BEHAVIOURAL TEMPERATURE REGULATION: THE EFFECT OF THERMAL AND NON-THERMAL FACTORS

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Doctoral Dissertation
Jožef Stefan International Postgraduate School
Ljubljana, Slovenia, April 2015

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VEDENJSKA TERMOREGULACIJA: VPLIV TERMALNIH IN NETERMALNIH DEJAVNIKOV

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Ljubljana, Slovenia, April 2015

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Abstract

The aim of the present thesis was to investigate the contribution of thermal and non-thermal factors to the perception of thermal comfort, and thus on behavioural temperature regulation. This was evaluated in four separate studies, each addressing specific factors, potentially affecting the thermal comfort.

Common to the main studies of the thesis (Studies 1 to 4) is the methodology used to determine the range of skin temperatures considered within the comfort zone. The characteristics of the thermal comfort zone (TCZ) in these studies were assessed with a water-perfused suit (WPS). Subjects donned the WPS, in which the temperature of the water perfusing the suit changed in a sinusoidal manner from 10° to 50°C. Subjects were instructed to maintain the temperature of the suit within a thermally comfortable range by depressing a switch. Over the 1-hr period of each trial, the temperature of the water perfusing the suit described a damped oscillation due to the subjects' regulation of thermal comfort. The width of this damped temperature oscillation was defined as the TCZ.

Study 1 tested the hypothesis that hypoxia alters the boundary temperatures of the thermal comfort zone, and that exercise training modifies any hypoxia-induced alterations. Fourteen healthy men were confined for 10 days in normobaric hypoxia, the fraction of oxygen (FO_2) in the air being maintained at 0.139 ± 0.003. Subjects were randomly assigned to either a Live High (LH) group, which did not conduct any exercise during the 10-d exposure (N=6), or a Live High-Train High (LH-TH) group, which conducted two bouts of exercise daily (N=8), each comprising 60-min cycle exercise at 50% of their pre-determined, hypoxic peak power output. A subset of participants also completed a control trial in normobaric normoxia (Control; N=5). Behavioural thermoregulation was assessed in the Control group under normoxic conditions and in the LH and LH-TH groups after 24-h and 216-h of confinement in hypoxia. The upper and lower temperatures of the thermal comfort zone were determined with WPS described above. Skin temperatures and proximal-distal skin temperature gradients (2 sites: forearm-fingertip, calf-toe) were recorded at minute intervals during the trials. There were no significant differences in the characteristics of the TCZ between the LH and LH-TH groups, and no difference between the Control, 24-h and 216-h hypoxic exposures. Long-term exposure to normobaric hypoxia does not affect the boundary temperatures of the TCZ, nor its magnitude.

Study 2 investigated separate and combined effects of inactivity and hypoxia (target altitude of 4000m) on behavioural temperature regulation in 11 healthy normothermic young male subjects. Subjects participated in three 21-day interventions separated by at least three months: 1) Hypoxic ambulatory confinement (HAMB; fraction of oxygen in the inhaled gas, F_iO_2=0.141 ± 0.004; partial pressure of oxygen in the inspired gas, P_iO_2=90.0 ± 0.4; ~4000 m simulated altitude), 2) Hypoxic bedrest (HBR; F_iO_2=0.141 ± 0.004; P_iO_2=90.0 ± 0.4; ~4000 m simulated altitude), and 3) Normoxic bedrest (NBR; F_iO_2=0.209; P_iO_2=133.1 ± 0.3). During each experimental campaign, measurements were performed 5 days prior to the intervention (D-5), and on days 10 (D10) and 20 (D20) of the intervention. No statistically significant differences were found in any of the

characteristics of the TCZ between the interventions (HAMB, HBR and NBR), or between different measurement days (D-5, D10, D20) within each intervention. Rectal temperature remained stable, whereas skin temperature (Tsk) increased in all experimental conditions throughout the one hour trial. No differences in Tsk between D-5, D10 and D20 and between HAMB, HBR and NBR were revealed. Subjects perceived the regulated temperature as thermally comfortable, and neutral or warm. Regulation of thermal comfort is not compromised by hypoxic inactivity.

Study 3 As part of a study investigating the effect of nonthermal factors (i.e. hypoxia and inactivity) on temperature regulation, subjects were required to conduct three trials over a period of one year. It has been reported that the seasonal variation in length of day and light intensity can affect the circadian rhythm as well as some characteristics of temperature regulation. We investigated the characteristics of the TCZ during two seasons. Twelve subjects participated in three trials conducted in October 2012 (T1), April 2013 (T2) and October 2013 (T3). We monitored skin and rectal temperature at a minute intervals and recorded subjects' ratings of thermal comfort and thermal sensation every 10 min. TCZ varied from 6.0 °C in T1, to 8.3 °C in T2 and 11.4 °C in T3, the difference between T1 and T3 being significantly different. The differences observed in the thermal perception between T1 and T3 were for trials conducted during the same season. We conclude that perception of thermal comfort does not seem to be affected by the seasonal variation, when comparing the data obtained in spring and autumn as in the present study.

Study 4 evaluated regional thermal comfort in males (N=8) and females (N=8). Subjects were requested to regulate the temperature of the water delivered to a water-perfused suit, such that it was thermally comfortable. In separate trials, subjects regulated the temperature of the whole suit, or specific regions of the suit covering different skin areas. The TCZ was determined for the whole body, and for the following regions: arms, legs, front torso, back torso, hands, feet, and head. The range of regulated temperatures did not differ between genders and skin regions. Females maintained higher mean skin temperature (Tsk) during the legs (females: 35.2 ± 0.7°C, males: 34.6 ± 0.8°C; $p<0.05$) and whole body (females: 35.1 ± 0.4°C, males: 34.3 ± 0.6°C; $p<0.01$) temperature regulation (TR), compared to males, who showed greater variation in preferred temperature for these areas. Females perceived thermal comfort at a higher mean Tsk during the legs and whole body TR.

Study 5 (in Appendix D) demonstrates the importance of including assessment of thermal comfort perception when evaluating protective clothing for extreme environments. The study evaluated the efficiency of two heat dissipation strategies under simulated desert patrol missions. Ten males participated in four trials, during which they walked on a treadmill (45°C, 20% relative humidity), carrying a load of 35 kg; two 50-min walks were separated by a 20-min rest. Active cooling (AC) provided by reduced clothing in combination with a ventilated vest was compared with passive cooling (PC), achieved by reduced clothing combined with partly undressing, water spraying and fanning body surfaces during the rest. Cooling strategies were compared to naked condition (NC) and full protective equipment (FP) with no supplemental cooling. During the walks, skin temperature (Tsk) was lower and heat flux (Q) higher in AC, whereas during the rest, Q was greater in PC. Tsk was higher during NC, compared to other conditions. In the FP, rectal temperature and heart rate were higher than in other conditions. Subjects perceived the ambient temperature as warmer and reported about greater thermal and wear discomfort during FP, compared to other conditions. Cooling strategies substantially improved evaporative cooling, and thus subjects' thermal status and thermal comfort. Four subjects terminated the trial prematurely due to signs of heat exhaustion in the FP condition.

Povzetek

Pričujoča teza je ovrednotila prispevek termalnih in netermalnih dejavnikov k zaznavanju toplotnega udobja in dalje vpliva na vedenjsko termoregulacijo. Vsaka od štirih ločenih študij je bila osredotočena na proučevanje specifičnega dejavnika, ki bi potencialno lahko vplival na spremembo toplotnega udobja.
Glavnim študijam teze (študije 1 do 4) je bila skupna metodologija, s katero je bilo določeno območje kožnih temperatur, zaznanih kot toplotno udobnih. Karakteristike območja toplotnega udobja v študijah so bile ocenjene s pomočjo termoregulacijskega oblačila. Subjekti so bili med eksperimentom oblečeni v termoregulacijsko oblačilo, v katerem se je temperatura vode spreminjala sinusoidno med 10 do 50°C. Preiskovancem je bilo naročeno, da vzdržujejo temperaturo vode znotraj termoregulacijskega oblačila v območju toplotnega udobja s pritiskanjem na daljinski upravljalnik. Temperatura vode je tekom enournega eksperimenta z vzdrževanjem toplotnega udobja ustvarjala nihanje. Razmik v nihanju temperature je bil definiran kot območje toplotnega udobja.

Študija 1 je proučevala hipotezo o vplivu hipoksije na območje toplotnega udobja in o vplivu telesne aktivnosti na modifikacijo sprememb, povezanih s hipoksijo. 14 zdravih moških preiskovancev je bilo izpostavljenih 10-dnevni normobarični hipoksiji s frakcijo kisika (FO2) v zraku, vzdrževano na 0,139 ± 0,003. Preiskovanci so bili naključno dodeljeni skupini (LH), ki ni izvajala nobene telesne aktivnosti med 10-dnevno izpostavitvijo hipoksiji (N=6), ali skupini (LH-TH), ki je trenirala dvakrat dnevno po eno uro, na 50% vnaprej določene hipoksične maksimalne zmogljivosti (N=8). Podskupina preiskovancev (N=5) je izvedla eksperimente med izpostavitvijo normobarični nomoksiji (kontrolna skupina). Vedenjska termoregulacija je bila proučevana v kontrolni skupini v normoksičnih pogojih ter v LH in LH-TH skupini po 24 urah ter 216 urah izpostavitve hipoksiji. Zgornje in spodnje meje območja toplotnega udobja so bile določene s pomočjo termoregulacijskega oblačila, kot opisano zgoraj. Med eksperimentom je bila merjena kožna temperatura in temperaturni gradient med podlaktjo ter konico kazalca na roki ter temperaturni gradient med meči in palcem na nogi. Nobena od karakteristik območja toplotnega udobja ni razkrila signifikantnih razlik med skupinama LH ter LH-TH ter med kontrolno skupino, 24-urno ter 216-urno izpostavitvijo hipoksiji. Dolgotrajna izpostavitev hipoksiji ne vpliva na meje in razpon območja toplotnega udobja.

Študija 2 je ocenjevala ločen in skupen vpliv neaktivnosti in hipoksije na vedenjsko termoregulacijo s proučevanjem na enajstih zdravih, mladih in normotermičnih moških preiskovancih. Preiskovanci so sodelovali v treh 21-dnevnih intervencijah, z vsaj trimesečnim razmikom med vsako: 1) Hypoxic ambulatory confinement (HAMB; fraction of oxygen in the inhaled gas, F_iO_2=0,141 ± 0,004; partial pressure of oxygen in the inspired gas, P_iO_2=90,0 ± 0,4; ~4000 m simulated altitude), 2) Hypoxic bedrest (HBR; F_iO_2=0,141 ± 0,004; P_iO_2=90,0 ± 0,4; ~4000 m simulated altitude), and 3) Normoxic bedrest (NBR; F_iO_2=0,209; P_iO_2=133,1 ± 0,3). V vsaki od treh kampanj so bile meritve izvedene 5 dni pred intervencijo ter na 10. in 20. dan intervencije. Nobena od karakteristik območja toplotnega udobja ni pokazala signifikantnih razlik med hipoksično ambulatorno skupino, hipoksično neaktivno skupino in normoksično neaktivno skupino ali razlik med različnimi dnevi izvedbe meritev. Rektalna temperatura je bila med

eksperimentom stabilna, medtem ko je kožna temperatura narasla v vseh eksperimentalnih pogojih. Kožna temperatura ni razkrila nobenih razlik med eksperimentalnimi pogoji in različnimi dnevi izvedbe meritev. Preiskovanci so zaznavali regulirano temperaturo kot udobno in nevtralno ali toplo. Hipoksična neaktivnost ne predstavlja ogrožajočega dejavnika za toplotno udobje.

Študija 3 Študija je proučevala vpliv netermalnih dejavnikov (hipoksije in neaktivnosti) na termoregulacijo. Preiskovanci so bili vključeni v serijo eksperimetnov v obdobju enega leta. Rezultati preteklih raziskav nakazujejo, da lahko sezonska nihanja v dolžini dneva in jakosti svetlobe vplivajo na cirkadiarni ritem in tudi na določene karakteristike termoregulacije. Pričujoča študija je proučevala značilnosti vedenjske termoregulacije tekom dveh letnih časov, še posebno vpliv na zaznavanje toplotnega udobja. Dvanajst preiskovancev je sodelovalo v treh eksperimentih, ki so se odvijali v oktobru 2012 (T1), aprilu 2013 (T2) in v oktobru 2013 (T3). Rektalno temperaturo in temperaturo kože smo spremljali vsako minuto, prav tako so preiskovanci vsakih 10 minut podali oceno toplotnega udobja in temperaturne percepcije. Območje toplotnega udobja je variiralo od 6,0 °C pri T1, 8,3 °C pri T2 in 11,4 °C pri T3, s signifikantno razliko med T1 in T3. Razlika v toplotni percepciji med T1 in T3 je bila poročana za eksperimenta, ki sta bila izvedena v istem letnem času. Zaključujemo, da letni čas ne vpliva na percepcijo toplotnega udobja, v kolikor so meritve izvedene v spomladanskem in jesenskem času, kot v primeru pričujoče študije.

Študija 4 je ocenjevala regionalno toplotno udobje moških (N=8) in žensk (N=8). Preiskovancem je bilo naročeno, da vzdržujejo toplotno udobje z reguliranjem temperature vode znotraj termoregulacijskega oblačila. Preiskovanci so v ločenih eksperimentih regulirali temperaturo celotnega telesa ali specifičnih telesnih regij, kot so roke, noge, sprednji del trupa, zadnji del trupa, dlani, podplatov in glave. Razpon reguliranih temperatur ni razkril razlik med moškimi in ženskami ter proučevanimi telesnimi regijami. Ženske so med reguliranjem temperature nog (ženske: 35,2 ± 0,7 °C, moški: 34,6 ± 0,8 °C; $p<0,05$) in celotnega telesa (ženske: 35,1 ± 0,4 °C, moški: 34,3 ± 0,6 °C; $p<0,01$) vzdrževale višjo povprečno kožno temperaturo v primerjavi z moškimi, pri katerih je bila odkrita večja variabilnost želenih temperatur za ti regiji. Toplotno udobje žensk je bilo povezano z višjo povprečno kožno temperaturo, izmerjeno med reguliranjem temperature nog in celotnega telesa.

Študija 5 (dodatek D) je proučila pomen toplotnega udobja pri ocenjevanju zaščitnih oblačilnih sistemov za ekstremne razmere. Ocenila je učinkovitost dveh ohlajevalnih strategij med simulirano izvidniško misijo. Deset moških preiskovancev je sodelovalo v štirih eksperimentih, med katerimi so med hojo po tekoči preprogi (45 °C, 20 % relativne vlage) nosili tovor skupne teže 35 kg; dve 50-minutni hoji sta bili prekinjeni z 20-minutnim počitkom. Aktivno ohlajanje (AC), doseženo z modifikacijo oblačil v kombinaciji z ventilacijskim jopičem, je bilo primerjano s pasivnim ohlajanjem (PC), doseženim z modifikacijo oblačil v kombinaciji z delnim odstranjevanjem oblačil, pršenjem z vodo in ventiliranjem telesnih površin med počitkom. Ohlajevalni strategiji sta bili primerjani z golim pogojem (NC) in pogojem, pri katerem so preiskovanci nosili polno bojno opremo brez dodatnega ohlajanja (FP). Med hojama je bila kožna temperatura nižja in toplotni tok višji v AC pogoju, medtem ko je bil med počitkom toplotni tok višji v PC pogoju. V NC pogoju je bila kožna temperatura višja kot v ostalih eksperimentalnih pogojih. V FP pogoju sta bila rektalna temperatura in srčni utrip višja kot v ostalih pogojih. Preiskovanci so pri FP pogoju v primerjavi z ostalimi pogoji temperaturo okolja zaznavali kot višjo ter temperaturno manj udobno, kot tudi manj udobno z vidika nošenja oblačil. Ohlajevalni strategiji sta bistveno izboljšali evaporativno ohlajanje ter s tem toplotno stanje in udobje. Štirje preiskovanci niso zaključili FP pogoja zaradi znakov vročinske izčrpanosti.

Abbreviations

AC	=	Active cooling
ANOVA	=	Analysis of variance
BDC	=	Baseline data collection
BR	=	Bedrest
CI	=	Confidence Interval
Control	=	Control group
DAP	=	Diastolic Arterial Pressure
$\Delta T_{calf\text{-}toe}$	=	Calf-toe skin temperature gradient
$\Delta T_{forearm\text{-}fingertip}$	=	Forearm-fingertip skin temperature gradient
D-5	=	Measurements performed 5 days prior to the intervention
D10	=	Measurements performed on day 10 of the intervention
D20	=	Measurements performed on day 20 of the intervention
E	=	Evaporation
EVA	=	Extra-vehicular activity
FO_2	=	Fraction of oxygen
F_iO_2	=	Fraction of inspired oxygen
FP	=	Full protection condition
HAMB	=	Hypoxic ambulatory confinement
HBR	=	Hypoxic bedrest
HR	=	Heart rate
LH	=	Live high regimen
LH-TH	=	Live high-Train high regimen
MSR	=	Modular Signal Recorder
NBR	=	Normoxic bedrest
NC	=	Naked condition
PC	=	Passive cooling
P_iO_2	=	Partial pressure of inspired oxygen
Q	=	Heat flux
Qt	=	Torso heat flux
RH	=	Ambient relative humidity
RPE	=	Rating of perceived exertion
SAP	=	Systolic Arterial Pressure
SpO_2	=	Capillary oxyhemoglobin saturation
Sw	=	Sweat secretion
Ta	=	Ambient temperature
Tc	=	Core temperature
TCZ	=	Thermal comfort zone
T_{high}	=	Upper limit of the thermal comfort zone
T_{low}	=	Lower limit of the thermal comfort zone
TR	=	Temperature regulation
Tre	=	Rectal temperature
Tsk	=	Skin temperature

Ttsk	=	Torso skin temperature
T_{TY}	=	Tympanic temperature
Twps	=	Water perfused suit temperature
VAS	=	Visual analogue scale
V_E	=	Minute ventilation
VO_2	=	Oxigen uptake
VO_{2peak}	=	Peak Oxigen uptake
WPS	=	Water perfused suit
24-h	=	Short-term hypoxic exposure
216-h	=	Long-term hypoxic exposure

1 Introduction

Regulation of body temperature within narrow limits is achieved by autonomic and behavioural responses, which maintain a balance between the heat produced and dissipated. The autonomic responses of shivering and sweating are activated at lower (LCT) and upper core threshold (UCT) temperatures, respectively. Heat retention and heat loss is achieved by the vasomotor responses of vasoconstriction and vasodilatation, which are also activated at specific LCT and UCT, respectively (Benzinger, 1961; Cabanac and Massonnet, 1977; Mekjavic and Bligh, 1987). Under normal conditions, body temperature is maintained within the temperature range bound by LCT and UCT, termed the null-zone (Mekjavic et al., 1991), or interthreshold zone. Behavioural responses are primarily initiated by the perception of thermal (dis)comfort, which is the result of cortical integration of thermal afferent information from the skin thermoreceptors, responding to the surrounding environmental conditions. Behavioural responses are the first line of defence against displacements in body temperature (Schlader et al., 2010; Schlader, 2014), and include readjustments of clothing, moving to a cooler or warmer environment, regulating the heating within homes, opening windows, and changing posture to reduce or to encourage the heat loss, etc. Appropriate heat exchange between the body and the environment ensures that an individuals' health is not jeopardized due to overheating or cooling of the body.

Thermal comfort is a subjective evaluation of perceived temperature, which differs among individuals exposed to the same ambient conditions. In the past, the term »pleasure« was suggested by Cabanac (1971) to define a sensation indicating the presence of a stimulus helping to correct an internal problem; in this manner »pleasant« was equated to »useful«. Temperature sensation was shown to differentiate between different skin regions (Nadel et al., 1973) and to depend on the combination of the thermal resistance of the clothing, activity level and ambient conditions (Fanger, 1970). Most importantly, behavioural, like autonomic, temperature regulation depends not only on thermal factors, but is also affected by a number of nonthermal factors. Thermal comfort is optimal when heat balance is achieved; when core temperature is within the autonomic interthreshold zone (Mekjavic and Eiken, 2006). The establishment of such a condition of thermal comfort is the principal aim of behavioural responses.

Some working conditions require the use of protective clothing, which significantly reduces heat exchange with the environment (Havenith, 1999; Montain et al., 1994; Nunneley, 1988). In such conditions thermal comfort cannot be optimal with the safety and health of an individual being of primary concern. Since thermal discomfort can negatively affect performance, protective clothing should be designed to insure safety, but also to include the highest level of thermal comfort as possible.

The present thesis investigates the contribution of thermal and nonthermal factors to the perception of thermal comfort, and thus on behavioural temperature regulation. The specific issues addressed are discussed below.

1.1 Regional differences in thermal sensation and thermal comfort

The contribution of different skin areas to the perception of thermal comfort is a result of different thermosensitivities of these areas. The face has been shown to display strong sensitivity to thermal stimuli (Brown and Williams, 1982; Cotter and Taylor, 2005; Crawshaw et al., 1975; Mekjavic and Eiken, 1985; Nadel et al., 1973). Also, areas such as the torso were identified as more thermosensitive than others (Arens et al., 2006; Nakamura et al., 2008; Nakamura et al., 2013; Zhang et al., 2004). The extremities usually display poor thermosensitivity and therefore contribute less to an overall thermal comfort (Cotter and Taylor, 2005; Nakamura et al., 2013).

The distinction in skin thermosensitivity can provide behavioural modifications of the areas that contribute greatly to reduction of the thermal discomfort (Palmer et al., 2001), and reduce physiological strain (Barwood et al., 2009), especially when the body is exposed to extreme environmental conditions. It has previously been reported that thermal comfort is enhanced whenever the thermal stress is relieved by local cooling or warming, re-establishing homeostasis (Arens et al., 2006). This is initiated when a warm stimuli is applied to cold skin area and vice versa, when a cold stimuli is applied to warm skin area (Cabanac, 1971).

In the design of comfortable ambient conditions, the knowledge about regional differences in thermal sensation and their contribution to overall thermal comfort should not be neglected, since it is evident that uniform ambient conditions in fact do not produce as much comfort as asymmetrical environments (Arens et al., 2006).

1.2 Gender

Although a number of studies have reported no difference in thermal sensation between males and females exposed to the same thermally neutral conditions (Lan et al., 2008; Parsons, 2002; Zhai et al., 2014), a study of the indoor thermal comfort for males and females indicated that females are generally less satisfied with room temperatures, perceive the temperatures as uncomfortable more often (too warm or too cold), and prefer higher temperatures than males (Karjalainen, 2007). During cool ambient conditions, females seem to perceive the temperatures cooler and less comfortable than males (Hashiguchi et al., 2010; Parsons, 2002; Zhai et al., 2014), with a preference for warmer ambient conditions (Lan et al., 2008; Zhai et al., 2014).

Studies on the gender specific differences in thermosensitivity indicate that lower thresholds for warm and cold sensation (Golja et al., 2003), and stronger thermal warmth sensation to a warm stimulus (Gerrett et al., 2014) are detected and perceived by females. Thermosensitivity also appears to be affected by the menstrual cycle. Sensitivity to cold stimuli decreases in the luteal phase of the menstrual cycle (Kocjan, 2007), whereas the sensation to the same peripheral cool stimulus has been reported to be perceived as very pleasant during the follicular phase and unpleasant during luteal phase (Cunningham and Cabanac, 1971). How these gender-related differences in thermosensitivity and thermal comfort are reflected in the preferred temperatures, obtained by behavioural responses, remains unresolved.

1.3 Age

A study performed on elderly and younger males reported similar preferred ambient temperature for both groups, but poor temperature discrimination and lack of precision in temperature adjustment for the elderly (Collins et al., 1981). A longitudinal study on an elderly population revealed an age-related decline in autonomic nervous function such as increase in the threshold temperature for sweating, orthostatic hypotension, and a diminished core-shell temperature gradient (Collins et al., 1977). Ageing has been associated with diminished cold-induced thermoregulation (Florez-Duquet and McDonald, 1998), with reduced intensity of the vasoconstriction and shivering responses, and decreased subjective thermal perception (Frank et al., 2000). In contrast, it has also been observed that vasoconstriction response in the hands is maintained with age (Kingma, 2011).

The reduced efficiency of thermoregulation in combination with other age-related diseases has been shown to have a detrimental effect on the health of this population (Collins, 1987). If the thermoregulatory responses are attenuated in the elderly, then this should be taken into consideration when designing appropriate thermal environments for the elderly, especially in the presence of other age-related diseases.

1.4 Hypoxia and reduced activity/inactivity

Dysfunction of the cardiorespiratory system results in deficiencies in the amount of oxygen delivered to tissues, resulting in tissue hypoxia. Similarly, lower than normal levels of partial pressure of oxygen in the environment causes hypoxia. The latter type of hypoxia is experienced by alpinists at high altitudes. Recently, it has been suggested that future planetary habitats will maintain a hypobaric hypoxic environment, to allow Extra-vehicular activities (EVAs) to be conducted without the need for decompression procedures. Slow decompression concomitant with oxygen pre-breathing is required during the transfer from the normobaric environment in the International Space Station to the hypobaric environment within the space suits used for EVAs. In addition to the hypoxia, astronauts will also be exposed to a reduced gravity environment in future Lunar and Mars habitats (Bodkin et al., 2006; Norcross et al., 2013). It has previously been reported that autonomic responses are altered due to the exposure to either hypoxia (Johnston et al., 1996), or inactivity/unloading (Michikami et al., 2004; Mekjavic et al., 2005). Studies using the bedrest experimental model to simulate the effects of reduced gravity however, reported no effect on the thermal comfort zone (Yogev et al., 2010). Accordingly, no change in the thermal comfort zone was observed after the short-time exposure to hypoxia (Golja and Mekjavic, 2003; Golja et al., 2005).

Apart from astronauts, the combined effects of hypoxia and inactivity/unloading are also experienced by specific patient populations. Namely, patients with chronic obstructive pulmonary disease or cardiovascular disease are hypoxic, and as a consequence also inactive. How the combination of hypoxia and unloading/inactivity affects the perception of thermal comfort and moreover behavioural thermoregulation, is yet unknown.

1.5 Physical training

The human body is able to adapt to a variety of ambient conditions as well as to different levels of physical activity by altering thermoregulatory responses as a result of these changes (Chen et al., 2013; Houmard et al., 1990; Machado-Moreira et al., 2005). During

exercise, heat balance is maintained by heat loss mechanisms of vasodilatation, conduction/convection, radiation, and evaporation. It is well known that prolonged physical training initiates the sweat response at a lower core temperature and improves the capacity for useful sweating during exercise in a hot environment (Araki et al., 1981). It has been suggested that the rise in body core temperature, which is initiated by regular exercise, is necessary for the training-induced alterations in autonomic thermoregulatory functioning, which improve tolerance to a hot environment (Avellini et al., 1982). As evident, training can modify the thermoregulatory responses, reflected in improved heat exchange mechanisms.

If the thermoregulatory responses are altered due to hypoxic exposure, as discussed in the previous chapter, physical activity may prevent these alterations by maintaining normal thermoregulatory functioning.

1.6 Ambient conditions and seasonal variation

Everyday alterations in ambient conditions, including temperature, relative humidity, air velocity and partial pressure of oxygen, affect our thermoregulatory system, establishing thermal balance through different responses of the organism. During exposure to extreme environments, physiological responses need to be re-established, as evident during the process of acclimatization (Magalhães et al., 2010), as an improved response to a new environment. The autonomic thermoregulatory responses however, only have a limited capacity (Flouris, 2011). Behavioural thermoregulatory responses are therefore crucial in maintaining the body temperature within safe limits especially in such conditions. Since the skin is the first contact with the surrounding environment, the information regarding the ambient conditions produces an immediate behavioural response (Schlader, 2014). Behavioural thermoregulatory responses are therefore the first line of defence of the core temperature, are more efficient, and energetically less costly for the organism than autonomic responses.

Seasonal variations can however affect the thermoregulatory responses. Similar to the photoreceptors in the retina, melanopsin cells also detect changes in ambient light (Berson et al., 2002; Güler et al., 2008; Hattar et al., 2002). It has been reported that the changes in day length and light intensity affect the human circadian rhythm, interthreshold zone, as well as metabolic rate (Honma et al., 1992; Kakitsuba et al., 2011; Kakitsuba et al., 2013; van Ooijen et al., 2004). Whether seasonal variations also affect the perception of thermal comfort and moreover behavioural temperature regulation in humans, is unresolved.

