical Activity levels (PAL) 1.4 for the HAMB and PAL 1.2 for the HBR and NBR campaigns to account for different activity levels. All five daily meals, (breakfast, morning snack, lunch, afternoon snack and dinner (Appendix 12.10)), were prepared by the kitchen staff at the Olympic Sport Centre, Planica, where the components of each meal were weighed to an accuracy ± 1g. The meals were served at the same time of the day throughout the confinement period. The participants were instructed to consume all the food provided. In case of any leftovers, the unconsumed food was weighed and the measured amount deducted from the initial weight, thus providing a net value of the participants' actual food intake. The energy, macronutrient and fluid intake analysis was performed subsequently using the OPKP for each individual participant.

Fluid intake was individually monitored and recorded throughout the study. The calculated total daily fluid intake comprised of the daily fluid intake and the daily food-water content. To assess the urinary output and fluid balance; the 24-hrs urine was collected on Day 1, Day 4 and Day 10. On each of those days, the urine collection started in the morning and was performed for the next 24-hrs. Urine was collected and pooled throughout the 24-hrs period in plastic collection containers (3-Liter). Fluid balance was calculated by deducting 24-hrs daily urine output from total daily water intake. During the three interventions, participants were not allowed to drink any beverages containing alcohol and were not supplemented for vitamins and minerals.

3.2.2 Regional and whole body composition

Body mass and composition was determined before (Pre) and immediately after (Post) each campaign with dual-energy X-ray absorptiometry (DEXA) using a fan-beam densitometer (Discovery W - QDR series, Hologic, Bedford USA). The DEXA technique relies on body tissue absorption measurements made at two photon energies to allow for quantification of lean, fat and bone mineral masses. Excellent precision of DEXA for in vivo body composition measurements has been demonstrated previously (Hind et al., 2011). The participants were instructed to lie supine on the DEXA table for the duration of the scanning procedure. The hands were positioned on the table separately from the trunk while the legs were separated using a standard holder. An experienced researcher performed the analysis of the obtained scans using Hologic APEX System Software (version 3.1.2). The software enables whole-body, as well as standard regional analysis, such as the lower and upper leg, gynoid and android regions that are automatically defined by specific anatomical landmarks. The regions of interest (ROI) were, whenever necessary, individually adjusted during the analysis process by the same researcher to precisely reflect the targeted areas. The ROI's used for the regional analysis were (Figure 4.1):

- Upper arm: Elbow-joint center (lower boundary) - Gleno-humeral joint center (upper boundary)
- Thigh: Knee-joint center (lower boundary) - Acetabulo-femoral joint center (upper boundary)
- Lower leg: Ankle-joint center (lower boundary) – Knee-joint center (upper boundary)
- Abdomen (Android): Pelvis cut (lower boundary) - Lumbar vertebrae (upper boundary)
- Hip (Gynoid): Middle of femur (50%) (lower boundary) - Acetabulo-femoral joint center (upper boundary)

3.2.3 Statistical analysis

One-way ANOVA was used to compare the average HR and S_aO_2 responses and LLMS between the three experimental campaigns. Differences in participants' body mass and composition before and after the campaigns as well as changes in energy and fluid intake, urine output and fluid balance were analysed using one-way repeated measures ANOVA (campaign × time). Tukey HSD post hoc test was used to define the specific differences when ANOVA analysis revealed a significant F-ratio for the main effect. Pearson's correlation analysis was used to define the possible relationships between the initial whole body mass, fat mass and whole body mass/fat mass ratio values and subsequent changes in these variables. The level of significance was set a priori at 0.05. Data are reported as means and standard deviations unless otherwise indicated. All statistical analyses were performed using Statistica 5.0 package (StatSoft, Tulsa, USA).

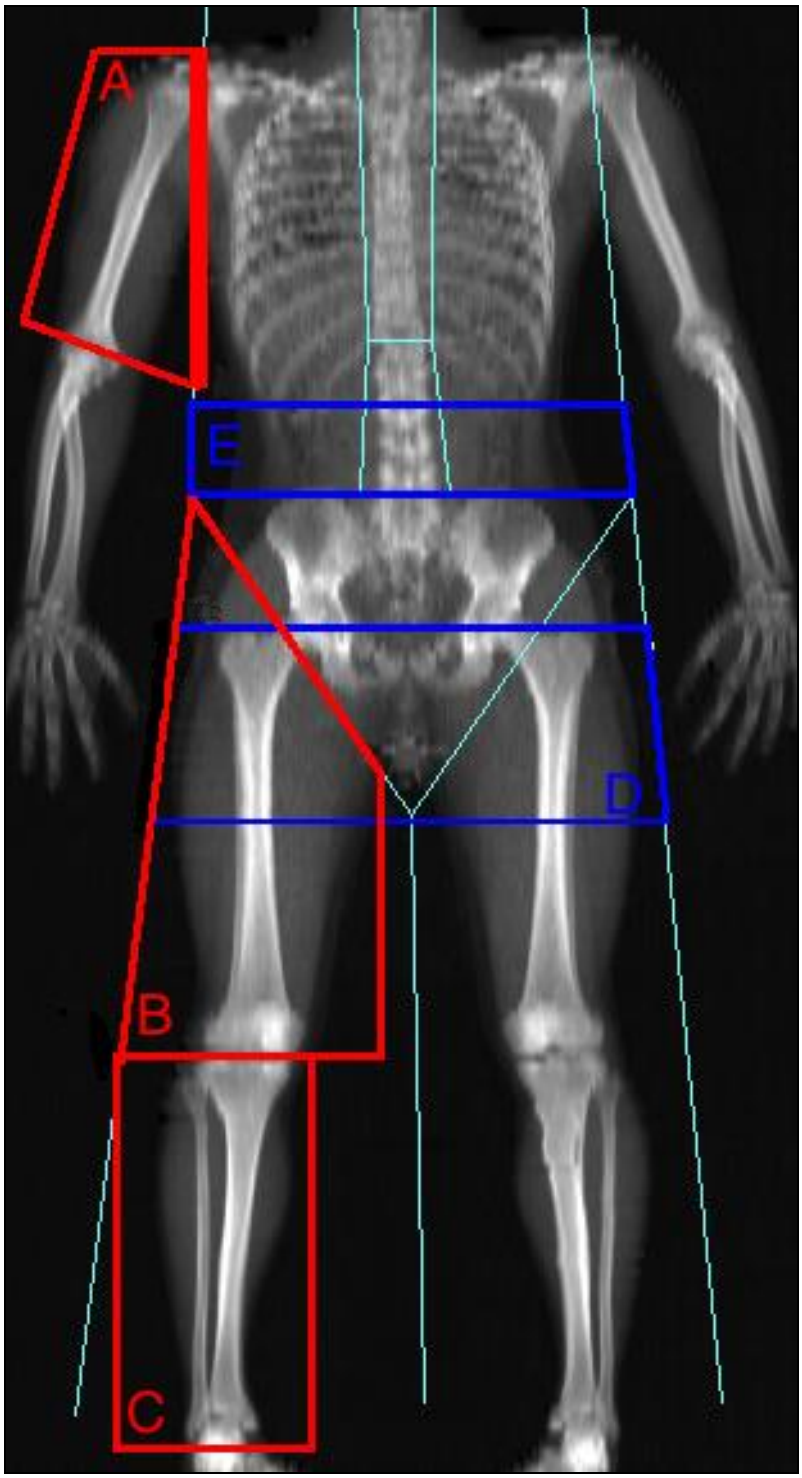


Figure 3.1: *DEXA ROI.* Regional areas of interest used for DXA body composition analysis (A - upper arm; B – thigh; C – lower leg; D – gynoid; E – android).

3.3 Results

3.3.1 General hypoxia and bed rest adaptation

The participants successfully completed all three campaigns in good health. The average LLMS scores were significantly higher ($p < 0.01$) during the HAMB and HBR trials: $LLMS_{HAMB} = 0.7 \pm 0.3$ and $LLMS_{HBR} = 1.0 \pm 0.4$ than during the NBR trial: $LLMS_{NBR} = 0.3 \pm 0.2$. During HAMB, but not HBR, the average LLMS significantly decreased ($p < 0.05$) ($LLMS_{HAMB} = 0.6 \pm 0.1$, $LLMS_{HBR} = 0.9 \pm 0.4$) during the last five days of the confinement compared to the first five ($LLMS_{HAMB} = 0.9 \pm 0.3$, $LLMS_{HBR} = 1.2 \pm 0.4$. Average HR was significantly higher ($p < 0.01$) during the HAMB campaign (80 ± 10bpm) than during NBR (68 ± 7bpm). No significant differences in HR responses were noted between the two hypoxic campaigns (HBR: 75 ± 6bpm). The average S_aO_2 values across the 10-day period were significantly lower ($p < 0.01$) during both HAMB and HBR campaigns than during the NBR campaign (values of S_aO_2 were 91.2 ± 1.2%, 91.6 ± 1.5% and 96.5 ± 1.1%, respectively).

Table 3.1: *Energy Intake.* Average daily (D1 to D10) energy intake (kcal per) and % of the daily targeted intake achieved (upper panel) and fluid intake (ml per day; lower panel) during normobaric hypoxic bed rest (HBR), normoxic bed rest (NBR) and normobaric hypoxic confinement (HAMB) campaigns. Data are presented as means (standard deviation).

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
					Energy Intake					
HBR	1957 (346) 94%	2349 (425)* 113%	2246 (212) 108%	2061 (333) 100%	2225 (276) 107%	2264 (396) 109%	2386 (350)* 115%	2211 (428) 106%	1967 (257) 95%	1968 (352) 95%
NBR	1885 (316) 91%	2286 (297) 110%	2172 (225) 105%	2356 (288)* 113%	2147 (506) 103%	2097 (313) 101%	2001 (247) 97%	2045 (469) 99%	2099 (364) 101%	1930 (582) 93%
HAMB	2041 (470)* 84%	2300 (420) 95%	2350 (344) 97%	2275 (387) 94%	2252 (382) 93%	2341 (346) 97%	2368 (348) 98%	2112 (403)* 87%	2488 (405) 103%	2197 (351)* 90%
					Fluid intake					
HBR	3214 (789)	3458 (846)	3364 (747)	3314 (753)	3287 (754)	3347 (612)	3400 (918)	3564 (563)	2931 (500)	3214 (789)
NBR	3515 (697)	3197 (513)	3060 (472)	3340 (746)	3815 (978)	3848 (433)	3487 (690)	3404 (697)	3301 (699)	3490 (692)
HAMB	3360 (887)	3379 (989)	3675 (925)	3879 (856)	3465 (967)	3606 (987)	3565 (845)	3306 (798)	3437 (911)	3583 (654)

3.3.2 Energy and nutrient intake

The average pre-calculated targeted daily energy intakes were 2071 ± 170kcal for HBR and NBR campaigns and 2417 ± 200kcal for HAMB campaign, respectively. The actual daily energy intake levels for all campaigns are presented in Table 3.1. In both the HBR and NBR campaigns the average energy intake was within the targeted level (2110 ± 372 and 2070 ± 356kcal, respectively). However, in the HAMB campaign the participants' average energy intake was lower than targeted (2245 ± 400kcal; $p < 0.05$). As can be seen in Table 3.1 the daily intake tended to be lower during the first and the last days of each campaign. Table 3.2 shows absolute and relative quantities of carbohydrate, fat and protein daily intake of the participants during the three campaigns.

3.3.3 Whole body mass and composition

As noted in Figure 3.2, body mass significantly decreased ($p < 0.05$) in all three campaigns by an average of 1.5 ± 0.9, 2.0 ± 1.8 and 1.4 ± 1.3kg after the HAMB, HBR and NBR trials, respectively. These changes were accompanied by significant decreases ($p < 0.05$) in lean mass of 2.1 ± 1.6, 2.1 ± 2.4 and 2.4 ± 2.1kg, and a slight, albeit not significant increase ($p > 0.1$), in total fat mass of 0.7 ± 1.2, 0.1 ± 1.6 and 1.0 ± 2.2kg after the HAMB, HBR and NBR, respectively (Figure 3.2 B, C, D). No significant correlation was noted between initial values of whole body mass, fat mass and whole body/fat mas ratio and subsequent changes in these variables following HAMB (0.11, 0.32, 0.13 ($p > 0.1$)), HBR (0.22, 0.51, 0.29 ($p > 0.08$)) and NBR (0.39, 0.31, 0.35 ($p > 0.1$)), respectively.

Table 3.2: *Macronutrients.* Daily average macronutrient intakes during normobaric hypoxic bed rest (HBR), normoxic bed rest (NBR) and normobaric hypoxic confinement (HAMB) campaigns. Data are presented as means ± standard deviation (% diet composition).

Campaign	Macronutrients		
	Carbohydrate g (%)	Fat g (%)	Protein g (%)
HBR	282 ± 65 (64)	71 ± 21 (16)	86 ± 20 (20)
NBR	277 ± 64 (65)	68 ± 21 (16)	83 ± 17 (19)
HAMB	299 ± 68 (64)	74 ± 23 (16)	92 ± 22 (20)

3.3.4 Fluid balance

Daily fluid intakes, calculated as consumed fluid plus food water content, are presented in Table 1. No significant differences were observed between the three campaigns in fluid intakes. The urine output was significantly lower ($p < 0.05$) on day 4 during HBR and on day 10 during both HAMB and HBR campaigns (Figure 3.3). As shown in Figure 3.3, no significant differences were observed in the urine output during the NBR campaign as

well as in the fluid balance during all three campaigns.

Table 3.3: *ROI composition.* Regional body composition before (PRE) and after (POST) the normobaric hypoxic bed rest (HBR), normoxic bed rest (NBR) and normobaric hypoxic confinement (HAMB) campaigns. Data are presented as means ± (standard deviation).

	HBR		NBR		HAMB	
	PRE	POST	PRE	POST	PRE	POST
Upper arm						
Lean mass (kg)	2.01 (0.37)	1.95 (0.32)	2.02 (0.36)	1.99 (0.33)	1.99 (0.23)	1.94 (0.28)
Fat mass (kg)	0.56 (0.24)	0.55 (0.22)	0.55 (0.24)	0.57 (0.25)	0.53 (0.23)	0.57 (0.24)
Fat mass (%)	21.2 (5.3)	21.5 (5.5)	20.8 (6.0)	21.6 (5.9)	20.6 (6.2)	22.0 (6.5)
Thigh						
Lean mass (kg)	6.15 (0.83)	5.90 (0.99)	6.44 (1.16)	6.17 (1.04)	6.30 (0.81)	6.11 (0.83)
Fat mass (kg)	2.03 (0.91)	2.07 (0.78)	2.07 (0.81)	2.10 (0.92)	1.98 (0.81)	2.15 (0.88)
Fat mass (%)	23.9 (6.2)	25.2 (5.0)	23.7 (5.3)	24.5 (5.2)	23.2 (5.1)	25.2 (6.3)
Lower leg						
Lean mass (kg)	2.34 (0.27)	2.23 (0.37)	2.41 (0.41)	2.32 (0.33)	2.37 (0.30)	2.31 (0.34)
Fat mass (kg)	0.71 (0.30)	0.72 (0.25)	0.69 (0.27)	0.69 (0.25)	0.68 (0.32)	0.75 (0.29)
Fat mass (%)	22.6 (7.0)	23.5 (6.4)	21.7 (5.7)	22.3 (5.8)	21.5 (7.7)	24.0 (7.5)
Abdomen (Android)						
Fat mass (kg)	1.18 (0.60)	1.21 (0.59)	1.19 (0.58)	1.22 (0.66)	1.20 (0.57)	1.23 (0.58)
Fat mass (%)	22.7 (6.6)	24.7 (7.0)	22.8 (6.4)	24.1 (8.1)	23.4 (6.5)	24.7 (6.7)
Hip (Gynoid)						
Fat mass (kg)	3.08 (1.21)	3.13 (1.10)	3.12 (1.15)	3.14 (1.27)	3.07 (1.19)	3.20 (1.19)
Fat mass (%)	25.2 (5.8)	26.6 (4.9)	25.2 (5.0)	25.7 (6.2)	25.1 (5.8)	26.6 (6.3)

3.3.5 Regional body composition changes

As shown in Table 3.3, no significant inter-condition differences in the composition of the investigated regions were noted before, or after each campaign, or between the campaigns. During the course of each intervention, a trend, though not significant, of a decrease in lean mass, and an increase in fat mass was noted in all regions ($p > 0.15$).

3.4 Discussion

The principal finding of the present study is that the targeted energy and nutrient intakes during the bed rest campaigns were insufficient to prevent a significant decrease in body mass. Furthermore, during the hypoxic ambulatory campaign the participants' intakes were lower than targeted. The observed decrease in lean body mass in all campaigns would unlikely be prevented by an increase in energy intakes. Especially, since the fat mass levels showed a tendency for an increase in both HAMB and NBR campaigns, reflecting a positive energy balance. Altering the macronutrient composition for future studies seems warranted to concomitantly reduce the observed lean mass reductions and minimize the fat accumulation, especially during longer exposures.

3.4.1 Hypoxia, bed rest and ambulation

Based on the results of the NBR and HBR interventions and contrary to our initial hypothesis, no specific effect of hypoxia *per se*, could be identified, since the reductions in body mass were similar (Figure 3.2). Furthermore, as no significant differences between the observed reductions in whole body and regional lean body mass were noted between the HBR and HAMB conditions, we conclude that there was no specific effect of unloading/inactivity, particularly on the postural segments. One possible explanation for the lack of differences in body mass between both, HBR and HAMB campaigns might be the relatively short duration of the experimental campaigns (Pavy-Le Traon et al., 2007b). Indeed, studies investigating bed rest exposures of longer duration have consistently reported substantial decreases in body mass, mostly as a result of decreases in lower limb lean mass (Blanc et al., 1998; LeBlanc et al., 1992). On the other hand, data from shorter bed rest studies is more inconclusive, the majority of which report no significant changes in regional as well as whole body composition (Blanc et al., 2000; Kanikowska et al., 2010). Even though significant decreases in muscle mass in response to unloading can be expected within 10-14 days (Trappe, 2009) of bed rest, the obtained regional composition data does not indicate any specific regional effect (Table 3.2). The body composition data from the present study concur with the findings of Krebs et al., (1990), who observed significant decreases in lean mass and increases in fat mass following 5 weeks of bed rest. The slightly higher fat mass accumulation in their study could be explained by the greater average daily energy intakes ($\geq$ 2500 kcal) and longer bed rest duration.

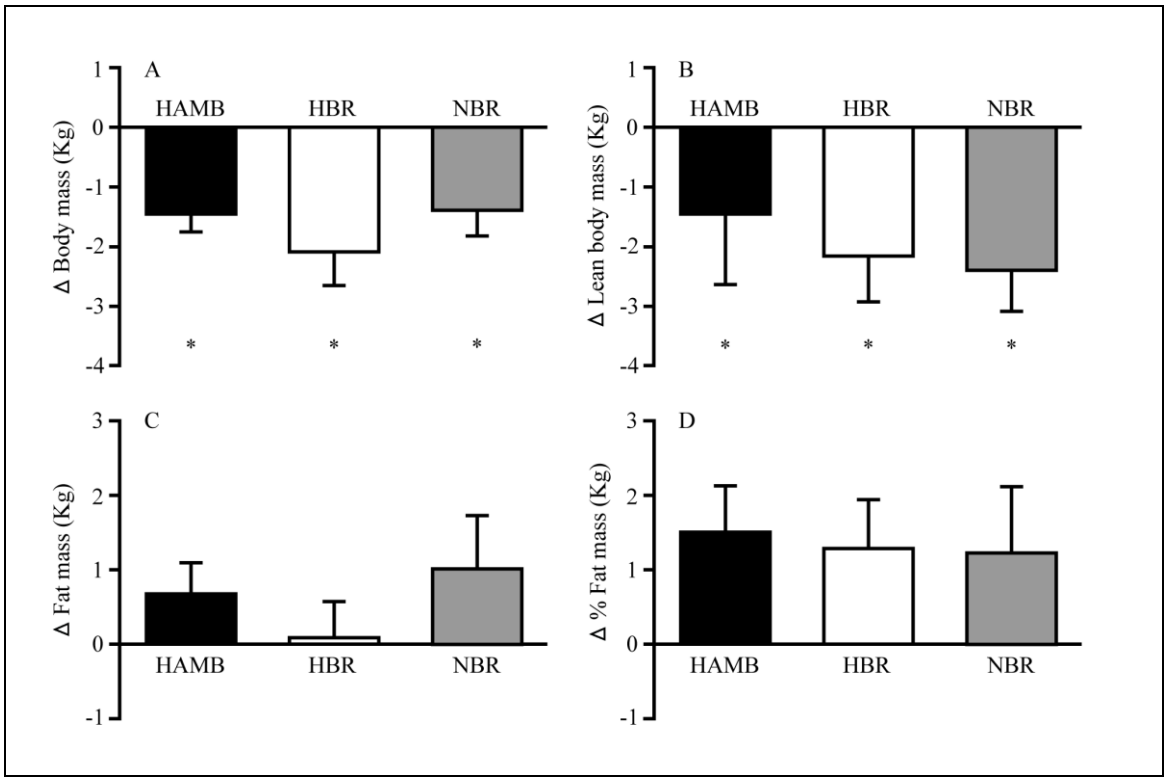


Figure 3.2: *Body Composition.* Changes in Total body mass (A; Upper panel - left), Lean Body mass (B; Upper panel - right), Fat mass (C; Bottom panel - left) and percentage Fat mass (D; Bottom panel - right) following the Hypoxic bed rest (HBR), Normoxic bed rest (NBR) and Hypoxic ambulatory confinement (HAMB) campaigns. Data are presented as means (SEM). * Denotes statistically significant differences from Pre ($P < 0.05$).

Significant weight loss following altitude exposures appears to be a consistent finding (Boyer and Blume, 1984; Quintero et al., 2010). Hypoxia has been shown to significantly affect appetite through modulation of appetite-regulating hormones (Lieverse et al., 1994; Shukla et al., 2005; Tschop and Morrison, 2001). However, we did not observe any significant additional effect of hypoxia *per se* on decreases in body mass during the HBR and HAMB as compared to NBR campaigns. This is especially interesting since a recent study, although in obese individuals, identified hypoxia as an independent stimulus for weight reduction (Lippl et al., 2010). In addition, our data provide further support to the notion that the decrease in lean mass and not fat mass is accountable for the observed decreases in total body mass following hypoxic exposures (Westerterp et al., 1994; Westerterp-Plantenga et al., 1999). Interestingly, the participants' energy intake was only significantly lower than targeted during the HAMB campaign and this could at least partly explain the weight loss, secondary to reduction in lean mass observed during the HAMB. However, other hypoxic exposure-related factors might have also contributed to the observed body mass reduction, such as increased resting energy expenditure (Reynolds et al., 1999).

As noted earlier, investigations regarding the combined effects of microgravity and hypoxia are scarce. To date, only one long-term study has investigated the combined

effects of hypoxia and inactivity/unloading on different physiological systems (Stevens et al., 1966). They observed a significant (1.5 - 2kg) decrease in participants' body mass following 4 weeks of combined hypoxia and bed rest, though the bed rest protocol was not as strict as in the present study. Although regional changes and energy intakes were not reported, the participants in the hypoxic condition were reportedly mildly anorectic and needed encouragement to complete their meals (Stevens, 1966). Thus confirming, that hypoxia may have affected their appetite. Conversely, Loeppky et al., (1993a), who investigated the effects of HBR, found no significant changes in body mass after a 7-day protocol. There was a significant decrease in body mass on day one of bed rest, most probably due to fluid shifts, but the participants regained their body mass thereafter. The fact that the participants were allowed to eat the food ad libitum, could explain the different outcomes although the average energy intakes were comparable (2000 – 2100kcal per day) to the intakes of the participants during the HBR campaign in our study.

3.4.2 Energy intake and macronutrient diet composition

To date, nutritional studies conducted during bed rest protocols have regulated the energy intake, in order to prevent changes in body mass and body composition. It is assumed that allowing ad libitum energy intake would cause an increase in body mass, primarily due to gains in fat mass outweighing the loss in lean mass as has already been shown (Gretebeck et al., 1995). Therefore, studies employ PAL for dietary intake calculations during the ambulatory and bed rest studies, with the PAL usually ranging between 1.4 to 1.6 for the ambulatory and 1.1 to 1.3 for bed rest conditions (Lane and Feeback, 2002; Smith et al., 2001; Zwart et al., 2009a). The PAL factors used in the present study (1.4 & 1.2) are hence, at the lower part of the spectrum. Indeed, other recent studies investigating the effects of microgravity and/or weightlessness on body composition changes have employed higher activity factors; 1.6 and 1.3 for ambulatory and bed rest conditions, respectively (Inniss et al., 2009; Zwart et al., 2009a). These studies also showed that body mass was, at least during the first 10 days of the studies, maintained when using higher targeted energy intake values under normoxic bed rest conditions. It is also worth noting, that the PAL levels used in this study (1.4 & 1.2) are below the estimated threshold (~ 1.8) that enables weight control in healthy males through physiological appetite modulation (Schoeller, 1998). It was suggested that no or very little reduction in energy intake is noted with transition from normal to reduced levels of physical activity (PAL $\leq$ 1.7), consequently leading to a positive energy balance (Melzer et al., 2005). We can thus hypothesize that the previously reported hypoxia-induced appetite reduction (Tschop and Morrison, 2001) might have been masked in our study by insufficient physical activity/energy demand. Collectively, the insufficient energy intakes could not account for the observed decreases in whole-body mass, since the fat mass did tend to increase in all campaigns. One of the factors that could have influenced the similar outcomes in all three campaigns might be the diet macronutrient composition.

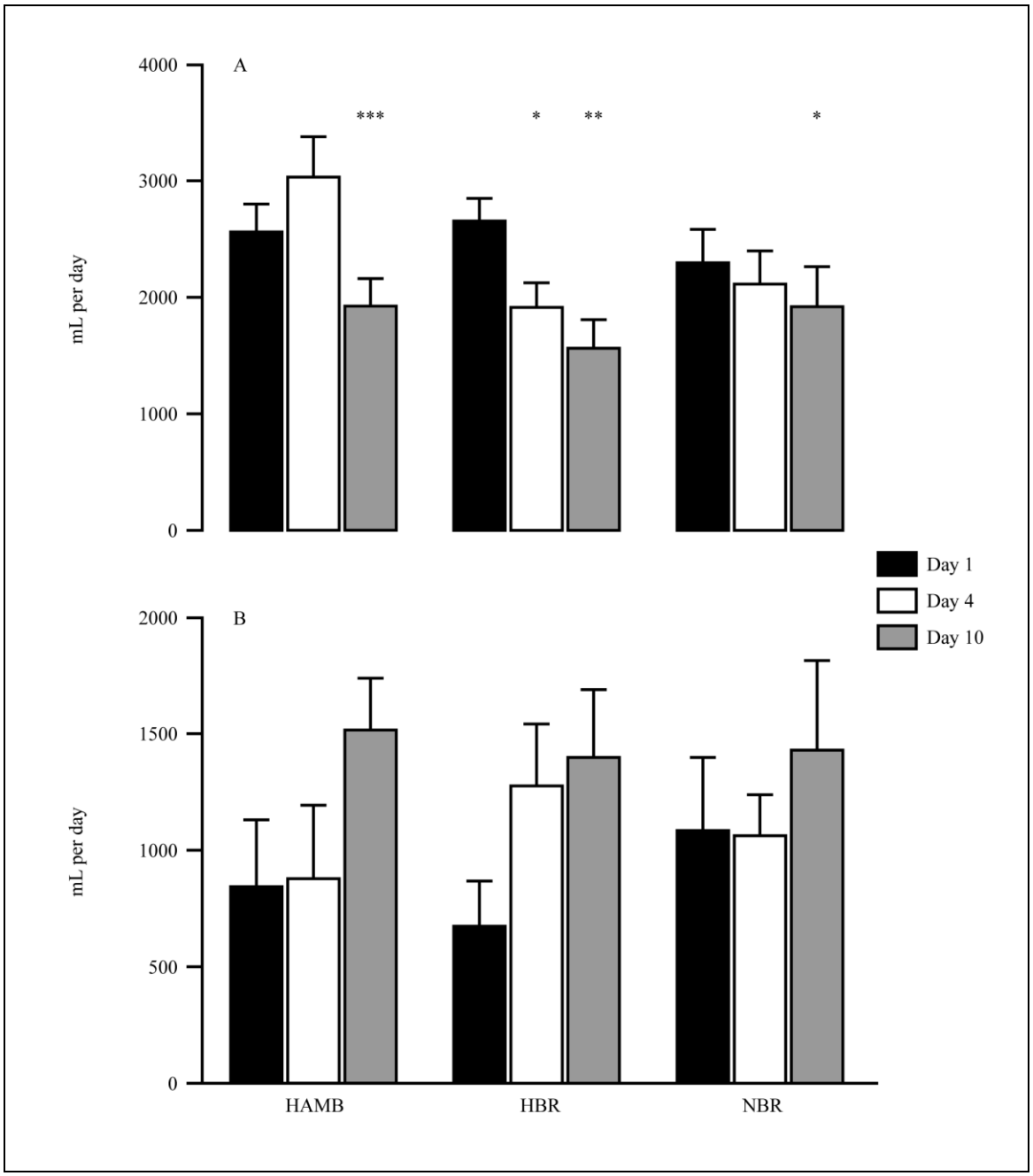


Figure 3.3: *Fluid Balance*. Whole day urine output (A; upper panel) and water balance (B; bottom panel; calculated as the urinary output deducted from the daily fluid intake) during Day 1, Day 4 and Day 10 of the Hypoxic bed rest (HBR), Normoxic bed rest (□□□) and Hypoxic ambulatory confinement (HAMB) campaigns. Data are presented as means (SEM). * Denotes statistically significant differences compared to HAMB ($P < 0.05$); ** Denotes statistically significant differences compared to Day 1 ($P < 0.05$); *** Denotes statistically significant differences compared to Day 4 ($P < 0.01$).

