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Daniel Yogev (Wolowske)

ASSESSMENT OF BEHAVIOURAL THERMOREGULATION IN HUMANS: with particular reference to mild narcosis and prolonged bed rest

Doctoral Dissertation

OCENA VEDENJSKE TERMOREGULACIJE PRI ČLOVEKU: Učinek simulirane dušikove narkoze in brezteţnosti

Doktorska disertacija

Supervisor: Prof.dr. Igor B Mekjavic

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MEDNARODNA PODIPLOMSKA ŠOLA JOŽEFA STEFANA
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Ljubljana, Slovenia

I dedicate this dissertation to Maja, who I love very much.

Index

Abstract

Behavioural responses are the most efficient thermoregulatory responses, since relatively simple actions can prevent the need to activate the more metabolically costly autonomic responses. Dependence on behavioural responses may increase in environmental conditions where the prevailing nonthermal factors (NTF) attenuate autonomic responses and alter thermal perception (Mekjavic et al., 2003; Mekjavic and Eiken, 2006). Thermal (dis)comfort is considered to be the driving force for the initiation of behavioural thermoregulatory responses (Weiss and Laties, 1961). Thus, alteration in the perception of thermal comfort by an NTF might jeopardize normothermia by preventing the initiation of appropriate behavioural responses (Mekjavic et al., 1994). This was confirmed in animal studies (Pertwee et al., 1986; Macdonald et al., 1989; Pertwee et al., 1990), but data in humans are lacking. Furthermore, there is currently no accepted method to evaluate behavioural thermoregulation in humans. The studies presented in this dissertation address both the need for a new method of assessing behavioural thermoregulation in humans (Study 1), and the need to evaluate how NTFs may influence this ability. Particular reference was given to the effects of mild narcosis (Study 2) and prolonged bed rest (Study 3). Both of these NTFs represent conditions that humans may encounter when performing in extreme environments (e.g., deep sea and space).

Study 1: A new method for assessing thermal comfort and behavioural thermoregulation in humans

There being no accepted method for assessing thermoregulatory behaviour in humans, the aim of the first study was to develop an experimental procedure for assessing behavioural thermoregulation. It is well accepted that behavioural thermoregulatory responses are initiated once the surrounding environment is perceived as thermally uncomfortable. The correlation between these two events, namely the change in thermal perception and initiation of a behavioural response to counteract it, were assessed by the new procedure. The experimental set-up comprised a water-perfused suit (WPS) with a manual control system designed to allow both the experimenter and the subject to control the temperature of the water perfusing the WPS (T_{wps}). The zone of thermally comfortable T_{wps} (TCZ) was evaluated in 12 subjects (6 males and 6 females) in two trials. In each trial, a control unit changed the T_{wps} in a sinusoidal manner from 27°C to 42°C, at a rate of 1.2°C/min. In the first trial, the subjects could only report their perception of the fluctuating T_{wps} and in the second, they could change the direction of T_{wps} when they perceived it to be slightly uncomfortable. The subjects regulated the T_{wps} within a preferred range for a total duration of one hour. The results demonstrate that the subjects reproducibly identified the boundaries of their TCZ on three separate occasions. The threshold T_{wps} perceived as slightly uncomfortable was highly correlated with the threshold T_{wps} at which the subjects initiated a behavioural response to counteract it. It was concluded that thermal preference could be reliably detected by evaluating the manner in which subjects behaviourally regulate their surrounding temperature. Furthermore, gender-related differences were found when the

subjects controlled the WPS temperature. Females preferred higher T_{wps} than males for thermal comfort. This suggested that the method could be used to determine the effect of nonthermal factors on behavioural temperature regulation.

Study 2 investigated how inert gas narcosis, a nonthermal factor that humans might encounter in hyperbaric environments, influences temperature perception, thermal comfort, and behavioural temperature regulation, based on the methodology that was developed in study 1. Twelve subjects (the same as in study 1) participated in two trials. During the trials, subjects wore a water-perfused suit (WPS). The temperature of the WPS (T_{wps}) fluctuated sinusoidally from 27°C to 42°C, at a heating and cooling rate of 1.2°C/min. In the first trial, the subjects had no control over the T_{wps}, they determined their thermal comfort zone (TCZ) by providing a subjective response whenever they perceived the temperature changing from a comfortable to an uncomfortable level and vice versa, and provided subjective ratings of temperature perception and thermal comfort on 7-point and 4-point scales, respectively, at each 3°C change in T_{wps}. As in study 1, in the second trial, subjects could change the direction of T_{wps} whenever it became uncomfortable by depressing a button on a manual control. The protocols were conducted with subjects breathing either room air (AIR), or a normoxic breathing mixture containing 30% N_2O. Subjects perceived increasing T_{wps} as equally warm and the decreasing T_{wps} as equally cold with AIR or N_2O. However, equal changes in T_{wps} were perceived as significantly less discomforting ($P<0.05$) in the presence of N_2O and the magnitude of the TCZ significantly ($P<0.01$) increased. Thus, narcosis did not alter thermal sensation, but it significantly changed the perception of comfort. These changes were not reflected in the behavioural response. Subjects produced similar T_{wps} damped-oscillation patterns in the AIR and N_2O trials. It was concluded that the narcosis-induced alteration in the peception of thermal comfort does not change either the preferred temperature, or the ability to behaviourally maintain thermal comfort.

Study 3 evaluated the utility of the developed methodology in assessing changes in behavioural temperature regulation due to overall deconditioning. The musculoskeletal and cardiovascular deconditioning that is observed with microgravity or prolonged inactivity, was established by maintaining subjects in a horizontal position for 21 days (bed rest experiment). Previous thermal studies using a bedrest model suggested that changes in thermal sensitivity and comfort might contribute to the elevated core temperature observed during spaceflights (Rimmer et al., 1999). Thus, in addition to assessing behavioural thermoregulation, cutaneous cold and warm sensitivity were also evaluated. Healthy male subjects (n=10) were accommodated in a hospital ward for the duration of the study, and were under 24-hr medical care. All activities (eating, drinking, hygiene, etc.) were conducted in the horizontal position. On the 1st and 22nd days of the bed rest cutaneous temperature sensitivity was tested by applying cold and warm stimuli of different magnitudes to the volar region of the forearm via a Peltier element thermode. As in studies 1 and 2, behavioural thermoregulation was assessed by having the subjects regulate the temperature of the water within a WPS they were wearing. In this study, however, the T_{wps} varied from 27°C to 42°C at a faster rate (2.1°C/min) and required that the subjects alter the direction of the change in T_{wps} more frequently. The magnitude of the oscillations towards the end of the trial was assumed to represent the upper and lower boundaries of the TCZ. The results demonstrate that there were no significant differences in the TCZ or the pattern of the regulated T_{wps} after bedrest. Furthermore, a higher rate of thermal changes at the WPS did not significantly change the preferred temperature or its behavioural control. In contrast, the cutaneous

threshold for detecting cold stimulus decreased ($p<0.05$) from 1.6 (1.0) ºC on Day 1 to 1.0 (0.3) ºC on Day 22. No effect was observed on the ability to detect warm stimuli. It was concluded that although cold sensitivity increased after bed rest, it was not of sufficient magnitude to cause any alteration in behavioural thermoregulatory responses.

The contribution of the present study is the development of a new, reliable, experimental procedure for evaluating behavioural thermoregulation in humans. As opposed to obtaining subjective scale ratings of thermal comfort, with the new method behavioural responses can be evaluated directly with minimal intervention of the observer. The results indicate that the proposed experimental protocol enabled subjects to identify the thresholds of warm and cold discomfort in a reproducible manner and that a higher rate of change in T_{wps} did not alter this ability. The upper and lower peaks of the T_{wps} pattern that are obtained when subjects maintain thermal comfort provide a valid measure of the threshold temperature eliciting warm and cold discomfort.

With respect to the influence of NTFs on behavioural thermoregulation, the results of the present research do not support the hypothesis that altered perception of comfort attenuates behavioural thermoregulation. Mild narcosis and prolonged bedrest did not significantly change thermoregulatory behaviour despite their significant influence on thermal sensation and comfort.

Povzetek

Vedenjski odzivi so najučinkovitejši termoregulacijski odzivi, saj relativno preprosta vedenjska dejanja lahko preprečijo aktivacijo metabolno bolj potratnih avtonomnih odzivov. Odvisnost od vedenjskih odzivov se lahko poveča v okoljskih pogojih, kjer prevladujoči netermalni faktorji (NTF) zmanjšujejo avtonomne odzive in spremenijo termalno zaznavanje (Mekjavic in Eiken 2006, Mekjavic et al. 1996, 2003). Termalno (ne)udobje velja kot sprožilec pričetka aktivacije vedenjskih termoregulacijskih odzivov (Weiss in Laties 1961). Sprememba zaznavanja termalnega udobja zaradi delovanja NTF lahko prepreči pričetek aktivacije primernih vedenjskih odzivov in s tem lahko ogrozi normotermijo (Mekjavic et al. 1996). Slednje je bilo potrjeno pri študijah z živalmi (Macdonald et al. 1989, Pertwee et al. 1986), medtem ko je podatkov za ljudi bore malo. Nadalje, trenutno še ne obstaja primeren postopek za ocenjevanje vedenjske termoregulacije pri ljudeh. V tej disertaciji predstavljene raziskave so bile namenjene oceni vedenjske termoregulacije pri človeku (prva raziskava), in sicer pri delovanju blage narkoze (druga raziskava) in pri dolgotrajni simulaciji učinka breztežnosti (tretja raziskava). Oba slednja NTF predstavljata pogoje, s katerimi se lahko ljudje soočajo, ko se nahajajo v ekstremnih okoljih (npr. globoko morje in vesolje).

Prva raziskava : Nov raziskovalni protokol za merjenje termalnega udobja in vedenjskih termoregulacijskih odzivov pri ljudeh.

Ozirajoč se na dejstvo, da trenutno primernega postopka za ocenjevanje vedenjske termoregulacije pri ljudeh ni, je bil cilj prve raziskave razviti le-tega. Dobro je znano, da se termoregulacijski odzivi sprožijo, ko se okoliščine sprejmejo kot termalno neudobne. Z novim postopkom je bila ocenjena povezava med spremembo v termalnem sprejemanju in pričetkom vedenjskega odziva na to spremembo.

V raziskovalnem postopku je bila uporabljena posebna obleka (t.i. water perfused suit; WPS). WPS je dovoljevala ali subjektu ali pa raziskovalcu možnost ročnega upravljanja temperature vode, pretakajoče se v njej (T_{wps}). Območje termalno udobne T_{wps} (t.i. thermally comfortable zone; TCZ) je bilo ocenjeno pri 12 preiskovancih (6 moških in 6 žensk) v dveh preizkusih. V obeh preizkusih je kontrolna enota sinusoidno spreminjala T_{wps} in sicer v mejah med 27°C in 42°C ter s hitrostjo 1.2°C/min. V prvem preizkusu so preiskovanci lahko le poročali o njihovem občutenju nihajoče T_{wps}, v drugem preizkusu pa so lahko sami spreminjali smer T_{wps}, ko so jo zaznali kot rahlo neudobno. Subjekti so upravljali T_{wps} v časovnem razponu ene ure. Rezultati kažejo, da so preiskovanci vedno enako določili meje njihove TCZ. Prag T_{wps}, ki so ga preiskovanci zaznali kot rahlo neudobnega je bil zelo podoben pragu T_{wps}, kjer so preiskovanci sami pričeli spreminjati smer T_{wps} . Zaključeno je bilo, da se termalno udobje lahko zanesljivo določi tako, da se oceni način, s katerim preiskovanci vedenjsko upravljajo temperaturo okolja. Odkrite so bile tudi razlike med spoloma. Ženske so izbirale višjo T_{wps} za termalno udobje kot moški.

Druga raziskava je, naslanjajoč se na metodologijo razvito v prvi raziskavi, preiskovala kako dušikova

narkoza kot netermalni faktor, s katerim se ljudje lahko srečajo v hiperbaričnih okoljih, vpliva na zaznavanje temperature, termalno udobje in vedenjsko termoregulacijo. Dvanajst preiskovancev je sodelovalo v dveh preizkusih. Med preizkusi so bili preiskovanci oblečeni v posebno obleko (t.j. water perfused suit; WPS). Temperatura v WPS (T_{wps}) se je sinusiodno spreminjala od 27°C do 42°C s hitrostjo 1.2°C/min segrevanja in ohlajanja. V prvem preizkusu preiskovanci niso imeli kontrole nad T_{wps}, svoje območje termalnega udobja (t.j. thermal comfort zone; TCZ) so določili s subjektivnimi odgovori, in sicer kadarkoli so zaznali, da se je temperatura spremenila iz njim udobne v njim neudobno območje in obratno. Preiskovanci so ocenjevali svoje temperaturno zaznavanje na 7 točkovni, termalno udobje pa na 4 točkovni lestvici, in sicer pri vsaki 3°C spremembi T_{wps}. V drugem preizkusu so preiskovanci z ročnim upravljalnikom lahko sami spreminjali smer $T_{wps,}$ kadarkoli je le-ta postala neudobna. Preiskovanci so med preizkusom vdihovali bodisi sobni zrak (AIR) ali pa normoksično dihalno mešanico, ki je vsebovala 30% N_2O. Preiskovanci so tako pri vdihavanju N_2O kot pri vdihavanju zraka povišano temperaturo WPS začutili enako toplo in tudi znižano temperaturo WPS enako hladno. Kljub temu so enake spremembe v T_{wps} začutili kot manj neudobne ($P<0.05$), kadar so vdihovali N_2O, in velikost TCZ je signifikantno ($P<0.01$) narasla. Narkoza ni spremenila termalnega zaznavanja, temveč je signifikantno spremenila zaznavanje udobja. Te spremembe pa se niso pokazale pri vedenjskem odzivu. Preiskovanci so ustvarili podobne T_{wps} vzorce pri AIR in N_2O preizkusih. Ugotovljeno je bilo, da zaradi narkoze spremenjeno zaznavanje termalnega udobja ne vpliva na zmožnosti vedenjskega ohranjanja termalnega udobja.

Tretja raziskava je raziskovala uporabnost v prvi raziskavi razvite metodologije pri ocenjevanju sprememb v vedenjski termoregulaciji kot posledice dolgotrajne neaktivnosti. Mišična, skeletna in krvožilna adaptacija, ki je opažena pri mikrogravitaciji ali podaljšani neaktivnosti, je bila vzpostavljena tako, da so preiskovanci 21 dni ležali v horizontalnem položaju (bed rest experiment). Prejšnje termalne raziskave, ki so uporabljale model bedrest so predvidevale, da lahko spremembe v termalnem občutenju in udobju prispevajo k povišanju telesne temperature med letom v vesolju (Rimmer et al.1999). Tako je bila v pričujoči raziskavi, poleg ocenjevanja vedenjske termoregulacije, raziskovana tudi kutana hladna in topla občutljivost. Zdravi moški preiskovanci (n=10) so bili nastanjeni v bolnišnici pod 24 urno medicinsko oskrbo. Vse aktivnosti (hranjenje, pitje, higiena itd.) so bile opravljene v horizontalni legi. Na 1. in 22. dan je bila testirana kutana temperaturna občutljivost in sicer z uporabo različnih velikosti hladnih in toplih dražljajev nameščenih na volarni regiji roke. Uporabljena je bila Peltier element termoda. Tako v prvi kot drugi raziskavi je bila vedenjska termoregulacija ocenjena tako, da so preiskovanci lahko sami spreminjali temperaturo vode v oblečeni WPS. Kljub temu pa se je v tej raziskavi T_{wps} med 27°C in 42°C spreminjala z večjo hitrostjo (2.1°C/min) in zaradi tega so morali preiskovanci spreminjati smer T_{wps} veliko hitreje. Velikost oscilacij proti koncu preizkusa je predpostavljala višje in nižje meje TCZ. Rezultati kažejo, da ni bilo nobenih signifikantnih sprememb v TCZ ali v vzorcu upravljanja T_{wps} po končanem bedrest eksperimentu. Večja hitrost spreminjanja temperature v WPS ni signifikantno spremenila subjektom najudobnejše temperature ali vedenjskega upravljanja le-te. Nasprotno pa se je kutani prag za zaznavanje hladnih dražljajev znižal ($p<0.05$) z 1.6 (1.0) ºC izmerjene prvi dan na 1.0 (0.3) ºC izmerjeno dvaindvajseti dan. Noben učinek ni bil opažen pri zaznavanju toplih dražljajev. Ugotovljeno je bilo, da kljub povišanju hladne občutljivosti po bedrest eksperimentu, le-ta ni bila dovolj velika, da bi povzročila spremembo v vedenjski termoregulaciji.

Glavni dosežek pričujoče študije je razvoj novega, zanesljivega, raziskovalnega postopka za ocenjevanje vedenjske termoregulacije pri ljudeh. S tem novim postopkom se vedenjske odzive lahko oceni neposredno in z minimalnim vplivom opazovalca. Rezultati kažejo, da so subjekti vedno enako ugotovili pragove toplega in hladnega neudobja, in da hitrejše spremembe T_{wps} niso vplivale na te ugotovitve. Zgornji in spodnji vrhovi krivulje T_{wps}, kjer subjekti sami obdržijo temperaturno udobje, nudijo veljavno meritev temperaturnih pragov, ki vzbudijo toplo in hladno neudobje.

Kljub temu, da NTF vplivajo na vedenjsko termoregulacijo, pa rezultati pričujoče raziskave ne podpirajo hipoteze, ki pravi, da spremenjeno dojemanje udobja vpliva na vedenjsko termoregulacijo. Blaga narkoza in dolgotrajen bedrest nista signifikantno spremenila termoregulacijskega vedenja, kljub njunemu signifikantnemu vplivu na termalno občutenje in udobje.

Abbreviations

BR	=	bed rest
CNS	=	Central nervous system
DMH	=	Dorso-medial hypothalamus
IGN	=	Inert gas narcosis
MDT	=	Minimal detectable temperature
MnPO	=	Median pre-optic nucleus
N_2O	=	Nitrous oxide
NTF	=	Nonthermal factors
OC	=	Operant conditioning
P_b	=	Barometric pressure
PO/AH	=	Pre-optic anterior hypothalamus
PS	=	Parastrial nucleus
RH	=	Relative humidity
T_a	=	Ambient temperature
T_c	=	Core temperature
TCV	=	Thermal comfort vote
TCZ	=	Thermal comfort zone (range of thermally comfortable T_{wps})
T_{high}	=	Upper boundary of TCZ
T_{in}	=	Water perfused suit temperature at inlet
T_{low}	=	Lower boundary of TCZ
T_{out}	=	Water perfused suit temperature at outlet
T_{sk}	=	Skin temperature
TSV	=	Thermal sensation vote
T_{wps}	=	Water perfused suit temperature (average of $T_{in}$and T_{out})
WPS	=	Water-perfused suit

1 Introduction

In healthy humans, the thermoregulatory system maintains the core body temperature (Tc) within a few tenths of a degree Celsius. In extreme thermal environments, where heat is rapidly exchanged with the environment, the capacity of autonomic thermoregulatory responses to maintain stable Tc is very limited. Thus, thermal stability in such environments is maintained, to a large extent, by behavioural thermoregulation. Behavioural thermoregulatory responses (e.g., change in insulation, choice of preferred environment) reduce the load on the metabolically more demanding autonomic responses and, therefore, enable the body to maintain thermal balance with minimal utilization of valuable resources (e.g., water, nutrients).

The physiological mechanism underlying behavioural thermoregulation is not as well understood as the neural processes involved in autonomic thermoregulation. Unlike the reflexive nature of autonomic thermoregulatory responses, behavioural responses are conscious, voluntary, and subjective. Thus, in the process of behavioural thermoregulation, the subjective perception of the thermal environment (e.g., as comfortable or uncomfortable) plays an important role. Behavioural thermoregulatory responses are initiated once the surrounding environment is no longer perceived as thermally comfortable (Weiss and Laties 1961, Hensel 1976, Cabanac 1971). There is currently no accepted method to evaluate the correlation between these two events. To date, assessment of behavioural thermoregulation in humans relies mainly on evaluation of changes in the perception of thermal comfort as expressed on a rating scale. However, such qualitative tools may be influenced by various psychological factors (Parsons 2002) and they do not reflect the behavioural responses initiated in response to thermal discomfort. Such methods can therefore be considered inadequate for evaluating the risk of thermal imbalance in extreme environments. The first aim of the present study was therefore to develop a new experimental procedure for reliably assessing behavioural thermoregulation in humans. It was hypothesised that the manner in which subjects regulate the ambient conditions to maintain thermal comfort would reliably reflect their thermal preferences.

Activity in extreme environments such as space flight and deep sea diving exposes the human body to unique nonthermal factors (NTFs) such as microgravity, inert gas narcosis, hypercapnia, hypoxia. Maintaining thermal balance in such conditions depends on the appropriate functioning of all thermoregulatory responses. It is now well established that several NTFs attenuate autonomic thermoregulatory responses (Mekjavic and Eiken 2006) and alter thermal sensation and thermal perception (Fowler et al. 1980; Collins et al. 1981; Coleshaw et al. 1990; Mekjavic et al. 1994; Golja et al. 2004). Passias et al. (1996) suggested that if thermal perception is altered by an NTF (e.g., nitrogen narcosis), maintenance of normothermia might be jeopardized, because behavioural responses are not activated appropriately. Although changes in thermoregulatory behaviour due to NTFs have been demonstrated in animal studies (Macdonald et al. 1989; Pertwee et al. 1990), data in humans are still lacking. Furthermore,

it is still unclear whether NTFs that alter thermal perception also attenuate thermoregulatory behaviour. Using the new experimental procedure, the hypothesis that NTFs that alter thermal perception attenuate behavioural thermoregulation in humans, was tested.

2 Behavioural thermoregulation in humans. A review.

Humans, as all homeotherms, have the remarkable ability of maintaining a stable core body temperature in a wide range of environmental conditions. To maintain thermal balance, the body activates behavioural and autonomic thermoregulatory responses that match the rate of heat gained by the body to that lost to the environment. The influence of different thermal and nonthermal factors on autonomic thermoregulatory responses has been studied extensively in the last few decades (Mekjavic and Eiken 2006). These studies have undoubtedly increased our understanding of the automatic process that underlies body temperature regulation. However, thermoregulation in humans is mostly a behavioural process (Bligh 1998). Thus, a better understanding of human thermoregulation requires that behavioural responses are also considered. Assessing behavioural thermoregulatory responses in humans under controlled experimental conditions is difficult since they may involve a wide variety of actions (e.g., moving to a different thermal environment, changing posture, voluntary exercise, and adjustments in clothing and/or in air-conditioning systems) and their activation may be automatic (reflex) or voluntary (conscious). Consequently, behavioural thermoregulation has received only slight attention in human studies and little is known about the underlying mechanism or the factors influencing this important means of regulating body temperature. Some of the human studies concerned with thermal perception, thermal comfort, and behavioural temperature regulation as well as the manner in which they are modified by nonthermal factors (NTFs) are reviewed in this chapter.

2.1 The role of behaviour in physiological thermoregulation

Maintaining homeostasis, and therefore survival, depends on behaviour as well as on physiological (autonomic) regulation. Homeostatic behaviours such as drinking and eating provide the organism with the essential materials for maintenance of fluid and energy balance by the appropriate physiological regulatory system. Similarly, seeking a hospitable thermal environment, a behavioural thermoregulatory act provides the conditions necessary for maintaining thermal balance by the physiological thermoregulatory system.

The *Glossary of Terms for Thermal Physiology* defines behavioural thermoregulatory responses as “a set of conscious, voluntary and coordinated movements ultimately tending to establish a preferred thermal environment for heat exchange with the environment” (Mercer 2003). In humans, such responses may involve a wide variety of actions that alter the microclimate surrounding the body including moving to a different thermal ambiance, changing posture, changing clothing, and activating climate control systems (air-conditioning in dwellings, heating/cooling systems under protective clothing, etc.) The motivation and driving force for thermoregulatory behaviour involves the perception of thermal discomfort, which is subjective and affective. The ability of humans to verbally report when a thermal environment is no longer perceived as comfortable is the basis for the qualitative assessment methods (e.g., scale votes) used to study

thermal perception and comfort under various thermal and nonthermal conditions (Fanger 1970; ISO 7730 2005). The expression of thermal (dis)comfort is a perceptual phenomenon related to the psychological changes that occur in response to changes in the temperature of the body core or of its surroundings. Such perceptual changes, however, do not necessarily reflect the manner by which humans would behaviourally respond to similar thermal changes. In addition, without an appropriate method to directly evaluate behavioural thermoregulation, the influence of various NTFs on this ability can only be speculated. Evaluating changes in human behavioural thermoregulation is particularly important for better understanding the thermal risks associated with exposure to the prevailing NTFs in extreme environments. In such conditions, the reliance of humans on behavioural responses is increased and survival may depend on appropriate initiation of a behavioural action.

2.2 The behavioural thermoregulatory system

Humans maintain their internal body temperature near 37ºC by generating metabolic heat within the cells and exchanging heat with the environment. Three types of processes are responsible for maintaining the thermal equilibrium of the human body: heat generation in the body, heat transfer (heat exchange), and heat storage (Equation 1). The metabolic rate of the body (M) provides energy to enable the body to do mechanical work (W) and the remainder is released as heat (i.e., M – W). Heat exchange with the environment can be by conduction (K), convection (C), radiation (R), and evaporation (E) through the skin surface and the lungs. When combined, all the rates of heat production and loss provide a rate of heat storage (S). For the body to be in heat balance (i.e., constant temperature), the rate of heat storage should be zero (S=0). If there is a net heat gain, storage will be positive and body temperature will rise. If there is a net heat loss, storage will be negative and body temperature will fall (Equation 2).