1.7 Determination of the perception of thermal comfort

The studies evaluating thermal comfort of different groups and in different conditions have usually been conducted using visual analogue scales (VAS), which refer to a subjective evaluation of perception of the surrounding environment. In the event that different VAS are used in different studies, then the results of such studies cannot be compared. Studies on regional thermosensitivity have usually been conducted by applying cold or warm stimuli to different skin sites, resulting in differential contribution to an overall thermal comfort. However, thermal comfort is not achieved at a single skin temperature, but it is likely established for a range of skin temperatures.

Since it is evident that thermal comfort is not uniform and that it is also not achieved at a single skin temperature, the present thesis evaluated thermal comfort objectively by using

a novel method described previously by Yogev et al. (2010). The method requires that subjects don a water perfused suit (WPS) comprising small-diameter PVC tubes woven in the mesh of the suit and connected to a manifold, as shown in Figure 1.1. The WPS is designed so different regions of the body can be regulated together or separately. The WPS covers the whole body except the hands, feet, neck and head. A test commences with a 10-min baseline period during which water pumped through the suit is maintained at a temperature (Twps) of 27°C. Thereafter, the temperature of the water perfusing the suit is regulated such that it describes a sinusoid that can potentially deliver water at temperatures ranging from 10° to 50°C.

The subjects are instructed to regulate the temperature of the suit within a range that they consider thermally comfortable. By depressing a button on a remote switch, they can initiate a change in the direction of the Twps once it becomes either uncomfortably warm during heating or uncomfortably cool during cooling. The control unit does not allow a steady-state position and therefore Twps has to be regulated at a preferred range during experiment with subjects naïve regarding the actual Twps. In this manner, the subjects determine their thermal comfort zone – a range of temperatures, perceived as thermally comfortable.

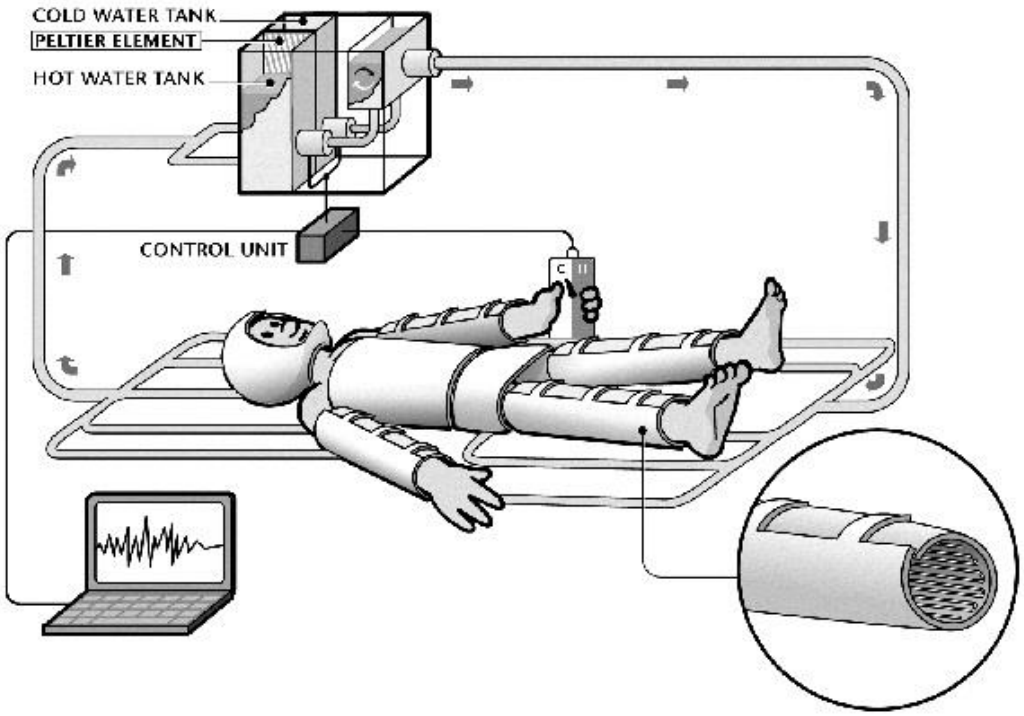


Figure 1.1: *Experimental set-up used for determination of the perception of thermal comfort (from Yogev, 2010).*

1.8 Practical implications

In Western societies, people spend most of their lives in uniform thermoneutral environments. Consequently, the thermal challenges the body has to cope with are limited (Kingma, 2011), potentially leading to obesity and pathologies related to obesity (Johnson et al., 2011). Also, thermal comfort in such environments does not seem to be optimal (Arens et al., 2006). A study performed in Finland revealed that people perceive greater thermal discomfort in offices where temperatures are kept constant, by feeling colder or

hotter more often than at home. It was concluded that the likely reason for this is that they have fewer opportunities to control the thermal environment at work (Karjalainen, 2009). It has been suggested that higher levels of thermal comfort can be achieved in asymmetrical environments, where temperatures can be individually controlled (Arens et al., 2006). Adaptive thermal comfort is now considered a behavioural adaptation to a surrounding environment (Linden et al., 2006). Such adaptations are possible in naturally ventilated buildings, ensuring greater control over thermal comfort (Raja et al., 2001).

By determining the thermal comfort zone for different groups of individuals, appropriate thermal environments can be designed. Since the thermal comfort zone presents a range of temperatures, perceived as thermally comfortable, this allows fluctuating ambient temperatures, which provide a constant stimulus to the skin thermoreceptors and higher level of thermal comfort. The knowledge about the thermal comfort zones can also be applied in the development of thermal manikins and furthermore different clothing systems. This is especially important when the design of protective clothing is considered. The necessity of wearing protective clothing attenuates not only autonomic, but also behavioural responses, since clothing modifications can only be limited in order to assure one's safety (Bligh, 1988). Even individuals working in a cold environment may experience heat stress due to clothing (Havenith, 1999). From this perspective, the complete absence of thermal discomfort cannot be achieved. As such, thermal comfort should be presented in stages, with optimal thermal comfort presented as the best possible situation, followed by slight discomfort which is almost unrecognized by people used to severe conditions, thermal discomfort which can already affect the performance, great thermal discomfort due to severe physical and psychological strain, and thermal discomfort which can affect individuals` health or even jeopardise their lives. In situations where autonomic thermoregulatory responses are limited, more attention should be focused on behavioural modifications. If simple and limited behavioural modifications can improve the evaporative heat loss while wearing protective clothing, this would be potentially reflected in improved thermal comfort and performance.

In conclusion, knowledge of the whole body and regional thermal comfort zones may assist in the design of appropriate thermal environments and clothing systems. For specific target groups such as patients, elderly, and children, such knowledge would be valuable in the optimal design of hospitals, retirement homes, and schools, respectively.

2 Aims and Hypotheses

The overall aim of the present thesis was to evaluate different factors that could influence thermal comfort and consequently modify behavioural thermoregulatory responses. This was addressed through a series of experiments conducted on human volunteers.

Study 1 (Chapter 4) compared the thermal comfort zone of two groups of subjects confined to a hypoxic environment for 10 days; one group was sedentary (Live High, LH), and the other conducted daily exercise training (Live High-Train High, LH-TH). The results provide an insight into the effect of hypoxic training on thermal comfort.

Study 2 (Chapter 5) evaluated the separate and combined effects of hypoxia and activity, as two non-thermal factors, on thermal comfort. Hypoxic bed rest experiments have implications for individuals confined to a hypoxic environment at reduced gravity in future planetary habitats, and also provide insight into the changes associated with certain chronic illnesses rendering patients hypoxic, and as a consequence also inactive, more commonly evident in elderly population. Any alterations in thermal perception could potentially affect the health of these people due to changes in behavioural responses. Information can also be used in the design of appropriate environments, ensuring the safety of these groups.

Study 3 (Chapter 6) evaluated if the seasonal variations in day length and light intensity affect the thermal comfort zone. When experiments are conducted on a longer time scale, potential influence of seasonal variation on a given thermoregulatory response must also be considered. Any seasonal influence would need to be taken into account when comparisons are done between studies conducted in different seasons, or conversely, only the results of studies conducted within the same season should be compared.

Study 4 (Chapter 7) evaluated regional thermal comfort in males and females. Both factors, gender and skin regions, represent important information that needs to be incorporated in the design of algorithms for heated manikins, simulating human thermoregulatory responses. Since thermal comfort is not uniform, local cooling/heating implementation in buildings and creation of asymmetrical environments (Arens et al., 2006) is a logical step forward, providing better thermal comfort for an individual, concomitantly reducing energy consumption.

Study 5 (Appendix D) evaluated strategies that could be beneficial in terms of reducing heat strain for the soldiers performing an activity in simulated desert conditions. The focus was on simple cooling techniques, which could be beneficial in terms of improved performance and thermal comfort. Such conditions require knowledge of behavioural modifications that can contribute the most to an improved thermal status without affecting the safety of an individual.

A number of studies already investigated different aspects of thermal comfort, and common to majority of these studies is that they evaluate thermal comfort using subjective rating scales, while subjects are passively exposed to different thermal conditions. In order to evaluate the thermal comfort in an objective manner the subjects in the present study determined their thermal comfort zone using a water perfused suit. This was the common methodology of the first four studies (Chapters 4 to 7). The effect of heat stress on thermal status, thermal comfort and ultimately performance was the focus of the fifth study (Appendix D).

The specific hypotheses addressed by this thesis are the following:

Study 1:
$H_0$1: Thermal comfort is not affected by hypoxic exercise training.

Study 2:
$H_0$2: Physical inactivity in a hypoxic environment does not affect the perception of thermal comfort.

Study 3:
$H_0$3: The perception of thermal comfort is not affect by seasonal variation.

Study 4:
$H_0$4: The thermal comfort is maintained at a single skin temperature (and not within a range of temperatures).
$H_0$5: There is no difference in the thermal comfort zone of different skin regions.
$H_0$6: There is no difference in the preferred skin temperature of males and females.

Study 5:
$H_0$7: Different cooling strategies cannot reduce heat strain and improve thermal comfort.

3 Thesis Structure

The hypotheses outlined in the previous section were tested in the present thesis within the framework of five separate research studies:

Study 1 (Chapter 4) addresses the effect of a Live High-Train High regimen on the thermal comfort zone.

Study 2 (Chapter 5) addresses the effect of normobaric hypoxic bedrest on the thermal comfort zone.

Study 3 (Chapter 6) addresses the seasonal effect on the thermal comfort zone.

Study 4 (Chapter 7) addresses the regional thermal comfort zone in males and females.

Study 5 (Appendix D) addresses the efficacy of different strategies for increasing evaporative cooling during simulated desert patrol mission.

The findings of these studies are summarized in Chapter 8.

5 The Effect of a Live High-Train High Regimen on Behavioural Temperature Regulation

During the summer months, winter athletes normally live and train at ski resorts situated at high altitudes, where appropriate snow conditions are guaranteed. Such Live High-Train High (LH-TH) regimens induce an adaptation to hypoxia, which may be modified by the exercise training. Cold injury is not uncommon among winter athletes, since thermal insulation of clothing is sacrificed for aerodynamics and reduced weight (Brownlie et al., 1987). The present study assessed whether the hypoxic component of such LH-TH training regimens modified the boundaries and magnitude of the thermal comfort zone, which would be reflected in altered behavioural modifications to maintain thermal comfort. This, in turn, may increase the risk of cold injury among such athletes. In addition, the present study evaluated whether exercise *per se* modified any hypoxia-induced changes in the perception of thermal comfort.

Accurate perception of thermal stimuli is a critical component in the neural process assessing the quality of a stimulus, namely whether it is considered 'comfortable' or 'uncomfortable', and ultimately resulting in a behavioural action to the perceived thermal stimuli. Behavioural reactions to thermal stimuli are initiated to achieve a state of thermal comfort, which is defined as: "a subjective indifference to one's thermal environment" (Mercer, 2001). As with autonomic thermoregulatory responses, behavioural thermoregulatory responses are also dependent on thermal and non-thermal factors (Mekjavic and Eiken, 2006). Since behavioural actions are essential in the maintenance of thermal comfort, they are sensitive to skin temperature, its rate of change, and the area exposed to a thermal stimuli (Burke and Mekjavic, 1991; Kenshalo, 1976). The present study investigated the manner in which hypoxia affects the behavioural actions to a stimulus applied to the skin. This was assessed during a short- (24-h) and long-term (216-h) exposure of sedentary and active individuals to a normobaric hypoxic environment.

Hypoxia-induced behavioural hypothermia has been observed in experimental studies on several animal species, reporting alterations of the thermal comfort, despite normal autonomic nervous functions (Gordon and Fogelson, 1991; Hicks and Wood, 1985). Behavioural responses in some of these animals were observed by initially placing the animals in a thermal gradient environment (unrestricted movement), and then slowly decreasing the oxygen content of the environment in a controlled manner. At a given level of hypoxia, these animals volitionally moved from a warm area to a colder environment, and despite whole-body hypothermia, remained in the colder area for as long as hypoxia persisted. Since a substantial reduction in core temperature did not result in voluntary movement back to a warmer environment, it was suggested that hypoxia-induced hypothermia affects animal behaviour, possibly serving to promote temperature-induced reductions in cellular damage, reduced enzymatic activity, or exposure to toxic metabolites (Gordon, 2001). In humans, acute exposure (~14-22 min exposure time) to moderate hypoxia does not appear to affect the boundaries of the thermal comfort zone (Golja et al., 2005), although it is known to reduce perfusion of the distal regions (Golja, 2004).

During normoxic exercise, heat-loss mechanisms are activated at specific threshold core temperatures. Physical training is known to improve heat loss responses, reflected in increased cutaneous blood flow (Fritzsche and Coyle, 2000) and sweat rate (Araki et al., 1981; Nadel et al., 1974), which are also initiated at a lower threshold core temperature (Roberts et al., 1977). Improvements in circulation as a consequence of physical training could be beneficial in terms of counteracting the hypoxia-induced reduction of perfusion of distal regions (Golja, 2004). Recently, Keramidas et al. (2014a,b) reported that acute and prolonged exposure to normobaric hypoxia *per se* does not alter the hand temperature responses to local cooling, but it impairs the vasomotor response during rewarming after a cold exposure. They also reported that the combined stimuli of continuous hypoxia and exercise training enhance finger cold-induced vasodilatation and hand rewarming responses. Thus, maintaining an appropriate level of training in hypoxic conditions may also minimise the risk of cold injury.

Therefore, in view of the reported effects of hypoxia on peripheral vasomotor responses and skin temperature, the purpose of this study was to determine whether short- and/or long-term exposure to simulated high altitude (normobaric hypoxia) modulates behavioural reactions to thermal stimuli applied to the skin of humans, and whether moderate exercise training affects these responses.

5.1 Methods

4.1.1 Subjects

Sixteen active, healthy men were recruited to participate in this hypoxic confinement study, and were assigned to either a non-exercising live-high (LH; N=8) or exercising live high-train high (LH-TH; N=8) group. Two subjects in the LH group terminated the study prematurely due to medical reasons and thus the study was completed with eight subjects in the LH-TH group, and six in the LH group. Additionally, a subset of participants (N=5) completed a control trial (CON; N=3 LH, N=2 LH-TH) in normobaric normoxia.
The study was approved by the National Committee for Medical Ethics at the Ministry of Health of the Republic of Slovenia, and conducted in conformation with the guidelines of the Declaration of Helsinki. Written informed consent was obtained from each subject prior to participation in the study. During the study, subjects were not allowed to consume any alcohol and caffeine. All subjects were non-smokers, sea-level dwellers, with no history of any known cardiorespiratory, musculoskeletal or circulatory disease. All testing was performed at the Olympic Sports Centre Planica (Ratece, Slovenia) situated at an altitude of 940 m above sea level.
The present study was part of a larger group-design research project conducted to determine the combined effect of activity and hypoxia on various aspects of human physiology, including metabolic adaptations to hypoxia (Debevec et al., 2014b), oxidative stress (Debevec et al., 2014a), cold-induced vasodilation (Keramidas et al., 2014b), the psychophysiology of confinement and circadian rhythm, among others.

5.1.2 Experimental protocol

Subjects arrived at the Olympic Sport Centre Planica for baseline data collection (BDC) in pairs, three nights prior to entering the hypoxic confinement condition (Figure 4.1). Subjects were allowed to move freely between their own rooms and the common living area (~200 m^2) during the entire experimental campaign. The environmental conditions in

the confinement area were: air temperature: 23 ± 1˚C; relative humidity: 57 ± 8%, barometric pressure: 682 ± 4 mmHg. The normobaric hypoxic condition in the facility was maintained using a Vacuum Pressure Swing Adsorption system (b-Cat, Tiel, The Netherlands) that generated and maintained the fraction of oxygen in the inhaled gas (FO_2) in the hypoxia area at 0.139 ± 0.003, resulting in a partial pressure of oxygen in the inspired gas (P_IO_2) of 88.2 ± 0.6 mmHg, corresponding to ~4000m simulated altitude. A sample of air was drawn from each room at 15-min intervals throughout the confinement period, and analysed for oxygen and carbon dioxide content, by oxygen and carbon dioxide analysers, respectively. Prior to each gas sample analysis, the analysers were calibrated automatically with a calibration gas. In the event that the FO_2 in a given room was higher than the pre-set level, then further delivery of hypoxic gas to this room was terminated, until the next gas analysis. In the event that the oxygen level was too low, then a fan would deliver external normoxic air to that room. Failure of the system to maintain the FO_2 resulted in an alarm alerting the attendants to the problem. As a safety measure, all participants were issued personal oxygen analysers (Rae PGM-1100, California, USA) for the duration of the hypoxic confinement. These analysers displayed the prevailing FO_2, and alerted the participants to any sudden decreases in FO_2.
Throughout the study, subjects were provided with five meals (breakfast, snack, lunch, snack, dinner) each day, with the total caloric intake corresponding to their daily energy requirements. Daily meal caloric ratios corresponded to 60:25:15 for the carbohydrate, fat and protein macronutrients, respectively. Individual caloric intake values were calculated by multiplying the subjects' resting energy expenditure by an activity factor (LH=1.4; LH-TH=1.8). Subjects were allowed to drink water *ad libitum*, and were actively encouraged to drink a minimum of 2 L per day throughout the study. Each morning, subjects' heart rate (HR), arterial pressure and capillary oxyhemoglobin saturation (SpO_2; 3100 WristOx, Nonin Medicals, Minnesota, USA) were measured prior to them standing. The self-assessment portion of the Lake Louise Mountain Sickness questionnaire was completed daily to obtain a Lake Louise score (LLMS, 0-15); an indirect index of acute mountain sickness occurrence and severity. Subjects were required to maintain consistent sleep-wake cycles, with "lights out" at 23h00 and "lights on" at 7h00 each day.

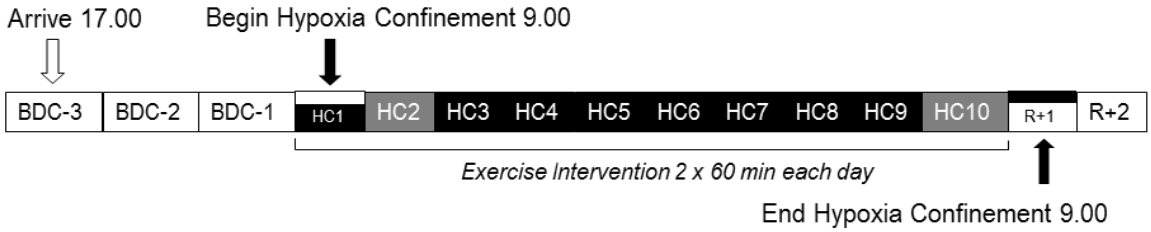


Figure 4.1: *Experimental Design.* Subjects entered the study at 17h00 three nights prior to the start of the intervention period. They entered the hypoxia confinement at 9h00 the morning of HC1, and remained in condition for 10 days. Behavioural thermoregulation was assessed after 24 (HC2) and 216-hours (HC9) of continuous exposure to simulated ~4000 m. The exercise intervention began on the morning of HC1 in the simulated high-altitude environment. Notes: BDC, baseline data collection day; HC, hypoxia confinement condition; R, recovery post-testing day.

5.1.3 Exercise intervention

The LH-TH subjects were required to complete two moderate intensity cycling exercise bouts per day; one hour in the morning (10h00), and one hour in the afternoon (15h00). All exercise training took place in a hypoxic laboratory where the environmental conditions matched those in the confinement area (FO_2=0.139 ± 0.003 and P_IO_2=88.2 ± 0.6 mmHg). Exercise intensity was adjusted so that HR was maintained at 50% of their

hypoxic peak O_2 uptake (VO_{2peak}), determined prior to the initiation of the hypoxic confinement using an incremental cycle test to volitional exhaustion (Ergo Bike Premium, Daum electronics, Fürth, Germany).
HR and SpO_2 were measured continuously by a researcher who maintained individuals' HR to within ± 4 beats per minute of their target peak power output HR by adjusting the cycling load accordingly. After each exercise session, subjects were required to follow a re-hydration protocol (consisting of drinking 1.5 x kg lost during exercise) prior to the start of the afternoon exercise session, or prior to going to sleep, respectively. Subjects in the LH group were not allowed to perform any kind of static or dynamic exercise during the course of the study.

5.1.4 Behavioural temperature regulation

The Behavioural thermoregulation protocol was conducted in normobaric normoxia and after short- (24-h) and long-term (216-h) exposure to hypoxia confinement (i.e. long-term measurements were obtained on the morning of Day 10 (HC10; Figure 4.1)). Behavioural thermoregulation (Figure 4.2) was assessed by having the subjects regulate the water temperature circulating through a custom-made, water-perfused suit (WPS), using methods described elsewhere (Yogev et al., 2010; see Section 1.7).

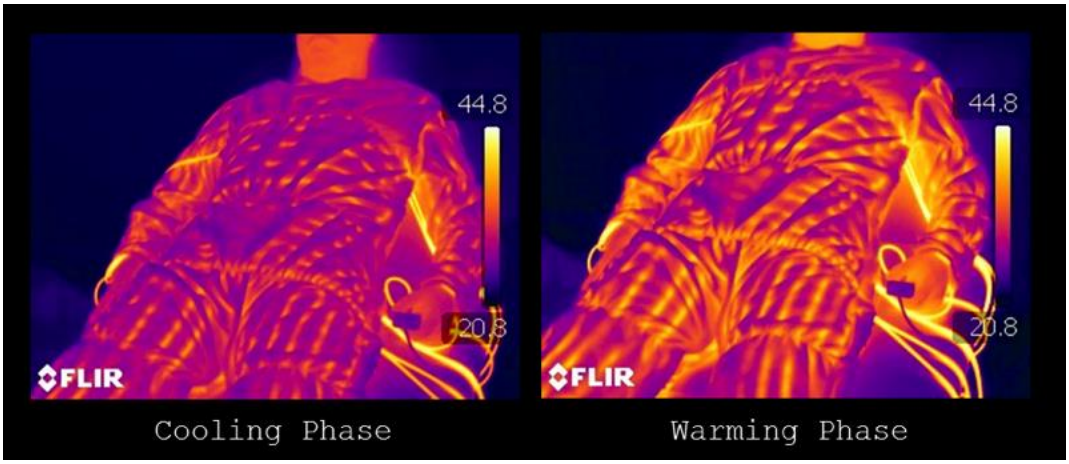


Figure 4.2: *Infrared thermographs depicting the subjects' control of water temperature direction of the water circulating through the custom-built thermal control suit during cooling (left) and warming (right).*

5.1.5 Skin temperature measurements

Skin temperatures were measured at 1-min intervals on the right side of the body at 7 sites (dorsal side of middle finger, forearm, bicep, chest, thigh, calf, great toe at nail bed) using T-type thermocouples (Almemo, 5990-2, Ahlborn, Holzkirchen, Germany). Thermocouples were affixed to the skin using thin, transparent tape (Tegaderm™, 3M). Mean skin temperature (Tsk) was calculated using estimated area weightings of 0.3 for bicep and chest, and 0.2 for thigh and calf (Ramanathan, 1964). Additionally, changes in cutaneous vasomotor tone were calculated from the proximal-distal differences between the calf: toe and the forearm: fingertip skin temperatures (Rubenstein and Sessler, 1990).
Changes in core temperature were estimated during the course of an experiment by tympanic temperature (ThermoScan IRT 3020, Braun, Kronberg, Germany) immediately before and after the testing sessions.

5.1.6 Statistical analysis

Mean Tsk and proximal-distal hand and foot Tsk gradients were averaged into 5-min bins for the entire 60-min protocol. To determine the participants' TCZ, the peak and nadir at which subjects chose to change the direction of water temperature were identified and averaged across the trial. On all descriptive data, an independent t-test was conducted to determine potential differences between the LH and LH-TH. On all dependent variables, a two-way repeated measure ANOVA was conducted (with two within subjects' factors [time, hypoxia exposure] and one between subjects' factor [group]) to determine any differences between the Control, short- (24-h) and long-term (216-h) hypoxic exposure and the exercise intervention. If there were no significant differences by training group observed, data were pooled (within-group) and paired t-tests were employed post-hoc (Bonferroni correction). Statistical analyses were limited to reduce the likelihood of Type I error, and family-wise significance was set at an alpha level of 0.05. Data were analysed using SPSS (v 17.0, Chicago, IL, USA) and are expressed as means ± standard deviations, with 95% Confidence Intervals (CI) for effects of interest.

5.2 Results

Experimental groups were equivalent in age, height, mass and VO_{2peak} at the beginning of the study (Table 4.1).

Table 4.1: *Subjects' characteristics separated by groups.*

	Age (y)	Height (cm)	Mass (kg)	Body Fat (%)	VO_{2peak} $mL \cdot kg^{-1} \cdot min^{-1}$
Control (N=5)	25 ± 4	178.0 ± 2.0	70.3 ± 8.7	22.1 ± 2.4	47.4 ± 3.5
NoEX (N=6)	24.8 ± 3.1	177.7 ± 3.5	70.4 ± 10.0	21.9 ± 4.7	42.2 ± 5.0
Ex (N=8)	25.8 ± 2.4	182.7 ± 5.9	76.6 ± 6.3	22.8 ± 6.2	42.6 ± 6.1

Notes: Control, control group; NoEx, non-exercising group; Ex, exercising group; VO_{2peak}, peak aerobic fitness score.

Daily morning values of participants' resting arterial pressure, HR, mass, SpO_2, and tympanic temperature are reported for the training groups. SpO_2 values were greater in the LH group (SpO_2 =95%; CI: 85.5 to 88.9%) compared to the LH-TH group (SpO_2= 95%; CI: 83.3 to 86.2%; p=0.035) throughout the confinement protocol (Table 4.2).

Table 4.2: *Subjects' daily morning measurements across the hypoxia-confinement intervention protocol.*

	Status	**BDC-1**	**HC1**	**HC10**
SAP	*LH*	118±14	115±11	117±10
(mm Hg)	*LH-TH*	124±6	124±8	121±8
DAP	*LH*	71±9	71±9	74±9
(mm Hg)	*LH-TH*	72±10	73±7	73±14
HR	*LH*	73±20	59±4	74±10
($min^{-1)}$	*LH-TH*	66±9	60±8	68±12
Mass	*LH*	71±10	70±10	69±9
(kg)	*LH-TH*	77±6	77±6	75±6
SpO_2	**LH*		84.7±4.0	88.3±1.8
(%)	**LH-TH*	N/A	80.9±2.8	87.3±3.4
Tty	*LH*	36.1±0.6	36.1±0.5	36.2±0.4
(°C)	*LH-TH*	36.3±0.2	36.2±0.3	36.3±0.2

Notes: NoEx, non-exercising group; Ex, exercising group; BDC-1, baseline data collection; HC, hypoxic confinement days; R, recovery day; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; HR, heart rate; SpO2, finger pulsed oxymetry; TTY, tympanic temperature. (*) Significant difference from BDC-1, (†) different from HC1, (§) significant interaction between-group. Values are means ± standard deviations ($p<0.05$).