The macronutrient diet composition differs substantially between studies investigating microgravity effects. However, the actual macronutrient diet composition in the present study (Table 2) was higher in carbohydrate (~ 65%) and protein (~ 20%) levels and correspondingly lower in fat (~ 16%) intake as compared to the diet compositions used in

other recent studies (~ 55/30/15 for the % carbohydrate, fat and protein, respectively) investigating the effects of weightlessness and/or simulated microgravity on nutritional status (Inniss et al., 2009; Smith et al., 2001; Smith et al., 2005; Zwart et al., 2009a). It can be speculated that the low fat intake levels prevented significant accumulation of fat mass in our participants. Conversely, relatively high intake levels of protein, suggested to prevent the otherwise reported unloading-related reductions in protein synthesis (Stuart et al., 1990), did not prevent a significant decrease in lean body mass during any of the experimental campaigns. It has also been suggested that calorie reduction *per se* can enhance lean mass catabolism (Biolo et al., 2007) and subsequently reduces body mass. However, the observed reductions in body mass cannot be explained by the possible low energy intake levels, as fat mass was maintained in all three campaigns (Figure 3.2).

3.4.3 Water balance

Fluid balance alterations in response to simulated microgravity/weightlessness have already been extensively studied and it seems that following the initial renal/endocrine adaptations, positive water balance is typically maintained (Drummer et al., 2001; Leach and Rambaut, 1975). However, only one study to date (Loeppky et al., 1993b) investigated the combined effects of hypoxia and simulated microgravity on body-fluid alterations and reported significantly greater changes in fluid balance during short-term hypoxic bed rest when compared to normoxic bed rest. Similarly to the present study, the participants' fluid intake did not change during the 7-day investigation, whereas the urinary output was significantly higher during the latter stage of the experiment in the HBR group only (Loeppky et al., 1993b). Conversely, in our study, the urinary output was significantly decreased during the end of both hypoxic campaigns (HAMB & HBR) (Figure 3.3). Thus, exposure to normobaric hypoxia induced a significant decrease in urinary output regardless of the participants being bedridden or ambulatory.

It is well documented that during prolonged exposure to hypoxia/altitude, the initial augmented diuresis is followed by reduced urinary output (Hildebrandt et al., 2000). Such a time sequence may in part explain the drop in urine production towards the end of the present hypoxia exposures. However, urine production dropped also during the course of the NBR. Thus the drop in urine production between D1 and D10 in the HBR and NBR conditions probably also reflected a transient initial increase in urinary flow induced by the redistribution of blood volume to the intrathoracic space upon the change in posture from upright to recumbent (Guell et al., 1982). Regardless of the underlying mechanisms, the reduction of urinary flow during the course of each campaign resulted in a tendency (albeit not significant) for an inverse increase in water balance towards the end of each campaign (Figure 4.3). It cannot be excluded that these changes in hydration could have affected the Post DEXA scan results, since hydration and regional fluid distribution have been shown to affect both fat and lean tissue energy attenuation on which the DEXA method is based (Pietrobelli et al., 1996). However, since the changes in water balance were comparable across all three campaigns (slight increase towards the end (Figure 4.3)), this factor could not significantly affect the reported relative body composition

changes.

3.4.4 Methodological considerations

The potential effects of confinement *per se* also have to be noted as confinement has also been shown to significantly affect body weight and hormonal status (Custaud et al., 2004). Thus, this might have also played a role in the present study, contributing to the similar changes observed following all three conditions. In particular, since the participants were confined for 10 days in a rather small area during all experimental campaigns. Although the majority of studies to date investigating the effects of confinement have focused on the effects of long-term (> 100 days) confinement on stress responses (Chouker et al., 2002) it has also been demonstrated that even shorter confinements (i.e. 10 days) result in altered immune and psychological responses (Shimamiya et al., 2004). However, as noted in (Chouker et al., 2002) the duration of the confinement seems to largely determine the subsequent outcome and shorter confinement periods (i.e. 10 days) do not likely result in significant changes in body weight and composition.

We also need to mention the limitations of the present study. Obviously, the duration of the campaign (10 days) was relatively short and longer exposures (as anticipated during actual space habitation) might make it possible to elucidate potential additive effects of hypoxia that might have been masked in our study due to insufficient exposure time. Second, the feeding strategy used in the present study (i.e. prescribed intake levels) significantly contributed to the outcome and different results could be anticipated if the participants would have ad libitum access to food and multiple food choices. Finally, additional measures of appetite-regulating hormones, assessment of appetite/satiety sensations and resting energy expenditure measurements before, during and after the campaigns would have provided valuable additional information.

3.5 Conclusion

We show that significant decreases in whole-body mass, secondary to lean-mass loss, occur following 10-day confinement in all of the tested conditions. Based on the results of this study, it does not seem reasonable to increase the energy intake levels during short-term bed rest above the ones used in this protocol (basal metabolic rate multiplied by PAL 1.2 or 1.4 for bed rest and ambulatory confinement, respectively) since positive energy balance was noted, even with hypoxia as an additional stressor. Alterations in macronutrient diet composition and other possible nutritional interventions seem warranted for future studies and missions incorporating combined unloading/inactivity and hypoxia.

Part B: The Effects of a Lunar Habit Simulation on Peripheral Perfusion and Emotional Strain

4 Circadian Rhythm of Peripheral Perfusion During 10-Day Hypoxic Confinement and Bed Rest

4.1 Introduction

Sustained bed rest (BR) is an experimental model utilised in ground-based studies investigating the effect of microgravity on physiological systems, as it induces similar physiological changes as noted in astronauts during sojourns in space and aboard the ISS. The inactivity and unloading associated with BR results in hypotrophy of postural muscles and loss of mass in weight-bearing bones (Berg et al., 2007a). The maintenance of a horizontal position also results in changes in the structure and function of vascular beds in the lower body (Eiken et al., 2008; Kölegård et al., 2009). One purported difference between future and current space-flight environments concerns the ambient oxygen partial pressure. It is envisaged that astronauts living in future lunar and Mars habitats will not only be exposed to reduced gravity, but in order to minimise the risk of decompression sickness during frequent extra-vehicular activities (EVA), also to hypobaric hypoxia (Bodkin et al., 2006). At present, EVA preparation includes oxygen pre-breathing concomitant with a slow reduction in the pressure within the suit to prevent the occurrence of decompression sickness. Since each EVA requires upwards of 6 hours of preparation, the solution to frequent daily excursions on the Moon and Mars will be to have astronauts living in a hypobaric environment, which would not require any decompression prior to exiting the habitat. To maintain an appropriate oxygen partial pressure and still keep the fire risk to a minimum, oxygen will be added to the air. Despite an increased fraction of oxygen in the air, the anticipated ambient pressure would render the habitat environment hypobaric and hypoxic (Bodkin et al., 2006). In such environments, several nonthermal factors may influence thermoregulation. Specifically, breathing a hypoxic gas mixture acutely leads to proximal vasodilatation and distal vasoconstriction (Golja et al., 2002). The circadian rhythm of body core temperature (Tb) is a result of an altered relationship between autonomic heat-production and heat-loss mechanisms. Heat loss aided through peripheral vasodilatation exhibits a peak rate late in the evening, a phenomena that is particularly prominent in the hands and the feet (Krauchi et al., 1997; Krauchi et al., 1999; Krauchi et al., 2004; Krauchi and Wirz-Justice, 1994, 2001). This nocturnal peripheral vasodilatation has been suggested to be essential for initiating sleep onset. Thermal afferent information, particularly from the warm sensors of the lower extremities converges onto a subpopulation of thermosensitive neurons in the preoptic anterior hypothalamus, and this has been postulated to initiate sleep (Raymann et al., 2008; Van Someren, 2004).

Previously, Golja et al., (2002) reported, a progressive peripheral vasoconstriction during 35 days of horizontal BR. Daytime infrared thermograms taken during the course of the BR indicated a progressive decrease in the skin temperature (Tsk) of the big toe from 30°C at the onset, to 24°C upon completion of BR. Considering the theory by Krauchi et al., (1999) regarding the contribution of peripheral vasodilatation to sleep onset, sleep disturbances observed among BR participants and astronauts on the ISS (Pavy-Le Traon et al., 2007b) may be, in part, due to the reduced toe Tsk, a consequence of a bed rest/inactivity-induced reduction in perfusion. Furthermore, it is well established that hypoxia causes peripheral vasoconstriction, thus it may also contribute to the occurrence of sleep disturbances at altitude.

The reported reductions in peripheral perfusion during BR have only been noted during the daytime. We therefore tested the hypothesis that the peripheral vasoconstriction caused by hypoxia persists at night and is augmented by sustained BR.

4.2 Materials and methods

This study was part of a larger research programme investigating the separate and combined effects of hypoxia and inactivity/unloading on the cardiovascular and musculoskeletal systems.

4.2.1 Participants

As outlined in Chapter 2, male participants were recruited through local advertisement and selected according to the ESA recommendation regarding the standardisation of bed rest study conditions. All participants gave their written informed consent to participate in the study, which was approved by the National Committee for Medical Ethics at the Ministry of Health (Republic of Slovenia) and performed according to the guidelines of the Declaration of Helsinki.

4.2.2 Experimental design

This repeated measures crossover design study was performed in the hypoxic facility of the Olympic Sport Centre, Planica (Rateče, Slovenia). The participants were requested to take part in three 10-day interventions in a randomised order: 1) mild hypobaric normoxic bed rest trial (NBR; actual altitude of 940m), 2) mild hypobaric hypoxic bed rest trial ((HBR), target simulated altitude equivalent to ~4000m) and 3) mild hypobaric hypoxic ambulatory confinement ((HAMB), target simulated altitude equivalent to ~4000m). In this third trial, participants were ambulatory, but confined to the hypoxic area of the Planica facility. All participants were confined to the Planica facility 24 hours per day for 10 days.

4.2.3 Procedures

Twenty-two hour continuous measurements of proximal to distal skin temperature gradient (ΔTp-d) (Rubinstein and Sessler, 1990), an index of peripheral vasoconstriction, were recorded on D1 and Day 10 (D10) of the three confinement periods. As the protocol followed the mountaineering guidelines (Bartsch and Saltin, 2008) the measurements recorded on D1 were obtained at a lower target simulated altitude of 3000m compared to the 4000m of altitude on D10. Measurements commenced at 09:00 hrs. Modular Signal Recorder (MSR) data loggers (MSR145, Henggart, Switzerland) were secured to four sites using a breathable transparent film (Tegaderm, 3M, Minnesota, USA) and micropore tape (3M, Minnesota, USA). The ΔTp-d areas chosen for study were forearm-fingertip (ΔTfo-fi) and calf-toe (ΔTc-t) skin temperature (Tsk) gradients. The Tsk measurement sites for determination of ΔTc-t were: medial aspect of soleus, mid-way between ankle and knee, and the toe pad of the big toe. For the assessment of ΔTfo-fi the measurement sites were: the volar surface of the forearm midway between wrist and elbow and the finger pad of the index finger. The measurement sites were on the non-dominant hand or foot so as to cause the least amount of disturbance to the participant's daily routine.

The LLMS questionnaire (please see appendix 11.3) was administered and completed each evening between 20:00 and 21:00 to monitor for signs and symptoms of AMS. In addition to the subjective rating of AMS, eye examinations were conducted to screen for the presence of high altitude retinopathy, which is considered to be a precursor of altitude sickness (Wiedman and Tabin, 1999). Each participant had a complete ophthalmological examination including retinal photographs that were taken with a Topcon fundus camera (ImageNet 2000; Topcon, Tokyo, Japan). Ophthalmological examinations were performed approximately one week prior to each intervention and on the third day of recovery (R3).

Previous work from our laboratory has shown that over the course of 35 days of horizontal bed rest there is no significant difference in either mean daily aural temperature (Taural) or in the magnitude of the diurnal Taural oscillations, (Golja et al., 2002). Therefore, it was deemed appropriate to measure Tb once per day. Taural was measured as an index of Tb at 4pm daily using a ThermoScan Ear Thermometer (BRAUN 6023, BRAUN, Frankfurt, Germany).

Baseline measurements of erythropoietin (EPO, $mU.mL^{-1}$) and red blood cell count (RBC, $10^{12}/L$) were obtained at the Planica facility prior to entry into the experimental intervention. Post-intervention measurements of EPO and RBC were sampled in the morning on D11 just prior to the participants exiting the intervention. All blood samples were taken from the antecubital vein in the morning, at approximately 07:00, after an overnight fast and prior to the participants rising from bed. EPO samples were collected in EDTA vacutainers, whereupon the blood was immediately centrifuged and the plasma was frozen at −80 °C for subsequent analysis. The concentration of EPO was determined via sandwich enzyme-linked immunoassay (Quantikine IVD EPO ELISA; R&D Systems,

Minneapolis, MN, USA). Optical density was quantified on a microplate reader Quant (Bio-Tek Instruments, Winooski, VT, USA) set at 450nm and corrected at 600nm. The coefficient of variation of the analysis was 5%. A 2ml whole blood sample was immediately transferred to a nearby medical institution for analysis of the complete blood count. RBC count was obtained with an automated laser-based haematology analyser (Advia 120; Siemens, Munich, Germany). The apparatus was calibrated before each measurement. All samples were assayed in duplicate.

4.2.4 Heart rate, capillary oxyhaemoglobin saturation and arterial pressure

In order to monitor the participants' well-being during all interventions, heart rate (HR) and arterial oxyhaemoglobin saturation (S_aO_2) responses were monitored daily at 16:00 using personal finger oximetry devices (3100 WristOx, Nonin Medicals, Minnesota, USA) at regular intervals throughout all interventions. Additionally, arterial pressure (AP) was measured daily at 16:00.

4.2.5 Data analysis

The MSR data logger recorded the forearm, fingertip, calf and toe skin temperatures in 1-minute intervals. The data was then averaged into 60-minute bins. A change in posture can affect Tb, as well as leg, foot and hand skin temperatures for up to 2 hours (Krauchi et al., 1997; Tikuisis and Ducharme, 1996). The participants changed position from standing to supine on entering the bed rest condition at 09:00 on D1, therefore the first 2 hours of data from 09:00 until 11:00 were removed. Additionally, in order to avoid masking a circadian rhythm of temperature, the hour when the participants ate lunch, 13:00 to 14:00, and dinner 19:00 to 20:00 was removed. Furthermore, in HAMB the participants exercised for 30-minutes at 10:00 and at 15:00; therefore the hour from 15:00 to 16:00 was also removed from the data. Recording was terminated the morning following D1 and D10.

In order to analyse the differences in peripheral perfusion between daytime and nighttime, the data were further collapsed to give three time points for the day (T1, T2, T3) and one for the night (Ni). The data was collapsed as follows: T1 = 11:00 to 13:00, T2 = 16:00 to 19:00, T3 = 20:00 to 23:00, Ni = 23:00 to 03:00. While data was recorded throughout the night, there were no statistical changes in Tsk observed during the nighttime. Therefore, the timeframe of 23:00 to 03:00 was chosen to represent nighttime Tsk changes.

4.2.6 Statistics

Values are reported as arithmetic mean ± standard deviation unless otherwise indicated.

Toe and finger temperature changes represented by Tsk gradients were analysed using a 3-way mixed effects analysis of variance (ANOVA). A 3-way fixed effects ANOVA (Condition (HAMB, HBR, NBR) X testing period (D1, D10) X time (T1, T2, T3, Ni)). A 3-way mixed effects ANOVA was used to analyse the Tsk gradients as the same subjects completed all three conditions in a cross over manner and within each condition there were multiple time points and repeated measures that required such a statistical test. When a main effect or an interaction was noted, a Tukey post hoc test was used to define the specific differences. The average HR, AP and SaO2 responses, the LLMS score, the facility ambient temperature and humidity data and the Taural changes were all analysed using a repeated measures ANOVA (Condition (HAMB, HBR, NBR) X time (D1, D10)). Finally, an ANOVA was utilised in all other tests as the cardiovascular and ambient data was not as complex or as detailed as the Tsk gradients. The alpha level of significance was defined a priori at 5 %. All statistical analyses were performed using Statistica 5.0 (StatSoft, Inc., Tulsa, Oklahoma, USA).

4.3 Results

Eleven male participants successfully completed the three 10-day confinements. Their physical characteristics are presented in Table 2.2

4.3.1 Ambient conditions

The ambient conditions have been reported in section 3.13.1. Briefly, the F_IO_2 in the hypoxic trials was maintained stable at 0.1629 ± 0.0005 on Day 1 (simulated equivalent altitude of 2,990m), 0.1549 ± 0.0005 on Day 2 (simulated equivalent altitude of 3,380m) and 0.144 ± 0.0002 (simulated equivalent altitude of 3,881m) throughout the remaining period of the hypoxic interventions. The equivalent altitudes were derived from the data of Gallagher and Hackett, (2004), please see appendix 11.7. The partial pressure of oxygen (P_IO_2) was calculated according to Equation 3: Pressure of Inspired Oxygen. Therefore, the P_IO_2 in the HAMB and HBR trials was 103mmHg on Day 1, 98mmHg on Day 2 and 92mmHg for the remaining days. The P_IO_2 in the NBR intervention was 133 ± 1mmHg.

4.3.2 Altitude sickness

The average LLMS scores recorded during the HAMB (LLMS = 0.7 ± 0.3) and HBR (LLMS = 1.0 ± 0.4) campaigns were significantly higher ($p < 0.01$) than the NBR scores (0.3 ± 0.2). During the HAMB intervention, the average LLMS score was higher during the first five days of exposure than during the last five days (Days 1-5: 0.9 ± 0.3, Days 6-10: 0.6 ± 0.1), ($p < 0.05$). There was no change in the LLMS score in the HBR intervention (Days 1-5: 1.2 ± 0.4, Days 6-10: 0.9 ± 0.4)

The average LLMS scores were less than 3, indicating the absence of AMS. However, an analysis of individual results over the first four days revealed that one participant presented with mild AMS symptoms in both the HAMB and HBR interventions. Five participants presented with mild AMS symptoms during the HBR campaign. Of the 11 participants, 5 also complained of a headache during the NBR condition. The attending medical officer cleared all participants exhibiting symptoms of mild AMS for continuation in the study. Therefore, it was not necessary to invoke the safety procedures to alleviate the participant's symptoms of AMS. Results of the eye examinations revealed no symptoms or signs of high altitude retinopathy, normally present in individuals suffering from altitude sickness. None of the participants had reduced visual acuity or clinically visible engorgement of retinal vessels or intra/pre retinal haemorrhages.

Table 4.1: *Cardiovascular Variables.* Heart Rate (HR), Capillary Oxyhaemoglobin (SaO2), Arterial Pressure (AP), Systolic Arterial Pressure (SAP), Diastolic Arterial Pressure (DAP) and Aural Temperature (Taural) recorded at 16:00 on days D 1 and D 10. The interventions are labelled as follows: Hypoxic Ambulatory (HAMB), Hypoxic Bed Rest (HBR) and Normoxic Bed Rest (NBR).

	Day	HR	S_aO_2 (%)		AP (mmHg)		Taural
		(bpm)	Day	Night	SAP	DAP	(°C)
HAMB	D1	67.9 ± 13 *	93.5 ± 1.9 *§	90.3 ± 0.9 *§	116.6 ± 8.9 *	66.5 ± 6.2 *	36.7 ± 0.4
	D10	88.8 ± 9.9 #	90.5 ± 2.7 #	86.9 ± 1.3 #	126.8 ± 16	75.5 ± 7.2 #	36.7 ± 0.3
HBR	D1	68.5 ± 8 *	93.6 ± 2.5 *§	90.6 ± 1 *§	111.8 ± 12.3 *	66.4 ± 6.7	36.6 ± 0.3
	D10	79.2 ± 8.2 ^	90.6 ± 2.7 #	86.8 ± 1.1 #	123.6 ± 12.7	72.7 ± 8.2	36.8 ± 0.4
NBR	D1	67.1 ±7.9 *	96.9 ± 1.4	94.4 ± 0.8	113.6 ± 9.2	66.4 ± 6	36.6 ± 0.3
	D10	74.4 ± 12.7	96.5 ± 1.7	94.3 ± 0.8	118.2 ± 11.5	66.4 ± 7.8	36.8 ± 0.4

* Significant difference within condition between D1 and D 10, § Significant difference on D 1 when compared to NBR, # Significant difference on D 10 when compared to NBR, ^ Significant difference between HAMB and HBR on D 10. Significant difference between daytime (16:00) and nighttime (average throughout the night) are not marked within the table as there were differences at all time points in all conditions. Data are presented as mean ± standard deviation.

4.3.3 Heart rate, capillary oxyhaemoglobin saturation and arterial pressure

The average heart rates across the 10-day HAMB, HBR and NBR interventions were 80.8 ± 9.9, 75.4 ± 6.3 and 68 ± 7.2bpm, respectively, with the HAMB and HBR heart rates being higher ($p < 0.01$) than those observed for the NBR trials. The differences between HBR and HAMB were not significant. Similarly, average S_aO_2 values were lower ($p < 0.01$) during the hypoxic conditions (HAMB = 91.2 ± 1.2%; HBR = 91.6 ± 1.5%) than during normoxia (NBR = 96.5 ± 1.1%). The heart rate, S_aO_2 and AP values recorded

specifically on D1 and D10 are presented in Table 4.1. Systolic AP was significantly increased in both the HBR and HAMB interventions on D10 compared to D1 ($p < 0.05$). Additionally, diastolic AP in HAMB was increased on D10 such that there was a significant difference compared to D10 NBR ($p < 0.05$).

4.3.4 Haematology

As a result of the continuous 10-day (240 hour) exposure to hypoxia, there was a significant ($p < 0.01$) increase in erythropoietin concentration (Table 4.2), during the HAMB and HBR interventions, compared to the NBR intervention. Additionally, there was a significant increase in red blood cell content ($p < 0.05$) in the HBR intervention compared to NBR. RBC content did increase during the HAMB trial but was not significantly different compared to NBR ($p = 0.059$). There were no statistically significant differences between the HBR and HAMB conditions.

Table 4.2: *Haematology.* Change in erythropoietin (ΔEPO) and red blood cell content (ΔRBC) from the baseline measurement to completion of each of the three interventions (240 hours). The interventions are labelled as follows: Hypoxic Ambulatory (HAMB), Hypoxic Bed Rest (HBR) and Normoxic Bed Rest (NBR).

Measurement	Δ EPO (mU.mL-1)			Δ RBC (1012/L)		
Condition	HAMB	HBR	NBR	HAMB	HBR	NBR
Average	3.87	3.16	-0.09 **	0.59	0.70	0.28 *
SD	3.45	2.86	0.75	0.29	0.38	0.23
N	10	10	9	6	7	7

* Significant difference between NBR and HBR. ** Significant difference between NBR and both HAMB and HBR. No difference was noted between the hypoxic conditions (HAMB and HBR).

4.3.5 Daily physical activity

There was no difference in any of the variables measured during the morning and afternoon exercise bouts. The heart rate during the morning and afternoon session were 115.9 ± 3.3 and 112.5 ± 2.7bpm, respectively. The S_aO_2 values were 88.3 ± 0.4 and 88.2 ± 0.4% during the morning and afternoon exercise bouts, respectively. The TRIMP values were 2.2 ± 0.1 and 2.1 ± 0.1 during the morning and afternoon exercise bouts, respectively.

4.3.6 Calf-toe (ΔTc-t, °C) Tsk gradients

There was no statistical difference between the interventions with regard to the perfusion of the toes, as reflected in ΔTc-t at any individual time point (T1, T2, T3 or Ni). That is to say, while there were differences in the toe perfusion on D1 compared to D10, the effect that each intervention had was similar (Table 4.3).

Table 4.3: *Skin Temperature Gradients*. Forearm-fingertip skin temperature gradient (ΔTfo-fi, °C) and Calf-toe skin temperature gradients (ΔTc-t, °C) are presented during four time periods on days D 1 and D 10 in the three interventions. T1 = 11:00 to 13:00; T2 = 16:00 to 19:00; T3 = 20:00 to 23:00; Ni = 23:00 to 03:00. The interventions are labelled as follows: Hypoxic Ambulatory (HAMB), Hypoxic Bed Rest (HBR) and Normoxic Bed Rest (NBR).

Forearm-fingertip skin temperature gradient (ΔTfo-fi, °C)					
Intervention	Day	T1 (°C)	T2 (°C)	T3 (°C)	Ni (°C)
HAMB	D1	-0.2 ± 1.7 *	1.8 ± 2.6 §	0.2 ± 2.0	-1.1 ± 0.9
	D10	0.9 ± 3.3	1.4 ± 3.1 §	0.4 ± 1.7	-0.8 ± 0.7
HBR	D1	0.9 ± 2.6	1.3 ± 1.4	0.4 ± 1.1	-0.4 ± 1.0
	D10	1.0 ± 2.1	0.4 ± 1.2	0.0 ± 1.4	-0.2 ± 1.6
NBR	D1	0.2 ± 2.1	0.6 ± 1.6	-0.6 ± 0.7	-0.5 ± 0.6
	D10	1.9 ± 3.1 **	0.2 ± 1.0	-0.4 ± 0.7	-0.9 ± 1.1
Calf-toe skin temperature gradient (ΔTc-t, °C)					
HAMB	D1	2.9 ± 3.3	4.3 ± 2.5	3.3 ± 2.5	0.8 ± 0.7
	D10	6.2 ± 4.0 ^ **	4.1 ± 4.6 §	2.1 ± 3.7	1.0 ± 0.8
HBR	D1	5.4 ± 3.6 ***	2.5 ± 2.7 §	1.7 ± 2.9 §	0.2 ± 1.2
	D10	8.7 ± 0.5 ^ **	6.0 ± 2.7 ^ §	4.2 ± 2.5 §	1.0 ± 0.8
NBR	D1	3.7 ± 3.0 §	4.9 ± 3.0 §	3.8 ± 4.0 §	0.4 ± 1.1
	D10	7.1 ± 2.2 ^ **	5.9 ± 4.1 §	3.4 ± 2.9	1.0 ± 1.2

* Denotes a significant difference between T1 and T2, ** Denotes a significant difference between T1, and both T3 and Ni, *** Denotes a significant difference between T1, and T2, T3 and Ni, ^ Denotes a significant difference between D 1 and D 10, § Denotes a significant difference from Ni. Data are presented as mean ± standard deviation.

4.3.6.1 Normoxic bed rest (NBR)

The perfusion of the toe during the NBR intervention decreased so that ΔTc-t was larger at T1 than at Ni ($p = 0.01$). Of note, this reduced perfusion persisted on D1 in the NBR intervention resulting in a difference from the time points T2 ($p < 0.01$) and T3 ($p < 0.05$) to Ni. During the NBR intervention, the ΔTc-t recorded on D10 was also larger at T1 ($p < 0.01$) and T2 ($p < 0.01$) than at Ni. These results indicate significant vasoconstriction in

the toe during the daytime until 19:00 whereby the Tsk increased in the toe during NBR from 20:00 (Table 4.3).

4.3.6.2 Acute hypoxia (D1)

The perfusion of the toe was acutely depressed on D1 during the HBR intervention only at time point T1 (Figure 4.1), which resulted in a difference compared to T2 ($p < 0.05$), T3 ($p < 0.01$) and Ni ($p < 0.01$). In contrast, there was no difference observed in the HAMB intervention in the ΔTc-t gradient on D1 between T1, T2, T3 or Ni.