Equations 1, 2: Heat balance equation of the human body.

$$M - W = E \pm R \pm C \pm K + S \quad (1)$$

In thermal balance (S=0)

$$M - W - E \pm R \pm C \pm K = 0 \quad (2)$$

In practice, due to constant environmental and physiological changes, a steady thermal state of the body cannot be achieved, but rather a dynamic equilibrium of the internal body temperatures is maintained or “regulated”.

Metabolic heat produced in the cells is conducted along the prevailing thermal gradient to the surrounding tissue from where it is convected by movement of extracellular fluids, e.g., blood. This results in an effective temperature gradient between the internal body and the skin and a net heat transfer from the cells of the body to the surface where it can be lost to the environment. The thermal properties of blood, muscle, fat, bone, etc. are therefore important for internal heat transfer and hence body heat exchange.

However, to regulate temperature in a changing environment, this 'passive' system must be controlled by a dynamic, "active", system of thermoregulation.

As in all physiological regulatory systems, the thermoregulatory system consists of a complex network of neurons and effector organs. Neural coded information from thermo-sensitive neurons is sent through specific pathways to neural centres in the central nervous system (CNS). There it is integrated, processed, and conveyed to specific organs, blood vessels, muscles, or sweat glands that act as thermoregulatory effectors. Their activation produces thermoregulatory activity, which counteracts the displacements of deep body temperature and maintains a stable body temperature. The thermoregulatory system can be considered in two forms: behavioural and autonomic (Mercer 2003). Autonomic thermoregulation is capable of fairly precise adjustments of heat balance but is effective only within a relatively narrow range of environmental conditions. Behavioural thermoregulation, on the other hand, is effective in a wide range of thermal environments, however the control of heat balance it provides is more coarse (Webb et al. 1970; Kuznetz 1980; Hexamer and Werner 1995).

Activation of autonomic and behavioural thermoregulatory responses relies on similar afferent information emanating from thermo-sensitive receptors. The mechanism and anatomical loci of the central processing of this information, however, is much better understood for autonomic responses than for behavioural responses.

The following sections review the components and pathways of the thermoregulatory system.

2.2.1 Thermal reception

Thermoafferent information is transduced by thermoreceptors: nerve endings that are sensitive to either warm or cold stimuli. A change in temperature in the thermoreceptive field of thermoreceptors (i.e., in their immediate environment) is converted into a graded potential. The size of the graded potential depends on the magnitude and rate of temperature change. When the graded potential is large enough to exceed a certain threshold, action potentials will be triggered. The action potentials convey neurally-encoded temperature information to the CNS. It has been shown that thermoreceptors may be bi-modal, i.e., sensitive to other stimuli besides temperature, or mono-modal, i.e., sensitive either to cold (cold receptors) or to warm (warm receptors) (Hensel et al. 1960; Pierau and Wurster 1981). Cold receptors increase their activity in response to cooling, and decrease their firing rate during warming, whereas warm receptors increase their activity during warming, and decrease their firing during cooling.

The spatial distribution of thermoreceptors is unequal throughout the body. Cold receptors are located predominantly in the skin. In contrast, warm receptors are abundant in deep body tissues, but less frequent in the periphery (Bligh 1998). It was suggested that the skin areas containing the greatest density of thermoreceptors are the facial skin (Pierau 1996) and upper torso (Burke and Mekjavic 1991). Variation in distribution of thermoreceptors may explain the differences in sensitivity to cold and warm when different body areas are stimulated (Tipton and Golden 1987; Burke and Mekjavic 1991).

The majority of thermoreceptors in the periphery are mono-modal cold-sensitive neurons, i.e., they respond exclusively to cold stimulation. They lie in the skin near the interface between the epidermis and dermis (Hensel et al. 1974; Adair 1999) and in the mucous membranes of the superficial part of the

respiratory system. The velocity of conduction through their afferent fibres is between 5 and 30 m/s (Schmidt and Thews 1989; Parsons 1999). Peripheral warm receptors are located in deeper layers of the skin and have slower conduction velocities of 0.5 to 0.75 m/s (Hensel 1976). Two separate neural pathways, one transmitting information from cold thermoreceptors and the other from warm thermoreceptors, ascend via spinal tracts towards the hypothalamus.

Differently than the peripheral thermoreceptors, central thermoreceptors are located in deep body tissues. They are often warm, multimodal receptors, i.e., they respond to warm as well as to signals from other regulatory control systems. Although much less common, central cold-sensitive neurons also exist (Jiang et al. 2002). The majority of central receptors have been located in the preoptic anterior hypothalamic (PO/AH) region (Boulant 1981; 1998; 2000). Other sites where central warm- and cold-sensitive receptors have been identified include: lower brain stem (midbrain and medulla oblongata; Jessen et al. 1983), spinal cord (Simon et al. 1986; Simon et al. 1998), abdominal region (Riedel et al. 1973; Riedel 1976; Gupta et al. 1979), skeletal muscles (Jessen et al. 1983; Mercer and Simon 2001) and the urinary bladder (Jiang et al. 2002). Central thermoreceptors provide most of the thermal information obtained from the body core (Simon 2000). Their sensitivity to nonthermal signals was suggested to provide a physiological basis to the mechanism by which other control systems may interact with the thermoregulatory system (Simon 2000).The activity of thermoreceptors depends on the dynamics of thermal change in its thermoreceptive field. A constant steady-state temperature will evoke a static (tonic) response and a temperature change will result in a dynamic (phasic) response.

Static activity

The characteristics of the tonic firing rate in response to constant thermal stimuli at the receptive field of thermoreceptors depend on the specific receptor type and on the steady-state temperature of a thermoreceptive field prior to the application of a thermal stimulus, i.e., the adapting temperature. The static frequency response of cold and warm receptors as a function of temperature describes a bell-shaped curve. The nature of the curve varies between individual receptors, but in general static activity can be observed at temperatures between approximately 5°C and 40°C in a cold receptor and between 30°C and 50°C in a warm receptor (Pierau 1996). The maximal static activity of thermoreceptors is evoked at temperatures between 25ºC and 30°C for cold receptors and between 40°C and 47°C for warm receptors (Pierau and Wurster 1981), (Fig. 1). The maximum discharge is much lower for cold receptors than it is for warm receptors (Hensel and Wurster 1970). The static activity declines as the stimulating temperature rises above or falls below the temperature of maximal response. The activity ceases immediately when a warm stimulus is applied to a cold receptor's receptive field and vice versa. When the temperature at the thermoreceptive field stabilises at a new steady level, the static activity of the thermoreceptor, appropriate to the new adapting temperature, reappears (Pierau 1996). The static activity of a cold thermoreceptor also reappears when the thermoreceptor is stimulated with temperatures greater than 45°C, a level where heat pain occurs. This phenomenon has been termed the static paradoxical discharge response (Dodt and Zottermann 1952) and reaches its maximum at 50°C.

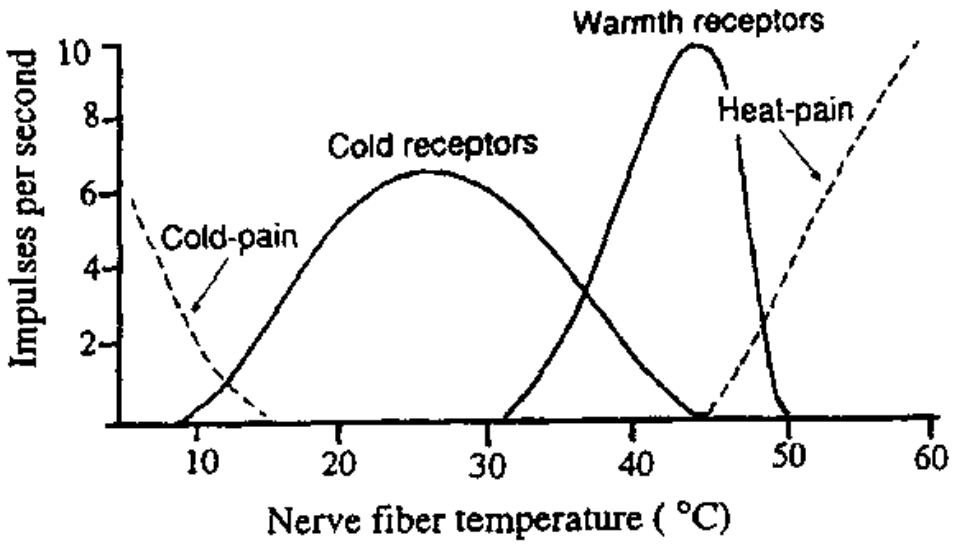

Figure 1. *Static responses of cold and warm thermoreceptors* (Guyton 1991).

Dynamic activity

The dynamic (phasic) response of thermoreceptors enables different directions and magnitudes of temperature changes to be detected by the thermoregulatory system. For example, the response of a cold receptor to a transient cooling of its receptive field will be an immediate, although transient, increase of the firing rate and simultaneous inhibition of the activity of the warm receptors. In contrast, warming increases the warm thermoreceptors' firing rate and inhibits the activity of cold thermoreceptors. After transient cooling or warming has been completed and a new adapting temperature has been established, the dynamic response ceases and the activity rapidly returns to a steady state level (Fig. 2). The magnitude of a thermoreceptor's dynamic response is related to the intensity (magnitude) of the applied thermal stimulus (ΔT), to the rate of temperature change ($\Delta T/s$), and to the adapting temperature of the skin (Koenietzy and Hensel 1977). For all adapting temperatures, a greater and/or faster change of temperature in a thermoreceptive field will results in a more vigorous response from the thermoreceptor (Kenshalo 1976, Koenietzy and Hensel 1977). By applying identical cooling/warming steps to a thermoreceptor at various adapting temperatures, a dynamic response curve can be produced (Fig. 3). The latter is similar to the bell-shaped curve of the thermoreceptor's static activity (Pierau 1996), with the obtained maximal dynamic response close to the adapting temperature of the maximal static response (Kenshalo 1976). As with the static responsiveness, the dynamic response of warm thermoreceptors seems to be greater than that of cold thermoreceptors.

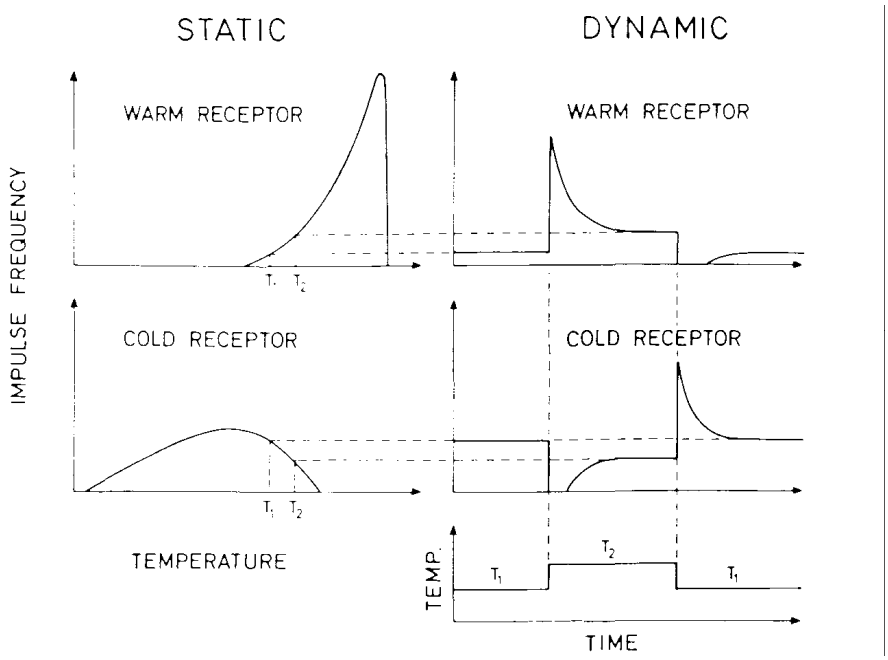

Figure 2. *Static and dynamic responses of thermoreceptors to constant temperature and transient temperature changes.* From: Hensel and Wurster (1970).

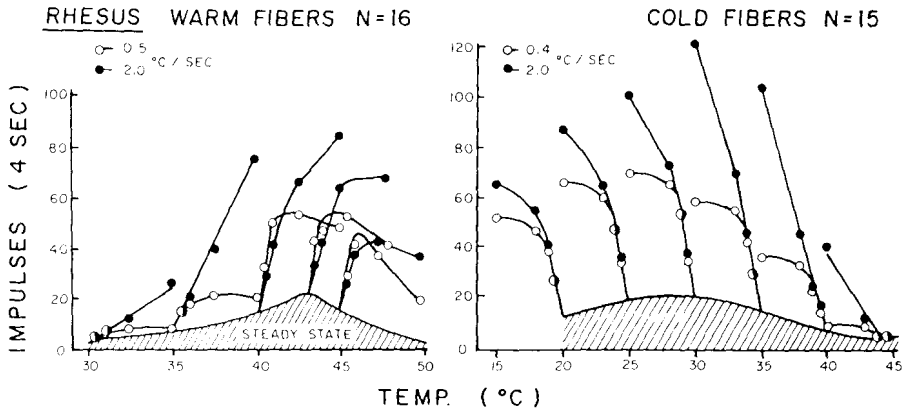

Figure 3. *The dynamic response of warm (left) and cold (right) receptors to different temperature change intensities (0.5, 1.2, and 5°C) at two rates (0.5 and 2°C/sec).* From Kenshalo (1976).

Simon et al. (1998) suggested that dynamic activity is mainly a property of peripheral, rather than central, thermoreceptors. In contrast to peripheral warm and cold thermoreceptors, dynamic thermosensitivity has been observed in only a few spinal cord thermoreceptive neurons, and has not been observed at all in hypothalamic neurons receiving thermoregulatory input from the spinal cord (Simon et al. 1998). Similarly, when different rates of core cooling were used at simultaneously clamped skin temperature, the activation of thermoregulatory effector responses was not proportional to the rate of change of core temperature, but rather to the degree to which core temperature deviated from the thermoneutral level (Roos and Jessen 1987). Rawson and Quick (1970, 1972), on the other hand, provided

evidence for thermoreceptor sensitivity as well as initiation of thermoregulatory responses (panting, sweating) in response to intra-abdominal heating of a ewe. Similarly, Riedel (1976) recorded warming-induced discharge in splanchnic units in the intestine of rabbits. These units were divided into two groups, one with static and dynamic maxima centered around 40°C and the other around 46°C. Dynamic responses are, therefore, not relevant only for the thermosensory function of peripheral thermoreceptors as suggested by Simon et al. (1998), but also represent a phenomenon of the core thermoreceptors.

An abundance of warm-sensitive sensors in central body regions and their increased static as well as dynamic responsiveness compared to cold-sensitive sensors suggests that the core is more sensitive to heating than it is to cooling. This is not surprising considering that humans are endothermic organisms (generating heat) and that their body core temperature is regulated closer to the upper survival limit than to the lower limit. In contrast, the abundance of cold-sensitive receptors at peripheral regions of the body and their increased rate of signal progression compared to warm-sensitive sensors suggest that the peripheral regions of the body are primarily concerned with defence against external cooling. This, however, does not imply that the thermoregulatory behaviour in response to heating is greater than the response to cooling. Initiation of thermoregulatory responses (autonomic and behavioural) depend on central integration of all afferent signals emanating from the core and the periphery.

2.2.2 Central processing of thermal information

Peripheral and core thermoafferent information is conveyed to thermoregulatory foci, which are situated in the CNS. The role of these foci is to integrate and process the thermoafferent information as well as mediate the neural responses to the thermo efferent neural pathways.

Central thermoreceptors involved in the control of thermoregulatory responses are concentrated mainly in the hypothalamus; however, other sites, such as the spinal cord and medulla also seem to participate in thermoregulation (Boulant 1996). It is largely agreed that the main focus of thermoregulation, particularly the one involved in autonomic responses, is the preoptic area of the hypothalamus (Satinoff 1978). The anterior part of the preoptic area of the hypothalamus (PO/AH) contains a large number of neurons that increase their firing rate in response to either warming or cooling. Animal studies show that even small temperature changes (e.g., less than 1°C) in the PO/AH evoke a variety of behavioural and physiological thermoregulatory responses (Boulant 1996, Boulant 1999). Warming of the PO/AH was shown to evoke behaviours such as moving to a cooler environment or postural changes that increase heat loss. Conversely, PO/AH cooling produces heat retention behavioural responses, cutaneous vasoconstriction and metabolic heat production by either shivering or nonshivering thermogenesis (Boulant 1999). PO/AH neurons not only sense hypothalamic temperature but also integrate inputs from thermoreceptors in the skin and various organs in the body core (Boulant 1996). In addition to the temperature-sensitive neurons, the PO/AH contains a large portion (about 70%) of temperature-insensitive neurons, which show little or no change in their firing rate during hypothalamic warming and cooling and are morphologically different than their thermo-sensitive counterparts (Boulant 2006). In addition, both types of neurons may be excited or inhibited by other modalities such as osmolality and glucose level (Silva and Boulant 1984) or by synaptic inputs from nearby neurons (Boulant 2006). Thus, preoptic neurons may play an important role in the interaction between the thermoregulatory system and other systems responsible for osmotic and metabolite

regulations.

Whether the anatomic areas in the CNS that are involved in autonomic thermoregulation are also responsible for behavioural thermoregulation is still unclear. According to Satinoff (1978), the neural foci for behavioural and autonomic temperature regulation are functionally and, at least in part, neuroanatomically separate. Satinoff (1987) proposed that the hypothalamus coordinates and adjusts the activity of subordinate thermoregulatory foci, which lie outside the preoptic area at lower levels of the thermoregulatory neuraxis, such as midbrain, pons, medulla, and spinal cord (Satinoff 1978, Satinoff 1983). Satinoff suggested that subordinate foci are capable of initiating the thermoregulatory responses independently, but they normally act in a concerted manner, because they are controlled, i.e., suppressed or activated, by the hierarchically higher foci (Satinoff 1983). When only the subordinate thermoregulatory foci are activated the thermoregulatory control they provide is less precise (Satinoff 1978), possibly because the threshold for their activation is greater than that of the hierarchically superior foci, or because the effector responses they evoke are only fragmentary. As more levels of the nervous system are involved in thermoregulatory control, the regulation of core temperature becomes more accurate (Satinoff 1983).

The existence of such hierarchically lower foci has been confirmed in numerous animal studies, which usually involved lesions at different levels of the central nervous system (Walther et al. 1971, Mercer and Simon 2001). In rats, for example, a lesion in the PO/AH extinguishes autonomic, but not behavioural, thermoregulatory responses (Satinoff 1978, 1983, Almeida et al. 2006). If so, the foci for behavioural thermoregulation, or at least some of them, must lie outside the preoptic area of the hypothalamus. One possible location is the lateral hypothalamus, as lesions in this area do not affect autonomic, but do abolish behavioural, thermoregulatory responses (Satinoff 1978). Other studies using various behavioural responses confirmed that several brain areas besides the PO/AH may play an important role in behavioural thermoregulation. These areas include the parastrial nucleus, the dorsomedial hypothalamus, and median preoptic nucleus (Maruyama et al. 2003, DiMicco and Zaretsky 2007).

Behavioural thermoregulatory responses are conscious, voluntary actions. Therefore, the afferent thermoregulatory information must be transferred not only to hypothalamic foci, but also to foci in the cortical regions involved in eliciting a thermal perception. Thermal perception involves different cortical and subcortical regions that are a part of the somatosensory pathway (medial dorsal midbrain, thalamus, cingulated cortex, S2 and S1 cortex, and insula) (Casey et al. 1994, Romanovsky 2007). Although neurophysiologic studies confirmed that some cortical somatosensory neurons have thermoreceptive properties (Dong et al. 1994), only a small proportion of them were multimodal neurons. Thus, it is still unclear whether multimodal cerebral structures also integrate thermal perceptions (Candas and Dufour 2005).

Behavioural thermoregulatory effectors

Thermoregulatory effectors (thermoeffectors) are defined as “Organ systems and their actions that affect heat balance in a controlled manner as part of the processes of autonomic and behavioural temperature regulation” (Mercer 2003). Bligh (1998) specified that thermoeffectors modulate either the rate of heat production or heat flow between the organism and its environment. Behavioural heat production responses

consist of voluntary exercise in which heat is generated by muscular contractions creating movement of the body against its environment. Exercise can generate sufficient heat to elevate body temperature by 1°C in 4 min (Bradford et al. 2007). However, the efficiency of exercise thermogenesis is limited to short periods of time by the gradual development of fatigue. This can lead to severe consequences for a person unexpectedly caught in cold terrestrial (Pugh 1966; 1967) or aquatic (Steinman et al. 1987) environments. On the other hand, exercising in a cold environment may prevent local cold injuries by increasing peripheral circulation. The heat produced by exercise, even in cold environments, can also lead to overheating if heat loss to the environment is limited (e.g., by protective clothing). In such conditions, maintaining thermal stability depends on the heat exchanged between the body and the direct microclimate under the protective garment. Much effort has been invested in optimizing the design and control of automatic cooling/warming systems underneath protective clothing (Nunneley 1970, Flouris and Cheung 2006) in order to maintain the body in thermal comfort. However, for multiple reasons, such automatic control has not yet been implemented in most of the systems used in space (Nunneley 1970, Flouris and Cheung 2006) or in diving (Mekjavic et al. 2001). Human behavioural responses are motivated by their subjective assessment of the thermal environment rather than actual exposure time, physiological status, prior acclimation to the cold, or deep body temperature.

Subjective assessment of the thermal environment as well as the factors influencing it will be summarised in the following sections.

2.2.3 Thermal perception and thermal comfort

Behavioural thermoregulation in humans is governed by thermal perception and thermal comfort. Thermal perception refers to a rational experience, which can be described with terms such as “warm” and “cold”. Thermal comfort, on the other hand, is an emotional experience, an affective judgment, characterized by terms such as “comfort” and “discomfort” and “pleasant” and “unpleasant” (Cabanac 1971). It is well accepted that the perception of thermal discomfort motivates thermoregulatory behaviour (Weiss and Laties 1961, Cabanac and Hardy 1969, Cabanac 1981). According to Cabanac (1981), the motivation for thermoregulatory behaviour is thermal pleasure, a transient phenomenon that results from relief of thermal discomfort and does not necessarily occur at thermal neutrality. Therefore, when a person feels “too cold”, a stimulus that causes heat loss will produce “displeasure” and will motivate the preservation or gain of heat “pleasure”. Conversely, if a person feels “too hot”, heat gain is perceived as “unpleasant” and heat loss “pleasant”. This phenomenon was termed alliesthesia, namely the dependence of thermal perception and thermal comfort on both skin and core temperatures. Positive alliesthesia indicates a change to a more pleasurable sensation, negative alliesthesia a change to a less pleasurable one (Mercer 2003).

Thermal perception and thermal comfort respond much earlier than any other physiological thermoregulatory response to changes in environmental temperature (Cunningham et al. 1978) and thus appear to anticipate future changes in the body’s thermal state. Such an anticipatory feature presumably reduces the need for frequent small behavioural adjustments. Hensel and Schafer (1981) found that temperature perception (especially local cold perception) depends mainly on the activity of thermoreceptors in the skin, whereas thermal comfort or discomfort reflects a general state of the thermoregulatory system. However, as mentioned above, feeling thermally comfortable does not ensure that the body temperature is

within its "normal" range, but rather that it is changing towards normothermia. The difference between thermal perception and thermal comfort was clearly demonstrated by Gagge et al (1967). They exposed subjects for one hour to neutral thermal conditions (29°C), then a step change to a much colder (17.5°C) or warmer (48°C) environment for a two-hour exposure, which was followed by a step change back to neutral conditions. On returning to the neutral environment discomfort almost immediately disappeared, while temperature perceptions lagged considerably behind the comfort reports in all subjects (Gagge et al. 1967).

The range of ambient temperatures associated with specified mean radiant temperature, humidity, clothing, and air movement, perceived as thermally comfortable defines the thermal comfort zone (TCZ). Within the boundaries of one's TCZ a sensation of "indifference" to the thermal environment will occur (Mercer 2003), no urge to behaviourally modify the thermal environment will arise (Cabanac 1971, Hensel 1976).

According to Fanger (1970), human intellectual, manual, and perceptual performance is in general highest when he is thermally comfortable. Thus, maintaining thermal comfort is not only pleasurable, but is also physiologically significant. In a thermally challenging environment, a change in thermal perception may serve as the first line of defence against alteration in deep body temperature. Maintaining thermal stability in extreme thermal conditions would also be dependent on the ability to maintain thermal comfort, identifying when discomfort occurs.