5.2.1 Hypoxic exercise confinement adaptations

All subjects assigned to the LH-TH group were able to complete all trainings, and adhered to the post-exercise hydration routine without incident. After the hypoxic confinement protocol, the LH-TH group had higher peak power output (Pre-intervention: 259 ± 34W; Post-intervention: 276 ± 34 W; p=0.0001) compared to no differences observed in the LH group (Pre-intervention: 244 ± 37 W; Post-intervention: 247 ± 41 W; p=0.414). The LH-TH group also exhibited a decrease in percent body fat (Pre-intervention: 22.8 ± 6.2%; Post-intervention: 21.2 ± 5.8%; p=0.0001), which was not evident in the LH group (Pre-intervention: 21.6 ± 4.4; Post-intervention: 21.3 ± 4.8%; p=0.103).

5.2.2 Behavioural temperature regulation

There were no significant differences in the boundary temperatures and magnitude of the TCZ in either the LH or LH-TH groups. The average width of the TCZ did not differ between the Control (9.0 ± 6.9°C), short-term (7.2 ± 4.2°C), and long-term (10.2 ± 7.5°C) hypoxic trials (p=0.256). There was no significant effect of hypoxic exposure on the upper boundary temperature of TCZ (p=0.418). For the Control group it was 37.0 ± 3.8°C, and for the combined LH-TH and LH groups it was 36.0 ± 3.4°C and 37.7 ± 6.1°C after 24-h and 216-h hypoxic exposure, respectively. Similarly, there was no significant difference in the lower boundary temperature of TCZ (p=0.356). For the Control group of subjects it was 28.0 ± 3.7°C, whereas for the combined LH and LH-TH groups it was 29.1 ± 2.1°C after 24-h, and 27.9 ± 3.0°C after 216-h of hypoxic confinement.

5.2.3 Mean skin (Tsk) and tympanic (Tty) temperature measurements

Volitionally-selected Tsk and Tty were not affected by the exercise intervention. The maximum Tsk volitionally sustained during the entire 60-min protocol in the combined LH-TH and LH groups (Figure 4.3) was higher (p=0.040; CI: 0.02 to 0.78°C) after 24-h of hypoxic exposure (Tsk=35.9 ± 1.0°C), compared to Control group (Tsk=34.9 ± 0.8°C), and after 216-h of hypoxic exposure (Tsk=35.6 ± 1.0°C). Maximum volitional Tsk was not significantly different after 216-h of hypoxic exposure from those in the Control group. There was no change in tympanic temperature during the trials (Tty at onset of trials: 36.2 ± 0.4: Tty at end trials: 36.3 ± 0.3°C; p=0.615).

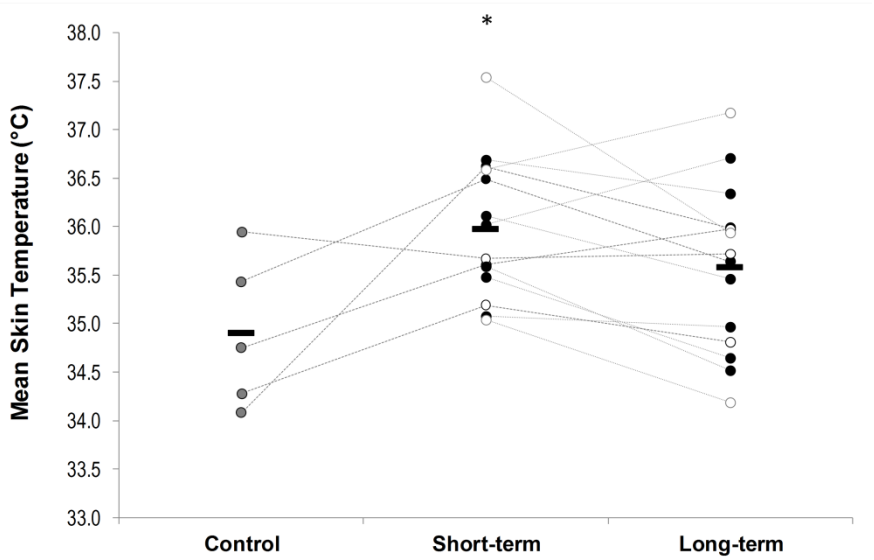


Figure 4.3: *Maximum mean skin temperature volitionally maintained by subjects attained during the behavioural thermoregulation protocol.* Mean group data (standard deviation error bars) are depicted using closed circles. (*) Significant difference between acute and both Control and long-term time-points (p<0.05).

5.2.4 Peripheral cutaneous vasoconstriction

Rubinstein and Sessler (1990) suggested that a skin temperature difference between the distal portion of a limb and the tip of a digit of more than +4 °C is indicative of vasoconstriction, whereas as House and Tipton (2002) suggest that vasoconstriction is present already at a gradient of 2.3°C. Accordingly, the observed baseline values (Table 6.3), for the forearm-fingertip skin temperature gradient for the combined LH and LH-TH groups (ALL) indicate a difference (p=0.009) between the Control (0.6 ± 2.3 °C) and Short-term exposure (4.2 ± 4.0 °C), indicative of vasoconstriction in the latter trial. Long-term exposure to hypoxia reduced this forearm-fingertip skin temperature gradient (1.1 ± 2.6°C), such that there was no significant difference between the Long-term and Control group values. A similar response was observed for the first 5 min of the trial. Upon completion of the test (last 5 minutes), there were no longer any differences in the forearm-fingertip skin temperature gradient. Whereas there were no significant differences between the LH and LH-TH groups for forearm-fingertip skin temperature gradient, the calf-toe skin temperature gradient revealed a significant reduction in the Long-term compared to the Short-term trial in the LH group, for measurements during both Baseline (Short-term: 7.5 ± 2.5°C; Long-term: 4.3 ± 2.9°C) and in the first 5 minutes (Short-term: 8.6 ± 2.3 °C; Long-term: 4.7 ± 3.2°C) of the test (Table 4.3).

Table 4.3: *Proximal-to-distal changes in peripheral cutaneous temperature gradients for the control (Control), non-exercising (NoEx), and exercising (Ex) group.* Larger values indicate greater difference in temperature gradient (vasoconstriction), whereas values closer to 0 indicate little change in the tissue perfusion gradient.

	Forearm-Fingertip (°C)			Calf-Toe (°C)		
	Control (N=5)	Acute (24-h)	Long-term (216-h)	Control (N=5)	Acute (24-h)	Long-term (216-h)
Start 5 min						
NoEx	---	4.7 ± 4.2	0.7 ± 3.1	---	7.5 ± 2.5	4.3 ± 2.9 +
Ex	---	3.8 ± 4.2	1.5 ± 2.4	---	5.1 ± 4.6	5.3 ± 2.0
ALL	0.6 ± 2.3	4.2 ± 4.0 *	1.1 ± 2.6	3.1 ± 2.3	6.1 ± 3.9	4.8 ± 2.4
First 5 min						
NoEx	---	5.5 ± 3.9	0.7 ± 3.3	---	8.6 ± 2.3	4.7 ± 3.2 +
Ex	---	4.3 ± 4.0	1.2 ± 2.5	---	5.5 ± 4.9	6.0 ± 2.0
ALL	0.2 ± 1.5	4.8 ± 3.8	0.9 ± 2.8 *	3.3 ± 3.2	6.8 ± 4.2	5.4 ± 2.6
Last 5 min						
NoEx	---	3.6 ± 5.3	1.5 ± 1.2	---	11.3 ± 4.1	7.9 ± 5.2
Ex	---	1.9 ± 3.5	0.5 ± 1.7	---	5.8 ± 7.8	5.4 ± 4.0
ALL	1.3 ± 1.3	2.6 ± 4.2	1.0 ± 1.5	4.0 ± 3.7	8.2 ± 6.9	6.6 ± 4.6

Notes: (*) Indicates a significant difference from the Control and Long-term exposure, (+) significant within-group difference from acute exposure. Values are means ± standard deviations ($p<0.05$).

4.3 Discussion

The main finding of the present study is that both short- and long-term hypoxic exposure did not alter the boundary temperatures and magnitude of the thermal comfort zone in sedentary individuals (LH), and in individuals participating in daily exercise training (LH-TH).

4.3.1 Thermal comfort zone and temperature measurements

Acute hypoxic exposure has been shown to decrease cutaneous sensitivity to cold in the feet (Golja et al., 2004), suggesting that alterations in thermal perception may affect an individual's ability to self-regulate thermal comfort during cold exposure at altitude. In contrast to these findings, the TCZ does not seem to be affected (Golja and Mekjavic, 2003; Golja et al., 2005). The present study observed no difference in or between the LH and LH-TH training groups. The preferred water temperature regulated within the suit resulted in significantly higher measured maximum Tsk after the short-term hypoxia exposure, compared to Control group and long-term hypoxia exposure, which is in contrast to other studies performed on animals exposed to acute hypoxia environments (Gordon and Fogelson, 1991; Hicks and Wood, 1985). In fact, it has been observed that short-term hypoxia causes modest vasodilatation in nonacral (Simmons et al., 2007) and vasoconstriction in acral skin regions in humans (Golja, 2004). Higher maximum Tsk after 24-h hypoxic exposure in the present study could be an indicator of vasodilatation in nonacral skin regions as reported elsewhere, since it was measured on skin parts such as calf, thigh, chest and arm (Ramanathan, 1964). Also, due to a greater vasoconstriction in the extremities after 24-h hypoxic exposure, participants could have maintained a higher maximum Tsk in order to reduce the thermal discomfort of the cooler hands and feet.

Since the maximum Tsk was observed to be higher after 24-h, compared to 216-h hypoxic exposure and Control, it is likely that differences in Tsk affected by the short-term exposure were gradually reduced after 216-h hypoxia exposure, reaching similar levels as in Control.

Forearm-fingertip skin temperature gradient was not different between the LH and LH-TH groups. Combining the data of both LH and LH-TH groups revealed a significantly

greater vasoconstriction after 24-h, compared to the long-term (216-h) hypoxic exposure and Control. The calf-toe skin temperature gradient indicated a significant reduction in the vasoconstriction observed after 216-h, compared to 24-h hypoxia exposure in the LH group, whereas in the LH-TH group it remained similar after short- and long-term hypoxic exposure, indicating vasoconstriction. This is in agreement with the study performed by Golja (2004) who also observed vasoconstriction of the distal regions after acute exposure to hypoxia. In addition, research conducted on subjects rendered inactive and confined to bedrest, also in a hypoxic environment, McDonnell et al. (2014) reported decreased perfusion of the toe from day 1 to day 10, but with no corresponding differences in the perfusion of the fingers. Interestingly, a similar calf-toe skin temperature gradient was observed after acute and long-term hypoxia exposure in the LH-TH group, which was also maintained at the same level during the behavioural temperature regulation trial (~5°C). Though differences between the LH-TH and LH groups were not significant, there was a tendency for less vasoconstriction in the lower extremities in the LH-TH group after the short-term hypoxic exposure, suggestive of an exercise-induced improvement in the circulation in this region. Indeed, Keramidas et al. (2014b) observed improved finger cold-induced vasodilation and hand rewarming responses after 10-day normobaric hypoxic confinement, combined with exercise protocol. Physical training is well known to encourage heat-loss mechanisms (Nadel et al., 1974; Roberts et al., 1977) including the increase in blood flow (Fritzsche and Coyle, 2000). Accordingly, the LH-TH group in the present study could have maintained a stable, albeit reduced blood flow in their lower extremities across the 10-day hypoxic confinement and during the one-hour behavioural temperature regulation protocol due to the alterations, induced by daily exercise.

4.3.2 Limitations and study considerations

The non-exercising group comprised six subjects, and the control group of only five subjects. Therefore, the small sample size reduced the statistical power of the test to detect statistical significance. Due to the study design, a greater sample size was not feasible at the time of performing the study, but should be considered in the future.

The characteristics of the thermal comfort zone, namely its boundaries and magnitude, were not affected by exposure to 10-d normobaric hypoxia in sedentary and exercise training subjects. We conclude that in the absence of any hypoxia and/or exercise effect on the perception of thermal comfort, it is unlikely that hypoxia alters behavioural reactions to thermal stimuli, and thus the possibility of an increased risk of cold injury for athletes training at altitudes simulated in the present study is also unlikely.

The combined effect of hypoxia and inactivity on the thermal comfort zone is, as yet, unresolved, and will be discussed in the next chapter.

6 Effects of Normobaric Hypoxic Bedrest on the Thermal Comfort Zone

In contrast to the International Space Station, which maintains a normobaric normoxic environment, it is envisaged that future Lunar and Mars habitats will maintain a hypobaric hypoxic environment to minimise the risk of decompression sickness during the preparation for extra-vehicular activity (EVA). Namely, the pressure within the space suits during EVAs is approximately one third of normal atmospheric pressure, and astronauts preparing for EVAs must conduct a lengthy decompression procedure in their space suits to prevent venous gas emboli. Maintaining hypobaric hypoxic environment in future planetary habitats will eliminate the need for lengthy decompression procedures, and consequently the risk of decompression sickness (Bodkin et al., 2006; Norcross et al., 2013). Due to exposure to reduced gravity and hypobaric hypoxia, the main concern is how these conditions will affect the function of physiological systems.

It is well documented that hypoxia alters behavioural and autonomic thermoregulation in a variety of species, such that they choose a cooler environment during hypoxic exposure and lower their preferred body temperature (Barros et al., 2004; Dupre and Wood, 1988; Gordon and Fogelson, 1991; Hicks and Wood, 1985; Pertwee et al., 1990; Tattersall and Milsom, 2009). The reduction in body temperature seems to be mediated by two autonomic thermoregulatory responses: peripheral vasodilation, which occurs as the circulatory system, attempts to deliver more blood to the oxygen-starved tissues, and a reduction in metabolic rate, reflecting the reduced activity of aerobic respiration. Lowering body temperature reduces tissue oxygen requirements, thus prolonging survival and affording protection to the hypoxic tissues (Gordon, 1993). Numerous studies observing the effects of hypoxia have also been performed on humans. The autonomic thermoregulatory responses were shown to be altered, with enhanced core cooling due to a delay in the onset of vasoconstriction and shivering and increased respiratory heat loss (Johnston et al., 1996). Malanda et al. (2008) investigated the effects of hypoxia in overweight altitude acclimatized subjects, using a thermal stimulator, and reported that exposure to normobaric hypoxia (4200 m) induces slowing of neural activity in the sensor-to-effector pathway, but does not affect cutaneous sensation threshold for either warm or cold detection, suggesting it is unlikely that the thermal comfort zone is affected by hypoxia under normobaric circumstances. The effect of hypoxia on the cutaneous thresholds for thermal sensation of the toes of unacclimatised subjects was also evaluated by Golja et al. (2004). They reported a reduced sensitivity to cold during the hypoxic exposure, suggesting that alteration in thermal perception may affect the individual's perception of thermal comfort and consequently attenuate thermoregulatory behaviour during cold exposure at altitude. The few studies that have investigated the effect of hypoxia on behavioural thermoregulatory responses in humans have reported that hypoxia does not seem to affect human behavioural thermoregulation (Golja and Mekjavic, 2003; Golja et al., 2005).

The bedrest experimental model is commonly used as a ground-based simulation model for studying the effects of microgravity on certain physiological systems (Fortney et al., 1996). Exposure to sustained bedrest has been shown to alter autonomic thermoregulatory responses. Specifically, it has been shown to induce a progressive decrease in skin

temperature of the distal regions, reflecting reduced skin blood perfusion (Golja et al., 2002), attenuated responses of sweating and cutaneous vasodilation, with increased threshold core temperature for sweating onset and decreased sweating sensitivity (Michikami et al., 2004), attenuated cold-induced vasoconstriction and consequently greater heat loss, and attenuated shivering after the exposure (Mekjavic et al., 2005). As regards behavioural thermoregulation, we have previously reported that responses were not affected after 21 days of normoxic inactivity/unloading achieved with the bedrest experimental model (Yogev et al., 2010).
The present study extends the work of Yogev et al. (2010), by investigating the effect on behavioural thermoregulation of living in a hypoxic environment during sustained bedrest. Even though these two non-thermal factors, both known to induce peripheral vasoconstriction during the exposure, do not seem to affect behavioural thermoregulation when studied separately, information is lacking regarding their combined effect on the perception of thermal comfort in humans.

5.1 Methods

5.1.1. Subjects

This study was part of a larger study investigating the separate and combined effects of inactivity/unloading and hypoxia on the cardiovascular, musculoskeletal, neurohumoural, and thermoregulatory systems. The study protocol was approved by the National Committee for Medical Ethics at the Ministry of Health of the Republic of Slovenia, and conformed to the guidelines of the Declaration of Helsinki. Fourteen healthy male subjects participated in the study. Prior to the onset of the study they had a medical examination, and their participation was subject to physician's approval. They were familiarised with the protocol of the study and gave their written consent for participation in the study. Their mean (SD) age, weight and height were 27 ± 6 years, 76.7 ± 11.8 kg and 179 ± 3 cm. The study was conducted at the Olympic Sport Centre Planica (Rateče, Slovenia) situated at an altitude of 940 m.

5.1.2 Experimental protocol

The study was designed as a repeated measures study, such that the subjects participated in three separate trials conducted on separate occasions. During the first trial subjects were assigned to one of three experimental groups: 1) Hypoxic ambulatory confinement (HAMB; fraction of oxygen in the inhaled gas, F_iO_2=0.141 ± 0.004; partial pressure of oxygen in the inspired gas, P_iO_2=90.0 ± 0.4; ~4000 m simulated altitude), 2) Hypoxic bedrest (HBR; F_iO_2=0.141 ± 0.004; P_iO_2=90.0 ± 0.4; ~4000 m simulated altitude), and 3) Normoxic bedrest (NBR; F_iO_2=0.209; P_iO_2=133.1 ± 0.3). In the remaining two trials the interventions were crossed over. The three experimental trials were conducted over a one-year period. The interval between the experimental trials was a minimum of 3 months to ensure that the subjects' physical condition returned to baseline, pre-trial, values. The simulation of the target altitude of 4000 m was achieved by reduction of the oxygen fraction in the air (normobaric hypoxia), while the effects of bedrest studied by confining the subjects to a horizontal position in bed, with all daily routines performed in this position for 21 days. To ensure the safety of the subjects, staff and researchers, the oxygen level was continuously monitored. Once subjects arrived at the Olympic Sport Centre, they were assigned to one of the three experimental groups, with 7 days of baseline measurements before starting the assigned 21 day intervention. Following the 21-day confinement, subjects remained at the Olympic Sport Centre for a further 4 days

of (post-) testing. During the study, subjects were provided with five meals (breakfast, snack, lunch, snack, dinner) each day with the total caloric intake corresponding to their daily energy requirements (Debevec et al., 2014c). The same menu with identical meal order for each day was repeated in each campaign, in order to exclude the effects of the diet on measured variables. All experiments were performed on the same days in all three campaigns. A staff of nurses and physicians ensured the health and well-being of the subjects 24/7 during each 21-day intervention. The three confinement trials were conducted on one floor of the Olympic Sport Centre, which comprised 10 double bedrooms, living and dining areas. A barrier separating the floor into two parts, allowed both normoxic and hypoxic interventions to be conducted simultaneously.

5.1.3 Behavioural temperature regulation

The assessment of behavioural temperature regulation was achieved with a water-perfused suit (WPS) as described in Section 1.7. The suit was constructed of mixed blend of synthetic fibres and mashed inner layer. Eight small-diameter (inner dia. = 4 mm; outer dia. = 5 mm) PVC tubes were woven in the mash of the suit and connected to a manifold. The length of each tube was 9 m, so that when the pump was activated, the equal length of the tubes ensured equal water flow (22 L/min) in all tubes connected to the manifold. Two of the 9 m tubes were woven into the arm segments, two in torso (one in front and one in back) segment and four in the leg (two in front and two in back) segments. The WPS covered the whole body except the hands, feet, neck and head. Each segment of the WPS was designed with a front and back segment that could be separated. A snug fit of the two parts on any given segment was achieved with Velcro® straps. Once instrumented, the subjects donned the WPS, and remained in the supine position for the duration of the test. The test commenced with a 10-min baseline period during which water pumped through the suit was maintained at a temperature (Twps) of 27°C. Thereafter, the temperature of the water perfusing the suit was regulated such that it described a sinusoid that could potentially deliver water at temperatures ranging from 10° to 50°C.

During each experimental campaign, measurements were performed 5 days prior to the intervention (D-5), and on days 10 (D10) and 20 (D20) of the intervention. In each experimental campaign, the measurements were performed at the same time of day.

5.1.4 Measurements

Each morning, subjects' heart rate (HR), and blood pressure were monitored using the iBody App (iBody, Wahoo Fitness, Atlanta, USA) running on subjects' iPads (Apple Inc., Cupertino, California, USA), and oxygen saturation (S_pO_2) with a finger pulse oximeter (3100 WristOx, Nonin Medicals, Minnesota, USA). Daily morning core temperature during the 21 day interventions was estimated with a tympanic temperature (ThermoScan IRT 3020, Braun, Kronberg, Germany).
During the assessment of behavioural thermoregulation, subjects' core temperature (T_{re}, °C) was measured with a rectal thermistor (MSR Electronics GmbH, Henggart, Switzerland) inserted approximately 12 cm beyond the anal sphincter. Skin temperature was assessed with thermistors (MSR Electronics GmbH, Henggart, Switzerland) secured on the calf, thigh, chest, biceps, forearm, finger and toe by thin air-permeable tape (Tegaderm, 3M, USA). All skin temperature sites were on the right side of the body. Heart rate (HR) was measured using a Polar t34 Heart rate Transmitter (Kempele, Finland). All the data were sampled and stored with an MSR12 (Modular Signal

Recorder, MSR Electronics GmbH, Henggart, Switzerland) data logger at 30-sec intervals. The average water temperature at the inlet and outlet of the WPS was measured using T-type thermocouples, and defined as Twps. The sinusoidal Twps pattern with its low (T_{low}) and high (T_{high}) peaks revealed a thermal comfort zone (TCZ) for each subject. The assessment of calf, thigh, chest, and arm skin temperature enabled the calculation of weighted mean skin temperature (Ramanathan, 1964). The proximal-to-distal skin temperature gradient was measured in the hand as the difference between skin temperature (T_{sk}) at the forearm and the second digit ($\Delta T_{forearm\text{-}finger}$), and in the foot as the difference between T_{sk} at the calf and big toe ($\Delta T_{calf\text{-}toe}$). These values were assumed to reflect cutaneous blood flow (Rubinstein and Sessler, 1990). Ambient temperature (Ta) and relative humidity (RH) were measured with transducers (MSR145, MSR Electronics GmbH, Henggart, Switzerland) situated in closed proximity to the subject. Ta and RH were maintained at 22-23°C and 50-60%, respectively.
Throughout the experiment, subjects provided ratings of their thermal comfort and thermal sensation every 10 min. Thermal comfort was evaluated on 7-point scale, with the assigned descriptors being: 0 – 0.5 = comfortable, 1 – 1.5 = slightly uncomfortable, 2 – 2.5 = uncomfortable and 3 = very uncomfortable. Thermal sensation was derived from a 7-point scale, with the following descriptors: 3 = hot, 2 = moderately hot, 1 = warm, 0 = neutral, -1 = cool, -2 = moderately cold and -3 = cold.

5.1.5 Data analysis

Only the second half of each 1-hr trial was included in the analysis unless otherwise stated. Yogev et al. (2010) demonstrated that the sinusoidal pattern of Twps during the course of the experiment described a damped oscillation, suggesting a temporal change in the magnitude of the thermal comfort zone. They concluded that the first oscillations regulated by the subject in such experiments most likely constituted the learning phase, since towards the latter half of the trials the sinusoidal pattern was stable, and no further changes in the magnitude of the thermal comfort zone noted.
Data are presented as means (SD) with the limit of statistical significance set at 0.05. A two-way repeated measures ANOVA (experimental condition (HAMB, HBR, NBR) x testing period (D-5, D10, D20, 21-D intervention)) was used to compare the WPS parameters (T_{low}, T_{high}, width of the TCZ) and daily morning measurements (Systolic Arterial Pressure, Diastolic Arterial Pressure, heart rate, tympanic temperature, oxygen saturation), whereas three-way repeated measures ANOVA (experimental condition (HAMB, HBR, NBR) x testing period (D-5, D10, D20) x time (30-min of the second half of the trial)) was used to compare T_{re}, T_{sk}, $T_{forearm\text{-}finger}$, $T_{calf\text{-}toe}$, HR and environmental conditions (Ta and RH). If a significant F value was found ($p < 0.05$), critical differences were analysed by Tukey`s procedure to locate the significant mean differences. Subjective ratings of temperature perception and thermal comfort were analysed with a Wilcoxon non-parametric test. Statistical analysis was performed using Statistica 5.0 (Statsoft Inc., Tulsa, OK, USA).

5.2 Results

Of the 14 subjects that were entered into the study, only 11 completed all three experimental conditions (HAMB, HBR, NBR), thus the data of only 11 subjects was included in the analysis and is presented in the results.

5.2.1 Water perfused suit temperature (Twps, °C)

Twps, controlled by subjects during the 1-hr experiment did not differ significantly between conditions and also no statistically significant differences were found within each condition, when D-5 measurements were compared with measurements obtained on D10 and D20. The mean (SD) values of the lower limits (T_{low}), upper limits (T_{high}) and width of the thermal comfort zone (TCZ) during all experimental trials are presented in Table 5.1.

Table 5.1: *Mean ± SD lower limits, upper limits and the width of the thermal comfort zone (°C) during Hypoxic ambulatory confinement (HAMB), Hypoxic bedrest (HBR) and Normoxic bedrest (NBR), 5 days prior to the intervention (D-5), and on days 10 (D10) and 20 (D20) of the intervention.*

	Lower limit of TCZ			Upper limit of TCZ			Width of TCZ		
Condition	D-5	D10	D20	D-5	D10	D20	D-5	D10	D20
HAMB	29.5± 3.3	29.4± 4.4	30.1± 6.3	36.7± 3.7	37.5± 4.7	37.3± 5.9	7.2± 5.2	8.1± 4.5	7.3± 5.0
HBR	28.0± 5.7	28.9± 5.0	28.1± 6.0	37.5± 6.6	37.8± 6.7	37.6± 7.8	9.5± 5.7	8.9± 4.9	9.5± 5.7
NBR	28.5± 5.5	27.4± 6.5	28.3± 7.6	36.3± 5.4	37.2± 8.1	38.5± 6.3	7.8± 5.2	9.8± 8.0	10.2± 6.2

5.2.2 Subjective ratings (thermal comfort, thermal sensation)

According to the ratings of thermal comfort and thermal sensation, subjects remained comfortable and warm during all trials with no significant differences between them, except during HAMB20 (Figure 5.2). In contrast to the other trials, during which subjects reported being comfortable and warm, in this trial the subjects reported being thermally comfortable, while rating the temperature as neutral.

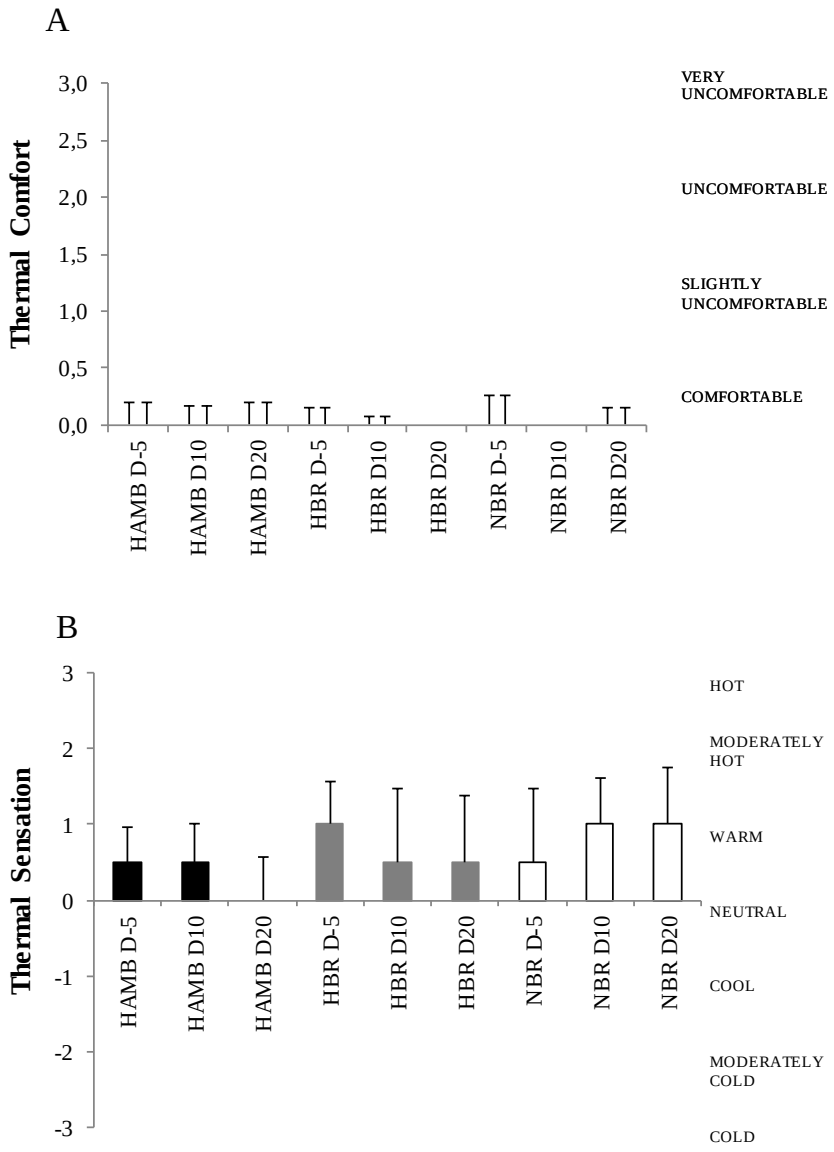


Figure 5.1: *Median ± SD subjective ratings of the thermal comfort and thermal sensation during Hypoxic ambulatory confinement (HAMB), Hypoxic bedrest (HBR) and Normoxic bedrest (NBR), 5 days prior to the intervention (D-5), and on days 10 (D10) and 20 (D20) of the intervention.*

5.2.3 Rectal temperature (Tre, °C)

A slight, albeit statistically nonsignificant, drop of 0.2°C (±0.2) in Tre was observed during baseline measurements (D-5) and in the HAMB condition (D10 and D20) throughout the 1-hr trial, while an almost steady state was noticed in the HBR and NBR conditions (D10 and D20). Rectal temperature (Table 5.2) did not differ significantly between baseline measurements and experimental conditions, except during the HAMB condition, where significantly higher Tre was reported during baseline HAMB (37.4°C, ±0.3) and lower during HAMB10 (37.1°C, ±0.3) and HAMB20 (37.1°C, ±0.2). Between condition comparison revealed no significant difference.

5.2.4 Skin temperature (Tsk, °C)

Tsk increased in all conditions during the 1-hr trial, the average increase being between 1.4°C and 2.5°C (Figure 5.3). No significant differences in Tsk were found within each

condition (D-5, D10 and D20) and also not between experimental conditions (HAMB, HBR, NBR).

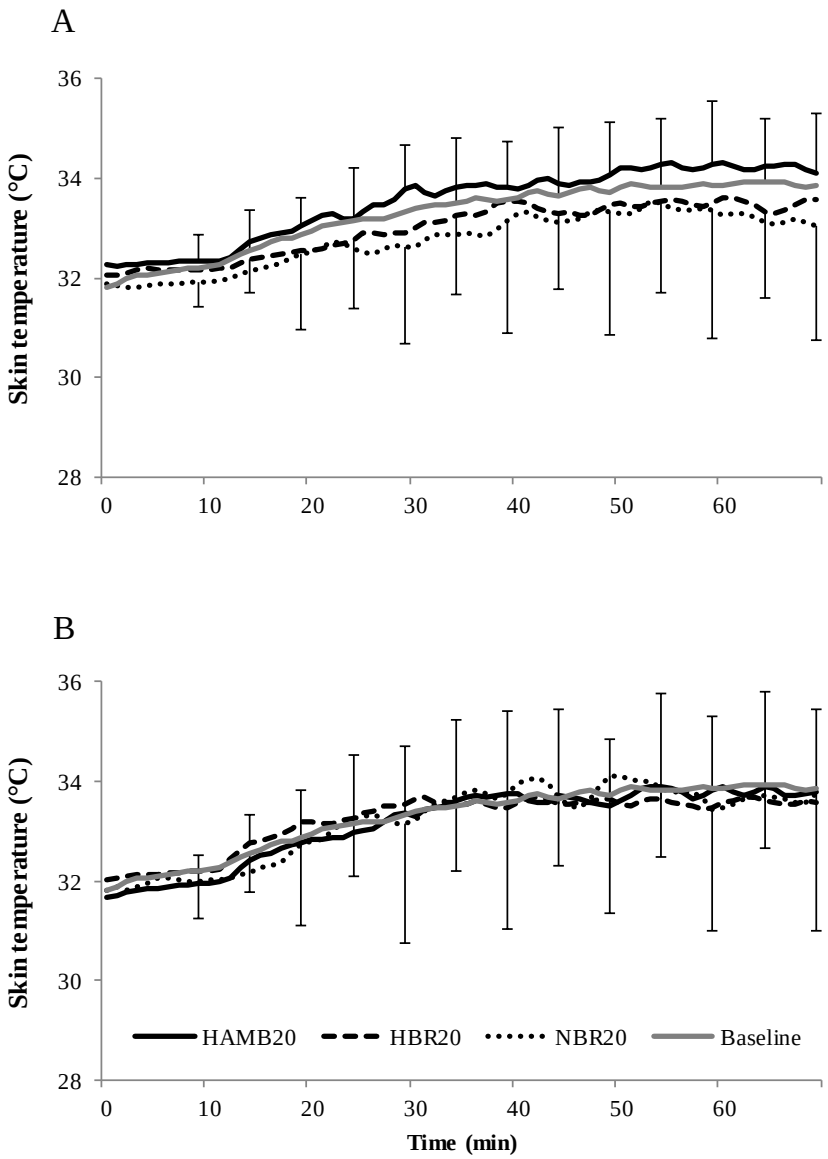


Figure 5.2: *Mean ± SD skin temperature (Tsk, °C) measured throughout the one hour trial, during Hypoxic ambulatory confinement (HAMB), Hypoxic bedrest (HBR), and Normoxic bedrest (NBR), on days 10 (A) and 20 (B), including baseline measurements.*

5.2.5 Proximal-to-distal skin-temperature gradient ($T_{forearm-fingertip}$, $T_{calf-toe}$, °C)

The differences in forearm-finger ($\Delta T_{forearm-fingertip}$) and calf-toe ($\Delta T_{calf-toe}$) skin temperatures measured during each trial were considered an index of cutaneous vasomotor tone, with values ≥ 2°C reflecting vasoconstriction, and ≤ 0°C vasodilation (House and Tipton, 2002). While $\Delta T_{forearm-fingertip}$ changed more extensively during the first half of the trial, $\Delta T_{calf-toe}$ remained stable during the whole experiment in all tested conditions (Table 5.2). Positive values of both indices throughout the 1-hr trials indicate the presence of vasoconstriction. There were no significant differences in $\Delta T_{forearm-fingertip}$ nor in $\Delta T_{calf-toe}$ within each experimental condition (D-5, D10 and D20) and also not between experimental conditions (HAMB, HBR and NBR).

Table 5.2: *Mean ± SD rectal temperature (Tre, °C), skin temperature (Tsk, °C), proximal-to-distal skin temperature gradients between the forearm and fingertip skin temperature ($T_{forearm-fingertip}$, °C), and between the calf and toe skin temperature ($T_{calf-toe}$, °C), and heart rate (HR, min^{-1}), during Hypoxic ambulatory confinement (HAMB), Hypoxic bedrest (HBR) and Normoxic bedrest (NBR), 5 days prior to the intervention (D-5), and on days 10 (D10) and 20 (D20) of the intervention.*

Condition	HAMB			HBR			NBR		
	D-5	D10	D20	D-5	D10	D20	D-5	D10	D20
Tre	37.4± 0.3 [a]	37.1± 0.3	37.1± 0.2	37.2± 0.2	37.2± 0.2	37.3± 0.3	37.3± 0.3	37.3± 0.1	37.2± 0.3
Tsk	34.5± 0.6	34.1± 1.1	33.7± 1.4	33.4± 2.3	33.4± 1.7	33.6± 2.0	33.6± 1.4	33.3± 2.4	33.8± 2.6
ΔTforearm-fingertip	2.4± 2.6	2.8± 2.6	3.5± 2.6	3.5± 2.8	2.0± 2.0	2.6± 2.4	3.7± 3.0	2.3± 1.5	3.2± 1.5
ΔTcalf-toe	4.8± 4.5	5.3± 3.0	6.0± 5.2	3.6± 2.3	4.4± 4.1	4.9± 4.0	4.0± 3.2	7.1± 3.0	5.6± 2.5
HR	79± 9 [a]	70± 10	68± 8	71± 9	72± 6	79± 7 [bc]	67± 10	67± 8	67± 7

[a] Significant difference between D-5 and measurements obtained on D10 and D20
[b] Significant difference within HBR condition between D-5 and D20
[c] Significant difference between HBR when compared to HAMB and NBR on D20

5.2.6 Heart rate (HR, min^{-1})

Heart rate (Table 5.2) was significantly higher during baseline HAMB (79±9 min^{-1}), when compared to HAMB10 (70±10 min^{-1}) and HAMB20 (68±8 min^{-1}). Significantly higher HR was also reported in HBR20 (79±7 min^{-1}), when compared to baseline HBR (71±9 min^{-1}). Between condition comparison resulted in a significantly higher HR in HBR20 condition (79 ±7 min^{-1}), when compared to HAMB20 (68±8 min^{-1}) and NBR20 (67±7 min^{-1}).

5.2.7 Daily morning measurements of systolic arterial pressure (SAP), diastolic arterial pressure (DAP), heart rate (HR), tympanic temperature (Tc), and capillary oxyhemoglobin saturation (SpO_2)

Daily morning measurements (Table 5.3) across 21-days of the intervention indicated higher SAP during HBR than during HAMB and higher DAP during HBR than during

HAMB and NBR. These differences were however not evident on D10 and D20 of the intervention, when the study of behavioural thermoregulation was performed. Arterial pressure (SAP and DAP) also did not differ between the measurements obtained prior to the intervention (D-5), and between day 10 and day 20 within each experimental condition. As evident in the present study of behavioural thermoregulation assessment the morning measurements also reflected higher HR values in HBR than in HAMB and NBR across 21-days of the intervention. On D10 and D20 HR in the HBR condition was significantly higher only when compared to the NBR condition. Tympanic temperature as an indicator of core temperature did not differ between or within experimental conditions across 21-days and on D-5, D10 and D20. As expected, SpO_2 was higher during NBR than during the hypoxic conditions (HAMB, HBR). Within condition comparison resulted in lower SpO_2 during D10 and D20 than during D-5 in both hypoxic conditions.

Table 5.3: *Mean ± SD values of daily morning measurements of Systolic Arterial Pressure (SAP), Diastolic Arterial Pressure (DAP), heart rate (HR), tympanic temperature (Tc), and capillary oxyhemoglobin saturation (SpO2) during Hypoxic ambulatory confinement (HAMB), Hypoxic bedrest (HBR) and Normoxic bedrest (NBR) prior the intervention (D-5), on day 10 and 20 of the intervention (D10 and D20) and across the 21 days of intervention (21-D).*

Condition	Day	SAP	DAP	HR	Tc	SpO_2
HAMB	D-5	114±13	65±5	63±9	36.2±0.4	97±1 [i]
	D10	119±9	73±9	68±8	36.3±0.2	88±2 [j]
	D20	117±13	73±11	63±9	36.5±0.5	89±2 [k]
	21-D	116±2	71±1 [b]	68±2 [d]	36.3±0.1	88± 0 [g]
HBR	D-5	114±11	66±5	64±12	36.2±0.5	97±1 [i]
	D10	121± 9	76±10	71±12 [e]	36.3±0.4	88±1 [l]
	D20	121±14	79±7	72±6 [f]	36.6±0.4	90±1 [m]
	21-D	121±2 [a]	76±2	72±2 [d]	36.5±0.1	88±1 [h]
NBR	D-5	111±7	61±5	64±11	36.4±0.4	98±0
	D10	116±10	67±8	60±13	36.4±0.3	97±1
	D20	119±11	70±7	59±8	36.3±0.3	97±0
	21-D	117±2	69±1 [c]	60±2 [d]	36.4±0.1	97±0

[a] Significant difference in SAP between HBR and HAMB across 21-days
[b] Significant difference in DAP between HBR and HAMB across 21-days
[c] Significant difference in DAP between HBR and NBR across 21-days
[d] Significant difference in HR between HAMB, HBR and NBR across 21-days
[e] Significant difference in HR between HBR and NBR on D10
[f] Significant difference in HR between HBR and NBR on D20
[g] Significant difference in SpO_2 between HAMB and NBR across 21-days
[h] Significant difference in SpO_2 between HBR and NBR across 21-days
[i] Significant difference in SpO_2 between D-5 and measurements obtained on D10 and D20
[j] Significant difference in SpO_2 between HAMB and NBR on D10
[k] Significant difference in SpO_2 between HAMB and NBR on D20
[l] Significant difference in SpO_2 between HBR and NBR on D10
[m] Significant difference in SpO_2 between HBR and NBR on D20

5.3 Discussion

This study investigated the separate and combined effects of hypoxia and bedrest on the perception of thermal comfort. The oscillatory response of Twps in the three interventions provided lower (T_{low}) and upper (T_{high}) limits of the thermal comfort zone (TCZ), with the difference between these temperatures reflecting the magnitude of TCZ. No statistically

significant differences were found between the interventions (HAMB, HBR and NBR), or between different measurement days (D-5, D10 and D20) within each intervention.

5.3.1 Water perfused suit temperature pattern

Yogev et al. (2010) reported that the first oscillations regulated by the subject in their experiments most likely constituted the learning phase, since towards the latter half of the trials the sinusoidal pattern was stable, and no further changes in the magnitude of the thermal comfort zone noted. However, this phenomenon was not noticed in our study, and this could partially be explained by the fact that subjects went through nine trials and therefore became very familiar with their preferred temperatures so no "learning" phase was noticed in later trials. Interestingly, the TCZ was significantly wider during the second than the first half of the trial in NBR10, and it exhibited a similar tendency ($p=0.05$) in the NBR20 trial. It can be hypothesised that this pattern, with similar lower limits and higher upper limits of regulated temperatures, was due to greater cutaneous vasoconstrictor response in the legs during the NBR10 than the baseline measurements. Since subjects were not wearing socks during the experiments, they might have been experiencing cold feet, especially towards the end of the experiment and hence might have started up-regulating the temperature in the WPS. For the subjects with particularly high or low preferred temperature or wider TCZ few changes in the Twps were made a given trial, especially during the initial phase when the preferred temperature had to be gradually reached from the starting point of 27°C. As a consequence, only the second half of the one-hour trial is presented in the results in the present study.

5.3.2 The combined and separate effect of inactivity/unloading and hypoxia on thermal comfort

Although a 35-day normoxic bedrest has been reported to affect not only autonomic temperature regulation during immersion in 28°C water, but also thermal perception with subjects perceiving given combinations of skin and core temperatures as warmer and thermally less uncomfortable after than before the bedrest (Mekjavic et al., 2005), the study of Yogev et al. (2010), reported no effect of a 21-day horizontal bedrest under normoxic conditions, on the thermal comfort zone. Although cold sensitivity increased, the change did not seem to be of sufficient magnitude to cause any alteration in behavioural thermoregulatory responses (Yogev et al., 2010).

Hypoxia has also been reported to alter autonomic thermoregulatory responses, such as cutaneous vasoconstriction (Golja and Mekjavic, 2002; McDonnell et al., 2014), and to decrease sensitivity to cold (Golja et al., 2004), hence potentially changing the perception of thermal comfort. It has been suggested that a normobaric hypoxic exposure induces slowing of neural activity in the sensor-to-effector pathway, but does not affect the threshold for cutaneous detection of either warmth or cold (Malanda et al., 2008). However, the results reported by Golja and Mekjavic (2003) and Golja et al. (2005) indicate that behavioural temperature regulation does not appear to be compromised by moderate hypoxia. Present results from the HAMB condition confirm previous findings (Golja and Mekjavic, 2003; Golja et al., 2005), and extend them to suggest that neither acute nor prolonged exposure to moderate hypoxia affects behavioural thermoregulation.

Moreover, previous findings that neither hypoxia nor inactivity/unloading *per se* alter behavioural thermoregulatory responses (Golja and Mekjavic, 2003; Golja et al., 2005; Yogev, 2010) are confirmed by present results, and extended to include that the combination of hypoxia and inactivity/unloading does not affect behavioural thermoregulation. No statistically significant differences were found when observing the

separate and combined effects of normobaric hypoxia and/or inactivity/unloading. Even though not significant, there was a difference in the lower limits of the thermal comfort zone between conditions, with T_{low} higher during HAMB (D10 and D20), and lower during HBR and NBR (D10 and D20). Since the upper limits of the TCZ did not differ as much as the lower limits, the latter contributed to a widening, albeit not significant, of TCZ in the HBR and NBR conditions.

5.3.3 Thermal comfort rating

In the present study, subjects evaluated their perception of thermal comfort and thermal sensation every 10 min during the 1-hr trial. Although subjects actively chose their preferred temperature, with upper and lower limits presenting their TCZ, subjective ratings were also included in the study, ensuring that subjects followed the instructions of maintaining thermal comfort. It should be noted though that a rating could have been made in a moment, when the subject was about to change the direction of Twps, by pressing cooling or heating, suggesting that thermal comfort was not optimal at the moment of rating. Ratings therefore present only an approximation of subjects' perception during the trial. In the majority of experimental conditions subjects felt thermally comfortable when they perceived the Twps as warm, whereas after 20 days in the HAMB condition, a neutral thermal sensation was rated as thermally comfortable.
When a single body segment is exposed to uncomfortable ambient conditions, thermal comfort can be altered (Fanger, 1970). The subjects in our study did not wear socks during the experiment, and could hence have been experiencing regional discomfort by feeling cold during the course of the one-hour trial, especially due to exaggerated cutaneous vasoconstriction, induced by prolonged hypoxia and/or bedrest (Golja et al., 2002; McDonnell et al., 2014). It is therefore conceivable that, in all three conditions, they could have regulated Twps at a higher level than in a normoxic ambulatory condition to out-balance the discomfort associated with cold feet. Since a normoxic ambulatory confinement trial was not included in this study, we can assume that the results of the D-5 trials reflect the normoxic ambulatory conditions. Since there were no differences in the regulation of TCZ between this trial and TCZ regulation in at days 10 and 20 in the three interventions, a significant contribution of the exposed feet to overall TCZ is most likely minimal. Subjects were not asked to rate their regional thermal comfort, and also did not report any thermal discomfort of the feet.

5.3.4 Body temperature and proximal-to-distal skin temperature gradient

Significantly higher Tre was measured during baseline HAMB than after 10 and 20 days in the HAMB condition. No other significant differences in Tre and Tsk were observed within or between experimental conditions.
There was no difference between or during each of the interventions as regards the $\Delta T_{forearm\text{-}fingertip}$ and $\Delta T_{calf\text{-}toe}$, reflecting no inter-condition difference in skin perfusion, with the levels of the $\Delta T_{forearm\text{-}fingertip}$ and $\Delta T_{calf\text{-}toe}$ indicating a slight cutaneous vasoconstriction in all three conditions. That neither bedrest, hypoxia, nor the combination of these interventions substantially increased proximal-to-distal skin temperature gradient is somewhat surprising considering that previous studies indicate that prolonged exposure to both hypoxia and sustained bedrest induce cutaneous vasoconstriction. Golja et al. (2002) observed a progressive decrease in subjects' skin temperature during 35 days of horizontal bedrest, with changes particularly obvious in distal body regions, and with the most pronounced temperature decrements observed at

the tips of the toes, suggesting that cutaneous vasomotor tone increased gradually during the course of the bedrest. During sustained hypoxic exposure, cutaneous vasoconstriction was shown to increase from day 1 to day 10, especially in lower extremities (McDonnell et al., 2014). Yogev et al. (2010), on the other hand, observed no significant difference in $\Delta T_{forearm\text{-}fingertip}$ and $\Delta T_{calf\text{-}toe}$ between days 1 and 22 of bedrest, a finding which is in agreement with the observations in the present study when comparing days 10 and 20 with baseline values within all three experimental conditions.

5.3.5 Limitations and study considerations

Subjects in the present study were instructed to maintain the thermal comfort throughout the whole one hour trial. They were requested to change the direction of the Twps as soon as it was perceived as slightly uncomfortable (too cool or too warm). These changes established the boundaries (T_{low} and T_{high}) of the TCZ, perceived as thermally comfortable. Since T_{low} and T_{high} present the limits of the TCZ, they do not present an optimal thermal comfort. However, as evident from the subjective ratings, subjects followed the instructions of maintaining the thermal comfort throughout the trial.

Our homogenous group of healthy and fit subjects were similar in their age, weight and height, thereby exclude any potential effects of these factors on the TCZ during the three interventions. It is possible that behavioural temperature regulation could be affected in populations rendered physically inactive due to different nonthermal factors, such as chronic illnesses, old age, obesity etc., but the results of the present study suggest that inactivity *per se* would not have a significant contribution to any behavioural thermoregulatory dysfunctioning in such populations.

The study was designed as a repeated measures study and therefore it was performed in two different seasons – autumn and spring over a one year period. It has previously been reported that the seasonal variations in day length and light intensity can affect the circadian rhythm, core interthreshold zone, and metabolic rate (Honma et al., 1992; Kakitsuba et al., 2011; Kakitsuba et al., 2013; van Ooijen et al., 2004). Therefore, the next chapter evaluates if the seasonal variations also affect the thermal comfort and moreover behavioural temperature regulation.

7 Seasonal Effect on Behavioural Temperature Regulation

Studies investigating the thermoregulatory responses of subjects exposed during repeated trials need to ensure that the interval between trials is sufficient to prevent any adaptation to the thermal environment, unless such adaptation is the aim of the study. The choice of the time interval between experimental trials is therefore crucial, if adaptation is to be avoided. When experiments are conducted on a longer time scale, and the time interval between experimental trials is several months, then the potential influence of seasonal variation on a given thermoregulatory response must also be considered.
Any seasonal influence on thermoregulatory responses would need to be taken into account when comparisons are done between studies conducted in different seasons, or conversely, only the results of studies conducted within the same season should be compared. The seasonal variation in day length and light intensity has been shown to affect the human circadian rhythm as well as the core interthreshold zone (Honma et al., 1992; Kakitsuba et al., 2011; Kakitsuba et al., 2013). The regulation of circadian rhythm was discovered to be influenced by melanopsin cells – photoreceptors in the retinal ganglion cells – which are sensitive to changes in ambient light (Berson et al., 2002; Hattar et al., 2002; Güler et al., 2008). Seasonal variations were found in the timing of sleep with subjects going to bed earliest in summer, intermediate in spring and autumn, and latest in winter. Mean body temperature and the phases of circadian temperature and melatonin rhythms have also been shown to be affected by seasons (Honma et al., 1992). The temperature zone between the core temperature thresholds for onset of shivering and cessation of sweating, termed the core interthreshold zone (CIZ) was reported to be significantly wider in summer than in winter, as well as significantly wider in the presence of a greater level of light intensity (Kakitsuba et al., 2011). During cold exposure, an increase in metabolic rate was also reported as significantly greater in winter than in summer (van Ooijen et al., 2004).

The study was designed as a repeated measures study, such that the subjects participated in three separate trials conducted on separate occasions. Of the three trials, two were conducted during different seasons in the Northern hemisphere: autumn (October) and spring (May), to be able to discern the effect of season. The third trial was conducted again in autumn, but a year after the first trial, to discern the temporal effect on thermal comfort.

6.1 Methods

The present study was a part of a larger study investigating the separate and combined effects of inactivity/unloading and hypoxia on the cardiovascular, musculoskeletal, neurohumoural, and thermoregulatory systems. The overall study comprised three experimental campaigns separated by a minimum of 2.5 months. We took advantage of this opportunity to investigate the behavioural responses of subjects to the same thermal stimuli in different seasons of the year.
The study protocol was approved by the National Committee for Medical Ethics at the Ministry of Health of the Republic of Slovenia, and conformed to the guidelines of the

Declaration of Helsinki. Twelve healthy male subjects provided the baseline data that was collected before each confinement. Prior to the onset of the study they had a medical examination, and their participation was subject to physician's approval. They were familiarised with the protocol of the study and gave their written consent for participation in the study. Their mean (SD) age, weight and height were 27 ± 6 years, 76.7 ± 11.8 kg and 179 ± 3 cm. The study was conducted at the Olympic Sport Centre Planica (Rateče, Slovenia) situated at an altitude of 940 m.

6.1.1 Protocol

The study was designed as a repeated measures study. The first campaign was conducted from October 1st to November 6th 2012 (trial 1), the second campaign from April 15th to May 21st 2013 (trial 2), and the third campaign from September 23rd to October 29th 2013 (trial 3). Two campaigns (1st and 3rd) were therefore performed in the autumn, whereas one campaign was performed in the spring (2nd campaign).

6.1.2 Behavioural thermoregulation assessment

The assessment of behavioural temperature regulation was achieved with a water-perfused suit (WPS) as described in Section 1.7 and Chapter 5.1.3.

6.1.3 Measurements

During the assessment of behavioural thermoregulation, subjects' core temperature (Tre, °C) was measured with a rectal thermistor (MSR Electronics GmbH, Henggart, Switzerland) inserted approximately 12 cm beyond the anal sphincter. Skin temperature was assessed with thermistors (MSR Electronics GmbH, Henggart, Switzerland) secured on the calf, thigh, chest, biceps, forearm, finger and toe by thin air-permeable tape (Tegaderm, 3M, USA). All skin temperature sites were on the right side of the body. Heart rate (HR) was measured using a Polar t34 Heart rate Transmitter (Kempele, Finland). All the data were sampled and stored with an MSR12 (Modular Signal Recorder, MSR Electronics GmbH, Henggart, Switzerland) data logger at 30-sec intervals. The average water temperature at the inlet and outlet of the WPS was measured using T-type thermocouples, and defined as Twps. The sinusoidal Twps pattern with its low (Tlow) and high (Thigh) peaks revealed a thermal comfort zone (TCZ) for each subject. The assessment of calf, thigh, chest, and arm skin temperature enabled the calculation of weighted mean skin temperature (Ramanathan, 1964). Ambient temperature (Ta) and relative humidity (RH) were measured with transducers (MSR145, MSR Electronics GmbH, Henggart, Switzerland) situated in closed proximity to the subject. Ta and RH were maintained at 22-23°C and 50-60%, respectively.
Throughout the experiment, subjects provided ratings of their thermal comfort and thermal sensation every 10 min. Thermal comfort was evaluated on 7-point scale, with the assigned descriptors being: 0 – 0.5 = comfortable, 1 – 1.5 = slightly uncomfortable, 2 – 2.5 = uncomfortable and 3 = very uncomfortable. Thermal sensation was derived from a 7-point scale, with the following descriptors: 3 = hot, 2 = moderately hot, 1 = warm, 0 = neutral, -1 = cool, -2 = moderately cold and -3 = cold.

6.1.4 Data analysis

Only the second half of each 1-hr trial was included in the analysis unless otherwise stated. Yogev et al. (2010) demonstrated that the sinusoidal pattern of Twps during the

course of the experiment described a damped oscillation, suggesting a temporal change in the magnitude of the thermal comfort zone. They concluded that the first oscillations regulated by the subject in such experiments most likely constituted the learning phase, since towards the latter half of the trials the sinusoidal pattern was stable, and no further changes in the magnitude of the thermal comfort zone noted.
Data are presented as means (SD) with the limit of statistical significance set at 0.05. A one-way repeated measures ANOVA (trial 1, trial 2, trial 3) was used to compare the WPS parameters (Tlow, Thigh, width of the TCZ), whereas two-way repeated measures ANOVA (experimental condition (trial 1, trial 2, trial 3) x time (30-min of the second half of the trial) was used to compare Tre, Tsk, HR and environmental conditions (Ta and RH). If a significant F value was found ($p < 0.05$), critical differences were analysed by Tukey's procedure to locate the significant mean differences. Subjective ratings of temperature perception and thermal comfort were analysed with a Wilcoxon non-parametric test. Statistical analysis was performed using Statistica 5.0 (Statsoft Inc., Tulsa, OK, USA).

6.2 Results

6.2.1 Water perfused suit temperature (Twps, °C)

Regulated Twps indicated no difference in preferred minimum and maximum temperature between trials, with average values between 29.8 ± 3.7 °C and 35.8 ± 2.5 °C during trial 1, 29.3 ± 4.6 °C and 37.6 ± 5.0 °C during trial 2, and 27.2 ± 5.4 °C and 38.6 ± 6.9 °C during trial 3. The range between the minimum and maximum values however was reported as wider during trial 3 (11.4 ± 6.0 °C) when compared to trial 1 (6.0 ± 4.4 °C) and trial 2 (8.3 ± 4.8 °C).

6.2.2 Subjective ratings (thermal comfort, thermal sensation)

According to the ratings of the thermal comfort and thermal sensation, subjects remained comfortable and neutral to warm during all trials with no significant differences between them.

Table 6.1: *Median ± SD subjective ratings of thermal comfort and thermal sensation in three trials.*

	Baseline 1	Baseline 2	Baseline 3
Thermal comfort	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.3
Thermal sensation	1.0 ± 0.7	0.0 ± 0.8	0.5 ± 0.5

6.2.3 Rectal temperature (Tre, °C)

Throughout one hour trial, a slight drop in rectal temperature was observed during all three trials, with 0.3 ± 0.2 °C during trial 1, 0.1 ± 0.2 °C during trial 2, and 0.2 ± 0.1 °C during trial 3 (Figure 6.1). The second half of the 1-hr trial indicated higher Tre values during trial 1 (37.4 ± 0.2 °C), when compared to trial 2 (37.2 ± 0.2 °C; $p<0.05$) and trial 3 (37.2 ± 0.3 °C; $p<0.05$).

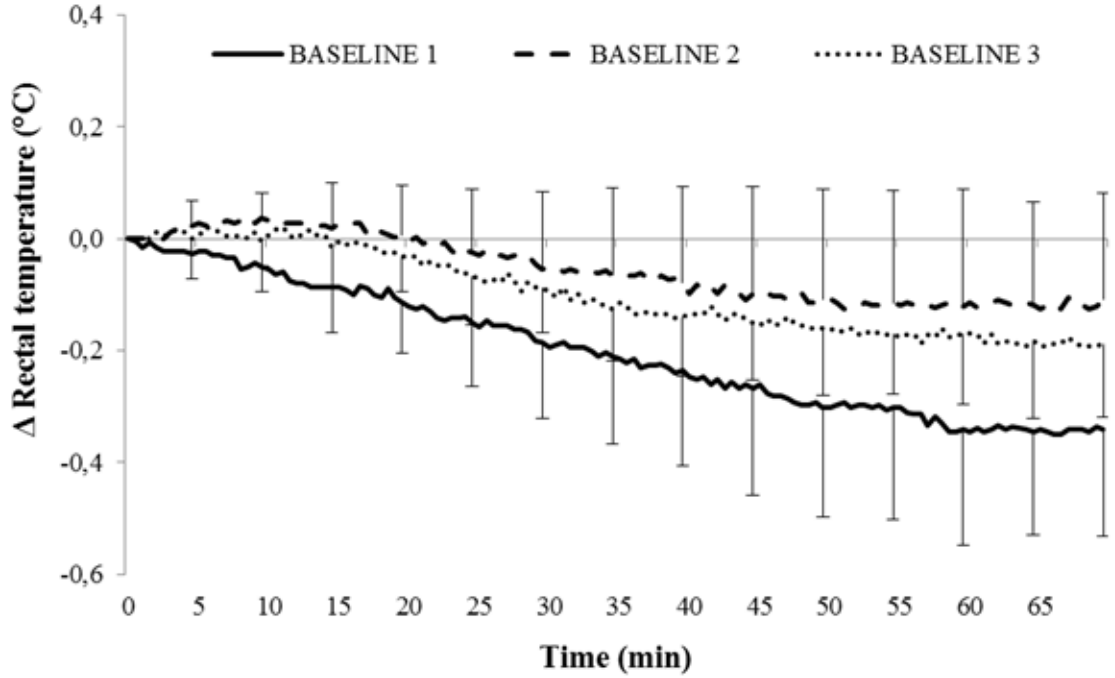


Figure 6.1: *Mean ± SD drop in rectal temperature throughout one hour trial.*

6.2.4 Skin temperature (Tsk, °C)

An increase in Tsk was observed in the first half of all three trials (Figure 6.2). Tsk was similar during the second half of trial 1 (34.4 ± 0.5 °C) and trial 2 (34.1 ± 1.3 °C). Tsk was higher during trial 1 when compared to trial 3 (33.1 ± 2.3 °C; $p<0.05$).

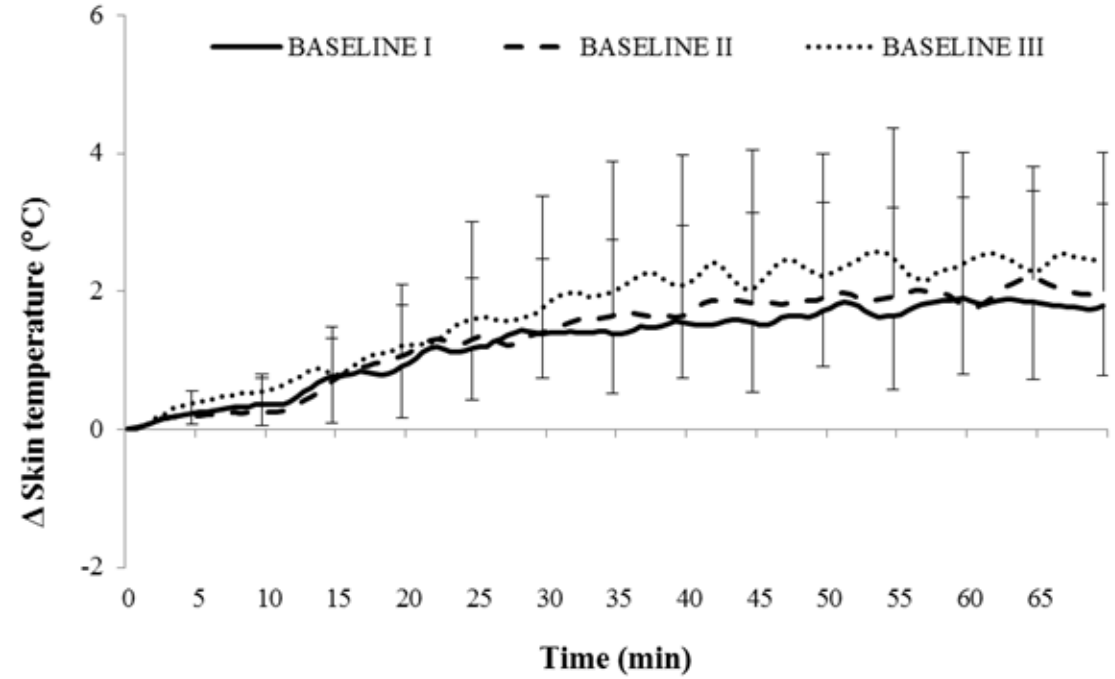


Figure 6.2: *Mean ± SD increase in skin temperature throughout one hour trial.*

6.2.5 Heart rate (HR, min-1)

HR was stable throughout all the one hour trials (Figure 6.3). It was reported as higher during trial 1 (80 ± 8 min-1) when compared to trial 2 (67 ± 8 min-1) and trial 3 (70 ± 9 min-1).

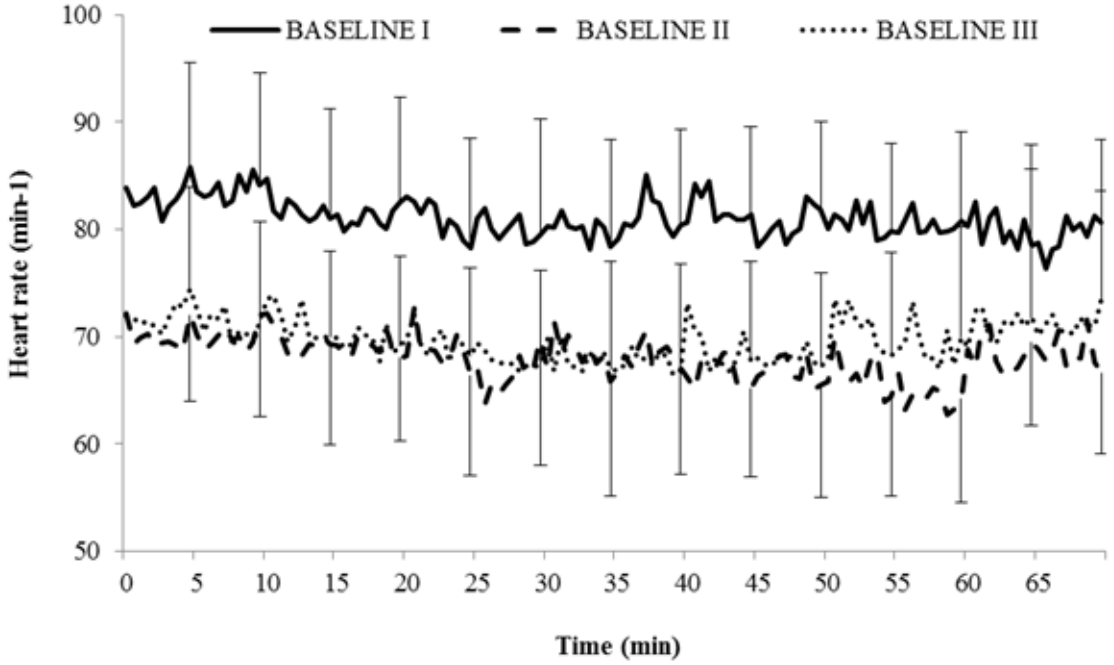


Figure 6.3: *Mean ± SD heart rate throughout one hour trial.*

6.2.6 Weather conditions

The weather conditions are presented in Table 6.2 (Naše okolje, Bilten Agencije RS za okolje, July 2012 – October 2013). Trials 1 and 3 were conducted at the start of the autumn, and presumably the summer exposure would have influenced any seasonal adaptation in the subjects. Similarly, the 2nd trial was conducted at the start of the spring season, and the conditions during the winter months would have influenced the subjects' thermoregulatory responses. For this reason, the summer and winter conditions are included in Table 2 since they could potentially affect the response observed in early spring and autumn. Data indicates no difference in the autumn measurements (trial 1 vs. trial 3), collected in the years of 2012 and 2013. As expected, more variations are observed between both autumn seasons when compared to spring season (trial 1 and trial 3 vs. trial 2).

Table 6.2: *Weather conditions during one year period.*

CAMPAIGN			Average air temperature	Hours of solar radiation	Number of cloudy days	Number of sunny days	Precipitation (mm)	Average air pressure	Average pressure of the water vapour
SUMMER 2012	Jul - Avg 2012	Average	20,0	250,5	5,6	9,4	115,2	985,2	15,6
		SD	1,9	16,9	1,2	3,9	44,8	28,4	1,5
1ST CAMPAIGN	Oct 1 - Nov 6 2012	Average	11,0	134,7	11,1	3,5	176,7	983,5	11,4
		SD	1,8	13,7	2,0	2,4	68,8	31,5	1,5
WINTER 2013	Jan - Mar 2013	Average	1,7	75,8	17,2	2,0	138,2	980,1	5,8
		SD	2,0	15,6	2,2	0,9	37,4	32,2	0,7
2ND CAMPAING	Apr 15 - May 21 2013	Average	12,8	174,3	12,1	2,4	122,9	982,4	10,9
		SD	1,7	20,0	2,6	1,0	36,4	31,6	1,1
SUMMER 2013	Jul - Avg 2013	Average	19,6	259,3	5,2	8,5	91,2	986,2	15,3
		SD	1,6	22,6	1,6	2,1	25,0	31,0	1,6
3RD CAMPAIGH	Sept 23 - Oct 29 2013	Average	12,3	122,5	12,5	2,5	74,7	988,7	11,9
		SD	1,6	22,0	2,4	1,3	41,0	31,9	1,4

6.3 Discussion

According to the results, no seasonal effects were observed during the study of behavioural temperature regulation. Significant differences were reported between different trials and measured parameters, but these differences were not evident only between the autumn and spring seasons, but also between both autumn campaigns. Therefore, it seems some other factors influenced the observed differences between the trials.

Day length was shorter during autumn (trial 1 and trial 3) with 10 hr and 58 min of daylight, and longer during spring (trial 2) with 14 hr and 13 min of daylight. Accordingly, more solar radiation was also present during the spring months (Naše okolje, Bilten Agencije RS za okolje, July 2012 – October 2013). Kakitsuba et al. (2011, 2013) reported that light intensity affects the core interthreshold zone, which they noted to be wider at greater light intensities. Therefore, it should also be wider during the summer compared to the winter months. Similarly, differences in metabolic response between these two seasons were also observed in the study by van Ooijen et al. (2004). Both studies were however performed in the summer and winter seasons, which differ in ambient conditions much more than the spring and autumn seasons investigated in the present study.

In the present study, experimental conditions were carefully controlled and all the trials were performed at the same time of the day. Significantly higher Tre and HR were measured during trial 1, when compared to trials 2 and 3. Trials 1 and 3 were however performed in the same season (autumn), but differed significantly. This could be explained by the fact that the subjects were not as comfortable during the first campaign, as in the next two campaigns, which could affect the measured parameters during the assessment of the behavioural temperature regulation. Tension due to a new and unknown environment could explain a higher Tre and HR, measured during trial 1. Significant differences between both autumn campaigns were also reported for Tsk, which was higher during trial 1 compared to trial 3. A lower Tsk could be due to a wider range in regulated temperatures during trial 3, when compared to trials 1 and 2 with subjects regulating Twps within narrower ranges. Interestingly, the range between the regulated temperatures was the widest in the last trial (trial 3), whereas it would be expected that the wider range would be chosen during the first trial, when subjects were less familiar with the protocol and could therefore show more variability in the regulated temperature. Yogev et al. (2010) reported larger oscillations in the Twps pattern during the first half of the trial and smaller oscillations in the second half. Smaller oscillations and narrower range would therefore be expected also after subjects had already performed a number of trials, but this did not prove to be true in the present study.

6.3.1 Limitations and study considerations

The present study did not observe any seasonal effect on behavioural temperature regulation, since the differences in measured responses were also evident between the measurements, collected in the same seasons. Due to the fact that all three campaigns were performed in either autumn or spring, which are more similar in ambient conditions than summer and winter, this could reduce the seasonal effect on the measured responses reported in other studies (Honma et al., 1992; Kakitsuba et al., 2011; Kakitsuba et al., 2013; van Ooijen et al., 2004). Accordingly, behavioural temperature regulation does not seem to be affected by the seasonal variation, when comparing the data obtained in spring and autumn. However, there can be other factors that affect the behavioural patterns, which also need to be considered.

Chapters 4-6 evaluated the thermal comfort zone of the entire body, whereas it is well known that the thermal comfort differs between body regions. The next chapter therefore evaluates the regional thermal comfort zone in males and females.

8 Regional Thermal Comfort Zone in Males and Females

In the somatosensory cortex, skin regions are presented proportionally, relative to the importance of their role for the organism (Anselme et al., 1999). Skin regions therefore differ in the perception of temperature sensation and contribution to local and overall thermal comfort (Arens et al., 2006; Nakamura et al., 2008, 2013; Stevens and Choo, 1998; Zhang et al., 2004). Central integration of regional skin temperature and subsequent cortical processing of this information provides an assessment not only of the sensation of temperature, but also a rating of the thermal comfort. The derived sensation of temperature and thermal comfort are the principal factors driving our behaviour in defence of the core temperature (Schlader et al., 2010). Thus, skin temperature has a relatively greater contribution to subjective thermal comfort than to the autonomic thermoregulatory responses, which serves to initiate behavioural thermoregulation before activation of the more metabolically demanding responses that maintain body temperature (Frank et al., 1999).
The studies investigating regional cutaneous sensitivity thresholds to warming and cooling have observed the face and the mouth to be the most sensitive, and the extremities least sensitive to thermal stimuli (Stevens and Choo, 1998). All skin regions appear to be more sensitive to cold than to warm stimuli (Stevens and Choo, 1998; Golja et al., 2003). The contribution of the trigeminal region in modulating autonomic and behavioural responses has been confirmed for cold stimuli by Mekjavic and Eiken (1985) and for warm stimuli by Cotter and Taylor (2005). Whereas cooling or warming the torso area has been shown to strongly influence overall temperature sensation and thermal comfort (Arens et al., 2006; Guéritée et al., 2014; Nakamura et al., 2008), the limbs and extremities have been reported to be less influential than the torso (Nakamura et al., 2013).

The nature of human autonomic and behavioural temperature regulation has been studied predominantly with male subjects, and particularly for thermal comfort there is a paucity of data for female subjects. Gender comparisons have often focused on physiological differences, such as greater body fatness and body surface area to mass ratio in females (Tikuisis et al., 2000), and in the contribution of the menstrual cycle (Kocjan, 2007). When matched for body mass and surface area, the immersion in cold (Tikuisis et al., 2000) and tepid (Anderson et al., 1995) water resulted in similar responses of body core temperature and metabolic rate for both genders, whereas sudomotor sensitivity was shown to be lower in females (Gagnon et al., 2013). However, it has recently been suggested that gender differences in sweat rate should be studied at fixed heat production (≤ 250 W.m^{-2}), providing more accurate data in terms of matching the groups (Jay, 2014). Differences in thermal sensation and thermal comfort between genders have been evaluated during exposure to a variety of ambient conditions (Hashiguchi et al., 2010; Karjalainen, 2007; Parsons, 2002; Schellen et al., 2012), indicating significant gender differences especially during exposure to cool conditions with greater thermal discomfort perceived by females. Furthermore, it was reported that females are more sensitive to warm and cold stimuli (lower thresholds for detection of the stimulus) than men (Golja et

al., 2003), and display a stronger warmth sensation to a warm stimulus (Gerrett et al., 2014).

Common to studies investigating thermal comfort is the manner in which subjective ratings of thermal comfort are derived. All use some manner of visual analogue scales (VAS), and it is not always possible to compare the results of different studies, if different VASs were used. To avoid this problem, the present study determined the thermal comfort zone of different body regions as the range of temperatures deemed thermally comfortable. This was achieved using a water-perfused suit (WPS). To date, all conclusions regarding variations in regional thermosensitivity, and the variation in the contribution of these regions to overall thermal comfort, have been based on subjective responses obtained with a variety of VASs. These studies have not considered that thermal comfort is not achieved at a single temperature, but that it is likely established for a range of skin temperatures. Similar to the autonomic regulation of body temperature, whereby deep body temperature is maintained within a range of core temperatures defined as the core interthreshold zone, it has been demonstrated that thermal comfort is also perceived over a range of skin temperatures. This range is defined as the thermal comfort zone in the present thesis. We tested the hypothesis that the thermal comfort zone is the same for different skin regions, and also the same for male and female subjects.

7.1 Methods

7.1.1 Subjects

Sixteen healthy Caucasian males (n=8) and females (n=8) participated in the study. Their mean (±SD) age, weight and height were 25 ± 3 yrs, 76 ± 7 kg and 177 ± 6 cm, respectively, for the male participants, and 25 ± 4 yrs, 60 ± 7 kg and 164 ± 3 cm, respectively, for the female participants. Following familiarisation with the equipment and study protocol, subjects gave their written consent for participating in the study, approved by the National Committee for Medical Ethics at the Ministry of Health of the Republic of Slovenia. Before entering the study they were requested that prior to each experimental trial they should not: i) participate in any heavy exercise, ii) have a large meal, iii) smoke, iv) drink coffee, and v) drink alcohol a day before the experiment. Due to the fluctuation of body temperature during the menstrual cycle, females provided information regarding their menstruation in order to avoid the period immediately after ovulation, when higher levels of progesterone induce elevations in the internal temperature (Baker et al., 2001).

7.1.2 Experimental protocol

Thermal comfort of seven skin regions (arms, legs, front torso, back torso, hands, feet and head) and the whole body was evaluated in the present study. In order to assess the temperature, perceived as thermally comfortable, subjects were requested to don a water perfused suit (WPS) and to regulate the temperature within the WPS for the duration of one hour trial. A sinusoidal change in the water perfused suit temperature (Twps), potentially delivering water of temperatures between 10 and 50°C, enabled no steady state position. The rate of change of temperature during the heating and cooling phases of the sinusoidal temperature variation was similar and approximately 2.2°C.min^{-1}. For the range of temperatures applied to the skin, this was expected to provide a substantial thermal afferent stimulus. Participants were instructed to re-adjust the water temperature whenever it was perceived as uncomfortably warm during warming phase or

uncomfortably cool during cooling phase. Depressing a button designated with a "+" symbol, initiated an increase in the temperature of the water perfusing the suit. Conversely, depressing a button designated with a symbol "-" initiated a decrease in water temperature. The lower and upper peaks of the sinusoidal pattern were considered to represent the boundaries of the thermal comfort zone (TCZ) for each subject (Figure 7.1). The WPS was constructed such that subjects could regulate the temperature of individual regions (arms, legs, front torso and back torso) separately. In this manner, TCZ for individual regions could be assessed. On one occasion, subjects regulated the water temperature within the whole suit. The WPS covered all body parts except the hands, feet, head and neck. The TCZ for hands, feet and head was assessed by subjects donning water perfused gloves, socks or hood on different occasions (three separate trials), with the remaining skin regions exposed to the ambient conditions (no WPS worn during these trials) in the climatic chamber maintained at 33°C.

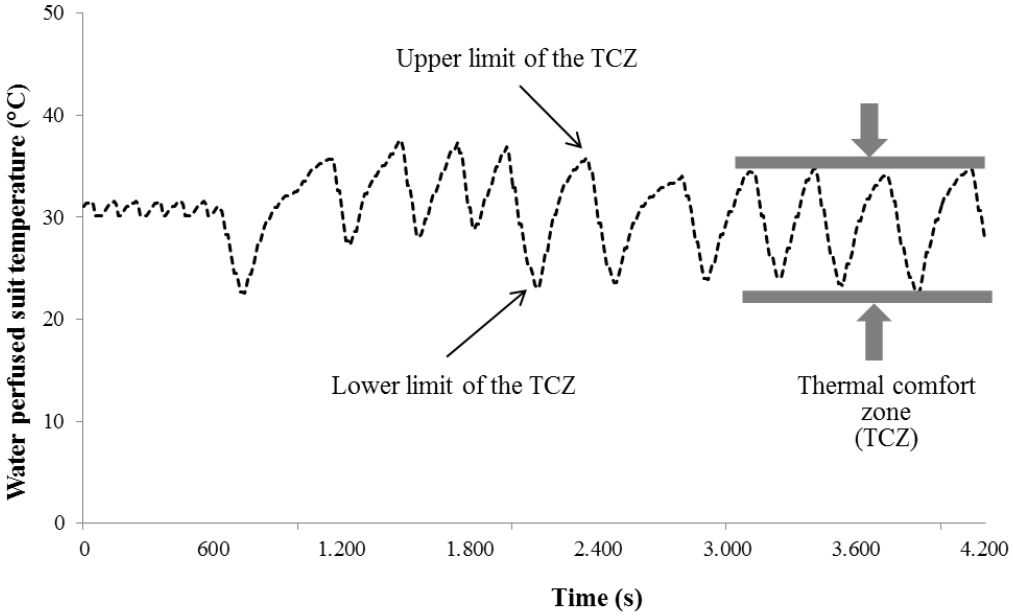


Figure 7.1: Typical example of the water perfused suit temperature regulation within a thermal comfort zone, obtained from one subject during a one hour trial (10-min baseline before commencing the trial).

Upon arrival at the laboratory, subjects rested for a minimum of fifteen minutes prior to being prepared for the test. The laboratory ambient temperature (Ta) was maintained at 25.2 ± 0.3°C, and 50% relative humidity (RH). Before entering the climatic chamber, males dressed in shorts, and females in shorts and bra. Thereafter they transferred to a climatic chamber, with ambient conditions maintained at 33°C (Ta) and 30 % RH. Before donning a WPS, gloves, socks or hood, they were instrumented with temperature sensors. They remained in a semi-supine position for the duration of the entire trial. Each trial commenced with a 10 min baseline, with the water temperature within the WPS, gloves, socks or hood, maintained at 33°C (equal to ambient temperature). The subjects were informed that in the absence of any regulation, the temperature of the suit would change in a sinusoidal manner from approximately 10 to 50°C. This sinusoidal temperature regulation commenced after the baseline measurements were completed. Subjects were given a remote control switch and started regulating the Twps within their preferred range for the duration of one hour. While the Twps of one region was being regulated by a subject, the remaining regions within the WPS were maintained at 33°C. During the temperature regulation of hands, feet or head, these regions were covered by water

perfused gloves, socks and hood, respectively. The remaining areas of the body were exposed to the ambient conditions within the climatic chamber (33°C, 30% RH).
For each subject, the trials were carried out at the same time of the day to avoid the effects of circadian rhythm. Subjects visited the laboratory on four consecutive days and completed two trials each day. During each of the four visits, the assessment of the TCZ of two regions (seven regions + whole body) was conducted. Two trials were separated by a ~10-min acclimation period, with all skin regions maintained at, or exposed to, 33°C. When the skin temperature (Tsk) returned back to the baseline values, a new trial was initiated.

7.1.3 Behavioural temperature regulation

Essential to the determination of the TCZ was the WPS. The torso segment of the WPS was designed as a poncho, with the sides fastened together with a series of Velcro® straps and buttons. The arms and legs of the suit were designed as water perfused sheets, dimensioned so that they could be wrapped around the arms and legs snuggly. Each segment of the WPS, including the gloves, socks and hood, was made of an outer layer comprised of a mixed blend of synthetic fibres, and an inner meshed layer. In each segment, small-diameter (inner dia. = 4 mm; outer dia. = 5 mm) PVC tubes were woven within the mesh lining, separated by 1 cm. Separate tubes were used in each studied region, enabling regional manipulation of water temperature. The length of each tube was 9 m, which ensured equal water flow. The sinusoidal change in temperature within the WPS was achieved by appropriate mixing of water from two baths maintained at 10 and 50°C with Peltier components.



Figure 7.2: *Experimental arrangement with a temperature regulating unit, connected to dedicated software, controlled by a subject.*

The water temperature regulating unit was controlled by dedicated software (MAK Elektronik, Medvode, Slovenia) on a computer connected to the unit. Once water circulated through the region(s) it was transferred back to the unit where it was either re-cooled or re-heated (Figure 7.2). One side of each tube in the suit provided the water mixture from the tanks while the other side transferred the circulated water from the suit again back to the unit.

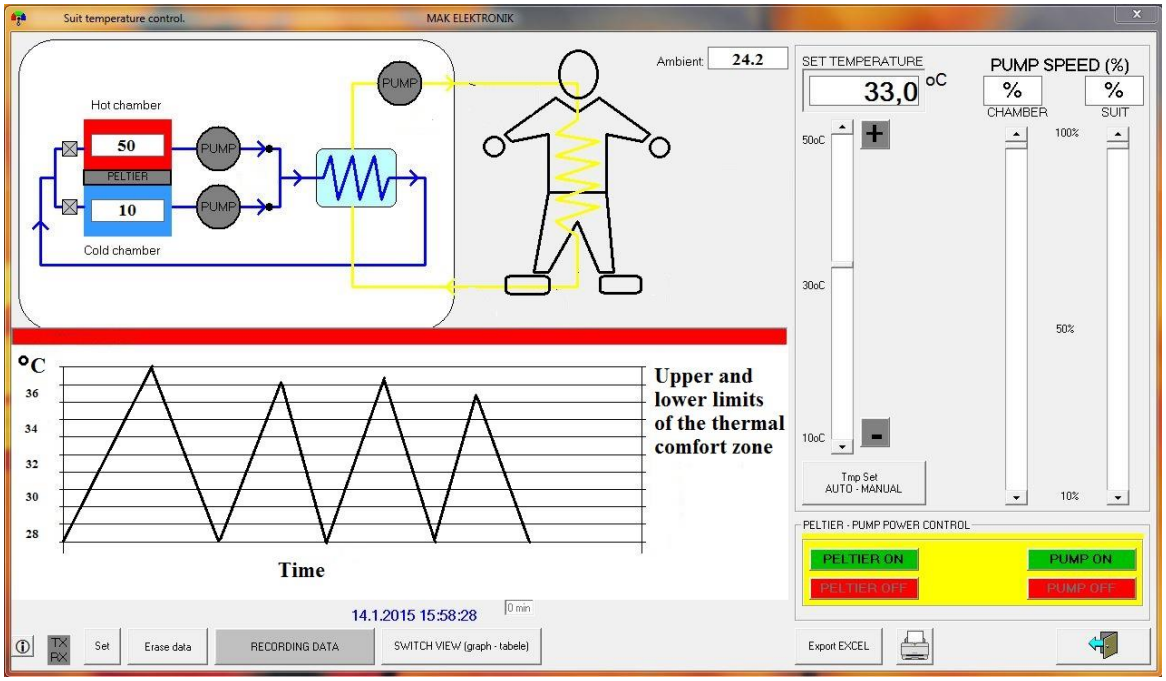


Figure 7.3: The user interface of the dedicated software developed for regulating the temperature of the water perfusing the suit.

The software controlling the units was designed to maintain the water temperature at a preferred level or to be regulated by a subject by a control switch (Figure 7.3). During temperature regulation (TR), every change in the direction of the Twps was recorded (change between heating and cooling). These changes provided the data of lower and upper limits of the TCZ, which were later analysed.

7.1.4 Research design

Ta and the Twps of 33°C (maintained during baseline and within the regions not controlled by a subject), were chosen as an approximation of a resting mean Tsk (Mairiaux et al., 1987; Mehnert et al., 2000), thus providing minimal heat transfer between the body and surrounding environment, and optimal thermal comfort during the baseline measurements. Applying cold or warm stimuli to skin areas (Arens et al., 2006; Gerrett et al., 2014; Nakamura et al., 2008, 2013; Zhang et al., 2004) has been shown to induce differential temperature sensation between skin regions, and also genders. The stimuli in these studies were always warmer or cooler than the skin temperature, in order to observe the magnitude of the change in the temperature sensation. In contrast, the present study exposed the subjects to ambient conditions, corresponding to their mean Tsk, while manipulating the water temperature of one of the regions within their thermal comfort zone.

In the present study the thermal stimulus was presented in a dynamic manner, whereas in most other studies the stimulus is static. The cutaneous temperature receptors exhibit a static and dynamic rate of action potential firing rate when a temperature stimulus is

applied to the skin. During cooling, it is the cold sensors that exhibit an overshoot, and the warm sensors the undershoot (Hensel, 1981). Regions such as the extremities and the head are exposed to greater variations in ambient conditions and present a smaller surface area, compared to the rest of the body. Therefore, it was decided that the TCZ for the extremities and the head would be evaluated independently of other skin areas, by using either water perfused gloves, socks, or hood on separate occasions, with the remaining of the skin regions exposed to the ambient conditions (Ta=33°C, RH=30%).

7.1.5 Equipment

Throughout the trial, core temperature (Tre) was measured with a rectal probe inserted approximately 12 cm beyond the anal sphincter (MSR Electronics GmbH, Henggart, Switzerland), sampled and stored at 30 s intervals by MSR12 data logger (Modular Signal Recorder, MSR Electronics GmbH, Henggart, Switzerland). Tsk at eleven sites (foot, calf, front thigh, abdomen, chest, upper back, lower back, biceps, arm, palm, and head) was measured with thermocouples (Model FRM-090-T, Wuntronic GmbH, Munich, Germany), sampled and stored at 30 s intervals by an Almemo Model 5990-2 data acquisition system (Ahlborn GmbH, Holzkirchen, Germany). Thermocouples were attached to the skin with thin air-permeable tape (Tegaderm, 3M, USA). By averaging Tsk, measured at eleven sites of the body, unweighted mean Tsk was assessed. Local Tsk for the arms, legs, front and back torso was assessed by averaging the Tsk measured by two sensors, placed on each region. Local Tsk for the hands, feet and head was presented by one sensor, placed on each of these regions. The temperature of the water at the inlet and outlet of the WPS and its segments (gloves, socks and hood), was measured using T-type thermocouples. Ambient conditions (Ta and RH) were monitored and controlled by a data logger MSR145, situated in close proximity to the subject (MSR Electronics GmbH, Henggart, Switzerland).
During each experiment, subjects provided the ratings of overall thermal comfort and thermal sensation every 10 min. Thermal comfort was evaluated on a 7-point scale, with the assigned descriptors being: 0 – 0.5 = comfortable, 1 – 1.5 = slightly uncomfortable, 2 – 2.5 = uncomfortable and 3 = very uncomfortable. Thermal sensation was derived from a 7-point scale, with the following descriptors: 3 = hot, 2 = moderately hot, 1 = warm, 0 = neutral, -1 = cool, -2 = moderately cold and -3 = cold.

7.1.6 Statistical analysis

The sinusoidal pattern of the regulated water temperature within the WPS in majority of cases described a damped oscillation. Yogev et al. (2010) suggested that the initial large oscillations in the regulated water temperature most likely constituted the learning phase, since towards the latter half of the trials the sinusoidal pattern was stable and no further changes in the magnitude of the thermal comfort zone noted. Thus only the latter half of the trial was included in the analysis in the present study, unless otherwise stated.
The points identified as the lower and upper limits of the TCZ, present the temperature points, at which the direction of the Twps was changed during the TR – the point when heating was changed to cooling and vice versa. The averaged lower and upper limit values were defined as the boundaries of the TCZ, and the difference between these limits defined as the magnitude of the TCZ for each subject.
Two-way repeated measures ANOVA (independent factor (males and females) x experimental condition (temperature regulation in arms, legs, front torso, back torso, hands, feet, head, and whole body) were used to compare the WPS parameters (upper and lower limits of the regulated temperature, and width of the TCZ). Three-way repeated

measures ANOVA (independent factor (males and females) x experimental condition (temperature regulation in arms, legs, front torso, back torso, hands, feet, head, and whole body) x time (30-min of the second half of the trial)) was used to compare Tre, Tsk, Ta and RH. If a significant F value was noted ($p < 0.05$), critical differences were analysed by Tukey`s procedure. Subjective ratings were analysed using Mann-Whitney U Test, comparing differences between independent factors (males vs. females) and Wilcoxon non-parametric test, comparing differences within each factor. Data are presented as means (±SD) with the limit of statistical significance set at 0.05. Statistical analysis was performed using Statistica 5.0 (Statsoft Inc., Tulsa, OK).
The study assessed the TCZ of different skin regions in two parts. By using the WPS, TCZ was assessed for the arms, legs, front torso, back torso, and whole body (Part 1). By using either water perfused gloves, socks or hood, with the rest of the body exposed to ambient conditions, TCZ of the hands, feet and head was assessed (Part 2). Data of the two parts were analysed separately. In the Results section data are presented as Part 1 and Part 2, with no between comparisons.

7.2 Results

During the baseline measurements, when the water temperature within all regions of the WPS, or water perfused gloves/socks/hood was maintained at 33°C, no difference in mean Tsk between genders and experimental conditions was observed. Also, no difference in measured local Tsk was reported between males and females. Baseline measurements indicated higher Tre for females, measured before initiation of the arms TR (females: 37.5 ± 0.2°C, males: 37.2 ± 0.2°C; $p<0.01$) and whole body TR (females: 37.5 ± 0.2, males: 37.2 ± 0.2; $p<0.05$). Irrespective of gender, higher head Tsk was reported during baseline (35.5 ± 0.5°C) when compared to hand Tsk (34.4 ± 0.6°C; $p<0.001$) and foot Tsk (34.2 ±1.4 °C; $p<0.05$). Behavioural temperature regulation was initiated at the same local and mean Tsk starting point for males and females.

7.2.1 Thermal comfort zone (TCZ, °C)

Part 1: Behavioural temperature regulation (TR) indicated no significant difference in the upper and the lower limits of the TCZ between genders (Table 7.1). The mean regulated temperature for the arms was: 24.3 ± 5.7°C for the females and 21.7 ± 6.7°C for the males ($p=0.41$); for the legs: 26.2 ± 3.5°C for the females and 22.1 ± 7.1°C for the males ($p=0.16$); for the front torso: 23.9 ± 5.7°C for the females and 20.1 ± 7.4°C for the males ($p=0.27$); for the back torso: 24.8 ± 5.9°C for the females and 22.8 ± 7.8°C for the males ($p=0.57$); and for the whole body: 29.3 ± 1.8°C for the females and 26.8 ± 3.7°C for the males ($p=0.12$). It is evident that females regulated higher temperature, albeit not significantly, within all studied regions (arms, legs, front torso, back torso, and whole body). The results indicated greater variation in controlled temperature for the males during leg TR ($p<0.05$), and whole body TR ($p<0.05$). Irrespective of gender no difference in the lower ($p=0.47$) and upper ($p=0.19$) limits of the TCZ for the arms, legs, front and back torso was noted. Whole body TR however resulted in higher levels of the lower ($p<0.001$), and upper ($p<0.01$) thermal limits of the TCZ for both genders when compared to TR within regions (arms, legs, front torso, and back torso).
Part 2: Gender had no significant effect on the regulated upper and lower limits of the TCZ for the hands, feet, and head (Table 7.1). The average regulated temperature for the hands was: 24.9 ± 5.8°C for females and 22.2 ± 7.6°C for males ($p=0.43$); for the feet: 24.2 ± 5.5°C for females and 21.6 ± 7.3°C for males ($p=0.43$); and for the head: 26.9 ± 6.4°C for females and 23.6 ± 6.0 °C for males ($p=0.32$). Again it was observed that

females regulated a higher, albeit non-significant, water temperature within all regions (hands, feet, and head). Irrespective of gender no difference in the upper (p=0.30) and lower (p=0.20) limits of the TCZ for the hands, feet and head was noted.

Table 7.1: *Mean ± SD lower limit, upper limit and the width of the thermal comfort zone (TCZ), and mean regulated water perfused suit temperature (Twps) for males and females during the temperature regulation (TR) in arms, legs, front torso, back torso, whole body, hands, feet and head.*

Condition		Arms TR	Legs TR	Front torso TR	Back torso TR	Whole body TR	Hands TR	Feet TR	Head TR
Lower limit of the TCZ	Males	18.5± 4.7	18.6± 5.8	17.2± 5.8	19.6± 7.3	23.2± 5.3	18.4± 7.6	17.0± 6.7	18.2± 6.0
	Females	19.8± 5.3	21.0± 4.3	18.8± 4.7	19.9± 6.2	26.0± 1.9	19.9± 5.6	19.1± 4.6	21.4± 5.1
	Both	19.1± 4.9	19.8± 5.1	18.0± 5.2	19.7± 6.6	24.6± 4.1	19.2± 6.5	18.0± 5.7	19.8± 5.6
Upper limit of the TCZ	Males	24.8± 8.8	25.7± 8.6	23.0± 9.7	26.0± 9.4	30.5± 3.4	25.9± 8.0	26.1± 8.5	29.1± 7.8
	Females	28.8± 7.6	31.5± 4.8	29.1± 7.4	29.8± 7.3	32.5± 2.9	29.9± 7.5	29.3± 7.8	32.4± 8.3
	Both	27.0± 8.0	28.7± 7.3	26.2± 8.6	28.0± 8.2	31.5± 3.3	27.9± 7.7	27.7± 8.1	30.7± 7.9
Width of the TCZ	Males	6.6± 4.4	7.3± 3.9	6.2± 5.5	6.6± 6.1	7.2± 5.1	7.5± 3.5	9.1± 4.9	10.9± 6.9
	Females	9.1± 6.5	10.6± 5.9	10.3± 4.7	9.9± 6.7	6.5± 3.3	9.9± 6.3	10.2± 6.5	11.0± 4.9
	Both	7.8± 5.5	8.9± 5.1	8.3± 5.4	8.2± 6.4	6.9± 4.2	8.7± 5.1	9.7± 5.6	10.9± 5.8
Mean Twps	Males	21.7± 6.7	22.1± 7.1	20.1± 7.4	22.8± 7.8	26.8± 3.7	22.2± 7.6	21.6± 7.3	23.6± 6.0
	Females	24.3± 5.7	26.2± 3.5	23.9± 5.7	24.8± 5.9	29.3± 1.8	24.9± 5.8	24.2± 5.5	26.9± 6.4
	Both	23.0± 6.1	24.2± 5.8	22.0± 6.7	23.8± 6.8	28.1± 3.1	23.5± 6.7	22.9± 6.4	25.2± 6.2

7.2.2 Rectal temperature (Tre, °C)

Part 1: Females' Tre was higher than the males' (Table 7.2) in all experimental conditions (arms, legs, front torso, back torso, and whole body TR) and significant during the arm TR (females: 37.5 ± 0.3°C, males: 37.2 ± 0.2°C; $p<0.001$), leg TR (females: 37.4 ± 0.2°C, males: 37.1 ± 0.2°C; $p<0.001$), and whole body TR (females: 37.4 ±0.2°C, males: 37.1 ± 0.2°C; $p<0.01$). During each one-hour trial, Tre remained stable or changed only slightly (±0.1°C). Irrespective of gender no difference in Tre during regional TR (arms, legs, front torso, and back torso TR) and whole body TR (p=0.17) was noted.
Part 2: Statistically insignificant, but higher Tre for females was also observed during hands, feet and head TR (Table 7.2). Tre changed mostly by ±0.1°C during the first half of the one-hour trial and remained unchanged thereafter, and was similar during all

experimental conditions. Irrespective of gender, no differences in Tre during hands, feet and head TR were observed (p=0.22).

Table 7.2: *Mean ± SD rectal temperature (Tre, °C) and skin temperature (Tsk, °C) for males and females during the temperature regulation (TR) in arms, legs, front torso, back torso, whole body, hands, feet and head.*

Condition		Arms TR	Legs TR	Front torso TR	Back torso TR	Whole body TR	Hands TR	Feet TR	Head TR
Rectal temperature	Males	37.2± 0.2 [a]	37.1± 0.2[a]	37.2± 0.1	37.3± 0.2	37.1± 0.2 [a]	37.2± 0.2	37.3± 0.2	37.2± 0.2
	Females	37.5± 0.3	37.4± 0.2	37.3± 0.3	37.4± 0.3	37.4± 0.2	37.3± 0.3	37.5± 0.3	37.4± 0.2
	Both	37.3± 0.3	37.3± 0.3	37.3± 0.2	37.4± 0.2	37.3± 0.3	37.3± 0.2	37.4± 0.3	37.3± 0.2
Skin temperature	Males	37.7± 0.4	34.6± 0.8 [a]	35.2± 0.3	35.1± 0.5	34.3± 0.6 [a]	35.3± 0.4	35.3± 0.3	35.4± 0.4
	Females	35.1± 0.7	35.2± 0.7	35.2± 0.4	35.3± 0.4	35.1± 0.4	35.4± 0.5	35.7± 0.3	35.7± 0.5
	Both	34.9± 0.6	34.9± 0.8	35.2± 0.4 [b]	35.2± 0.5 [b]	34.7± 0.7	35.4± 0.4	35.5± 0.4	35.5± 0.4
Local skin temperature	Males	35.3± 0.4	33.3± 2.0	34.5± 0.9	34.3± 1.2	-	34.6± 0.7	33.7± 1.7	35.5± 0.3
	Females	35.6± 0.6	34.5± 1.4	34.8± 1.1	34.6± 1.1	-	34.2± 1.1	33.8± 1.0	35.9± 0.5
	Both	35.4± 0.5 [c]	33.9± 1.8	34.7± 1.0	34.5± 1.1	-	34.4± 0.9 [d]	33.7± 1.3 [d]	35.7± 0.4

[a] Significant gender difference
[b] Significant difference compared to whole body TR
[c] Significant difference compared to legs TR
[d] Significant difference compared to head TR

7.2.3 Skin temperature (Tsk, °C)

Part 1: During each one hour trial, Tsk was, at most, altered by ~0.6°C. Throughout the second half of the one hour trial, females` mean Tsk was higher during leg TR (females: 35.2 ± 0.7°C, males: 34.6 ± 0.8°C; $p<0.05$) and whole body TR (females: 35.1 ± 0.4°C, males: 34.3 ± 0.6°C; $p<0.01$), compared to the males` mean Tsk (Table 7.2). Irrespective of gender, higher mean Tsk was reported during front torso TR (35.2 ± 0.4°C; $p<0.01$) and back torso TR (35.2 ± 0.5°C; $p<0.05$), compared to whole body TR (34.7 ± 0.7°C). Local Tsk (arms, legs, front torso, and back torso Tsk), influenced by regional TR (arms, legs, front torso, and back torso TR), was not affected by gender during these trials. However, irrespective of gender higher local Tsk was measured during arms TR (35.4 ± 0.5°C), compared to local Tsk during leg TR (33.9 ± 1.8°C; $p<0.001$). No difference in local temperature during front and back torso TR was noted (front torso: 34.7 ± 1.0°C, back torso: 34.5 ± 1.1°C; p=0.96).

Part 2: During hands, feet and head TR, mean Tsk was elevated mostly by ~1°C during the first half of the one hour trial and remained stable thereafter in all experimental conditions. This difference between the Tsk of males and females was not significant (Table 7.2). Local Tsk (hands, feet and head Tsk), influenced by regional TR (hands, feet, and head TR) did not differ between genders. Irrespective of gender, significantly higher head Tsk was observed during head TR (35.7 ± 0.4 °C), when compared to hand Tsk during hands TR (34.4 ± 0.9 °C; $p<0.01$) and foot Tsk during feet TR (33.7 ± 1.3°C; $p<0.001$).

7.2.4 Subjective ratings of thermal comfort and thermal sensation

Parts 1 and 2: No within or between gender differences in thermal comfort were observed with ratings indicating thermal comfort (Table 7.3). Thermal sensation was not influenced by gender or experimental condition, with subjects perceiving the temperatures between neutral and warm.

Table 7.3: *Median ± SD thermal comfort and thermal sensation ratings.* Thermal comfort: 0 – 0.5 = comfortable, 1 – 1.5 = slightly uncomfortable, 2 – 2.5 = uncomfortable and 3 = very uncomfortable. Thermal sensation: 3 = hot, 2 = moderately hot, 1 = warm, 0 = neutral, -1 = cool, -2 = moderately cold and -3 = cold.

Condition	Thermal comfort		Thermal sensation	
	Males	Females	Males	Females
Arms TR	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.7	0.1 ± 0.9
Legs TR	0.0 ± 0.2	0.0 ± 0.2	0.3 ± 0.8	0.5 ± 0.4
Front torso TR	0.0 ± 0.2	0.0 ± 0.2	0.5 ± 0.4	0.8 ± 0.5
Back torso TR	0.0 ± 0.2	0.0 ± 0.2	0.3 ± 0.5	0.8 ± 0.5
Whole body TR	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.5	0.0 ± 0.7
Hands TR	0.0 ± 0.3	0.0 ± 0.2	0.4 ± 0.4	0.8 ± 0.6
Feet TR	0.1 ± 0.2	0.0 ± 0.4	0.3 ± 0.9	1.0 ± 0.8
Head TR	0.1 ± 0.4	0.0 ± 0.1	0.5 ± 0.8	1.0 ± 0.4

7.2.5 Ambient temperature (Ta, °C) and relative humidity (RH, %)

Due to controlled conditions in the climatic chamber, Ta and RH remained stable with no significant differences between genders and experimental conditions (*Part 1*: Ta = 32.2 ± 0.2°C, RH = 32.9 ± 0.9%; *Part 2*: Ta = 33.3 ± 0.2°C, RH = 32.7 ± 0.6%).

7.3 Discussion

The principal finding of the present study is that females regulated Twps within different regions slightly, albeit non-significantly, higher. Females regulated the Twps to establish significantly higher mean Tsk during the legs and whole body TR. They thus perceived thermal comfort at a higher mean Tsk during these two conditions.

7.3.1 Thermal comfort zone

TR within the lower and upper limits of the TCZ reflected individual patterns of regulation throughout the one-hour trial. Gender had no significant effect on the range of temperatures perceived as thermally comfortable for different skin regions. In agreement

with these observations, local Tsk, influenced by TR at a specific region, was also similar for both genders. However, mean Tsk was significantly higher for females, measured during legs and whole body TR. Before initiating the whole body TR, baseline measurements indicated significantly higher Tre for females when compared to males. In contrast, no difference in Tre between males and females was observed before commencing TR of the legs. It has previously been reported that Tsk contributes relatively more to perception of thermal (dis)comfort and therefore to the initiation of behavioural responses, than does the core temperature, which is due to more rapid and greater changes of Tsk during environmental variations (Frank et al., 1999). Therefore it is less likely that higher mean Tsk, maintained during whole body TR, was affected by a higher baseline Tre for females. Further, TR of the legs was initiated at a similar Tre for both genders, and also resulted in significantly higher measured mean Tsk for females. Gerrett et al. (2014) reported greater temperature sensations for females, by applying warm stimuli (40 °C) across 31 body locations. During convective and radiative cooling, it was shown that females perceive greater thermal discomfort, with local sensation greatly influencing whole body thermal sensation (Schellen et al., 2012). The approach of the present study was however different since the subjects were not exposed to a non-controlled thermal stimulus, and did not provide subjective ratings of their temperature sensation. Instead, they were asked to actively regulate the temperature of different skin regions within the limits they perceived as thermally comfortable.

The exposure to neutral ambient conditions has been shown to induce no changes in temperature sensation and thermal comfort between genders (Lan et al., 2008; Zhai et al., 2014). Partly, this was also evident in the present study with no difference in regulated lower and upper limits of the TCZ between genders. It has previously been reported that females are more critical of their thermal environments (Karjalainen, 2007) and perceive the thermal discomfort sooner than males, especially in cool conditions (Hashiguchi et al., 2010). In the present study, this was evident during the legs and whole body TR, with the female group maintaining a higher mean Tsk, with less variation in preferred temperatures than the male group. Irrespective of gender, a higher mean Tsk maintained during front and back torso TR, when compared to whole body TR, is in agreement with the observations that humans prefer warmth on front and back torso, and that these two regions contribute greatly to an overall thermal comfort (Nakamura et al., 2013).

Similar ranges of regulated temperatures in the hands, feet, and head by both genders corresponded to a similar local Tsk, measured at these regions. Significantly higher head Tsk was observed for both males and females during baseline measurements, compared to hands and feet Tsk. These differences also persisted when subjects were provided with the ability to behaviourally modify the temperatures of these regions. Arens et al. (2006) reported that consistently cooler foot sensation, compared to whole body sensation, was perceived as comfortable by the subjects in their study. Apart from a greater area devoted to hands in the somatosensory cortex, hands do not seem to contribute much to an overall thermal sensation and comfort, as reported by a number of studies (Arens et al., 2006; Nakamura et al., 2013; Zhang et al., 2004). A greater facial thermosensitivity for sweating and whole body thermal discomfort was reported by Cotter and Taylor (2005), especially evident during the cooling, when compared to other skin sites. Water-perfused stimulation of the face (Nakamura et al., 2008) during mild heat exposure resulted in ratings of “most comfortable” during local cooling, and ratings of “most uncomfortable” during local warming of this area. In the present study, only thermal comfort of the head was included, but it has previously been reported that head cooling improves the overall thermal comfort and reduces the strain during heat exposure (Massey, 2002; Palmer et al., 2001).

Since head Tsk is usually higher than hands and feet Tsk (Olesen, 1982), this is perceived as thermally comfortable, evident in the present study, with subjects maintaining temperature difference between these regions even when given a possibility for modifications.

7.3.2 Subjective ratings

Apart from subjects regulating their preferred temperature in one of the skin regions or whole body, with upper and lower limits presenting their TCZ, subjective ratings were also included in the study. Perception of thermal comfort and thermal sensation was reported every 10 min during the 1-hr trial, ensuring the instructions of being thermally comfortable were followed. It should be noted though that a rating could have been made in a moment, when the subject was about to change the direction of the Twps, by pressing cooling or heating, suggesting that thermal comfort was not optimal at the moment of rating. Ratings therefore present only an approximation of subjects` perception during the trial. The regulation of water temperature within a TCZ was reflected in subjective ratings, with subjects reporting of being thermally comfortable and neutral or warm.
The ratings of thermal comfort and thermal sensation were not done separately for the regulated region and body as a whole, as in the studies of Arens et al. (2006), Nakamura et al. (2008, 2013), and Zhang et al. (2004). However, the design of these studies was different, with stimulation of different skin sites and exposure to different ambient temperatures, not under control of the participants, in order to distinguish the regional difference in temperature sensitiveness and its contribution to an overall thermal comfort. In the present study the ratings of thermal comfort and sensation were not a priority, but served as orientation, with the main focus of the study on the regulation of the temperature of individual skin regions and on one occasion the whole body. Arens et al. (2006) reported that subjects in their study perceived neutral conditions as comfortable, but not as “very comfortable”. The “very comfortable” votes happened only when application of local cooling or heating helped to relieve a thermal stress and establish homeostasis. Regional adjustments therefore contribute significantly to an overall thermal comfort. We do not exclude the possibility that local and overall thermal comfort and thermal sensation were not equal throughout the trial, but with subjects actively changing the temperature to maintain the thermal comfort, any local or overall thermal stress could have potentially been removed.

7.3.3 Limitations and study considerations

Though the WPS was snuggly wrapped around the participants’ bodies, the lower back temperature could have been perceived differently and more intensely due to the compression of this region, while subjects were lying semi-supine on their backs. The head was however supported by a thick pillow during the trial which created a bit of space between the back and the surface, reducing the pressure. The back has been shown to be one of the dominant regions, contributing substantially to an overall thermal sensation (Zhang et al., 2004). Gender differences have also been identified for this region, with females perceiving a greater thermal sensation to a warm stimulus (Gerrett et al., 2014). In the present study a similar number of changes in the direction of the Twps (changes between cooling and heating) was noted for front and back torso (front torso: 17 ± 8, back torso: 20 ± 9; $p=0.13$). If subjects experienced the back torso TR more intense, this would potentially be reflected in greater number of changes in the direction of the Twps (change between heating and cooling), but this was however not evident. Also no difference in local Tsk during the front and back torso TR was noted.

Since the skin regions were covered by a WPS proportionally to their sizes, the surface areas between regions were unequal. Therefore the contribution per unit area of skin of each region to an overall thermal comfort could not be evaluated.

9 Conclusions

The main findings and conclusions of the present thesis were:

Study 1 The characteristics of the thermal comfort zone, namely its boundaries and magnitude, were not affected by exposure to 10-d normobaric hypoxia in sedentary and exercise training subjects. We conclude that in the absence of any hypoxia and/or exercise effect on the perception of thermal comfort, it is unlikely that hypoxia alters behavioural reactions to thermal stimuli, and thus the possibility of an increased risk of cold injury for athletes training at altitudes simulated in the present study is also unlikely ($H_0$1 is accepted; see Section 2).

Study 2 Whilst the results from the present hypoxic bedrest experiments may have implications for people living in hypoxic environments at reduced gravity in future planetary habitats, they also provide insight into the changes associated with certain chronic illnesses rendering patients hypoxic (i.e. chronic obstructive pulmonary disease), and as a consequence also inactive. Based on the results of this study, it can be concluded that hypoxic inactivity will most likely not compromise behavioural temperature regulation in such patients ($H_0$2 accepted; see Section 2).

Study 3 Behavioural temperature regulation does not seem to be affected by the seasonal variation, when comparing the data obtained in spring and autumn. However, there can be other factors that affect the behavioural patterns, which also need to be considered ($H_0$3 accepted; see Section 2).

Study 4 When exposed to a thermally comfortable environment (33°C), thermal comfort is not perceived at a single temperature of the skin, but for a range of temperatures defined as the thermal comfort zone ($H_0$4 is rejected). No regional differences in the boundaries and magnitudes of the thermal comfort zones were evident ($H_0$5 accepted; see Section 2) in male and female subjects ($H_0$6 accepted; see Section 2).

Study 5 Ambient air-ventilated vests can enhance heat loss, as long as the circulating air is not hindered by the weight of the backpack collapsing the air passages in the mesh layer. Intermittent cooling by wetting and fanning the dry skin areas is also efficient in removing the heat away from the body. The two cooling strategies were equally beneficial in terms of reducing the heat strain and improving the thermal comfort during simulated desert patrol mission ($H_0$7 is rejected; see Section 2).

The results of studies 1 to 4 provide an insight into the range of temperatures, perceived as thermally comfortable by adult males and females, as well as the potential for non-thermal factors to alter this perception of thermal comfort. Using this knowledge, ambient thermal environments could be designed with fluctuating rather than uniform temperatures, providing a constant thermal stimulus to the skin, thus providing better thermal comfort, leading to a reduction in the energy requirements. Future studies should investigate the thermal comfort zone of other target groups, such as children and elderly.

Since it has previously been reported that males and females perceive the cold and hot temperatures differently, a future focus should be in determining the thermal comfort zone of both genders during exposure to more extreme environmental conditions.

In Study 5 (Appendix D), simple cooling techniques have been shown to substantially improve the thermal comfort and reduce heat strain. In future, similar approaches should be tested in conditions, which do not allow removal of the protective clothing. Moreover, cooling techniques should also be tested under different ambient conditions, especially at higher ambient relative humidity, in order to determine if they are also efficient in such conditions.

10 Acknowledgements

This dissertation would not have been possible without the help of a number of people, supporting me in so many ways!

I would like to express the deepest appreciation to my supervisor Prof. Igor B. Mekjavic. Without his patience, guidance and support this thesis would not have been possible.

I would like to thank Prof. Ola Eiken for his expertise and valuable guidance.

I am grateful to Dr. Stelios Kounalakis for his clear explanation of the things that were completely new to me.

I would like to express my gratitude to Dr. Daniela Zavec Pavlinic, for assisting in the design and manufacture of the revised water perfused suit used in the assessment of behavioural temperature regulation. She shared a lot of her knowledge with me during the first years of my research work.

I am thankful to Miro Vrhovec and Adi Košenina for their technical assistance and valuable practical advice during my studies.

I would like to thank the subjects for their willingness to participate in my experiments.

Most of all I would like to thank my family … There are no words to express the gratitude for their support over these years! You mean the world to me!

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Appendix A

Dissemination

The results of studies reported in Chapters 4-7 of this thesis have been presented at International Conferences and submitted to peer-review journals for publication. The following details the dissemination activity:

Study 1 (Chapter 4):

1. Morrison, A.S.; Ciuha, U.; Zavec-Pavlinic, D.; Eiken, O.; Mekjavic, I.B.
   The effect of a Live High-Train High regimen on behavioural temperature regulation.
   Journal of Sports Science. Submitted.

Study 2 (Chapter 5):

2. Ciuha, U.; Mekjavic, I.B.; Eiken, O.
   Effect of normobaric hypoxic bedrest on the thermal comfort zone.
   Journal of Thermal Biology. Accepted for publication (doi: 10.1016/j.jtherbio.2015.02.001).

3. Ciuha, U.; Mekjavic, I.B.; Eiken, O.
   The effect of hypoxic bedrest on behavioural temperature regulation.
   International Society for Gravitational Physiology. 15 – 20 June 2014, Waterloo, Ontario, Canada.

Study 3 (Chapter 6):

4. Ciuha, U.; Kounalakis, S.; Bali, T.C.; Noakes, T.; Schabort, E.; Mekjavić, I.B.
   Seasonal variation in autonomic and behavioural temperature regulation.
   Environmental Ergonomics and Physiology Symposium. 17th International multiconference, 6th -10th October, 2014. Jožef Stefan Institute, Ljubljana, Slovenia.

Study 4 (Chapter 7):

5. Ciuha, U.; Mekjavic, I.B.
   Regional thermal comfort zone in males and females.
   Applied Ergonomics. Submitted (Ms. Ref. No.: JERG-D-15-00022).

6. Ciuha, U; Mekjavic, I.B.
   The effect of gender and regional thermal comfort.

Environmental Ergonomics and Physiology Symposium. 17th International multiconference, 6th -10th October, 2014. Jožef Stefan Institute, Jamova cesta 39, Ljubljana, Slovenia.

Study 5 (Appendix D):

7. Ciuha, U.; Grönkvist, M.; Mekjavic, I.B.; Eiken, O.
Strategies for increasing evaporative cooling during simulated desert patrol mission. *Ergonomics.* Submitted (Ms. Ref. No.: TERG-2015-0012).

8. Ciuha, U. Strategies for increasing evaporative cooling during simulated desert patrol missions.
3rd International Congress on Soldiers` Physical Performance. August 18 – 21 August 2014, Boston, USA.

9. Ciuha, U.; Grönkvist, M.; Mekjavic, I.B.; Kölegård, R.; Zavec Pavlinic, D.; Eiken, O.
Thermal strain in soldiers performing patrol missions in a desert climate: effect of the two different cooling strategies.
XIV International Conference on Environmental Ergonomics, 2011, Nafplio, Greece (*Final programme and book of abstracts*. [S. l.]: Paschalidis Medical Publisher, 2011, 71). [COBISS.SI-ID 25016359]

10. Zavec Pavlinic, D.; Ciuha, U.; Grönkvist, M.; Mekjavic, I.B.; Eiken, O.
Vpliv spodnjega perila na mikrookolje oblačilnega sistema med vadbo v simuliranem puščavskem okolju = Effect of underwear on clothing system microclimate during exercise in simulated desert environment.
In Proceedings of combined meetings: 42nd symposium on new technologies in textile science "New Technology – Yes or no? & 6th symosium on new technologies in graphics "New ideas!", June 2nd, 2011, Ljubljana (*Nove tehnologije - da ali ne?*. Editors: Simončič, B., Gregor-Svetec, D., Forte-Tavčar, P.
Ljubljana: Naravoslovnotehniška fakulteta, Oddelek za tekstilstvo, 2011 [COBISS.SI-ID 25006375]

11. Ciuha, U.; Zavec Pavlinic, D.; Grönkvist M.; Mekjavic, I.B.; Eiken, O.
Uporaba toplotnega manikina pri razvoju protokola ohlajanja za primer vojaških oblačilnih sistemov = The use of thermal manikin for development of cooling protocol in case combant clothing ensemble.
In Proceedings of combined meetings: 42nd symposium on new technologies in textile science "New Technology – Yes or no? & 6th symosium on new technologies in graphics "New ideas!", June 2nd, 2011, Ljubljana (*Nove tehnologije - da ali ne?*. Editors: Simončič, B., Gregor-Svetec, D., Forte-Tavčar, P.
Ljubljana: Naravoslovnotehniška fakulteta, Oddelek za tekstilstvo, 2011 [COBISS.SI-ID 25006119]

Appendix B

KOMISIJA REPUBLIKE SLOVENIJE ZA MEDICINSKO ETIKO

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

Štev.: 205/02/11
Datum: 11.3. 2011

Spoštovani gospod prof. dr. Mekjavic,

Komisiji za medicinsko etiko (KME) ste poslali 14. 2. 2011 v oceno predlog raziskave z naslovom:

"Breztežnost in zmanjšana težnost: vpliv na srčnožilni in mišično-skeletni sistem."

KME je na seji 15. februarja 2011 ocenila, da je raziskava etično sprejemljiva, in Vam za izvedbo izdaja svoje soglasje.

Lep pozdrav,

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

Naslov:
Prof. dr. Jože Trontelj, Inštitut za klinično nevrofiziologijo, Univerzitetni klinični center Ljubljana
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Spletni naslov: http://www.kme-nmec.si/

Appendix C

REPUBLIKA SLOVENIJA
MINISTRSTVO ZA GOSPODARSKI RAZVOJ IN TEHNOLOGIJO
URAD RS ZA INTELEKTUALNO LASTNINO

Kotnikova 6, 1000 Ljubljana

T: 01 620 31 00
F: 01 620 31 11
E: sipo@uil-sipo.si
www.uil-sipo.si

Urad Republike Slovenije za intelektualno lastnino (v nadaljnjem besedilu: urad) izdaja na podlagi prvega odstavka 107. in šestega odstavka 108. člena Zakona o industrijski lastnini (Uradni list RS, št. 51/2006 - uradno prečiščeno besedilo in 100/2013, v nadaljnjem besedilu: zakon) ter zahteve, ki jo je vložil Biomed d.o.o., Raziskovanje in profesionalno svetovanje, Tugomerjeva 2, 1000 Ljubljana, Slovenija, ki ga zastopa dr. Jure Marn, univ.dipl.inž., univ.dipl.prav., Maribor, za vpis spremembe v register patentov pri patentu št. 23161, naslednjo

O D L O Č B O

V register patentov se pri patentu št. 23161 vpiše naslednja sprememba:
- poleg dosedanjega izumitelja, ki je bil že vpisan v register patentov in sicer: MEKJAVIĆ Igor, 1000 Ljubljana, Slovenija se vpiše še izumiteljica: CIUHA Urša, 1000 Ljubljana, Slovenija, tako da sta sedaj v register patentov vpisana: MEKJAVIĆ Igor, 1000 Ljubljana, Slovenija in CIUHA Urša, 1000 Ljubljana Slovenija.

Posebni stroški v tem postopku niso nastali.

O b r a z l o ž i t e v

Dne 06.03.2015 je imetnik Biomed d.o.o., Raziskovanje in profesionalno svetovanje, Tugomerjeva 2, 1000 Ljubljana, Slovenija, ki ga zastopa dr. Jure Marn, univ.dipl.inž., univ.dipl.prav., Maribor, zahteval vpis spremembe v registru patentov pri patentu št. 23161, in sicer vpis dodatnega izumitelja. Imetnik je predložil tudi pooblastilo zastopniku, potrdilo o plačilu pristojbine za vpis spremembe in Izjavo o vpisu dodatnega izumitelja v originalu, z dne 5.3.2015.

Urad ugotavlja, da je vpis spremembe v register zahteval imetnik in da so zato izpolnjeni pogoji iz tretjega odstavka 108. člena zakona za vpis te spremembe v register, zato je v skladu s tretjim odstavkom 108. člena ter na podlagi prvega odstavka 107. člena in šestega odstavka 108. člena zakona, odločil kot je navedeno v izreku.

Urad ugotavlja, da v tem postopku niso nastali posebni stroški, zato je odločil kot je v izreku navedeno.

Pouk o pravnem sredstvu:

Ta odločba je dokončna v upravnem postopku. Zoper njo ni pritožbe, možna pa je tožba v upravnem sporu, ki se vloži pri Upravnem sodišču Republike Slovenije v Ljubljani v 30 (tridesetih) dneh od vročitve te odločbe.

Številka: 31200-323/2009-17
Datum: 13.3.2015

REPUBLIKA SLOVENIJA URAD RS ZA INTELEKTUALNO LASTNINO 75 Ljubljana

Marjana Gorkič
višja svetovalka II

Vročiti:
- imetniku na naslov za obveščanje, osebno

Appendix D

Strategies for Increasing Evaporative Cooling during Simulated Desert Patrol Mission

The general requirement for thermal protective clothing is that it should maintain a thermal barrier between the wearer and the external environment allowing normal activity in extreme conditions, and survival in accident scenarios. Protective clothing designed for hot environments should allow adequate heat exchange for a range of activities and environmental conditions, thus preventing or minimising the increase in core temperature. Failure of a protective clothing system to establish a thermal equilibrium within the body may have fatal consequences (Leithhead and Lind, 1964).

Ideally, protective clothing should establish conditions of thermal comfort of the wearer for a range of activities, in a range of environments. Factors that influence the thermal comfort can be grouped into three categories: ambient conditions, metabolic heat production and the clothing characteristics (Fanger, 1970). During heat exposure, thermal comfort can also be influenced by a number of other factors, such as acclimatization level, aerobic capacity, circulation and hydration state, body size and composition, gender, age, circadian rhythm, hypertension and usage of drugs (Havenith, 1997; Kenney, 1985). Maintenance of thermal comfort during work in extreme environmental conditions is technically difficult to achieve, and may be unnecessary. The design of a protective clothing system should, in general, consider the impact of the clothing ensemble on thermal and ergonomic comfort, long-term health, and core temperature (Nunneley, 1988).

Some working conditions including military scenarios require the use of protective clothing in order to ensure the safety of individuals. The need to wear protective clothing in hot ambient conditions can lead to intolerable heat strain, as the clothing will reduce the ability to lose heat to the environment (Havenith, 1999), shifting body temperatures to higher levels. The adaptation to heat can significantly improve physiological responses, evident in increased sweating rate (Machado-Moreira et al. 2005), decreased core temperature and heart rate (Gill and Sleivert, 2001; Houmard et al., 1990) and increased endurance time (Chen et al., 2013). The usage of microclimate cooling systems has also been shown to successfully reduce the signs of heat stress and thus prolong the activity in such conditions (Barwood et al., 2009; Bomalaski et al., 1995; Cadarette et al., 1990; Caldwell et al., 2012; Chinevere et al., 2008; Hadid et al., 2008; McLellan et al., 1999; Shapiro et al., 1982).

The present study investigated two field strategies that could be beneficial in terms of reducing heat strain and thus prolonging activity in desert conditions. A principal question addressed by the present study concerns the efficacy of increasing evaporative cooling from the skin surface using ventilated vests (active cooling, AC) versus partially undressing (removing the helmet and body armour, unbuttoning the shirt and trousers) and manually spraying water on the exposed dry skin during rest breaks (passive cooling, PC). This is of practical concern, since a ventilated vest that does not provide any benefit in terms of evaporative cooling, only adds an additional barrier to heat loss, not to mention the noise generated by the fans within the vest, the need for maintaining the

batteries charged for optimal use, and the additional physical burden to the soldier. Manually spraying water on the skin surface during rest breaks in the field provides significant increases in evaporative cooling during the breaks only. From a tactical perspective undressing body armour and clothes in the field will increase the vulnerability of the wearer, and can thus only be done in a secured area. In the event that the benefit of a constantly elevated evaporative cooling, as provided by the ventilated vests, is similar to the intermittently enhanced evaporative cooling, as provided by manually spraying water on the skin surface, then the latter would be an acceptable option for managing heat strain in the field. Both of these strategies were also compared to two conditions considered worst-case and best-case scenarios: in the former subjects conducted the exercise with no supplemental cooling, and in the latter condition they conducted the exercise wearing only shorts and whilst presumed partly heat acclimatized.

Methods

Subjects

Ten healthy heat-unacclimatized male volunteers participated in the study. Their mean ± SD age, weight, and height were 25 ± 2.9 yrs, 75.9 ± 8.3 kg, and 178 ± 9 cm. The study was performed in a climatic chamber (IZR d.d., Skofja Loka, Slovenia) and the protocol of the study approved by the Committee for Medical Ethics at the Ministry of Health (Republic of Slovenia). Subjects were informed about the experimental protocol before giving their consent to participate in the study and were instructed that they could terminate any given trial at any time, and were also free to withdraw from the study at any time.

Experimental protocol

Subjects were requested not to eat or drink coffee three hours prior to a trial, and not to participate in any heavy exercise during the entire study. Since each subject participated in a total of four trials, this meant that they were requested to refrain from heavy physical activity for a minimum of 2 weeks. For each subject, all trials were carried out at the same time of the day, with at least one day of rest in between to avoid effects of fatigue and circadian influences on the measured responses. The last trial was conducted with the subject wearing only swimming shorts, while the order of the remaining three trials was randomized. Once instrumented with sensors and fully equipped, the subject rested outside the chamber for 10 min (Ta=25ºC, RH=40%), and baseline measurements were obtained. Thereafter, he entered the climatic chamber, where ambient conditions were maintained at pre-set levels: ambient temperature (Ta)=45ºC, relative humidity (RH)=20%, wind speed and treadmill speed=3.2 km/h. In each trial, the subject completed two 50-min walk cycles, separated by a 20-min period of rest, during which the treadmill and fans were switched off and he was placed on a chair that also provided support for the backpack.

During the 20-min rest phase, the subject drank 0.5 L of water, previously conditioned in the chamber, such that the temperature of the water was the same as the temperature of the air in the climatic chamber (45°C). The total amount of sweat produced during each trial was calculated from the difference in nude body weight before and after the trial, corrected for water intake. The subjects' weight was also recorded at the beginning and the end of the rest period. Sweat evaporated was calculated from the difference in the weight of each clothing item before and after the trial. The amount of water used to spray the skin surface was recorded and taken into account for calculation of sweat evaporation.

The trial continued until rectal temperature (Tre) reached 39.0ºC, or until the subject attained his individual maximum heart rate (HR), and/or when subjective tolerance limits were noted (exhaustion, dizziness, nausea, or other subjective complaints).

Clothing worn during the trials

The conditions tested were two cooling strategies (Active and Passive Cooling; AC, PC), a full-protection (FP) and a naked control (NC) trial. In both cooling experiments (AC and PC), the clothing worn was similar, and comprised underpants, shirt, trousers, body armour, combat vest, backpack, helmet, gloves, socks, boots and a dummy rifle (Table 7.1). The shirt was unbuttoned at the neck with rolled up sleeves, and the zippers on the trousers (crotch and around ankles) were open. In the PC trial, a T-shirt was worn under the combat shirt, while in the AC trial an ambient air-ventilated vest (Entrak, Germany), with fans running during the whole experiment, was worn under the combat shirt, and was thus in contact with the skin. Before initiation of the study, the clothing ensemble worn during AC was tested on a heated manikin (Biomed d.o.o., Ljubljana, Slovenia), in order to evaluate the potential differences between the T-shirt worn and not worn under the ambient air-ventilated vest. Ambient temperature in the climatic chamber was set at 15°C, while the manikin shell was maintained at 35°C. The manikin software regulated the manikin shell temperature, and measured the electrical power required to maintain the manikin shell temperature at the pre-set temperature. During these tests, heat flux (Q) from the manikin surface was measured on the front torso, back torso, arms and legs with an Almemo (Model 5990-2) data acquisition system (Ahlborn GmbH, Holzkirchen, Germany). Based on the above measurements the software provided values of clothing insulation (Clo: m^2K/W). Since Q was greater when no T-shirt was worn under the ambient air-ventilated vest, it was expected that this scenario would provide more evaporative heat loss and better thermal comfort to the subjects.

The purpose of the ambient air-ventilated vest was to provide a flow of ambient air over the surface of the torso, which should markedly increase the evaporation of sweat from the skin surface, and consequently provide a greater cooling benefit. It was made of air-permeable and compression-proof spacer fabric**.** The outer material consisted of 72% polyamide and 28% polyester, and the inner material spacer fabric of 100% polyester. Ambient air was circulated by small battery-operated fans, placed in outer pockets on each side, through the spacer fabric. The fans could be controlled at three settings, together providing a volume flow through the vest of: setting 1=200 $L.min^{-1}$, setting 2=320 $L.min^{-1}$, and setting 3=400 $L.min^{-1}$. On one charge the fans could operate for 6 hours at the highest setting (setting 3). The total weight of the ventilated vest was around 1 kg (±100 g), depending on the size of the vest. During the AC trial, the fans in the vest were set at the highest setting, presumably providing airflow of 400 $L.min^{-1}$.

In the FP trial, the subject was fully equipped, wearing underpants, T-shirt, shirt, jacket, trousers, body armour, combat vest, backpack, helmet, gloves, socks, boots and a dummy rifle. Trouser zippers were closed, the shirt was buttoned at the neck and its sleeves were rolled down and buttoned. In the NC the subject wore shorts, socks, boots, dummy rifle and backpack (Table 7.1).

Table 1: *Clothing items, worn in the naked condition (NC) trial, with subjects wearing only swimming trunks and the rest of the clothing packed in the backpack, and during the trials with either Active Cooling (AC), Passive Cooling (PC), or Full Protection body armour with no supplemental cooling (FP).*

	NC	FP	AC	PC
Shorts	√	√	√	√
T-Shirt	X	√	X	√
Cooling vest	X	X	√	X
Shirt	X	√	√ (short sleeves)	√ (short sleeves)
Jacket	X	√	X (in the backpack)	X (in the backpack)
Trousers	X	√	√ (zippers open)	√ (zippers open)
Socks	√	√	√	√
Boots	√	√	√	√
Body armour	X	√	√	√
Combat vest	X	√	√	√
Combat bag	√ (29.3 kg)	√	√	√
Helmet	X	√	√	√
Gloves	X	√	√	√
Dummy rifle	√	√	√	√
TOTAL EQUIPMENT WEIGHT (kg)	35.2	35.2	35.2	35.2

√: item worn during the trial
X: item not worn during the trial

Experimental trials

The four experimental trials were designed such that the comparison of the results of the trials would address two specific issues:

The benefit of two cooling strategies

In the AC trial the subject wore an ambient air-ventilated vest, whereas, during the rest period in the PC trial, he was partly undressed (backpack, helmet, gloves, body armour and combat vest were removed, while the shirt was unbuttoned, and trousers undone), the drier surfaces of the body and clothing sprayed with water pre-conditioned to the same temperature as in the chamber, whilst the surfaces were fanned. Comparison of the results of the AC and PC trials would indicate whether one cooling strategy is superior, in terms of cooling efficiency and walking performance, to the other. In the AC trial the fans within the vest provided ventilation for the full duration of the trial, whereas in the PC trials, the cooling period was limited to the rest cycles. This was taken into account in the determination of the sweat secreted and evaporated.

Comparison of both cooling strategies with the “best case” (NC) and “worst-case” (FP) scenarios

The NC condition, providing maximum skin surface area for evaporative heat loss, was from a thermal point of view considered “best case scenario”. In contrast, the FP condition, providing minimal skin surface area for heat exchange and no supplemental cooling, was considered the “worst case scenario” for a soldier on a desert patrol mission. Comparison of the results of the NC and FP trials with those of the AC and PC trials would further indicate the efficacy of the cooling strategies.

Load carriage during the trials

In all four trials subjects carried the same weight (±100 g), but with different distribution of the weight on the body. The weight difference in conditions was corrected with the load carried in the backpack. The total weight of the clothing and equipment was similar for all subjects (approximately 35 kg); any slight differences in the weights were due to different shoe and clothing sizes. In the NC condition most of the weight was carried in the backpack (29.3 ± 0.02 kg).

Equipment

Each subject walked on a treadmill (Woodway Model PPS Med treadmill, Woodway GmbH, Weil am Rein, Germany) with a walking area of 56 x 173 cm. To simulate a walk-dependant headwind, two fans with wing diameter of 85 cm (Biomed d.o.o., Ljubljana, Slovenia) were positioned at a distance of about 80 cm in front of the treadmill. A laminar constant flow of air from the walking surface of the treadmill to a height of approximately 200 cm above the treadmill in a straight headward direction was achieved by placing a frame containing 36 pieces of PVC tubing, each with an inner diameter of 15 cm, and length of 50 cm in front of the fans. Wind velocity at each tube was then measured with an anemometer. The tubes were arranged to completely cover the area of the two fans, and to direct the air flow from the fans towards the treadmill.
Skin temperature (Tsk) and heat flux (Q) were measured at minute intervals at 12 different sites of the body with combined Tsk/Q sensors, and the data were sampled and stored with a data logger Almemo Model 5990-2 data acquisition system (Ahlborn GmbH, Holzkirchen, Germany). Sensors were positioned on the foot, calf, front thigh, back thigh, abdomen, chest, lower back, upper back, upper arm, forearm, palm and forehead, on the right side of the body. The assessment of the Tsk and Q enabled the calculation of unweighted mean Tsk and Q for the whole body and for the torso, with the latter including the measurements for the abdomen, chest, lower back and upper back. Core temperature (Tre) was assessed with a rectal thermistor (MSR Electronics GmbH, Henggart, Switzerland), inserted 12 cm beyond the anal sphincter. An Almemo 2590-9 weather station (Ahlborn, Holzkirchen, Germany) provided information about ambient temperature (Ta) and relative humidity (RH) in the chamber during the trials. Subjects' weight and the weight of the heavier clothing items, such as body armour and backpack, were measured with a model TPT 5N Libela Elsi (Celje, Slovenia) weight scale, with range and resolution of 300 ± 0.25 kg. The rest of the clothing items were weighed on a model UWE HGM-4000, Universal Weight Enterprises (Hsin Tien City, Taiwan) weight scale, with range and resolution of 4000 ± 0.2 g. Oxygen uptake (VO_2; $ml.min^{-1}$) and expired volume (V_E; $ml.min^{-1}$) were monitored with a COSMED K4b2 system (COSMED Srl, Pavona di Albano, Rome, Italy), with measurements obtained during the 10-min rest period outside the climatic chamber, and during the first five and the last five minutes of each walking phase. The last minute of each recording was averaged and used for analysis.
During all four conditions, the subject evaluated his perception of thermal comfort (TC), wear comfort (WC), thermal sensation (TS) and perceived exertion (RPE). He provided ratings of thermal and wear comfort from a 7-point scale, with the assigned descriptors being: 0 – 0.5 = comfortable, 1 – 1.5 = slightly uncomfortable, 2 – 2.5 = uncomfortable and 3 = very uncomfortable. Similarly, each subject provided ratings of thermal sensation on a 7-point scale, with the following descriptors: 3 = hot, 2 = moderately hot, 1 = warm, 0 = neutral, -1 = cool, -2 = moderately cold and -3 = cold. Perceived exertion (RPE) was rated using the 15-point Borg scale (ratings ranging from 6 to 20) for the whole body: 6 –

7 very very light; 8 – 9 very light; 10 – 11 fairy light; 12 -13 somewhat hard; 14 – 15 hard; 16 – 17 very hard; 18 – 19 very very hard (Borg, 1970). The first rating was provided outside the chamber during the baseline measurements. The subject was asked to provide the next rating after 15-min of walking, after 30-min and again after 45-min of walking. He also provided ratings at the beginning of rest period, and a few minutes before finishing the rest period.

Statistical Analysis

Statistical significance of differences for temperature measurements (Tre, Tsk, and Q), HR, metabolic variables (VO_2, V_E) and ambient conditions over time and between the four experimental conditions were calculated with two-way repeated measures ANOVA. If a significant F value was found ($p < 0.05$), critical differences were analysed by Tukey's procedure to locate the significant mean differences. Differences in sweat production and evaporation and the final end-point between experimental conditions were compared using one-way repeated measures ANOVA. For the subjectively rated variables, differences were evaluated using the non-parametric Wilcoxon matched-pairs test, with P-values < 0.05 regarded as statistically significant. Measurements, recorded at minute intervals, are presented as means ±SD, unless otherwise stated. Statistical analysis was performed with Statistica 5.0 (Statsoft Inc., Tulsa, OK) software.
Since all subjects who terminated a trial prematurely were either heat exhausted or experienced severe pain at the back and the shoulders, evident also in the obtained measurements, it was assumed that they provided a maximum effort. Therefore the last value for all subjects, independent at which time point they terminated the trial, was used as the final value for comparison between experimental conditions. The final comparable time point was set by the trial with the shortest endurance time – for all the subjects that terminated the trial prematurely, this point was determined by the FP condition. Thus, values obtained at the final time point in the FP condition were compared with corresponding time-point values obtained in the other conditions.

Results

All subjects were able to complete the NC and AC trials, whereas two subjects could not complete the second walk period in the PC condition due to back and shoulder pain. The majority of early terminations of a trial were in the FP condition. Four subjects were not able to complete the FP trial, reporting nausea, dizziness, headache, and pins and needles in the arms. Three of these subjects managed to complete the first walk and rest, but withdrew during the second walk, while one of the subjects did not manage to complete the rest period. Since only six subjects were able to complete the FP condition, this was taken into account when analysing and interpreting the data (details explained in Statistical analysis section).

Ambient temperature (T, °C) and relative humidity (RH, %)

Baseline measurements were recorded during a 10-min rest period conducted in the laboratory environment (Ta=25ºC, RH=40%) external to the climatic chamber. The ambient conditions within the climatic chamber were similar in all trials and the average ambient temperatures (Ta) and relative humidities (RH) during each condition are presented in Table 7.2.

Table 2: *Mean ± SD ambient temperature (Ta) and relative humidity (RH) during each experimental condition.*

	Ta	RH
NC	45.6 ± 0.3	22.5 ± 0.2
FP	45.6 ± 0.2	21.9 ± 0.3
AC	45.6 ± 0.2	22.1 ± 0.5
PC	45.5 ± 0.3	22.2 ± 0.5

Rectal temperature (Tre, °C)

Tre was stable during the 10-min baseline period as shown in Figure 7.1. It increased progressively, starting at 37.2 °C in AC and FP, and 37.3 °C in PC and NC, attaining an average of 37.7 ± 0.3°C in AC, 37.9 ± 0.3°C in FP, and 37.8 ± 0.3°C in PC and NC by the end of the 1st walk period. During the rest and second walk, Tre tended to stabilise or increased at a slower rate in the NC, AC, and PC than in the FP condition ($p<0.05$), in which it continued to increase at a similar high rate as during the second half of the 1st walk; the Tre response was similar in the NC, AC and PC conditions. At the final comparable time point (i.e. the FP end-point) values indicate that Tre was higher in the FP condition (38.6 ± 0.4°C; $p<0.001$) than in the AC (37.9 ± 0.4°C), PC (38. 1 ± 0.4°C) and NC (38.1 ± 0.5°C) conditions.

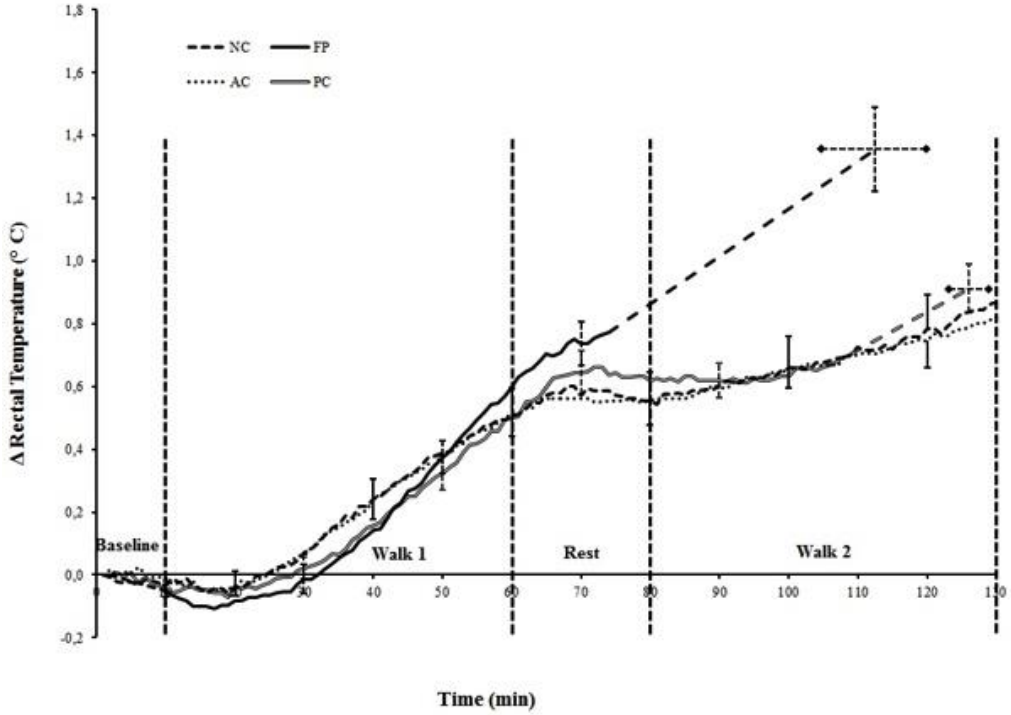


Figure 1: *Mean change in core temperature (°C) ± SD in the active (AC) and passive cooling (PC), naked condition (NC) and full protection condition (FP).* For the two conditions where subjects terminated prematurely (full protection and passive cooling), the last data point for these subjects was averaged with the final data point, obtained from the subjects completing the trial. The solid line presents all ten subjects' data whereas the hatched line indicates predicted course of the trial, based on the final data point.

Heart rate (HR, min^{-1})

During the 1st walk period HR in all conditions increased, with the responses in the NC and AC conditions being significantly lower ($p<0.05$) than those in the FP condition at the end of the first walk, as seen in Figure 7.2. Indeed, the HR response in the FP condition was the highest, attaining values of 126 ± 22 min^{-1} by the end of the 1st walk, compared to 124 ± 10, 119 ± 9, and 112 ± 5 min^{-1} in the PC, NC, and AC conditions, respectively. During the 1st rest period, HR decreased to levels similar to those measured during the start of the 1st walk in the NC (96 ± 7 min^{-1}), PC (90 ± 9 min^{-1}) and AC (89 ± 6 min^{-1}) conditions, but not in the FP condition (113 ± 15 min^{-1}). During the 2nd walk period HR increased steadily, attaining final end-point values of 145 (12), 121 ± 17, 113 ± 18 and 110 ± 17 min^{-1} for FP, PC, NC and AC, reported as higher in FP ($p<0.001$), compared to the rest of conditions.

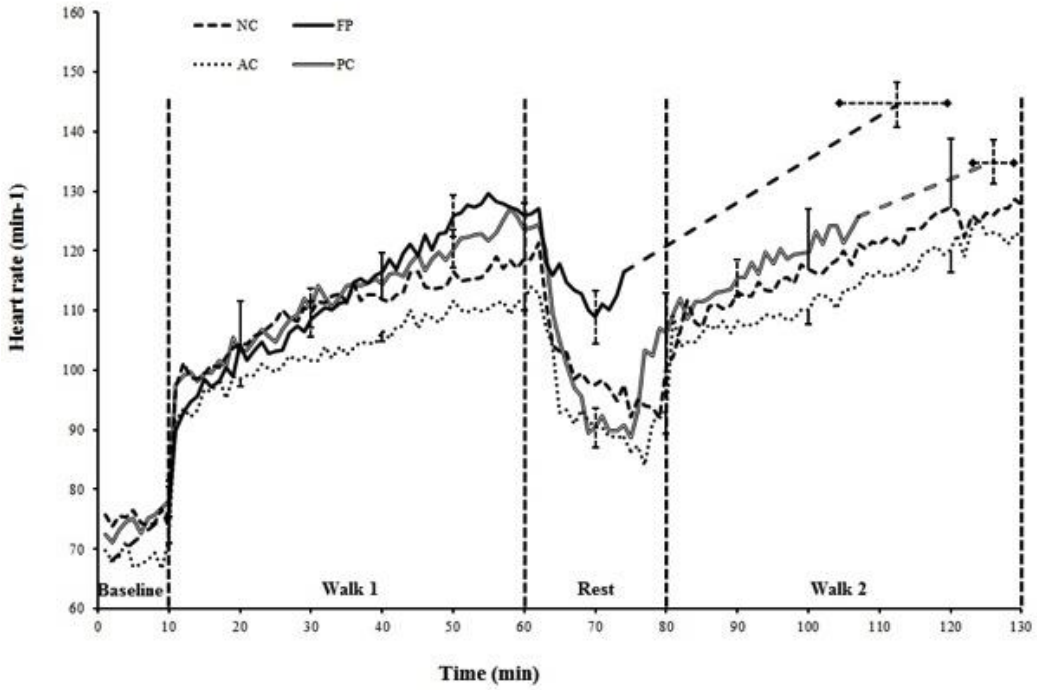


Figure 2: *Mean heart rate (min-1) ± SD in active (AC) and passive cooling (PC), naked condition (NC) and full protection condition (FP).* For the two conditions where subjects terminated prematurely (full protection and passive cooling), the last data point for these subjects was averaged with the final data point, obtained from the subjects completing the trial. The solid line presents all ten subjects' data whereas the hatched line indicates predicted course of the trial, based on the final data point.

Torso skin temperature (Ttsk, °C)

Since the cooling strategies focussed on the torso, with the air ventilated vest providing cooling in the AC condition, and wetting/fanning of exposed skin providing cooling in the PC conditions, the main difference in Tsk and Q were anticipated in the torso region.

With the exception of the AC condition, an increase in Ttsk of 0.5−0.9°C was observed during the 10-min baseline period (Figure 7.3). Thereafter, during the 1st walk period, Ttsk increased rapidly towards asymptotic values in all conditions. At the end of the 1st walk the highest Ttsk was observed in the NC condition (37.4 ± 0.4°C), followed by the FP (37.0 ± 0.4°C), PC (36.6 ± 0.5°C) and AC (35.9 ± 0.7°C) conditions.

In contrast to the FP, AC and NC trials, in which there was a minimal (AC) or no change (FP and NC) in Ttsk, a drop in Ttsk was observed during rest in the PC condition ($p<0.05$).

During the 2nd walk period, Ttsk in the PC condition increased towards end-values observed in the 1st walk period. With the exception of the rest period, Ttsk in the AC condition was lower than in the other three conditions ($p<0.001$). The final end-point values during the 2nd walk were 37.6 ± 0.5°C, 37.2 ± 0.4°C, 36.4 ± 0.9°C, and 35.6 ±0.8°C in the NC, FP, PC, and AC conditions, respectively (Figure 7.3), with Ttsk higher in NC ($p<0.01$) compared to other three conditions, and lower in AC ($p<0.05$), compared to NC and FP conditions. The final end-point values were also measured for the overall Tsk (NC: 37.9 ± 0.4°C; FP: 37.3 ± 0.5°C; PC: 36.9 ± 0.8°C; AC: 36.7 ± 0.6°C), indicating higher Tsk in NC ($p<0.001$), compared to other conditions.

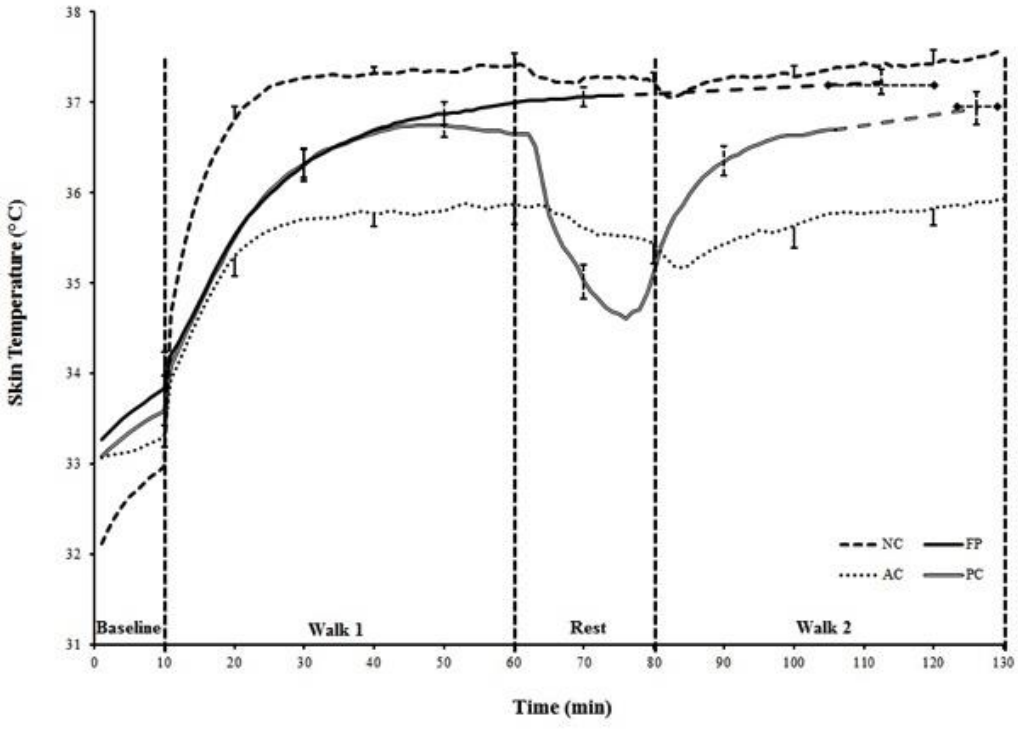


Figure 3: *Mean torso skin temperature (°C) ± SD in active (AC) and passive cooling (PC), naked condition (NC) and full protection condition (FP).* For the two conditions where subjects terminated prematurely (full protection and passive cooling), the last data point for these subjects was averaged with the final data point, obtained from the subjects completing the trial. The solid line presents all ten subjects' data whereas the hatched line indicates predicted course of the trial, based on the final data point.

Torso heat flux (Qt, W·m^{-2})

Upon entering the climatic chamber, the temperature of the clothing layers increased in all conditions, thus reducing the heat flux from the skin. From Figure 7.4 it is evident that the AC condition provided the greatest loss of heat, whereas there was delayed response in the FP and PC conditions, with values of Qt in these two conditions reaching similar levels as in the AC condition by the end of the 1st walk. In contrast to the conditions with a clothing barrier, in the NC condition, Qt was directed from the ambient to the skin. With the exception of the PC condition, Qt remained stable at end-values observed for the 1st walk during the rest period. Removing the body armour in the PC condition, opening up the shirt and dropping the trousers to the ankles at the start of the rest period caused a substantial transient increase in Qt ($p<0.01$), compared to other conditions. Throughout the rest period body surfaces that appeared dry were sprayed with water, the total amount of water used was on average 113 ml. Despite the water spraying, Qt began to decrease during later part of the rest period, as evident in Figure 7.4. Final end-point for Qt values during the 2nd walk were 46.1 ± 36.8, 34.6 ± 16.9, 33.1 ± 31.3 and -42.4 ± 20.3 W.m^{-2} in the AC, FP, PC, and NC conditions, respectively (Figure 7.4) with Q lower in NC ($p<0.001$), than in the other conditions. Similar was observed for the overall Q with the final end-point values of 18.3 ± 16.2, 2.3 ± 30.7, 0.8 ± 24.4, and -59.5 ± 9.2 W.m^{-2} in the FP, PC, AC and NC, respectively.

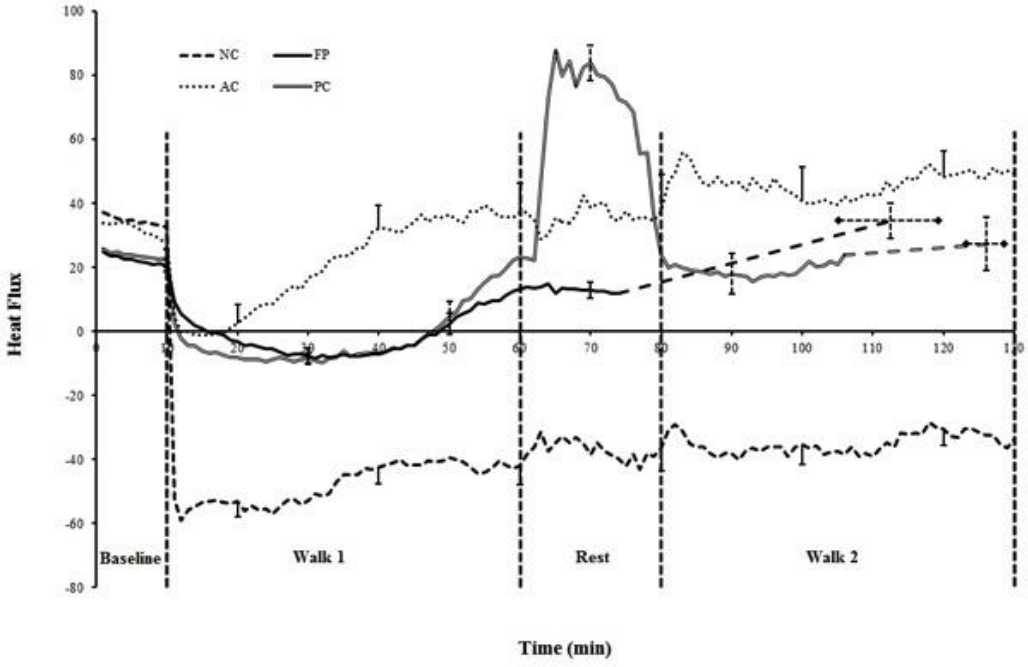


Figure 4: *Mean torso heat flux (Q) ± SD in active (AC) and passive cooling (PC), naked condition (NC) and full protection condition (FP).* For the two conditions where subjects terminated prematurely (full protection and passive cooling), the last data point for these subjects was averaged with the final data point, obtained from the subjects completing the trial. The solid line presents all ten subjects' data whereas the hatched line indicates predicted course of the trial, based on the final data point.

Sweat secretion (Sw, g) and evaporation (E, g)

There was no significant difference in the total amount of sweat secreted in the AC (1949 ± 221 g) and PC (2064 ± 314 g) conditions. Accounting for the amount of sweat absorbed by the clothing layers, there was also no significant difference in the amount of sweat that was evaporated in the AC (1823 ± 170 g) and PC (1849 ± 232 g) conditions. Greater sweat loss was observed in the NC (2364 ± 267 g; $p<0.05$) than the AC and PC conditions. Evaporation was greater in the NC (2220 ± 198 g; $p<0.01$) than the FP (1372 ± 407 g), AC and PC conditions (Table 7.3). Evaporation was lower in FP than the other three conditions ($p<0.001$).

Table 3: *Mean sweat secretion (kg) ± SD, corrected for the water intake, and evaporation (kg), excluding the sweat contained in the clothing, in the naked condition (NC), full protection condition (FP), active (AC) and passive cooling (PC).*

	NC	FP	AC	PC
Sweat secretion (kg)	2.4 ± 0.3 [a]	2.1 ± 0.5	1.9 ± 0.2	2.1 ± 0.3
Evaporation (kg)	2.2 ± 0.2 [b]	1.4 ± 0.4 [c]	1.8 ± 0.2	1.8 ± 0.2

Oxygen uptake (VO_2, ml.min^{-1}) and minute ventilation (V_E, ml.min^{-1})

The comparison of VO_2 and V_E between experimental conditions was done for the two measurements, obtained in the first walk (start and the end of the 1st walk period).
Greater VO_2 was measured in NC ($p<0.05$) with 1330 ± 111 ml.min^{-1}, compared to PC, FP and AC, with 1247 ± 143, 1239 ± 124, and 1211 ± 102 ml.min^{-1}, respectively. V_E was significantly greater in NC ($p<0.05$) with 31 ± 4 ml.min^{-1}, compared to AC with 28 ± 4 ml.min^{-1}. V_E in FP and PC was 30 ± 7 and 29 ± 5 ml.min^{-1}, respectively.

Subjective ratings of thermal comfort, wear comfort, thermal sensation and perceived exertion (RPE)

The final end-point values indicated greater thermal and wear discomfort during the FP condition, compared to other conditions ($p<0.05$). Subjects perceived the temperature as warmer in the FP than in other conditions ($p<0.05$). RPE was also greater in FP condition, when compared to other conditions ($p<0.05$). No other differences between experimental conditions were observed (Table 7.4).

Table 4: *Median ± SD subjective ratings of thermal comfort (TC), wear comfort (WC), thermal sensation (TS), and perceived exertion (RPE) as the final end-point.* Detailed explanation of subjective ratings is given in the Methods section.

	TC	WC	TS	RPE
NC	1.3 ± 0.8	1.8 ± 0.9	2.0 ± 0.7	13.0 ± 2.1
FP	2.5 ± 0.8*	2.5 ± 0.7*	3.0 ± 0.5*	15.5 ± 3.1*
AC	1.0 ± 0.7	1.5 ± 0.8	1.5 ± 1.0	12.0 ± 2.8
PC	1.3 ± 0.9	1.5 ± 0.9	2.0 ± 0.6	13.0 ± 1.9

* Significant difference from other experimental conditions

Discussion

A principal finding of the present study is that only 40% of the subjects were able to complete the simulated desert mission without signs and symptoms of heat exhaustion while wearing full protective clothing with no cooling applied, and that the two cooling strategies tested were both efficient in reducing heat strain. The NC condition resulted in increased sweat evaporation, but higher Ttsk, Tsk and VO_2 when compared to FP and to the two cooling strategies.

Heat loss during the naked condition

The NC trial was considered optimal for heat loss. Since it was the last trial, subjects would have had the benefit of some degree of acclimatisation. Gill and Sleivert (2001) reported about minimal adaptation following intermittent exposure to heat, with daily heat exposure as the most effective in heat acclimatization. In contrast, Fein et al. (1975) observed similar reduction in heat strain during daily (10 consecutive days) and intermittent (total of 10 exposures every 3rd day) exposures to heat.
There being no thermal barrier between the body and the surrounding ambient air, heat loss by sweat evaporation was expected to be optimal. However, due to direct exposure of the body to the warmer environment, concomitant with the high evaporative heat dissipation in the naked condition, heat was also gained from the ambient via radiation and convection, as evident from high Tsk and negative Q values, indicating a transfer of the heat towards the body.
Due to the weight being distributed on the back and partly on the hips, subjects reported back and shoulder pain and problems with the gait. This discomfort of load carriage was, in part, also due to the light pain induced by the straps of the backpack on the skin. Presumably, the disadvantageous distribution of the carried weight, with 29 kg in the backpack, was also the reason for the significantly reduced mechanical efficiency during the walks (higher VO_2), in the NC than in the other conditions, and in particular than in the AC and PC conditions. Thus, at any given time during the walks, oxygen consumption was ≈ 0.1 l/min higher in the NC condition than the AC and PC conditions, corresponding to an added heat production rate in the NC condition of 35W, or an added endogenous heat of 0.2 MJ in total over the 2 * 50 min walks. Thus, that Tre increased at a similar rate in the NC as in the AC and PC conditions, despite optimal provisions for evaporative heat loss in the former condition, was most likely due to both higher endogenous heat production and heat gain via radiation and convection in the NC condition.

Ambient air ventilated vest and water spraying

In the AC condition, the ambient air-ventilated vest resulted in a lower Ttsk and higher Qt during the walks when compared to PC condition, whereas the opposite was observed during the rest, when the removing of the clothing, spraying and ventilating dry skin surface areas in PC decreased the Ttsk and increased Qt. The measurements on a heated manikin indicated greater heat loss on the torso with no T-shirt worn under the vest (Q=180 $W.m^{-2}$) compared to a T-shirt worn under the vest (Q=120 $W.m^{-2}$). As a consequence, the trials were conducted with the vest worn directly on the skin. However, the weight of the backpack on the shoulders (body armour, combat vest and backpack) pressed the spacing-mesh in the vest and irritated the skin on the shoulders, which resulted in reduced wear comfort. In addition, it appeared that the weight of the body armour and backpack compressed the spacing mesh to the extent that the airflow was hindered through some parts of the vest. Thus, most of the subjects felt the wind from the

vest fans blowing more during the rest period, when the backpack was supported by a chair, positioned behind the seated subject, as also observed in the study by Barwood et al. (2009). This position made a space between the ambient air-ventilated vest and body armour, so the air could circulate better. Based on the Qt data, most of the reduction of air flow whilst the backpack was carried on the shoulders was noted on the back. Heat loss was better in the lower and frontal parts of the torso, with the highest Q observed on the abdomen, less on the chest, even less on the lower back and a negligible amount on the upper back, where the vest was compressed the most. These findings were supported by Ttsk data, with front torso Ttsk lower than back torso Ttsk, and also a greater Ttsk difference between AC and PC when comparing front and back torso (with Ttsk lower on the front torso in AC condition). Apart from the skin being irritated by the spacer mesh, the use of the ambient air-ventilated vest at high ambient temperatures did not damage the skin (cf. Shapiro et al., 1982) or induce any other unwarranted effects.

A number of studies have investigated the differences between water and air-cooled vests, and have evaluated different commercial microclimate cooling systems, continuous and intermittent microclimate cooling, the use of microclimate cooling vest in comparison with no vest (Barwood et al., 2009; Bomalaski et al., 1995; Cadarette et al., 1990; Caldwell et al., 2012; Davey et al., 2013; McLellan et al., 1999; Pimental et al., 1987; Shapiro et al., 1982) and ventilation flow-rate requirement for air-cooling systems (Kaufman, 2001). One concern when using air-ventilated cooling vests in operational settings is the action time of the battery driven fans, since the consequence of a battery failure is added thermal strain resulting from the added water vapour resistance induced by the fabric layers of the vest. Throughout the present study, there was no account of battery failure or any other problem with the fans. It has been suggested that an intermittent flow, preferably with gradually increasing/decreasing flow rate, should be implemented when a battery life-span is a concern, since the thermal balance and thermal comfort do not seem to be compromised during intermittent cooling (Davey et al., 2013).

The PC condition provided comfort to the subjects during the rest period when parts of the clothing were removed, and the exposed surfaces of the body and underwear were sprayed with water and fanned. This improved Q and reduced Tsk, especially on the front torso, arms and front thighs, but only during the rest period.

Cooling strategies compared to the no clothing and full protective clothing conditions

The NC as the “best case scenario” with naked and presumably slightly heat acclimatized subjects (the NC condition was always the last trial conducted by all subjects), and the FP as the “worst case scenario” with subjects wearing all protective clothing on their bodies (*i.e.* shirt sleeves down and buttoned at the neck, with jacket, and all zippers in the trousers closed), and two cooling strategies with AC and PC were compared in the present study in order to evaluate the strategies which could potentially be beneficial for a soldiers’ performance in a desert patrol mission. The “worst-case” FP condition could not be completed by 40% of the subjects, predominantly due to signs and symptoms of heat exhaustion. Improvements in physiological responses have been associated with work intensities, allowing permeation of the sweat through the clothing (Aoyagi et al., 1998), which was very limited in FP. Thus, the responses of Tre and HR, indices of heat strain, were significantly higher in the FP condition than in the other three conditions. The main reason for the increased heat strain in the FP condition was presumably the compromised ventilation of the clothing microclimate, severely limiting the provisions for evaporative heat loss. This notion is supported by the findings of reduced evaporation of sweat in the FP compared to in the other conditions and high Ttsk and low Q compared to the cooling

conditions. Noteworthy is the observation that the "best-case" NC scenario provided similar thermal (Tre) response as the AC and PC conditions, despite that, as expected, the absence of a clothing barrier significantly enhanced the evaporation of sweat in the NC condition. However, in this condition, heat balance was as mentioned affected by a slightly elevated endogenous heat production. The benefits to evaporative cooling provided by the air-ventilated vest throughout the simulated patrol were similar to that provided by wetting/fanning during the rest break in the PC condition. That wetting and fanning the body during about 15 min of the 120-min simulated patrol mission proved an equally efficient cooling strategy as continuous use of the ventilated vest is probably attributable to the dry ambient air and vigorous fanning, providing near optimal conditions for evaporation. Assuming that all of the 0.1-0.2 l of water sprayed on the body surface during the rest period evaporated, then it can be estimated that about a fifth of the total endogenous heat produced during the 100-min walk (≈2.1 MJ) dissipated during the wetting/fanning procedure.

Whereas the 20-min rest period provided some recovery in HR, and stabilised Tre in the NC, AC and PC conditions, it provided minimal HR recovery and no stabilisation of Tre in the FP condition. While Tre attained quasi steady state during the first half of the 2nd walk in the NC, AC in PC conditions, with a slight elevation during the latter part of the walk, Tre increased steadily and at a similar high rate in the 1st and 2nd in the FP condition.

The benefit of the air-ventilated vest can also be discerned from the Ttsk response (Figure 7.3), as Tsk was lowest in the AC condition, and highest in the NC condition. Notably, the Ttsk responses were similar in FP and PC during the 1st walk, suggesting that the extra clothing layer by the jacket in FP did not add much extra thermal insulation of the torso, i.e. the insulation of body armour must thus be efficient enough to mask the thermal burden by the jacket. Following the transient decrease in Ttsk during the rest period in the PC condition, Tsk increases towards similar asymptotic values during the 2nd walk as observed in the FP condition.

Limitations

In analysing the Qt response from the skin surface during the 1st walk, it should be considered that the clothing in the AC, PC and FP conditions was pre-conditioned to the ambient environment of the laboratory. Consequently, during the baseline period, the clothing layers acted as a heat sink.

The delayed and subsequent increase in Qt during the latter half of the 1st walk period most likely reflects the progressive sweating-induced wetting of the clothing layers and heat-flux discs, thus inducing greater heat loss.

The study approach was practical, aiming at evaluating two cooling strategies that might be implemented under field conditions. Since both the AC and PC condition comprised a combination of two means of cooling (reduced clothing in combination with either the ventilated vest or with wetting/fanning), it can be argued that the data do not allow us to discern if, or to what extent, the vest and wetting/fanning contributed to the overall cooling effect. However, judging from previous experiments, it appears obvious that both the vest and the wetting/fanning procedure contributed considerable shares to overall cooling (Eiken et al., 2010). Notwithstanding, it is warranted to in future studies separate cooling effects induced by the vest and wetting/fanning procedure from those induced by the reduced clothing. Likewise, it is warranted to also compare AC and PC strategies with a naked control condition in which the endogenous heat production is clamped at an identical level as in the AC and PC conditions.