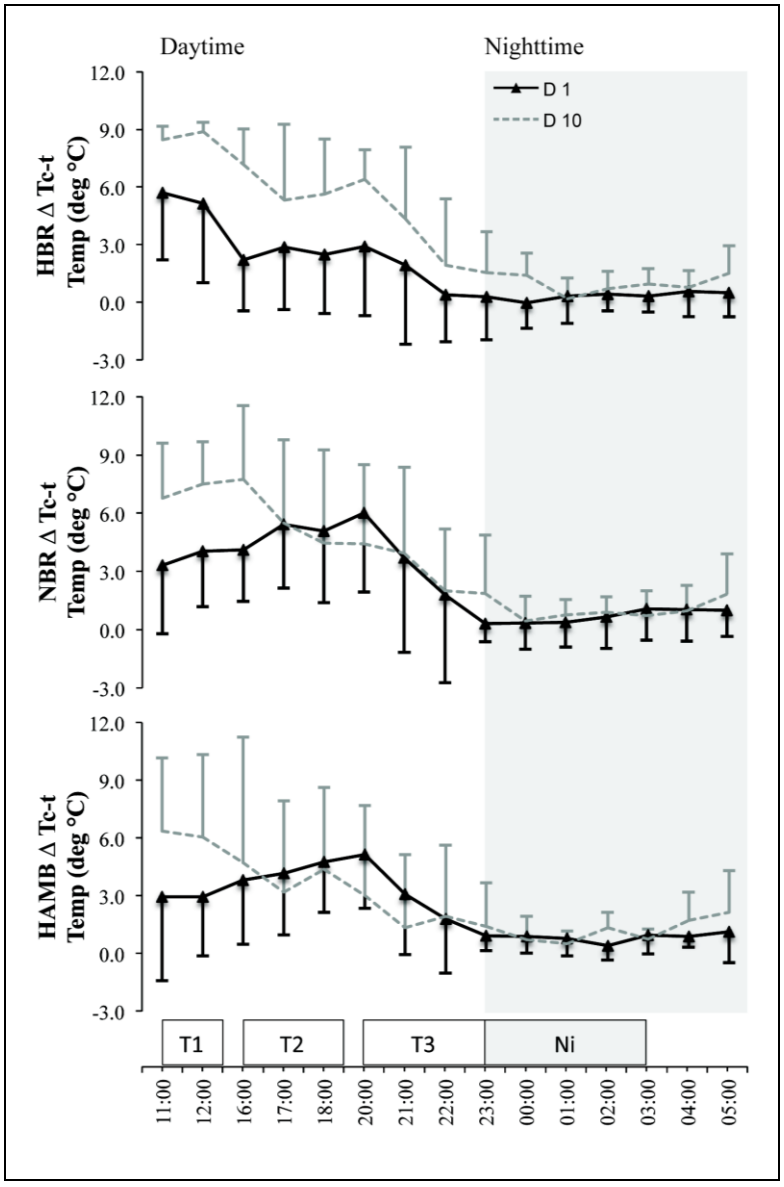


Figure 4.1: *Calf – Toe Temperature Gradients (ΔTc-t).* Changes in the 60 minute averages of peripheral skin temperature (Tsk, °C) for the calf – toe gradients (ΔTc-t) on Day 1 (D1, Solid black line) and Day 10 (D10, Dashed grey line) are presented for each intervention; Hypoxic Bed Rest (HBR, Upper panel), Normoxic Bed Rest, (NBR, Middle panel) and Hypoxic Ambulatory (HAMB, Bottom panel). Time points T1, 2, 3 and Ni are noted along the x-axis. Each data point represents the average temperature gradient for the previous 60 minutes. Data are presented as means ± standard deviation.

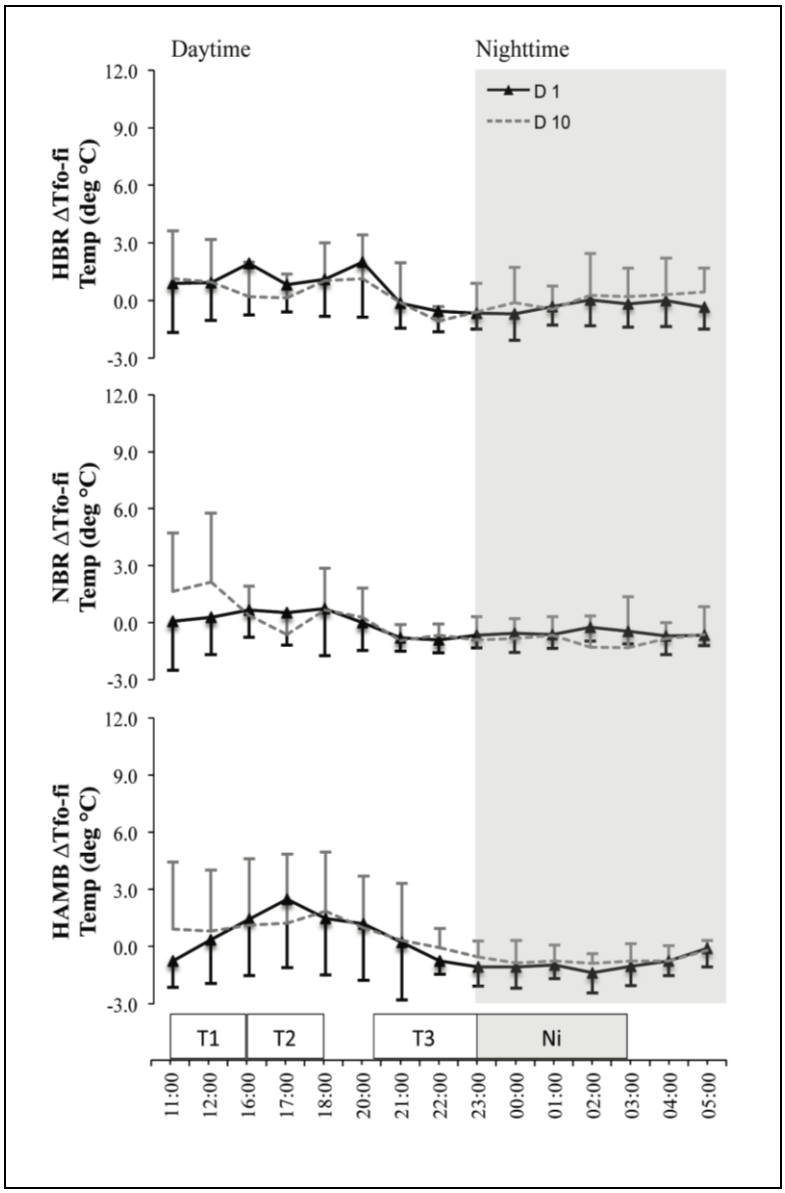


Figure 4.2: *Forearm – Fingertip Temperature Gradients (ΔTfo-fi).* Changes in the 60 minute averages of peripheral skin temperature (Tsk, °C) for the forearm – fingertip gradients (ΔTfo-fi) on Day 1 (D1, Solid black line) and Day 10 (D10, Dashed grey line) are presented for each intervention; Hypoxic Bed Rest (HBR, Upper panel), Normoxic Bed Rest, (NBR, Middle panel) and Hypoxic Ambulatory (HAMB, Bottom panel). Time points T1, 2, 3 and Ni are noted along the x-axis. Each data point represents the average temperature gradient for the previous 60 minutes. Data are presented as means ± standard deviation.

4.3.6.3 10 days of hypoxia (D10)

There was a significant increase in the vasoconstriction of the toes from D1 to D10 at time points T1 ($p < 0.01$) and T2 ($p < 0.01$) in HBR (Figure 4.1). Moreover, on D10 of HBR, the vasoconstriction persisted culminating in a significant decrease in toe perfusion measured during T1 ($p < 0.01$), T2 ($p < 0.01$) and T3 ($p < 0.01$) when compared to Ni.

Perfusion of the toe decreased significantly from D1 to D10 as a result of the HAMB intervention, $p < 0.01$. Accordingly, T1 ΔTc-t gradient was significantly greater compared to T3 ($p < 0.01$) and Ni ($p < 0.01$). Furthermore, T2 ΔTc-t gradient was also significantly larger than Ni ($p < 0.05$). Again, similar to bed rest, the results of 10-days hypoxic exposure indicate daytime vasoconstriction.

4.3.6.4 Daytime versus nighttime

While significant daytime vasoconstriction was noted, the ΔTc-t gradients were the same in all interventions on D1 at Ni (Table 4.3 and Figure 4.1). Furthermore, there was no difference in the ΔTc-t gradients on D10 at Ni and these gradients were not different from D1.

4.3.7 Forearm-fingertip (ΔTfo-fi, °C) Tsk gradients

Similar to in the lower limbs, there was no difference between the interventions with regard to the perfusion of the fingers. Additionally, no difference was noted in the perfusion of the fingers during the HBR intervention between any time points (Table 4.3). Nor was there any difference as a result of the intervention from D1 to D10 (Figure 4.2).

4.3.7.1 Normoxic bed rest (NBR)

As can be seen in Figure 4.2, there was no difference in the ΔTfo-fi during the NBR intervention between time points on D1. On D10 of the NBR intervention, the ΔTfo-fi gradient was higher at T1 than at T3 ($p < 0.01$) and at Ni ($p < 0.01$).

4.3.7.2 Acute hypoxia (D1)

There was a decrease ($p < 0.05$) in the perfusion of the fingertip on D1 from T1 to T2 during the HAMB campaign such that T2 was different from Ni (Table 4.3).

4.3.7.3 10 days of hypoxia (D10)

A similar difference ($p < 0.01$) was noted on D10 during the HAMB intervention as on D1, the fingertip being significantly less perfused at T2 than at Ni (Table 4.3).

4.3.7.4 Daytime versus nighttime

There was a decrease in the perfusion of the fingertips noted during the NBR intervention at T1 and at T2 during the HAMB intervention. However, the ΔTfo-fi gradient at Ni during all intervention was the same on D1 and D10.

4.4 Discussion

The main finding of the present study is that hypoxia-induced daytime vasoconstriction observed in the hypoxic bed rest (HBR) and hypoxic ambulatory (HAMB) interventions did not persist at night. Our results also support previous findings (Golja et al., 2002) of a progressive inactivity-induced reduction of daytime perfusion over the course of the normoxic bed rest intervention (NBR). Nocturnal ΔTp-d index, reflecting perfusion of the fingers and toes, was the same in all three conditions, suggesting that a nonthermal mechanism mediating nocturnal vasodilatation overrode the vasoconstrictive effect of hypoxia and bed rest observed during the daytime.

According to the LLMS score, retinal examination, RBC and EPO results all participants adequately acclimatised to the hypoxic conditions. Therefore, it is possible to interpret the results in the current study as the effects of hypoxia and bed rest, rather than as any confounding effects from maladaptation.

4.4.1 Altitude sickness and ΔTp-d

Subjective (LLMS) and objective (signs of high altitude retinopathy on retinal photographs) measures of altitude sickness were monitored to account for any potential affect of altitude sickness on the ΔTp-d response. Namely, altitude sickness has been reported to affect peripheral perfusion (Loeppky et al., 2003), thus the ΔTp-d response may have been affected in participants experiencing altitude sickness. Mild symptoms of altitude sickness were noted in several participants, particularly during the first days of exposure to hypoxia, although this was not reflected in their ΔTp-d responses. Measurements conducted before and after each intervention revealed no significant differences in the retinal photographs. In particular, a detailed analysis of the retinal vessels (De Boever et al., 2013) confirmed that there was no effect from the hypoxic exposure and/or bed rest on vessel diameter. Consequently, it is concluded that altitude sickness did not contribute to the ΔTp-d responses observed. Logistically, it was not possible to monitor retinal vessel diameter during the hypoxic or bed rest exposures. Thus, the possibility that any mild AMS affected retinal vessel diameter cannot be ruled out with the results of the present study. However, in cases of severe AMS, altitude retinopathy persists for days following return to sea level and any haemorrhages require weeks to be resorbed (Bosch et al., 2012). The absence of any signs of high altitude retinopathy during the ophthalmological examination conducted 3 days after the intervention confirms that the several cases of AMS observed were not severe.

4.4.2 Elevation in skin temperature as a precursor to sleep onset latency

The circadian rhythm of body temperature is a consequence of the variations in heat production (HP) and heat loss (HL). The relative contribution of HP and HL to the circadian rhythm of body temperature is different for the daytime and nighttime. Whereas

the daytime transient increase in Tb is a consequence of changes in both HP an HL, the nocturnal transient decrease in Tb is attributable primarily to an increase in HL, with HP remaining at basal levels throughout the night. The decrease in Tb during the late evening hours commences prior to the onset of sleep (Campbell and Broughton, 1994; Murphy and Campbell, 1997) and is preceded by the nocturnal elevation in HL (Krauchi and Wirz-Justice 1994). The elevated heat loss causes an increase in the peripheral Tsk, which provides thermal afferent information converging on the thermoregulatory centre in the preoptic anterior hypothalamus (Gilbert et al., 2004). It is the view of Krauchi and Wirz-Justice, (2001) and Raymann et al., (2008) that the vasodilation of the periphery is a key factor in the orchestration of the events leading to and regulating sleep. When manipulating the participants' knowledge of bedtime Rogers et al., (2007) observed a significant variability in the onset of peripheral vasodilatation, which is assumed necessary to achieve optimal heat loss in preparation for sleep. The results of the present study demonstrate that toe and finger Tsk were elevated during the night, reflecting increased peripheral perfusion and that there was no difference in the elevated nocturnal finger and toe Tsk either between the first and last nights in all three interventions, or between the three interventions. Thus, in preparation for sleep the increased HL - mediated by peripheral vasodilatation elevating skin temperature - was unaffected both by hypoxia and sustained bed rest. Sleep architecture in the three interventions was assessed in a separate study (Rojc et al., 2014) and was found to be unaffected by the three interventions. Combined, the similar sleep architecture and the similarly elevated nocturnal extremity Tsk in the three interventions, lends further support to the theory of a functional link between extremity digit temperatures and sleep onset.

4.4.3 Difference in extremity digit perfusion (hands versus feet)

The basic tenet of the current study is that the proximal to distal Tsk gradients represented by ΔTc-t and ΔTfo-fi reflect vasomotor tone in the toes and fingers respectively. According to Rubinstein and Sessler, (1990), a skin temperature difference between the distal portion of a limb and the tip of a digit (ΔTp-d) of more than +4°C is indicative of significant vasoconstriction, although a more conservative range of +2.3 to +2.7°C was suggested by House and Tipton, (2002). While significant variability was noted in the fingertip temperatures of the participants in the current study, during the HAMB and NBR interventions, at no point did the ΔTfo-fi values exceed either of these suggested thresholds for vasoconstriction. There was no variability measured during the HBR intervention. The largest measured ΔTp-d in the HAMB and NBR campaigns of the upper extremity were +1.8°C and +1.9°C, respectively. Indeed, the ΔTfo-fi did show significant differences between time points during the daytime, however, it appears that the fingertips were continuously well perfused. In contrast, ΔTc-t exhibited significant circadian changes, suggesting that a significant elevation in perfusion is only observed in the toes prior to sleep and may emphasise a greater contribution of thermal afferent information from this region in sleep onset latency. This concurs with the conclusion of Krauchi et al., (1999) and Krauchi and Wirz-Justice, (2001), who also demonstrated that the greater the vasodilatation of the feet in the evening, the greater reduction in the time to

sleep onset.

4.4.4 The effect of bed rest on extremity perfusion

Presumably, the vasoconstriction observed in the feet during bed rest is initially caused by simple fluid dynamics, as a consequence of the change in posture. The craniad displacement of blood from the capacitance vessels in the lower extremities is a passive response, which assists in maintaining cardiac output in the horizontal position. In contrast, the increase in nocturnal digit Tsk must be mediated by vasodilatation, which persists throughout the night and ceases only in the morning in preparation for waking. This active vasodilatation could be a consequence of thermal factors. The extremities, particularly the feet, are covered at night and this will activate local vasodilatation. In the present study however, during the HBR and NBR interventions, participants were in bed and covered with a duvet throughout the 10-day period. Thus, the microenvironment surrounding the lower extremities was similar throughout the bed rest exposures. Also, Krauchi and Wirz-Justice, (1994) showed that while recumbent with the feet covered and the hands exposed to the room environment, the circadian ΔTp-d response in the hands and feet will be similar in terms of time-course and amplitude. Thus, the covering of the feet during the day or in preparation for bedtime (HAMB intervention) is not a factor affecting our results.

Another factor that may affect ΔTp-d of the extremities is core temperature. Increases in deep body temperature will also initiate heat-loss mechanisms, which would be reflected in ΔTp-d. However, since core temperature decreases during the night and bed rest has been reported to cause a reduction in Tb (Monk et al., 1997), this factor can also be neglected as contributing to the nocturnal heat loss.

Since the present study demonstrates that nocturnal digit vasodilatation is not affected by bed rest, it is therefore not surprising that no bed rest-induced changes are observed in sleep onset latency (Monk et al., 1997) or in sleep architecture (Rojc et al., 2014).

4.4.5 ΔTp-d and hypoxia

Hypoxia-induced peripheral vasoconstriction, may, as will a transition from upright to supine, cause a redistribution of blood from the periphery to the central organs (Kuwahira et al., 1993; Mortola, 2007). If, or to what extent, hypocapnea resulting from hypoxia-induced hyperpnea also influenced peripheral vasomotor tone during HAMB and HBR remains unsettled (Heistad and Wheeler, 1970). Regardless, the vasoconstriction seen during the daytime on D1 in the HAMB and HBR campaigns might be expected to persist into the evening and nighttime. Our results show, for the first time, that this is not the case: both the hands and the feet are vasodilated in the evening resulting in increased heat loss. The discussion above has concluded that the mechanism responsible for overriding the hypoxia- and bed rest-induced vasoconstriction is most likely not a thermal factor. It

is therefore concluded that a nonthermal factor associated with the regulation and maintenance of a circadian rhythm is most likely implicated in counteracting the vasoconstrictive drive.

4.4.6 Limitations

The present data does not allow us to discern the mechanisms underlying the nighttime increase in toe perfusion. It might be warranted to include measurements of circadian changes in blood concentrations of catecholamines and melatonin in future studies. Another shortcoming of the present study was that we did not measure blood volume. Thus, we can only speculate whether the increase in RBC content observed in HBR reflected a hypoxia-induced augmented reduction in plasma volume or was due to hypoxia-induced erythropoiesis or decreased erythrocyte lysis (West et al., 2012); or was caused by a combination of these mechanisms. Considering the relatively short duration of the present BR and the fact that neocytolysis has mainly been observed during long-term BR interventions (De Santo et al., 2005) it appears unlikely that neocytolysis played a major role in the HBR-induced increase in RBC. Finally, the present study was not designed as a high-fidelity experiment in terms of simulating the exact conditions anticipated in future planetary habitats. Thus, extrapolation of our findings to future planetary habitats must be conducted with caution considering the difference between the present intervention and those anticipated in such habitats as regards duration and magnitude of both the hypogravity and hypoxic stimulus. Nevertheless, as stated below in the conclusions section, we feel it warranted suggesting that present findings might have a few implications for physiological responses in planetary habitats. In this connection it should also be noted that present hypoxia exposures were conducted under normobaric or slightly hypobaric conditions, whereas the ambient gas in planetary habitats is anticipated to be markedly hypobaric (Bodkin et al., 2006). However, judging by preliminary results from an on going study, there is no reason to believe that ambient pressure *per se* affects cutaneous vasomotor tone (McDonnell et al., 2014b). Furthermore, during space missions, factors other than cutaneous vasoconstriction may interfere with the onset of sleep and/or the sleeping pattern. Thus, sleep disturbances have been observed in astronauts on the ISS (Pavy-Le Traon et al., 2007b), on which the ambient air is normoxic. In fact, there is evidence to suggest systemic vasodilatation in ISS astronauts (Norsk et al., 2006).

4.5 Conclusion

The present study tested the hypothesis that two nonthermal factors known to induce vasoconstriction during the daytime, hypoxia and bed rest, might cause sleep disruption, should their vasoconstrictive effect persist during the night. Although there is no evidence of any sleep disruptions during bed rest, sleep disruption is a symptom of high altitude sickness. Hypoxia-induced sleeplessness has been considered an effect of hypoxia on central mechanisms and not local vascular effects. The present results reveal that

extremity digit skin temperature is not reduced at night as a result of hypoxia and thus the likelihood of a local vascular effect (i.e. digit vasoconstriction) contributing to the sleeplessness at altitude is negligible.

With regards to future short-term missions to the moon, where astronauts will be exposed to the combined effects of reduced gravity and hypoxia, the present results suggest that these two nonthermal factors should not influence circadian factors mediating sleep onset.

5 Examining the Effect of Hypoxic Bed Rest and Confinement on Psychological Strain

5.1 Introduction

Due to the normal ambient gas pressure within the International Space Station (ISS), preparation for extravehicular activity (EVA) – during which astronauts are enclosed in a space suit at about one third of an atmosphere (Norfleet and Butler 2001) – requires several hours of slow decompression and oxygen prebreathing to minimize the risk of decompression sickness. To reduce the preparation time for EVAs the ambient gas within future planetary habitats will be hypobaric and hypoxic.

Studies have confirmed that the changes observed in several physiological systems over a given period of time in participants rendered inactive and confined to a horizontal position (bed rest), are similar in nature and magnitude to those observed in astronauts during a sojourn in space of equal length (Pavy-Le Traon et al., 2007b). The principal effect of the bed rest model is the unloading of the weight-bearing bones, postural muscles and the withdrawal of the head-to-foot gravity vector with its resultant effect on the pressure gradients in blood vessels. Consequently, the bed rest ground-based analogue for studying microgravity effects on physiological systems has also been used to study countermeasures that would minimise the adaptive changes noted in astronauts during their space missions. In addition to physiological changes, previous research on the psychological effect of bed rest has shown a detrimental effect on mood (Ishizaki et al., 2002; Winget and DeRoshia, 1986) and cognitive function. However, the lack of consistency among these findings (Lipnicki and Gunga, 2009) supports the need for further research. On the other hand, exercise has a positive effect on participants' mood, affect and mental health (Yeung, 1996) increasing the positive emotions (e.g. vigor) while decreasing the negative ones (e.g. tension, anger). The effect of exercise on individuals' mood and affect is highly dependent on exercise (type, duration, intensity) and on participants' characteristics (age, physical condition).

Recently, the estimated 500-day confinement anticipated during a return mission to Mars has been simulated by confining a group of participants to the living quarters envisaged on the space vehicle to Mars. The main aim of the MARS 500 study was to investigate the interaction of the crew, performance of various tasks and also to assess the indices of psychological strain. The present study adds a new dimension to such confinement studies, by introducing deconditioning induced by sustained inactivity and physical unloading in a hypoxic environment.

Understanding the psychobiological mechanisms of successful adaptation to adverse environments provides important information regarding human function and needs. Several studies have examined the psychological and affective responses to high altitude exposure (Bahrke and Shukitt-Hale, 1993). Exposure to high altitude with a consequent reduction of oxygen supply to the central nervous system is associated with a variety of neuropsychological impairments (Davidson, 2001) including alterations in a person's psychological mood, behaviour and cognitive function. The symptoms that appear during exposure to hypoxic environments include impairments of coordination, vision, cognitive function, alertness and vigor. Concomitantly, these symptoms are usually accompanied by increases of apathy, anxiety, negative mood, fatigue, confusion, hostility and generally an increase of a person's negative psychological characteristics (Bahrke and Shukitt-Hale, 1993; Banderet and Burse, 1991; Missoum et al., 1992).

The effects of hypoxia on mood, behaviour and cognitive function seem to be unclear, presumably due to different characteristics measured, methods of measurement as well as in the duration and degree of the hypoxic exposure. In addition, although significant changes in mood, behaviour and cognition are revealed during exposure to high altitude, to a great extent these changes appear related to the substantial individual variation of adaptation to altitude. Thus, large inter-individual differences might reflect differences in the cognitive appraisal of the situation, which is important for one's adaptation to a stressful environment (Acevedo and Ekkekakis, 2001).

To better capture participants' psychological changes in the present study, the Profile of Mood State (POMS) (McNair et al., 1971) (please see appendix 11.4) and the Positive Affect – Negative Affect Schedule (PANAS) (Watson et al., 1988) (please see appendix 11.5) were used. The POMS and PANAS are two well-established instruments measuring psychological responses in sport and exercise settings (Watson and Clark, 1997). Far from the wide use of the specific instruments in previous research, the reasons for selecting the POMS and PANAS are based on the fact that the choice can provide the opportunity to compare the current findings with previous ones avoiding methodological issues or contradictory results because of the different instruments used. In addition to the above, the construction of the two instruments has been based on the different theoretical background providing a broad approach and comprehensive assessment, which includes "scales assessing fearful/anxious, sad/depressed, and anger/hostile mood, as well as some type of positive affect" (Watson and Clark, 1997).

In order to understand the singular and combined effects of hypoxia and reduced gravity on indices of psychological strain, the present study took advantage of two experimental paradigms. Thus, the purpose of the present research was to examine the psychological responses of participants rendered inactive in hypoxic and normoxic environments. The responses observed during hypoxic bed rest were compared with responses observed during a condition where the subjects were ambulatory and active, but confined to the hypoxic living quarters. Based on previous research, we expected

hypoxia, as well as, bed rest to have a detrimental effect on the participants' psychological profile (an increase of negative and a decrease of the positive psychological characteristics), while we expected exercise or physical activity to counteract these effects.

5.2 Material and methods

This study was part of the larger research programme described in Chapter 3, investigating the effects of future lunar and planetary habitats on different physiological systems. The current study focussed on indices of psychological strain.

5.2.1 Participants

Participants were recreationally active lowland Slovene residents (<500m). Eleven healthy males successfully completed the study. Their baseline characteristics are available in Table 2.2.

5.2.2 Experimental design

This repeated measures crossover design study was performed in the hypoxic facility of the Olympic Sport Centre, Planica (Rateče, Slovenia). The participants were requested to take part in three 10-day interventions in a randomised order: 1) mild hypobaric normoxic bed rest trial (NBR; actual altitude of 940m), 2) mild hypobaric hypoxic bed rest trial ((HBR), target simulated altitude equivalent to ~4000m) and 3) mild hypobaric hypoxic ambulatory confinement ((HAMB), target simulated altitude equivalent to ~4000m). As aforementioned, the targeted simulated altitude for the hypoxic conditions was 4000m. As the actual pressure of inspired oxygen is weather dependent the calculated altitude may vary with changes in atmospheric pressure. Please see Chapter 4.3.1 for ambient conditions and altitude calculation (appendix 11.7).

5.2.3 Measurements

5.2.3.1 Profile of Mood States (POMS)

The participants' mood state was measured using the POMS - Short Form (McNair et al., 1971)(Please see appendix 11.4). The POMS is a 37-item self-evaluation questionnaire of six subscales: tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, and confusion-bewilderment. Participants' description of their feelings were provided based on a 5-point scale with anchors of 0 representing "not at all" and 4 referring to "extremely". Several validation studies (Cronbach, 1951) have supported the internal consistency for the POMS subscales, as well as, for the test-retest reliability of the instrument (McNair et al., 1971).

5.2.3.2 Positive and Negative Affect Schedule (PANAS)

The Positive and Negative Affect Schedule (PANAS) (Watson et al., 1988) is a self-report instrument comprising two 10-item moods and was developed to provide measures of positive affect (PA; 10 items) and negative affect (NA; 10 items), (Please see appendix 11.5). Respondents are asked to rate the extent to which they experience each of the emotions that are described in PANAS items. Participants answer each item based on a 5-point scale with anchors of "very slightly or not at all" (1) to "very much" (5), with intermediate points of 2 repressing "a little", 3 "moderately" and 4 "quite a bit". Each affect factor's (PA and NA) total score, ranging from 10 to 50, was produced by adding the 10-items' score in each factor together.

The POMS and PANAS questionnaires were presented to the participants in hardcopy format and they were asked to complete the questionnaires promptly on completion of their other tasks or activities. The participants completed the POMS and the PANAS during the baseline measurement days, specifically, two days before the onset of each experimental condition (Pre). Thereafter, they completed the questionnaires on the 5th (D5) and 10th (D10) day of each condition and on the third day of recovery (Post) following each condition. The participants completed the questionnaires based on how they felt at the exact time of answering. The instruments indicated acceptable reliability in the current study (POMS Cronbach's α range = 0.65 - 0.90 and PANAS Cronbach's α range = 0.66 - 0.75).

5.2.3.3 Lake Louis Mountain Sickness (LLMS)

Due to the possibility that acute mountain sickness (AMS) might occur on the ascent to altitude, its presence was assessed with the Lake Louis Mountain Sickness (LLMS) questionnaire (Roach et al., 1993), which was administered and completed daily at 21:00 during the three campaigns (please see 12.3). The LLMS questionnaire is self-reported and involves the participants rating their own perception of five symptoms. The participants were asked to rate each of the following on a scale from 0 to 3: headache, gastrointestinal symptoms, fatigue and/or weakness, dizziness or light-headedness and difficulty sleeping.