2.3 Factors influencing thermoregulation

2.3.1 Thermal factors

A suprathreshold thermal stimulus evokes neural activity in the thermoreceptors. The afferent information that is provided by the thermoreceptors is integrated in cortical somatosensory foci and produces conscious thermal perception (Cabanac 1971). The neural activity of thermoreceptors is not always consciously detected, however. When only a small temperature change is presented to a small area of skin, the excitation of cold or warm receptors does not result in a conscious thermal perception (Hensel 1976). Similarly, when the rate of temperature change applied to the skin is very small (0.007°C/s), no thermal perception is evoked within a certain range of skin temperatures termed the zone of physiological zero (Kenshalo 1976). For a thermally stimulated area of approximately 75cm^2, the zone of physiological zero extends over a range of one or two degrees near 34°C (Hensel 1976). When smaller areas of skin are stimulated, the zone of physiological zero becomes wider. Thus, it was proposed that a thermal sensation is only evoked when a certain number of impulses per unit time reach the central nervous system (Hensel 1976). Any factor that affects the afferent input from thermoreceptors can also alter thermal perception. Indeed, the adapting temperature of the skin and the surface of the thermally stimulated area, both of which affect the number of afferent impulses per unit time reaching the CNS, have a pronounced effect on thermal perception. Thermal sensitivity is highest when bigger surface areas are stimulated and when the adapting temperature coincides with the temperature that evokes maximal activity in warm or cold receptors. Alternatively, smaller stimulation areas and adapting temperatures that are changed in either direction of

the maximal response of the thermoreceptors will reduce thermal sensitivity (Melzack et al. 1962, Kenshalo 1976). Thermoregulatory characteristics also vary considerably between different regions in the body. For example, cutaneous vessels of the limbs exhibit vasoconstriction in response to cold, while the head and the neck lack such a mechanism (Granberg 1991). The potential heat exchange with the environment for a particular region depends on the skin surface area, tissue morphology, vascularity, and local heat production. Recent evidence suggests that the face displays stronger thermal sensitivity than the forearm, thigh, leg, and foot for sudomotor and discomfort responses (Cotter and Taylor 2005)—the subjective feeling of thermal (dis)comfort changes when distinct regions on the body are cooled or warmed. When Webb et al. (1970) allowed subjects to select the preferred temperature at different regions of their body, they chose asymetrical skin temperature distribution to provide optimal comfort (Webb et al. 1970) depending on the task (e.g., rest, light or heavy work) they performed. Later, studies by Xu et al. (1999), Huizenga et al. (2004), and Arens et al. (2006) confirmed that general (dis)satisfaction can be caused by local (dis)comfort and asymmetries as a result of heating or cooling of a particular part of the body.

2.3.2 Nonthermal factors

The manner in which thermal signals from the core and periphery are integrated centrally has been the focus of many studies in the past. However, attention is shifting towards a better understanding of how nonthermal factors (NTFs) influence temperature regulation. It is well documented that several NTFs interfere with the function of the autonomic thermoregulatory system (Mekjavic et al. 2003, Mekjavic and Eiken 2006). NTFs can act as inhibitory or excitatory stimuli at various locations along the thermoregulatory neural pathway. Some NTFs, such as aging for example, have been shown to affect all thermoregulatory responses (Kenney and Munce 2003), whereas others only affect individual autonomic responses. Hypoglycemia, for example, only attenuates shivering, and dehydration only the sweating response (Mekjavic and Eiken 2006). In addition, it has been mentioned in several studies that in addition to the effect they exert on autonomic responses, thermal perception is also altered. Altered perception of thermal comfort and thermal sensation has been documented with various NTFs including gender (Anderson et al. 1995, Paulson et al. 1998, Sarlani et al. 2003, Chao et al. 2007) , ageing (Collins et al. 1981, Taylor et al. 1995, Anderson and Mekjavic 1996, Anderson et al. 1996), inert gas narcosis (Fowler et al. 1985, Coleshaw et al. 1990, Mekjavic et al. 1994), hypoglycaemia (Passias et al. 1992), hypoxia (Golja and Mekjavic 2003, Golja et al. 2004), and bed rest (Greenleaf 1989, Mekjavic et al. 2005). In most of these studies, however, the behavioural assessment is secondary to the assessment of autonomic thermoregulatory responses. The control of such studies with respect to behavioural thermoregulation is often poor. Therefore, the influence of NTFs on thermal perception, thermal comfort, and particularly on behavioural responses is still unclear.

The following section provides a brief review on the literature related to the influence of NTFs on the thermoregulatory system.

Effects on autonomic thermoregulation

Regulation of skin blood flow

The initial autonomic defence of body temperature is achieved by changes in the vasomotor tone, responding to the prevailing ambient temperature. During heat stress, elevated T_c and T_{sk} lead to cutaneous vasodilatation. Conversely, during cold stress, reduced temperatures lead to cutaneous vasoconstriction. Regulation of skin blood flow is expected to be most affected by NTFs because of its involvement in other autonomic regulatory processes; e.g., in maintaining cardiac output, blood pressure, and tissue oxygen delivery. Vasomotor responses are activated not only due to thermal stress, but also during postural changes, haemorrhage, and exercise. The range of ambient temperatures where no autonomic response is activated is defined (Mercer 2003) as the thermoneutral zone (TNZ). It is well recognized that the width of the TNZ will vary as a consequence of the influence of NTFs on the vasomotor response (Johnson 1986, Romanovsky et al. 2002). In response to local increases in T_{sk}, cutaneous blood vessels dilate by local temperature-dependent mechanisms in addition to neural mechanisms. Maximal local skin blood flow is reached when local T_{sk} is held at 42°C for 35–55 min (Taylor et al. 1984). It has been reported that the vasodilatation caused by local warming can be also evoked in denervated skin (Aulick et al. 1977), suggesting that the vasomotor responses to heat stress are mediated, at least in part, by non-neural factors. Recently, nitric oxide (NO), a vasodilating substance produced by the vascular endothelium has attracted much attention with respect to active vasodilatation. When NO synthase, an enzyme responsible for the NO production, is inhibited, locally induced vasodilatation is attenuated (Kellogg et al. 1998) and skin blood flow during normothermia decreases (Coffman 1994). Although NO seems to maintain a basal vasodilator tone during normothermia, it does not seem to contribute to the regulation of blood flow in a cool environment, as the inhibition of NO synthase under cool conditions does not diminish local blood flow any further (Coffman 1994). In contrast, when NO synthase is inhibited during heat stress, active vasodilatation is substantially diminished, yet not completely abolished (Kellogg et al. 1998, Shastry et al. 2000). The production of NO thus seems to be required for the full expression of active vasodilatation during heat stress (Kellogg et al. 1998). In the elderly, the responses of hand blood flow to cooling and warming are attenuated (Collins et al. 1977). It was reported that in approximately 20% of the elderly subjects tested, skin cooling did not result in vasoconstriction (Collins et al. 1977, Collins and Exton-Smith 1983). Aged people have a smaller core to periphery temperature gradient, as the deep body temperature is lower and the skin temperature higher than in the young. A smaller core to periphery temperature gradient supports the observations of abnormalities in the cold-induced vasoconstriction mechanism and suggests that the ability to maintain body heat stores by vasoconstriction is impaired with ageing.

Shivering and sweating

Once the capacity of the vasomotor response to maintain a stable T_c is exceeded, the appropriate autonomic responses of sweating or shivering are activated. The range of temperatures bound by the T_c thresholds for shivering and sweating is defined as the interthreshold zone (Mercer 2003). The characteristics of the sweating and shivering responses include not only the T_c values at which they are activated, but also the gains of these responses. NTFs may influence both of these characteristics, namely the thresholds and gains. It has been reported that the inhalation of N_2O in normobaric conditions does not affect the core temperature at which sweating ceases, but does decrease the core temperature at which shivering is initiated (Mekjavic and Sundberg 1992). Similarly, during hypoglycaemia the core temperature threshold for the

cessation of sweating remains unchanged, and the core temperature for the onset of shivering decreases (Gale et al. 1981, Passias et al. 1996). The sweating response does not seem to be affected by NTFs such as hypoglycaemia or N_2O-induced narcosis, but the shivering response diminishes. Conversely, during dehydration only the sweating response is affected (Montain et al. 1995, Mekjavic et al. 2003). As opposed to NTFs affecting only individual autonomic responses, ageing, for example, has been shown to affect all autonomic thermoregulatory responses; shivering is initiated at a lower core temperature (Frank et al. 2000), sweating at a higher core temperature (Collins et al. 1977, Anderson et al. 1996), and the intensity of the sweating response is reduced (Anderson et al. 1996). Neurophysiological studies have provided neuroanatomical evidence explaining the manner in which some of these nonthermal factors alter the thermoregulatory responses. In the case of hypoglycemia and dehydration, it has been demonstrated that the majority of the hypothalamic cold and warm sensors are bimodal, responding to more than one stimulus modality. Some of the cold sensors are also glucosensitive, and some of the warm sensors are osmosensitive (Silva and Boulant 1984). During cooling, the cold sensors will be progressively more active, but their activity will be dependent on the level of plasma glucose. In the same manner, the activity of the warm sensors will be dependent on the plasma osmolality. The attenuated activity of the cold and warm sensors will be reflected in the shivering and sweating responses, respectively.

Effects of NTFs on behavioural thermoregulation

It is now becoming increasingly evident that NTFs that attenuate autonomic responses also affect thermal perception, and therefore may also alter behavioural thermoregulatory responses. Fanger (1970) suggested that a wide range of factors affect the conditions required for thermal comfort including: national-geographic location, age, sex, body build, menstrual cycle, ethnic differences, food, circadian rhythm thermal transients, unilateral heating or cooling of the body, and colour . In more recent years, it was found that also ageing (Collins et al. 1977, Collins et al. 1981, Collins and Exton-Smith 1983, Anderson and Mekjavic 1996, Anderson et al. 1996), inert-gas narcosis (Pertwee et al. 1986, Mekjavic and Sundberg 1992, Mekjavic et al. 1994, Cheung and Mekjavic 1995, Mekjavic et al. 1995), and hypoglycaemia (Passias et al. 1996) alter the range of temperatures perceived as thermally comfortable. Results of studies investigating the influence of NTFs on thermal comfort are often contradictory and, due to different experimental approaches, are also difficult to compare. The effect of ageing, for example, on thermal comfort received much attention, because of the frequent occurrence of hypothermia among the older population (Exton-Smith 1973); however, the debate regarding the mechanism underlying the effects still persist. Fanger (1970) stated that thermal comfort temperature is identical for the old and the young. On the other hand, Young (1991) showed that aging causes differences in comfort conditions in men while no difference is observed in those of women. Collins et al. (1981) showed that the mean preferred environmental temperature chosen by elderly to maintain thermal comfort is similar to that of the young; however, control of the preferred temperature was much less precise. The fluctuations around the mean preferred environmental temperature are much wider in the elderly than in the young. The inability to adequately regulate temperature behaviourally was associated with development of mild hypothermia in the elderly (Fox et al. 1973) and was attributed to a decreased ability to detect thermal changes with age (Collins et al. 1977). In the study by Collins et al. (1977), behavioural thermoregulatory responses were

attenuated with ageing; however, the perception of thermal comfort did not change; the great majority (76%) of mildly hypothermic people with a core temperature of less than 35.5°C felt thermally comfortable or even warm.

Although most of the scientific literature on thermoregulation focuses mainly on males, several studies investigated the differences in thermal comfort requirements the genders or between women at different phases of the menstrual cycle. In Fanger's experiments (1970), no difference in comfort conditions was observed between males and females. Chung and Tong (1990), on the other hand, found that females felt cooler than males in ambient temperatures between 20 and 30°C and associated it with an increased sensitivity to temperature changes. Changes in thermal perception have also been described at different stages of the menstrual cycle. In the luteal phase, women sense changes in skin temperature more quickly than during the follicular phase (Kenshalo 1966) and have a higher skin temperature preference (Cunningham and Cabanac 1971).

Similarly, narcosis either induced by inert gases or by anaesthetic agents has been shown to alter thermal perception in humans and modify thermoregulatory behaviour in animals. During normobaric N_2O narcosis (Mekjavic et al. 1994) or hyperbaric N_2 narcosis (Mekjavic et al. 1995), equal decrements in core temperature at clamped skin temperature evoke less thermal discomfort in humans than without narcosis. Animal studies have demonstrated that exposure to normobaric N_2O or hyperbaric N_2 results in a preference for a cooler environment (Pertwee et al. 1986), which was associated with a progressive fall of deep body temperature towards hypothermia. Evidence exists that the perception of thermal comfort during hypoglycaemia is altered in a similar way as that seen during inert gas narcosis (Passias et al. 1996).

Interaction between nonthermal factors

In a broader sense, studies investigating the effects of NTFs on thermoregulation provide an insight on how the homeostatic systems regulating body temperature and other physiological parameters interact. Alterations in thermal perception are most likely due to an effect on the neural mechanisms involved in transducing thermal stimuli into a perception of thermal comfort. The effect of NTFs on neural function has been established in many studies. The influence of hypoxia, for instance, on neural function has also been implicated in the observed hypoxia-induced decrements in visual perception (Fowler et al. 1993), auditory perception (Fowler and Lindeis 1992), cognitive function (Kennedy et al. 1989), and increments in reaction time (Fowler and Prlic 1995). As mentioned earlier, the neurophysiological studies by Silva and Boulant (1984) have demonstrated that some preoptic sensors also respond to modalities other than temperature. They reported that a fraction of the population of cold-sensitive preoptic neurons is also glucosensitive and that similarly a fraction of the warm-sensitive preoptic neurons is also sensitive to plasma osmolality. It is, therefore, important to evaluate not only the influence of individual NTFs, but also the results of the interaction between them. For example, in the diving environment, the function of thermoregulatory responses will be influenced directly by the increase in hydrostatic pressure (HP) and, indirectly, by the HP-induced narcosis associated with inhaling breathing mixtures containing inert gases. It has been demonstrated that the effect of pressure could be antagonized by narcotic gas (Johnson and Flagler 1950, Bennett 1963, Kendig 1984, Balon et al. 2002). This pressure “reversal” of anaesthesia was first described by Johnson et al. (1942) who noted that ”Under physiological conditions the narcosis of bacterial

luminescence by alcohol, urethane and certain other drugs may be virtually abolished by an increase in hydrostatic pressure." Later, Johnson and Flagler (1950) demonstrated that a similar relationship also occurs in higher animals. It would be interesting to know if a similar opposing effect of HP and narcosis (or other NTFs) also exist in the influence they exert on thermoregulatory responses. Considering the highly complex neural network that involves conscious activities compared to reflexive activities, and evidence demonstrating an effect of several NTFs on synaptic transmission, it can be concluded that such interaction will first influence conscious thermal perception and behavioural responses and only later, autonomic responses. Thus, a method that would accurately evaluate the influence of NTFs on behavioural thermoregulation can be a useful tool for also studying the combined influence of different NTFs on the thermoregulatory system as a whole.

Quantifying the thresholds for thermoregulatory behaviour requires that the limits of warm- and cold-induced discomfort are evaluated. In the sections below, the considerations and different approaches in measuring behavioural thermoregulatory responses are reviewed.

2.4 Measurement of behavioural thermoregulation in humans

2.4.1 Qualitative methods

According to a simple model of human response to the environment, a given set of environmental conditions will be followed by a thermal perception or behaviour. This model is the basis for the widely used method for evaluating thermal perception and thermal comfort – the scale rating approach. With this method the intensity of thermal perception or comfort is expressed on point-scales or, as recently described, on visual analogue scales (Zhang et al., 2004). The most widely used methods today are still the 7-point scales that were described during the 1930s by Bedford (1936) and later by the American Society of Heating, Refrigerating and Air Conditioning Engineers (ASHRAE, 1997). Voting on such scales requires that a subject interpret its sensation by a limited set of terms offered by the scale (Fig. 4). MacIntyre (1981) suggested that people of different climatic origins that are tested at the same time of the year might interpret words such as warm/cold and comfortable differently. MacIntyre stresses that it is highly unlikely, for example, that people from cold regions will describe their sensation as "comfortably cool" during winter. Results from the studies of Gagge et al. (1967) futher support the role of the wording used in the scale on comfort assessments. They demonstrated that equal changes in the ambient temperature are not rated similarly when scales of pleasure, comfort, or temperature are used. This implies that the subjective assessment of thermal comfort depends not only on physiological but also on psychological aspects such as interpretation, expectation, and personality. When subjects are directly asked if they are comfortable, too hot or warm, or if they wished the temperature changed, it is almost impossible to avoid some degree of suggestion by the observer.

Value	Description
+3	Much too warm
+2	Too warm
+1	Comfortably warm
0	Neutral
-1	Comfortably cool
-2	Too cool
-3	Much too cool

Value	Description
+3	Hot
+2	Warm
+1	Slightly warm
0	Neutral
-1	Slightly cool
-2	Cool
-3	Cold

Figure 4. *Commonly used scales for assessing thermal perception. The "Bedford" combined sensation and comfort scale (left) and the thermal sensation scale used by the ASHRAE (right).*

Suggestability caused by leading questions, language, and ego might significantly influence the results from scale votes. Interpretation of results from scale votes would also depend on the type of scale, depending on the appropriate analytical tools used; e.g., data obtained by a point-type scale would be analysed using non-parametric statistics, whereas parametric tests can be used to analyse data from a continuous-type scale. Lastly, subjective votes supposedly correspond to behavioural modifications, which would be initiated by a subject, though there is, as yet, no evidence for this. All that can be concluded on the basis of subjective ratings of thermal discomfort is that a subject would probably initiate some sort of behavioural modification.

2.4.2 Quantitative methods

Various approaches for measuring behavioural thermoregulation in humans have been suggested throughout the years. Observational methods such as monitoring the way internal home temperatures are controlled and adaptation of clothing during heat stress using time-lapse photography, and two-way mirrors (Parsons, 2003) have been suggested. Other approaches such as measuring clothing or dressing behaviour were used in the laboratory to evaluate the effects of factors such as age (Jeong, 1999), menstrual cycle (Kim and Tokura, 1995), and light intensities (Kim and Tokura, 1995; Kim and Jeong, 2002; Parsons, 2002). However, as mentioned earlier, even if such behavioural measures correlate well with changes in thermal conditions, their experimental control is low and the cause for such behaviour might not depend entirely on thermal conditions (Parsons, 2003). For instance, controlling a home's temperature might be related also to increased expenses for fuel or electricity, and clothing adjustments might be influenced by modesty, acceptability, and design (Parsons, 2002). Nevertheless, such methods are practically useful when they represent an actual, everyday, behavioural thermoregulatory activity.

Direct measurement of thermoregulatory behaviour in controlled conditions has been described using operant conditioning (OC) methods. In OC methods the subject is given voluntary control over some aspect of his thermal environment. For example, animals may learn to press a lever for warm stimuli in a cold environment (Hardy, 1970) or to select a preferred thermal environment in the presence of a NTF (Weiss and Laties, 1961; Satinoff, 1964; Macdonald et al., 1989; Pertwee et al., 1990; Gordon, 1997; Almeida et al., 2006). Squirrel monkeys, for example, can learn to alter the ambient temperature within their chamber while their core temperature (at the hypothalamus) is being controlled by implanted thermodes. In these

studies, the ambient temperature selected was linear and inversely proportional to core temperature (Adair, 1977). Changes in ambient temperature initiate nest building, huddling, or postural changes in animals, all with the apparent purpose of regulating body temperature (Satinoff, 1996). Although OC methods have been largely used in studies of animal subjects, several studies also found them applicable for studying the effects of various factors on human subjects. Collins et al. (1981), for example, suggested an experimental protocol to investigate the effect of ageing on behavioural thermoregulation. In his studies, young and old subjects controlled room temperature to their preferred level using a remote controller. Although age did not affect the mean preferred temperature chosen by the subjects, the control of ambient temperature among the elderly was much less precise and resulted in large fluctuations. This was attributed to a reduced ability to detect thermal changes and increased thermal comfort zone (Collins et al., 1981), and was later confirmed by Natsume et al. (1992) and Taylor et al. (1995), who concluded that elderly people require a more intense thermal stimulus to elicit an appropriate behavioural response. Cabanac et al. (1971) provided subjects with control over the temperature of the water perfusing a hand glove while the rest of the body was immersed in 28°C water. In their experiments, subjects preferred that the temperature of the water perfusing the glove increased with decreasing core temperature. They concluded that the perception of skin temperature as comfortable or not provides an indication of whether normothermia is compromised. Thus, discomfort of the skin will elicit an appropriate behaviour to resist changes of body temperature. Golja and Mekjavic (2003) used a similar experimental protocol to investigate the influence of hypoxia on behavioural thermoregulation. Hypoxia is an NTF that has been shown to interfere with normal thermoregulatory responses in animals (Gordon, 1997; Gordon et al., 1998) as well as in humans (Cipriano and Goldman, 1975; Blatteis and Lutherer, 1976). Their results indicated that hypoxia did not significantly alter the preferred hand temperature. However, they observed high inter-individual differences in the relative, as well as the absolute, preferred hand temperature subjects chose with or without hypoxia. In fact, only three of the eight subjects that were examined preferred a higher hand temperature with their core temperature decreased, as suggested by Cabanac (1971). The authors concluded that since subjects had to make their evaluation of thermal comfort according to the small surface area of the hand they might have failed to discern subtle changes in thermal comfort. Thus, such a protocol may not be sufficiently sensitive to investigate the effects of NTFs on behavioural thermoregulation.

Studies on thermal comfort and its behavioural control are not only of physiological interest, but also of practical importance. Early studies of thermal comfort in the 1930s were mainly concered with providing satisfactory conditions for people in built, indoor areas. With the rapid improvement in optimizing the design of protective clothing, the number of people that will work, recreate, or live in progressively more hazardous environments is expected to increase. Modern studies of thermal comfort will have to consider not only the thermal conditions required for thermal stability and comfort under protective clothing, but also the influence of the unique, nonthermal factors that exist in such environments on the ability to maintain thermal comfort.

The studies described in this thesis were performed in order to provide the methodology to systematically investigate the manner by which humans can regulate their thermal comfort. Furthermore, it provides direct evidence of the influence of NTFs on the control of thermal comfort and the preferred temperature range.

2.5 Conclusions

The present chapter reviewed the literature concerning behavioural thermoregulation in humans, the factors that might influence it, and the available methods used to evaluate it. From the literature review, it can be concluded that behavioural responses play an important role in human thermoregulation. In extreme thermal environments, this role is further increased. Activation of behavioural thermoregulatory responses is closely related to thermal perception and the onset of thermal discomfort. However, there is yet no systematic information on the degree of correspondence between the onset of thermal discomfort and initiation of an appropriate behavioural thermoregulatory response. Several NTFs that attenuate autonomic thermoregulatory responses might also influence thermoregulatory behaviour. However, current knowledge on the influence of NTFs on behavioural thermoregulation is speculative and based largely on analysis of scale votes. The influence of various NTFs on thermal perception and comfort must be substantiated using a more rigorous procedure. Furthermore, with the absence of an accepted method for assessing thermoregulatory behaviour in humans, there is still a need to describe a reliable experimental procedure for this purpose.

3 Aims and Hypotheses

The aims of the studies presented in this thesis were:

1. To develop a new experimental procedure for reliably assessing behavioural thermoregulation in humans (Chapter 4).[1]
2. To test the hypothesis that a subanaesthetic level of N_2O alters thermal perception and consequently attenuates behavioural thermoregulation in humans (Chapter 5).[2]
3. To test the hypothesis that prolonged bed rest alters behavioural thermoregulation in humans and evaluate its effects on cutaneous thermal sensitivity (Chapter 6).[3]

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[1] The study was presented at the 12th International Conference on Environmental Ergonomics (Yogev and Mekjavic, 2007).

[2] The study was presented at the 32nd Annual Scientific Meeting of the European Underwater and Baromedical Society (EUBS) on Diving and Hyperbaric Medicine (Yogev and Mekjavic, 2006) and has been submitted for publication in *Undersea and Hyperbaric Medicine*.

[3] The study was presented at the 12th International Conference on Environmental Ergonomics (Yogev et al., 2007) and received the W.L. Gore & Associates award for best poster presentation. The study has been accepted for publication in the *European Journal of Applied Physiology*.

4 A new method for assessing thermal comfort and behavioural thermoregulation in humans

4.1 Introduction

Maintaining thermal balance is an important consideration for humans working in extreme thermal environments. Such environments are characterized by excessive heat exchange (loss or gain) between the human body and its surroundings. The rate of heat exchange is determined by a combination of factors including thermal conductivity, specific heat per unit volume, and density of the particular surrounding medium (e.g., gas/air). Thermal balance is achieved when the rate of heat generated by the body equals the rate of heat loss to the environment. Behavioural and autonomic thermoregulatory responses continuously regulate the physiological processes associated with heat production and heat loss of the body. In high heat loss environments, the capacity of autonomic thermoregulatory responses to maintain thermal balance is very limited. Thus, in such conditions behavioural thermoregulation becomes of paramount importance. Behavioural thermoregulation refers to actions that modify heat exchange between the body and the surrounding environment. Heat exchange is altered by modifying the insulating layer around the body and/or by selecting a suitable, i.e., preferred, ambient temperature. In practice, people working in extremely hostile environments (e.g., diving in deep cold water, executing extravehicular activities in space) must wear protective clothing. Due to high insulation and low vapour permeability of protective clothing, and excessive heat load from the environment and endogenous factors, thermal stress is a major problem for the user of protective clothing. Accordingly, much effort is being invested in optimizing the design and temperature control systems underneath protective clothing (Nunnely 1970, Flouris and Cheung 2006). Ideally, such control systems should automatically maintain the body in thermal comfort. However, for multiple reasons, such an automatic control has not yet been implemented in the systems used in space (Nunnely 1970, Flouris and Cheung 2006) or in diving (Mekjavic, 2003). Therefore, to achieve thermal balance astronauts and divers still depend, to a large extent, on their ability to control the temperature subjectively. The basis for behavioural regulation of body temperature is the perception of thermal (dis)comfort, an affective judgment of the thermal environment (Mercer 2001). The range of ambient temperatures, associated with specified mean radiant temperature, humidity, and clothing perceived as thermally comfortable defines the thermal comfort zone (TCZ). When a boundary of the TCZ is exceeded, an urge to behaviourally counteract it arises (Cabanac, 1969, Hensel, 1976, Cabanac, 1981). The degree of correspondence between the perception of thermal (dis)comfort and initiation of a behavioural response has not been systematically studied (Hardy, 1970). Thus, it is still unclear, whether different thresholds exist for expressing thermal discomfort and for initiating a behavioural action to alleviate it. Moreover, in thermally extreme environments, behavioural responses should be maximally efficient. This, however, is not always the case. It is becoming increasingly evident that in addition to the thermal factors a variety of nonthermal

factors (NTF) influence the capacity of the thermoregulatory responses to maintain thermal balance (Mekjavic and Eiken 2006). Thus, in any given environment, the efficiency of the thermoregulatory responses will also be dependent on the presence of NTFs that may influence thermoregulation.

Our first aim was to develop a method that would enable reliable evaluation of behavioural thermoregulation in humans. Specifically, the developed method should be designed, such that it could assess the behavioural responses initiated to maintain thermal comfort in humans exposed to various NTFs in a laboratory setup.

4.2 Methods

4.2.1 Experimental arrangement

The experimental set-up comprised a water-perfused suit (WPS) with a temperature control unit designed to allow the subject and the experimenter control over the water temperature perfusing the WPS (T_{wps}). A schematic representation of the experimental set-up is presented in Figure 1.

Water perfused suit (WPS)

The WPS consisted of five components: one covering the head and upper part of the back, one for each leg, one surrounding the torso and one for both arms and the lower back. The WPS did not cover the hands, feet, neck, or face. All five components of the WPS were fed from a common manifold and consisted of identical lengths (25 meters) of small-diameter (inner = 4 mm, outer =5 mm) PVC tubing that were woven in the meshed lining of the suit. This ensured equal flow of water in all five segments of the suit of approximately 3.5 L/min. The total volume of water contained by the tubes in the suit was approximately 1.25 L. The WPS was designed to fit various body sizes using Velcro stripes.

Temperature control

Heating and cooling of the T_{wps} was achieved by activating and de-activating two 12 V/DC pumps (Conrad electronic, BARWIG) connected to 5-litre warm and cold water reservoirs. The water entered a 1-litre mixing container before it was circulated to the suit by a third (identical) pump. Computer software (TempObleka™, Mak Elektronik Ltd. Slovenia) specifically written for this study, enabled the experimenter to control the range of oscillations and rate of T_{wps} change at the WPS. It could also be set to automatically maintain sinusoidal warming and cooling oscillations in T_{wps}.

By depressing a manual control switch, the subjects could alternate between cooling and heating of the Twps whenever they felt the suit becoming uncomfortably warm or cold.

4.2.2 Protocols

Two experimental protocols were used. In the first, the subjects passively reported their perception of the changing temperature of the WPS; in the second, they could control the heating and cooling processes to maintain thermal comfort. A total of 12 healthy subjects (6 males, 6 females) participated in the study.

Their physical characteristics (mean(SD)) were: age = 25.2 (3.9), height = 176.4 (9.8) cm, Weight = 68.0 (14.6) kg. Subjects are naive regarding the absolute temperature of the water perfusing the WPS.

a. Protocol 1

This protocol was used to evaluate how subjects perceived the thermal changes of the WPS and determine the range of thermal comfort (and threshold for discomfort). After an initial 10 minutes at baseline temperature (T_{wps}=27°C) the T_{wps} started to fluctuate within the range 27°C to 42°C at a rate of 1.2°C/min. The subjects were asked to report when they perceived the temperature changing from a comfortable to an uncomfortable level and *vice versa*, thus indicating the boundaries, T_{low} and T_{high}, and the range of their thermal comfort zone (TCZ) during heating and cooling.

Subjects repeated this trial on three separate occasions to assess the reproducibility of their reports.

b. Protocol 2

In the second protocol, the 10 min period at baseline temperature (T_{wps}=27°C) was followed by a 60 min comfort regulation trial in which subjects' behavioural thermoregulatory response to maintain thermal comfort was assessed. The temperature of the water perfusing the WPS varied in a sinusoidal manner from 27°C to 42°C at a rate of 1.2°C/min, as in the first trial. However, rather than passively reporting their perception of the changing temperature of the WPS, in this trial subjects could control the heating and cooling processes. Subjects were instructed to change the direction of the temperature of the water perfusing the WPS once it became either uncomfortably warm during heating, or uncomfortably cool during cooling, by depressing a button on a remote control switch. The control unit did not enable a steady state position, thus it could only alternate between heating and cooling. Subjects were instructed to maintain T_{wps} within a preferred range for a total duration of one hour. Subjects were not coached or given any further instructions during the trial. After the trial concluded, the resultant characteristics of the sinusoidal T_{wps} pattern with its low peaks (T_{low}), high peaks (T_{high}) and range were analysed.

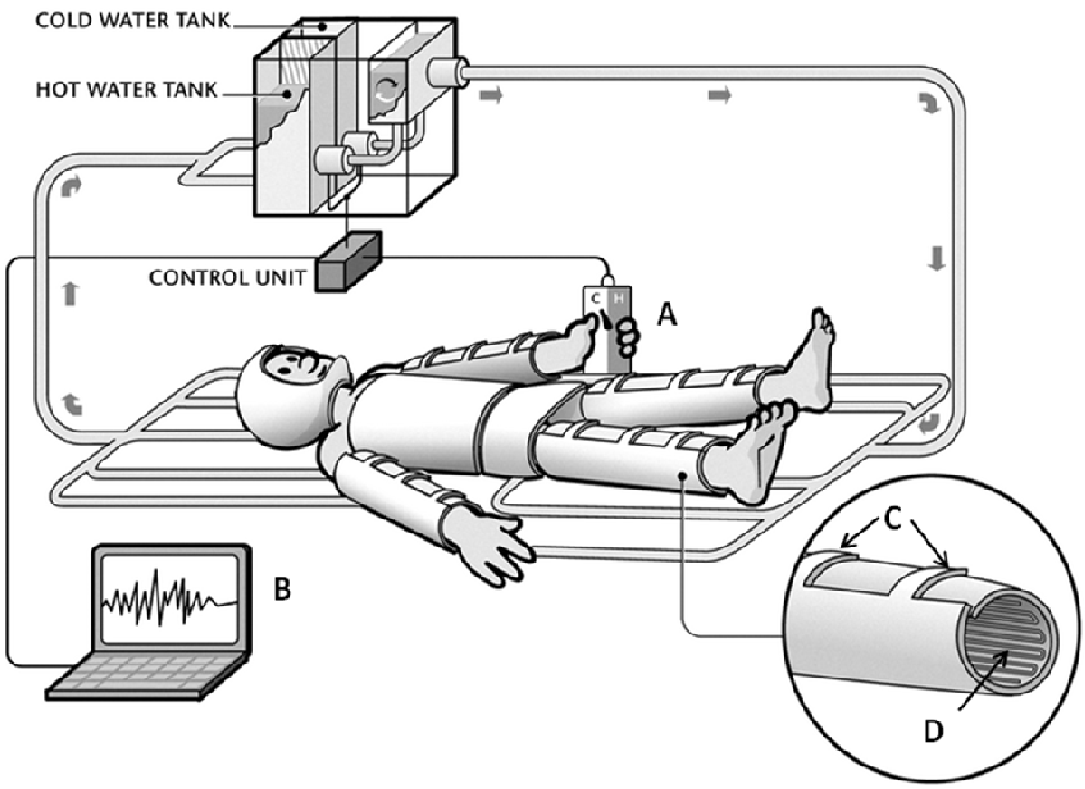


Figure 1: *A schematic representation of the experimental arrangement used to assess behavioural regulation of thermal comfort. By depressing a control switch (A), the subject could invert the direction of temperature change in a water-perfused suit they were wearing when it became uncomfortably warm or cold. Computer software (B) controlled the rate and magnitude of oscillations in the temperature of the water perfusing the suit. Velcro strips (C) embedded in the suit ensured a good fit of the water tubes (D) to the body surface.*

4.2.3 Instrumentation

The temperature of the WPS was measured using T-type thermocouples positioned at the inlet and the outlet of the suit. The average of the water temperature at the inlet and outlet of the WPS was considered to represent the overall WPS temperature (T_{wps}). Ambient temperature (T_a) and relative humidity (RH) were measured with a portable weather station (BAR 938 HG OS, Huger, Germany). Skin temperature (T_{sk}) was measured using thermocouples (Concept Engineering, Old Saybrook, CT, USA) which were attached at eight sites covered by the WPS (calf, thigh, abdomen, back, forehead, chest, forearm, and arm) and four sites that were not covered by the WPS (neck, second finger of the hand, big toe, and foot). The thermocouples were attached to the skin on the right side of the body using a thin, breathable transparent film dressing (3M Tegaderm™). Skin temperature data were collected at 1 second intervals with a data logger (Almemo 5990-2, Ahlborn, Holzkirchen, Germany). Core temperature (T_c) was estimated by measuring the tympanic temperature using an infrared tympanic thermometer (ThermoScan IRT 3020, Braun, Kronberg, Germany).

4.2.4 Data analysis

The reproducibility of identifying the boundaries of the TCZ was evaluated using a 2×3 [(males/females) × (Trial 1/Trial 2/Trial 3)] two-way analysis of variance (ANOVA) with repeated measures on the lower limit (T_{low}), the upper limit (T_{high}) and the width of the TCZ. A paired t-test was used to compare the average T_{wps} at the moment subjects reported feeling uncomfortable (protocol 1) and the moment they initiated a behavioural response (Protocol 2). A Pearson correlation test was used to evaluate correlation between these values. Mean T_{sk} was calculated as an unweighted mean of the T_{sk} from all twelve sites. Three consecutive measurements of T_c were performed before each trial, at the peaks of warming and cooling and immediately after each trial, and the highest value was considered representative of the core temperature. Data were expressed as mean (SD) and the limit of statistical significance was set to 0.05.

4.3 Results

4.3.1 Characteristics of temperature changes

The temperature control system changed the temperature of the water in the WPS at a rate of 1.2°C/min during both the heating and the cooling phases. The T_{wps} pattern and underlying mean T_{sk} of a representative subject while the T_{wps} automatically fluctuated between 27°C and 42°C (Protocol 1) are shown in Figure 2. Arrows indicate the moment that the subject reported feeling the T_{wps} becoming slightly uncomfortable. As evident from Fig. 2 , thermal fluctuations of the WPS resulted in fluctuations of 2–3°C in mean T_{sk}. The T_{wps} pattern and underlying mean T_{sk} produced by the same subject when given control over the T_{wps} and requested to maintain comfort for 60 minutes (Protocol 2) are shown in Figure 3. Arrows indicate the moment when the subject turned the manual control switch. The results demonstrate that rate of T_{wps} change was stable throughout the trial. Furtheremore, the stimulus presented by the WPS was uniform and there were no large differences between the temperature at the outlet and inlet of the WPS during the dynamic changes. Due to the thermal inertia of the system, there was an approximately 1°C over- and undershoot of T_{wps} once a change was initiated in the direction of T_{wps} to cooling and heating, respectively. The exchange between heating and cooling lasted approximately 5–7 minutes. By regulating the T_{wps}, subjects maintained their mean T_{sk} within a narrow range around 33°C. The fluctuations in T_{wps} did not change the T_c during the trials. T_c was maintained in the range 36.7 (0.4) to 36.9 (0.5) at all stages of the trials.

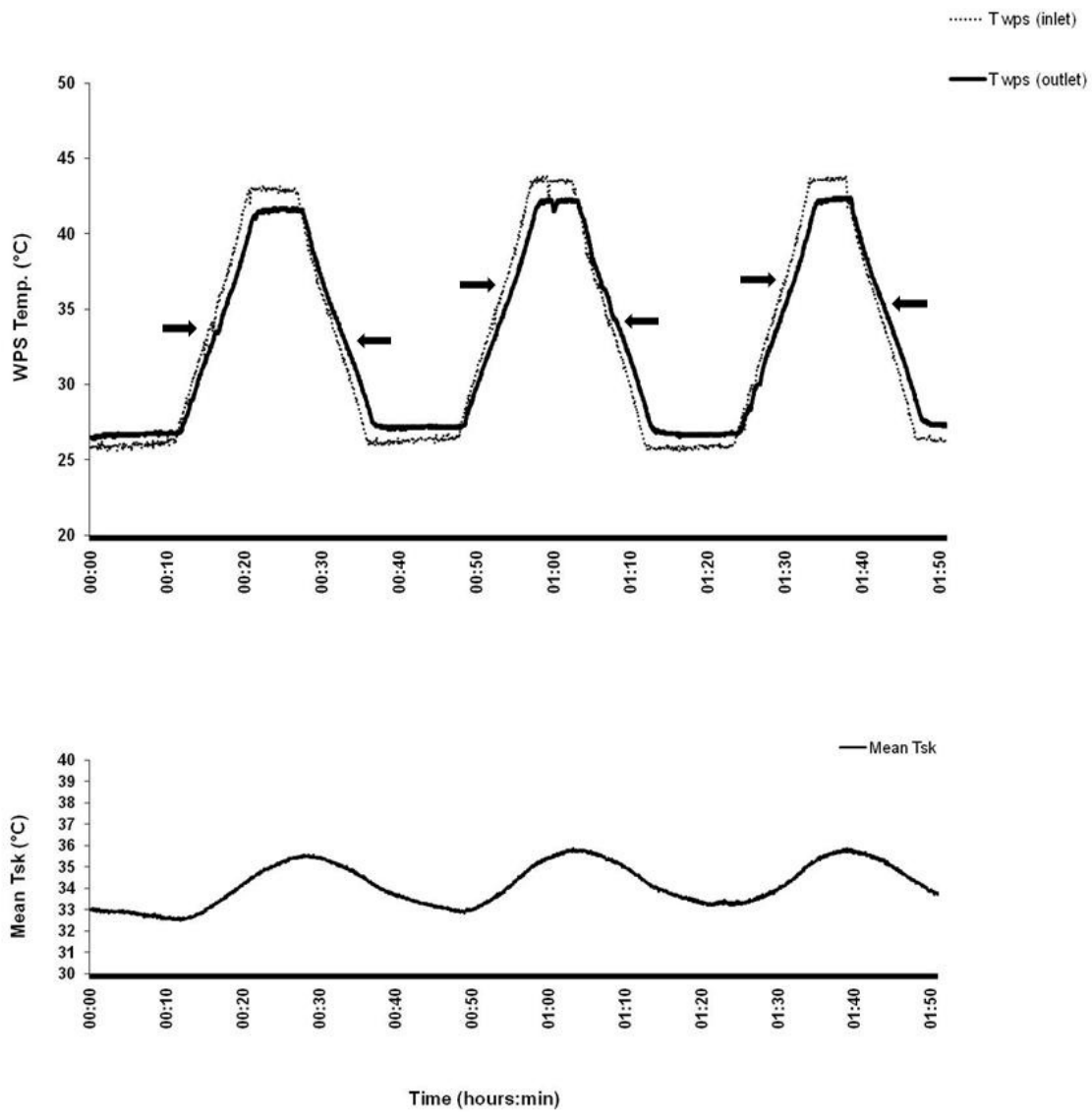


Figure 2: *Representative example of the pattern of temperature changes at the inlet (Tin) and outlet (Tout) of the WPS and the underlying mean skin temperature of one male subject during automatic fluctuations in the WPS temperature (Protocol 1). Arrows indicate each moment subject felt the* T_{wps} *becoming uncomfortable.*

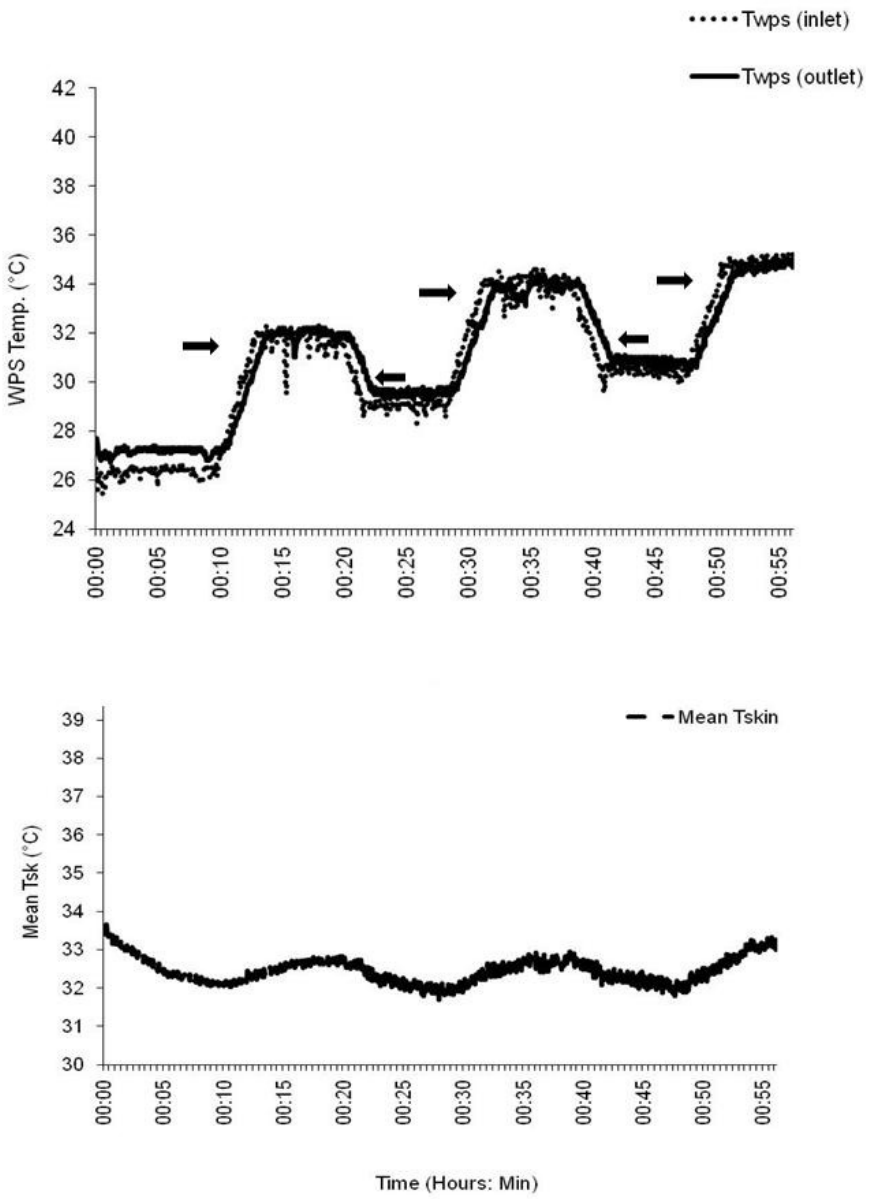


Figure. 3: *Representative example of the pattern of temperature fluctuations in T_{in}, T_{out}, and underlying mean T_{sk} while a subject regulated the temperature at the WPS to maintain comfort (Protocol 2). Arrows indicate each time subjects turned the control switch to avoid further warming or cooling.*

4.3.2 Detection of the thermal comfort zone (Protocol 1)

Subjects identified the boundaries of their TCZ reproductively in three separate heating and cooling processes. There were no significant differences ($P > 0.05$) between T_{low}, T_{high}, and width of TCZ the subjects chose in Trials 1–3 (Table 1). The upper and lower boundaries of the TCZ (mean ± SD) during warming were 30.0±1.5°C – 35.1±2.9°C, and 35.4±1.9°C – 38.7±2.3°C during cooling (Fig. 3). The coefficient of variation, used here as a measure of accuracy, of the T_{wps} eliciting discomfort was 2.32% for cold discomfort (T_{low} during cooling) and 5.5% for warm discomfort (T_{high} during heating).

The results indicate that in the experimental conditions described, subjects could reproducibly identify the boundaries of their TCZ. The T_{wps} at which discomfort commenced was similar during heating and cooling (approximately 35°C), however subjects detected discomfort more accurately during cooling compared to heating.

Table 1. *Characteristics of the TCZ during heating and cooling of the WPS as reported in 3 trials using Protocol 1.*

	T_{low} **- T_{high}** (comfortable → **uncomfortably warm)**			**Width of TCZ (°C)**		
	Males	**Females**	**Both**	**Males**	**females**	**Both**
Heating trial						
1	29.8±1.2 - **35.3±3.0**	30.6±2.0 - **35.7±2.6**	30.1±1.6 - **35.5±2.7**	5.6±2.5	5.5±3.5	5.5±2.7
2	29.4± 1.0 - **34.4±3.0**	30.1±1.3 - **34.7±1.6**	29.7±1.1- **34.6±2.3**	5.1±2.2	4.6±1.1	4.9±1.7
3	29.8± 1.8 - **34.2±2.5**	30.4±2.0 - **36.3±4.4**	30.1±1.8 - **35.2±3.6**	4.4±1.6	5.9±3.5	5.1±2.7
AVG	29.6±1.3 **- 34.6±2.8**	30.4±1.8 **- 35.6±2.9**	30.0±1.5 **- 35.1±2.9**	5.0±2.1	5.3±2.7	5.2±2.4
Cooling trial	**T_{low} -** T_{high} **(uncomfortably cold** ←comfortable)			**Width of TCZ (°C)**		
1	**34.9± 1.6** - 38.1±2.3	**36.4±1.9** - 39.7±1.4	**35.6±1.8** - 38.8±2.0	3.2±0.8	3.3±1.3	3.2±1.0
2	**34.0± 1.9** - 37.3±2.3	**36.1±1.2** - 40.2±1.0	**35.0±1.9** - 38.5±2.3	3.2±1.2	4.1±1.1	3.6±1.2
3	**35.0± 2.1** - 37.8±1.9	**36.1±2.0** - 39.9±2.8	**35.6±2.0** - 38.8±2.5	2.8±0.9	3.8±1.0	3.3±1.0
AVG 3	34.6±1.9 - 37.7±2.1	36.1±1.7 - 39.9±1.7	35.4±1.9 - 38.7±2.3	3.1±0.9	3.7±1.1	3.4±1.1

Data are expressed as Mean ± SD. Values in bold represent the threshold T_{wps} at which discomfort was reported.

4.3.3 Behavioural regulation of thermal comfort (Protocol 2)

The threshold T_{wps} at which subjects initiated a behavioural response to counteract thermal discomfort was determined by calculating the average T_{wps} at which the subjects turned the control switch during warming and cooling; i.e., the high and low peaks of the T_{wps} pattern. The results show that subjects inverted the direction of T_{wps} change 5.5 times (usually 3 high peaks and 2–3 low peaks) during the trial. The average T_{wps} (mean ± SD) at which the subjects interrupted with heating (upper regulated zone) was 34.7 ± 1.0 and the T_{wps} at which they responded to cooling (lower regulated zone) was 34.1± 0.8.

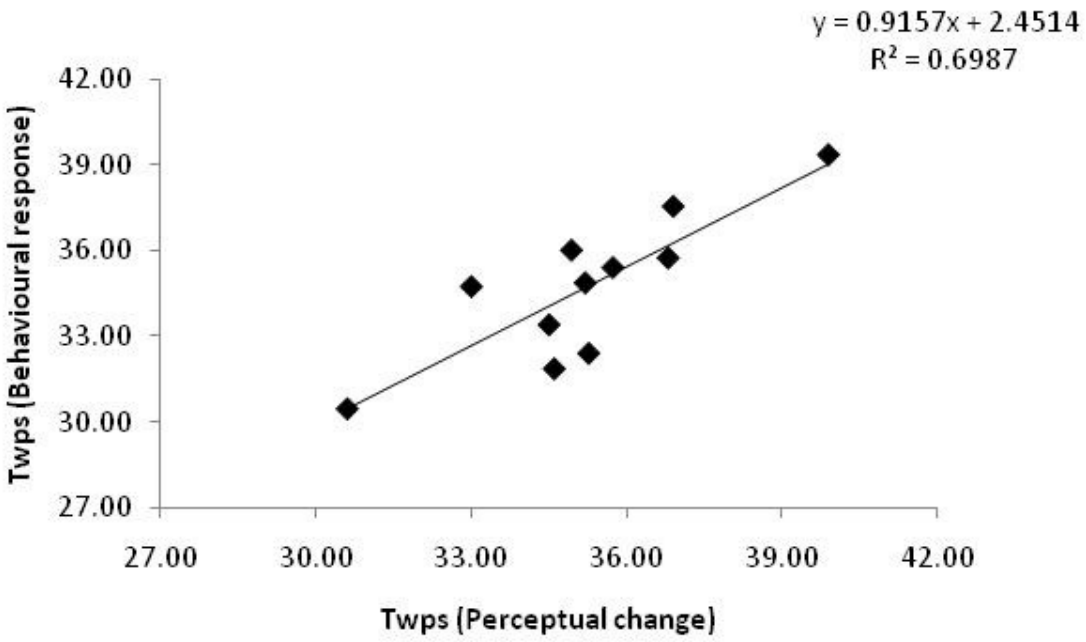
4.3.4 Effects of gender

Although small groups of subjects were tested from both genders (6 males, 6 females), small differences between the groups could be detected. In Protocol 1, there was a tendency for an upward shift in the TCZ among female subjects compared to their male counterparts during cooling. The temperature at which cold discomfort was reported by females was greater (albeit not significantly) during the cooling processes (Table 1). Accordingly, when given control over the temperature of the WPS, female subjects maintained the T_{wps} at a significantly higher temperature ($P < 0.05$) compared to male subjects. The results indicate that the female participants in our study preferred higher WPS temperature for thermal comfort compared to males and were able to regulate the T_{wps} to their preferred level.