5.2.3.4 Cardiovascular variables

In order to monitor the participants' well-being, daily measurements of pertinent cardiovascular variables were carried out at 16:00. The staff nurse on duty measured arterial pressure (AP) with a manual sphygmomanometer. Heart rate (HR) and capillary oxyhaemoglobin saturation (S_aO_2) responses were monitored using a personal finger oximetry device (3100 WristOx, Nonin Medicals, Minnesota, USA) throughout all experimental conditions.

5.2.4 Statistical analysis

Two 3 (Conditions: NBR, HBR, HAMB) X 4 (Time: PRE, D5, D10, POST) multivariate analysis of variance, with repeated measure on the second factor (RMANOVA) were computed. One RMANOVA was conducted for PANAS subscales and one for POMS subscales. Although, the assumption of equality of covariance matrices was satisfactory at the univariate level (Levene's test), it was violated in some cases at the multivariate level (Box's M test). Therefore, Pillia's trace was chosen as the appropriate multivariate test statistic due to its robustness over test violations (Field, 2009; Tabachnick and Fidell, 2012).

Based on the results of the RMANOVA, separate mixed analysis of variance were performed, 3 (Conditions: NBR, HBR, HAMB) X 4 (Time: Pre, D5, D10, Post), on each of the affect (PANAS) and mood (POMS) factors to examine the between-subject condition differences and the within-subject repeated measures of the experimental conditions. In cases where the assumptions of sphericity were not met in the within-subjects repeated measure analyses, based on the results of Mauchly's test of sphericity, the Green-House Geisser correction and the corresponding degrees of freedom, were applied for subsequent F statistic estimation (Field, 2009; Tabachnick and Fidell, 2012). Bonferroni corrected t-tests followed any significant between and within effects in the ANOVA models testing pairwise comparisons (Field, 2009; Tabachnick and Fidell, 2012). The significance level was set at $p<0.05$. The Statistical Package for Social Sciences (SPSS 19.0 Win) was used for the statistical analyses.

5.3 RESULTS

5.3.1 Psychological responses

The participants' responses to the PANAS (PA: positive affect, NA: negative affect) and POMS subscales (tension, vigor, anger, fatigue, confusion and depression) in the three experimental conditions (HBR, NBR and HAMB), were examined for significant difference at the baseline measure before the experimental protocol. The results indicated no significant differences among the three experimental conditions on either the PANAS (Pillai's Trace V = 0.494, η^2_p = 0.032) or the POMS (Pillai's Trace V = 0.690, η^2_p = 0.137) subscales.

5.3.1.1 Positive – Negative Affect (Positive and Negative Affect Schedule, PANAS)

A significant Condition (3) x Time (4) interaction was detected ($F = 2.592$, $p < 0.01$, η^2_p = 0.374) for both of the PANAS subscales (PA and NA). The means and standard deviations of the PANAS subscales are presented in Figure 5.1.

The results indicated a significant Condition X Time interaction for PA ($F = 2.470$, $p < 0.05$, $\eta^2_p = 0.141$). There was a significant change revealed in the HBR condition ($F = 13.961$, $p < 0.001$, $\eta^2_p = 0.583$), specifically, a reduction of PA from PRE to D5 ($p < 0.001$) and from Pre to D10 ($p < 0.01$). While PA was increased on Post compared to on D5 ($p < 0.001$) and on D10 ($p < 0.01$). Differences were also noted in the NBR condition ($F = 5.557$, $p<0.01$, $\eta^2_p = 0.357$), which indicated a decrease of PA from Pre to D10 ($p < 0.05$). However, PA was increased at Post compared to on D5 ($p < 0.05$) and on D10 ($p < 0.01$). Finally, no significant changes appeared over time in the HAMB condition ($F = 0.759$, η^2_p $p = 0.071$). Furthermore, no significant differences were revealed between the three experimental conditions in the four time measures.

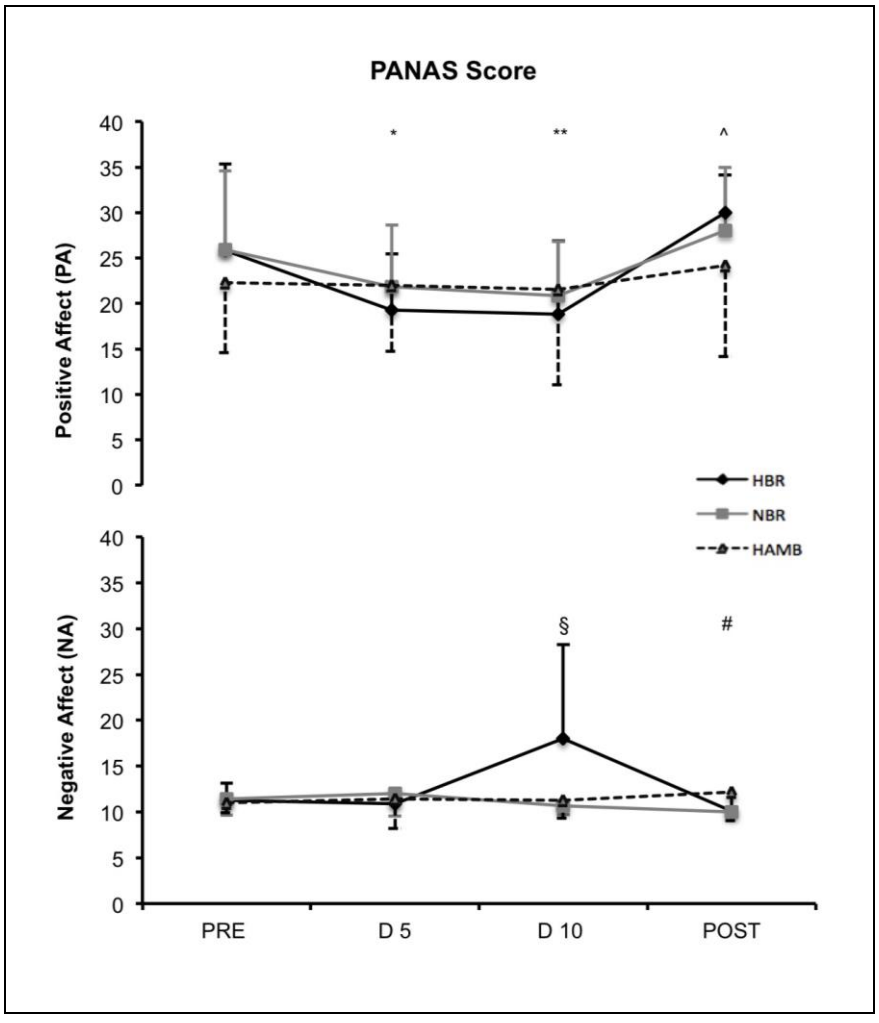


Figure 5.1: *Positive Affect Negative Affect Schedule.* The participants' subjective ratings of the Positive Affect Negative Affect Schedule (PANAS) are presented for each intervention; Hypoxic Bedrest (HBR: Solid black line), Normoxic Bedrest (NBR: Solid grey line) and Hypoxic Ambulatory (HAMB: Broken black line). Positive Affect (PA) is presented in the upper panel, while Negative Affect (NA) is presented in the lower panel for each time point (PRE, D5, D10 and POST) throughout each condition. Data are means ± standard deviation. * Denotes a significant difference on D5 compared to Pre in HBR. ** Denotes a significant difference on D10 compared to Pre in both HBR and NBR. ^ Denotes a significant difference from D5 and D10 to Post in both HBR and NBR. § Denotes a significant difference on D10 compared to D5 and Pre in HBR. # Denotes a significant difference between HAMB and both HBR and NBR on Post.

With regard to the analysis of the PANAS NA subscale, the results indicated that there was a significant Condition X Time interaction ($F = 5.082$, $p < 0.01$, $\eta^2_p = 0.253$). A significant change for NA appeared in the HBR condition ($F = 5.396$, $p < 0.05$, $\eta^2_p = 0.350$), indicating an increase of NA from Pre to D10 ($p < 0.05$) and from D5 to D10 ($p < 0.05$). NA subsequently decreased from D10 to Post ($p < 0.05$). Finally, no significant changes over Time appeared in the NBR ($F = 3.519$, $p = 0.052$, $\eta^2_p = 0.260$) or the HAMB conditions ($F = .567$, $p = 0.61$, $\eta^2_p = 0.054$). On D10, the participants in the HBR condition indicated higher NA compared to those in NBR ($p < 0.05$) and HAMB ($p < 0.05$), while during the Post measure the HAMB condition indicated higher NA compared to the HBR ($p < 0.05$) and NBR ($p < 0.05$).

5.3.1.2 Mood (Profile of Mood States, POMS)

The Condition (3) x Time (4) interaction for the POMS subscales was significant ($F = 2.196$, $p<0.05$, $\eta^2_p = 0.767$), suggesting that mood-state changes over time differed depending on the condition. The means and standard deviations of the POMS subscales are presented in Table 5.1

The analysis of tension showed a significant Condition X Time interaction ($F = 5.383$, $p < 0.001$, $\eta^2_p = 0.277$). The tension values in the HBR trial ($F = 9.089$, $p < 0.05$, $\eta^2_p = 0.502$) changed significantly over time. Tension on D10 was higher than Pre ($p < 0.05$), D5 $p < 0.01$) and Post ($p < 0.05$) measures, as well as in Post compared to the Pre ($p < 0.05$) measure. Finally, no significant tension-level changes appeared over time in NBR ($F = 0.741$, $p = 0.45$, $\eta^2_p = 0.076$) or HAMB ($F = 1.961$, $p = 0.18$, $\eta^2_p = 0.164$). Examining the differences between the three experimental conditions, significant differences were only revealed on D10, where HBR indicated higher tension compared to the NBR ($p < 0.01$) and HAMB ($p < 0.01$) conditions.

There was a significant Condition X Time interaction for vigor ($F = 2.470$, $p < 0.05$, $\eta^2_p = 0.150$). The results indicated that vigor significantly decreased in the HBR condition ($F = 18.455$, $p < 0.001$, $\eta^2_p = 0.672$) from Pre to D5 ($p < 0.01$) and from Pre to D10 ($p < 0.01$). On the other hand, an increase of vigor was detected on D10 ($p < 0.001$) and at Post ($p < 0.001$) compared to D5. Differences were also revealed during NBR ($F = 6.606$, $p < 0.01$, $\eta^2_p = 0.423$), showing an increase of vigor in the Post measure compared to Pre measure ($p < 0.05$). Additionally, vigor was significantly lower on D5 ($p < 0.01$) and D10 ($p < 0.01$) compared to the Post measure. Finally, no significant changes over time appeared in the HAMB experimental condition ($F = 0.496$, $p = 0.62$, $\eta^2_p = 0.047$), or between the experimental conditions across the three time measures.

The results for the anger subscale indicated a significant Condition X Time interaction ($F = 6.643$, $p < 0.001$, $\eta^2_p = 0.322$). A significant change in the HBR condition appeared across time ($F = 8.110$, $p < 0.01$, $\eta^2_p = 0.474$). Specifically, anger was increased on D5 ($p < 0.05$) and on D10 ($p < 0.01$) compared to Pre and that anger then decreased from D10 to

Post ($p < 0.05$). Additionally, on D10 of the HBR condition anger was higher when compared to the NBR ($p < 0.01$) and HAMB ($p < 0.05$). The participants' anger in the HAMB condition was higher in the Post measure compared to the HBR ($p < 0.05$) and NBR ($p < 0.01$) conditions. No significant differences were found across the four time measures for anger in NBR ($F = 2.508$, $p = 0.11$, $\eta^2_p = 0.218$) or HAMB ($F = 3.056$, $p = 0.09$, $\eta^2_p = 0.234$).

In the confusion subscale, a significant Condition X Time interaction was detected ($F = 2.623$, $p < 0.05$, $\eta^2_p = 0.158$). The results indicated a significant change in the HBR condition ($F = 4.841$, $p < 0.01$, $\eta^2_p = 0.350$). Post hoc analysis indicated that confusion decreased from D10 to Post ($p < 0.05$) during HBR. No significant main effect was found for NBR ($F = 2.591$, $p = 0.07$, $\eta^2_p = 0.224$) or HAMB ($F = 0.360$, $p = 0.68$, $\eta 2p = 0.035$). No significant Condition X Time interaction for depression ($F = 1.394$, $p = 0.28$, $\eta^2_p = 0.091$) or fatigue ($F = 0.679$, $p = 0.62$, $\eta^2_p = 0.046$) was detected.

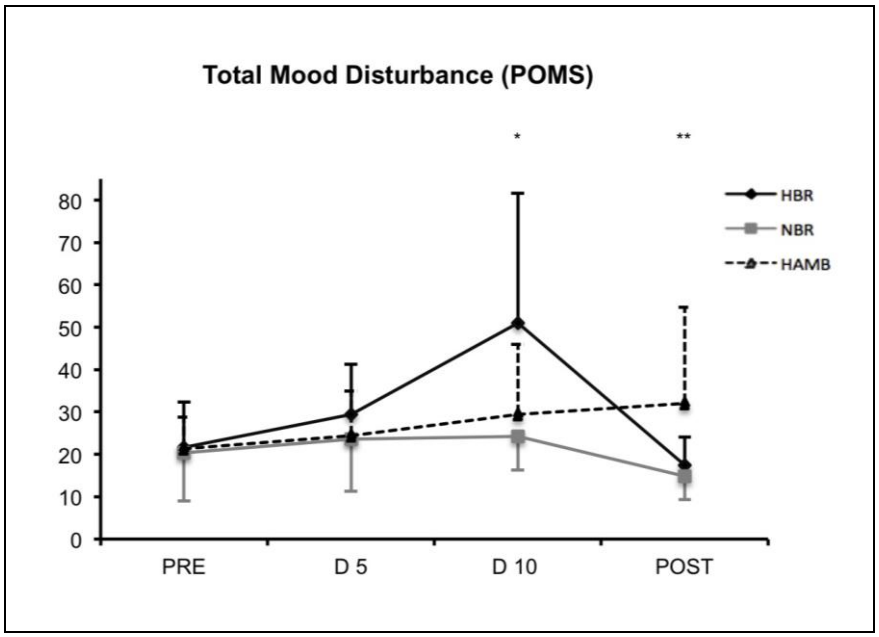


Figure 5.2: *Total Mood Disturbance.* The participants Total Mood Disturbance (TMD) scores are presented for each intervention; Hypoxic Bedrest (HBR: Solid black line), Normoxic Bedrest (NBR: Solid grey line) and Hypoxic Ambulatory (HAMB: Broken black line). The TMD score is calculated from the POMS subscales and are presented for each time point (Pre, D5, D10 and Post) throughout each condition. Data are means ± standard deviations. * HBR significantly different to NBR. ** HAMB significantly different to HBR and NBR.

Equation 4: Total Mood Disturbance

$$TMD = tension + depression + anger + confusion + fatigue + (24 - vigor)$$

Finally, the total mood disturbance score (TMD) (Curran et al., 1995), calculated according to equation 4, was examined across the four time measures among the

experimental conditions, signifying a significant Condition X Time interaction ($F = 4.438$, $p < 0.01$, $\eta^2_p = 0.241$) (Figure 6.2). A significant change in the HBR condition was detected ($F = 8.909$, $p < 0.01$, $\eta^2_p = 0.497$). The TMD was significantly increased on D10 when compared to Pre ($p < 0.01$) and D5 ($p < 0.05$), while there was a tendency for TMD to increase from Pre to D5 ($p = 0.059$). On the other hand, a decrease of TMD appeared from D5 to Post ($p < 0.01$) and from D10 to Post ($p < 0.01$). Regarding the NBR condition a significant change of the TMD was revealed across time ($F = 2.966$, $p < 0.05$, $\eta^2_p = 0.248$). A significant decrease of TMD was detected in the Post measure compared to the D5 ($p < 0.05$) and D10 ($p < 0.01$). Finally, no significant differences were revealed across time in the HAMB condition ($F = 1.421$, $p = 0.266$, $\eta^2_p = 0.124$). Examining the differences among the experimental conditions the results indicated that on D10 ($F = 4.755$, $p < 0.05$) significant differences were revealed between the HBR and NBR conditions ($p < 0.05$), while the differences between the HBR and HAMB ($p = 0.06$) conditions approached statistical significance. There were no significant differences were also revealed in the Post measure ($F = 4.882$, $p < 0.05$) between HAMB and HBR ($p < 0.05$), as well as, between the HAMB and NBR conditions ($p < 0.05$).

Table 5.1: *Profile of Mood State.* Subjective ratings of Tension, Vigor, Confusion, Depression, Anger and Fatigue derived from the Profile of Mood State (POMS) questionnaire during the second day of baseline measurements (Pre), during days 5 (D5) and 10 (D10) of the intervention and on the third day of recovery (Post) during the Normoxic Bedrest (NBR), Hypoxic Bedrest (HBR) and Hypoxic Ambulatory (HAMB) experimental conditions. Data are presented as means ± standard deviation.

Variable	Condition	Pre	D5	D10	Post
Tension	HBR	2.00 ± 1.27	1.1 ± 1.66	7.00 ± 5.72	1.09 ± 1.58
	NBR	1.64 ± 1.75	2.00 ± 2.53	1.10 ± 1.37	1.18 ± 1.25
	HAMB	1.09 ± 1.22	1.45 ± 1.92	2.00 ± 1.79	4.27 ± 6.17
Vigor	HBR	10.09 ± 6.58	4.80 ± 3.55	4.70 ± 4.30	13.00 ± 5.23
	NBR	10.73 ± 5.64	8.27 ± 6.68	7.10 ± 5.22	14.73 ± 3.41
	HAMB	8.09 ± 5.38	7.00 ± 6.00	7.27 ± 7.14	9.00 ± 7.16
Anger	HBR	0.91 ± 2.39	2.40 ± 3.24	8.70 ± 7.93	0.36 ± 0.92
	NBR	1.73 ± 3.64	2.27 ± 2 .57	0.50 ± 0.85	0.09 ± 0.30
	HAMB	1.00 ± 1.48	1.18 ± 2.14	2.45 ± 3.91	5.27 ± 6.44
Fatigue	HBR	2.55 ± 2.58	2.80 ± 2.97	7.40 ± 6.36	4.00 ± 2.90
	NBR	1.91 ± 2.55	1.91 ± 2.81	3.90 ± 3.03	3.36 ± 3.53
	HAMB	1.73 ± 1.49	2.64 ± 2.84	4.73 ± 4.00	4.09 ± 3.81
Confusion	HBR	1.55 ± 2.02	2.00 ± 2.79	3.20 ± 2.57	0.45 ± 0.69
	NBR	1.55 ± 1.21	0.73 ± 1.27	1.00 ± 1.76	0.82 ± 1.25
	HAMB	1.09 ± 0.83	1.63 ± 1.96	1.45 ± 2.25	1.82 ± 3.06
Depression	HBR	0.73 ± 1.42	1.90 ± 2.81	5.3 ± 8.77	0.55 ± 0.82
	NBR	0.27 ± 0.90	0.91 ± 1.30	0.90 ± 1.52	0.09 ± 0.30
	HAMB	0.45 ± 1.04	0.55 ± 1.51	2.09 ± 3.39	1.63 ± 4.15

Table 5.2: *Cardiovascular variables.* Heart rate (HR), capillary oxyhaemoglobin saturation (S_aO_2) and Arterial Pressure (AP) recorded at 16:00 on D1, D5 and D10.

Condition	Day	HR (bpm)	S_aO_2 (%)	AP (mmHg) Systolic	AP (mmHg) Diastolic
HAMB	D1	68 ± 13 *	93.5 ± 1.9 *	117 ± 9	67 ± 6
	D5	82 ± 9	89.9 ± 2.3	114 ± 10	71 ± 7
	D10	89 ± 10 **	90.5 ± 2.7	127 ± 16	76 ± 7
HBR	D1	68 ± 8	93.6 ± 2.5	112 ± 12	66 ± 7
	D5	77 ±10	92.1 ± 2.6	120 ± 9	71 ± 6
	D10	79 ± 8	90.6 ± 2.7	124 ± 13	73 ± 8
NBR	D1	67 ± 8	96.9 ± 1.4 ^	114 ± 9	66 ± 6
	D5	67 ± 9 §	96.4 ± 1.9 §	113 ± 13	66 ± 9
	D10	74 ± 13 #	96.5 ± 1.7 #	118 ± 11	66 ± 8

* Denotes a significant difference between D1 and D5, ** Denotes a significant difference between D1 and D10. ^ Denotes a significant difference on D1 compared to NBR, § Denotes significant difference on D5 compared to NBR, # Denotes significant difference on D10 compared to NBR. Data are presented as mean (SD).

5.3.2 Lake Louise Mountain Sickness (LLMS)

The LLMS scores recorded on D1, D5 and D10 are presented in Figure 5.3. There were no statistically significant differences noted between the HAMB, HBR or NBR conditions on D1, D5 or D10. However, taking an average of the LLMS scores over the first four days revealed a significant difference between the hypoxic conditions (HAMB (0.9 ± 1.4) and HBR (1.2 ± 1.2)) and the NBR condition (0.2 ± 0.2), $p<0.05$. The average LLMS scores were less than 3, indicating an absence of AMS. Thus, an analysis of individual results over the first four days was carried out and revealed that one participant presented with mild AMS symptoms in both the HAMB and HBR conditions. Five participants presented with mild AMS symptoms during the HBR campaign. Of the 11 participants, 5 also complained of a headache during the NBR condition.

5.3.3 Heart rate, arterial saturation and pressure

The HR, S_aO_2 and AP values recorded on D1, D5 and D10 are presented in Table 5.2. There was a main effect for Condition ($p < 0.01$), Time ($p < 0.01$) and a Condition X Time interaction ($p < 0.01$) for HR. Tukey post hoc analysis indicates that HR increased from D1 to D5 ($p < 0.01$) and from D1 to D10 ($p < 0.01$) in the HAMB condition. Additionally, HR on D5 ($p < 0.01$) and D10 ($p < 0.01$) were significantly reduced in the HAMB condition compared with NBR. There were no changes in the HBR or NBR

conditions.

S_aO_2 showed significant main effects for Condition ($p < 0.01$) and Time ($p < 0.01$), however there was no significant Condition X Time interaction. S_aO_2 significantly decreased from D1 to D5 in the HAMB condition ($p < 0.01$). Furthermore, S_aO_2 in the HAMB condition was significantly lower on D1 ($p < 0.05$), D5 ($p < 0.01$) and D10 ($p < 0.01$) than during the NBR condition.

There was a main effect for Time on systolic AP ($p < 0.05$), however Tukey post hoc analysis revealed no significant difference between the time points D1, D5 and D10. Suggesting that systolic AP is increasing over the 10-days of condition, but there was no difference between the conditions (HAMB, HBR, NBR). Finally, there were no significant effects for diastolic AP.

5.3.4 Daily physical activity

There was no difference in any of the variables measured during the stepping exercise performed in the morning and afternoon during the HAMB condition. HR during the morning and afternoon activity were 116 ± 3 and 113 ± 3bpm, respectively. The SaO2 values were 88.3 ± 0.4 and 88.2 ± 0.4% during the morning and afternoon activity, respectively. The TRIMP values did not vary significantly between the morning (2.2 ± 0.1) and afternoon (2.1 ± 0.1).

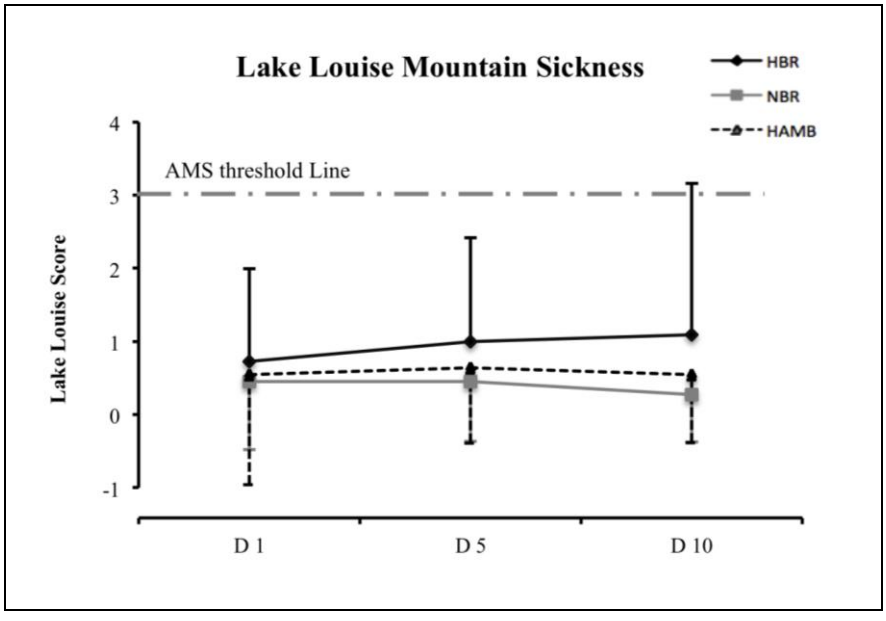


Figure 5.3: *Lake Louise Mountain Sickness.* The participants self perceived rating of Lake Louise Mountain Sickness Score (LLMS) is presented for day 1 (D1), day 5 (D5) and after the tenth day (D10) of each intervention: Hypoxic Bedrest (HBR: Solid black line), Normoxic Bedrest (NBR: Solid grey line) and Hypoxic Ambulatory (HAMB: Broken black line. No difference was noted between conditions at these time points. Data are means ± standard deviation.

5.4 Discussion

The principle finding of the present study is that the changes in positive and negative emotions associated with bed rest are exacerbated by hypoxia. During the ambulatory condition participants did not suffer the same psychological changes, suggesting that ambulation counteracts the effects of hypoxia. The factors that may have contributed to these psychological changes are discussed below.

5.4.1 Acute mountain sickness (AMS)

Mountain sickness normally occurs upon arrival at altitude, particularly if and when, the ascent rate is fast. At higher altitudes, AMS may develop into high altitude pulmonary oedema or high altitude cerebral oedema. This is unlikely to occur at an altitude of 3881m as simulated in the present study. More likely, mountain sickness may develop within the first days of ascent, but will subside with continued exposure to this altitude. The first measures of POMS and PANAS after baseline were derived on day 5, at which point any occurrence of AMS was resolved according to our LLMS score (Figure 5.3). Mountain sickness will undoubtedly have an effect on the traits rated by the POMS and PANAS scales, but this correlation was not the aim of the present study. Instead, we wished to examine the effect of hypoxia, in the absence of mountain sickness on the indices of psychological strain. Signs and symptoms of mountain sickness were observed in some participants upon arrival at altitude, but the symptoms were not so severe that a descent to a lower altitude was required. During the second half of the intervention period, some of the participants in the NBR condition reported slight headaches, as did participants in the HBR condition. While such transient occurrences of headaches can influence the LLMS score, they are not consistent with AMS and were therefore attributed to bed rest rather than hypoxia *per se*.

5.4.2 The effect of bed rest

Positive affect was decreased during NBR on D5 and D10 compared to the Pre measure, while a “rebound” in the positive affect appeared in the Post measure. The same pattern of changes was found in the vigor subscale, whereas the TMD improved in the Post measure compared to during the condition. These results indicate that positive emotions decreased during bed rest and then returned to the initial levels following the conclusion of the condition. On the other hand, it seems that bed rest does not elicit negative emotions (e.g., anger, tension), as indicated by non-significant alterations in these variables. The present findings are in accordance with previous results (Ishizaki et al., 2002; Liu et al., 2012). Presumably, the bed rest-induced reductions in positive 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 of or a lack of social

interaction (Winget and DeRoshia, 1986).

Results reported in the literature regarding the effects of bed rest on cognitive functions and psychological aspects lack consistency and further studies are needed to address the reasons behind these findings (Lipnicki and Gunga, 2009). Some studies support a detrimental effect of bed rest (Edwards et al., 1981; Ryback et al., 1971), while others found an improvement during bed rest (DeRoshia and Greenleaf, 1993; Ishizaki et al., 2002) or no change (Shehab et al., 1998). Such contradictory findings might be due to the different instruments used, research methodologies applied, participants' characteristics (age, gender) and duration of bed rest exposure. It seems that having a baseline measure in order to examine the potential impact of sustained bed rest compared to the upright position, as the current study did, will resolve some of the methodological problems inherent in previous studies (Lipnicki and Gunga, 2009). Understanding the effects of reducing physical activity below that of a sedentary lifestyle has implications for individuals with severely restricted physical activity (Gaul et al., 2006; McDonald, 2002; Park et al., 2001).