4.3.5 Correlation between perceptual change and behavioural response

There was no significant difference ($P > 0.05$) between the threshold T_{wps} at which discomfort was reported and the threshold T_{wps} at which a behavioural response was initiated. Furthermore, there was a strong correlation between the perceptual and behavioural T_{wps} thresholds. The Pearson correlation coefficient was 0.73 during heating (Fig. 4A) and 0.84 for during cooling (Fig. 4B).

A.



B.

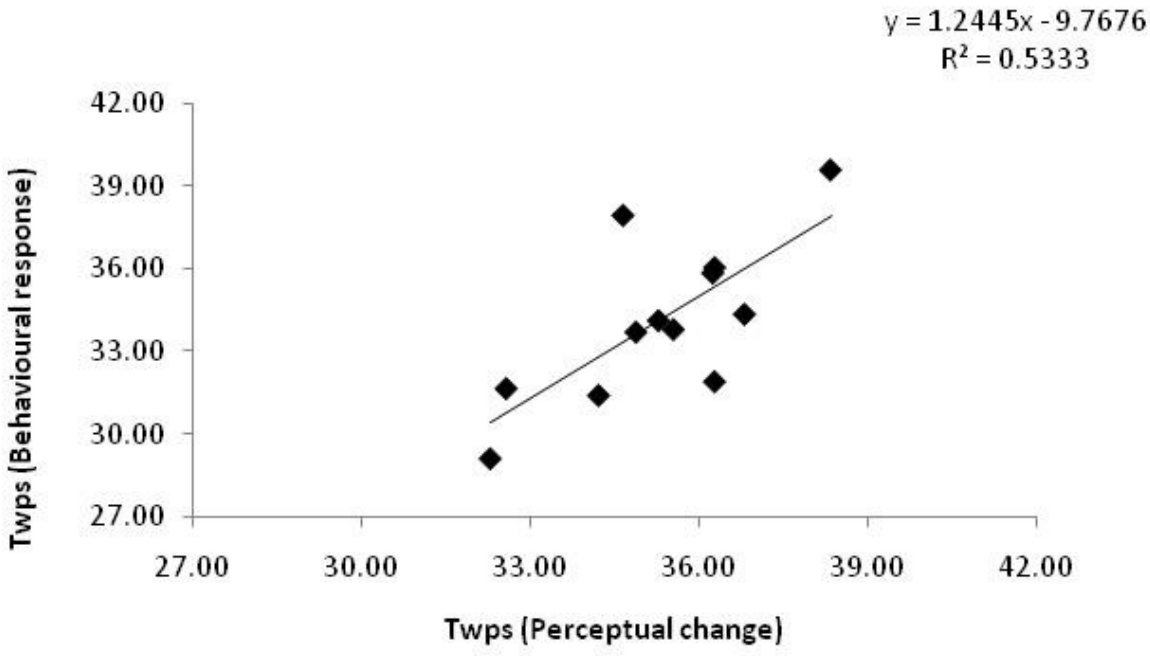


Figure 4. *Correlation between the threshold T_{wps} during warming (A) and cooling (B) at which discomfort was reported, and the threshold T_{wps} at which a behavioural response to counteract discomfort was initiated.*

4.4 Discussion

The main contribution of the current study is the development of a new experimental procedure that enables assessment of the manner by which humans activate behavioural responses to maintain thermal comfort. With this approach, thermal comfort is reflected in the limits at which the subject initiates a behavioural response rather than the limit where s/he expresses a change in perception. Such assessment of comfort is less likely to be influenced by factors such as intellect, ego, language, etc. that may influence the manner by which a subject would vote on a rating scale. Furthermore, since the method requires that subjects respond behaviourally to perceptual changes in their thermal environment (i.e., activate a control switch when the WPS becomes uncomfortably warm or cold), it provides direct evidence on the behavioural responses initiated in response to thermal discomfort.

The influence of other, concomitant, thermoregulatory responses (e.g., shivering, sweating, postural changes) on thermal comfort was minimised. Sweating, for example, by increasing skin wettedness has been associated with increased heat discomfort (Havenith 2009), whereas shivering with cold discomfort (Frank et al 1999). Thus, T_{wps} was set to fluctuate in a range slightly above and below the thermoneutrality (T_{wps} = 27°C–42°C). Furthermore, subjects were tested at rest and after having sufficient time to equilibrate to room conditions. Indeed, the results show that there was no significant alteration in the core temperature during the trial. Thus, it was concluded that in the experimental design, dynamic changes in skin temperature were the main cause for changes in thermal perception and initiation of behavioural responses. The influence of other forms of behavioural thermoregulatory adjustments (e.g., clothing, postural changes) were minimised by (1) applying the stimulus directly to the skin of minimally dressed subjects and (2) by testing the subjects in a supine position, thus minimising their ability to perform posture adjustments.

In order to verify that the T_{wps} pattern obtained by individual subject's regulation of T_{wps} represented their TCZ, it was necessary to characterise the subjective range of T_{wps} perceived as thermally comfortable for each subject in the range 27°C–42°C. Subjects repeated this assessment three times to assure that a reliable value is obtained. The results show that the accuracy in detecting cold discomfort was greater than that of warm discomfort, as indicated by a lower coefficient of variation in the former compared to the latter. This may suggest that cooling of the skin in the protocol used in our study presented a larger discomforting stimulus compared to the warm , presumably due to the higher density of cold receptors compared to warm receptors at the skin surface (Bligh 1998). Nevertheless, during both warming and cooling subjects detected the boundaries of the TCZ with a precision of approximately 1°C. This suggests that the boundaries of the TCZ can be reliably defined using the current protocol.

It is largely accepted that thermal discomfort is the driving force behind behavioural thermoregulation. However, the correlation between these two events has not yet been systematically studied. The results of the present study demonstrate that the threshold T_{wps} at which each subject reported feeling slightly uncomfortable was highly correlated with the T_{wps} at which s/he interrupted the heating or cooling processes to avoid discomfort. Thus, the results suggest that, in normal conditions (e.g., at normothermia, in normal ambient conditions), a change in thermal perception and activation of a behavioural response occur at similar T_{wps} and are highly correlated events. This, however, does not imply that the same is true

for more extreme environmental conditions where the prevailing non-thermal factors attenuate the normal function of the thermoregulatory system (Mekjavic and Eiken 2006). The impact of factors that alter thermal perception on the activation of behavioural responses still needs to be clarified. Gender is a non thermal factor that has been previously associated with improved thermal perception (Cunningham and Cabanac 1971). In contrast, other studies argue that differences in thermal perception between males and females result from differences in body mass and surface area (Glickman-Weiss et.al. 2000). Due to the use of various evaluation methods and lack of appropriate control, it is still unclear whether differences in thermal preference do exist between males and females. In the current study, male and female subjects were recruited to examine whether gender-related differences in thermal perception can be detected using the new method. The purpose was not to investigate the influence of gender *per se* on thermal comfort, but rather to determine whether differences in thermal perception caused by a NTF such as gender, if existing, could be identified using the new experimental procedure.

In the present study, the boundaries of the TCZ did not significantly differ between males and females. However, when subjects were given control over their surrounding temperature, females interrupted the heating and cooling processes at a significantly higher T_{wps} compared to males ($P < 0.01$). Although the phase of the menstrual cycle or body characteristics were not controlled, the results of the current study support the hypothesis that differences between genders in thermal perception may exist and that females require higher temperatures for thermal comfort. From a methodological point of view, the fact that gender-related differences in thermal preference were detected suggests that, in future studies, the influence of other NTFs that influence thermal perception (e.g., narcosis, alcohol) should also be evaluated.

4.5 References

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5 Behavioural temperature regulation in humans during mild narcosis induced by inhalation of 30% nitrous oxide

5.1 Introduction

In study 1, we developed a new method for assessing behavioural temperature regulation in humans. We concluded that the method provides some advantage over the currently used practice of obtaining subjective scale ratings of thermal comfort. Furthermore, we suggested that it could be used to determine the effect of nonthermal factors on behavioural temperature regulation. Thus, in this study we evaluate the effect of inert gas narcosis, a nonthermal factor known to influence thermoregulation in humans during exposure to hyperbaric environments, on behavioural temperature regulation using the methodology developed in study 1.

The special characteristics of the marine environment, specifically the high heat conductance of water and alteration in hydrostatic pressure with changing depth, impose considerable physiological burdens on the human body. Divers are at a constant risk of hypothermia. Even mild hypothermia can predispose divers to accidents by impairing their psychomotor abilities (Baddeley et al., 1975) and by increasing decompression risks (Dunford and Hayward, 1981).

Despite increased awareness of the hazards of hypothermia and use of modern protective clothing, numerous incidents of undetected, 'insidious', hypothermia have been reported during diving (Hayward and Keatinge, 1979), and 'feeling cold' is still one of the most prominent complaints in dives that result in accidents (Vann et al., 2003).

Maintaining thermal balance during diving depends on the appropriate functioning of thermoregulatory responses. Previous studies demonstrated that various nonthermal factors that exist in the diving environment decrease the efficiency of thermoregulatory responses. Nonthermal factors associated with diving include factors such as hypercapnia, hypoxia, hydrostatic pressure, and nitrogen narcosis (Mekjavic and Eiken, 2006). Nitrogen narcosis refers to the euphoric sensation, retardment of the higher mental processes, and impaired neuromuscular coordination that result from breathing gas mixtures (most commonly air) under elevated pressure (Bennett and Rostain, 2003). The signs and symptoms of nitrogen narcosis are first noticed at about 30 meters of sea water (4 ATA) and become increasingly severe with increasing depth (Bennett and Rostain, 2003).

Nitrogen narcosis, associated with compressed air diving, causes alterations in numerous important functions including delayed responses to visual (Hamilton et al., 1993), auditory (Fowler et al., 1980), olfactory and tactile stimuli (Hamilton et al., 1993), impaired memory (Fowler et al., 1980), and reduced ability to perform arithmetic calculations and fine movements (Fowler et al., 1985). In general, it seems that intellectual functions are more severely affected by narcosis than functions such as manual dexterity

(Fowler et al., 1985; Bennett and Rostain, 2003).

The combination of thermal stress and nitrogen narcosis may therefore lead to a quick deterioration in the physiological and psychological status of a diver at depth, and consequently to drowning and other injuries. Mekjavic and co-workers (Mekjavic and Sundberg, 1992; Mekjavic et al., 1995) demonstrated that nitrogen narcosis and its behavioural analogue nitrous oxide (N_2O) attenuate autonomic thermoregulatory responses. The onset of shivering is shifted to lower levels and its magnitude significantly attenuated (Mekjavic and Sundberg, 1992; Cheung and Mekjavic, 1995; Mekjavic et al., 1995). The sweating response, however, is unaffected (Mekjavic and Sundberg, 1992). These alterations might occur even at mild narcotic levels, i.e., small elevations in PN_2 at shallow depth (Mekjavic et al., 1995). Cheung and Mekjavic (1995) evaluated the effects of increasing levels of inert gas narcosis on shivering thermogenesis in hypothermic subjects breathing mixtures containing 10, 15, 20, and 25% N_2O. They reported that N_2O does not influence shivering thermogenesis in a dose-dependent manner, with thermoregulation in hypothermic conditions being significantly impaired even at low levels of inert gas narcosis.

Under normal conditions, small shifts in core temperature are initially counteracted by changes in peripheral vasomotor tone and by behavioural responses; if core temperature is shifted further, then activation of the metabolically more demanding autonomic responses, shivering or sweating, ensue. The influence of narcosis on behavioural thermoregulatory responses received less attention in previous studies. Cheung and Mekjavic (1995) reported that narcosis induced changes not only in autonomic thermoregulatory responses, but also altered thermal perception. Unlike its effect on the shivering response, N_2O affected thermal comfort in a dose-dependent manner with each increase in N_2O concentration resulting in a warmer median vote on a thermal sensation scale. Later, it was confirmed that during normobaric N_2O narcosis (Mekjavic et al., 1994) or hyperbaric N_2 narcosis (Mekjavic et al., 1995), equal decrements in core temperature at clamped skin temperature evoke less thermal discomfort than without narcosis. Thus, during progression towards hypothermia, narcosis increases thermal comfort and alters thermal sensation so that the environment is perceived as warmer and more comfortable. Mekjavic et al. (1994) suggested that alterations in temperature perception might jeopardize thermal balance by preventing the initiation of appropriate behavioural responses. Such changes in behavioural thermoregulation were observed in animal studies, where mice given a choice between a cold or warm environment during mild narcosis chose the cold environment, which resulted in substantial core cooling (Pertwee et al., 1986). There is, however, no direct evidence for a narcosis-induced attenuation of behavioural thermoregulation in humans. Furthermore, it is still unclear whether narcosis alters thermal perception and comfort before significant changes in core temperature occur (i.e., when the signal to initiate a behavioural response first appears).

In this study, we tested the hypothesis that a subanaesthetic level of N_2O alters thermal perception and consequently attenuates behavioural thermoregulation in humans. The experimental approach used in the current study was designed to provide evidence not only of narcosis-induced changes in temperature perception and thermal comfort, but also on the behavioural responses associated with maintaining thermal comfort.

5.2 Methods

The experimental protocol was approved by the National Medical Ethics Committee of the Slovene Ministry of Health, and conformed to the Declaration of Helsinki. Subjects gave their informed consent to participate in the study. A total of 12 healthy subjects (6 males, 6 females) participated in these trials conducted at the Jozef Stefan Institute. The physical characteristics of the subjects are presented in Table 1.

5.2.1 Experimental set-up

As described previously (Chapter 4), the experimental set-up comprised a water-perfused suit (WPS) with a manual control unit designed to allow the subject (or the experimenter) control over the temperature of water perfusing the WPS. By depressing the control switch, the subjects could change the direction of the temperature change of the water perfusing the WPS. The temperature control unit had no steady-state position, thus, the temperature of the WPS alternated sinusoidally between a cooling and a heating mode according to the subjects' control. During the entire duration of the trial, subjects were unaware of the actual temperature of the water perfusing the suit. The WPS was made of five components covering the entire surface of the body, with the exception of the hands, feet, neck and face. All five components were fed from a common manifold and consisted of identical length of small-diameter (inner =4 mm, outer=5 mm) PVC tubing which were woven in intervals of 1cm in the meshed lining of the suit. Velcro® stripes embedded in the suit ensured a good fit to the surface of the skin.

5.2.2 Protocol

Subjects participated in two trials. In the first, we examined the influence of mild narcosis on temperature perception and thermal comfort, and in the second we evaluated whether narcosis alters thermoregulatory behaviour.

To minimize the effects of circadian rhythm, subjects were tested at the same time of the day. Subjects were asked to refrain from the following for at least 2 hours prior to testing: strenuous exercise, large meals, cigarette smoking, caffeine or alcohol containing beverages. On each occasion, after 30 minutes acclimation to room conditions (Ta=25°C), subjects were instrumented with skin sensors, donned the WPS, and assumed a supine position on a gurney. Male subjects were dressed in shorts, and females in shorts and a bikini top.

In each trial the temperature of the water perfusing the WPS (T_{wps}) was first set at a temperature of 27°C for 10 minutes. Thereafter, it fluctuated sinusoidally between 27° and 42°C at a rate of 1.2°C/min. Both trials comprised two sessions, in which subjects inspired either room air (AIR), or a normoxic breathing mixture (PN2O=0.3ATA, PO2=0.2ATA, PN2=0.5ATA) containing 30 % nitrous oxide (N2O). The level of narcosis induced by inhalation of 30% N2O is often used as a behavioural analogue equivalent to the narcotic effect of breathing air at 40-70 meters of sea water (Biersner, 1972; Fowler et al., 1985; Mekjavic et al., 1994). The N_2O mixture was decompressed from high-pressure cylinders and directed to a

humidification circuit, where it was passed through water at room temperature and pressure, and finally collected in a meteorological balloon. Subjects inspired N_2O or AIR via an oro-nasal mask (Hans Rudolph, Kansas City, MO, USA).

Effects of narcosis on thermal perception and comfort

In the first trial, changes in thermal perception and comfort were assessed by having subjects provide a thermal sensation vote (TSV) on a 7-point scale (-3 – very cold, -2 – cold, -1 – slightly cold, 0 – neutral, +1 slightly warm, +2 warm, +3 very warm) and thermal (dis)comfort vote (TCV) on a 4-point scale (0 – comfortable, 1 – slightly uncomfortable, 2 – uncomfortable, 3 – very uncomfortable) scale, at each 3°C change in T_{wps}. Furthermore, subjects were asked to report when they perceived the temperature changing from a comfortable to an uncomfortable level and vice versa, thus indicating the boundaries, Tlow and Thigh, and the range of their thermal comfort zone (TCZ).

Effects of narcosis on behavioural thermoregulation

In the second trial, the 10-min period at baseline temperature (T_{wps}=27°C), was followed by a 60-min comfort regulation trial in which subjects' behavioural thermoregulatory response to maintain thermal comfort was assessed. The temperature of the water perfusing the WPS varied in a sinusoidal manner from 27°C to 42°C at a rate 1.2°C/min, as in the first trial. However, rather than passively reporting their perception of the changing temperature at the WPS, in this trial, subjects could control the heating and cooling processes. Subjects were instructed to change the direction of the temperature of the water perfusing the WPS once it became either uncomfortably warm during heating, or uncomfortably cool during cooling, by depressing a button on a remote control switch. The control unit did not enable a steady state position, thus it could only alternate between heating and cooling. Subjects were instructed to maintain T_{wps} within a preferred range for a total duration of one hour. subjects were not coached or given any further instructions during the trial. After the trial concluded, the resultant characteristics of the sinusoidal T_{wps} pattern with its low peaks (T_{low}), high peaks (T_{high}) and range were analysed.

In the first trial, subjects conducted the two sessions (AIR and N_2O) at the same day (separated by minimum 15 minutes), whereas in the second trial, the sessions were conducted on different days, separated by a minimum of 48 hours. The order of these trials was randomized between the subjects to eliminate any order effects.

5.2.3 Instrumentation

The temperature of the WPS was measured using T-type thermocouples positioned at the inlet and the outlet of the suit. The average between the water temperature at the inlet and outlet of the WPS was considered to represent the overall WPS temperature (T_{wps}). Ambient temperature (T_a) and relative humidity (RH) were measured with a portable weather station (BAR 938 HG OS, Huger, Germany). Skin temperature (Tsk) was measured using thermocouples (Concept Engineering, Old Saybrook, CT, USA) which were attached at eight sites covered by the WPS (calf, thigh, abdomen, back, forehead, chest,

forearm, and arm) and four sites that were not covered by the WPS (neck, second finger of the hand, big toe, and foot). The thermocouples were attached to the skin on the right side of the body using a thin, breathable transparent film dressing (3M™ Tegaderm™). Skin temperature data were collected at 1 second intervals with a data logger (Almemo 5990-2, Ahlborn, Holzkirchen, Germany). Core temperature (T_c) was estimated by measuring the tympanic temperature using an infrared tympanic thermometer (ThermoScan IRT 3020, Braun, Kronberg, Germany).

5.2.4 Data analysis

A paired t-test was used to compare the environmental conditions (T_a and RH) and the WPS parameters (average T_{wps}, T_{low}, T_{high} and range) between AIR and N_2O conditions. The patterns of T_{wps} were analysed from the moment subjects started controlling the T_{wps} (after the first 10 minutes of the trial). Ratings of temperature perception and thermal comfort were collected at each 3°C change during dynamic changes in T_{wps}. Due to the thermal inertia of the system, there was an approximately 1°C over- and undershoot of T_{wps} once the subject initiated a change in the direction of T_{wps} to cooling and heating, respectively. Median values of votes were analysed using the Wilcoxon non-parametric test.

Mean skin temperature (T_{sk}) was calculated as an unweighted mean of the T_{sk} from all twelve sites. Three consecutive measurements of T_c were performed before and after the trial, and the highest value was considered representative of the core temperature. T_c was also measured during the trial immediately after the subject depressed the button on the remote control switch. Data were expressed as mean (SD) and the limit of statistical significance was set to 0.05.

5.3 Results

The ambient conditions in the experimental room were similar in all trials; the mean (SD) Ta was 25.5(0.8)°C and RH was 23.9 (8.4)%. All subjects reported feeling thermally comfortable before they donned the WPS, and slightly uncomfortably cold after 10 minutes at the initial baseline temperature (T_{wps}=27°C). Inhalation of the N_2O mixture resulted in an intoxicated-like feeling and subjects reported feeling generally more relaxed.

5.3.1 Effects of mild narcosis on thermal perception

In the first trial, the influence of narcosis on thermal perception was determined with the subjects having no control over the T_{wps}. Subjects could only report on how they perceived the T_{wps} while it fluctuated sinusoidally between 27° to 42°C. They were requested to subjectively define their thermal comfort zone and provide subjective ratings on a thermal sensation and thermal perception scales.

i. Changes in the thermal comfort zone

With the exception of one (female) subject, all subjects could determine the boundaries of their TCZ within the examined range. The results from this subject were not included in the analysis of this section (n=11, 5 females, 6 males).

Narcosis significantly (P<0.01) increased the TCZ during warming from 4.5(1.3)°C to 6.7 (2.4)°C. The boundaries of the TCZ were between 30.2 (1.3)°C and 34.6 (2.1)°C during AIR and between 29.1(1.2)°C and 35.9 (2.8)°C during N_2O. Figure 1 illustrates the characteristics of the TCZ reported by the subjects during AIR and N_2O trials. There were no significant differences in the TCZ reported by males and females, and narcosis similarly broadened this range in both genders (Fig. 2).

ii. Changes in scale rating of thermal comfort and thermal perception

Analysis of the subjective ratings of temperature perception and thermal comfort did not reveal significant effect of narcosis on the median thermal sensation votes subjects provided at each 3°C change in T_{wps}. Subjects perceived the increasing T_{wps} as equally warm and the decreasing T_{wps} as equally cold during AIR or N_2O (Fig. 3A). The median votes on the thermal (dis)comfort scale, on the other hand, were significantly lower (P<0.05) indicating that subjects perceived equal changes in T_{wps} as significantly less uncomfortable (Fig. 3B).

5.3.2 Effects of mild narcosis on behavioural thermoregulation

All the subjects reported that they could successfully maintain T_{wps} at their preferred level for the duration of the experiment. Unlike its effect on the perception of comfort, narcosis did not significantly influence

the manner in which subjects maintained their thermal comfort. There were no significant differences between AIR and N_2O in any of the characteristics of the T_{wps} pattern including average T_{wps}, T_{high}, T_{low} and range. The preferred T_{wps} (average T_{wps}) was 34.5 (2.6)°C in the AIR, and 34.2 (2.3)°C in the N_2O trial. Interestingly, male and female subjects produced similar T_{wps} patterns while breathing AIR or N_2O however, females maintained the T_{wps} at about 3°C higher than their male counterparts to achieve thermal comfort. The characteristics of the sinusoidal T_{wps} pattern produced by males and females during AIR and N_2O trials are summarized in Table 2.

5.3.3 Dependent variables

Core temperature

There were no significant changes in Tc during the trials. For all subjects Tc was maintained in the range 36.7 (0.4) –36.9 (0.5)°C in all AIR and N_2O trials.

Core temperature was assessed with an infrared tympanic thermometer for several reasons. First, behavioural thermoregulatory responses are influenced by thermal discomfort, and the discomfort associated with the continuous measurement of rectal/oesophageal temperature might have interfered with the ability of the subjects to report the discomfort associated with the thermal environment. Second, Mekjavic et al. (1992) reported that changes in tympanic temperature are well correlated with changes in oesophageal temperature during core cooling and warming of subjects. The correlation was not as good with rectal temperature. Furthermore, Daanen (2006) reported that tympanic temperature underestimated oesophageal temperature by about 0.4°C. Thus, for the purpose of the present study, we assumed that the tympanic temperature measured with an infrared tympanic thermometer would reflect any significant change in core temperature. The variation of tympanic temperature experienced by subjects during the trials was in the range ±0.3°C. Thus, we considered that subjects experienced no significant changes in their core temperature, and that their reports of discomfort as well as their behavioural responses resulted primarily, if not solely, as a consequence of the thermal stimulus at the skin.

Skin temperature

There were no significant differences in mean Tsk between the AIR and N_2O trials. Subjects reported feeling thermally comfortable at a mean (SD) Tsk of 33.9 (1.2)°C in the AIR, and at 34.1 (1.0)°C in the N_2O trials. As demonstrated in Figures 4 and 5, when given control over the T_{wps}, female subjects maintained mean T_{wps} (Fig. 4) and Tsk (Fig. 5) at significantly higher ($P<0.05$) levels compared to male subjects during both AIR and N_2O trials.

5.4 Discussion

The main finding of the current study is that breathing a narcotic mixture containing 30% N_2O does not significantly change thermoregulatory behaviour despite its significant influence on the perception of thermal comfort.

It has been previously demonstrated that narcosis attenuates thermal perception and thermal comfort during decrements in core temperature (Mekjavic et al., 1994; Cheung and Mekjavic, 1995). However, dynamic changes in core temperature at the level described in these studies, resulted in significant shivering that, by itself, increases thermal discomfort (Gagge et al., 1967). Thus, it could be argued that the increase in thermal perception reported by Cheung and Mekjavic (1995) and Mekjavic et al. (1994) was influenced by the narcosis-induced attenuation of the shivering response (Cheung and Mekjavic, 1995). In the current study, narcosis significantly increased thermal comfort while subjects were thermally stable (e.g., no change in T_c) and only their skin temperature changed. Thus, mild narcosis increases thermal comfort not only during dynamic changes in core temperature, but also in normothermic subjects during changes in skin temperature.

Ratings of thermal sensation showed a smaller effect of N_2O; there were no significant changes in the median TSV between N_2O and AIR conditions during cooling or warming. This suggests that thermal sensation (the perception of temperature as cold or warm) is not equally affected by narcosis as thermal comfort (the affective judgment). Thus, changes in behavioural thermoregulatory responses, if observed, are not likely to be due to an inability to detect thermal changes, but rather due to a decrease in the motivation to do so; i.e reduced thermal discomfort. This assumption is supported by the findings of De Jong and Nace (1967) who found no changes in nerve impulse conduction and cutaneous receptor responses during general anaesthesia.

The narcosis-induced changes in the perception of thermal comfort that were observed in the current study seem to be too small to significantly attenuate the ability to maintain thermal comfort behaviourally. When given control over the temperature of the WPS, subjects interfered with the heating and cooling processes at similar T_{wps} while breathing AIR or N_2O. There were also no significant differences between the AIR and N_2O trials either in the preferred temperature, or in the range of the T_{wps} pattern. Two possible mechanisms might explain why narcosis affects perception of thermal comfort, but not thermoregulatory behaviour: 1) dose-dependent global neural inhibition, and 2) a regionally specific suppression of neuronal activity. Davis et al. (1957) suggested that the effect of inhaled anaesthetics depends on the complexity of the neural network (i.e. number of synaptic connections) involved in the pathway. A larger narcotic affect on thermal perception compared to the affect on behavioural responses would suggest that the underlying neural mechanism of the former is more complex than the mechanism underlying the latter. Satinoff (1983) suggested that the central processing involved in thermoregulation occurs at several interconnected levels of the central nervous system (CNS), rather than at a single location. Such a hierarchical arrangement of the thermoregulatory brain foci, provides the means of fine-tuning of body temperature regulation. Thus, the higher processing foci involved in thermal perception modulate the more coarse behavioural foci in the

CNS. This concept, that behaviour and perception are controlled by different regions in the brain was recently supported by the study of Heinke and Koelsch (2005), who demonstrated that the brain as a whole is not affected to the same degree by anaesthetics, but that specific brain regions (and particular cognitive processes mediated by these regions) are more sensitive to anaesthesia and sedation than others. Heinke and Koelsch (2005) explain that inhibition of activity in multimodal association cortices (such as parietal and prefrontal association cortices) by sedative concentrations of anaesthetics produces amnesia and attention deficits, whereas activity in unimodal cortices and in the thalamus remains largely unaffected by low doses of anaesthetics. Activity in the midbrain reticular formation, thalamus, and unimodal cortices appears to be suppressed only by anaesthetic concentrations causing unconsciousness.

The smaller influence of narcosis during the cooling periods in our study is in contrast to the results of Mekjavic et al. (1994) and Cheung and Mekjavic (1995) indicating that narcosis increased the perception of comfort while subjects progressed to hypothermia. This difference cannot be explained on the basis of the dose of narcosis used since the breathing mixture that was used in this study was similar to the dose used by Mekjavic et al. (1994) and more potent than the doses used by Cheung and Mekjavic (1995). A possible explanation for this discrepancy may be in the different protocols that were used to cool the subjects. The subjects in the studies of Mekjavic et al. (1994) and Cheung and Mekjavic (1995) were cooled by immersing them in cold water (15°C and 28°C, respectively), which resulted in a gradual fall in their core temperature, while skin temperature was clamped at a level slightly higher than that of the water temperature. In contrast, Tc in the current study remained unaltered while Tsk fluctuated in the range of 32°C–36°C. Keatinge et al. (1986) demonstrated that constancy of skin temperature produced by immersion in cool water is associated with reduced discomfort. Thus, it seems that clamping the temperature of the skin during core cooling in the studies of Mekjavic et al. (1994) and Cheung and Mekjavic (1995) had a positive effect on thermal comfort that was further exaggerated by narcosis. The constantly changing skin temperature in the current study provided a greater discomforting stimulus and was therefore less affected by narcosis.

The different effect of narcosis on thermal comfort during the warming and cooling periods in the current study might be explained by the relative sensitivity of cold and warm receptors; the bell shaped characteristic of thermoreceptor activation and the role they play in maintaining thermal stability. The temperatures used in our study fluctuated approximately between the peaks of the cold and warm sensor activity; 25°C and 42°C, respectively (Hensel and Iggo, 1971). The dynamic as well as static response of the peripheral warm receptors is larger than that of cold receptors (Kenshalo, 1976). However, the spatial distribution of thermoreceptors is unequal throughout the body. Cold receptors are located predominantly in the skin. In contrast, warm receptors are abundant in deep body tissues, but less frequent in the periphery (Bligh, 1998). Furthermore, the conduction velocity is greater in cold afferent fibres than in peripheral warm receptors (Hensel, 1976). This functional-morphological arrangement suggests that the body is "designed" to protect itself particularly from external cooling and internal warming. Thus, it makes physiological sense that for a normothermic person, cooling the skin within a certain range provides a stronger discomforting stimulus, than does a warming stimulus.

In the current study, gender-related differences in thermal perception and behaviour were analysed by comparing the results obtained from male (n=6) and female (n=5) subjects. Although no significant

differences were found in the TCZ reported by males and females, when given control over T_{wps}, females maintained T_{wps} about 3°C higher than their male counterparts to achieve thermal comfort. This tendency was not influenced by narcosis (Figs. 4 and 5).

Horstman and Christensen (1982) demonstrated that while exercising in hot environments, sweat rate is greater in males compared to females. In the cold, Cunnigham et al. (1978) reported that the threshold for shivering is higher for females compared to males. However, when differences in surface area and body mass between the genders are taken into consideration, gender-related differences are usually negligible (Glickman-Weiss et al., 2000). Indeed, also in the present study the Dubois surface area (1927) of male subjects was greater ($P<0.001$) than that of females (surface area 1.99 and 1.70, respectively). Furthermore, there is evidence that changes in behavioural thermoregulation in females might be modulated by the menstrual cycle. In the luteal phase, women are more sensitive to skin temperature changes than during the follicular phase (Kenshalo, 1976). Cunningham and Cabanac (1971) demonstrated that females have a higher skin temperature preference in the luteal phase. Since the majority of females in the current study were using contraceptive pills, we could not determine the phase of their menstrual cycle. Nevertheless, the results of the present study are consistent with a gender-related increase in thermal preference.

Interestingly, the preference for higher temperatures among the female subjects in our study was related to the ability to control the temperature. Although no gender-related differences were found in the trial, during which subjects reported their perceptions (detecting the TCZ and subjective ratings) during the sinusoidal thermal stimulus to the skin. In contrast, when females could control T_{wps}, they preferred a significantly higher temperature compared to males. In fact, this discrepancy resulted in the exclusion of one subject from the analysis. This subject (S8, female) did not feel the T_{wps} becoming uncomfortable during the entire heating process (warming stopped at Twps=45°C). However, when the same subject could control T_{wps} to her preferred range, she maintained it within the range 35.4–40.2°C. Whether differences associated with gender exist in the preferred temperature or the ability to behaviourally maintain thermal comfort remains to be elucidated.

Assuming that the effect of N_2O is similar to that of hyperbaric N_2, then the practical implication of the results of the present study are that any inert gas narcosis-induced increase in thermal comfort experienced by a normothermic diver will unlikely affect their ability to respond behaviourally to changes in their thermal environment.

5.5 Figures & Tables

Table 1. *Subjects' physical characteristics.*

Name code	Gender	Age (years)	Height (cm)	Weight (kg)
S1	Male	25	181.0	74.3
S2	Male	35	183.0	101.0
S3	Male	27	172.0	73.6
S4	Male	27	190.9	74.9
S5	Male	20	178.4	68.1
S6	Male	21	191.6	80.0
S7	Female	25	162.6	47.4
S8	Female	25	159.5	52.9
S9	Female	25	179.3	67.6
S10	Female	28	169.9	66.6
S11	Female	26	175.0	61.0
S12	Female	24	177.5	61.6
Mean (SD)		25.2 (3.9)	176.4 (9.8)	68.0 (14.6)

Table 2. *Characteristics of the sinusoidal T_{wps} pattern in the AIR and N_2O trials.*

	AIR			N2O		
	Males	Females	Both	Males	Females	Both
Preferred T_{wps}	33.4 (2.1)	36.0 (2.1)*	34.5 (2.6)	33.0(1.6)	36.0 (1.6)*	34.2 (2.3)
T_{high}	33.5 (2.2)	36.3 (2.3)	34.7 (2.6)	33.9 (1.8)	37.0 (2.0)	35.3 (2.4)
T_{low}	32.3 (2.3)	36.2 (2.5)	34.1 (3.1)	32.4 (2.4)	36.0 (2.0)	34.0 (2.8)
Range	5.1 (1.3)	4.8 (1.3)	5.0 (1.0)	5.7 (3.2)	6.9 (3.2)	6.2 (3.2)

* $P<0.05$ (Males vs. Females). N=11 (6 males, 5 females). Data is expressed as mean (SD).

The preferred temperature of the water perfusing the suit (T_{wps}) was derived by averaging T_{wps} from the moment subjects started controlling the T_{wps} (after the first 10 minutes of the trial). Similarly, T_{high} and T_{low} were obtained from the peaks and nadirs of the sinusoidal temperature pattern in this period, the difference between the two being the range of the thermal comfort zone (TCZ).

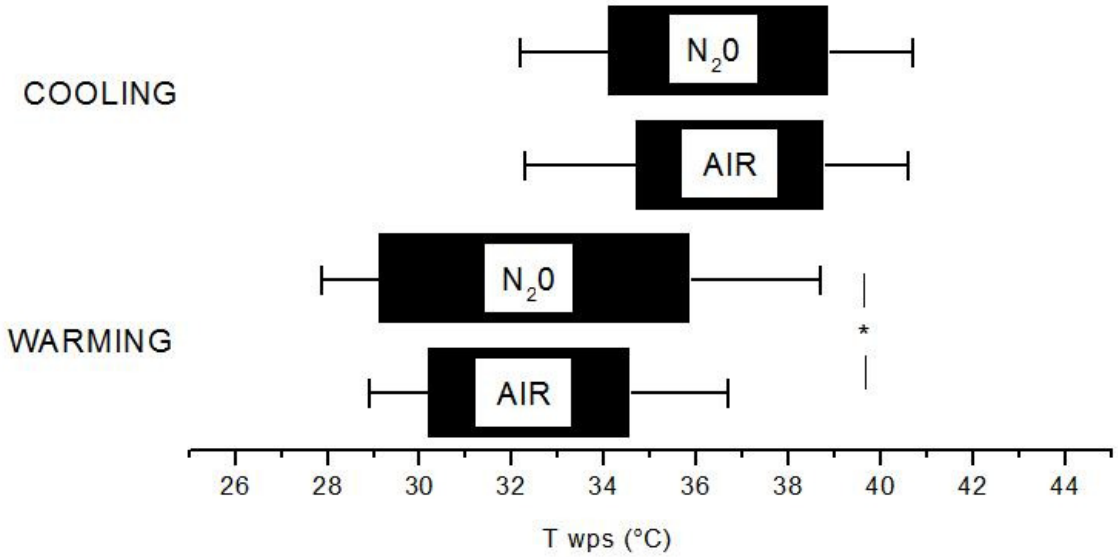


Figure 1: *Mild narcosis significantly broadened the width of the TCZ during warming, but it had no significant effect on the TCZ during cooling.*

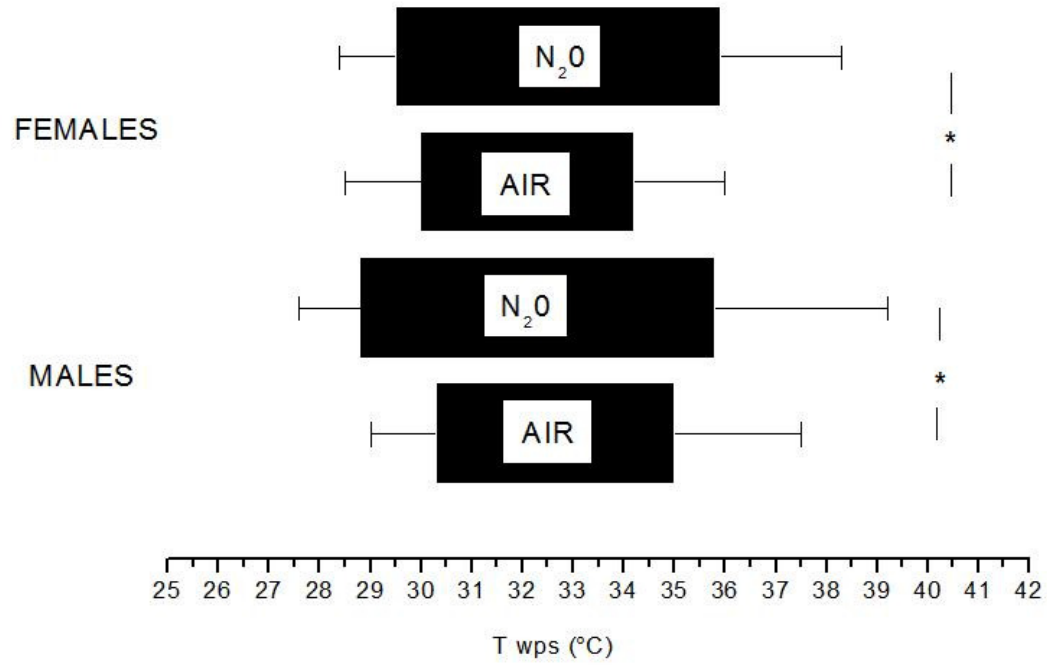
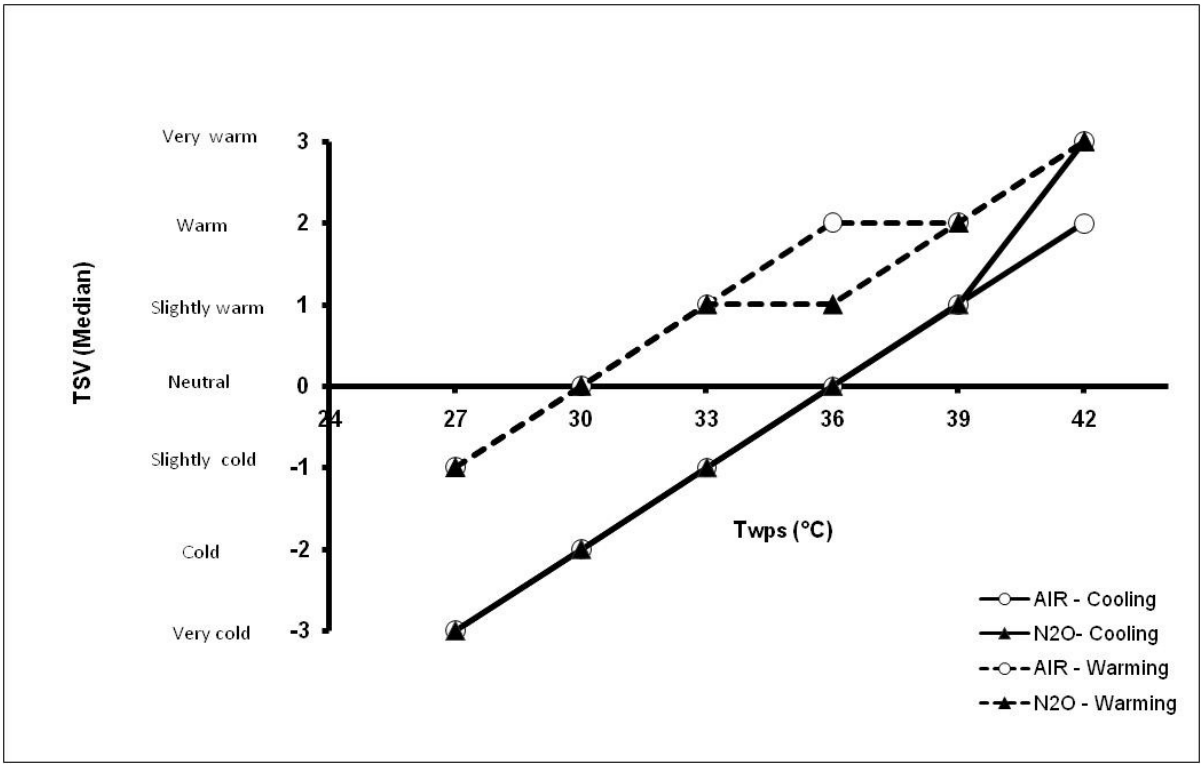


Figure 2: *Influence of gender on the thermal comfort zone during warming of the water-perfused suit (WPS). Narcosis similarly increased the range of T_{wps} perceived as thermally comfortable in males and females.*

A.



B.

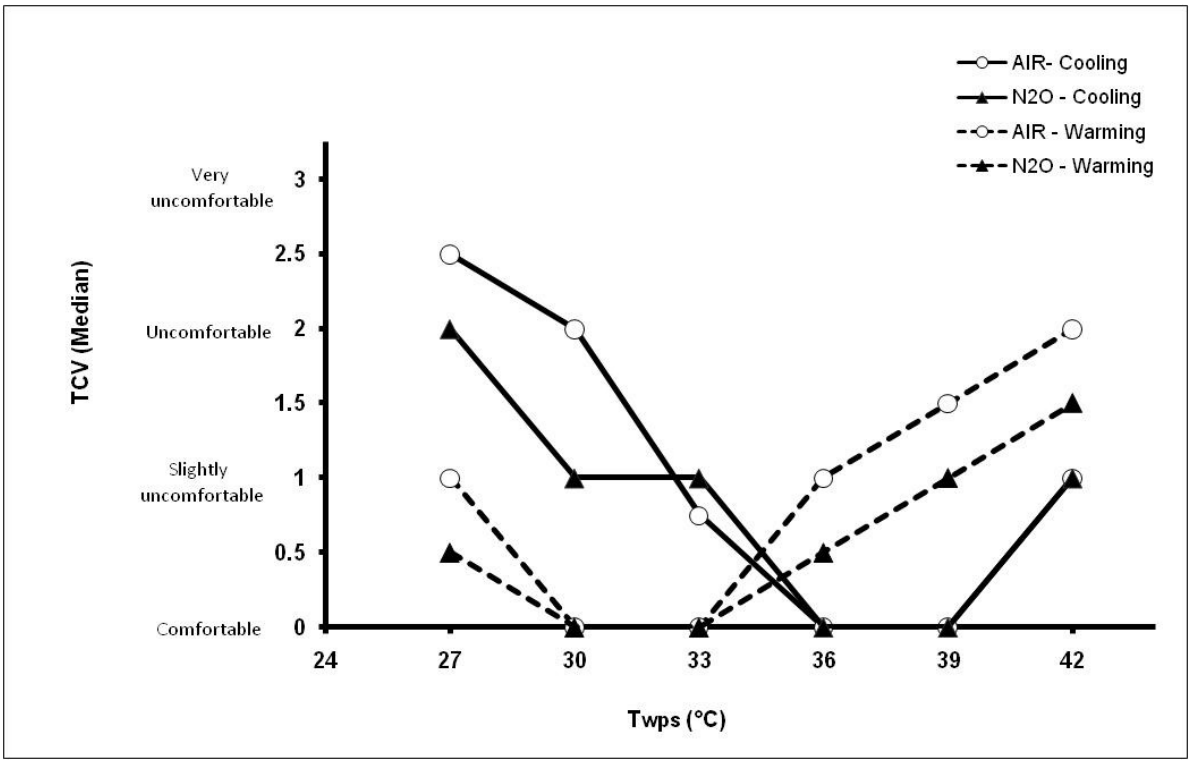


Figure 3: *Effect of narcosis on subjective ratings of (A) thermal sensation and (B) thermal (dis)comfort during warming (dashed line) and cooling (solid line). A: Subjects perceived the increasing T_{wps} as equally warm and the decreasing T_{wps} as equally cold during AIR (open circles) and N_2O (closed triangles). B: With narcosis, subjects perceived equal changes in T_{wps} as significantly less uncomfortable.*

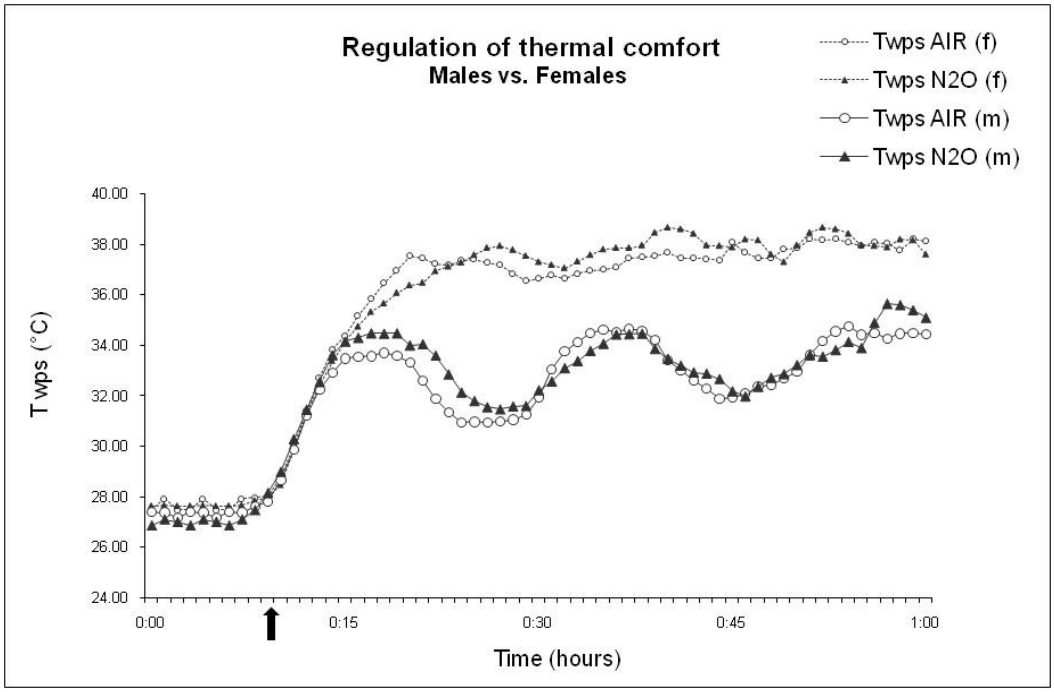


Figure 4: *Average pattern of the T_{wps} response of female (f, dashed line) and male (m, solid line) subjects maintaining thermal comfort during AIR (circles) and N_2O (triangles) trials. Arrow indicates when subjects started controlling Twps*

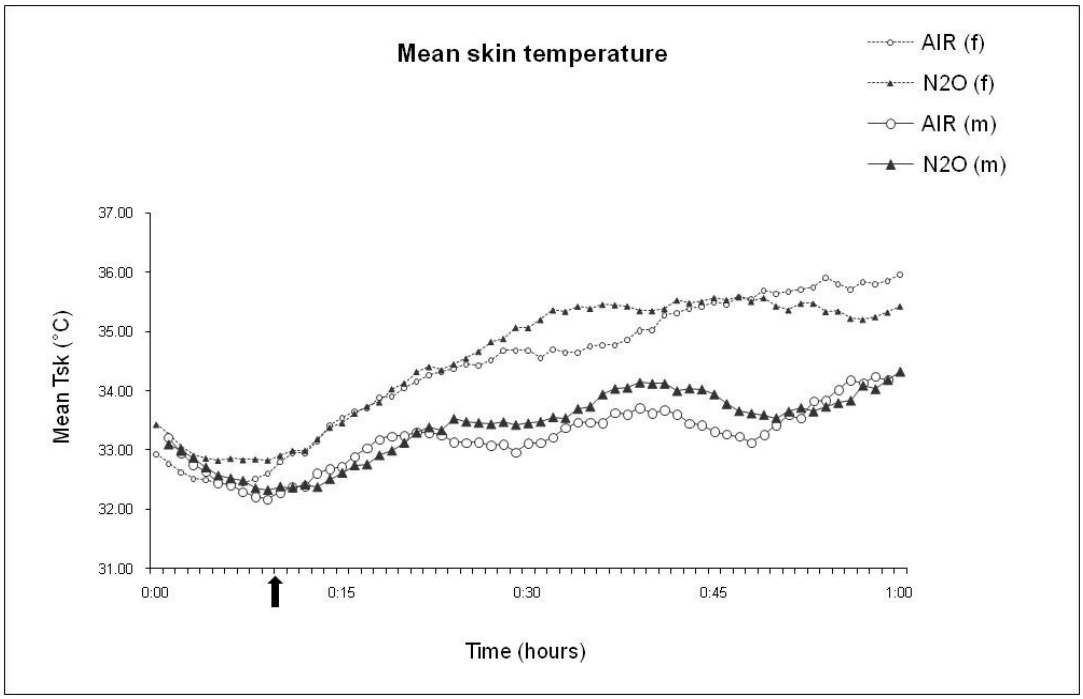


Figure 5: *The mean Tsk of males (solid line) and females (dashed line) maintaining thermal comfort in the AIR (circles) and N_2O (triangles) trials. Arrow indicates when subjects started controlling the T_{wps}.*

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6 Effect of 21 days horizontal bed rest on behavioural thermoregulation

6.1 Introduction

In Study 2 (Chapter 5), we demonstrated that the methodology developed for assessing the characteristics of behavioural temperature regulation (Study 1, Chapter 4) can be used to evaluate the effect of a nonthermal factor on behavioural temperature regulation. Specifically, the effect of mild narcosis on behavioural temperature regulation was investigated. Narcosis induced by inhalation of nitrous oxide alters neural function and consequently influences the conveyance of neural-coded thermal afferent information to the central nervous system, and its subsequent central integration, which ultimately results in the modulation autonomic and behavioural temperature regulation. The present study investigated the utility of the developed methodology (Chapter 4) in assessing changes in behavioural temperature regulatiom due to overall deconditioning, as may be observed with microgravity or with prolonged inactivity due to ageing or injury.