5.4.3 The effect of hypoxia

The differences in emotional state on D10 between the NBR and HBR conditions indicated that the HBR participants had higher negative affect, confusion, tension, anger and total mood disturbance compared with the NBR condition. It thus seems that HBR elicits negative emotions compared to NBR, whereas no significant effect seems to be exerted on positive emotions such as positive affect and vigor. This further supports the notion that positive and negative emotions do not follow an opposite pattern and are not reciprocally affected by hypoxia, since alterations appeared only on negative emotions. Although research findings in bed rest conditions are lacking, the results of the present study are in accordance with previous research findings, indicating that exposure to high altitude with a consequently reduced oxygen supply to the central nervous system causes a variety of neuropsychological impairments (Bahrke and Shukitt-Hale, 1993; Banderet and Burse, 1991; Davidson, 2001). A possible physiological explanation of the deterioration of psychological responses may be the effect of the hypoxia on the prefrontal cortex and amygdala. Based on previous findings (Ochsner et al., 2002), it seems that the prefrontal cortex does not exert a direct mediation of emotional responses, but mainly seems to have a moderating pattern of the activity of other parts of the neural circuit that control emotional responses, such as the modulation of the amygdala (Davidson, 2004). According to Davidson and colleagues (Davidson, 2002; Davidson et al., 2000; Winget and DeRoshia, 1986) the prefrontal cortex exerts an inhibitory control of the amygdala during exposure to aversive stimuli, helping to regulate and shorten the duration of the negative affective responses, while the time course of the positive emotions is accentuated. Davidson et al., (2000) conclude, "in the absence of this normal inhibitory input, the amygdala remains unchecked and continues to remain activated". Furthermore, as regards the effect of altitude exposure on psychological indices, additional research is needed to assess whether the observed changes are linked to

alterations in an individual's behaviour and cognitive function, factors crucial for performance in adverse environments (e.g., high altitude).

5.4.4 The effect of activity

Examining the differences in the emotional state between HBR and HAMB indicated that the participants in the HBR condition revealed their most negative psychological profile on D10. Specifically, the participants in the HBR condition indicated more pronounced negative affect, tension, anger and total mood disturbance than those in the HAMB condition. In the HAMB condition, the participants were not confined to the horizontal position as they were in the HBR condition, meaning that activities such as personal hygiene and eating could be completed in the upright position. It seems that the regular daily 30-minutes of low to moderate physical activity or indeed other activities performed in the HAMB condition reduced the psychological strain noted in the HBR condition. However, no significant differences were found for positive emotions between these two experimental conditions, supporting the notion that the positive and negative emotions did not follow an opposite and inverse pattern during exposure to the experimental conditions.

It is also noteworthy that the participants' emotional state continuously deteriorated during the HBR condition, as indicated by significant differences between the baseline measure (Pre) and the measures observed during the hypoxic exposure (D5, D10). On the other hand, the emotional status in HAMB remained stable with no differences during the hypoxic exposure, since no differences emerged for positive or negative emotions from Pre or Post measures to D5 and/or D10.

The factors associated with the HAMB condition, including the daily physical activity, were sufficient to ameliorate the psychological strain of the participants observed during HBR (Bodkin et al., 2006). The purpose of the activity during the HAMB condition was to simulate the cumulative daily activity of the subjects during their normal lifestyle. The activity was not meant to provide a training stimulus. However, future research should focus on the effects of various exercise training programmes on psychological status during hypoxic confinement. The current result is in accordance with previous research findings confirming the positive effects exercise has on mood, emotion, cognitive performance, well-being and brain function (De La Torre et al., 2012). Yeung, (1996) review 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 (Ekkekakis and Lind, 2006; Lind et al., 2005). Data from the MARS105 (Van Baarsen et al., 2009) study show that mood was highly correlated with the overall brain cortical activity and that even short exercise programmes were able to restore mood and brain cortical function to pre-isolation values. Presumably, this could be attributed to the increased blood flow and oxygenation in the prefrontal cortex during exercise, as measured with near-infrareds spectroscopy (Ekkekakis, 2009), causing an inhibition of the amygdala. Ekkekakis and

Acevedo, (2006), based on neuroanatomical and neurophysiological findings, indicated that the amygdala appears to be a key component in the coordination and regulation of the affective responses during exercise. However, other studies showed no effect of exercise on mood and emotion. DeRoshia and Greenleaf, (1993) indicated that exercise training during 30-day bed rest experiments did not counter the deterioration of psychological mood, additionally, Ishizaki et al., (2002) also found similar results. The contradictory results suggest that further research is needed to clarify the effect of exercise during bed rest and confinement. Presumably, these inconsistencies could be attributed to the participants' characteristics (age and / or physical condition), types of exercise (duration, frequency, intensity), and the instruments used to examine participants' emotional state (mood, affect, emotion). Ultimately, further work is also required to examine the effect of exhaustive exercise, as would be performed by athletes during training on psychological strain.

5.4.5 Implications for patients with chronic illnesses which would render them hypoxic

Patients with a chronic disease rendering them hypoxic, such as chronic obstructive pulmonary oedema, may not only have hypoxia-induced elevated levels of psychological strain, but a substantially reduced exercise capacity. Whether the ability of these patients to become more active would also reduce their psychological strain remains unresolved.

5.4.6 Implications for Planetary Habitats

During exposures to microgravity on the International Space Station (ISS) and moderately reduced gravity in future habitats on the Moon and Mars, astronauts will conduct regular exercise to prevent muscle atrophy, loss of bone mass and cardiovascular deconditioning. It is also anticipated that the atmosphere in Moon and Mars habitats will be moderately hypoxic (Bodkin et al., 2006). Whether the levels of hypoxia and unloading / reduced physical activity will be severe enough to increase psychological strain remains to be settled.

The level of unloading / inactivity is more pronounced in the present study than is anticipated in future lunar and Mars habitats, as is the level of hypoxia. Lower levels of hypoxia and higher levels of exercise in future space missions, will potentially diminish the hypoxia-induced negative psychological profile observed in the HBR condition.

5.5 Conclusion

The participants did not demonstrate significant differences between the pre- and post-intervention measurements, since the emotions (positive and negative) returned to the initial levels on recovery following BR and / or hypoxia. The results indicate that bed rest

has a detrimental effect on positive emotions and hypoxia augments the negative affect in the inactive, bed rest participants. This augmented negative psychological mood was not evident in the hypoxic ambulatory participants.

The activity level in the current study was specifically designed to mimic a low level of daily physical activity and not acting as a training stimulus. While there may be other factors involved, i.e. simply not being confined to a horizontal position, the introduction of a normal level of daily activity while under a hypoxic stimulus appeared to reduce the negative psychological strain of the participants. While bed rest may be a useful model of microgravity in certain situations, it does not provide a good representation of an astronauts' workload or the volume of exercise training that they complete as a countermeasure to muscle atrophy and cardiovascular deconditioning. Given that a small volume of physical activity in the upright position can negate the hypoxia-induced increase in negative emotional status, it is germane to both astronauts and athletes to further investigate the potential for exercise to improve the psychological state within a hypoxic environment.

In conclusion, the study suggests that inactivity and unloading induced by sustained bed rest has a detrimental effect on positive emotions and that hypoxia provokes negative emotions in bedridden, but not ambulatory participants.

Part C: Hypoxic Exercise Confinement and its Effects on Peripheral Perfusion and Emotional State

6 The Effect of a Live-High Train-High Regimen on Peripheral Perfusion

6.1 Introduction

Due to the potential benefits to aerobic performance at sea level and at altitude, hypoxic training has become popular among athletes (Bonetti and Hopkins, 2009). Hypoxia is used in a variety of dose-duration combinations. Intermittent hypoxic training, for example, requires athletes to intermittently inspire progressively lower concentrations of oxygen (Debevec and Mekjavic, 2012; Mekjavic et al., 2012). Some advocate training in a hypoxic environment and living under normoxic conditions, but this regimen has not proven to provide any benefit (Debevec et al., 2010). The Live-High Train-Low regimen, whereby athletes train in normobaric normoxic environments, but live and sleep at altitude (Stray-Gundersen and Levine, 2008; Kounalakis et al., 2012) appears to provide improvement of aerobic performance (Stray-Gundersen and Levine, 2008), in part by stimulating erythrogenesis. For some athletes, this type of training schedule is logistically impractical. In particular, winter athletes live and train at altitude during the summer, to take advantage of the snow conditions at these elevations. This regimen is referred to as Live-High Train-High (LH-TH; (Bonne et al., 2014). The disadvantage of this training protocol is that the intensity of physical training that can be performed by the subjects on arrival is impeded due to the hypobaric hypoxia. With time and progressive adaptation to the altitude, the quality of training, or rather the training intensity can be increased.

Thus, athletes training and living at high altitude, particularly during winter months, are exposed to the combined effect of hypoxia and cold: both factors contributing to the vasoconstriction of the peripheral circulation, particularly of the hands and feet. We recently reported that the nocturnal, peripheral vasodilatation observed in normoxic conditions and functionally linked with sleep onset latency (Krauchi et al., 1999; Raymann et al., 2008), is not counteracted by either normobaric (McDonnell et al., 2014a) or hypobaric (McDonnell et al., 2014b) hypoxia. These studies were conducted with inactive and sedentary subjects and may not reflect the effect of hypoxia on nocturnal vasodilatation in athletes training in a hypoxic environment.

Assuming that thermal afferent information contributes to sleep onset (Krauchi et al., 1999) and depth (Raymann et al., 2008), then nocturnal peripheral vasodilatation is a necessary requirement for normal sleep. The two major components of the LH-TH regimen, namely hypoxia and exercise training, both affect peripheral blood flow (Fritzsche and Coyle, 2000; Leuenberger et al., 2001; Simmons et al., 2011; Simmons et

al., 2007b) and combined may play a role in the maintenance or disturbance of sleep during athletic training camps. Namely, regular exercise training improves endothelial function, skeletal blood flow and leads to vasorelaxation (Delp, 1995; Fritzsche and Coyle, 2000; Wang, 2005). Thus, we hypothesised that provided that endurance exercise training improves peripheral perfusion, then endurance exercise at altitude may mitigate the daytime hypoxia-induced vasoconstriction previously noted (Golja et al., 2002; McDonnell et al., 2014a).

The second hypothesis tested in the present study was therefore, whether exercise training would reduce the daytime vasoconstriction and shorten the time to sleep onset. Finally, we monitored the circadian rhythm of peripheral skin temperature in subjects confined to a hypoxic facility for 10 days, either with, or without daily exercise training. Comparison of the results at the onset and at the end of the 10-day hypoxic exposure would reveal the degree to which adaptation to hypoxia modifies the peripheral blood flow, which is reflected in the skin temperature.

6.2 Materials and methods

6.2.1 Participant Selection

Participant recruitment followed the same methodology implemented in Chapter 2. Local advertisements were placed and male participants were invited to attend an information session. Following that, individual interviews were held for those maintaining an interest in the study. All participants gave their written informed consent to participate in the study, which was approved by the National Medical Ethics Committee of the Republic of Slovenia and performed according to the guidelines of the Declaration of Helsinki.

Participants were low-land residents (<500m) and were recreationally active, healthy, males. Prior to their involvement in the study, all participants spent a full weekend at the hypoxic confinement facility being familiarised with the testing procedures. Following this familiarisation and the initial screening, sixteen participants were selected and randomly assigned to either a Live-High Train-High (LH-TH) group, or to a group that was only exposed to hypoxia, but not required to undergo daily physical exercise. This latter group was the Live-High (LH) group. Two participants from the LH group did not complete the entire experimental protocol due to medical issues. A complete data set was obtained from fourteen participants: eight participants in the LH-TH group and six participants in the LH group. There were no statistical differences observed between the groups for the aforementioned physical characteristics (Table 6.1)

6.2.2 Experimental Design

The experimental campaign was performed in the hypoxic facility of the Olympic Sport

Centre Planica (Rateče, Slovenia).

Table 6.1: *Participant characteristics*. Participant age, height, weight, body composition and maximal oxygen consumption (VO2max) are presented for the Live-High Train-High (LH-TH: N = 8) group and the Live-High (LH: N = 6) group. Data are presented as means ± standard deviation.

	LH-TH	LH
Age (yr)	25.8 ± 2.4	24.8 ± 3.1
Stature (cm)	182.7 ± 5.9	177.7 ± 3.5
Body mass (kg)	76.6 ± 6.3	70.4 ± 10.0
BMI (kg/m^2)	22.9 ± 1.2	22.3 ± 2.5
Body fat (%)	22.8 ± 6.2	21.9 ± 4.7
VO2max (ml/kg/min)	42.6 ± 6.1	42.2 ± 5.0

6.2.3 Ambient Conditions

The experimental campaign was conducted in May/June 2012. The ambient temperature and relative humidity within the facility remained stable throughout the experimental campaign at 23.1 ± 1.0°C and 56 ± 8%, respectively. The ambient pressure at the facility was 682 ± 4mmHg. The FO_2 in the facility was maintained throughout the intervention at 0.139 ± 0.003mmHg. The partial pressure of inspired oxygen (P_IO_2) was calculated according to Equation 3. Accordingly, P_IO_2 was maintained at 88.2 ± 0.6mmHg throughout the hypoxic intervention. This equates to a simulated altitude of approximately 4,175m within the Planica facility (Gallagher and Hackett, 2004) (Please see appendix 11.7).

The current study was part of a larger research programme designed to investigate the effects of hypoxia, exercise and confinement on various aspects of human physiology. As such, some of the participant characteristics, heart rate (HR), capillary oxyhaemoglobin saturation (S_aO_2), arterial pressure (AP) and peak power output (PPO) were also reported by (Debevec et al., 2014; Keramidas et al., 2014). The participants were accommodated in the hypoxic facility for three days prior to the start of the confinement protocol, to allow for baseline experimental testing. All participants were confined to the hypoxic facility 24 hours per day for 10 days, which commenced at 09:00 on day 1 (D1). The participants completed the confinement period at 09:00 on day 11, so that the cumulative exposure for each individual was exactly 240 hours. The participants then remained in the facility for a further two days following the completion of the intervention to allow for the collection of post-intervention experimental data. A strict sleep/wake cycle was enforced so that lights on and wake-up occurred at 07:00 and lights out and sleep at 23:00. The participants were allocated two per room and had free access to a recreation area of approximately $200m^2$. The ascent to the simulated altitude of 4,175m was achieved immediately on entry into the hypoxic confinement area at 09:00 on D1. Consequently,

the signs and symptoms of acute mountain sickness (AMS) were monitored at 20:00 daily with the Lake Louise Mountain Sickness (LLMS) questionnaire (Roach et al., 1993) (please see appendix 11.3). This questionnaire is used to determine whether the number and severity of symptoms reported by the respondent can be attributed to AMS

6.2.4 Exercise Training

During the 10-day hypoxic confinement period, the participants in the LH-TH group performed two 60-minute, moderate intensity exercise sessions, daily on an indoor cycle ergometer. The bicycle was fitted with an indoor tyre (Schwalbe Insider Turbo Trainer Tyre, Reichshof, Germany) designed specifically for use with an indoor cycle turbo trainer (Elite SuperChrono Forte, Fontaniva, Italy). The participants completed the first daily exercise session in the morning and the second in the afternoon. All exercise sessions were performed in a designated training room under the same environmental conditions as during the normobaric hypoxic confinement. Participants' HR and SaO2 were monitored throughout the exercise using the iPad (Apple Inc., Cupertino, California, USA) controlled iBody system (iBody, Wahoo Fitness, Atlanta, USA) and finger oximetry device (3100 WristOx, Nonin Medicals, Minnesota, USA), respectively. To assess the relative influence of each exercise session on the participants, the training impulse (TRIMP) was calculated according to Equation 1 (Banister et al., 1975).

Prior to and immediately after the confinement period, all participants performed an incremental-load cycle ergometer test (Ergo Bike Premium, Daum electronics, Fürth, Germany) to volitional exhaustion. During the exercise test, the participants breathed a humidified hypoxic gas mixture (fraction of inspired oxygen content (F_IO_2) = 0.144) from a 200l Douglas bag using a low resistance two-way valve (2700 NRBV, Hans Rudolph Inc., Shawnee, USA) and an oronasal mask (7920 AL, Hans Rudolph Inc., Shawnee, USA). S_aO_2 and HR were measured continuously using (Nellcor, BCI 3301, Boulder, USA) and (iBody, Wahoo Fitness, Atlanta, USA), respectively. Maximal power (peak power output: PPO) was calculated according to Equation 2.

During the training sessions, the LH-TH group exercised at an intensity corresponding to their individual HR recorded at 50% of their Pmax attained during the hypoxic maximal exercise test. A researcher constantly monitored the HR to ensure that it was within the participants' targeted level (± 4 beats/min) and adjusted the load accordingly. The participants in the LH group remained sedentary throughout the confinement period and were not allowed to perform any type of dynamic or static exercise.

6.2.5 Procedures

A 24-hr recording was made of the proximal to distal skin-temperature (ΔTp-d) gradient, an index of vasoconstriction (Rubinstein and Sessler 1990), on three separate days. The ΔTp-d gradient was calculated by subtracting the distal skin temperature (Tsk) from the

proximal Tsk. Measurements were recorded from 10:00 on the day prior to entering the confinement condition (Pre) and on the 1st and 10th day of confinement (D1 and D10, respectively). Thermistor probes from the Modular Signal Recorder (MSR) data loggers (MSR145, Henggart, Switzerland) were secured to two sites using a breathable transparent film (Tegaderm, 3M, Minnesota, USA) and micropore tape (3M, Minnesota, USA). The ΔTp-d area chosen for measurement was the calf-toe (ΔTc-t) Tsk gradient. The Tsk measurement sites utilised for determination of ΔTc-t gradient were: a) proximal: the medial aspect of soleus, mid way between the medial malleolus and the medial condyle of the tibia and b) distally: the toe pad of the great toe. The measurement sites were on the non-dominant side.

6.2.6 Zeo

Sleep onset latency (SOL) was assessed with the Zeo Sleep Manager device (Zeo Inc, Boston, USA). The Zeo device has previously been validated against several polysomnography scorers (Shambroom et al., 2012) and found to be a relatively accurate device for use when a full night's polysomnography recording is not possible. The device was placed on the participants' forehead prior to sleep and initiated precisely at 23:00 to coincide with lights out. The device can be used to report details of sleep stages and wake after sleep onset. In the present study, the only measure used was the time of sleep onset. Therefore, the Zeo instrument was used to gather information in the baseline condition, night 2 (N2) and night 9 (N9) of the hypoxic confinement. Due to experimental logistics, the Zeo recorded SOL in 12 participants (LH-TH, N=7; LH, N=5).

Previous work from our laboratory has shown that over the course of a 10-day, sustained horizontal bed rest and hypoxic exposure, there is no significant difference in mean daily aural temperature (Chapter 4 (McDonnell et al., 2014a)). The participants of the current study were accommodated in a thermoneutral environment and therefore, it was deemed appropriate to measure body core temperature (Tb) once per day. Aural temperature was measured as an index of body temperature (Tb) at 07:00 daily using a ThermoScan Ear Thermometer (BRAUN 6023, BRAUN, Frankfurt, Germany). To obtain resting values, other pertinent physiological variables were also recorded at 07:00 just after waking. The staff nurse on duty at this time measured AP and HR with a Withings (Issy Les Mounlineaux, France) blood pressure monitoring device and the participants' S_aO_2 was monitored with a finger pulse oximetry device (3100 WristOx, Nonin Medicals, Minnesota, USA). The participants were required to stay in their beds resting in a supine position for at least 10 minutes until these data were recorded.

6.2.7 Data Analysis

The MSR data logger recorded the calf and toe Tsk at 1-minute intervals. The data was then averaged into 60-minute bins. In order to avoid masking the circadian rhythm of Tsk with exercise induced thermogenesis, the periods of time when the LH-TH group was

exercising: 1000 to 1200 and 1500 to 1700, were compared to the same periods of time in the LH group, see Figure 7.1. A 3-way fixed effects factorial analysis of variance (ANOVA) revealed no significant main effect for condition (LH-TH compared to LH) p=0.56. Therefore, these time points were kept in the main data set for the overall analysis. Furthermore, the hours when the participants ate: lunch 13:00 to 14:00 and dinner 19:00 to 20:00 were removed from the main data set. Recording was terminated after 24 hours in the morning following on from PRE, D1 and D10, respectively.

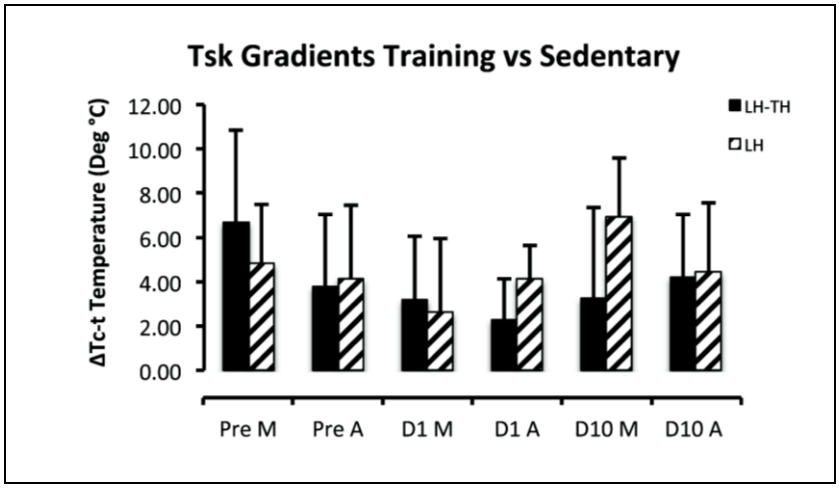


Figure 6.1: *Calf – Toe Temperature Gradients (ΔTc-t)*. The data presented are the average peripheral skin temperatures (Tsk, °C) for the calf – toe gradients (ΔTc-t). The ΔTc-t are presented for the time period during which the Live-High Train-High group (LH-TH: Black bar) completed their daily endurance training during either the morning (M: 10:00 to 12:00) or afternoon (A: 15:00 to 17:00). The same time periods are presented for the Live-High group (LH: Black and white striped bar) on the day before entering condition (Pre), Day 1 (D1) and Day 10 (D10) of the hypoxic condition. There were no significant differences between groups. Data are presented as means ± standard deviation.

To analyse the change in peripheral perfusion during the daytime, compared to the nighttime, the data were further collapsed into three time points, daytime (Day), transition period (Trans) and nighttime (Ni). The data was collapsed as follows: Day = 10:00 to 20:00 Trans = 20:00 to 23:00, Ni = 23:00 to 06:00. While data was recorded throughout the night until the following morning, there were no statistical changes in Tsk observed during the nighttime until 06:00 to 07:00 at which time some of the participants were in a pre-awake stage. Therefore, the timeframe of 23:00 to 06:00 was chosen to represent nighttime Tsk changes.

6.2.8 Statistics

Toe temperature changes represented by Tsk gradients were analysed using a 3-way mixed effects ANOVA (condition (LH-TH, LH) X testing period (Pre, D1, D10) X time

(Day, Trans, Ni)). Taural, HR, S_aO_2 responses, AP, LLMS and PPO (testing period: Pre, Post) were analysed using a repeated measures ANOVA (condition (LH-TH, LH) X testing period (D1, D10)). The Zeo SOL scores were analysed using a repeated measures ANOVA to compare the average on a daily basis between groups (condition (LH-TH, LH) X testing period (Pre, N2 and N9). Finally the TRIMPS values were analysed with a repeated measures ANOVA. When a main effect or an interaction was noted, Tukey post hoc test was used to define the specific differences. Values are reported as arithmetic mean ± standard deviation. The alpha level of significance was defined a priori at 5%. All statistical analyses were performed using Statistica 10.1 (StatSoft, Inc., Tulsa, Oklahoma, USA).

6.3 Results

6.3.1 Skin temperature gradients (ΔTc-t)

There were no differences noted in the ΔTc-t between the LH-TH and LH groups. Also, no differences were observed at any time across the intervention, i.e. Day, Trans and Ni ΔTc-t were the same regardless of the day (Pre, D1 or D10). However, there was a significant main effect found for changes in ΔTc-t from daytime to nighttime. Specifically, in the LH group, Ni ΔTc-t was significantly lower than Day ΔTc-t on all 3 days (Pre, D1 and D10; $p < 0.05$), indicating a significant vasodilatation of the toe from Day to Ni. In the training group (LH-TH), a similar difference was found between Day and Ni at the Pre measurement and on D10 ($p < 0.05$). There was no significant day-to-night difference measured on D1 in the LH-TH group. The Tsk gradients are presented in Figure 6.2.

Table 6.2: *Cardiovascular variables*. Heart rate (HR), capillary oxyhaemoglobin (S_aO_2), Lake Louise Mountain Sickness Score (LLMS, recorded at 20:00), arterial pressure (AP) and aural temperature (Taural) recorded at 07:00 on D1 and D10. Data are presented as mean ± standard deviation. Live-High Train-High group, N = 8 (LH-TH) and Live-High group, N = 6 (LH).

Condition	Day	HR (bpm)	S_aO_2 (%)	LLMS	AP (mmHg)		Taural (°C)
					Systolic	Diastolic	
LH-TH	D1	100.2 ± 12.9	80.9 ± 2.8	2 ± 1.4	124 ± 8.1	73.0 ± 7.0	36.2 ± 0.3
	D10	78.1 ± 12.6 *	87.3 ± 3.4 *	1.4 ± 0.9	120.5 ± 7.6	73.4 ± 13.7	36.3 ± 0.2
LH	D1	89.5 ± 13.9	84.7 ± 4	2.3 ± 2.3	114.8 ± 11.5	71.4 ± 9.3	36.1 ± 0.5
	D10	84 ±14.6	88.3 ± 1.8	1.7 ± 2.7	116.8 ± 10.3	73.5 ± 9.4	36.2 ± 0.4

* Denotes a significant difference between D1 and D10. There were no differences between groups.

6.3.2 Sleep onset latency

There was no difference in the time of sleep onset latency on any of the nights or between LH-TH and LH groups. The SOL for the LH-TH group were: Pre, 16 ± 15 minutes; N2, 8 ± 15 minutes; N9, 22 ± 19 minutes. For the LH group the SOL times were: Pre, 17 ± 20 minutes; N2, 7 ± 13 minutes; N9, 9 ± 8 minutes.

6.3.3 Lake Louise Mountain Sickness Score (LLMS)

The average LLMS scores are presented in Table 6.2 (please see appendix 11.3). All participants adapted well to the hypoxic stimulus and as such there were no differences between the groups at any measured time point.

6.3.4 Heart Rate, capillary oxyhaemoglobin saturation, arterial pressure and aural temperature

Heart rate decreased from D1 to D10 in the LH-TH group ($p < 0.05$), whereas there were no temporal differences in the LH group (Table 6.2). Furthermore, there were no differences noted between the LH-TH and LH groups. There were no changes in AP in either group (Table 6.2). A significant ($p < 0.05$) main effect was revealed for the S_aO_2 values across time (Table 6.2). Upon further analysis, it was noted that the LH-TH S_aO_2 values significantly increased ($p < 0.05$) from D1 to D10. S_aO_2 values did not change from D1 to D10 in the LH group. Additionally, there were no differences between the LH-TH and LH groups. There were no differences noted in the Aural Temperature in either group or across time (Table 6.2).

6.3.5 Daily Training Sessions

All exercise sessions were carried out under the same environmental conditions as during the normobaric hypoxic confinement in this study, $F_IO_2 = 0.140 \pm 0.004$ and $P_IO_2 = 88.8 \pm 0.6$ mmHg. No changes in HR were detected across the entire exercise intervention with an average HR of 142 ± 11bpm. The S_aO_2 values during the exercise were stable throughout the intervention at 84 ± 3%. Subsequently, the TRIMP values did not differ throughout the campaign; TRIMP values were 40.0 ± 0.5 per exercise session.

6.3.6 Incremental Exercise

As a result of the daily training, PPO measured during the incremental exercise test was higher in the LH-TH group after the intervention (Pre: 259 ± 34 and Post: 276 ± 34W, $p < 0.05$). There was no change in S_aO_2 (Pre: 75 ± 3 and Post: 77 ± 4%). However, there was a decrease ($p < 0.05$) noted in the peak HR of the LH-TH group from Pre to Post, 184 ± 4 and 177 ± 6bpm, respectively. Conversely, there was no change in the PPO of the LH group (Pre: 244 ± 37 and Post: 247 ± 41W) or in the peak values of S_aO_2 (Pre: 75 ± 5 and Post: 78 ± 5%) or HR (Pre: 188 ± 4 and Post: 184 ± 8bpm) following the hypoxic exposure. These values have also been reported by Debevec et al., (2014).