Studies on human thermoregulation conducted under conditions of musculoskeletal and cardiovascular deconditioning such as microgravity or prolonged experimental bed rest indicate that, although maintenance of normothermia is not jeopardized, the core temperature is elevated and some thermoregulatory responses are attenuated.

The most prominent changes were discovered in the autonomic thermoregulatory component, particularly during recovery from exercise. Exercise-induced heat loss (sweating and vasodilatation) and heat production responses (shivering) were shown to be modified after bed rest (Greenleaf, 1989; Fortney et al., 1996, 1998; Mekjavic et al., 2005; Pavy-Le Traon et al., 2007). Changes in the efficiency and/or sensitivity of these autonomic responses were suggested to contribute to the elevation in core temperature observed during spaceflights (Rimmer et al., 1999) and in bed rest experiments (Greenleaf, 1997; Fortney et al., 1998; Ertl et al., 2000).

However, in addition to autonomic control of body temperature, maintaining normothermia involves also behavioural thermoregulatory responses. Under normal conditions, the temperature of the body is not constant. Rather, a dynamic core-to-skin thermal gradient exists according to the shifts in body temperature at different times of the day, level of activity etc. Small shifts in core temperature are counteracted initially by changes in peripheral vasomotor tone and behavioural responses; if core temperature is shifted further, then activation of the metabolically more demanding autonomic responses, shivering or sweating, ensues.

Behavioural responses modulate heat flow from the body by adjusting insulation, and the effective surface area for heat exchange. In fact, where regulation of ambient temperature is possible, by actively adjusting an environmental control system (i.e. heating and cooling), the action of maintaining a preferred

ambient temperature is probably the most efficient means of maintaining thermal balance and in preventing any deleterious displacements of core temperature.

Such thermoregulatory behaviour depends on the normal functioning of the thermal sensory apparatus (thermal information from peripheral and core thermosensors) and is motivated by the perception of thermal discomfort, or rather an affective judgment of the thermal environment (Weiss and Laties, 1961; Cabanac, 1971; Hensel, 1976; Cabanac, 1981). Thus, if deconditioning induced by microgravity or bed rest impairs thermal sensation, or the perception of thermal (dis)comfort, initiation of appropriate behavioural responses might be modified. Inappropriate behavioural regulation of body temperature may contribute, at least in part, to the observed thermal imbalance during prolonged bed rest or microgravity. Changes in thermal comfort and thermal sensation have been reported in previous studies during hypokinesia (Panferova, 1976; Panferova et al., 1989) and experimental bed rest (Fortney et al., 1996; Greenleaf, 1989; Mekjavic et al., 2005). However, the effects of bed rest on behavioural thermoregulatory responses have not yet been investigated.

The present study tested the hypothesis that prolonged bed rest alters behavioural thermoregulation in humans. We also evaluated the effects of prolonged bed rest on cutaneous thermal sensitivity, to determine whether changes, if any, in the preferred thermal comfort zone are a consequence of an alteration in cutaneous temperature sensitivity.

6.2 Methods

Ten healthy male subjects volunteered for the study. Their average (SD) physical characteristics were: age = 22.2 (3.9) years, height = 182.2 (6.2) cm, and mass = 80.3 (11.2) kg.

6.2.1 Protocol

This study was part of a larger study investigating the effects of 35 days of horizontal bed rest on muscle atrophy and osteoporosis. The protocol of the study was approved by the National Ethics Committee of the Republic of Slovenia, and conformed to the Helsinki Declaration. Subjects were accommodated in a ward of the Orthopaedic Hospital Valdoltra (Ankaran, Slovenia). During the bed rest period, subjects were under 24-hr health care. Throughout the entire period of the bed rest, they were requested to remain in the horizontal position at all times and not to perform any exercises (e.g., undue isometric contractions). All activities were performed in the horizontal position. Subjects could support themselves on their elbows during eating, personal hygiene, and transfer between bed and gurney. Subjects received regular physiotherapy, comprising passive movements of all joints, and light massage of the neck and lower back regions. Active muscle contractions were avoided. Subjects received physiotherapy on request.

6.2.2 Cutaneous thermal sensitivity assessment

Minimum detectable temperature (MDT) was assessed on the 1st and 22nd day of bed rest. Testing was conducted in a temperature-controlled room at the same time of the day. Subjects were allowed to equilibrate to the conditions of the room (ambient temperature was 25°C) for 30 minutes. Following equilibration, two cutaneous temperature (cold and warm) sensitivity tests were performed in random order, to assess the influence of bed rest on MDT. The protocol used for assessing cutaneous thermal sensitivity with a Middlesex Thermal Testing System (Howe Institute, Canvey Island, Essex, UK) has been reported previously (Golja et al., 2004). Briefly, a controlling unit initiated the thermal stimuli, which were presented to the cutaneous region via a thermode (a Peltier element) with a surface area of 24 cm^2. The rate of temperature change during heating and cooling of the thermode was 1°C/s. The thermode was positioned on the volar side of the right forearm between the elbow and the wrist. A thermocouple attached to the copper surface of the thermode determined the magnitude and duration of the stimulus, and ensured that the adaptive temperature of the skin (temperature of the skin below the thermode prior to initiation of thermal stimuli) was similar in all trials.

The stimuli were presented to the skin immediately after an audible tone was generated by the computer. Each trial commenced with three stimuli of maximal intensity (either an increase or decrease in temperature of 3°C from the adaptive skin temperature) to familiarize the subjects with the procedure. Subjects were required to detect at least one familiarisation stimulus. Subjects that failed to detect the familiarisation stimuli three times (total of nine stimuli) were excluded from further analysis. Once the trial was initiated, subjects were requested to confirm whether they perceived the stimulus, or not. If a stimulus

was perceived, the next stimulus was smaller; if the stimulus was not perceived, the next stimulus was greater. Intermittently, the software also initiated a sham stimulus; no thermal stimulus was generated following the audible tone. We considered the ratio of false positive reports (i.e., number of positive reports to sham stimuli/total number of reports) as a measure of the reliability of the subject's assessments.

6.2.3 Behavioural thermoregulation assessment

Behavioural thermoregulatory function was assessed after the cutaneous thermal sensitivity tests concluded. Subjects remained supine while thermocouples were attached to their skin and a water-perfused suit (WPS) donned on them. The WPS was made of five components covering the entire surface of the body with the exception of the hands, feet, neck and face. All five components were fed from a common manifold and consisted of identical length of small-diameter (inner =4 mm, outer=5 mm) PVC tubing which were woven in intervals of 1cm in the meshed lining of the suit. Velcro stripes embedded in the suit ensured a good fit to the surface of the skin. Once fully instrumented, the temperature of the water perfusing the WPS (T_{wps}) was set at 27°C, which was perceived by the subjects as being cool. Thereafter, their behaviour in maintaining thermal comfort was evaluated. T_{wps} varied in a sinusoidal manner from 27°C to 42°C. The rate of change of the water temperature was 2.1°C/min and each warming and cooling phase lasted approximately 15 minutes. Subjects were instructed that by depressing a button on a manual control, they could initiate a change in the direction of T_{wps} once it became either uncomfortably warm during heating, or uncomfortably cool during cooling. Subjects were requested to maintain T_{wps} within a preferred range for a total duration of one hour. We did not coach the subjects or give them any further instructions during the trials. The resultant magnitude of the sinusoidal T_{wps} pattern defined as the regulated thermal comfort zone (TCZ), and its lower (T_{low}) and upper (T_{high}) temperature boundaries, were compared between days 1 and 22 of bed rest.

Subjects were unaware of the actual temperature of the water perfusing the suit during the trial. The effects of circadian rhythm were minimized by testing the subjects at the same time of the day.

Ambient conditions

The conditions in the experimental room were monitored by portable weather station (BAR 938 HG OS, Huger, Germany). The ambient room temperatures were 25-26°C in all testing days and the relative humidity 40-50%.

Skin temperature

Skin temperature (Tsk) was measured with T-type (copper-constantan) thermocouples attached to six sites covered by the WPS (calf, thigh, abdomen, chest, forearm, and arm) and two sites that were not covered (foot and hand). The distance between the tubes in the WPS was approximately 1cm, which ensured a uniform stimulation of the skin areas covered by the suit. The thermocouples were attached to the skin at the right side of the body using a thin, breathable transparent film dressing (3M™, Tegaderm™). Skin temperatures were continuously recorded with a data logger (Almemo 5990-2, Ahlborn, Holzkirchen,

Germany). The proximal-to-distal skin temperature gradient was measured in the hand as the difference between Tsk at the forearm and the second digit (ΔTforearm-fingertip), and in the foot as the difference between Tsk at the calf and toe (Δ Tcalf-toe). These values were assumed to reflect peripheral blood flow (Rubinstein and Sessler, 1990).

Core temperature

Core temperature (Tc) was estimated from measurements of tympanic temperature using an infrared thermometer (ThermoScan IRT 3020, Braun, Kronberg, Germany). Three consecutive measurements of Tc were performed before and immediately after each trial, and the highest value was considered representative of the core temperature. We did not continuously measure Tc during the trial since we did not expect it to change while the subjects were at the vicinity of their TCZ. We previously showed that the tympanic temperature remains unchanged during automatic oscillations of T_{wps} in the range 27°C-42°C, and that the preferred T_{wps} for thermal comfort is within this range (Yogev and Mekjavic, 2007). Thus, in the current experimental design, changes in Tsk rather than in Tc were the driving force for the behavioural responses involved in maintaining the WPS thermally comfortable.

6.2.4 Data analysis

A paired t-test was used to compare differences in ambient room temperature and relative humidity between at Day1 and Day22 of bedrest. Measurements were taken during the first 10 minutes (equilibration time) before testing. Mean Tsk was calculated as the unweighted mean of the Tsk at eight sites (forearm, arm, abdomen, chest, neck, forehead, back, thigh, calf, foot, finger and toe) measured at the right side of the body. A paired t-test was used to compare the T_{wps}, Tsk, ΔTforearm-fingertip and ΔTcalf-toe as measured at the 1st and the 22nd day of bedrest. Data are expressed as means (SD) and the limit of statistical significance was set at 0.05.

Assessment of cutaneous thermal sensitivity

The MDT was calculated as the smallest of the last five positive responses. A paired t-test was used to compare differences in the cutaneous thresholds for detecting warm and cold stimuli between days 1 and 22 of bedrest.

Assessment of behavioural thermoregulation

The lower and upper peaks of the oscillation pattern obtained during the last 30 minutes of each trial were considered to represent the boundaries of the subject's TCZ (Tlow and Thigh respectively). Pre- and post BR values were averaged and compared using a paired t-test. Changes in the accuracy of the behavioural response (i.e. in the ability to behaviourally maintain thermal comfort) were estimated by calculating the coefficient of variation (%) within the series of T_{high} and T_{low} made by the subject during each half of the 60 minutes trial.

6.3 Results

6.3.1 Effects of bed rest on cutaneous thermal sensitivity

Familiarisation trial: All subjects (n=10) could detect the initial 3°C decrease in their adaptive Tsk during familiarisation. The warm stimuli, however, were harder to detect and most subjects (seven on Day1 and five on Day22) required more than one familiarisation trial until a 3°C increase in their adaptive T_{sk} was detected.

Adaptive temperature: Testing of cutaneous thermal sensitivity initiated at similar adaptive T_{sk} before and after bedrest. Due to the familiarisation pre-trial, warm testing was initiated at a slightly higher adaptive T_{sk} compared to cold testing. During the warm trials, the adaptive T_{sk} was 32.9 (0.7) ºC on Day1 and 32.1 (1.6) ºC on Day 22 and, during the cold trials, it was 31.6 (0.7) ºC on Day1 and 30.7 (1.4) ºC on Day 22.

Cutaneous thermal sensitivity: The 21 days of horizontal bed rest resulted in an increase in cold sensitivity. The MDT during cold stimuli significantly decreased ($P<0.05$, power = 0.71) from 1.6 (1.0) ºC on Day1 to 1.0 (0.3) ºC on Day22. In contrast, the sensitivity to warmth tended to decrease, albeit not significantly, after bedrest; three subjects were unable to detect any of the warm stimuli after bed rest (compared to one subject before bedrest) and the MDT among the rest of the subjects did not significantly change after bed rest ($P>0.05$, power=0.20). It was 1.3 (0.5) ºC on Day1 and 1.6 (0.9) ºC on Day22. Individual differences in the MDT during warming and cooling are presented in Fig. 1.

Reliability: The percentage of false positive reports was not evenly distributed between the subjects. As demonstrated in Fig. 2, two subjects (S6, S7) had a particularly low reliability score (over 50% false positive reports). If the results from these subjects are not considered in the analysis of MDT (n=8), a more homogeneous effect can be observed. Seven subjects experienced a decrease in warm sensitivity (i.e. increase in MDT/ unable to detect stimuli) and six subjects experienced an increase in cold sensitivity (i.e. decrease in MDT) after bed rest.

6.3.2 Effects of bed rest on behavioural thermoregulation

The comfort regulation trials on Day 1 and 22 were conducted with minimal experimenter supervision and/or feedback. The subjects received a through explanation about the purpose of the trial before it started; however, they were not coached or given any further instructions during the trial. Consequently, three subjects completed the trials without regulating Twps during the entire duration of the trial. The boundaries and width of the TCZ could not be determined for these subjects.

Characteristics of comfort regulation behaviour

Subjects (n=7) used different approaches to regulate thermal comfort. Some preferred to regulate T_{wps} within a narrow range (Fig. 5A) while others allowed T_{wps} to fluctuate within a wider range (Fig. 5B).

Nevertheless, 21 days of horizontal bedrest did not significantly alter the manner subjects controlled the T_{wps} to maintain thermal comfort during a 60 min trial. A typical learning curve was noted in the T_{wps} pattern with larger oscillations in the first half of the trial followed by smaller oscillations in the second. The mean (SD) frequency of oscillation was similar before and after bedrest. Subjects inverted the direction of the T_{wps} change 21 (9.2) times on day 1 and 24.9 (11.5) times on day 22. The coefficient of variation of T_{high} and T_{low} chosen in the last half of the trial was lower (1.7%) than that observed in the first half (3.5%), indicating a greater accuracy in controlling the temperature towards the end of the trials. Similar accuracy in T_{wps} control was observed after 22 days of bedrest.

Characteristics of the thermal comfort zone

As shown in Table 2, the mean (SD) T_{wps}, T_{high} and T_{low} were not significantly different after 22 days of bed rest as compared to the first day. Nevertheless the decrease in the width of the TCZ after bed rest was significant ($P<0.05$).

6.3.3 Effects of bedrest on skin and core temperatures

The core temperature of the subjects was slightly increased ($p<0.05$) from 36.7 (0.1)°C on Day 1 to 37.0 (0.2)°C on day 22. However, upon conclusion of the trial it was not significantly different anymore. Mean skin temperature (T_{sk}) was slightly higher ($P<0.05$) higher after bedrest. It increase from 32.1(0.7)°C on Day1 to 32.9 (0.9)°C on Day22. In contrast to previous reports (Golja et al., 2002), in the current study, the skin temperature measured at the extremities (the toe and the 3rd digit of the hand) was not significantly different between the 1st and 22nd day of BR. Accordingly, the differences in skin temperature between the forearm and fingertip ($\Delta T_{forearm\text{-}Fingertip}$) and between the calf and toe ($\Delta T_{calf\text{-}toe}$), indices of blood flow in the extremities, were not significantly different between Days 1 and 22.

6.4 Discussion

The main finding of the present study is that following three weeks of horizontal bed rest, there were no significant changes in thermal comfort or its behavioural control despite small changes in cutaneous sensitivity.

6.4.1 Effects of bed rest on thermoregulation

Previous studies have reported that the physiological adaptation following prolonged periods of inactivity attenuate thermoregulatory responses (Greenleaf, 1989; Fortney et al., 1998). Changes in the sensitivity (gain) and threshold of activation of autonomic heat loss and heat gain responses have been suggested as possible mechanisms for these observations. In addition, since activation of behavioural thermoregulatory responses relies on similar thermal afferent neural information as autonomic thermoregulatory responses, it was hypothesised that behavioural responses might also be affected in such conditions.

Mekjavic et al. (2005) reported that subjects perceived similar combinations of skin and the core temperatures as warmer and thermally less uncomfortable after 35 days of horizontal bed rest. Reports from earlier studies indicate that some changes in thermal sensitivity occur (Fortney et al., 1996; Panferova, 1976) and that subjects in bed rest studies often complain of cold feet (Taylor et al., 1949; Fortney et al., 1996; Mekjavic et al., 2005). Using weekly infrared thermograms during a 35-day bed rest, Golja et al. (2002) showed a significant decrease in skin temperature at the feet and concluded that cold sensation was due to an actual cooling of extremities rather than alteration in thermal sensory function. In the current study, after a 21-day bed rest, the mean Tsk and the core temperature were slightly increased ¸however there were no significant changes in extremity Tsk. The results from the current study are in agreement with the studies demonstrating that changes in peripheral thermal sensitivity might occur after bed rest (Panferova, 1976; Fortney et al., 1996, 1998). In the current study, the changes in cold sensitivity were not due to actual cooling, since the adaptive skin temperature was not significantly different after bedrest and the mean Tsk tended to increase. Furthermore, increased sensitivity to cold can also explain why bedrest did not increase, but rather tended to decrease the regulated TCZ. These changes in local thermal sensitivity, appear to be too small to significantly affect the preferred temperature or its behavioural regulation.

It therefore seems unlikely that the increase in core temperature observed after bedrest and spaceflights is the result of an inability to activate behavioural thermoregulatory responses near thermal neutrality. This is in agreement with previous bed rest studies where core temperature only occasionally increased in resting subjects (Greenleaf, 1997). More consistent are reports of a bedrest-induced "excessive" increase in core temperature during exercise (Greenleaf, 1989; Greenleaf, 1997; Golja et al., 2002; Mekjavic et al., 2005), or greater decrements in core temperature during immersion in 28°C water (Mekjavic et al., 2005). The former may be attributed to attenuation of the sweating and/or vasodilatatory responses consequent to a reduced circulating blood volume (Crandall et al., 2003; Wilson et al., 2003), and the latter to attenuation of shivering and/or cold-induced vasoconstriction.

In the present study, there was no change in Tc after 60 minutes of comfort regulation. Furthermore, we have previously demonstrated that oscillations in T_{wps} in the range 27-42°C leads to fluctuations of 3-4°C in mean Tsk, without significantly changing the tympanic temperature (Yogev and Mekjavic, 2007). As previously, regulation of the Twps in the current study was limited to the same range. We therefore assumed that the core temperature was unaltered during the entire duration of the trials. Therefore, any change in thermal perception or thermal sensitivity could not reflect changes in the function of either the sweating or the shivering responses.

As there were no significant differences in any of the parameters related to vasomotor tone (skin temperature at the extremities, ΔTforearm-finger and ΔTcalf-toe), the observed changes in our study most likely resulted from changes in the perception of thermal stimuli. The latter incorporates not only the transduction of thermal energy into neural coded information, but also the central integration of thermal afferent information from the core and the skin that results in a subjective perception of thermal (dis)comfort.

6.4.2 The effects of bedrest on cutaneous thermal sensitivity

An important factor affecting thermal sensitivity is the adaptive temperature of the skin; the steady-state temperature of the skin prior to the application of a thermal stimulus. Alteration in skin blood flow can alter this temperature and consequently affect cutaneous thermosensitivity. Previous bedrest-studies show that the naturally occurring central-to-peripheral thermal gradient that enables heat flow from the core to the periphery is altered after lying down (Golja et al., 2002). Such alteration in the thermal gradient may be attributed to redistribution of blood from peripheral to central regions caused by increased peripheral vasoconstriction (Convertino, 2002).

The adaptive temperature in the cutaneous sensitivity tests was not significantly different after bedrest, however, the mean Tsk was about 1°C lower on day 1 compared to day 22. Such a change in the skin temperature may have contributed to the reduction of the warm- and exaggerate the cold-sensitivity, since it approaches the temperature corresponding to the maximum activity of the cold thermal sensors (Kenshalo, 1976). Thus, we cannot exclude the possibility that the observed increase in cold sensitivity and reduced warm sensitivity were influenced by a small bed rest-induced change in skin temperature.

6.4.3 The effect of bed rest on behavioural regulation of thermal comfort

It was previously reported that thermal perception changes after bed rest (Mekjavic et al., 2005). Since thermal perception is the basis for behavioural thermoregulation, it was suggested that these changes might lead to inappropriate behavioural thermoregulatory responses. Assuming that similar changes would occur in microgravity this might be of practical importance to astronauts during space exploration missions. Namely, during extravehicular activity (EVA), the liquid cooling garment (LCG) worn next to the skin and designed to maintain a thermoneutral microenvironment, is controlled manually. In this study, rather than asking the subjects to passively evaluate the degree of thermal (dis)comfort using a rating scale (a non-

parametric variable), we assessed the manner by which they actively regulate the temperature of the WPS according to their comfort. We have previously shown that the T_{wps} at which discomfort perception appears, and the T_{wps} at which subjects initiate a behavioural response to counteract it, are highly correlated (Yogev and Mekjavic, 2007).

The fact that no significant differences were observed in the preferred skin temperature, the upper and lower boundaries of the TCZ, and in the characteristics of the control of T_{wps} suggests that the changes that occur after 3 weeks of bed rest do not significantly attenuate the ability to behaviourally maintaining thermal comfort in the WPS.

A limitation to the current study is that we did not estimate the contribution of local discomfort. Thus, we cannot determine the relative contribution of certain skin regions to the resultant thermoregulatory behaviour. Previous studies have demonstrated that different skin regions do not equally contribute to thermal sensation and comfort (Burke and Mekjavic, 1991; Zhang et al., 2004; Cotter and Taylor, 2005) it would be interesting to examine how subjects, given control over their skin temperature, would regulate the different regions of the skin to maintain an overall thermal comfort.

Our results are in line with previous observations indicating that prolonged inactivity may lead to changes in thermal sensation (Mekjavic et al., 2005), and suggest that it is primarily the peripheral sensation of cold. Future studies on thermal sensitivity after bedrest will have to eliminate any change in adaptive temperature of the skin, a consequence of the bed rest-induced increase in peripheral vasoconstriction. The ability to behaviourally maintain thermal comfort was not compromised under the prevailing, relatively mild, conditions of this study (vicinity of thermal neutrality, rest, no additional tasks). However, the results of this study indicate that small changes in thermal perception after bed rest may occur not only as a result of alteration in core temperature (induced by exercise or immersion in cold water), but also at rest, within the boundaries of TCZ. Although these changes seem to be too small to result in significant changes in the behavioural thermoregulatory response, it would be premature to dismiss the role of behavioural thermoregulatory responses when thermal balance in actual microgravity is considered. In such conditions, an astronaut might have to perform accurate tasks after strenuous activity over long periods. Even a small shift from thermal comfort might increase the metabolic demand and exhaust an astronaut thus jeopardising his safety. Future studies will have to widen the scope of conditions under which behavioural thermoregulation is being examined.

6.5 Figures & Tables

Table 1. *Average (SD) subjectively chosen temperature of the water perfusing the suit (Twps), the temperatures of the water perfusing the suit at the upper (Thigh) and lower (Tlow) borders of the Thermal comfort zone (TCZ), and the magnitude of TCZ, on the 1st and 22nd day of bed rest.*

	Day 1	Day 22
Average WPS Temperature	36.8 (6.2)	35.9 (0.7)
Upper boundary of TCZ	38.9 (4.8)	37.7 (1.3)
Lower boundary of TCZ	34.7 (8.4)	34.3 (1.0)
Width of TCZ	4.2 (2.0)	3.2 (1.2)*

* Indicates statistical significant difference (P<0.05) compared to Day1.

Table 2. *Average (SD) mean skin temperature (Tsk), and the proximal-to-distal skin temperature gradient in the hands (ΔTforearm-fingertip) and feet (ΔTcalf-toe), and index of peripheral blood flow, at the upper (Thigh) and lower (Tlow) boundaries of TCZ (at the 1st and 22nd day of bed rest.*

	Day 1		Day 22	
	Thigh	Tlow	Thigh	Tlow
Mean skin temperature	35.0 (0.7)	34.4 (1.6)	35.9 (0.8)*	35.5 (0.5)*
ΔTforearm-fingertip	-1.4 (2.4)	-1.4 (1.4)	-1.0 (1.4)	-0.9 (1.1)
ΔTcalf-toe	3.6 (3.6)	3.1 (3.5)	4.1 (8.4)	3.7 (7.7)

* Indicates statistical significant difference (P<0.05) compared to Day1.

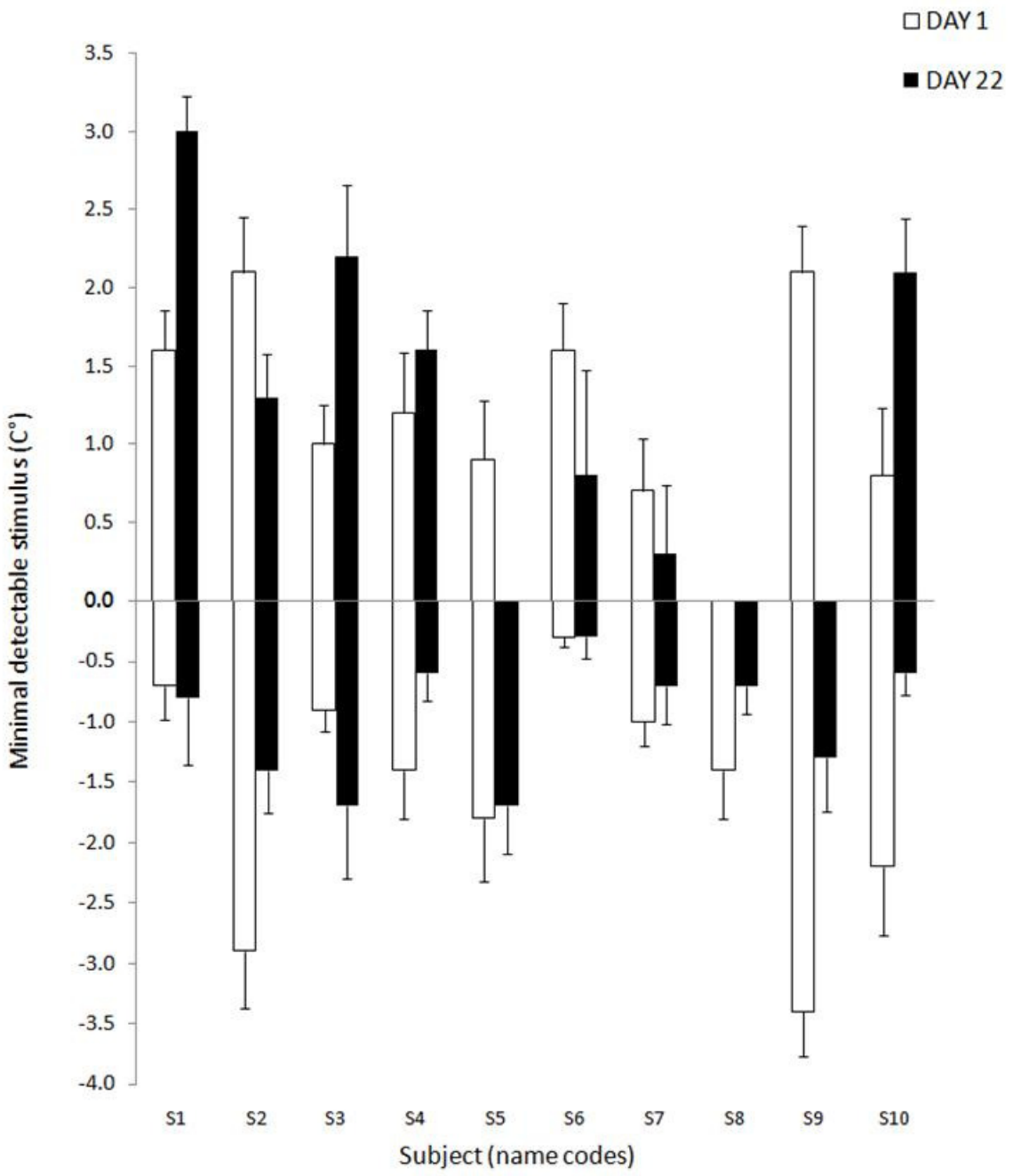


Figure 1: *Individual thresholds for cutaneous thermal sensation of cold (negative values) and warm (positive values) stimuli. Zero-point (missing bars) indicate that the threshold could not be determined (e.g. MDT>3°C).*

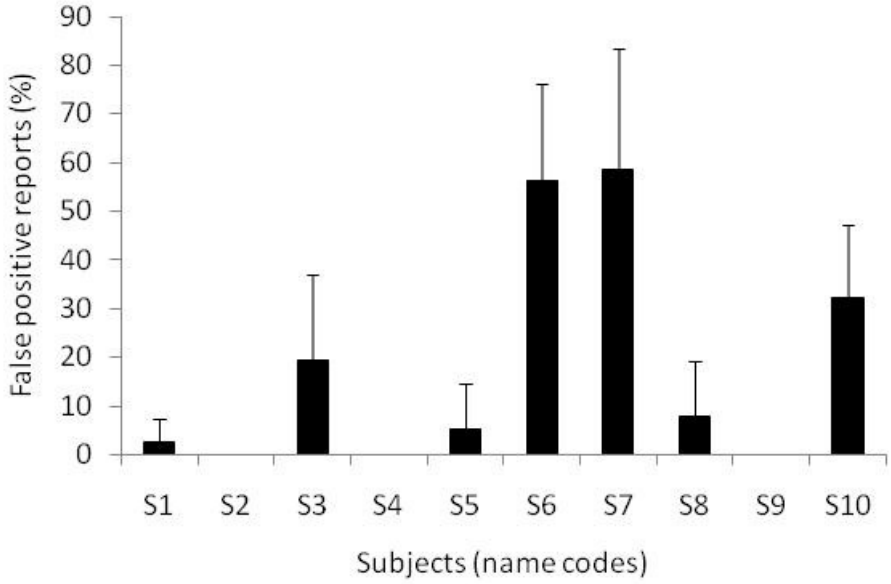


Figure 2: *False positive reports during cutaneous sensitivity testing.*

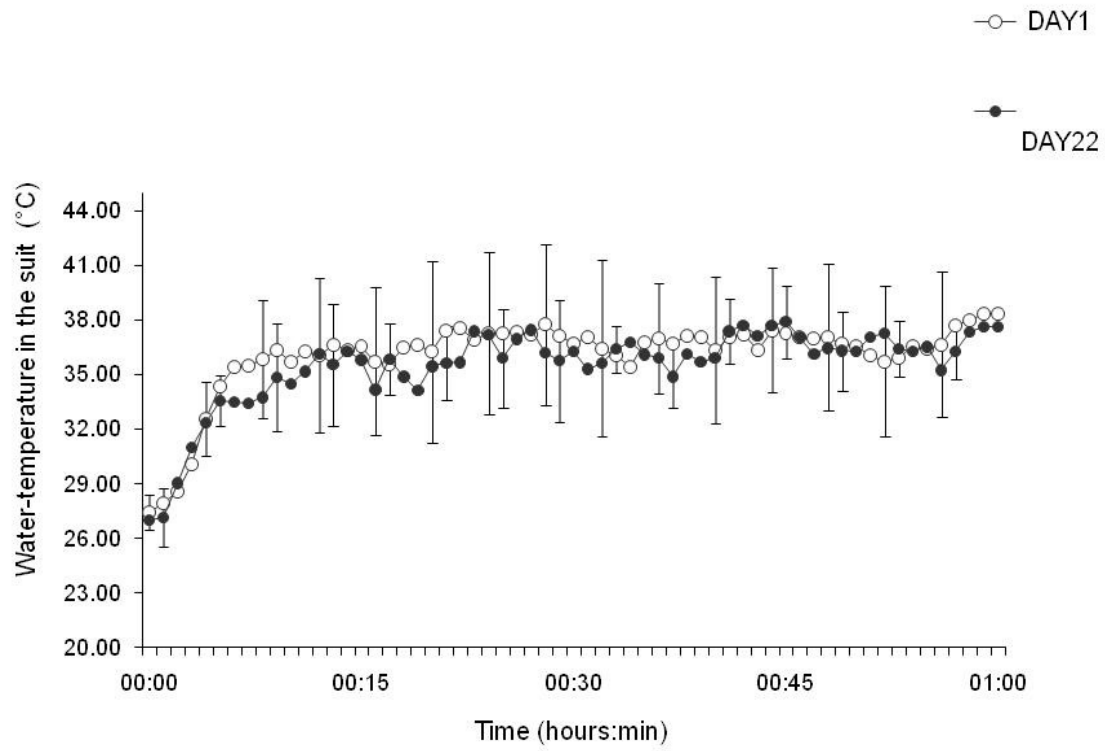


Figure 3: *The average (SD) Twps pattern of the subjective regulation of the temperature of the water perfusing the suit (Twps) to maintain thermal comfort was similar on the 1st (open circles) and 22nd day of bed rest (closed circles).*

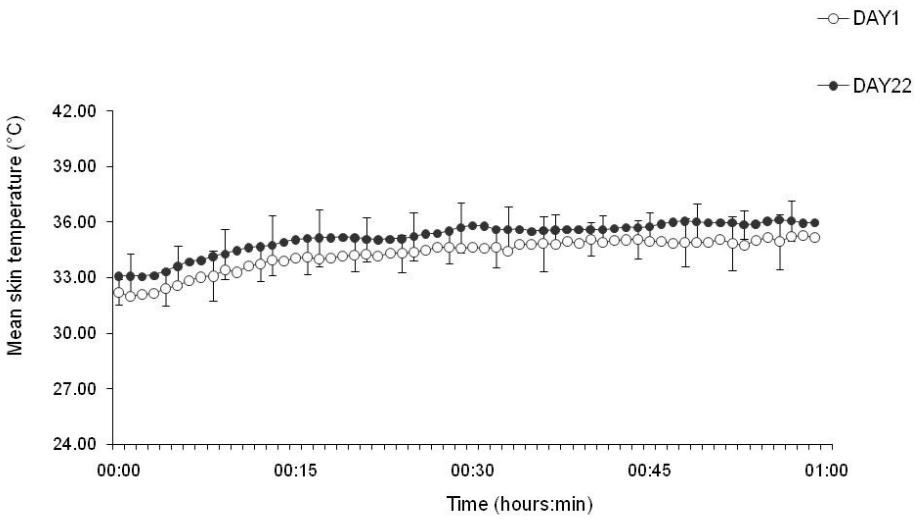
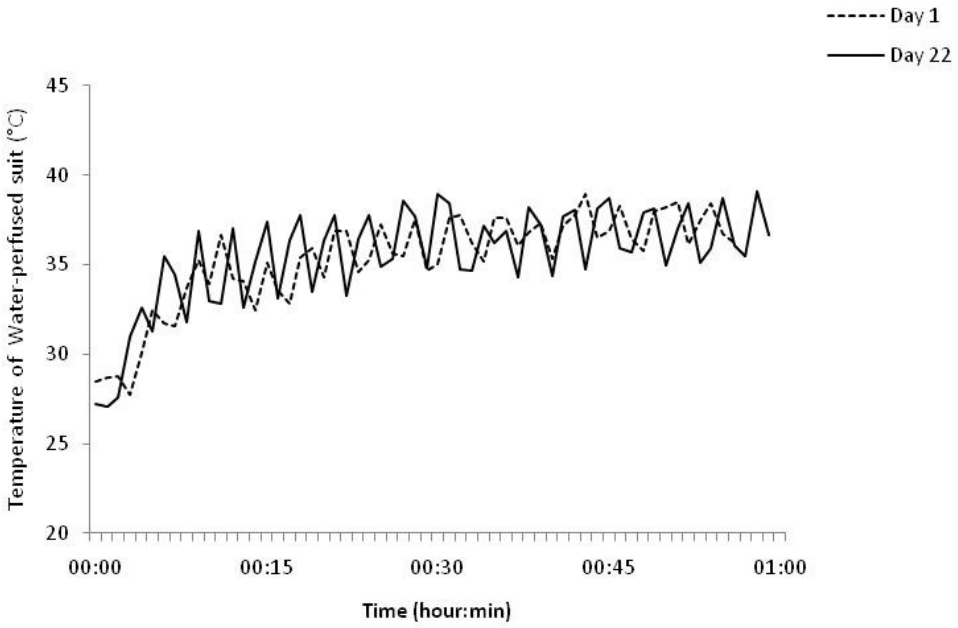


Figure 4: *Average (SD) mean skin temperature during regulation of thermal comfort on the 1st (open circles) and 22nd day of bed rest (closed circles).*

A.



B.

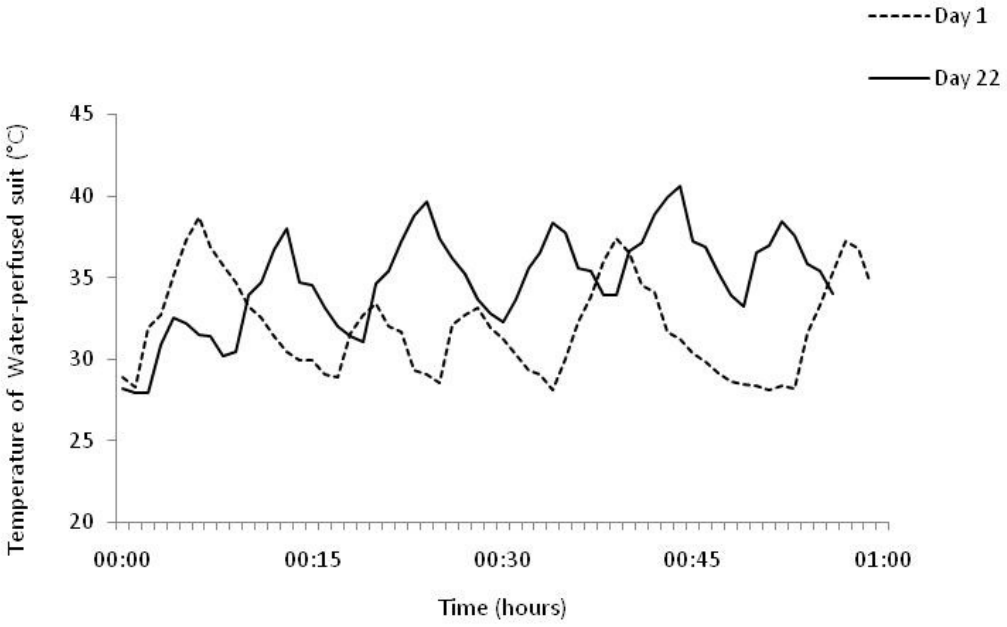


Figure 5: *Representative T_{wps} patterns obtained by two subjects regulating the temperature of a water-perfused suit to maintain thermal comfort on the 1st and 22nd day of bedrest.*

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7 Summary and conclusions

The studies presented in this thesis addressed the need to assess behavioural thermoregulation in humans and particularly, to evaluate whether NTFs that alter thermal perception also influence behavioural thermoregulation. In the present work, particular reference has been given to examining the influence of narcosis and prolonged bedrest on behavioural thermoregulation. These NTFs represent the conditions that humans may encounter while performing in extreme environments such as deep sea or space. It was previously hypothesized that since these NTFs alter thermal perception, they may consequently jeopardise thermal stability by preventing appropriate activation of behavioural thermoregulatory responses.

In the absence of an accepted method for assessing thermoregulatory behaviour in humans, the main achievement of the present study was the development of a new experimental procedure for assessing human thermoregulatory behaviour. The results of the present study do not support the hypothesis that altered perception of comfort attenuates behavioural thermoregulation. Mild narcosis and prolonged bedrest did not significantly change thermoregulatory behaviour despite their significant influence on thermal sensation and comfort.

Specifically, the main findings of the present thesis are:

1. The perception of thermal discomfort and the initiation of a behavioural response to counteract it are highly correlated events.
2. Females compared to males tend to prefer higher temperature for thermal comfort.
3. Narcosis does not significantly change thermoregulatory behaviour despite its significant influence on the perception of comfort.
4. Thermal sensation (the perception of temperature as cold or warm) is not equally affected by narcosis as is thermal comfort (the affective judgment).
5. Following three weeks of horizontal bed rest, there were no significant changes in thermal comfort or its behavioural control despite small changes in cutaneous sensitivity.
6. Three weeks of horizontal bed rest resulted in an increase in cold sensitivity; however, the sensitivity to warmth tended to decrease.

New experimental approach for assess behavioural thermoregulation

The results indicate that the proposed experimental protocol enabled subjects to identify the thresholds of warm and cold discomfort in a reproducible manner, and that the threshold for initiation of behavioural thermoregulatory responses can be reliably measured. The upper and lower peaks of the T_{wps} pattern that is obtained when subjects maintain thermal comfort, provide a valid measure of the threshold temperature eliciting warm and cold discomfort.

The current experimental approach assesses the behaviour directly rather than assuming how

behaviour will change according to changes in the perception of comfort. With this procedure we directly evaluate the manner by which humans initiate behavioural responses to regulate their ambient temperature. This is a significant improvement to the common scale rating approach where actual behaviour can only be assumed according to indirect assessment of changes in the perception of comfort.

Assessment of thermoregulatory behaviour in this method is less likely to be influenced by factors that are not associated with the regulation of body temperature. In this approach the subjects do not have to respond to a question of the experimenter (which might be leading) or report their sensation according to the wording dictated by the scale (which might not represent their actual sensation). It leaves much less room for the influence of psychological and sociological factors and focuses their assessment on thermal factors. This was clearly demonstrated when scales were used under narcosis. The reports subjects provided were significantly altered by narcosis; however, when the temperature was controlled by the subject there was no difference.

Using a WPS that uniformly changes the skin temperature provides a controlled and defined input stimulus of the microclimate of the skin (whole body or in a specific region) that can be correlated to a defined behavioural response. Such a practice can allow testing subjects that are almost naked, without raising issues of embarrassment and modesty, which limit methods assessing dressing behaviour. In addition, the control over the microclimate surrounding the body is more complex when subjects are dressed. When subjects are clothed the design and fit of the garment can influence the microclimate surrounding the skin. In the current procedure, the WPS could be adjusted to fit different body sizes and shapes by using Velcro strips, thus ensuring that an equal thermal stimulus is provided to the skin of all the subjects.

Thermoregulatory behaviour can be assessed on the basis of data that are analysed by parametric statistical tools, whereas non-parametric tools must be used when scale votes are analysed. Using parametric tools for the analysis is more adequate when conclusions should be drawn regarding the risk associated with improper initiation of behavioural responses in extreme environments.

By comparing the results from the study 1 and 2, the influence of rate of change in the periphery can be investigated. Subjects (different groups) were requested to maintain thermal comfort using two protocols, in the first (n=6, males only) the water temperature changed at 1.2 °C/min and in the second (n=10 males) 2.1°C/min. The results of the two studies show that with higher rate of T_{wps} the preferred temperature is initially increased; however, these differences are less evident towards the end of the trial (Fig. 5).

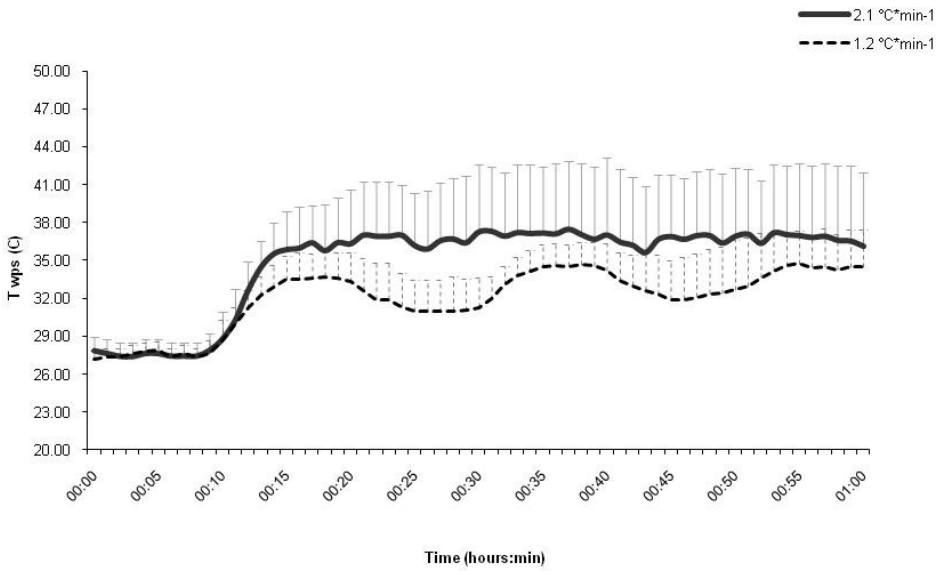

Figure 5: *Differences in the T_{wps} pattern obtained at a faster (solid line) and slower (dashed line) rate of temperature change at the WPS.*

Influence of NTFs on thermal perception and behavioural thermoregulation

The results of the current study confirm that mild narcosis induced by inhaling a mixture containing 30% Nitrous oxide alters the perception of thermal comfort. The perception of temperature as cold or warm, on the other hand, was not equally affected by narcosis, suggesting that narcosis does not influence the ability to detect thermal changes, but rather the motivation to respond to them.

The narcosis-induced increase in comfort occurs not only during dynamic changes in core temperature as reported by Cheung and Mekjavic (1995) and Mekjavic et al. (1994), but also in normothermic subjects during changes in skin temperature. Thus, the effects of narcosis on thermal perception do not depend on its effect on the shivering response.

In contrast to the well documented effect of narcosis on behavioural thermoregulation in animals (Pertwee et al., 1986; Macdonald et al., 1989; Pertwee et al., 1990) and its significant influence on thermal comfort (Mekjavic et al., 1994; Cheung and Mekjavic, 1995), the ability of humans to behaviourally maintain thermal comfort was not significantly changed by mild narcosis. Considering that brain regions associated with cognitive processes are more sensitive to anaesthesia than other regions (Heinke and Koelsch, 2005), this finding supports Satinoff's (1983) suggestion that the central processing involved in thermoregulation occurs at several levels of the CNS, rather than at a single location.

Gender-related differences in behavioural thermoregulation were found. When given control over the T_{wps}, females maintained the T_{wps} about 3°C higher than their male counterparts to achieve thermal comfort. The results are in line with previous studies demonstrating that females prefer higher temperatures for thermal comfort (Cunningham and Cabanac, 1971; Golja et al., 2005). Further studies using appropriate control for body mass, surface area, and the menstrual cycle should be performed in order to clarify whether differences in thermal preference do exist between males and females.

Three weeks of horizontal bed rest resulted in an increase in cold sensitivity; however, the sensitivity to warmth tended to decrease. The results are in agreement with the studies demonstrating that changes in peripheral thermal sensitivity might occur after bed rest (Panferova, 1976; Fortney et al., 1996, Fortney et al., 1998); however, these changes appear to be too small to result in significant changes in the behavioural thermoregulatory responses. It therefore seems unlikely that the increase in core temperature observed after bedrest and spaceflights is the result of an inability to activate behavioural thermoregulatory responses near normothermia. Since alterations in the thermoregulatory process after bedrest has been associated with dynamic changes in Tc (Greenleaf, 1989; Greenleaf, 1997; Golja et al., 2002; Mekjavic et al., 2005), future studies should also examine thermoregulatory behaviour in situations where core temperature is shifted, e.g., during exercise.

The present thesis demonstrated that behavioural thermoregulation in humans can be reliably studied in the laboratory. The threshold at which behavioural thermoregulatory responses are initiated is not as sensitive to NTFs as is thermal comfort. There is a practical need to determine the type of NTFs, and the threshold level (dose) that would attenuate the ability to maintain thermal stability by behaviour. Particularly, examining thermoregulatory behaviour in increasing doses of narcosis and longer bedrest periods is still to be performed. This is essential information for assessing the thermal risks associated with activities in extreme environments.

In addition to studying the effects of NTFs, the influence of thermal factors on thermoregulatory behaviour should be clarified. For example, the influence of core temperature on behavioural thermoregulation can be studied by examining how mildly hyperthermia (e.g., after exercise) or hypothermia (e.g., after water immersion) maintain thermal stability and comfort.

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Appendix – Ethics approval

All experimental protocols used in this thesis were previously approved (below) by the National Medical Ethics Committee at the Slovene Ministry of Health and conformed to the standards set by the Declaration of Helsinki (2002). All volunteers gave written informed consent to participate in the study.

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Datum: 14. 12. 2004

Spoštovana gospod prof. Mekjavič in gospa dr. Felicijan,

Komisiji za medicinsko etiko ste 12. 11. 2004 naslovili prošnjo za oceno študije z naslovom:

"Vpliv inertnih plinov na občutenje toplotnih dražljajev."

Komisija za medicinsko etiko je na današnji seji ugotovila, da zoper vaš načrt nima etičnih ugovorov. Glede na to Vam izdaja svoje soglasje.

Z lepimi pozdravi in dobrimi željami,

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