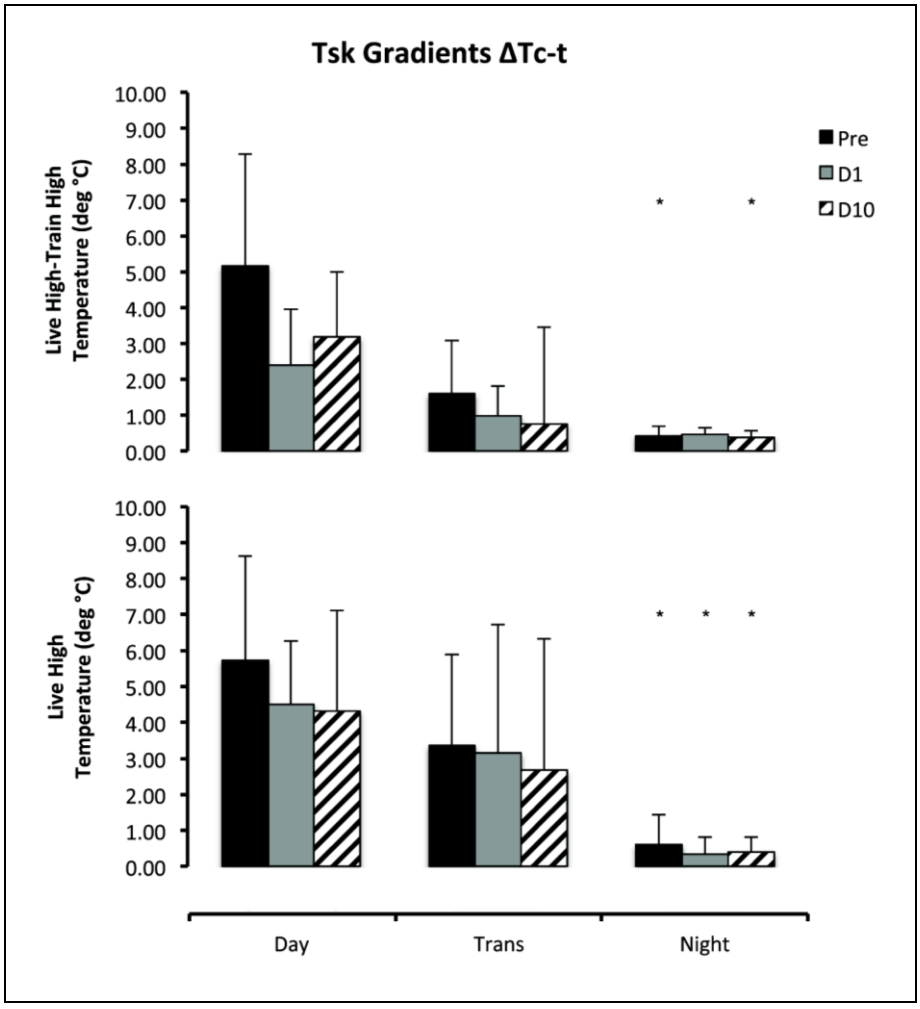


Figure 6.2: *Calf – Toe Temperature Gradients (ΔTc-t)*. The data presented are the peripheral skin temperatures (Tsk, °C) for the calf – toe gradients (ΔTc-t) collapsed into three time points, daytime (Day: 10:00 to 20:00), transition period (Trans: 20:00 to 23:00) and nighttime (Ni: 23:00 to 06:00). ΔTc-t gradients are presented for the Live-High Train-High group (LH-TH) upper panel and the Live-High group (LH) lower panel on the day before entering condition (Pre, Solid black bar), Day 1 (D1, Solid grey bar) and Day 10 (D10, Striped black and white bar). * Denotes a significant difference between Day and Ni. Data are presented as means ± standard deviation.

6.4 Discussion

The main finding from the present study was that the toe perfusion significantly increased from daytime to nighttime, regardless of condition (LH-TH or LH). Secondly, there was no difference in toe perfusion between the sedentary group (LH) and the training group (LH-TH), either during acute (D1) or prolonged exposure to hypoxia (D10). Finally, there was no difference in the time to sleep onset in either group.

We have previously shown that in a hypoxic confinement scenario there was a significant nocturnal vasodilatation of the toe (Chapter 4 (McDonnell et al., 2014a)). The results from the current study confirm our previous findings, that there is a significant increase in the perfusion of the toe prior to sleep (Figure 6.2). There was no difference between the Pre measure and D1 indicating that there was no acute affect of hypoxia on toe perfusion. Additionally, there was no change in ΔTc-t as a result of the adaptation to hypoxia (D1 to D10).

6.4.1 Sleep

Insomnia and poor sleep are among the main symptoms of AMS. At altitudes above 2500m (Moderate Altitude, (Bartsch and Saltin, 2008)), the occurrence of AMS is likely and the incidence enhanced by increased rate of ascent and total altitude gained (Beidleman et al., 2014). Basnyat and Murdoch, (2003) reported that between 50 and 84% of individuals travelling to altitude of 3,800m to 4,000m are likely to develop AMS symptoms. Additionally, Honigman et al., (1993) indicate that approximately 30% of tourists at altitude suffer some form of sleep disturbance above 2,000m. While the current results show both groups (LH-TH and LH) were under the threshold for AMS according to the LLMS questionnaire (Table 6.2), 4 participants in the LH-TH and 3 in the LH group did exhibit signs of AMS on D1.

Morrison et al., (2013) carried out a full nights' polysomnography on the participants in the current study on the 1st and the 10th night of the hypoxic exposure. They indicated that there was no difference between the groups (LH and LH-TH) in total sleep time on night 1 or on night 10. Krauchi et al., (1999) stated that the degree to which the toe temperature increases prior to sleep is the best indicator of a rapid sleep onset. Also it is well established that SOL is a good predictor of the quality of sleep throughout the night particularly related to total sleep time (TST) and the number of awakenings after sleep onset (WASO). Additionally, Rojc et al., (2014), found that following 10-days of hypoxic bed rest or hypoxic confinement, there was no effect of hypoxia on TST, WASO, sleep efficiency, or the number of stage shifts. With regard to the current study, we found no differences in SOL, measured with the Zeo on N2 or N9. While there were other changes in the sleep architecture noted i.e. increased number of apnoeas (Morrison et al., 2013), these data provide evidence that the factors related to toe temperature (TST, WASO and SOL) are not affected by a hypoxic influence on toe perfusion (Krauchi et al., 1999; Raymann et al., 2008).

6.4.2 Exercise

Endurance exercising at altitude will induce greater hypoxaemia than will endurance exercising at sea level, which has a direct link to AMS and the severity of AMS symptoms (Roach et al., 2000; Roach et al., 2007; Roach et al., 2014). However, the current results are in agreement with Schommer et al., (2012), as we found no difference in the AMS symptoms reported by our participants between the LH and LH-TH groups. Our results indicate the LH-TH group adapted to the exercise stimulus over the course of the 10-day confinement; HR decreased significantly over time and S_aO_2 increased to the same level as that of the LH group.

There were no differences found in the ΔTc-t during the LH-TH exercise period (10:00 - 12:00 and 14:00 - 16:00) compared to the same time period in the LH group (Figure 7.1). While PPO increased only in the LH-TH group, indicating an adaptation to training, it can be assumed there were other physiological changes as a result of training. However, we did not observe an increase in cutaneous blood flow in the LH-TH as suggested by Fritzsche and Coyle, (2000). Thus, we can conclude that exercise training for 2 hours per day did not have an effect on ΔTc-t and did not reduce sleep onset latency at altitude.

6.4.3 Hypoxia

There is an increase in sympathetic drive during exposure to hypoxia, which, however, as regards forearm or calf blood flow, may be masked by locally mediated hypoxic vasodilatation (Weisbrod et al., 2001). Sagawa et al., (1986) found decreased finger perfusion in response to hypoxia during heat exposure, suggesting that there are regional variations in the hypoxic control of blood flow. Hypoxia stimulates the carotid bodies, which in turn increases sympathetic activity. Additionally, Leuenberger et al., (2001) found that hypoxia increased sympathetic drive to skeletal muscle as a result of the stimulation of the arterial chemoreceptors, whereas, Pollock et al., (2014) and Heffernan and Muller, (2014) found differential controls of vasomotor tone in the muscle versus the skin while breathing a hypoxic gas. Finally, Simmons et al., (2011), report that during prolonged hypoxic exposure (45 minutes), cutaneous vasoconstriction was increased while core cooling rate was not consistently affected, compared to only 5 minutes of hypoxia where Simmons et al., (2007b) found cutaneous vasodilation. It is possible that this trend continues with increased time in a hypoxic environment (McDonnell et al., 2014a).

Krauchi and Wirz-Justice, (1994) demonstrated that whether or not the hands and feet are covered or uncovered they follow the same pattern of nocturnal vasodilatation. Thus suggesting that a nonthermal factor was influencing and controlling the nocturnal vasodilatation noted in the current study. As regards the hypoxic effects on the regulation of cutaneous blood flow, these appear to be mediated by peripheral rather than central

chemoreceptors (Heffernan and Muller, 2014). Furthermore, the nocturnal vasodilatation noted in the present study must certainly have involved a release of the hypoxic vasoconstriction noted during the daytime. Since, in the context of sleep pattern, toe temperature predominantly influences the initiation of sleep (Krauchi et al., 1999, 2000) and because in the present hypoxia exposures we noted normal nocturnal vasodilatation and no change in SOL, one could argue that hypoxia-induced sleep disturbances are likely induced on a central nervous level and not by disturbances in cutaneous vasomotor tone.

With regard to alpinists, or those taking part in a hypobaric LH-TH programme where ambient temperature will not be thermoneutral, our finding of a preserved, nocturnal cutaneous vasodilatation in hypoxia alleviates some concerns over nocturnal risk of cold injury.

6.4.4 Circadian Rhythm

Previously, we asserted that a nonthermal factor associated with the regulation and maintenance of the circadian rhythm of toe perfusion was driving nocturnal vasodilatation. Based on the literature one such factor is melatonin. The ingestion of melatonin significantly increases peripheral Tsk, reduces Tb and increases subjective sleepiness (Krauchi et al., 1997). Moreover, the decrease in Tb is instigated via distal vasodilatation (Krauchi et al., 2000). Melatonin reaches a maximum blood concentration close to the nadir of Tb, around 04:00, with approximately 50% at midnight. However, melatonin has a vasodilatory effect (Markwald et al., 2009), thus suggesting that only small amounts of naturally secreted, rather than ingested, melatonin is required to initiate nocturnal heat loss prior to sleep onset.

While there was no statistical difference noted in ΔTc-t during the daytime from PRE to D1, the participants tended to be more vasoconstricted in Pre. This is most likely the result of a time constraint and an interactional effect with other studies carried out on the participants. They were involved in a cold stress test during the morning of Pre for two periods of 30 minutes over the course of 4 hours (Keramidas et al., 2014). This seems to be the most likely reason that we see ΔTc-t values of between 5 and 6°C during our Pre measure.

6.5 Conclusions

The present study tested the hypothesis that increased activity in a normobaric hypoxic facility replicating a LH-TH scenario would reduce the daytime hypoxia-induced vasoconstriction and thus limit the risk of cold injury to individuals exposed to high altitude and cold. Furthermore, we tested the concept that increased exercise and training during confinement would reduce the daytime vasoconstriction and have a positive effect on sleep onset. Compared to extreme inactivity such as sustained bed rest, simply being ambulatory and performing light daily physical activity will decrease any hypoxia-

induced vasoconstriction. However, further increasing the level of activity appears not to be of any further benefit. Finally, there was no change in SOL times, which may indicate that any change in sleep architecture throughout the night is mediated through central mechanisms rather than peripheral.

7 The Effect of a Live-High Train-High Regimen on Emotional State

7.1 Introduction

Winter athletes regularly train at high altitude ski resorts during the Summer months to take advantage of the available snow conditions. This type of regimen is termed Live-High Train-High (LH-TH) and has also been used in normobaric hypoxic facilities to elicit adaptations that may benefit physical endurance performance not only at altitude, but also at sea level (Bonne et al., 2014; Friedmann-Bette, 2008; Saunders et al., 2009). Adaptation to altitude is normally assessed utilising criterion tests of performance, which evaluate primarily the autonomic responses and ignore the contribution of LH-TH to psychological strain, if any. A study on the effects of a 31-day exposure to hypobaric hypoxia, (Bolmont et al., 2000) has shown deterioration of mood, particularly dysthymia, as manifested by impaired cognition, alertness and vigor, combined with increased negative mood, fatigue and confusion (Bahrke and Shukitt-Hale, 1993). The level of physical activity will also have an effect on the emotional state of an athlete; whereas, moderate activity may have a positive effect (Yeung, 1996), high-intensity activity may potentiate negative emotions (Ekkekakis et al., 2011; Steptoe and Cox, 1988). The principal aim of the present study was therefore to assess this interaction of hypoxia and the level of activity during a LH-TH regimen.

While it is well established that hypoxia reduces endurance exercise performance, by way of several physiological mechanisms (Lundby and Damsgaard, 2006), the effects of hypoxic exposure on psychology have largely been ignored. It seems that examining the effect of hypoxia on neuropsychological pathways is important, since athletes during their performance require high levels of physical exertion, coordination, stability and emotional control (Acevedo and Ekkekakis, 2001; Virues-Ortega et al., 2004).

Since the process of altitude acclimatisation varies substantially between individuals, the level of emotional disturbances in hypoxic environments may also be highly individualised, regarding the level and type of emotional disturbance as well as the time duration needed to adapt. For this reason a range of psychological indices were assessed in the present study using the Profile of Mood State (POMS; (McNair et al., 1971) and Positive and Negative Affect Schedule (PANAS; (Watson et al., 1988). The POMS and PANAS instruments provide information on various emotional responses as well as the interaction of these responses (Abraini et al., 1998; Bolmont et al., 2000). Notably, Bolmont et al., (2000) acknowledged the importance of examining the interplay between

mood dimensions in hypoxic environments and demonstrated significant interaction between psychomotor ability, mental efficiency and POMS mood dimensions, including confusion, fatigue and tension. Providing further support for the theory of mood interaction proposed by Bolmont et al., (2000), Lane et al., (2001) who dichotomised participants into low and high depression groups, concluded that small indicators of depressed mood have a large impact on other mood states. Furthermore, Lane et al., (2004) reported that the role of depression appears to be critical to the formulation of the other negative moods, even when the level of depression is low.

The present study assessed the effect of a LH-TH regimen on emotional state, as well as on the interaction between psychological indices. We tested the hypothesis that hypoxia would have a detrimental effect on mood which would be counteracted by the beneficial effect of exercise, *per se*.

7.2 Materials and methods

7.2.1 Participant selection

The present study was carried out on the same male participants as described in Chapter 7, however, the focus of the current study was on the participants psychological indices. All participants gave their written informed consent to participate in the study. The study was approved by the National Medical Ethics Committee of the Republic of Slovenia and performed according to the guidelines of the Declaration of Helsinki.

As aforementioned, participants were low-land residents (<500m) and were recreationally active healthy males. A complete data set was obtained from fourteen participants: eight participants in the LH-TH group and six participants in the LH group. There were no statistical differences observed between the groups for the aforementioned physical characteristics (Table 6.1).

7.2.2 Experimental Design

The experimental protocol was performed in the hypoxic facility of the Olympic Sport Centre Planica (Rateče, Slovenia). The details of the basic experimental design have been outlined in Chapter 6.2.3 and 6.2.4. Further details, specific to testing the emotional status of the participants can be found below.

7.2.3 Procedures

7.2.3.1 Profile of Mood States (POMS)

The participants' mood state was measured using the POMS-Short Form (McNair et al., 1971; Shacham, 1983). The POMS instrument (please see appendix 11.4) is a 37-item self-evaluation questionnaire of six subscales: tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia and confusion-bewilderment. Participants' provided a description of their feelings based on a 5-point Likert scale, where anchors of 0 and 4 represent "not at all" and "extremely", respectively. The total mood disturbance (TMD) was calculated according to Equation 4 (Curran et al., 1995).

7.2.3.2 Positive and Negative Affect Schedule (PANAS)

PANAS is a self-report instrument comprising two 10-item moods and was developed to provide measures of Positive Affect (PA: 10 items) and Negative Affect (NA: 10 items) (Watson et al., 1988)(please see appendix 11.5). Respondents were asked to rate the extent to which they experienced each of the emotions described in the PANAS. Participants answer each item based on a 5-point Likert scale with anchors of 1 "very slightly or not at all" to 5 "very much", with intermediate points of 2 repressing "a little", 3 "moderately" and 4 "quite a bit". Each affect factors (PA and NA) total score was produced by adding the 10-items' score in each factor together. Therefore, a total score for each affect factor could range from 10 to 50.

The POMS and PANAS are two well-established instruments in the sport and exercise setting and were used to provide comparable results with previous studies, thus avoiding methodological concerns of different instruments used. The POMS and PANAS questionnaires were presented to the participants using a bespoke iPad application called PlanHab (Pilon, Ljubljana, Slovenia). The PlanHab application delivered the questionnaires to the iPad and prompted the participants to complete the POMS and PANAS questionnaires at the appropriate times. Should the participants fail to answer the questionnaires in a timely fashion, the iPad sounded a reminder tone and the researcher overseeing the PlanHab application (questionnaires) was notified. The results were then stored on a secure server for later analysis. The participants were asked to complete the questionnaires immediately on completion of any prevailing experimental tasks or activities. The POMS and the PANAS were recorded during the baseline measurement days, specifically two days before the onset of the intervention (Pre). Thereafter, the questionnaires were completed on the 3rd (D3), 7th (D7) and 10th (D10) day of the intervention and on the second day of recovery (Post). The participants completed the questionnaires based on how they felt at the exact time of responding to the assessment tool.

7.2.3.3 Lake Louis Mountain Sickness (LLMS)

Due to the possibility that acute mountain sickness (AMS) might occur on the ascent to altitude, its presence was assessed with the Lake Louis Mountain Sickness (LLMS) (please see appendix 11.3) questionnaire (Roach et al., 1993), which was administered and completed daily at 20:00 from D1 to D10. The LLMS questionnaire is self-reported and involves the participants rating their own perception of five symptoms. The participants were asked to rate each of the following on a scale from 0 to 3: headache, gastrointestinal symptoms, fatigue and/or weakness, dizziness or light-headedness and difficulty sleeping.

7.2.3.4 Cardiovascular variabless

In order to monitor the participants' well-being, cardiovascular parameters were measured daily at 07:00. The staff nurse on duty measured arterial pressure (AP) and Heart Rate (HR) with a Withings (Issy Les Mounlineaux, France) blood pressure monitoring device. Measurements of AP, HR and S_aO_2 were recorded at 07:00 just after waking and prior to the participants getting out of bed.

7.2.4 Safety Procedures

For safety reasons, all participants wore a personal clip-on oxygen analyser (Rae PGM-1100, California, USA) throughout the whole experimental period. An audible alarm would be activated in the event that an individual's oxygen level decreased below a pre-set level. A diagnosis of AMS can be given if the individual has ascended to altitude within the last four days with the presence of a headache, plus one other symptom and a total score of 3 or more. A total score of 6 or more represents severe AMS. The individual presenting with AMS should not ascend further and, if symptoms deteriorate, an immediate descent is warranted. As mentioned, the ascent to the simulated altitude of 4,000m was achieved immediately on D1 at 09:00. In the event that a participant presented with an adverse reaction to altitude, the provision was made for the project physician to assess their AMS symptoms. If the symptoms continued or deteriorated, the project physician would make a decision whether to maintain the current altitude, to descend or ultimately exclude the participant from the study. If the decision was to descend, the participant could be moved to a separate room where the altitude level could be controlled individually via the VPSA control unit, thereby allowing continued involvement in the study but at a lower safer altitude until the AMS symptoms subsided. In order to monitor the participants' well being, HR and S_aO_2 responses were monitored using personal finger oximetry devices (3100 WristOx, Nonin Medicals, Minnesota, USA) throughout the intervention.

7.2.5 Statistics

Data are reported as mean ± standard deviation, unless indicated otherwise. Differences

between the two experimental conditions were examined using independent-samples t-test. The PANAS and POMS subscales were analysed using a one-way repeated ANOVA. Tukey HSD post hoc test was used to define the differences among the five time measures (Pre, D3, D7, D10, Post) when the ANOVA analysis indicated a significant F-ratio. In cases where the assumptions of sphericity were not met in the within-subjects repeated measures analyses, based on Mauchly's test of sphericity results, the Green-House Geisser correction was applied for subsequent F statistic estimation, as well as, for the corresponding degrees of freedom (Field, 2009; Tabachnick and Fidell, 2012). The significance level was set at $p<0.05$. The Statistical Package for Social Sciences (SPSS 21.0 Win) was used for the statistical analyses.

7.3 Results

7.3.1 Ambient Conditions

The experimental campaign was conducted in May/June 2012 at the Olympic Sports Centre, Planica, situated at an altitude of 940m. The ambient temperature and relative humidity within the facility remained stable at 23.1 ± 1.0°C and 56 ± 8%, respectively. The average ambient pressure at the facility was 682 ± 4mmHg. The ascent to a simulated altitude was achieved immediately at 09:00 on D1 such that FO_2 on D1 and for the remainder of the intervention was 0.139 ± 0.003mmHg. The partial pressure of inspired oxygen was calculated according to Equation 3. Accordingly, P_IO_2 was maintained at 88.2 ± 0.6mmHg throughout the hypoxic intervention. This equates to a simulated altitude of 4,175m within the Planica facility (Gallagher and Hackett, 2004).

7.3.2 Heart rate, capillary oxyhaemoglobin saturation and arterial pressure

HR was significantly lower on D7 and D10 than on D1 and D3 in the LH-TH group, Figure 7.1A. There were no differences in the LH group. There were also no differences between the two groups. S_aO_2 values were different on D3 between the groups; for the LH-TH it was 82.9 ± 3.3% and significantly lower than that of the LH group, which had average values of 87.7 ± 1.7% for the LH group ($p < 0.05$). S_aO_2 increased from D1 to D7 and to D10 in the LH-TH group ($p < 0.05$). S_aO_2 values did not change from D1 to D10 in the LH group, Fig 7.1B. Systolic arterial pressure was increased on D3 in the LH-TH group compared to the LH group, $p = 0.054$. Systolic arterial pressure was reduced on D10 compared to the values on D3 in the LH-TH group ($p < 0.05$). There were no other changes in arterial pressure, (Figure 7.2).

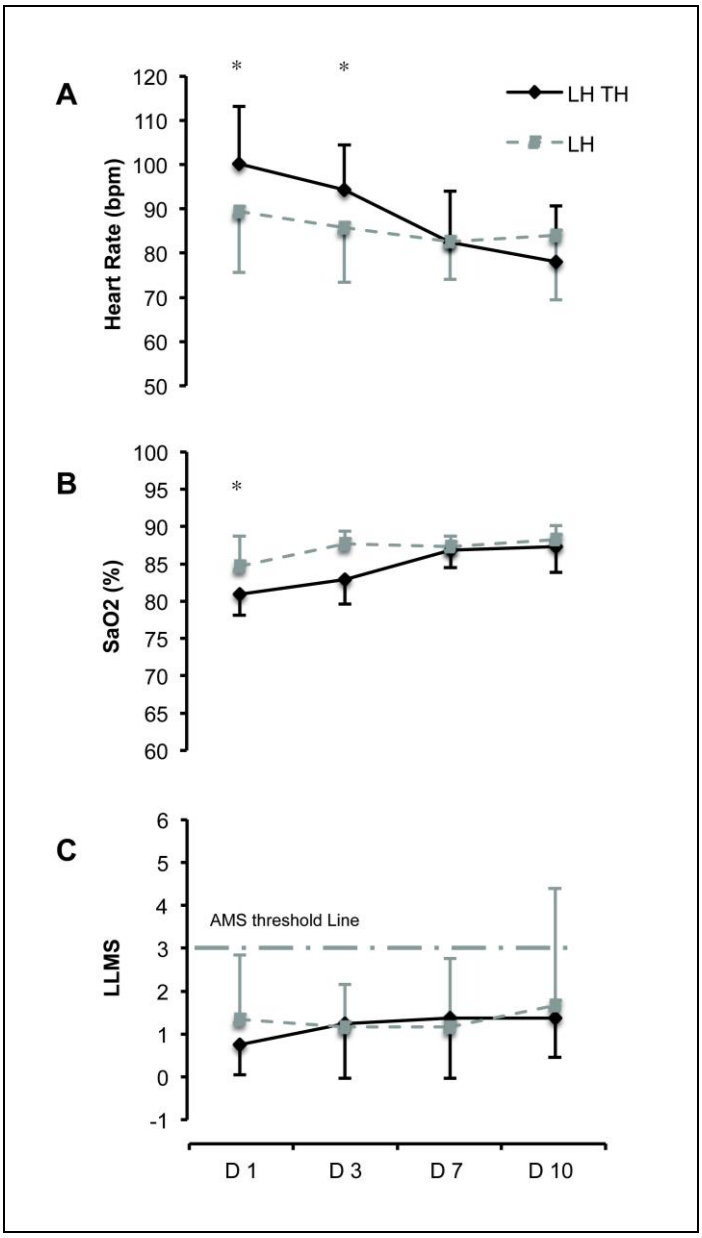


Figure 7.1: *Cardiovascular Variables*. **(A)** Heart Rate (HR); **(B)** Capillary oxyhaemoglobin saturation (S_aO_2); **(C)** Lake Louise Mountain Sickness score (LLMS) are presented for day 1 (D1), day 3 (D3), day 7 (D7) and day 10 (D10) of the confinement period. Live-High Train-High (LH-TH: Solid black line); Live-High (LH: broken grey line). Data are means ± standard deviation. * Significant difference compared to D7 and D10 in the LH-TH condition.

7.3.3 Lake Louise Mountain Sickness (LLMS)

The average LLMS scores recorded across the 10-day intervention for the exercise group (LH-TH) were 1.2 ± 0.3 and for the sedentary group (LH) 1.3 ± 0.2. All participants adapted well to the hypoxic stimulus and as such there were no differences between the groups at any measured time point. For more detail, LLMS scores are presented in Figure 7.1C. On D10 one participant in the LH group registered a 7 on the LLMS scale and complained of headache. As these symptoms presented on D10 of the campaign they were attributed to confinement *per se* and not to acute mountain sickness. The attending

medical officer cleared all participants exhibiting any symptoms of mild AMS for continuation in the study. Therefore, it was not necessary to invoke the safety procedures to alleviate the participant's symptoms of AMS.

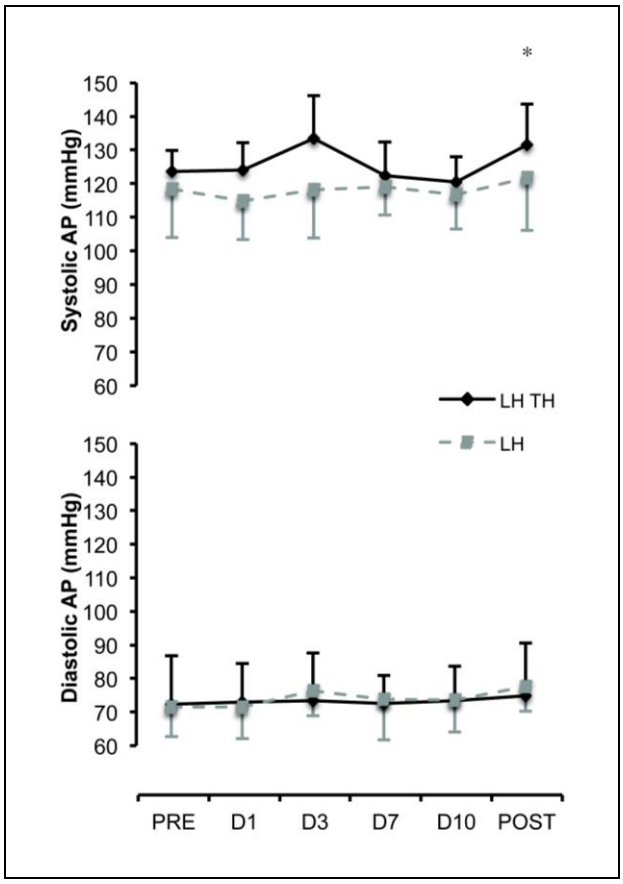


Figure 7.2: *Arterial Pressure*. The participants' arterial pressure (AP) is presented for baseline (Pre), day 1 (D1), day 3 (D3), day 7 (D7) and day 10 (D10) of the confinement period and for the post intervention measure (Post). Systolic AP is presented in the upper panel and Diastolic AP is presented in the lower panel. Live-High Train-High (LH-TH: Solid black line); Live-High (LH: broken grey line). Data are means ± standard deviation. * Significant difference compared to D3 in the LH-TH condition.

7.3.4 Daily exercise training sessions

All exercise sessions were performed in a designated training room under similar environmental conditions as during the normobaric hypoxic confinement, F_IO_2 = 0.140 ± 0.004 and P_IO_2 = 88.8 ± 0.6mmHg. There were no differences in any of the variables measured during the morning and afternoon exercise training. No changes in HR were detected across the entire exercise intervention, with an average HR of 142 ± 11bpm. The S_aO_2 values during the exercise were stable throughout the intervention at 84 ± 3%. As expected the TRIMP values did not differ throughout the intervention: the average TRIMP value per training session was 40 ± 0.5.

7.3.5 POMS and PANAS subscales differences

There were no differences in the PANAS subscales (PA and NA) across the five measures in either the LH (PA: F = 0.309, p = 0.788 or NA: F = 0.748, p = 0.496) or in the LH-TH groups (PA: F = 1.052, p = 0.357 or NA: F = 0.360, p = 0.700), Figure 7.3. Regarding the POMS subscales, there were no differences measured at any time points for depression, (LH-TH: F = 0.191, p = 0.706; LH: F = 1.000, p = 0.363), vigor (LH-TH: F = 1.159, p = 0.345; LH: F = .909, p = 0.416), confusion (LH-TH: F = 0.123, p = 0.833; LH: F = 0.406, p = 0.608), tension (LH-TH: F = 0.186, p = 0.760; LH: F = 1.210, p = 0.325), anger (LH-TH: F = .278, p = .735; LH: F = 0.505, p = 0.578) or fatigue (LH-TH: F = 0.821, p = 0.470; LH: F = 0.357, p = 0.718) in either group, Figure 7.4.

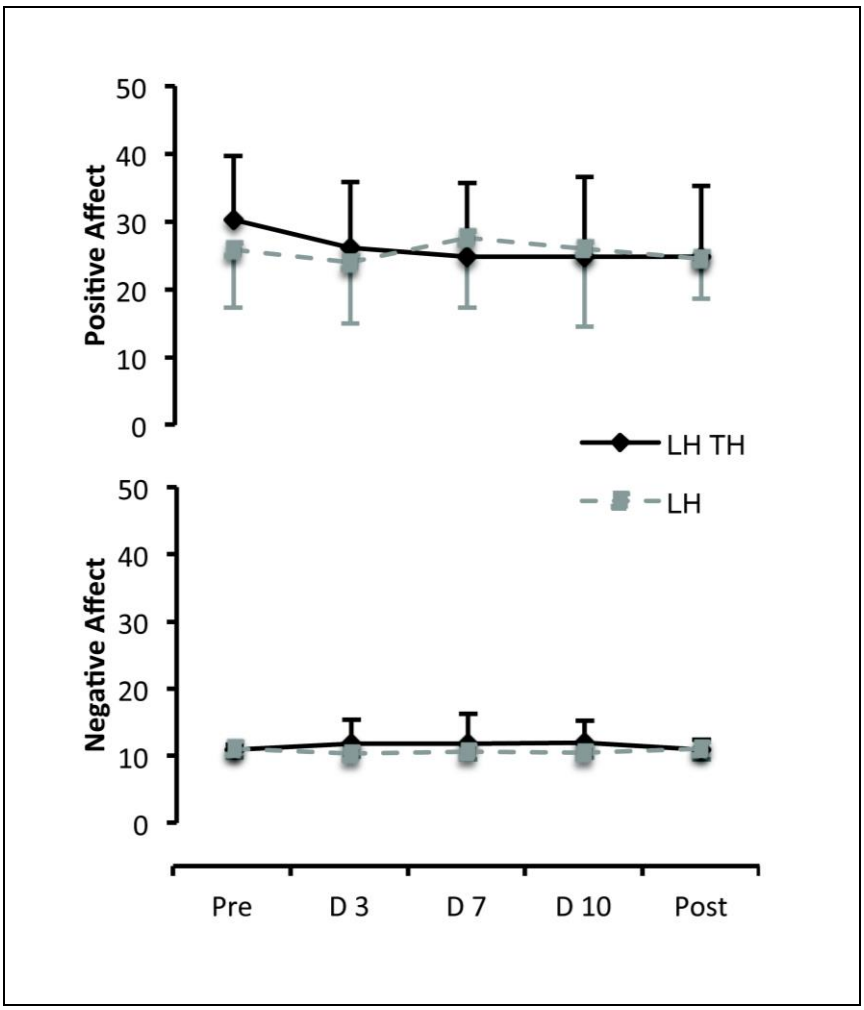


Figure 7.3: *Positive Affect Negative Affect Schedule.* The participants' subjective ratings of the Positive Affect Negative Affect Schedule (PANAS) are presented for each group: Live-High Train-High (LH-TH: Solid black line); Live-High (LH: broken grey line). Positive Affect (PA) is presented in the upper panel, while Negative Affect (NA) is presented in the lower panel for each time point (PRE, D3, D7, D10 and POST). Data are means ± standard deviation.

Additionally, no differences were revealed in the TMD (LH-TH: F = 0.309, p = 0.647; LH: F = 0.1325, p = 0.808) of either group. Consequently, there were no differences

between the LH and LH-TH groups in PANAS and POMS subscales at any of the five time measures (Pre, D3, D7, D10 and Post), Figure 7.5.

Table 7.1: *Profile of Mood State*. The data presented are the correlation values among the POMS subscales.

Variable		Depression	Vigor	Confusion	Tension	Anger
Vigor	PRE	-0.14				
	D3	-0.38				
	D7	-0.39				
	D10	-0.45				
	POST	-0.52				
Confusion	PRE	0.71	-0.28			
	D3	0.92	-0.36			
	D7	0.73	-0.51			
	D10	0.69	-0.51			
	POST	0.84	-0.49			
Tension	PRE	0.10	-0.02	0.32		
	D3	0.94	-0.45	0.92		
	D7	0.99	-0.43	0.72		
	D10	0.81	-0.42	0.48		
	POST	0.74	-0.53	0.76		
Anger	PRE	0.93	-0.10	0.61	0.02	
	D3	0.97	-0.39	0.94	0.94	
	D7	0.98	-0.45	0.70	0.99	
	D10	0.56	-0.59	0.50	0.56	
	POST	0.52	-0.37	0.42	0.18	
Fatigue	PRE	0.10	-0.39	0.15	0.21	0.09
	D3	0.80	-0.58	0.82	0.80	0.74
	D7	0.79	-0.45	0.57	0.80	0.83
	D10	0.71	-0.46	0.71	0.57	0.45
	POST	0.35	-0.42	0.26	0.29	0.59

Value correlations above ± 0.78 significant at $p<0.001$, above ± 0.66 at $p<0.01$ and above ± 0.54 at $p<0.05$.

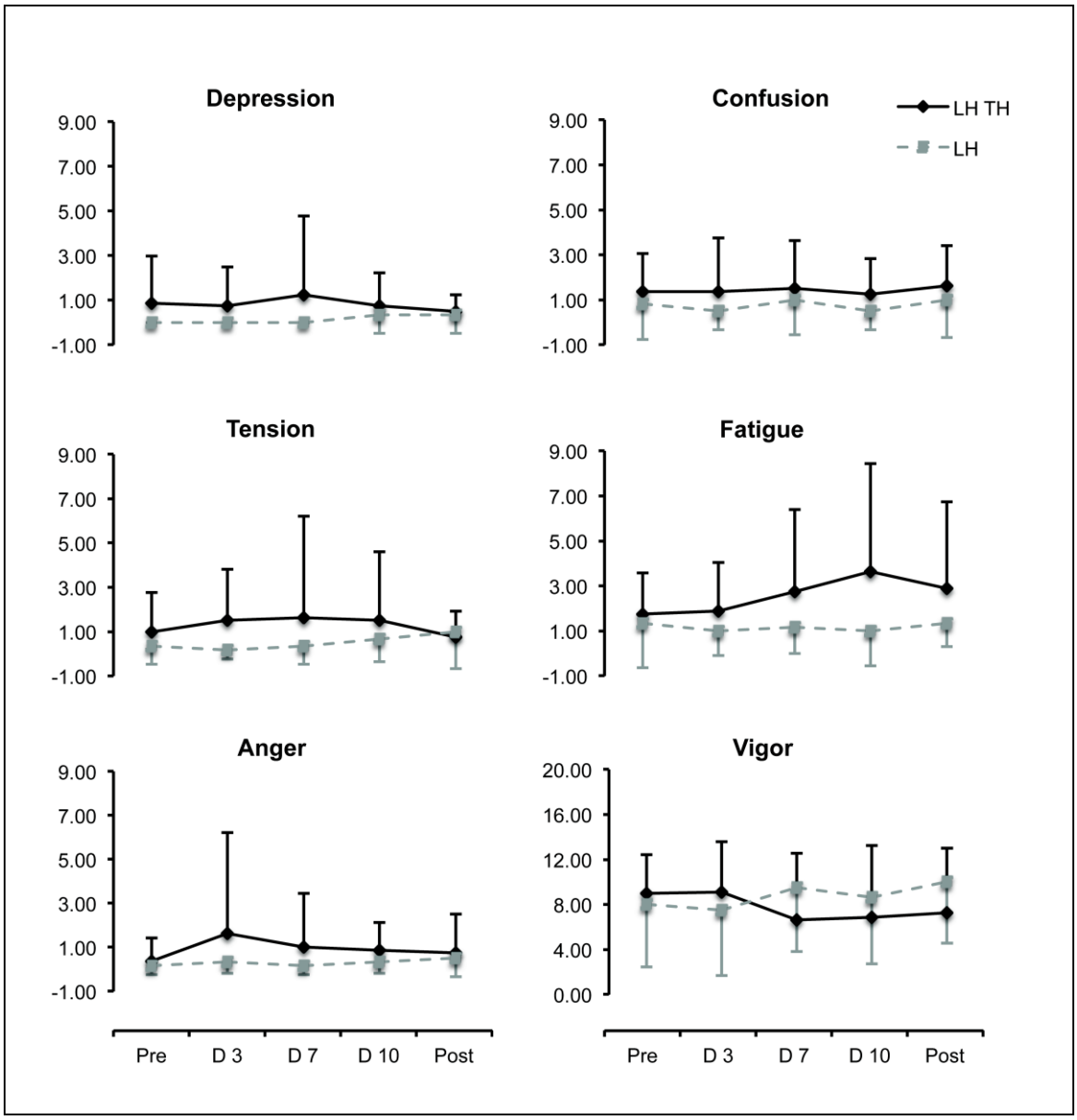


Figure 7.4: *Profile of Mood State*. The participants' subjective ratings for each of the Profile of Mood States (POMS) subscales (Depression, Confusion, Anger, Tension, Fatigue and Vigor) are presented for each condition: Live-High Train-High (LH-TH: Solid black line); Live-High (LH: broken grey line). Data are means ± standard deviation and are presented for each time point (Pre, D3, D7, D10 and Post).

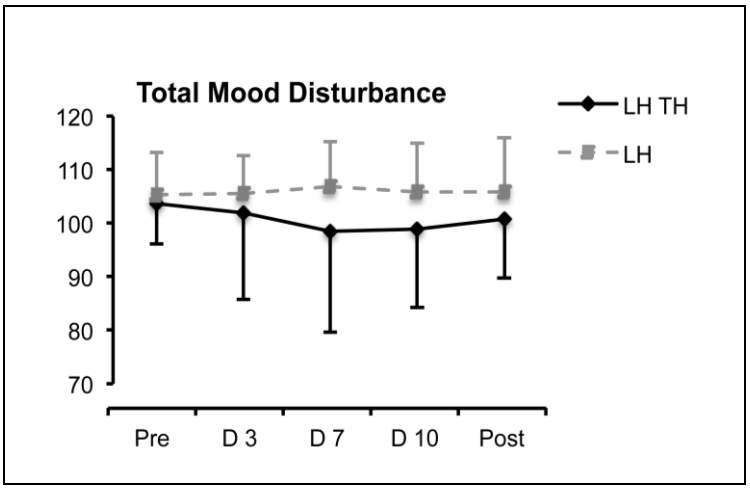


Figure 7.5: *Total Mood Disturbance*. The participants Total Mood Disturbance (TMD) scores are presented for each group: Live-High Train-High (LH-TH: Solid black line), Live-High (LH: broken grey line. The TMD score is calculated from the POMS subscales and are presented for each time point (PRE, D3, D7, D10 and POST). Data are means ± standard deviation.

7.3.6 POMS and PANAS subscales correlations

There were positive correlations among the negative POMS subscales (depression, confusion, tension, anger, and fatigue), Table 7.1. Furthermore, the correlations among the five negative POMS factors increased from Pre [r mean = 0.32, shared variance (SV)% = 10%] to D3 [r mean = 0.88, SV% = 77%] and D7 [r mean = 0.81, SV% = 66%]. The correlations began to decrease on D10 [r mean = 0.60, SV% = 36%] and were further reduced at Post [r mean = 0.50, SV% = 25%]. On the other hand, the vigor subscale showed negative correlations with the other POMS factors. In more detail, vigor indicated low negative correlations with the other POMS factors at Pre (r mean = -0.19). Thereafter, increasing to a medium correlation during the hypoxic confinement on D3 (r mean = -0.43), D7 (r mean = -0.45), D10 (r mean = -0.49) and also following the intervention at the Post measurement (r mean = -0.47). Finally, there were no significant PANAS correlations between NA and PA subscales at Pre (r = -0.02), D3 (r = -0.34), D7 (r = -0.35) and D10 (r = -0.32) or at Post (r = -0.27).

7.4 Discussion

The main finding of the present study is that there was no difference in the participants' emotional state during hypoxic confinement, irrespective of the level of their physical activity. The emotional state (as measured with the POMS or PANAS instruments) was the same during acute (D1) as prolonged (D10) hypoxic exposure both in the LH-TH and LH group. There was a correlation between depression and the other negative POMS factors observed on D1 and this correlation increased with prolonged hypoxic exposure.

The results of the current study indicate that, while not significant, there was a

tendency for fatigue to be higher and for vigor to be lower, in the LH-TH compared to the LH group. This is not surprising given the level of activity the LH-TH participants performed during each day of the confinement. As previously mentioned, low-intensity exercise may alleviate a hypoxia-induced negative mood state, whereas, an exercise regimen of moderate to vigorous intensity may not (Ekkekakis et al., 2011). In the present study, the questionnaires were not administered immediately upon completion of the exercise, but at a time when the subjects had presumably recovered from the training session. Thus, the results should be interpreted with caution, they reflect the overall emotional state during the LH-TH condition and do not necessarily reflect the mood during the performance of the exercise.

The correlation analysis of the POMS subscales indicated that a significant interaction existed between depression and the other negative mood scales during the hypoxic exposure. Before the hypoxic exposure, depression showed significant positive correlations to anger ($r = 0.93$) and confusion ($r = 0.71$). Of note, during the hypoxic conditions, significantly higher intercorrelations were evident among the negative moods, particularly depression, tension and anger, followed by confusion and fatigue as compared to the pretrial correlations, Table 7.1. Thus, exposure to hypoxia may be associated with an intimate relationship among the negative mood factors, such that an otherwise innocuous event could trigger a negative mood. A possible explanation for this close interaction among the negative moods can be ascribed to a limitation in the crucial role of appraisal in cognitive and affective responses to adverse environments (Lane et al., 2004). An individual's coping resources might be limited during exposure to adverse environments (hypoxia), resulting in that individual experiencing a wide range of negative psychological responses during the hypoxic exposure (Lazarus and Folkman, 1984).

Initial exposure to high altitude may lead to acute mountain sickness (AMS), if the rate of ascent is fast or the individual's tolerance to high altitude is poor (Hackett et al., 1976). The current results show that both groups (LH-TH and LH) were under the threshold for AMS (Figure 7.1C). Thus, any contribution that AMS may have had to the POMS and PANAS responses was negligible. While AMS is most likely not of concern to athletes training at altitudes up to 2,800m, it may contribute to the psychological state of recreational alpinists or tourists travelling to higher altitudes than currently investigated.

Exposure to adverse environments, in this case high altitude, causes a variety of changes in mood states (Shukitt and Banderet, 1988) and impairments in motor and cognitive performance (Bahrke and Shukitt-Hale, 1993). The effect of altitude on psychological factors is associated with the impact of hypoxia on the central nervous system (Davidson et al., 2000). Shukitt-Hale et al., (1990) mentioned that exposure to hypoxia produced graded effects on symptoms, moods and performance as altitude level and duration were increased. Additionally, changes in mood and impairments to motor and cognitive function all show a degree of individual variation, as does adaptation to altitude. The perception of the psychological demands imposed by the stressful situation

and the individuals' perceived capability to cope with such demands is a process that can dramatically alter the intensity of the ensuing physiological activation (Acevedo and Ekkekakis, 2001). An individual's coping resources might be limited during exposure to adverse environments resulting in the inability to cope with the stressful situation. Thus a wide range of negative psychological responses can be experienced during hypoxic exposure, particularly due to an individual's inexperience with this type of environment or unexpected person-environment interaction. These inter-individual differences suggest the importance of one's cognitive appraisal of the situation (Acevedo and Ekkekakis, 2001), particularly as adverse changes are increased with higher altitudes and longer durations (Davidson et al., 2000). Additionally, depression, tension and anger appear to share a close relationship (D3: rrange = 0.94 to 0.97 and D7: rrange = 0.98 to 0.99) suggesting that maybe these three dimensions are significant in modulating the rest of the psychological experience or share common attributes during hypoxia. However, it seems clear that more research and/or information regarding the emotional disturbance during hypoxic exposure is warranted, with particular focus on the interaction among psychological indices, and the duration, intensity and frequency of exercise as well as the type of activities of hypoxic exposure.

Social factors and group dynamics during a confinement study may also be quite important in terms of emotional state. Previously, our group noted (Stavrou et al., 2015) that in a bed rest scenario, where participants were confined to bed in rooms designated for 2, with limited social interaction during the day, hypoxia had a profoundly negative affect on mood. All of the participants in the present study could freely interact with each other throughout the day (07:00 to 23:00). Furthermore, each participant was allocated his own iPad for the duration of the study. The social interaction, group dynamics and access to social media and other Internet tools may have played a large role in mediating the participants' mood. According to Shehab et al., (1998), astronauts confined to small areas and under a large amount of stress with a high level of task management, tolerate short duration space flight well. Currently astronauts are not subjected to a hypoxic environment and therefore the information from Shehab et al., (1998), may only have partial bearing on changes of alpinists' and athletes' mood during hypoxic training with a particular focus on confinement. However, the main aim of an athletic or alpinist sojourn at altitude is to either improve physiological performance or to make an attempt on a summit. The main focus is therefore exercise based, as such, free time in a hypoxic facility for athletes may need more structure to maintain ones emotional state. Similarly, while alpinists wait at base camp (essentially hypoxic confinement) for ideal weather and snow conditions to attempt a summit push, structured free time may delay the onset of hypoxia-induced negative emotions. The current results may also be pertinent to coaches in determining treatment or motivational techniques to use with their athletes, given that our participants had good social interaction and showed no hypoxia-induced negative mood. Individual athletes may require more distraction and time outdoors during their training camp compared to those involved in team sports, where the numbers of individuals confined to the hypoxic facility will in, and of, itself negate the influence of hypoxia on mood.

8 Discussion and Concluding Remarks

This thesis set out to address some of the primary concerns associated with future interplanetary travel along with hypoxic confinement scenarios. In order to address the physiological and psychological changes coupled with hypoxia, confinement, inactivity/unloading and exercise, the framework of this thesis was divided into 3 sections:

- **Part A**: The development and maintenance of a hypoxic lunar habitat simulation facility.
- **Part B:** The effects of hypoxic bed rest and confinement on peripheral perfusion and emotional state.
- **Part C:** The effects of hypoxic confinement with and without exercise on peripheral perfusion and emotional state.

8.1 Part A

Part A set out to establish a Lunar Habitat Simulation facility. The main findings from this section are that normoxic bed rest induced similar physiological changes in our participants as observed in other studies of similar duration. Secondly, the addition of hypoxia to the 10-day bed rest paradigm did not induce any additional physiological deconditioning.

This section also addressed the nutritional aspect of hypoxic confinement and bed rest. It was found that when participants were fed according to a menu as opposed to *ad libitum*, there were significant decreases in whole-body mass, secondary to lean-mass loss, following 10-day confinement regardless of intervention. It was also found that based on the results of Chapter 4, it does not seem reasonable to increase the energy intake levels during short-term bed rest above the ones used in this protocol in order to maintain body mass, as a positive energy balance was noted, even with hypoxia as an additional stressor.

With regards to Part A, the following conclusions can be made regarding the Null-Hypotheses in Chapters 3 and 4.

Chapter 3: Establishing a Lunar Habitat Simulation.

$H_0$1: Normoxic bed rest will not result in deconditioning of the musculoskeletal and cardiovascular system.

We can reject this null-hypothesis.

$H_0$2: 10-day hypoxic bed rest will not result in a further degradation of the musculoskeletal and cardiovascular system than normoxic bed rest alone.
It is possible to accept this Null-Hypothesis

Chapter 4: Whole body and regional body composition changes following 10-day hypoxic confinement and unloading/inactivity.

$H_0$3: 10-day normoxic bed rest will result in no change to the regional or whole body composition.
We can reject this null-hypothesis.

$H_0$4: Targeted energy intake levels and macronutrient composition will be sufficient to maintain regional or whole body composition.
We can reject this null-hypothesis.

$H_0$5: The addition of a hypoxic exposure to bed rest will not induce any reduction in whole body mass compared to bed rest only.
It is possible to accept this Null-Hypothesis

8.2 Part B

Following on from Part A, which addressed the basic set up and methodology of Lunar Habitat Simulation, Part B investigated more specific questions relating to how hypoxia and bed rest affected the circadian rhythm or peripheral perfusion and the change in emotional state.

The main finding of the work carried out in Part B was that while in a hypoxic confinement scenario, representing a simulated planetary habitat, hypoxia-induced daytime vasoconstriction observed in the hypoxic bed rest (HBR) and hypoxic ambulatory (HAMB) interventions did not persist at night. Our results also indicated a progressive inactivity-induced reduction of daytime perfusion over the course of the normoxic bed rest intervention (NBR). Nocturnal ΔTp-d index, reflecting perfusion of the fingers and toes, was the same in all three conditions. While it is evident that bed rest and hypoxia are strong factors mediating peripheral perfusion, nocturnal vasodilatation was maintained in all conditions, suggesting that the temporal relationship between sleep and peripheral perfusion was also maintained.

The second main finding is that psychological parameters may be altered as a direct result of the hypoxic stimulus. Our results indicated that there were distinct changes in the psychological indices as a result of bed rest and / or hypoxia. Specifically, a detrimental effect on positive emotions and an increase in negative was associated with bed rest and

exacerbated by hypoxia. Additionally, we noted that the participants involved in the HAMB intervention who underwent daily physical activity, did not demonstrate the same increased level of negative mood seen in the NBR and HBR trials. Finally, positive and negative emotions returned to initial levels following the recovery phase from either bed rest or hypoxic bed rest.

At the completion of the studies set out in Part B, the following conclusions can be made regarding the Null-Hypotheses in Chapters 5 and 6.

Chapter 5: The Circadian Rhythm of Peripheral Perfusion During a 10-day Hypoxic Confinement and Bed Rest.

$H_0$6: 10-d inactivity/unloading induced vasoconstriction will be equal during the day and night.
We can reject this null-hypothesis.

$H_0$7: 10-d exposure to hypoxia will not result in an increase of peripheral vasoconstriction.
We can reject this null-hypothesis.

$H_0$8: The synergistic effect of combining hypoxia with inactivity/unloading will not induce a greater vasoconstriction than either factor alone.
It is possible to accept this Null-Hypothesis

Chapter 6: Examining The Effect of Hypoxic Bed Rest and Confinement on Psychological Strain

$H_0$9: Hypoxia will not have a detrimental effect on the participants' psychological profile (an increase of negative and a decrease of the positive psychological characteristics).
We can reject this null-hypothesis.

$H_0$10: Inactivity/unloading will not have a detrimental effect on the participants' psychological profile.
We can reject this null-hypothesis.

$H_0$11: Exercise will have no effect on the negative psychological profile.
We can reject this null-hypothesis.

8.3 Part C

The primary focus of Parts A and B of this thesis was on bed rest and confinement as it relates to lunar habitat simulation. These sections simulated the habitat conditions with no exercise countermeasures, apart from during the HAMB intervention, which mimicked

daily physical activity (not bedridden). The present standard aboard the International Space Station (ISS) is for astronauts to conduct a daily aerobic and resistance training programme to prevent cardiovascular and musculoskeletal deconditioning. Perhaps a more suitable bed rest protocol mimicking the conditions on the ISS should include exercise. Furthermore, hypoxic confinement is routinely utilised by athletes and alpinists and thus exploring a hypoxic confinement scenario combined with exercise may illuminate other physiological and psychological changes we had not previously noted exclusively in bed rest.

Chapter 7 tested the hypothesis that increased activity in a normobaric hypoxic facility replicating a LH-TH scenario would reduce the daytime hypoxia-induced vasoconstriction. Chapter 7 also tested the concept that increased exercise and training during confinement would reduce the daytime vasoconstriction and have a positive effect on sleep onset. The main finding from this investigation was that the perfusion of the toe significantly increased from daytime to nighttime, similarly to that noted in Chapter 5. Regardless of condition (LH-TH or LH), toe perfusion increased from daytime to nighttime. Secondly, we did not find a difference in the perfusion of the toe as a result of either condition (with and without exercise training) following 10 days of exposure to a hypoxic confinement.

Compared to the level of extreme inactivity such as sustained bed rest (Chapter 5), increasing the level of physical activity – as we did in the current chapter - appears not to have any effect on nocturnal vasodilatation.

Chapter 8 dealt with the investigating the change in emotional state in two groups of participants, those partaking in daily exercise training and a sedentary group. The main finding of the present chapter was that there was no difference in the participants' emotional state during hypoxic confinement, irrespective of the level of their physical activity. While during the 10-day hypoxic bed rest we noted decreased positive emotions and increased negativity that was reduced in the HAMB group, the same pattern was not observed in this group of participants (Chapter 8). The emotional state in both groups (LH-TH and LH) was the same during acute (D1) as prolonged (D10) hypoxic exposure. However, an increase in the correlation between depression and the other negative POMS factors observed on D1 and on D10 of hypoxic exposure. There are likely differences in how social interaction may have taken place in the study compared to that in Chapter 6. Additionally, the number of participants was larger in the current study and the number of experiments was less than that of the bed rest study. These and possibly other factors may play a role in how little or how much hypoxia triggers an increase of the negative emotions.

Chapter 7: The Effect of a Live-High Train-High Regimen on Peripheral Perfusion.

$H_0$12: Endurance exercise at altitude will not cause any change in daytime hypoxia-induced vasoconstriction.

It is possible to accept this Null-Hypothesis

Chapter 8: The Effect of a Live-High Train-High Regimen on Emotional State.

$H_0$13: Compared to sedentary controls, increased endurance exercise will not counteract the detrimental effect hypoxia has on emotional state.
It is possible to accept this Null-Hypothesis

8.4 Limitations and final thoughts

As with any study, there are a number of limitations that need to be addressed. The studies that form this thesis are not considered to be "high-fidelity" of future planetary habitats. There are a number of unknown factors relating to future planetary travel including, but not limited to, the duration of travel and the magnitude of the hypoxic stimulus. Furthermore, there are still too many factors that are as yet uncertain regarding planetary habitat environments; thus in the context of planetary habitats the findings of the present thesis must be used with caution. The level of hypoxia chosen in the current thesis is beyond that expected in any future space habitats; firstly, the predicted level of hypoxia is unknown and secondly, we wished to use a level of hypoxic that would result in a significant stimulus upon on participants, thereby creating a worst case scenario.

The studies in the present thesis used a normobaric hypoxic stimulus, which has been argued (Conkin and Wessel, 2008) may have a different effect than a hypobaric hypoxic stimulus, as will exist in lunar habitats. To test the effect of an equivalent air altitude on circadian peripheral perfusion a study was conducted comparing the 24 hour proximal-to-distal skin temperature gradient in participants living in the Concordia Antarctic Research Station at an altitude of 4000m (hypobaric hypoxia) with the results of ambulatory subjects exposed to the hypoxic environment at the Planica facility (normobaric hypoxia). The results of this study suggest that ambient pressure *per se* during normobaric or hypobaric hypoxia does not play a role with regard to nocturnal peripheral vasodilatation (McDonnell et al., 2014b).

It cannot be excluded that the nocturnal Tsk elevations were brought about by different mechanisms in the different conditions (HAMB, HBR, NBR, LH-TH or LH), although it does not seem very likely. Potential mechanisms include altered sympathetic tone and change in the melatonin secretion. Unfortunately we did not measure blood levels of catecholamines, melatonin or any other hormonal responses and hence, we cannot discern, if hormonal / humoral mechanisms might have been involved. The studies reported in this thesis were part of larger collaborative studies with several other institutions, thus certain limitations were imposed by the protocols of other studies being performed.

Female astronauts play a major in Space missions, thus all Space life sciences studies

should include female subjects. Female participants were not included in the current studies, as it would have been necessary to either conduct the repeated trials at the same phase of the menstrual cycle, or include hormonal therapy to eliminate the menstrual cycle. Logistically, it was not possible to consider implementing these requirements and thus the study was conducted solely on male participants. Future studies must consider conducting such lunar habitat simulations with female subjects.

As a number of the studies within the thesis focused on the change in skin temperature, it should be noted that body core temperature was not measured during these studies. Bed rest and confinement studies are demanding and stressful protocols and as discussed elsewhere, these studies were large investigations placing a large demand on each participant. We felt it was an unfair burden to our participants to ask them to maintain a rectal thermometer probe for the full 24 hours of temperature recordings, which is also logistically problematic. Aural temperature was measured once a day at the same time of day, which is a good indicator of body temperature. If there was a shift in the temperature rhythm it would have been possible to detect. However, if there was a dampening of the circadian rhythm of body temperature as a result of our interventions, we would have missed it. Thus, for greater specificity in the future, core temperature, perhaps with a radio transmitting core pill should be utilised.

Finally, with regard to the duration of the current studies, ESA classifies 10 days of bed rest as short term. While, the duration of future lunar missions is as yet undefined, it will likely much longer. Therefore to gain a full appreciation of the effect of hypoxia on the physiological and psychological systems longer duration studies will need to be undertaken.

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11 Appendix

11.1 Select publications

11.1.1 Journal articles published

Monedero, J., McDonnell, AC, Keoghan, M, O'Gorman, D. J. Modified active videogame play results in moderate-intensity exercise. Games for health journal, 2014, vol. 3, no. 4, pp. 234-240.

Chapter 4
Debevec, T., McDonnell, AC., MacDonald, I., Eiken, O., Mekjavic, IB. Whole body and regional body composition changes following 10-day hypoxic confinement and unloading/inactivity. Applied physiology, nutrition and metabolism, 2014, vol. 39, no. 3, pp. 386-394

Chapter 5
McDonnell, AC., Jaki, P., Eiken, O., Mekjavic, IB. Circadian rhythm of peripheral perfusion during 10-day hypoxic confinement and bed rest. European journal of applied physiology, 2014, vol. 114, no. 10, pp 2093- 2104.

Chapter 6
Stavrou, N., McDonnell, AC., Eiken, O., Mekjavic, IB. Psychological strain : examining the effect of hypoxic bedrest and confinement. Physiology & behavior, 2015, vol. 139, pp. 497-504,

11.1.2 Extended abstracts

McDonnell, AC., Dolenc-Grošelj, L., Jaki, P., Eiken, O., Mekjavic, IB. Effect of hypoxia and bedrest on peripheral vasoconstriction. Proceedings of Life in space for life on earth, 18-22 June 2012, Aberdeen.

Stavrou, N., McDonnell, AC., Eiken, O., Mekjavic, IB. Indices of psychological strain during hypoxic bedrest and confinement. Proceedings of Life in space for life on earth, 18-22 June 2012, Aberdeen.

11.1.3 IJS reports

Debevec, T., McDonnell, AC., MacDonald, I., Eiken, O., Mekjavic, IB. Changes in body composition and dietary intake as a consequence of 10-day hypoxic confinement and unloading/inactivity. (IJS delovno poročilo, 11288). 2013.

Mekjavic, IB., Debevec, T., Morrison SA., Bali TC., McDonnell, AC., Eiken, O. Subject recruitment for bed rest and confinement studies, (IJS delovno poročilo, 11390). 2013.

11.1.4 Dissemination

M Mekjavic, IB., (interviewee), Eiken, O., (interviewee), McDonnell AC., (interviewee). Problem hujšanja v vesolju : gremo na Luno, gremo na Mars. Dnevnik online. 1. okt. 2011. http://www.dnevnik.si/objektiv/vec_vsebin/1042476805.

11.1.5 Photography

Suhadolnik, G., McDonnell, AC., (author, photographer). Ležanje z razlogom: raziskovalni center v Planici. Delo, 6. avg. 2011, 53, 181.

11.2 Nurse and assistant duties

Hypoxic, bed rest and confinement: Care of the participants

- Performing and monitoring standard physiological measurements (Arterial Pressure, Heart Rate, arterial Saturation, Tympanic Temperature, Daily Body Weight), (Medical staff only)
- Performing rounds (Regular check ups on participants)
- Maintaining a daily Work Log and completing a Critical Incident Log
- Venipuncture/ Intravenous cannulation (Medical staff only)
- Monitor and change biopsy dressings (Medical staff only)
- Assist with urine collection
- Sanitation of urine bottles and Bedpans
- Transfer participants to and from hygiene facility (Showering, toilet)
- Supervise Questionnaire Management System and ensure timely completion of all questionnaires
- Supervise daily exercise
- Monitor vitals (HR, SaO2)
- Socialising with the participants (talking, playing, spending time with them)
- Ensuring that rooms are maintained tidy by the facility cleaning staff
- Daily cleaning of rooms
- Changing linen, fresh towels
- Wipe down countertops, empty trash
- Collect laundry
- Provide assistance with the preparation, weighing & delivery of meals
- Provide drinks to participants when requested
- Monitor and note daily fluid intake
- Organise rooms
- Before and after each campaign, the hotel must be transformed into a hospital like setting. Hotel furniture removed and store, specialised bed rest equipment put in place
- Set up and dismantle all equipment and furniture associated with the operation of the study
- Monitor facility ambient condition
- Facility oxygen content
- Temperature and Humidity
- To assist project doctors with muscle biopsy procedures
- Maintaining an inventory of materials and supplies
- Transfer blood samples from the Planica facility to the local laboratory for analysis
- Collect and return participants to/from their homes
- Shopping for any required items (ie. drugs, food, materials, supplies, etc.) when required.
- Active participation in daily staff meetings

- Any other assistance that may be required

Work Schedule

Nurses and Assistants were required to work one shift per day. Each shift was a minimum of 8 hours in duration. The shifts were:

06:45 – 15:00
15:00 – 23:00
23:00 – 08:00

11.3 Lake Louise Mountain Sickness Score

Lake Louise Score (LLMS) for the Diagnosis of Acute Mountain Sickness (AMS)

A diagnosis of AMS is based on:
A rise in altitude within the last 4 days
Presence of a headache
PLUS
Presence of at least one other symptom
A total score of 3 or more from the questions below

SELF-REPORT QUESTIONNAIRE
Add together the individual scores for each symptom to get the total score.

Headache	No headache	**0**	
	Mild headache	**1**	
	Moderate headache	**2**	
	Severe headache, incapacitating	**3**	
Gastrointestinal symptoms	None	**0**	
	Poor appetite or nausea	**1**	
	Moderate nausea &/or vomiting	**2**	
	Severe nausea &/or vomiting	**3**	
Fatigue &/or weakness	Not tired or weak	**0**	
	Mild fatigue/ weakness	**1**	
	Moderate fatigue/ weakness	**2**	
	Severe fatigue/ weakness	**3**	
Dizziness/lightheadedness	Not dizzy	**0**	
	Mild dizziness	**1**	
	Moderate dizziness	**2**	
	Severe dizziness, incapacitating	**3**	
Difficulty sleeping	Slept as well as usual	**0**	
	Did not sleep as well as usual	**1**	
	Woke many times, poor sleep	**2**	
	Could not sleep at all	**3**	
	TOTAL		

Total score of:
3 to 5 = mild AMS
6 or more = severe AMS
Note:
Do not ascend with symptoms of AMS
Descend if symptoms are not improving or getting worse
Descend if symptoms of HACE or HAPE develop

11.4 POMS Questionnaire

Profile of Mood State (POMS) Questionnaire						
Code: _____		Condition: _____			Date: _____	
	Rate how you feel RIGHT NOW by circling ONLY ONE descriptive number following each of the feelings listed below					
	FEELING	Not at all	A little	Moderate	Quite a bit	Extremely
1	Tense	0	1	2	3	4
2	Angry	0	1	2	3	4
3	Worn Out	0	1	2	3	4
4	Unhappy	0	1	2	3	4
5	Lively	0	1	2	3	4
6	Confused	0	1	2	3	4
7	Peeved	0	1	2	3	4
8	Sad	0	1	2	3	4
9	Active	0	1	2	3	4
10	On edge	0	1	2	3	4
11	Grouchy	0	1	2	3	4
12	Blue	0	1	2	3	4
13	Energetic	0	1	2	3	4
14	Hopeless	0	1	2	3	4
15	Uneasy	0	1	2	3	4
16	Restless	0	1	2	3	4
17	Unable to concentrate	0	1	2	3	4
18	Fatigued	0	1	2	3	4
19	Annoyed	0	1	2	3	4
20	Discouraged	0	1	2	3	4
21	Resentful	0	1	2	3	4
22	Nervous	0	1	2	3	4
23	Miserable	0	1	2	3	4
24	Cheerful	0	1	2	3	4
25	Bitter	0	1	2	3	4
26	Exhausted	0	1	2	3	4
27	Anxious	0	1	2	3	4
28	Helpless	0	1	2	3	4
29	Weary	0	1	2	3	4
30	Bewildered	0	1	2	3	4
31	Furious	0	1	2	3	4
32	Full of pep	0	1	2	3	4
33	Worthless	0	1	2	3	4
34	Forgetful	0	1	2	3	4
35	Vigorous	0	1	2	3	4
36	Uncertain about things	0	1	2	3	4
37	Bushed	0	1	2	3	4

11.5 PANAS Questionnaire

PANAS, 1988

The PANAS

This scale consists of a number of words that describe different feeling s and emotions. Read each item and then mark the appropriate answer on the 1 ("Very slightly or not at all") to 5 ("Extremely") scale next to that word. Indicate to what extent you are feeling like that *now, at this particular moment*.

	Very slightly or not at all	A little	Modera-tely	Quite a bit	Extre-mely
1. Interested.	①	☐	☐	☐	☐
2. Distressed.	①	☐	☐	☐	☐
3. Excited.	①	☐	☐	☐	☐
4. Upset.	①	☐	☐	☐	☐
5. Strong.	①	☐	☐	☐	☐
6. Guilty.	①	☐	☐	☐	☐
7. Scared.	①	☐	☐	☐	☐
8. Hostile.	①	☐	☐	☐	☐
9. Enthusiastic.	①	☐	☐	☐	☐
10. Proud.	①	☐	☐	☐	☐
11. Irritable.	①	☐	☐	☐	☐
12. Alert.	①	☐	☐	☐	☐
13. Ashamed.	①	☐	☐	☐	☐
14. Inspired.	①	☐	☐	☐	☐
15. Nervous.	①	☐	☐	☐	☐
16. Determined.	①	☐	☐	☐	☐
17. Attentive.	①	☐	☐	☐	☐
18. Jittery.	①	☐	☐	☐	☐
19. Active.	①	☐	☐	☐	☐
20. Afraid.	①	☐	☐	☐	☐

Watson, Clark, & Tellegen (1988)

11.6 Ethical Approval

KOMISIJA REPUBLIKE SLOVENIJE ZA MEDICINSKO ETIKO

Prof. dr. Igor B. Mekjavič
Odsek za avtomatizacijo, biokibernetiko in robotiko
Institut Jožef Stefan
Jamova 39, 1000 Ljubljana

Štev.: 88/04/12
Datum: 28. 4. 2012

Spoštovani gospod prof. dr. Mekjavič,

Komisiji za medicinsko etiko (KME) ste 6. 4. 2012 poslali v oceno predlog raziskave z naslovom:

"Simulacija planetarnega habitata."

KME je na seji 17. aprila 2012 ocenila, da je raziskava etično sprejemljiva, in Vam s tem izdaja svoje soglasje.

Lep pozdrav,

prof. dr. Jože Trontelj
predsednik Komisije RS za medicinsko etiko

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Spletni naslov: http://www.kme-nmec.si/

11.7 Adapted Graph from Gallagher and Hacket (2006)

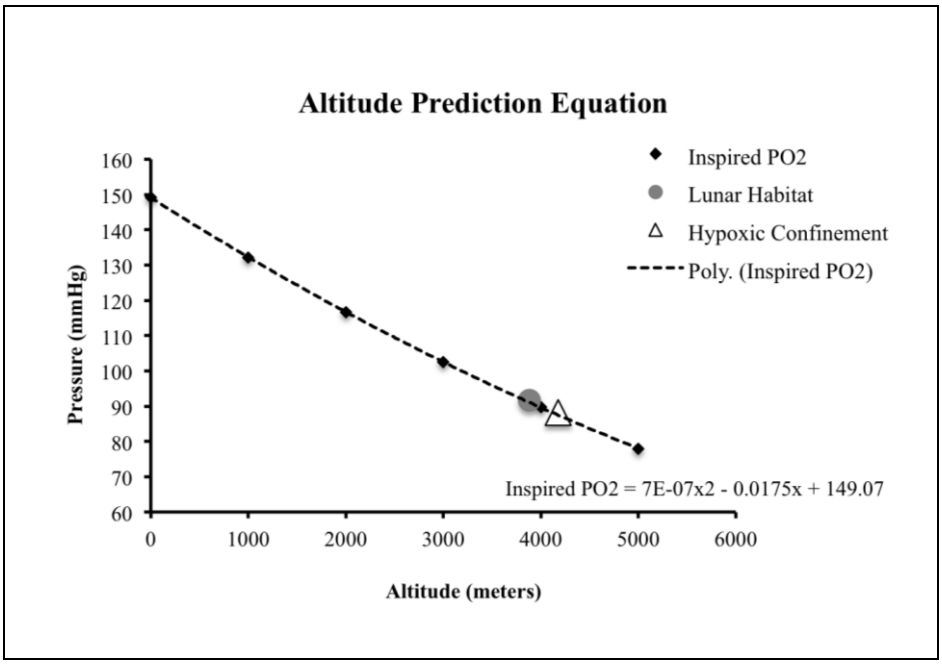


Figure 11.1: *Figure Adapted from Gallagher and Hacket (2004).* Data was plotted and a trendline fitted to generate the equation of the line. Inspired PO2 was calculated according to the formula: 7E-07x2 - 0.0175x + 149.07. The original data from Gallagher and Hacket (Inspired PO2) is plotted as the black diamond. The PIO2 for the Lunar Habitat Simulation Study (Part B) is plotted as the grey circle. The PIO2 for the Hypoxic Exercise Confinement Study (Part C) is plotted as a white triangle with a black outline.

11.8 Study Designs

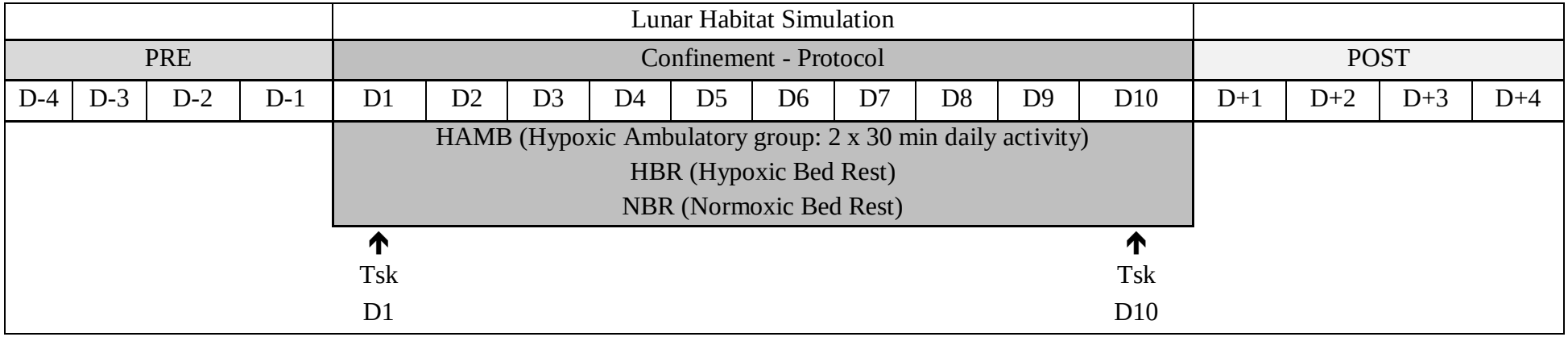


Figure 11.2: Lunar Habitat Simulation Study Design

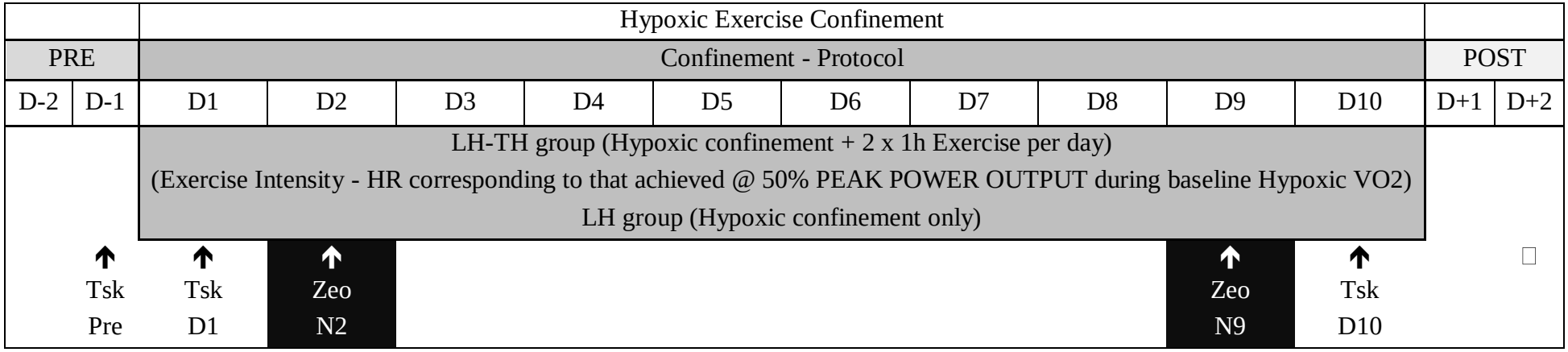
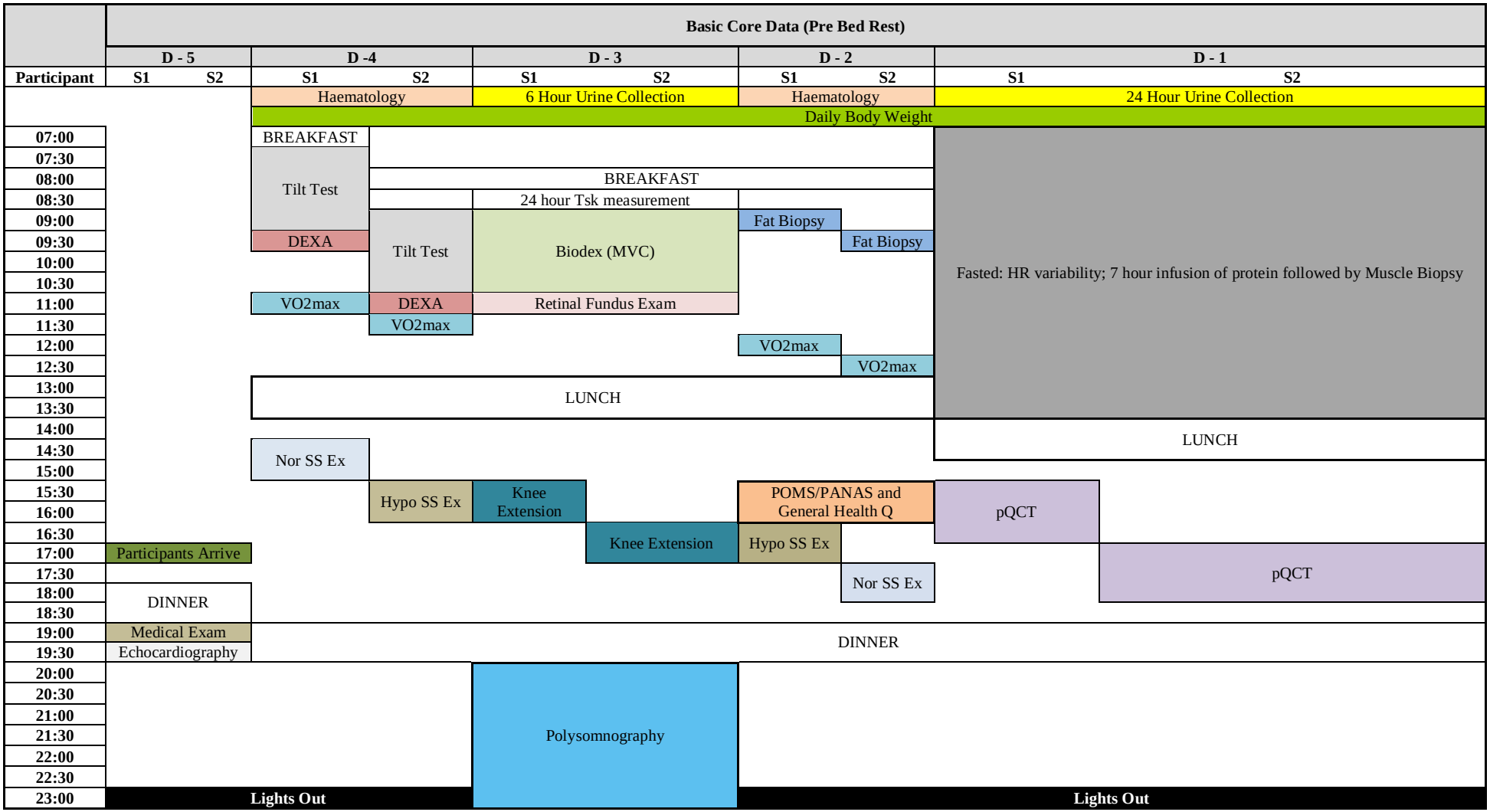


Figure 11.3: *Hypoxic Exercise Confinement Study Design*

11.9 Daily Schedule for Lunar Habitat Simulation Study (Part B)



	Bed Rest Phase (HAMB, HBR, NBR)									
	D1		D2		D3		D4		D5	
Participant	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
	24 Hour Urine Collection						24 Hour Urine Collection			
	Haematology		Haematology				Haematology			
07:00	Daily Body Weight									
07:30										
08:00	BREAKFAST									
08:30	24 hour Tsk measurement									
09:00	Enter Condition									
09:30										
10:00										
10:30	30 mins Physical Activity (HAMB group only)									
11:00	Snack									
11:30										
12:00										
12:30										
13:00	LUNCH									
13:30										
14:00										
14:30										
15:00	30 mins Physical Activity (HAMB group only)									
15:30	Snack									
16:00	Heart Rate, Arterial Saturation and Pressure and Aural Temperature									
16:30										
17:00										
17:30			Echocardiography							
18:00										
18:30									POMS/PANAS	
19:00	DINNER									
19:30										
20:00	Polysomnography									
20:30										
21:00			Lake Louise Mountain Sickness							
21:30										
22:00										
22:30										
23:00			**Lights Out**							

	Bed Rest Phase (HAMB, HBR, NBR)									
	D6		D7		D8		D9		D10	
Participant	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
			6 Hour Urine Collection						24 Hour Urine Collection	
									Haematology	
07:00	Daily Body Weight								24 hour Tsk measurement	
07:30									Fasted: HR variablity; 7 hour infusion of protein followed by Muscle Biopsy	
08:00	BREAKFAST									
08:30										
09:00					24 Hour Arterial Pressure		Fat Biopsy			
09:30								Fat Biopsy		
10:00										
10:30	30 mins Physical Activity (HAMB group only)									
11:00	Snack									
11:30										
12:00										
12:30										
13:00	LUNCH									
13:30										
14:00										
14:30									LUNCH	
15:00	30 mins Physical Activity (HAMB group only)									
15:30	Snack								pQCT	
16:00	Heart Rate, Arterial Saturation and Pressure and Aural Temperature									
16:30										
17:00										pQCT
17:30							Echocardiography			
18:00										
18:30									POMS/PANAS	
19:00	DINNER									
19:30										
20:00									Polysomnography	
20:30										
21:00	Lake Louise Mountain Sickness									
21:30										
22:00										
22:30										
23:00	**Lights Out**									

	Basic Core Data (Post Bed Rest)					
	D+1		D+2		D+3	
Participant	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**
	Haematology					
07:00	BREAKFAST					
07:30	Tilt Test					
08:00			BREAKFAST			
08:30						
09:00						
09:30	DEXA	Tilt Test				
10:00						
10:30						
11:00						
11:30	VO2max	DEXA				
12:00		VO2max				
12:30	Biodex (MVC)				VO2max	
13:00			LUNCH			VO2max
13:30						LUNCH
14:00						
14:30	Retinal Fundus Exam					
15:00						
15:30	Nor SS Ex		Knee Extension			
16:00						
16:30		Hypo SS Ex		Knee Extension		
17:00					POMS/PANAS	
17:30					Hypo SS Ex	
18:00						
18:30						Nor SS Ex
19:00	DINNER					
19:30						DINNER
20:00					Participants Depart	
20:30						
21:00						
21:30						
22:00						
22:30						
23:00	**Lights Out**					

11.10 Lunar Habitat Simulation Dietary Outline

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
B	bread	bread	bread	oat meal	bread	bread	meat pie	bread	cereals	bread
	processed cheese	tuna	scrambled egg	fruit yogurt	cooked egg	sausage	cheese slice	scrambled eggs	honey	butter and jam
	jam	honey	FJ	jam	honey	hazelnut spread	FJ	fruit yogurt	fruit yogurt	plain yogurt
	FJ/M	T		CM/T	FJ	M/CM/FJ		FJ	M/CM	M/CM
MS	apple	grape	pear	orange	apple	mixed fruit	apple	banana	orange	mixed fruit
	honey	banana	apple	rusk	kiwifruit	compote	honey	FJ	T	compote
	T	T	T	T	T		T			
L	**HAKE**	**TURKEY**	**CHICKEN**	**PORK CHOP**	**GNOCCHI**	**CHICKEN**	**BEEF**	**BARLEY SOUP**	**TURKEY**	**GNOCCI**
	mushroom soup	beef soup	carrot soup	pea soup	pea soup	tomato soup	spinach soup	barley soup	beef soup with	gnocchi with
	fried hake	turkey grilled	baked chicken	pork chop	gnocchi	roasted chicken	boiled beef	dried meat	grilled turkey	cheese
	cooked potatoes	stewed rice	mashed potatoes	cooked rice	tomato sauce	polenta	mashed potatoes	bread	cooked rice	mixed salad
	green salad	vegetables	mixed salad	fruit roll	apple pie	vanilla pudding	lettuce	chocolate pudding	vegetables	cake
	chocolate rolls T	chocolate cake FJ	cake T	FJ	T	FJ	chock bar T	FJ	apple pie T	FJ
AS	plain tea biscuit	croissant	fruit yogurt	plain yogurt	plain yogurt	dried fruit	cottage cheese	plain yogurt	plain tea biscuit	plain yogurt
	FJ	T		bun	FJ	T	pie		FJ	
D	**HAM SANDW.**	**SALAD**	**FISH SANDW.**	**WAFFLES**	**VEGETABLES**	**DUMPLINGS**	**SALAD**	**TUNA SANDW.**	**SALAD**	**BURGER**
	whole wheat	mixed salad	bread	imperial waffles	grilled vegetables	cheese	mixed salad with	tuna	green salad	grilled burger
	bread	yogurt	fish spread	fruit compote	grilled corn	dumplings	chicken	bread	tomatoes	bread
	slices of ham	grilled bread	cheese	T	FJ	compote	bread	corn salad tomato	cheese	potato salad
	plain yogurt FJ	FJ	T				T	FJ	ham FJ	FJ

Figure 11.4: *Lunar Habitat Simulation Dietary Outline.* A 10-day menu (5 meals per day) used during the normobaric hypoxic bed rest (HBR), normoxic bed rest (NBR) and normobaric hypoxic confinement (HAMB) campaigns. The individual food amounts were pre-calculated, precisely weighted (± 1g) and served accordingly. The possible leftovers were re-weighted and deducted from the final food amount used to report the actual daily intakes. In addition to the drinks served during the meals the participants had water and unsweetened tea available ad libitum throughout the campaigns.

B = breakfast; MS = morning snack; L = lunch; AS = afternoon snack; D = dinner; FJ = fruit juice; M = milk; CM = chocolate milk; T = tea; bread = whole wheat bread.

11.11 Declaration of authorship

DECLARATION

I the undersigned Adam C. McDonnell declare herewith that:

- The submitted Doctoral Dissertation has been prepared by myself based on my own research and using the sources listed, under the supervision of supervisor Prof. Dr. Igor B. Mekjavic and co-supervisor Prof. Ola Eiken
- This Dissertation has not been submitted either in whole or in part for obtaining any other degree at another university or faculty.
- I declare my own responsibility for ensuring that previously copyrighted material used in this work, beyond fair use, has been reproduced with written permission of the copyright owner.
- I agree with public access to this Dissertation in specialised libraries and the IPS homepage.

In Ljubljana, on 3rd April 2015 Signature of the candidate:



Signature of the supervisor:

