Humans and Warm Environments

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Physiology, Health and Behaviour

Hannah Pallubinsky

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Für Bea und Uli

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General introduction and outline

From naturally controlled to an engineering marvel – humans and their thermal environments

Ever since the existence of human life, our body needed to adapt to various thermal environments in order to survive. The natural thermal environment fluctuates a lot, not only over the different seasons, but also in the course of day and night. Evolutionary processes, the plasticity of the human physiological system and behavioural adjustments made it possible for the human species to adapt to a wide array of different climatic zones (1, 2). In this way, the human species was able to spread across the globe and now populates the greatest part of our planet.

Indigenous people are very well acclimatised to their natural environment. Over the course of thousands of generations, they have specialised to cope with even the most extreme conditions, like the Inuits in the Arctic, the Aboriginals in Australia and the Bedouins in the desert (3, 4). Morphological, functional, genetic and cultural adaptation play an important role for the beneficial adjustment to an environment. Moreover, the use of shelter and fire has always been crucial: already 4 million years ago, the *Australopithecus* species inhabited trees, and in early stone age, natural forms of shelter such as caves were used to brave the elements. The first evidence of men-made shelters, built from branches, stones and bones are probably some 500.000 years old and the control of fire by *Homo erectus* dates at least 600.000 years back (5).

Leaping to the situation nowadays in Western Europe, it might be more accurate to say that today, we manipulate the thermal environment to our wishes and desires, rather than adapt ourselves to a natural habitat. This is true for most developed, industrialized countries, where people nowadays are hardly ever exposed to the variation of outdoor conditions, as people spend the greatest part of their time indoors (6). This means in fact that indoor environments are our 'new' not-so-natural habitat.

The design of comfortable indoor environments and its pitfalls

The design of our indoor spaces has developed and changed tremendously over the past decades, inter alia, to make them as comfortable and optimally tempered as possible. We can heat, cool, humidify, dry and replace the indoor air to our liking, and thus, create a total 'weatherlessness'. The invention of air-conditioning by Willis Carrier in 1902 was one of the essential factors in favour of this development. Ever since, the use of air conditioning and heating in the Modern World has changed our life a lot. Based on the research of Fanger in the 1970s (7, 8), and the subsequently developed standards for indoor environments according to the American Society for Heating, Refrigerating and Air Conditioning Engineers (ASHRAE), it became the goal to strive for a thermally *neutral* environment in buildings. Thermal neutrality was assumed to be the most *comfortable* for the majority of building occupants (9). According to the ASHRAE Standard 55 (9) and ISO Standard 7730 (10), indoor temperature should only marginally fluctuate around the supposedly neutral temperature, causing an accepted variance of only ±0.5˚C around the targeted

set point. Although the guidelines are very strict, they have been assumed to be applicable in all types of buildings, across different climates, seasons and populations (9, 11).

Up until today, these standards are still retained, albeit some parts are criticised by many, and for several reasons. Firstly, operating a building on a tightly controlled set point, and not tolerating a reasonable amount of variation, costs a lot of energy. For naturally ventilated buildings, it might not even be feasible. In recent decades, the costs for 'Heating, Ventilating and Air-conditioning' (HVAC) systems have risen tremendously, leading to the expenditure of approximately 1/3 of our primary energy supply to condition the indoor environments of private, public and commercial buildings (12). Spending so much energy does not only cost a lot of money, but it also consumes a vast amount of other resources (13). Besides that, field studies on natural ventilation and personal control of thermal environments have led to the recognition that people tend to accept a much wider range of ambient temperatures than predicted by the conventional models (11, 14, 15). These were only some of the many important reasons why an alternative model, the 'Adaptive Comfort Standard', established by Humphreys and Nicol (16, 17) and later revised by De Dear and Brager (11, 18), was introduced to the recent ASHRAE Standard 55-2013 (19). The Adaptive Comfort Standard allows for more variation of the indoor temperature in relation to outdoor temperatures and the different seasons (20).

Secondly, reasonable doubt has been expressed about the healthiness of such uniform indoor environments (21-26). As mentioned above, the human thermoregulatory system used to be regularly stimulated by exposure to varying outdoor conditions. In order to maintain a stable core temperature, the body expends energy to warm up and dissipates heat to cool down. However, fewer calories might be burned to this end due to the lack of temperature variation indoors, which might contribute to a tipping of the fragile energy balance (22-26). It has therefore been hypothesized that the tight thermal indoor environment, alongside with oversupply of food and sedentary behaviour, might be one of the reasons for the global 'diabesity epidemic'.

Overheating of buildings and climate change

Measures taken to comply with the strict indoor air guidelines described in the above, combined with the endeavour to reduce energy costs, have led to the use of highly insulating construction materials. As a consequence, many modern buildings are nearly air tight, and the hermetic construction paired with a high internal heat load (due to technical equipment and occupants) puts those buildings at risk for overheating (27). Moreover, it becomes even more difficult to maintain a stable indoor temperature, as required by the aforementioned standards, when the outside temperature rises and radiation of the sun increases. Therefore, even more energy will have to be spent to condition our indoor spaces, especially in summer. Due to climate change and global warming, the scenario will progressively become more serious. The consequences of climate change include altered average and extreme temperature, precipitation, rise of sea level due to shrinking snow and ice and warmer oceans (28). These changes have led and will lead to

more impact on our ecosystems, socio-economic sectors and, importantly, human health (29). Della-Marta *et al.* (30) have shown that between 1880 and 2005, the frequency of hot days in Western Europe has almost tripled and summer heat waves nowadays last twice as long. By the end of the 21st century, countries in central Europe are expected to experience as many hot days as are currently encountered in Southern Europe (31). Hence, events such as the extreme and unusual European summer heat wave of 2003 will likely not be as unusual any more in the near future. Consequently, people living in Western and Central Europe will soon be exposed to warm environments more often and more frequently – both indoors and outdoors.

The human in a warm environment – aspects of thermophysiology, health and adaptation

There has been a lot of scientific interest in studying extreme temperature conditions and the impact thereof on human physiology and health. A vast amount of studies previously investigated the effect of intense, mostly exercise-induced heat acclimation programs on a variety of health-related outcomes and performance parameters (examples include (32-38)). However, the influence of passive exposure to only moderately increased temperatures, which we might encounter in day-to-day situations, is largely unknown. Therefore, it is of great importance to examine the effects of short-term and longer-term exposure to mild heat on human physiology, behaviour and health.

The human thermoneutral zone

In order to elucidate the effect of mildly increased ambient temperature on the human body, a logical first step is to indicate at which temperatures the human metabolism is actually in thermal balance, thus in a thermally neutral state. When viewing from a metabolic perspective, thermoneutrality means that no additional energy needs to be spent to cool or warm the body in order to keep a stable core temperature (Tcore). Hence, the rate of energy expenditure is at its lowest when the body is in a thermoneutral, resting and fasted state. The range of temperatures at which the body is in 'thermal neutrality' is called the thermoneutral zone (TNZ, Figure 1). Within the TNZ, temperature regulation is solely achieved by control of dry heat loss and no extra thermoregulatory heat production or additional evaporative heat loss (sweating) occurs (39, 40).

According to the classical model, above or below the TNZ, i.e. above or below the lower and upper critical temperatures (LCT and UCT), metabolic rate and evaporative water loss (only UCT) increases in proportion to the change of ambient temperature (Figure 1). To date, the TNZ has mainly been studied in animals. With respect to the human TNZ, limited studies are available (41-46). Few additional studies present data for the human LCT (47-49), but regarding the human UCT, hardly any information is available.

Data derived from the few human studies available indicate that the assumed human thermoneutral range lies between 28˚C and 32˚C (42). However, earlier research suggest that the TNZ differs between individuals, since it is influenced by, for example, body composition, age, and gender (50). Furthermore, acclimation to heat or cold might play a significant role, as previously shown in animal studies, where acclimation to heat shifted the LCT of the TNZ (51). Hitherto, the determination of the human thermoneutral zone remains problematic: in many animal studies, the TNZ has been measured at a range of fixed ambient temperatures in order to establish steady-state energy expenditure values for each temperature (52-54), which is relatively easy to accomplish in small mammals. However, due to the great thermal mass of the human body, it would take hours to reach a steady state - if possible at all. Therefore, a stepwise temperature protocol for the study of the human TNZ would be very difficult to implement and also extremely time-consuming. To examine the individual ranges of the human TNZ and to evaluate the impact that bodily characteristics and demographic variables have on its width and positioning, an alternative approach is needed.

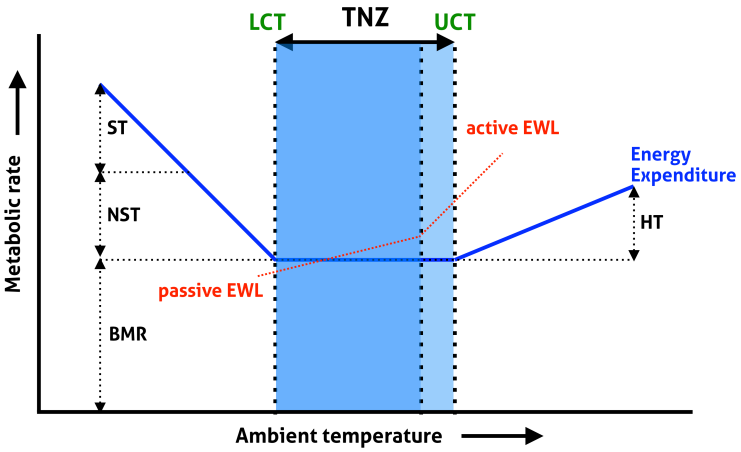

Figure 1 | The thermoneutral zone

The thermoneutral zone (TNZ) reflects the ambient temperature range at which temperature regulation is solely controlled by sensible heat loss, i.e. without any regulatory changes in metabolic rate (nonshivering thermogenesis NST, shivering thermogenesis ST or heat-related thermogenesis HT) or (active) evaporative heat loss (evaporative water loss EWL) (55). Within the TNZ, energy expenditure is at its basal level (basal metabolic rate, BMR). Below the lower critical temperature (LCT) and above the upper critical temperature (UCT), energy expenditure is expected to increase due to an increase or decrease of the ambient temperature. The red-dotted line indicates (the anticipated onset of) evaporative waterloss in humans. The inflection point of the red-dotted line, where passive EWL transforms into active EWL, has earlier been suggested to reflect the evaporative UCT (56, 57). The figure is derived from animal studies (57).

Thermoregulation in humans

The physiological processes of thermoregulation are meticulously controlled by the central nervous system and involve the integration and coordination of several bodily functions such as cutaneous circulation, metabolism, muscle activity, sweat gland activity and respiration (58). In general, humans employ three physiological processes to maintain temperature homeostasis.

Firstly, within the TNZ, thermal cutaneous vasomotor control narrowly regulates temperature through constriction and dilation of skin vessels (59, 60). This way, the body is able precisely control the core temperature through the retraction of blood towards the core, or, alternatively, increase heat dissipation via the skin.

Secondly, as shown in Figure 1, the metabolic rate increases when the ambient temperature falls below a certain assumedly individual critical level (46, 59). When temperature decreases, the increase of metabolic rate is needed to maintain Tcore. Non-shivering thermogenesis, in which brown adipose tissue has been shown to play an important role, is the first line of defence (61-63). If the ambient temperature continues to decline, and non-shivering thermogenesis does not

deliver enough capacity to stabilise Tcore, shivering commences (58). Shivering produces a lot of heat through involuntary contraction of skeletal muscle, which consumes a considerable amount of energy (up to 5 times as much as the basic metabolic rate) (58). However, shivering is uncomfortable and not easily endured for a longer period of time. To which extent shivering and non-shivering processes go hand in hand is still unresolved.

Thirdly, when temperature increases above a certain limit (which can be referred to as the evaporative UCT, Figure 1), increased passive (insensible) evaporation and active (sensible) evaporation or sweating is needed to maintain or reduce body temperature (64). Passive evaporation, characterised by transcutaneous diffusion across the skin and from respiratory surfaces, is most efficient when the water produced to the surface vaporizes completely. With an increasing need for cooling, however, perspiration intensifies and unevaporated sweat may become palpable as a sweat film or dripping sweat on the skin (64). In extreme situations, the adult body can produce up to 2-4 litres of sweat per hour for cooling purposes, which consumes a lot of liquid and salts (65). During and after such strong bouts of sweating, water and mineral replenishment is therefore crucial. The effectiveness of sweating is, amongst other things, determined by the relative humidity of the ambient air. In dry heat, sweat can evaporate easily whereas it is more difficult in humid heat due to the already high saturation of water in the air (37). Evaporation and sweating are, however, largely energy-neutral mechanism: when compared with the overall energy expenditure of the human body, the effect of evaporation and sweating on the metabolic rate is considered negligible.

As a matter of fact, an increase of metabolic rate in the heat is actually counter-intuitive and unfavourable, as even more heat is produced that needs to be released. This underpins the advantage of evaporation and sweating being largely metabolically independent. Interestingly though, if evaporation and sweating are not (alone) responsible for heat-related thermogenesis, other processes must contribute to the raise of energy expenditure in the heat. Here, it needs to be taken into consideration that the schematic depiction of the TNZ presented in Figure 1 has been derived from an animal model. It is not yet fully understood how and to what extent heat-related thermogenesis plays a role in human thermoregulation. However, an early study from 1950 (66) already described increased metabolic rates during high temperatures when compared with the same activity level at cooler temperatures.

A possible explanation for the increase of metabolism in the heat might be found in changed circulatory and respiratory activity: when maximal heat loss is required, the cardiovascular system is put under a considerable strain. Vasodilation of superficial blood vessels, especially in the distal body parts, promotes the dissipation of heat. The induced widening of the blood vessel diameter, however, decreases the total peripheral resistance and thereby significantly lowers blood pressure. In order to ensure optimal blood supply to the target organs and to balance the decrease of blood pressure, the heart rate needs to increase. Accompanied by a heightened demand of oxygen

supply, an increase of breathing rate is also evident. Consequently, both cardiovascular and respiratory responses come along with augmented muscle activity, thereby potentially adding to the energy equation.

Furthermore, another factor contributing to the raised metabolism might be the rate of change of biological or chemical reactions as a consequence to increasing ambient temperature. The so-called Q10-factor denotes a two- to three-fold increase of the metabolic rate when the temperature of the tissues increases by 10˚C. In other words, according to the Arrhenius law, a 1˚C change of mean body temperature might account for an increase of energy expenditure of as much as 8% (67).

Functional physiological adaptation to the thermal environment

When the body is repeatedly exposed to temperatures outside the TNZ, adaptive processes to the specific thermal challenge set in (65). For example, cold acclimation brings along an increase of brown adipose tissue activity, in order to improve the resistance to cold, to reduce the need for shivering and to facilitate the maintenance of Tcore (25, 47). As adaptation to cold is not the primary focus of this thesis, the reader is referred to a number of publications, which discuss the acute and longer-term responses to cold exposure (25, 47, 58, 68).

Whereas the tolerance for cold is relatively limited and largely depends on behavioural support, the human capacity to withstand heat is more advanced (65). A large variety of studies have evaluated the different forms of heat adaptation: 1) the naturally-induced form, which is referred to as *acclimatisation* and derives from long term residence in warm, tropical regions, and 2) the artificially-induced adaptation, referred to as *acclimation* (65).

A lot of heat acclimation studies were conducted in the context of deep mining (69-72), in the military (73, 74) or in elite athletes (75-77). Those target groups have in common that one way or the other, all have to perform physical exercise in sometimes uncomfortably warm or even hazardously hot environments. Essential goals of these (active) heat acclimation studies include making the stay in warm or hot environments for a longer period of time safe, but also optimizing physical performance in the heat. Therefore, physical work is an important part of the study methodology: in the majority of experiments, participants were asked to repeatedly exercise in warm or hot conditions in order to evoke physiological adaptation processes as a response to the physical strain. The combination of an exogenous (high ambient temperature) and endogenous stimulus (high metabolic rate) leads to a substantial increase of body temperature. This disturbance of the *milieu intérieur* is regarded as an important impulse for heat acclimation (65). Key physiological adaptations for a superior tolerance of heat include improved cardiovascular function (cardiac functioning as well as cutaneous vasomotor function), reduced body temperature and more efficient sudomotor function (less sweating on the longer term and better resorption of salts into the sweat gland duct). A detailed review of the key concepts of heat

acclimation and acclimatisation and the different theories, principles and practices of heat adaptation has recently been written by Taylor (65).

In contrast to exercise-induced heat acclimation, only few studies evaluate the influence of passive exposure to heat (78-83). These earlier laboratory studies in humans, examining the isolated effect of an exogenous temperature stimulus, also show significant reductions of core temperature, sweating and cardiovascular function, similar to those observed after active heat acclimation. Some studies used very high ambient temperatures between 45-55˚C (80-82), sometimes also combined with high relative humidity, whereas others applied hot water immersion (83) or vapour-barrier suits (78) in their methods. Recently, two papers with respect to passive heat therapy were added, showing that repeated hot water immersion improves cardiovascular functioning (84, 85).

In addition to laboratory studies, human field studies in naturally-acclimatised Pima Indians reported that the naturally habituated population has a lower sleeping core temperature than matched Caucasians, but the Caucasians exhibited the same change, namely a lower Tcore, following heat acclimation (86). Together, this shows the great plasticity of the thermophysiological system, which not only functions in genetically predisposed populations but also in those usually not residing in warm climates. Although the studies enumerated in the above used an external heat stimulus, they do not resemble temperature challenges encountered, for example, by a (sedentary) person in an overheated office space or dwelling. Due to their methodological nature, it is therefore difficult to draw direct conclusions with respect to a prolonged, repeated stay in a warm thermal environment at, for example, only 35˚C and moderate relative humidity (under 50%). It has been suggested that the homeostatic disturbance may not be great enough to serve as an adaptation impulse and will therefore not lead to physiological adaptations (65, 77). However, publications examining relatively mild conditions and their effect on human thermoregulation and health are lacking. Possibly, this is due to the less explicit pragmatic need for information in this domain. However, the scenario presented in the first part of this introduction clearly stresses the need for more sophisticated information on the effect of passive and relatively mild heat stress on the body: in the context of climate change and global warming, the frequent overheating of buildings and more severe and frequent summer heat waves, it is crucial to investigate the available coping mechanisms of the human body. Therefore, it is of particular interest to study the effect of *passive mild* heat acclimation on thermophysiology in humans.

Heat acclimation as a tool to improve cardiovascular and metabolic health?

In the Western World, we are currently facing a very high prevalence of overweight, obesity, obesity-induced insulin resistance and Type 2 Diabetes: since 1980, obesity has more than doubled worldwide and the number of people suffering from diabetes has risen from 108 million to 422 million in 2014 (87, 88). Overweight and obesity are major risk factors for the development of

Type 2 Diabetes. Current therapy standards fail to effectively tackle the problem: although exercise therapy, healthy diet or a combination of both have been shown to be very effective in preventing and treating metabolic diseases, therapy adherence is often low and long-term weight reduction and improved insulin sensitivity are rarely accomplished (89, 90). Therefore, it is of great importance to explore new horizons to prevent and treat obesity and metabolic diseases effectively, and to keep the rapidly increasing numbers of new cases at bay.

Recently, it has been suggested that regular exposure to warmth might have positive implications for metabolic and cardiovascular health (26). Regular bathing in hot water has been shown to significantly improve cardiovascular function in young, healthy volunteers (84, 85). In a study by Hooper (91) in 1999, it was shown that glucose handling improved significantly in T2DM patients after daily hot baths over the course of three weeks. Furthermore, literature has previously suggested an improvement of diabetes status in the warmer months of the year (92-97).

Hooper, in his first publication, did not indicate which mechanisms might be responsible for the improved glucose clearance. However, later on, an inverse relationship between the expression of 72-kDa heat shock proteins (HSP72) and insulin resistance was found (98, 99). Heat shock proteins (HSPs) are highly conserved polypeptides, so called 'cell chaperones' and play an important role in the protection of cell integrity (100-102). Chronic stress stimuli, for example ultraviolet light exposure, pharmacological stresses or electrical stimulation, have been shown to increase the expression of HSPs in cells (103). Importantly, elevated temperature also leads to an augmented expression of HSP72, which is how Ritossa in 1962 found out about their existence in the first place (104). Therefore, they were given the name 'heat shock proteins', which can be misleading as they not solely respond to extreme temperature disturbances but also to rather moderate temperature increases (100) and other non-temperature related stressors, as mentioned earlier.

A number of studies subsequently indicated the link between acute heat exposure, heat acclimation, elevated HSP72 expression and glucose metabolism (98, 99, 105-111). Alongside with the change in HSP72, also other factors such as c-Jun N-terminal kinases as well as interleukins 6 and 10, which play an important role in (low-grade) inflammation, are altered due to acute and longer-term heat exposure (99, 103, 107, 108, 110-112). However, there is yet no *in-vivo* data available displaying the direct effect of (any type of) heat acclimation on HSP72 expression (and other factors involved in the insulin signalling cascade) in humans and the anticipated impact thereof on insulin sensitivity. Taking all this into consideration, there seems to be a great potential for heat treatment as a new therapy for metabolic diseases, but more research is required to elucidate the underlying relationships. One promising approach in this context might be the design of healthier indoor environments, alongside with the development of tailor-made anti-obesity and insulin-sensitizing temperature interventions.

Behavioural adaptation and the retention of thermal comfort – how to keep a cool head in the heat

The foregoing paragraphs of this chapter have mainly focussed on autonomic thermoregulatory processes, thus *physiological* mechanisms, to maintain a thermal equilibrium of the body, which are controlled by the central nervous system. Another very important but frequently overlooked aspect of thermoregulation is the *conscious behavioural* regulation of body temperature. Interestingly, physiological mechanisms, such as vasomotion, sweating and shivering, have relatively limited capacity, whereas the capability of thermoregulatory behaviour is manifold. Thermoregulatory behaviour (TRB) can be described as the "avoidance of an impending thermal insult, acting to prevent activation of energy- and/or water-consuming autonomic responses" (113, 114). In other words, when we open or close a window, change clothes, seek shadow or retract to shelter, we are actively preventing an undesired shift of (core) body temperature.

Although TRB makes for a substantial part of the human thermoregulatory system, only very little is known about its driving forces and mechanisms (115). It has previously been described that changes of skin temperature and Tcore play an important role (115-118), however, it is not known which thresholds or limits must be reached or exceeded before TRB is actually initiated. Apart from these physiological determinants, subjective perception of a thermal environment is likely to be an important factor. Thermal comfort and thermal sensation are measures of subjective temperature perception and it has previously been shown that TRB is likely to occur, when an individual rates its thermal environment as uncomfortable, too warm or too cold (119).

Regarding the effects of temperature acclimation on thermophysiology, it is likely that also TRB changes due to the repeated exposure to a thermal environment. Strikingly, there is no information available in the literature with respect to the effect of heat acclimation on TRB. To gain important insights for the built environment sector, it is desirable to test the potential for behavioural adaptation to warm indoor conditions.

Local cooling strategies in warm office spaces

As earlier established, the indoor climate of a building has a significant effect on human metabolism, but it can also affect general wellbeing and satisfaction of a person. Uncomfortably warm environments might influence thermal sensation and thermal comfort, cause sleepiness and restrict productivity. Acclimation to a certain thermal environment can alter both conscious and autonomic thermoregulation, which ideally induces greater resilience to non-neutral temperatures. However, this does not necessarily mean that thermal comfort and satisfaction of a person in an overheated office are fully restored in the longer term. Interestingly, recent investigations have confirmed that individually-attuned comfort systems have the potential to not only restore thermal comfort and satisfaction, but simultaneously, they can also save a significant amount of energy (up to 50% compared with overall air-conditioning) (120). The application of

such individually-attuned local cooling may allow for an increase of the overall indoor temperature of a building, without negatively affecting thermal comfort. In order to find the most suitable and effective target body sites, different local cooling strategies need to be evaluated with respect to their effectiveness on thermal sensation and thermal comfort.

Outline of the thesis

The aim of the present thesis is to provide a broad overview of available literature and our new scientific studies on human physiological and behavioural coping and adaptability to warm thermal environments as well as the influence of prolonged exposure to elevated temperatures on human health. **CHAPTER 2** of this thesis addresses the human thermoneutral zone. Here, a new dynamic approach to evaluate the human thermoneutral zone is described. In **CHAPTER 3**, the effect of passive mild heat acclimation (PMHA) on human thermophysiology and metabolism is explored. **CHAPTER 4** describes the influence of PMHA on glucose metabolism and heat shock protein 72 in overweight elderly men. **CHAPTER 5** of this thesis deals with the topics of thermoregulatory behaviour and thermal comfort in the context of PMHA. In **CHAPTER 6**, local cooling, a practical approach on how to improve thermal comfort and thermal sensation in an overheated office environment, is examined. In **CHAPTER 7**, all findings of the previous parts will be discussed, and the overall conclusions of this thesis will be presented.

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Chapter 2

Exploring the human thermoneutral zone – a dynamic approach

In preparation

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ABSTRACT

To date, the human thermoneutral zone (TNZ) remains largely unexplored. Indications exist that the TNZ positioning and width might be influenced by age, body composition and the level of acclimatisation. The objective of the present study was to explore a method to evaluate the individual TNZ, characterized by metabolic lower and upper critical temperatures (LCT and UCT) and to test the effect of passive mild heat acclimation on the position and width of the human metabolic TNZ.

A dynamic protocol consisting of two experimental conditions was designed: starting from a thermoneutral condition (~28˚C), temperature gradually increased from 28.8±0.3 to 37.5±0.6˚C during warming (UP) over the course of 90 min and decreased from 28.8±0.3 to 17.8±0.6˚C during cooling (DOWN) over the course of 120 min. For six participants, temperature increased further to 41.6±1.0˚C during UP. Eleven healthy men (19-31y) underwent UP and DOWN twice, i.e. before and after passive mild heat acclimation (PMHA, 7 days at approximately 33˚C for 6h/day). During UP and DOWN, energy expenditure was measured by indirect calorimetry.

We show that the generally assumed LCT for an average male person of approximately 28˚C does not match the dynamically assessed LCTs in this study, as those were considerably lower in most cases. Distinct inter-individual variation of the positioning of the dynamic LCT was evident. Regarding the metabolic UCT, critical temperatures could not be determined for most participants, due to minor or no increases in energy metabolism during UP. PMHA did not significantly change the positioning of the LCTs. The applied method allowed for the determination of individual dynamic LCTs, however, distinct metabolic UCTs could not be established. For a better understanding of the human UCT, future studies should also include a measurement of evaporative heat loss to allow for a two-factor analysis of both metabolic and evaporative human UCT.

INTRODUCTION

The thermoneutral zone (TNZ) reflects the range of ambient temperatures at which internal temperature regulation is solely achieved by control of dry heat loss, which means that the metabolic rate is relatively constant without regulatory changes in heat production or evaporative heat loss (1, 2).

To date, the TNZ has mainly been studied in (small) animals (3-6). According to the Glossary of Terms for Thermal Physiology (2), the lower critical temperature (LCT) is defined as "the ambient temperature below which the rate of metabolic heat production of a resting thermoregulating tachymetabolic animal must be increased by shivering and/or non-shivering thermogenesis in order to maintain thermal balance". Hence, the relation between ambient temperature and metabolic rate below the LCT can be described according to the classical Newtonian cooling model for an endotherm (3).

For the upper critical temperature (UCT), a distinction between an evaporative UCT and a metabolic UCT is recommended, defined by either an increase of evaporative heat loss or an increase of metabolic rate (2). Gordon *et al.* (6-8) extensively investigated the thermoregulatory properties and TNZ of laboratory rodents. In the author's publications, a distinction has been made between two different UCTs for mice and rats: on the one hand, the UCT can be defined by an increase of evaporative water loss, and on the other hand, by metabolic rate (6). According to the author, evaporative water loss again can be divided in two components: a passive component characterised by passive evaporation and water loss via the skin, and an active component characterised by active water loss through sweating, panting or grooming moisture on the skin (6-8). Whereas the evaporative UCT has been defined as the ambient temperature where passive evaporative water loss increases considerably and additional active evaporative water loss commences, the metabolic UCT has been specified as the ambient temperature at which the metabolic rate increases significantly from the basic metabolic rate (6).

With respect to the human TNZ, limited studies are available (9-14). Few publications present data for the human LCT (15-17), but regarding the human UCT, hardly any information is available. Data derived from the few human studies indicate that the generally assumed human thermoneutral range lies between approximately 28˚C and 32˚C (9, 10). However, earlier research and theoretical considerations suggest that the TNZ differs between individuals, since it is likely to be influenced by body composition, age, and gender (18). Furthermore, temperature acclimatisation might play a significant role, as previously shown in animal studies (19). Despite the earlier efforts to define the human TNZ, its determination remains problematic to this day. In many animal studies, the TNZ has been measured at fixed ambient temperatures in order to establish steady-state energy expenditure values for each temperature, which is relatively easy to

accomplish in small mammals (5, 20, 21). However, due to the great thermal mass and depending on the thermal history, it might take hours to reach a steady state for the human body. Therefore, a stepwise temperature protocol for the study of the human TNZ in ambient air is practically very challenging and is likely to not be feasible for the participants. Other techniques, for example incorporating a water-perfused suit for superior conductivity and thus better temperature transfer, do not necessarily reflect human physiological responses in ambient air and the results of such experiments would thus be difficult to translate to daily living circumstances. Therefore, an alternative method is needed to allow for evaluation of the critical physiological temperatures in ambient air.

Being able to determine the individual TNZ is of relevance to various (scientific) fields. For example, it has recently been suggested that a causal relation might exist between the time spent in thermoneutral environments and increased adiposity (22). Excursions to ambient conditions outside the TNZ can increase energy expenditure and improve glucose metabolism, and subsequently may reduce susceptibility to obesity and Diabetes type 2 (23, 24). Our daily living environment, however, is often controlled very tightly. These tightly controlled indoor ambient conditions have their background in thermal comfort research. According to Hensel (25), the TNZ in resting humans is equal to the zone of thermal comfort. However, in an earlier study, we showed that thermoneutrality and thermal comfort are not necessarily the same (26). Moreover, indications exist that the TNZ can be shifted by acclimation to warm or cold ambient conditions (17, 27). From both health and building energy-use perspectives, it could be highly beneficial to extend the control range at which the indoor environmental temperature is regulated (14, 28, 29). With regard to the constant and undeniable progress of global warming and increasing indoor and outdoor temperatures, it is important to assess the influence thereof on human physiology and health. In order to design a more healthy, sustainable and comfortable indoor environment, it is relevant to obtain more insight into the individual human TNZ and the effect of acclimation to mildly elevated temperatures on the latter.

Also, with respect to the design of studies on metabolism, thermoregulation and cardiovascular aspects, it is relevant to control for the ambient temperature as a factor of influence and to account for individual differences in the TNZ. Ideally, the ambient temperature that participants are exposed to during an experiment should be individually attuned to each person, depending on the goal of the respective study (18). However, to be able to account for thermal neutrality and to potentially adjust the ambient conditions to an individual, it should be identifiable whether a person is actually situated within their TNZ.

In summary, the TNZ, based on metabolic rate, has been studied thoroughly in animals. For humans, however, the understanding of the TNZ as well as possible individual differences remains very limited (30). Therefore, the objective of the present study was to explore a practical method to determine the positioning and width of the individual human TNZ. As described in the above,

steady state situations are not easily met and practically challenging, which is why we chose to apply a pragmatic solution by measuring LCT and UCT during dynamic temperature conditions. Moreover, considering the possible influence of temperature acclimation on the width and positioning of the individual TNZ, we aimed to measure both individual LCTs and UCTs before and after passive mild heat acclimation (PMHA).

The goal of the present study was to explore the *metabolic* human TNZ (Figure 1). Hence, the limits determining the TNZ, i.e. the lower and upper critical temperatures (LCT and UCT), were studied according to the changes in metabolic rate upon changes in ambient temperature (Figure 1).

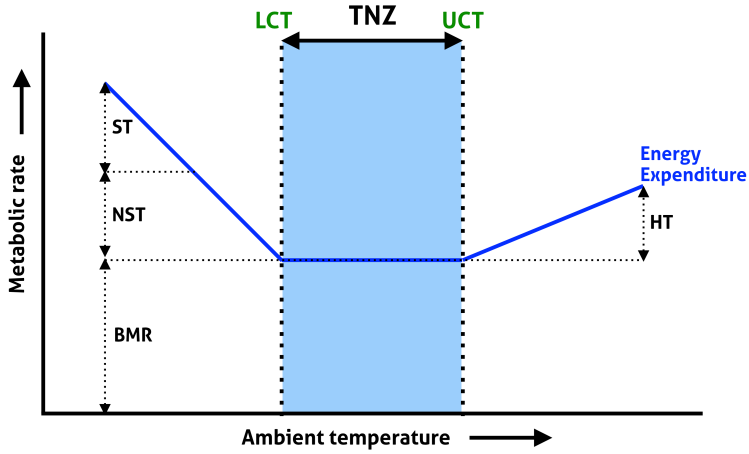

Figure 1 | The thermoneutral zone

The thermoneutral zone (TNZ) reflects the ambient temperature range wherein the energy expenditure is at basal level (basal metabolic rate, BMR) and there are no thermoregulatory changes in metabolic rate (non-shivering thermogenesis, NST; shivering thermogenesis, ST; heat-related thermogenesis, HT). In the present paper, metabolic rate is used to describe the TNZ. As shown in the graph, below the lower critical temperature (LCT) and above the upper critical temperature (UCT), metabolic rate (heat production) is expected to increase due to a respectively decrease or increase of the ambient temperature. The graph is derived from animal studies (adapted from (6)).

METHODS

Eleven healthy, white Western European male volunteers participated in this study. Participants were given detailed information regarding the purpose and the methods of the study before written informed consent was obtained. The protocol was approved by the ethics committee of Maastricht University Medical Center+ and designed and performed according to the Declaration of Helsinki (Fortaleza, Brazil, 2013). The study was conducted in the period of December 2014 till August 2015, as part of a larger experiment. In the following, only those methods and results with importance for the aim of this study are presented.

Table 1 | Participant characteristics

	Mean±SD
Age (year)	24.6 ± 2.7
Height (m)	1.79 ± 0.07
Area (m^2)	1.90 ± 0.13
Body mass (kg)	72.2 ± 8.9
BMI (kg/m^2)	22.6 ± 2.9
Body fat% (%)	19.7 ± 3.0
Fat mass (kg)	14.5 ± 3.3
Data is presented as mean±SD, N=11.	

Experimental conditions

Passive mild heat acclimation and dynamic temperature protocols

Participants underwent a passive mild heat acclimation (PMHA) protocol, which consisted of 7 consecutive days of PMHA at 33.3±1.6˚C for 4h at acclimation day 2 and 6h per day for the remaining 6 days (Figure 2). Before and after PHMA, subjects were exposed to two thermal conditions: an increasing and a decreasing temperature ramp, respectively UP and DOWN (Figure 3). For both conditions, the protocol started at a constant temperature of 28.8±0.3˚C for the first 60 minutes, which served as a baseline measurement. Given the boundary conditions, this baseline temperature has been assessed as neutral based on a literature review of Kingma *et al.* (18) for humans in a resting, semi-nude state. It has been adjusted for the insulation of the

stretcher on which participants lay in supine position. The first 30 minutes of both conditions were regarded as familiarization period.

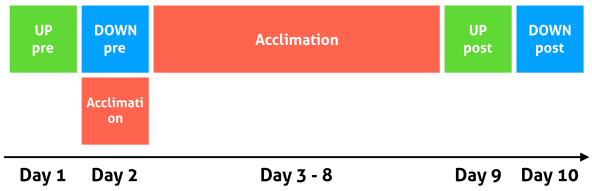

Figure 2 | Study time line

To cover a temperature range that expectedly includes the human TNZ (which has previously estimated to be positioned between approximately 28-32˚C (10)), the ambient temperature increased from 28.8±0.3 to 37.5±0.6˚C during UP over the course of 90 min and decreased from 28.8±0.3 to 17.8±0.6˚C during DOWN over the course of 120 min (Figure 3). For the last 6 study participants, an additional 30 min of ramp was added subsequently to the UP protocol to cover an even wider temperature range. This was due to the fact that during the experiments, it appeared that the higher temperature range did not induce increases of metabolic rate. On average, the final temperature that was reached for these last 6 participants during UP was 41.6±1.0˚C (averaged over the last 10 min). As the formal acclimation terminated after day 8 of the study protocol, the UP protocol at day 9 was regarded as an additional heat stimulus, which supposedly prevented a possible decay of potentially acquired physiological adaptation before the last DOWN measurement.

If severe shivering, paired with a noticeable increase of metabolic rate, occurred during DOWN, the measurement was terminated prematurely. Moreover, the measurement was terminated when participants reported strong discomfort, which only occurred during DOWN but never during UP. For the DOWN protocol, before PMHA six measurements were stopped early and five after PMHA. Due to technical problems with the climate chambers during DOWN in three cases, data of these participants was excluded from analysis.

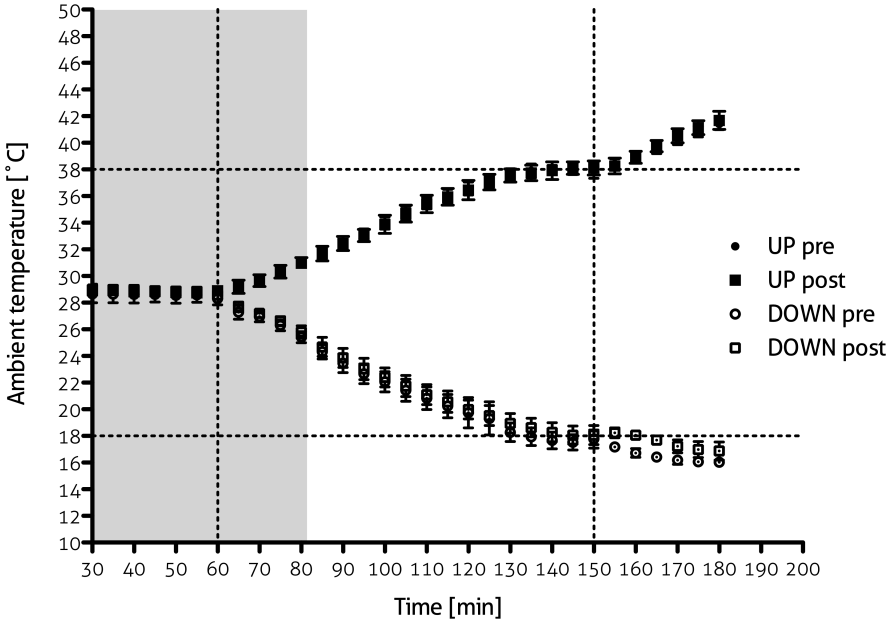

Figure 3 | Dynamic temperature ramp protocols
Data is presented as mean±SD over the course of 5min per time point. UP: N=11 until t=150 and N=6 until t=180, DOWN: N=8 until t=120, where after participants started to drop out due to severe shivering. For DOWN pre, 2 out of 8 participants completed the full 180 min and for DOWN post, 3 out of 8 participants reached t=180.

Air temperature and relative humidity (Hygrochron iButton, DS1923, Maxim Integrated Products, USA,) were measured according to EN-ISO 7726 (31) at 1-minute intervals at 0.1m, 0.6m, 1.1m and 1.7m height. Relative humidity (RH) was allowed to drift with changes in temperature, resulting in an average RH of 25.82±7.18% during UP and 35.31±8.71% during DOWN.

During the measurements, participants were situated in a semi-supine position on a stretcher with air-permeable fabric, wearing underpants only (0.05 clo (32)). They were allowed to watch television, but they were instructed to refrain from any movement. During one of the measurement days, body composition was determined by means of a DEXA-scan (Discovery A, Hologic Inc.).

Data analysis
The first 30 minutes of both conditions were excluded from the data analyses since this was regarded as a familiarisation period. For the analyses of energy expenditure, data was averaged

over one minute. The commercially available software package PASW Statistics 21.0 for Mac (SPSS Inc.) was used for the statistical data analyses. Differences in physiological parameters before and after passive mild heat acclimation, i.e. core temperature, mean skin temperature and proximal and distal skin temperatures, were tested using paired-sample t-tests. Correlations between energy expenditure, physiological parameters, and body characteristics were assessed using Pearson correlations. Significant effects are reported for $P<0.05$. Determination of the lower and upper critical temperatures of the TNZ was performed using MATLAB 2012a for Mac (The Mathworks Inc.).

Determination of the individual human TNZ and definition of LCT and UCT
The objective of this study was to explore the positioning and width of the individual human TNZ. The LCT and UCT were determined based on the individual metabolic rate.

According to the Newtonian cooling model (3) (assuming a constant body core temperature and 'perfect' thermoregulation), the steady state relationship between the ambient temperature (Ta) and the metabolic rate (MR) that are needed to sustain a stable body core temperature can be described by the application of two linear functions:

(1) For ambient temperatures lower than the LCT: $MR = -a_1 T_a + b_1$

(2) For ambient temperatures between LCT and UCT (TNZ): MR = constant

Function (1) describes a situation where the body increases its metabolic rate to maintain stable core temperature (below LCT) and function (2) is applicable when the body is capable of maintaining a stable core temperature only by the modification of tissue insulation (TNZ). Beyond the UCT, increased metabolic rate is in contradiction with the Newtonian cooling model. Nevertheless, as the metabolic rate is expected to increase above the UCT, we applied a similar approach:

(3) For ambient temperatures higher than the UCT: $MR= a_2 T_a + b_2$

Here, the LCT was assumed to be equal to the point of intersection of function (1) and (2) and the UCT as the intersection of function (2) and (3) respectively.

For each individual participant, the three functions were fitted through the measured data points simultaneously. The 'best fit' for each function was determined by the least squares method. For the determination of metabolic LCT and UCT, energy expenditure relative to the resting metabolic rate (RMR, as measured during baseline period) was applied, and presented as a moving average over a triangular window of 10 min. The resulting LCT and UCT were counter-checked by subjective observation of the measured data for each individual dataset.

Physiological measurements
For both protocols UP and DOWN, participants arrived at the laboratory in the morning after an overnight fast (as of 22:00h at the evening before). At all four days before the measurements took place (day 0, day 1, day 6 and day 7), participants consumed the same standardised evening meal, as chosen by them on the evening before the first measurement day.

Upon arrival at the laboratory, participants ingested a telemetric pill (Vital Sense, Philips Healthcare, NL) to measure core temperature. To detect the signal of the telemetric pill, an Equivital apparatus was attached to the participant's body using a chest strap (Equivital Hidalgo, UK). The same device was used to record heart rate. Wireless skin temperature sensors (iButton, Maxim Integrated Products, California, USA) were attached to 14 ISO-defined body sites with semi-adhesive tape (Fixomull stretch, BSN medical GmbH, GER) to measure mean skin temperature. After preparations, participants took place on a stretcher (approximately 0.15clo) in the climate chamber. A face mask was attached to measure energy expenditure continuously by means of indirect calorimetry using a facemask (Omnical, Maastricht Instruments, NL). Energy expenditure, i.e. metabolic rate, was calculated according to Weir (33), using the measured consumed oxygen and produced carbon dioxide which were multiplied by a factor 4.186 to convert data into kJ/min. Core temperature, heart rate and skin temperatures and energy expenditure were recorded at 1-min intervals. Physiological data for core and skin temperature as well as cardiovascular parameters for the UP protocol have been reported in an earlier publication (27).

RESULTS

Individual LCTs and UCTs

Table 5 provides an overview of all LCTs and UCTs. The individual critical temperatures were assessed before and after PMHA. Figure 4 shows examples of the energy expenditure curve of five representative participants, as measured during UP and DOWN, before and after PMHA.

Overall, in most participants, the LCT was observable as a strong inflection point in the relation between energy metabolism and air temperature, and, except for one occasion, LCTs could be calculated by the application of the functions as described in the above (Figure 4, Table 2). As for the UCT, most participants did not exhibit a clear inflection point of energy expenditure with increasing ambient temperature. Few participants show a gradual small increase, mostly starting from the baseline temperature. However, several participants did not show an increase of

metabolic rate during UP at all (Figure 4). One participant showed no detectable increase of energy expenditure - neither during UP, nor during DOWN (Table 2, Figure 4D).

Due to the very few identifiable UCTs, only LCTs were statistically analysed to evaluate the effect of PMHA. Interestingly, there was no significant influence of PMHA on the positioning of the LCT (P=0.962). RMR as measured during baseline was 4.82±0.52kj/min before PMHA and 4.79±0.61kj/min after PMHA. RMR was not significantly altered by PMHA (34).

To assess the influence of individual characteristics and body composition on the results of the LCT position, the correlation with age, fat percentage, fat mass, lean mass, height, weight, and BMI were analysed for LCT and UCT, both before and after PMHA. No significant correlations were found between demographic characteristics and the positioning of the LCT before or after PMHA.

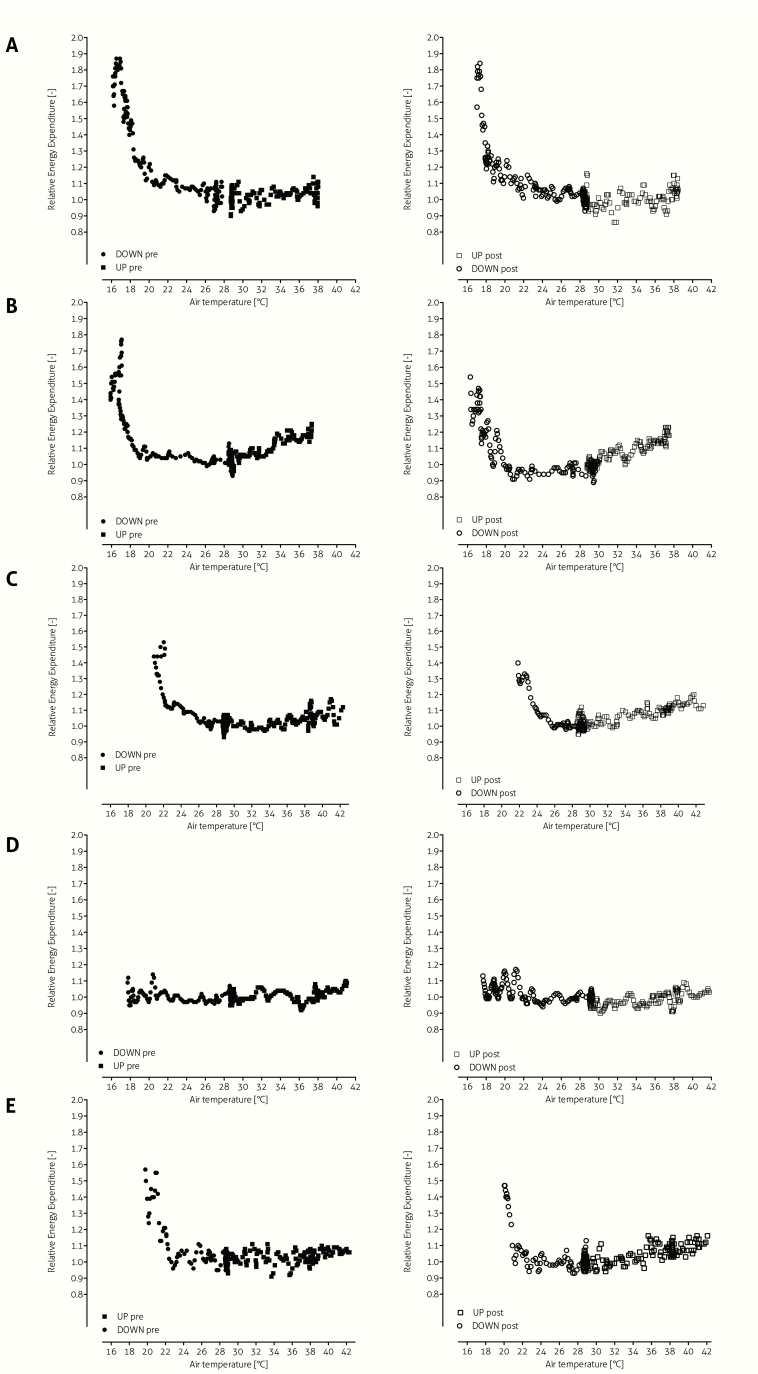

Figure 4 | Relative energy expenditure during UP and DOWN in relation to ambient temperature
Five representative participants (A, B, C, D and E) pre (left hand side) and post (right hand side) PMHA.

Table 2 | Critical temperatures before and after PMHA

Participant ID	LCT pre [°C]	LCT post [°C]	UCT pre [°C]	UCT post [°C]
1			/	/
2 (A)	20.4	24.0	/	/
3			/	/
4 (B)	19.2	21.2	29.0	29.8
5 (C)	24.6	25.0	/	/
6	22.8	22.1	/	/
7 (D)	/	/	/	/
8			/	/
9 (E)	22.9	21.2	/	31.2
10	28.9	23.4	/	/
11	24.3	26.6	/	/

Slash = no detectable inflection point, grey field = no data obtained, *LCT* lower critical temperature, *UCT* upper critical temperature. The letters next to the participant numbers refer to the graphs shown in Figure 4.

DISCUSSION

The objective of the present study was to explore and test a new dynamic method to identify the positioning of the lower and upper critical temperatures and the width of the human metabolic TNZ. Moreover, this study aimed to assess the effect of passive mild heat acclimation on the (dynamic) human TNZ, to evaluate the impact of acclimation to elevated temperatures on the critical temperatures.

The results of this study show that for the great majority of participants, a clearly observable and distinct increase of metabolic rate was evident when temperatures decreased, allowing for the calculation of the individual LCTs. However, during warming, several participants showed only a very slight and gradual increase of energy metabolism, whereas others did not exhibit observable changes of the metabolic rate at all. Hence, a clear inflection point for the metabolic UCT was not identifiable and therefore not quantifiable in most cases.

Interestingly, there appeared to be considerable differences in metabolic response to decreasing ambient temperatures *between* the individuals, denoted by the wide range of LCTs observed both before and after PMHA (temperature range: pre: 9.7˚C, post: 5.4˚; Table 2). Interestingly, the results furthermore show that repeated measurements lead to an individual characteristic metabolic response over time for both UP and DOWN protocols (Figure 4).

With respect to the effect of PMHA on the TNZ, no changes of the metabolic rate, neither basal nor during warming or cooling were evident after acclimation. For a detailed physiological analysis of the UP protocol see our earlier publication (27).

Assessing the individual thermoneutral zone in humans

The TNZ for small mammals has been described in detail by Scholander (3) and later modified by Gordon (6). However, hitherto, the width and positioning of the human TNZ has been difficult to measure. An earlier study by Mekjavic *et al.* (35) established that core temperature thresholds for shivering and evaporative heat loss are significantly different from each other. This indicates that a so-called 'null-zone' exists, a core temperature range with a magnitude of ~0.6˚C, at which no sweating or shivering occurs. Importantly, the present study aimed to identify the *metabolic* TNZ, which is not assessed by measuring the critical ambient temperatures at which core temperature changes, but those at which changes of the metabolic rate are observed. With respect to the *metabolic* TNZ, several attempts have been made to determine the critical limits (9-14), whereof one in water (12) and one in naked babies (13), but structural and sufficient data is lacking.

One of the major issues concerning determination of the human TNZ is the great thermal mass of the human body. Hypothetically, in order to determine the metabolic rate characteristic for a specific ambient temperature, an individual would have to remain in that particular ambient condition until thermal equilibrium is achieved. However, in order to test a wide range of ambient temperatures (e.g. 18˚C to 41˚C), a person would have to remain in a resting and fasted state for many hours, which is extremely time consuming and practically very difficult. Additionally, circadian physiological changes, such as increasing and decreasing core temperature during the course of the day, might affect energy expenditure, which would further complicate the procedure. Consequently, in order to bypass these practical issues, we explored a dynamic approach to test the human TNZ, consisting of two temperature ramp protocols as described in the methods section above (2.1.1).

In the literature, the metabolic TNZ for males in air has been suggested to range between 28-32°C (9, 10). Hill *et al.* (14) has reported a lower LCT of 26-27°C for naked humans. Furthermore, a study using a biophysical model has suggested that the steady state human TNZ might even span

from 26°C to 33°C (26). However, the ranges listed above are from experiments that were conducted with a very limited number of subjects and were not all specifically designed to uncover the human TNZ. Importantly, sex has been suggested to have a significant influence on both LCT and UCT, considering the fact that females are more susceptible to heat loss due to a larger surface to mass ratio and also have a lower metabolic rate (up to -35%) (36). Hence, the female TNZ might be shifted to higher ambient temperatures. Apart from gender, also body composition and age are strong determinants for the width and positioning of the individual TNZ (18).

LCT

As for the LCT, the applied protocol indeed allowed for the evaluation and calculation of the critical inflection points of metabolic rate during decreasing temperatures (Table 2). For 1 out of 8 participants, metabolic rate did not increase it was therefore not possible to determine an LCT, neither before nor after PMHA (Table 2, Figure 4D).

The great majority of LCTs measured in the present study were situated at considerably lower temperatures than those reported in the literature (Table 2). All but 2 calculated LCTs, for both pre- and post-PMHA, were lower than 26˚C. One possible explanation for this might be the insulation of underwear (0.05clo) and the stretcher (0.15clo) used during the ramp protocols. The estimated insulation value for underwear and stretcher together approximates 0.2clo, which might lower the anticipated LCT by as much as 3°C.

The most likely explanation is the dynamic nature of our protocols. During the ramps, temperature drifted with approximately 10K/h. During static exposure at a certain low temperature, heat loss may be still higher than heat production, which, over time, might results in extra heat production by NST or ST. The relatively short time span spent in one specific temperature due to the temperature ramp, therefore, assumedly shifts this metabolic increase to lower temperatures. In other words, the body might not face the same amount of heat loss when a specific ambient temperature is reached during the dynamic protocol as under static conditions at the same ambient temperature. In conclusion, the LCTs obtained in the present study must be considered as 'dynamic metabolic LCTs', as they might differ from hypothetical static values. The benefit of a dynamic protocol is, however, that it is relatively easy to implement (compared to static protocols) and it may moreover be a more realistic approach for the comparison with every-day life situation, which are often not static. For scientific purposes, characterisation of subjects and environments, and for comparisons between studies, standardisation of such dynamic protocols are desirable.

UCT

Interestingly, as opposed to the clearly observable LCTs, the increase of metabolic rate was much less pronounced or even completely absent during warming. Several participants exhibited a slight gradual increase of metabolic rate (for example Figure 4B (pre and post), but for other

participants, the metabolic rate was not affected by the increasing ambient temperatures (for example Figure 4A and 4D). Hence, using the calculation methods applied in this study, it was only possible to quantify one complete set of UCTs before and after PMHA (Table 2, Figure 4B) and for one more participant after PMHA only (Table 2, Figure 4E).

When considering the difference between humans and non-primate mammals, the latter only have eccrine sweat glands (the type of sweat glands primarily involved in thermoregulation) on their hand palms and foot soles (with only very few exceptions, for example horses). Therefore, their capacity for active evaporative water loss through sweating is much smaller than those for humans, considering the fact that humans have eccrine sweat glands spread over the greatest part of their skin surface (6, 7). Non-primate mammals, however, can still employ other mechanisms to actively wet the surface of their skin for cooling purposes, such as grooming moisture on the skin or panting. However, these behavioural activities inevitably affect the metabolic rate, which is why they also play a role for the metabolic UCT. On the contrary, the human evaporative UCT is predominately determined by active sweating and not by other behavioural (metabolically costly) strategies for active evaporative water loss as engaged by, for example, mice and rats.

Which mechanisms actually cause the metabolic increase in humans during warming is not completely understood. As sweating is a largely energy-neutral process, it can be concluded that sweating is not likely to influence the metabolic response to increasing ambient temperatures. Possibly, a combination of increased heart- and ventilation rate due to increasing ambient temperatures partly accounts for the increase of energy expenditure in humans. In this study, a significant increase of heart rate during the temperature ramp was not evident (27) and breath rate was not measured. Another possible factor of influence for the gradual and slow increase of energy expenditure during warming might be found in the Q10 effect. According to the Arrhenius law, a 1°C change in mean body temperature might account for an increase of the energy expenditure of as much as 8% (assuming Q10-factor = 2.3) (37). Hence, if observed, the increase of energy expenditure during warming might have been due to a combination of several factors, but the exact magnitude and mechanism of the energy expenditure increase remains uncertain.

It might therefore be concluded that the human (dynamic) LCT can be defined based on metabolic rate, whereas the human UCT should not solely be assessed based on energy metabolism, but by the evaluation of both the evaporative and metabolic UCT.

Individual variation of the LCT

Our data suggests that despite the relatively narrow inclusion criteria and comparable participant characteristics in the present study, metabolic responses to both decreasing and increasing temperatures varied greatly. The latter suggests that parameters other than body size, age, gender and acclimation are very likely to influence the individual TNZ. This is also supported by the fact

that no significant correlations could be obtained between the calculated LCTs and demographic characteristics such as height, weight, BMI, %body fat and fat mass.

Limitations

It was not possible to detect critical temperatures for all participants, which was due to one or more of the following reasons: Firstly, we encountered a technical problem with the air conditioning in our respiration chamber in three cases, which is why the data of the DOWN protocol of these three participants had to be excluded from analysis. Secondly, the ambient temperatures applied during the temperature ramps were, at least in some cases and specifically during UP, not extreme enough to exceed the critical temperatures. With respect to future studies of the human TNZ, it is essential to ensure that the applied temperature protocols are sufficiently broad to allow for an assessment of the full metabolic (and evaporative) response.

Future perspectives

In order to adequately measure metabolic LCT and metabolic as well as evaporative UCT of the human TNZ, future studies should broaden the covered range of air temperatures and attune the protocol to each individual, as such that for every measured participant, the critical temperatures, especially the UCTs, are reached and thus made identifiable. Future TNZ studies should also incorporate a measure of evaporative water loss additionally to the assessment of energy expenditure. Moreover, heart- and breath rate should be assessed to investigate their involvement in the metabolic response to increasing ambient temperatures. Additionally, a slower increase of the ambient temperature should be tested to evaluate the influence of the temperature slope (i.e. the effect of time in the respective ambient condition) on the metabolic response as well as the width and positioning of the human TNZ.

Future research is needed to gather important yet unavailable information on the TNZ of women, and, for example older age groups and obese persons or patients with type 2 diabetes mellitus (T2DM). The latter is of great significance as an increasing proportion of the World population suffers from metabolic diseases such as T2DM. Presumably, these conditions influence the range and position of the TNZ, due to, for example, altered thermophysiology and changes of body composition.

Conclusion

The results of the present study show that the generally assumed LCT of approximately 28˚C, which has earlier been indicated in the literature, does not match with the dynamically assessed LCTs in this study, as those were considerably lower in most cases. PMHA did not significantly change the positioning of the LCT, indicating that the relatively mild and passive acclimation to heat does not affect energy metabolism. Substantial individual variation of the positioning of the dynamic LCT was evident between the participants, both before and after PMHA. The latter could not solely be explained by age or body composition, as these parameters did not correlate with the obtained LCTs.

As for the measured metabolic UCT, a distinct inflection point could not be determined for the great majority of participants, which was due to the relatively small or even absent metabolic response to increasing ambient temperatures.

In conclusion, the applied dynamic method allowed for the determination of individual dynamic LCTs, however, distinct metabolic UCTs could not be established in the present study. Suggestions for protocol adjustments for the determination of the dynamic UCT are discussed.

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Chapter 3

Thermophysiological adaptations to passive mild heat acclimation

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ABSTRACT

Passive mild heat acclimation (PMHA) reflects realistic temperature challenges encountered in everyday life. Active heat acclimation, combining heat exposure and exercise, influences several important thermophysiological parameters; for example, it decreases core temperature and enhances heat exchange via the skin. However, it is unclear whether PMHA elicits comparable adaptations. Therefore, this study investigated the effect of PMHA on thermophysiological parameters.

Participants were exposed to slightly increased temperatures (~33˚C/22%RH) for 6 h/d over 7 consecutive days. To study physiologic responses before and after PMHA, participants underwent a temperature ramp (UP), where ambient temperature increased from a thermoneutral value (28.8±0.3˚C) to 37.5±0.6˚C. During UP, core and skin temperature, water loss, cardiovascular parameters, skin blood flow and energy expenditure were measured. Three intervals were selected to compare data before and after PMHA: baseline (minutes 30–55: 28.44±0.21˚C), T1 (minutes 105–115: 33.29±0.4˚C) and T2 (minutes 130–140: 35.68±0.6˚C).

After 7 days of PMHA, core (T1: -0.13±0.13˚C, P=0.011; T2: -0.14±0.15˚C, P=0.026) and proximal skin temperature (T1: -0.22±0.29 C, P=0.029) were lower during UP, whereas distal skin temperature was higher in a thermoneutral state (baseline: +0.74±0.77˚C, P=0.009) and during UP (T1: +0.49±0.76˚C, P=0.057 (not significant), T2: +0.51±0.63˚C, P=0.022). Moreover, water loss was reduced (-30.5±33.3ml, P=0.012) and both systolic (-7.7±7.7mmHg, P=0.015) and diastolic (-4.4±4.8mmHg, P=0.001) blood pressure was lowered in a thermoneutral state. During UP, only systolic blood pressure was decreased (T2: -6.1±4.4mmHg, P=0.003). Skin blood flow was significantly decreased at T1 (-28.35±38.96%, P=0.037), yet energy expenditure remained unchanged.

In conclusion, despite the mild heat stimulus, we show that PMHA induces distinct thermophysiological adaptations leading to increased resilience to heat.

INTRODUCTION

Heat acclimation studies typically report changes of physiological parameters, for example of core and skin temperature and sudomotor functions, in rest as well as during exercise (1-8). Different approaches of heat acclimation have been tested in the past, mostly to develop optimal heat adaptation models for miners, athletes or the military. It is generally believed that a relatively strong (heat) stimulus is needed to catalyse the anticipated changes, and therefore, most heat acclimation studies combine exposure to high ambient temperatures and exercise ('active heat acclimation'), to ensure the effectiveness of the intervention (8).

Passive heat acclimation, i.e. without exercise, is a phenomenon likely to occur in everyday situations, for example due to prolonged occupancy of an overheated building (9), during a holiday in a warm country or simply during a warm summer or a heat wave. Considering the progress of global warming, the occurrence of those events is likely to be more frequent, even in European oceanic and humid continental climates (Köppen climate classification) (10-12). However, only few studies have evaluated the effect of an external 'passive' heat stimulus on human thermophysiology alone (13-20), without additional elevated endogenous heat production.

Those earlier laboratory studies investigating passive heat acclimation applied, for example, a combination of heat exposure and vapour-barrier suits (13) or hot water immersion (18-20) to induce controlled hyperthermia. Other studies incorporated prolonged exposure to high ambient temperatures between 45˚C and 55˚C (15-17). Such passive heat acclimation results significant reductions of core temperature and sweating and improved cardiovascular function, indicating increased resilience to heat. A study in mice has, furthermore, shown that also prolonged passive exposure to a relatively mild ambient temperature (5 days, approximately 37˚C) elicits physiological changes such as a decreased core temperature during heat exposure (21). Human field studies show that naturally acclimatised Pima Indians have a lower sleeping core temperature than matched Caucasian counterparts (22). However, more structured information considering passive *mild* heat acclimation, without the induction of controlled hyperthermia and only induced by the exposure to warm ambient air, is lacking.

Recently, it has been suggested that regular exposure to warmth might also have important implications for metabolic and cardiovascular health (23). In rats, it has been shown that heat treatment improves glucose tolerance (24) and shifts obesity-induced insulin resistance back to normal, healthy insulin sensitivity (25). Heat therapy by means of water immersion in young healthy volunteers has been shown to significantly improve cardiovascular function (19, 20). However, it is not yet known if exposure to warm ambient air elicits comparable health effects in humans. It is therefore of particular interest to study the effects of passive heat acclimation on

energy metabolism and cardiovascular parameters, as it might help to understand how temperature exposure could possibly contribute to the treatment of metabolic and cardiovascular disorders.

Considering the very limited knowledge of the effects of passive mild heat acclimation on thermophysiology in humans, the main objective of the present study was to evaluate the effect of passive mild heat acclimation on core temperature, skin temperature, water loss, cardiovascular parameters, energy expenditure and skin blood flow.

METHODS

This study was conducted in the period of December 2014 till August 2015. In this period, the average day outdoor temperature as recorded 2 weeks previously to the start of each individual measurement ranged between 1.5˚C and 20.2˚C (11.5±7.1˚C mean±SD). The Medical Ethics Committee of Maastricht University approved the study and it was conducted conform with the Declaration of Helsinki (Fortaleza, Brazil, 2013).

Participant characteristics

In total, 11 healthy male Caucasian volunteers participated in the study (Table 1). All participants were normotensive, non-obese, non-smokers and did not take any medication that might have influenced the thermoregulatory system. Before commencing the study, all participants were provided with detailed information regarding the purpose and the methods of the study. All gave written informed consent.

Table 1 | Participant characteristics

	Mean	± SD
Age [years]	24.6	2.7
Height [m]	1.79	0.07
Weight [kg]	72.2	8.9
BMI [kg/m2]	22.6	2.9
Fat percentage [%]	19.7	3.0
Fat mass [kg]	14.5	3.3
Habitual physical activity [Baecke score total]	8.1	1.4
N=11, data is presented as mean±SD.		

Study design

Participants were exposed to 7 days of mild passive heat acclimation (PMHA) (Figure 1A). To study the physiological response to high temperatures, participants underwent an increasing temperature ramp before and after PMHA, which will be referred to as UP (Figure 1A and B).

UP protocol

For protocol UP, participants arrived at the laboratory in the morning after an overnight fast (as of 22:00h). Both evenings before the UP measurements took place, participants consumed a self-chosen standardised evening meal.

UP started with a baseline period of 60 minutes at 28.8±0.3˚C (Figure 1B). The baseline temperature was assumed to be the neutral temperature for a resting semi-nude person, based on the literature review of Kingma et al (26) and it was adjusted for the isolation of the stretcher that participants lay on during the measurements (Figure 2). After the baseline period, operative temperature increased over the course of 90 minutes to 37.5±0.6˚C (Figure 1B). Relative humidity drifted with changes in temperature, resulting in an average relative humidity of 25.8±7.2% during UP. Three time-intervals were selected to compare data before and after PMHA (protocol time and ambient temperature in brackets): baseline (minutes 30-55: 28.4±0.2˚C), T1 (minutes 105-115: 33.3±0.4˚C) and T2 (minutes 130-140: 35.7±0.6˚C) (Figure 1B).

Physiological measurements

Upon arrival at the laboratory, participants ingested a telemetric pill (Vital Sense, Philips Healthcare, NL) to measure core temperature. To detect the signal of the telemetric pill, an Equivital apparatus was attached to the participant's body using a chest strap (Equivital Hidalgo, UK). The same device was used to record heart rate. To calculate mean skin temperature, wireless skin temperature sensors (iButton, Maxim Integrated Products, California, USA) were attached to 14 ISO-defined body sites (27) with semi-adhesive tape (Fixomull stretch, BSN medical GmbH, GER). Proximal skin temperature was calculated as an average of the ISO-defined sites of scapula, lower back paravertebral, upper chest and abdomen. For the distal skin temperature, skin temperatures of hand and instep were averaged. Core temperature, heart rate and skin temperatures were recorded at 1-minute intervals.

In the climate chamber, participants took place on a stretcher with air-permeable fabric (Figure 2). Here, Laser Doppler Flowmetry (LDF) probes were fixated to the participant's thenar and ventral side of the underarm halfway between carpus and antebrachium, to continuously measure skin blood flow (10Hz; PeriFlux System 5000, Perimed, SE). A finger blood pressure cuff was attached to assess cardiac output (CO) (Finometer MIDI, Amsterdam, NL) at baseline, T1 and T2, and upper arm blood pressure was measured at the same time points on the other arm by auscultation (Medisana MTP, Medisana AG, GER). Energy expenditure was continuously measured using indirect calorimetry with a respiratory gas analyser. Metabolic rate was calculated using the method of Weir (28) from the consumption of oxygen and the production of carbon dioxide. Immediately before entering the climate chamber and after leaving it, participants were weighed to determine total water loss, using the difference in total body mass before and after the UP protocol.

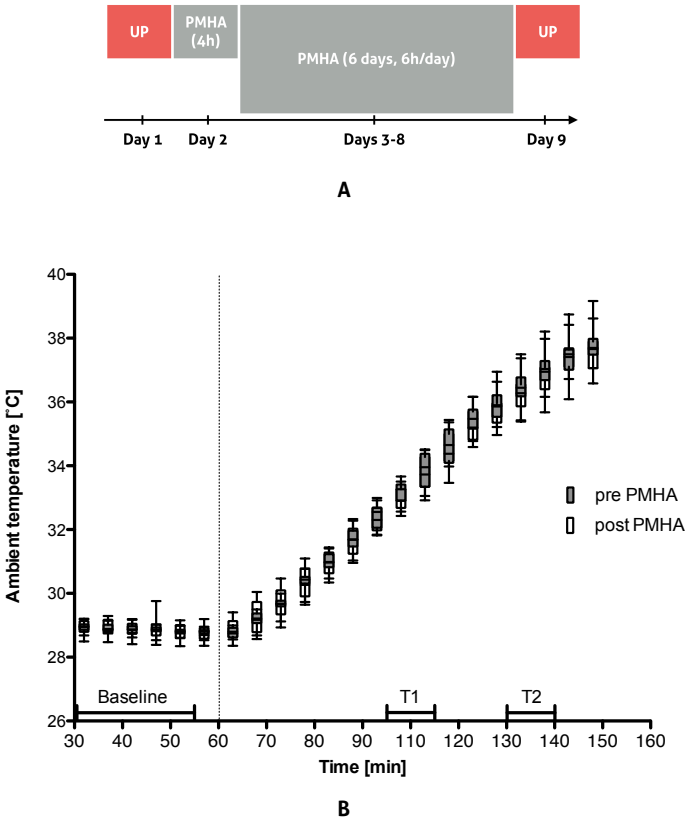

Figure 1A and B | Overview of the study procedures and experimental conditions during protocol UP

(A) PMHA refers to passive mild heat acclimation. UP refers to the temperature ramp protocol as described in the methods section. During UP participants were exposed to an incremental ambient temperature ramp (28.8±0.3˚C to 37.5±0.6˚C), (B) Each boxplot represents a 5-minute interval of the ambient temperature after the start of the temperature drift. Whiskers indicate ±1 SD. Baseline (28.44±0.21˚C mean±SD), T1 (33.29±0.4˚C) and T2 (35.68± 0.61˚C) represent the respective intervals used for data analysis. N=11.

Passive mild heat acclimation

PMHA commenced in the noon of study day 2 (Figure 1A). During the first sequence of PMHA, participants stayed in a 'warm chamber' for 4 hours. During the remaining 6 days of PMHA, participants acclimatised for 6 hours per day. From earlier active heat acclimation studies, we know that the most important changes are expected to occur within the first 4-6 days of heat exposure. Therefore, we applied a 7-day acclimation protocol (29). The operative temperature in

the warm chamber was kept constant at 33.3±1.6˚C; and the relative humidity was 22.3±6.6%, which classifies the ambient air as dry. All participants successfully completed the passive mild heat acclimation period.

During their stay, participants remained seated at a desk and were allowed to perform regular office work (1.2METs). Participants wore standardised clothing composed of underwear, T-shirt, shorts and socks/slippers. The total thermal resistance of the clothing ensemble plus the desk chair added up to approximately 0.41clo (30, 31). Participants had unlimited access to water; and food was provided upon request, to not influence habitual diet. Participants were allowed to leave the warm chamber for toilet breaks.

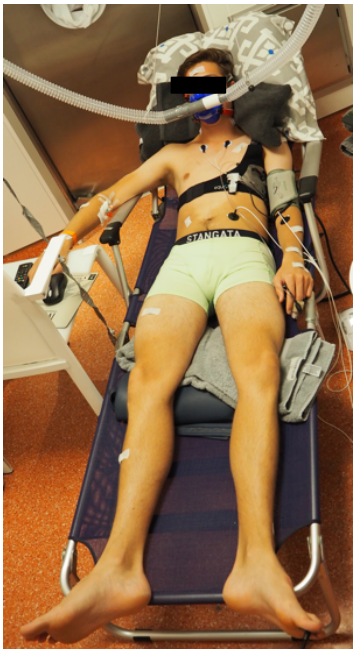

Figure 2 | Participant in a climate chamber during UP

Data analysis

The software package PASW Statistics 22.0 for Mac (SPSS Inc.) was used for the statistical analysis.

Physiological data
The first 30 minutes of protocol UP were regarded as familiarisation period, and therefore excluded from the data analysis. For the comparisons of core temperature, skin temperatures, energy expenditure and skin blood flow within each of the protocols and before and after PMHA, three periods were selected during UP: baseline (t=30-55 minutes, 28.81±0.40˚C), T1 (t=105-115, 34.81±0.50˚C) and T2 (t=130-140, 37.53±0.58˚C). Energy expenditure was normalised for body surface area (m^2). Since SkBF data was obtained in arbitrary units, the data measured during UP has been averaged per minute and has been analysed relative to the baseline period.

Paired-sample t-tests were used to compare the measured parameters before and after PMHA. Repeated Measures ANOVA was performed to test for significant changes within the protocols (from baseline to T1 to T2). If the Assumption of Sphericity for the general linear model was violated, Bonferroni correction was applied as a post-hoc test. Linear regression analysis was performed to test the potential influence of outdoor temperature on the outcome parameters. Statistical significance was considered for $P \leq 0.05$ and a statistical trend was considered if $0.05 < P < 0.10$.

RESULTS

Core temperature

After PMHA, core temperature was significantly lower during protocol UP at T1 (-0.13±0.13˚C, P=0.011) and T2 (-0.14±0.15˚C, P=0.026) (Table 2, Figure 3), but not at baseline (-0.12±0.23˚C, P=0.115)

Skin temperatures

After PMHA, average mean skin temperature was not significantly different from the pre-measurement at any time point. However, average proximal skin temperature significantly decreased at T1 (-0.22±0.29˚C, P=0.029) and average distal skin temperature increased at baseline (+0.74±0.77˚C, P=0.009), and T2 (+0.51±0.63˚C, P=0.022) and tended to be higher at T1 (+0.49±0.76˚C, P=0.057) upon warmth exposure during UP post PMHA (Table 2). Moreover, the gradient between average proximal and average distal skin temperature was significantly reduced

after PMHA at baseline (-0.84±0.94˚C, P=0.014), T1 (-0.71±0.79˚C, P=0.014) and T2 (-0.67±0.49˚C, P=0.001). The same was observed for the gradient between core temperature and distal skin temperature at baseline (-0.86±0.84˚C, P=0.007), T1 (-0.61±0.74˚C, P=0.021) and T2 (-0.56±0.54˚C, P=0.009).

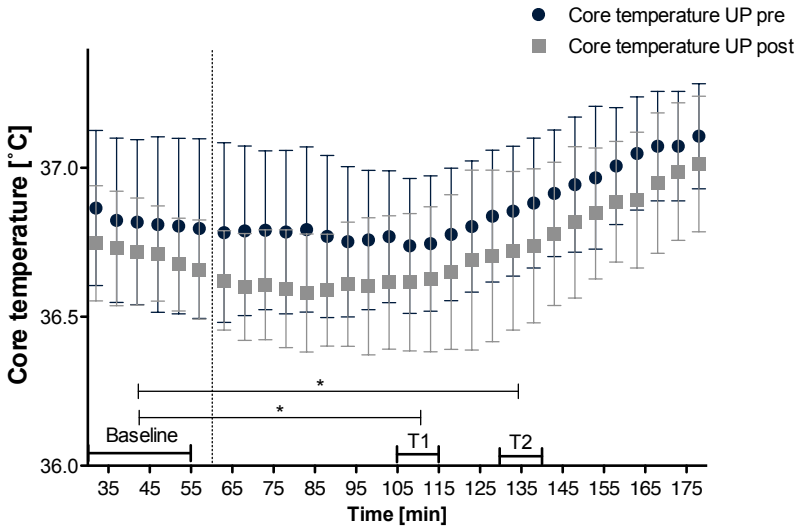

Figure 3 | Average core temperature during protocol UP pre and post PMHA
T1 and T2 represent the respective intervals used for data analysis. Data is presented as mean±SD. N=11, * indicates P<0.05 for changes after PMHA.

Total water loss

Total water loss during UP decreased from 217.3±62.1ml before PMHA to 186.81±50.18ml after PMHA (P=0.012).

Cardiovascular parameters

Heart rate, systolic and diastolic blood pressure, stroke volume and cardiac output were measured at baseline, T1 and T2 during UP, before and after PMHA. At baseline, both systolic and diastolic blood pressure were significantly lower after PMHA (systolic: Δ-7.7±7.7mmHg, P=.015; diastolic: Δ-4.4±4.8mmHg, P=0.001, Table 3). At T2 of UP post, systolic blood pressure was significantly lower than before PMHA (P=0.003), but diastolic blood pressure was no longer significantly

different from the pre-measurements (P=0.235, Table 3). Heart rate, stroke volume and cardiac output were not significantly affected post PMHA.

Table 2 | Body temperatures during UP pre and post PMHA

Protocol UP	Baseline pre	T1 pre	T2 pre	Baseline post	T1 post	T2 post
Core temperature [°C]	36.80±0.27	36.74±0.25	36.87±0.22	36.72±0.18	36.62±0.23*	36.73±0.26*
Mean skin temperature [°C]	33.89±0.50	35.08±0.42	35.72±0.37	33.97±0.30	35.08±0.30	35.79±0.25
Proximal skin temperature [°C]	34.41±0.49	35.60±0.35	36.18±0.28	34.30±0.43	35.38±0.38*	36.03±0.32
Distal skin temperature [°C]	32.44±0.75	32.11±0.52	34.97±0.52	33.18±0.55**	34.60±0.49$	35.48±0.46*
Proximal-distal skin temperature gradient [°C]	1.97±0.83	1.49±0.64	1.21±0.61	1.12±0.66*	0.78±0.58	0.54±0.53**
Core-distal skin temperature gradient [°C]	4.40±0.81	2.64±0.59	1.90±0.61	3.54±0.51*	2.02±0.49*	1.29±0.55**

Data is presented as mean±SD. N=11. $ indicates 0.05<P<0.1 for changes post PMHA, * indicates P<0.05 for changes post PMHA, ** indicates P<0.01 for changes post PMHA.

Energy expenditure

To assess the effect of the temperature drift on energy expenditure, baseline values were compared to T1 and T2 (Table 4). During the pre-measurement, energy expenditure tended to increase from baseline and T1 (+0.17±0.26kJ/min, P=0.056) and significantly increased from baseline to T2 (+0.25±0.20kJ/min, P=0.002). After PMHA, energy expenditure increased significantly from baseline to T1 (+0.19±0.24kJ/min, P=0.024) and a trend was evident for baseline to T2 (+0.20±0.31kJ/min, P=0.056).

To determine the effect of PMHA on energy expenditure, baseline, T1 and T2 were compared before and after PMHA. No significant changes, and thus no effect of PMHA on basic metabolic rate (baseline) and energy expenditure were observed.

Table 3 | Systolic and diastolic blood pressure pre- and post- PMHA

PRE	Baseline	T1	T2
Systolic [mmHg]	118±9	117±11	118±9
Diastolic [mmHg]	72±7	70±10	67±9
Heat rate [bpm]	68±17	70±18	72±16
Stroke volume [ml]	93±17	94±18	82±30
Cardiac output [l/min]	5.9±1.5	6.3±1.7	6.3±1.7
POST	**Baseline**	**T1**	**T2**
Systolic [mmHg]	113±7*	113±9	113±8*
Diastolic [mmHg]	68±6*	66±7	64±6
Heat rate [bpm]	61±9	63±10	64±9
Stroke volume [ml]	96±11	92±12	94±14
Cardiac output [l/min]	5.8±1.4	6.0±1.4	6.3±1.3
Data is presented as mean±SD. N=11, * indicates P<0.05 for changes post PMHA			

SkBF

Before PMHA, there was a significant increase from baseline to T1 (±36.83±11.66%, P=0.01), baseline to T2 (±74.18±21.37%, P=0.006) and from T1 to T2 (+37.35±12.63%, P=0.014). After PMHA, the increase of SkBF was only significant between baseline and T2 (+49.00±18.74%, P=0.026) (Table 4). Post PMHA, hand SkBF decreased significantly by 28.35±38.96% (P=0.037) at T1, but at T2, the decrease was no longer significant (P=0.208).

Table 4 | Energy expenditure and hand SkBF pre- and post- PMHA

	Baseline pre	T1 pre	T2 pre	Baseline post	T1 post	T2 post
Energy expenditure UP [kJ/min]	4.83±0.55	5.00±0.63	5.07±0.59*	4.79±0.73	4.97±0.84*	4.99±0.85
Relative hand SkBF UP [%]	1.00±0.00	1.30±0.36*	1.67±0.68*,#	1.00±0.00	1.02±0.21§	1.40±0.60*

Data is presented as mean±SD, N=11. * P<0.05 for changes compared to baseline within the same protocol; # P<0.05 for changes from T1 to T2 within the same protocol. § P<0.05 for differences between pre and post PMHA.

DISCUSSION

This study evaluated the effects of passive mild heat acclimation (PMHA), i.e. without exercise, on human thermophysiology. PMHA is of particular interest, as it represents temperature challenges encountered in everyday life, which are fundamentally different to those studied with active heat acclimation (AHA). Whereas AHA addresses the effect of exogenous and endogenous heat stimuli, PMHA focuses only on a relatively mild exogenous heat stimulus. We show that PMHA consisting of exposure to ~33˚C at 7 consecutive days indeed elicited a decrease of core temperature and a redistribution of skin temperature in warm ambient temperatures. Water loss and blood pressure were decreased post acclimation. Energy expenditure, however, was not affected by PMHA.

Core temperature

PMHA resulted in a decrease of core temperature during warming (-0.13˚C at T1 (P<0.01) and -0.14˚C at T2 (P=0.026)). This result is in line with many earlier studies that evaluated various models of heat acclimation (6, 13, 14, 32-35). Although the core temperature decrease was often more outspoken in studies inducing controlled hyperthermia (e.g. approximately -0.19˚C, orally measured core temperature (35, 36)), especially after acclimation to humid heat (e.g. ranging from -0.1 to -0.5˚C (6)) and after active heat acclimation (-0.3˚C to -0.4˚C lower resting core temperature (33, 37)), our results show that 7 days of PMHA also modulates core temperature. The observed decrease of core temperature combined with the increase of distal skin temperature also resulted in a reduced core-distal skin gradient post acclimation. The latter represents an effective adaptation mechanism for warm environments: a smaller temperature gradient between

core and distal skin helps to create a certain thermoregulatory 'buffer', as the total tissue temperature increases more slowly (8, 38, 39).

Skin temperature

There was no significant effect of PMHA on the course of mean skin temperature during warming, which is not in line with earlier findings from an AHA study (33). However, skin temperature distribution changed significantly: proximal skin temperature was decreased during warming, whereas distal skin temperature was increased during baseline and warming. As indicated above, the decreased temperature gradient between core and skin has the potential to decelerate the warming of the body. Additionally to the advantageous effect of higher distal skin temperatures on body warming, increased skin temperature also influences cutaneous water vapour pressure, which, in turn, facilitates evaporative cooling (40). Hence, the increase of distal skin temperature, together with the decreased core temperature as found in the present study, represent important functional adaptation to heat.

Water loss

After 7 days of PMHA, total water loss, as measured by the change of body mass before and after warming, was significantly decreased.

Changes of sudomotor functions after (short- or mid-term) active heat acclimation are commonly reported, indicating an increase of sweating capacity and an increased sudomotor sensitivity (8, 41). The decreased total water loss, which was evident in the present study, might, however, suggest the contrary, namely a reduction of evaporative heat loss due to mild heat acclimation. The reason for this result might be due to the applied methods: the heat stimulus during the acclimation period was kept constant and the temperature increments during the pre- and post-tests were identical. Considering the lowered core temperature after PMHA, it can be concluded that a certain level of heat habituation had, indeed, been acquired. As a consequence, the same exogenous heat stimulus during UP after PMHA became less severe than it was before PMHA. Thus, less evaporative heat loss and sweating was needed to maintain the target core temperature, which might explain why the total water loss after acclimation was less (8).

In the present study, no hydration assessment was performed to ensure comparable hydration status before and after PMHA, which might be considered as a limitation. However, since the decrease of water loss post PMHA was clearly significant (P=0.012), and participants served as their own controls, a confounding influence of hydration status is regarded as relatively unlikely.

Cardiovascular parameters

In a thermoneutral condition (baseline), both systolic and diastolic blood pressure were significantly decreased after PMHA and systolic blood pressure was also significantly decreased during warming. Heart rate, cardiac output and stroke volume were not affected by PMHA.

The regulation of blood pressure is challenged during heat exposure. Blood flow in the extremities remarkably increases in a warm environment (vasodilation), thereby decreasing total peripheral resistance and blood pressure. In contrast, blood flow to the extremities decreases to a minimum in a cold environment (vasoconstriction), which increases total peripheral resistance and increases blood pressure. There is not much literature available describing the effect of passive heat acclimation on blood pressure. Two studies did not find an effect of active heat acclimation on blood pressure, although they report distinct changes of heart rate, as well as cardiac output and stroke volume (42, 43). Both studies incorporated exercise training combined with heat acclimation; and acclimation effects were evaluated during exercise in a warm environment. Blood pressure values in a neutral thermal environment, however, were not reported. Recent studies by Brunt *et al.* (19, 20) found that cardiovascular function and blood pressure were remarkably improved after long-term passive heat therapy (daily hot baths over the course of 8 weeks). The decrease of blood pressure is in line with our findings, despite the fact that the acclimation strategy of the present study was less intense, much shorter and without the application of controlled hyperthermia.

Skin blood flow

As expected, we found that a warming thermal environment caused vasodilation and thereby an increased SkBF at the hand. Contrary to our expectations, PMHA resulted in an average *reduction* of the SkBF increment during the UP protocol of approximately 28%.

A decrease of SkBF in the heat after heat acclimation has earlier been reported in the literature, when different ethnic groups and acclimatised indigenous people to unacclimatised groups were compared (44, 45). Roberts *et al.* (2) and others (46) also found that after exercise training and heat acclimation, SkBF decreased. However, due to the increase of skin temperature at the extremities, a corresponding increase of SkBF would have been expected after PMHA. Moreover, considering the lowered core temperature and the decreased core-distal skin temperature gradient, earlier heat loss via the skin is stimulated, which would suggest an enhanced SkBF as well.

In this study, we measured SkBF using a Laser Doppler Flowmetry apparatus. Measurements with this technique scale linearly with changes in underlying blood flow velocity and blood volume,

producing an output of arbitrary units rather than absolute values. Since the angle of measurement relative to the skin blood flow can be different between measurements, a normalisation of the data to baseline is required. However, the applied normalisation of the data to baseline implies that it is not possible to detect an effect of heat acclimation on baseline flow measurements. In this study, distal skin temperature was significantly higher after PMHA, which is why we assume that the same was true for SkBF. However, due to the normalisation procedure of the SkBF data, the anticipated increase of SkBF at baseline is not observable. Moreover, as the absolute level of skin blood flow after PMHA may have been closer to its maximal value, the relative effect of SkBF increase relative to baseline during the temperature increment might have been blunted due to the methodology. The latter could therefore also account for the decreased SkBF measured post PMHA.

Energy expenditure

Whereas an energy expenditure increase observed during cooling can be attributed to non-shivering thermogenesis (probably by the activation of brown adipose tissue) and shivering thermogenesis (muscle tissue) (23, 47-50), it remains uncertain to which tissue or bodily function the increase of energy expenditure during warming is related. Although the reabsorption of electrolytes during sweating is an ATP-consuming process, the energy required for sweating is very small compared with the overall increase in energy expenditure. Possibly, an increase of heart rate and ventilation due to hyperthermia, which has earlier been shown, might partly explain the increase of energy expenditure (51, 52). However, an increase of heart rate was not detected in the present study and ventilation was not measured. Another explanation for the increase of energy expenditure might be the Q10 effect (53). According to the Arrhenius law, a 1°C change in mean body temperature, might account for an increase of the energy expenditure of as much as 8% (assuming Q10-factor = 2.3) (54). However, we only find this relation between the Q10 effect and the percentage of change of energy expenditure between baseline and T1 post PMHA ($r=0.610$, $P=0.046$). Presumably, an increase of energy expenditure with an increasing ambient temperature is due to a combination of several factors, but the exact magnitude and mechanism of the energy expenditure increase remains uncertain.

With respect to the effect of PMHA on energy metabolism, we did not detect an effect of PMHA on the course of energy expenditure. Generally, a slight decrease of metabolic heat production in a warm environment is very advantageous, as it concurs with a decreased need for (evaporative) body cooling. Earlier (field) researches found a relation between heat acclimation and lower energy expenditure (55-57), which is likely to represent an adaptation due to more long-term or more intensive (active) heat acclimation. Contrarily, a controlled study comparing heat acclimatised Pima Indians with matched Caucasians did not detect a difference of basal metabolic

rate (22, 58). It is, however, difficult to differentiate between actual metabolic adaptations and changes resulting from modified thermoregulatory behaviour.

Limitations and future perspectives

Due to practical reasons, the present study was conducted over the course of 9 month between December 2014 and August 2015. As mentioned in the methods section, the mean day outdoor temperature, recorded 2 weeks before the start of each individual measurement, during this period varied between 1.5˚C and 20.2˚C (11.5±7.1˚C mean±SD). To rule out possible confounding, we used linear regression analysis to test if the mean outdoor temperature influenced the effect of PMHA on the outcome parameters (data not shown). However, no significant effects of season were detected.

Since we have found significant effects of PMHA on thermophysiological parameters after a short intervention period of only 7 days, more research is warranted to evaluate the long-term effects of prolonged warmth exposure on human thermophysiology and its potential decay. Moreover, it is of great relevance to evaluate the impact of PMHA on other health parameters such as insulin sensitivity and other cardiovascular related parameters in populations with metabolic and cardiovascular risk factors.

Conclusion

This study evaluates the effect of passive mild heat acclimation (PMHA) on thermophysiology in humans. We show that PMHA induces adaptations of the human thermoregulatory physiology and cardiovascular system, leading to an improved resilience to warm ambient conditions. Energy metabolism is not affected by PMHA.

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Chapter 4

The effect of passive mild heat acclimation on glucose metabolism and heat shock protein 72 in overweight elderly men

In preparation

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ABSTRACT

Global incidences of obesity, insulin resistance and type 2 diabetes mellitus are alarmingly high and constantly rising. It has been shown that heat acclimation and the possibly thereby increased levels of heat shock protein 72 (HSP72), are positively correlated with insulin sensitivity. Regular exposure to elevated temperature might thus have positive implications for metabolic health. Therefore, the present study investigated the effect of passive mild heat acclimation (PMHA) on glucose metabolism, HSP72 levels in muscle, and thermophysiological parameters in an overweight elderly population.

11 overweight elderly men (65.7±4.9y, BMI 30.4±3.2kg/m^2) underwent PMHA (10 days, 34.4±0.2˚C, 4-6h/day). Pre- and post-PMHA, fasting plasma glucose (FPG) and insulin (FPI) were measured and insulin sensitivity was assessed by hyperinsulinemic-euglycemic clamps. Muscle biopsies were taken to indicate HSP72 levels in muscle pre- and post-PMHA. A temperature-ramp-protocol was conducted to evaluate adaptation of thermophysiological parameters (core temperature (Tcore), skin temperatures, sweating, mean arterial pressure (MAP) and heart rate) to PMHA. Four time-points during the temperature-ramp were selected to compare the thermophysiological parameters before and after PMHA: baseline (28.8±0.15˚C), T1 (35.4±0.40˚C), T2 (38.9±0.49˚C) and T3 (41.3±0.33˚C).

FPG and FPI decreased significantly after PMHA (ΔFPG: -0.27mmol/L, P=0.036; ΔFPI: -12.69pmol/L, P=0.026). Tcore decreased after PMHA, both during baseline and during warming (Δbaseline: -0.13±0.18˚C, P=0.035; ΔT1: -0.19±0.26˚C, P=0.036; ΔT2: -0.18±0.25˚C, P=0.041; ΔT3: -0.10±0.52˚C, P=0.073). Insulin sensitivity, HSP72 levels in muscle, skin temperature and sweating did not change. MAP decreased (Δ-2.91±2.67mmHg, P=0.007) and heart rate tended to decrease (Δ-2.98±3.50bpm, P=0.065) post PMHA.

This study shows that prolonged exposure to mild heat induces significant changes of FPG and FPI in an overweight elderly population. Moreover, PMHA induces thermophysiological and cardiovascular adaptations in this overweight elderly population, which confirms the findings of an earlier PMHA study in young healthy men. Although previous (more intense and often exercise-induced) heat acclimation studies report increases of insulin sensitivity and HSP72 levels, PMHA did not lead to such changes. More research is warranted to further elucidate underlying mechanisms of the relationship between heat exposure, glucose metabolism and HSP72 levels in humans.

INTRODUCTION

Overweight, obesity and obesity-induced insulin resistance are major global health problems and have been shown to strongly increase the risk for development of Type 2 Diabetes Mellitus (T2DM) (1-3). Commonly, obese humans are characterised by metabolic disturbances resulting in elevated circulating plasma levels of free fatty acids (FFA) and glucose as well as increased low-grade inflammation (1, 3-5). These metabolic disturbances are linked to the development of insulin resistance and T2DM, however, the exact underlying mechanisms or affected pathways are still focus of research. Alteration of lifestyle factors such as exercise and healthy diet have been shown to improve obesity-induced insulin resistance in obese humans with and without T2DM (6-9). Unfortunately, prolonged adherence to these beneficial changes in lifestyle is often low and recidivism very common (9-13). Therefore, the investigation of more adaptable alternative ways to improve insulin resistance is of great relevance. Recently, it has been suggested that environmental factors, specifically temperature, might play a significant role in the 'globesity' and diabetes epidemic (14-18). Moreover, ambient temperature has been shown to influence both energy intake as well as energy expenditure (15), making it an attractive target for further exploration.

Interestingly, seasonal variations of HbA1c (glycosylated haemoglobin, an indicator for severity of insulin resistance) have previously been described in the literature in both healthy adults (19) and T2DM patients (20-23). Most studies indicate that HbA1c is lower during the summer months and therefore suggests improved glycaemic control during warmer times of the year (19, 20, 22-24). However, one study could not confirm this finding (21). Possibly, weight gain and increased food intake during wintertime are important underlying factors. In 1999, Hooper (25) was the first to report that T2DM patients experienced significantly improved glycaemic control, i. e. lower mean fasting plasma glucose and HbA1c levels, after taking daily hot baths for 3 weeks. However, the mechanism(s) that might cause warmth to improve glucose metabolism, as seen during the seasonal comparisons and hot baths, remain(s) unclear.

In 2002, Kurucz *et al.* (26) described an inverse relationship between insulin resistance and the expression of 72-kDa heat shock protein (HSP72). Here, gene expression of HSP72 in skeletal muscle cells was significantly lower in T2DM patients compared with healthy controls. Subsequently, more studies confirmed this link (27-31) and a detailed review with respect to the potential therapeutic role of HSP72 has also been published (32). HSPs are so-called 'chaperones' and play an important role in the protection of cell integrity. Due to acute or chronic stimuli such as environmental or pharmacological stresses and an increase of body temperature, HSP expression in the cell rises, and the polypeptides bind to other cellular proteins to prevent from and/or repair damage and inappropriate folding (26, 33-36). The easily inducible HSP72 plays an

important cyto-protective and regulating role under both physiological and stress conditions of the cell (26, 34, 35, 37, 38).

Inducing increased expression of intracellular HSP72 can thus be achieved by different pharmacological and non-pharmacological stimuli. Heat exposure has been shown to effectively raise intracellular HSP72 levels and has widely been studied (33, 35, 37-45). Here, a distinction can be made between the HSP72 response to acute heat exposure and prolonged exposure (heat acclimation). Acute heat exposure causes a rapid increase of HSP72, which underpins the important role of HSPs for thermotolerance of the body. Different types of artificially induced heat acclimation in both rodents (35, 45) and humans (41, 42, 44, 46), however, go along with increased baseline levels of HSP72 in muscle. Additionally, the rapid increase of HSP72 expression due to acute thermal stress, is blunted after heat acclimation. Heat acclimation might therefore be a valid strategy to increase intracellular HSP72 expression and thereby elicit the beneficial cyto-protective effects as well as improve insulin sensitivity at the same time.

In conclusion, there are indications suggesting that heat acclimation might positively affect insulin resistance in humans, which might, amongst other things, be due to an increase of HSP72 levels. However, there is yet no study available assessing the effect of *in-vivo* whole-body heat acclimation on glucose metabolism and HSP72 levels in humans. Moreover, it is not clear if the earlier reported seasonal fluctuations of glucose metabolism are caused by temperature directly or if these are otherwise affected by confounding factors such as an altered seasonal diet. Therefore, we intended to study a passively induced heat acclimation protocol in order to assess the effect of heat exposure on glucose metabolism without the effect of confounding factors such as season-related changes in diet or physical activity.

In an earlier study, we have shown that passive and relatively mild heat acclimation (PMHA, ~33˚C ambient temperature for 6h a day, at 7 consecutive days), representing realistic Western and central European conditions during a warm summer or a heat wave, induces thermophysiological changes in young healthy men (47). A significant decrease of core temperature was evident after PMHA in the earlier study, which has been shown to be one of the most pronounced physiological adaptations to heat in many other, more intense heat acclimation studies (for example (48-52)). Earlier studies have attested the increased HSP expression in human muscle cells after active, exercise-induced heat acclimation and also in rodent heart and liver tissue after passive heat acclimation (41, 42, 44, 45), which is why it might be anticipated that PMHA evokes similar reactions in humans. In order to study the isolated effect of prolonged exposure to elevated ambient temperatures, a passive acclimation protocol is needed, which is why the applied PMHA protocol did not incorporate physical activity.

In summary, PMHA might be a potential strategy to improve glucose metabolism, possibly via an increase of HSP72 levels. Therefore, the primary goal of this study was to investigate the effect of

PMHA on glucose metabolism in overweight elderly men. In addition, we studied the effect of PMHA on HSP72 levels in muscle and the thermophysiological responses.

METHODS

The present study was conducted in the period of October 2016 till May 2017. Average day outdoor temperature during this period, as recorded two weeks previously to each individual measurement, ranged between 0.3˚C and 9.8˚C (8.1±2.8˚C mean±SD). The Medical Ethics Committee of Maastricht University approved the study and it was conducted in conformity with the Declaration of Helsinki (Fortaleza, Brazil, 2013).

Participant characteristics

In total, 11 elderly overweight, white Western European men volunteered in the study (Table 1). Participants were recruited via advertisements in local newspapers and gave their written informed consent. During screening, all were checked for their medical history. Exclusion criteria included uncontrolled hypertension, active cardiovascular disease, liver or kidney dysfunction, smoking and use of beta-blockers or other medication known to interfere with glucose metabolism. Fasting glucose and 2-h glucose during an oral glucose tolerance test (OGTT) were also determined during the screening. Participants with (previously undiagnosed) T2DM were excluded from the study based on the OGTT (2-h glucose > 11.1mmol/L). Three volunteers were using medication for hypertension, whereof one also used NSAID for arthritis. Two other volunteers were using proton pump inhibitors to treat gastroesophageal reflux disease. All participants were instructed to use their medication as usual during the study to avoid potential disturbances caused by irregularities.

Participants had not undertaken any sort of formal acclimation and had not spent time in a hot environment at least 2 months previous to their participation. To avoid an effect of biorhythm on the outcome parameters, all testing and acclimation days commenced at the same time in the morning.

Table 1 | Participant characteristics

	Mean	± SD
Age [years]	65.7	4.9
Height [m]	1.80	0.1
Weight [kg]	95.5	15.3
BMI [kg/m2]	30.4	3.2
Fat percentage [%]	28.5	4.6
Fat mass [kg]	27.9	9.0
Fasting glucose [mmol/L]	6.0	0.5
2-h Glucose [mmol/L]	7.6	1.9
N=11.		

Study design

Participants were exposed to 10 days of passive mild heat acclimation (PMHA) (Figure 1). Before and after PMHA, all participants underwent two tests: 1) a hyperinsulinemic-euglycemic clamp to evaluate insulin sensitivity and 2) a temperature ramp protocol to study the physiological response to increasing ambient temperatures (Figure 1 and 2). The increasing temperature ramp before and after PMHA will be referred to as UP in the following (Figure 1 and 2).

Before measurement day 1, 2, 11 and 12 (Figure 1), participants consumed self-chosen standardised evening meals, with a comparable composition of nutrients.

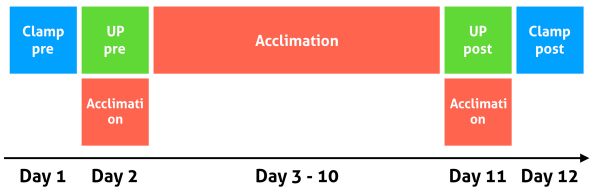

Figure 1 | Protocol time line

Clamp refers to the hyperinsulinemic-euglycemic clamp procedure as described in the methods section, *UP* refers to the temperature ramp protocol, *Acclimation* refers to the passive mild heat acclimation period as described in the methods section, *pre* before acclimation, *post* after acclimation.

Hyperinsulinemic-euglycemic clamp
To measure peripheral insulin sensitivity, a 2-h hyperinsulinemic-euglycemic (40mU/m^2/min) clamp, according to DeFronzo (53), was performed in the morning after an overnight fast (from 22.00h on at the previous evening). The clamp contained a basal phase (from t=0-120 min) and an insulin-stimulated phase (from t=120-270 min). Blood sampling (at t=0, 110, 120, 260 and 270 min) and measurements for indirect calorimetry using a ventilated hood system (Omnical, IDEE, Maastricht Instruments, Maastricht) were performed during the steady-state periods in the basal (t=90-120 min) and the insulin-stimulated phase (t=240-270 min). Glucose and fat oxidation rates from gaseous exchange were calculated according to Frayn (54).

Participants were clamped at glucose values round 5.0 mmol/L. The clamps were performed before and after PMHA. Insulin sensitivity was expressed as the M-value (mg/kg/min) calculated according to the DeFronzo protocol (53). The M-value denotes the amount of glucose infused over a certain period of time, in response to a fixed dose of insulin. A continuous infusion of insulin stimulates glucose uptake mainly exerted by skeletal muscle. Therefore, an increase of the M-value indicates an improvement of insulin-stimulated glucose uptake (insulin sensitivity), whereas a decrease of the M-value represents a reduction of insulin sensitivity. Due to a technical issue in one case, the hyperinsulinemic-euglycemic clamp results are reported for N=10 participants.

Blood plasma samples were centrifuged, plasma was snap-frozen in liquid nitrogen and stored at -80˚C for later analysis for glucose, insulin and free fatty acid concentrations.

Muscle biopsies
During the hyperinsulinemic-euglycemic clamp procedure, a muscle biopsy was taken from the m. quadriceps vastus lateralis according to Bergström et al. (55). The biopsy was taken during the basal steady-state of the clamp. The procedure was performed under local anaesthesia (1% lidocaine). Muscle tissue was directly frozen in melting 2-methylbutane. Protein expression was determined by Western Blotting according to standard procedures. Equal amounts of protein were loaded and controlled by a REVERTprotein-staining method (Licor, Westburg, Leusden, The Netherlands). After incubation with HSP70/72 antibodies (Enzolifesciences, Farmingdale, NY, USA), detection of the protein bands was performed with the Odyssey Near Infrared scanner (Licor, Westburg, Leusden, NL).

Heat shock protein 72 levels were determined by Western blotting and are expressed in arbitrary units (AU). Changes between pre- and post-measurements were calculated. Due to technical complications in one case, HSP72 analyses are also based on N=10.

UP protocol

For protocol UP, participants arrived at the laboratory in the morning after an overnight fast (as of 22:00h the previous evening). Upon arrival at the laboratory, participants ingested a telemetric pill (Vital Sense, Philips Healthcare, NL) to measure core temperature, which was monitored using an Equivital apparatus mounted to the participant with a chest strap (Equivital Hidalgo, UK). Heart rate was measured with the same Equivital device. To measure mean skin temperature, wireless skin temperature sensors (iButtons, Maxim Integrated Products, California, USA) were attached to 14 ISO-defined body sites (56) with semi-adhesive tape (Fixomull stretch, BDN medical GmbH, GER). Proximal skin temperature was composed as an average of the ISO-defined sites of the scapula, lower back paravertebral, upper chest and abdomen. For the distal skin temperature, hand and instep temperature were averaged. Core temperature, heart rate and skin temperatures were recorded at 1-min intervals.

In a climate chamber, participants took place on a stretcher with air-permeable fabric. Two Laser Doppler Flowmetry (LDF) probes were fixated to the participant's thenar and ventral side of the underarm halfway between carpus and antebrachium, to continuously measure skin blood flow (10Hz; PeriFlux System 5000, Perimed, SE). Next to the LDF probe, a ventilated capsule was mounted to the skin to continuously measure local sweat rate and to determine sweat onset (Qsweat, WR medical, Maplewood, USA). Using a finger blood pressure cuff (Finometer MIDI, Amsterdam, NL), cardiac output and stroke volume were measured. Additionally, upper arm blood pressure was recorded on the opposite arm by oscillometric principle (Omron M6 Comfort IT, Omron Healthcare, JPN). Immediately before entering the climate chamber and after leaving it, participants were weighed (after towelling themselves thoroughly) to determine total water loss, using the difference in total body mass before and after the UP protocol.

When preparations were finished, UP started with a baseline period of 60 min followed by an increase of the ambient temperature over the course of 120 min (Figure 2). The baseline temperature (28.8±0.15˚C) was assumed to be neutral for a resting semi-nude person, based on the literature review of Kingma *et al.* (57) and it was corrected for the isolation of the stretcher that participants rested on during the testing. Relative humidity was allowed to drift freely with the changes in temperature, resulting in an average relative humidity of 23.2±3.3% during the test.

The first 30 min of protocol UP were regarded as familiarisation period, and therefore excluded from the data analysis. For the comparisons of physiological variables during UP, three periods were selected: baseline, T1, T2 and T3 (Figure 2). Blood pressure, cardiac output and stroke volume were only measured at baseline, T2 and T3. Since SkBF data was obtained in arbitrary units, the data measured during UP has been averaged per minute and has been analysed relative to the baseline period. Sweat onset was determined as the minute of the first observable humidity increase during the measurement after which there was no return to baseline values in the curve. If a decrease to baseline values occurred after the first increase, the second explicit increase was

identified as point of sweat onset. The minute of sweat onset was then matched with the respective ambient temperature measured for the individual participant at the specific point in time. For one participant, the post measurement failed and therefore, sweat data is based on N=10.

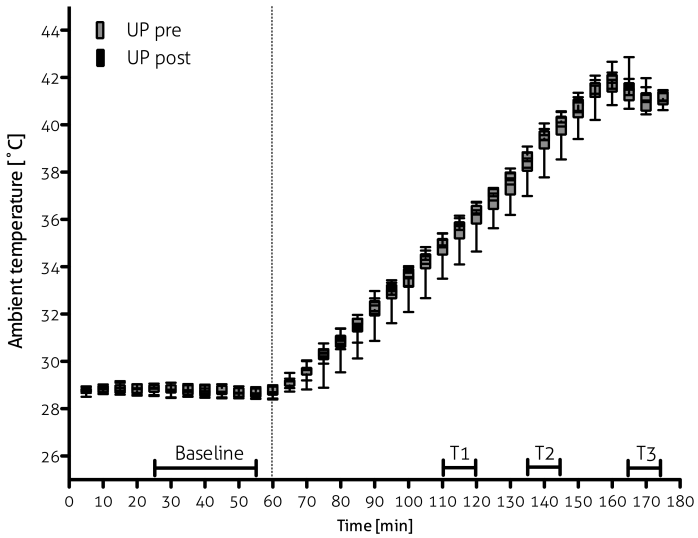

Figure 2 | Experimental conditions during UP before and after acclimation
Four time-intervals were selected to compare data before and after PMHA (protocol time and ambient temperature in brackets): baseline (minutes 25-55: 28.8±0.15˚C), T1 (minutes 110-120: 35.4±0.40˚C), T2 (minutes 135-145: 38.9±0.49˚C) and T3 (minutes 165-175: 41.3±0.33˚C). T1 represents 50% of the ramp and approximates the acclimation temperature of 34.5±0.16˚C, and T2 and T3 were the minutes just before the investigators entered the climate chamber to take additional measurements for blood pressure, cardiac output and stroke volume. N=11.

Passive mild heat acclimation

PMHA started in the afternoon of study day two (Figure 1). During the first and last sequence of PMHA (day 2 and 11), participants stayed in a 'warm chamber' for 4h. During the remaining 8 days of PMHA, participants acclimatised for 6h per day. From earlier heat acclimation studies (for example (39, 52)), we know that the most important changes with respect to thermophysiology and HSP72 expression are expected to occur within the first 2-7 days of repeated heat exposure. Therefore, we applied a 10-day acclimation protocol, expecting a sufficiently long acclimation period to induce the anticipated adaptive reactions (52).

Ambient temperature in the warm chamber was kept constant at 34.4±0.2˚C; and relative humidity was 22.8±2.7%, which classifies the ambient air as dry. All participants successfully completed PMHA.

During their stay in the acclimation chamber, participants remained seated at a desk and were allowed to perform regular office work (1.2METs). Participants wore standardised clothing composed of underwear, T-shirt, shorts and socks/slippers. The total thermal resistance of the clothing ensemble plus the desk chair added up to approximately 0.41clo (58, 59). Participants had unlimited access to water; and food was provided upon request, in order to not influence habitual diet. They were allowed to leave the chamber for toilet breaks.

Data analyses

The software packages Microsoft Office 2011 Excel (Microsoft) and SPSS 23 for Mac (SPSS Inc.) was used for data analyses. Mean±SD, delta values and relative changes were calculated for all parameters. Paired-sample t-tests were used to compare the measured parameters before and after PMHA. Pearson correlation coefficient was used to determine relationships between variables. Statistical significance was considered for $P \leq 0.05$ and a statistical trend was considered if $0.05 < P < 0.10$. Data is presented as mean±SD.

RESULTS

Hyperinsulinemic euglycemic clamp

Insulin sensitivity, measured by means of hyperinsulinemic-euglyceminc clamps before and after PMHA, did not change (M-value: pre 3.1±0.9 mg/min/kg, post 2.9±0.9 mg/min/kg, N=10). The individual changes in M-value between pre- and post PMHA showed a broad spread and ranged from -66% to 26% (see Supplement 1, Figure 3).

Although no differences in whole body insulin sensitivity were found, fasting plasma glucose (FPG, P=0.013) and fasting plasma insulin (FPI, P=0.026) were significantly lower post PMHA (Table 2). Free-fatty acids remained, however, unchanged.

Table 2 | Fasting plasma glucose, fasting plasma insulin and free-fatty acid levels before and after PMHA

	Pre PMHA	Post PMHA	Δ	P-value
Fasting plasma glucose [mmol/L]				
	6.0±0.50	5.8±0.4	-0.2±0.4	0.013*
Fasting plasma insulin [pmol/L]				
	96.7±54.7	84.0±49.3	-12.7±15.1	0.026*
Free-fatty acids [mmol/L]				
	0.7±0.2	0.6±0.2	-0.1±0.2	0.352
Data is presented as mean±SD. N=11. Δ denotes changes post vs. pre PMHA, * indicates P<0.05 for changes post PMHA.				

In Table 3, energy metabolism and substrate utilisation, obtained by indirect calorimetry during the hyperinsulinemic-euglycemic clamp procedures, are presented. After PMHA, the respiratory exchange ratio (RER) decreased, both in the basal (P=0.004) and insulin-stimulated state (P=0.035), which suggests a higher reliance on fatty acid oxidation. In line with the latter, glucose oxidation was lower in both basal (P=0.003) and insulin-stimulated state (P=0.018). Moreover, absolute fat oxidation increased after PMHA in the basal state (P=0.018) and tended to increase in the insulin-stimulated state (P=0.051). After PMHA, energy expenditure tended to decrease in the basal state (P=0.059) and was significantly lower during insulin-stimulation (P=0.010).

The changes from the basal to the insulin-stimulated condition (Δinsulin - basal) during the hyperinsulinemic-euglycemic clamp were not significantly affected by PMHA for neither RER, glucose oxidation and fat oxidation nor for energy expenditure (Table 3).

Table 3 | Energy expenditure, carbohydrate and fat oxidation before and after PMHA during the clamp

	Pre PMHA	Post PMHA	Δ post-pre PMHA	P-value
Respiratory exchange ratio				
Basal	0.80±0.05	0.76±0.05	-0.05±0.04	0.004**
Insulin-stimulated	0.85±0.05	0.82±0.05	-0.03±0.04	0.035*
Δ Insulin - basal	0.05±0.02	0.06±0.04	0.01±0.04	0.378
Glucose oxidation [umol/kg/min]				
Basal	6.58±3.43	3.35±3.20	-3.23±2.58	0.003**
Insulin-stimulated	10.04±3.32	7.52±3.00	-2.52±2.86	0.021*
Δ Insulin - basal	3.46±1.71	4.17±2.30	0.71±2.43	0.376
Fat oxidation [umol/kg/min]				
Basal	1.08±0.29	1.30±0.32	0.21±0.23	0.018*
Insulin-stimulated	0.78±0.29	0.95±0.29	0.16±0.23	0.051$
Δ Insulin - basal	-0.30±0.12	-0.35±0.22	-0.05±0.21	0.486
Energy Expenditure [kJ/min]				
Basal	5.31±0.87	5.14±1.00	-0.17±0.24	0.059$
Insulin-stimulated	5.28±0.89	5.13±0.90	-0.15±0.14	0.010*
Δ Insulin - basal	-0.03±0.19	-0.01±0.20	0.02±0.25	0.828

Data is presented as mean±SD. N=10. Δ post-pre PMHA denotes the changes between before and after PMHA, and Δ insulin –baseline denotes the changes between the basal and insulin-stimulated state during the hyperinsulinemic-euglycemic clamp. $ indicates 0.05<P<0.1 for changes post PMHA, * indicates P<0.05 for changes post PMHA, ** indicates P<0.01 for changes post PMHA.

Heat shock protein expression in human muscle

For the total group, HSP72 levels in muscle did not change significantly post PMHA (HSP72 pre: 4153±1139; post: 4574±1224, P=0.396). On individual level, the results varied considerably (Figure 3). Two participants exhibited a decrease of intracellular HSP72 expression (no. 3 and no. 6, -41% respectively -10%) whereas for the remaining 8 participants an increase of intracellular HSP72 expression was evident (Figure 3).

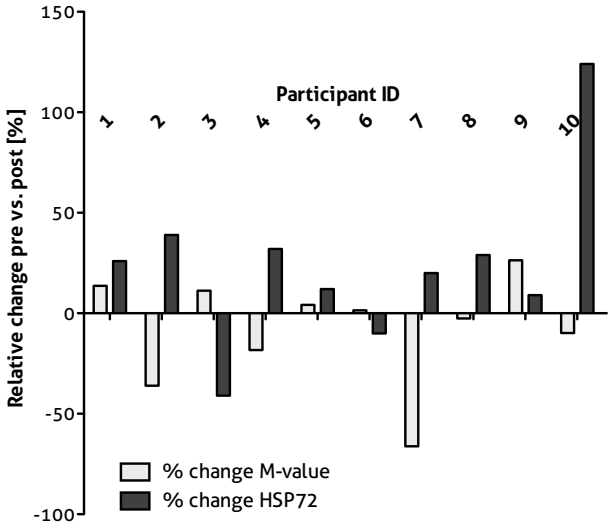

Figure 3 | Relative changes of HSP72 and M-value pre vs. post PMHA
N=10.

Thermophysiological responses

Body temperatures
After PMHA, Tcore was significantly decreased by 0.13±0.18°C at baseline (P=0.035), T1 (-0.19±0.26˚C, P=0.036) and T2 (-0.18±0.25˚C, P=0.041) and tended towards a decrease at T3 (-0.10±0.52˚C, P=0.073) during the UP protocol, compared with Tcore values before PMHA (Table 4). Mean, proximal and distal skin temperature (Supplement 2) remained unchanged after PMHA, but core-distal skin temperature gradient decreased significantly at T1 (P=0.008) and tended to decrease at T2 (P=0.076, Table 4) compared with the core-distal skin temperature gradient before PMHA. At T3, the trend was no longer present.

Table 4 | Body temperatures during UP pre and post PMHA

	Pre PMHA	Post PMHA	Δ	P-value
Core temperature [˚C]				
Baseline	36.81±0.20	36.67±0.17	-0.13±0.18	0.035*
T1	36.74 ±0.17	36.55±0.22	-0.19±0.26	0.036*
T2	36.88±0.18	36.70±0.23	-0.18±0.25	0.041*
T3	37.11±0.20	37.01±0.22	-0.10±0.52	0.073$
Core-distal skin temperature gradient [˚C]				
Baseline	2.95±0.84	2.59±0.43	-0.36±0.68	0.112
T1	1.47±0.62	1.04±0.22	-0.43±0.43	0.008**
T2	0.64±0.49	0.37±0.27	-0.26±0.44	0.076$
T3	0.26±0.37	0.24±0.28	-0.02±0.54	0.920

Data is presented as mean±SD. N=11. Δ denotes changes post vs. pre PMHA, $ indicates 0.05<P<0.1 for changes post PMHA, * indicates P<0.05 for changes post PMHA, ** indicates P<0.01 for changes post PMHA.

Cardiovascular parameters

Post-PMHA, MAP significantly decreased at baseline and T2 compared with MAP values before PMHA, but at T3 the decrease was no longer significant (MAP baseline: pre 93±8mmHg, post 90±8 mmHg, Δ-3±4, P=0.020; T2: pre 93±9 mmHg, post 90±10 mmHg, Δ-4±3, P=0.002, T3: pre 92±7 mmHg, post 90±8 mmHg, Δ-2±5, P=0.147). Heart rate was unchanged at baseline, T1 and T3 but significantly decreased at T2 (HR baseline: pre 64±11bpm, post 63±8 bpm, Δ-1±4, P=0.356; T1: pre 69±8 bpm, post 67±7 bpm, -2±3, P=0.107; T2: 71±11 bpm, post 67±10 bpm, Δ-4±4, P=0.011; T3: pre 72±11 bpm, post 70±10 bpm, Δ-2±5, P=0.160). Cardiac output and stroke volume as well as relative hand and underarm skin blood flow did not change significantly after PMHA (Supplement 2).

Sweating

Total sweat loss as measured by the change in weight before and after UP did not change with PMHA (Δpre 0.253±0.066kg, Δpost 0.237±0.049kg, P=0.175, Supplement 2), and also local sweat rate at the underarm as well as sweat onset remained unchanged post PMHA (Figure 4, Table 5).

Table 5 | Sweating before and after PMHA

	Pre PMHA	Post PMHA	Δ	P-value
Sweat rate underarm [nL/cm²/min]				
Baseline	4.3±0.7	6.6±6.0	2.2±6.0	0.247
T1	5.1±1.5	6.9±6.0	1.7±6.3	0.382
T2	11.1±9.1	14.8±13.6	3.7±11.9	0.320
T3	29.9±11.0	42.4±32.9	12.5±35.9	0.277
Sweat onset [°C]				
	39.0±2.1	39.7±1.6	0.7±1.5	0.173
Data is presented as mean±SD. N=10. Δ denotes changes post vs. pre.				

Correlations with insulin sensitivity

No significant correlation between the change in M-value (post- vs. pre-PMHA) and HSP72 levels (post- vs. pre-PMHA) was found in this study (r=-0.391, P=0.264, N=10).

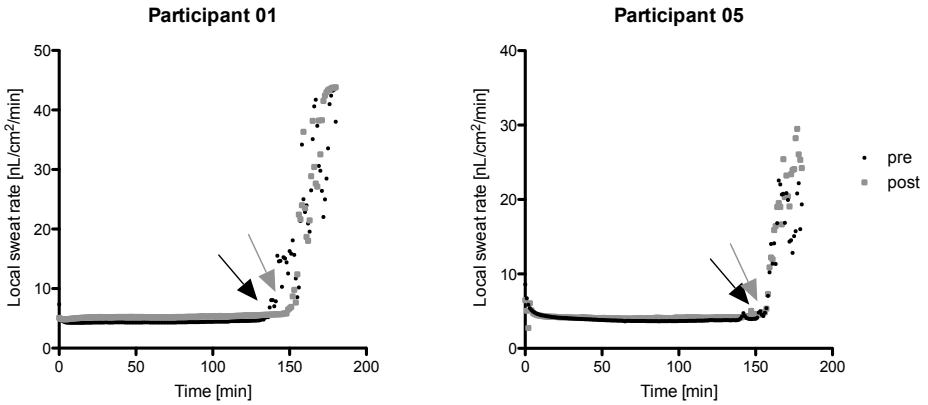

Figure 4 | Local sweat rate during UP of two representative participants
Arrows indicate the respective point of sweat onset (black pre PMHA, grey post PMHA).

The change in M-value post- vs. pre-PMHA showed a trend towards positive correlation with the change in Tcore post- vs. pre-PMHA at T2 and a significant positive correlation was evident at T3 (M-value vs. ΔTcore at T2: r=0.556, P=0.095, ΔTcore at T3: r=0.868, P=0.001, N=10, Figure 5).

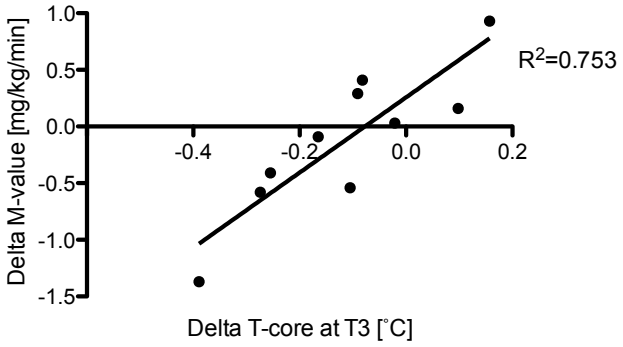

Figure 5 | The relationship between ΔM-value and ΔTcore at T3
Delta's denote changes post- vs. pre-PMHA.

DISCUSSION AND CONCLUSION

The present study investigated the effect of passive mild heat acclimation (PMHA) on glucose metabolism and heat shock protein (HSP) 72 levels in skeletal muscle as well as thermophysiological factors in overweight elderly men. We show for the first time that relatively mild, passively administered heat acclimation evokes substantial changes of glucose metabolism: fasting plasma glucose (FPG), and fasting plasma insulin (FPI) decreased significantly after PMHA. Mean insulin sensitivity and mean HSP72 expression were not significantly affected by PMHA. We confirmed earlier findings on thermophysiological effects of PMHA, showing that the applied acclimation model evoked significant changes of core temperature, which is an important indicator for successful acclimation.

Insulin sensitivity

In the present study, the applied mild heat acclimation method did not cause significant changes of insulin sensitivity at group level. Strikingly, although insulin sensitivity as determined by the clamps was not affected, both FPG and FPI significantly decreased upon PMHA. It has earlier

been suggested that FPG and FPI indicate the basal function "at the nadir of the dose-response curve, whereas clamps are an assessment of the stimulated extreme…" (60). Decreased FPG and FPI values post PMHA might be an indication for an improved balance of hepatic glucose output and insulin secretion in the basal state (60). Based on the latter, it might be cautiously suggested that PMHA improved hepatic insulin sensitivity but not peripheral insulin sensitivity, but additional measurements and analyses are required to verify this hypothesis.

Energy expenditure and substrate oxidation

In this study, indirect calorimetry was used to measure energy metabolism during the clamps. After PMHA, the respiratory exchange ratio (RER) decreased, which suggests a shift in substrate utilization towards more fat oxidation than prior to PMHA. The calculations of glucose and fat oxidation (based on formulae of Frayn (54) further support this assumption, showing significantly lower values of glucose oxidation as opposed to higher levels of fat oxidation post PMHA (Table 3). The latter was evident in both basal and insulin-stimulated states. Interestingly, earlier studies applying *active* exercise-induced heat acclimation found decreased muscle glycogen use post intervention, suggesting that heat acclimation leads to a reduced use of carbohydrate as a fuel during exercise (61-63). Although the results of these previous active heat acclimation studies are based on muscle glycogen concentration whereas the present results are obtained by whole-body indirect calorimetry, they point towards the same conclusion: heat acclimation, both active and passive, causes a substrate switch towards decreased glucose oxidation and increased fat oxidation.

HSP72 expression in muscle

Although (active) heat acclimation has earlier been shown to enhance HSP72 expression (42, 44), a significant increase of HSP72 expression in muscle was not observed in the present study. Similar to insulin sensitivity, relative changes of HSP72 expression also varied greatly between subjects; however, the changes of HSP72 did not correlate with changes in insulin sensitivity. The results of the present study do not support findings of earlier studies, which suggest that heat acclimation improves insulin sensitivity via the increase of HSP72 levels.

A possible explanation for the lack of change in HSP72 expression in muscle by PMHA might be the relatively mild thermal challenge employed in this study: passive exposure to ~34.5˚C for 6h per day, at 10 consecutive days. Earlier studies, reporting effective recruitment of HSP72, applied more intense heat treatments, using exercise *and* elevated ambient temperature simultaneously, which might have led to a superior stimulation of HSP72 expression (40-42, 44). As HSP72 is primarily involved in cyto-protective mechanisms, an overexpression is likely to be triggered when the equilibrium of a cell is considerably disturbed. Possibly, due to the mild form of

acclimation, although it effectively influenced other thermophysiological parameters such as Tcore, blood pressure and HR, this was not (always) the case.

Another contributing factor might lie in the fact that all participants were mildly or significantly insulin-resistant upon initiation of the study. It has earlier been shown that insulin resistance as well as T2DM go along with a considerably decreased content of HSP72 in muscle tissue compared with a healthy situation (26, 43, 64). Although PMHA indeed caused other beneficial metabolic and thermophysiological adaptations, intracellular HSP72 expression in muscle did not change and could therefore not contribute to the desired enhancement of peripheral insulin sensitivity.

Thermophysiological and cardiovascular adaptations to PMHA

PMHA elicited significant thermophysiological adaptations in the present study, causing a decrease of Tcore, core-distal skin temperature gradient and local sweat rate during thermoneutral conditions. Moreover, a distinct decrease of mean arterial pressure (MAP) was evident and partly a lowering of heart rate during warming.

In an earlier study, we have shown that in a young healthy population, a 7-day PMHA protocol elicited significant thermophysiological and cardiovascular changes, which were similar to those typically reported after more intense (often exercise-induced) heat acclimation studies (47). The present investigation confirms the effectiveness of PMHA also in an overweight elderly population. An average overall decrease of Tcore of approximately -0.17˚C in the present study is even more pronounced than in the earlier PMHA study in healthy participants, where Tcore decreased by approximately -0.14˚C post PMHA, which might, amongst other things, be due to the longer duration of acclimation (10 days vs. 7 days) and the slightly higher acclimation temperature (~34.5˚C vs. 33˚C). Moreover, in both studies, we observed a significant decrease of blood pressure. Especially in the present study, the observed reduced mean arterial pressure (MAP) during warming is a highly favourable result as hypertension represents a frequent medical issue in overweight and elderly individuals. Note that in the present study, four out of eleven participants were previously diagnosed with high blood pressure and three of those were using antihypertensive drugs during the study. Prolonged and more frequent exposure to warm thermal environments might help to alleviate hypertension and potentially even facilitate a reduced need for medication.

Interestingly, although Tcore and core-distal skin temperature gradient were affected by PMHA, a change of skin temperature distribution towards higher distal and lower proximal skin temperature as observed in the earlier PMHA study in health individuals, was not evident. A possible explanation for this dissimilar result might lie in the difference of body fat content

between the two study groups: whereas the healthy population had an average BMI of 22.6±2.9kg/m^2 and respectively 19.7±3.0% body fat (47), the average BMI of the overweight elderly group was as much as 30.4±3.2kg/m^2 with respectively 28.5±4.6% body fat. A thicker subcutaneous fat layer is known to more effectively isolate thermal exchange between the body and the surrounding environment and might therefore also impair or attenuate the capacity for heat loss via the skin. Moreover, age has been shown to be a crucial factor in reduced cutaneous circulatory response to heat (65, 66). Together, a thicker subcutaneous fat layer and reduced capacity for vasodilation might have attenuated or blunted the increase of blood flow, and thereby the dissipation of heat, to the distal body parts of the overweight elderly group.

Relationship between Tcore and M-value post PMHA

A rather unexpected result of the present study lies in the observed inverse relationship between ΔTcore and ΔM-value that was evident after PMHA: a large decrease of Tcore was linked to more insulin resistance. This result is opposed to many earlier studies showing an improvement of glucose clearance and a decrease of insulin resistance after short-term and longer-term heat exposure, which is why the opposite result, a greater improvement of insulin sensitivity paired with a larger decrease of Tcore, might have been expected.

Although PMHA effectively affected thermophysiological parameters, no increase of intracellular HSP72 expression was evident after PMHA and therefore no HSP72-mediated improvement of insulin sensitivity could have taken place. Lastly, most previous heat acclimation studies showing a decrease of insulin sensitivity were performed in small rodents, which might not be representative for whole-body physiological changes in humans.

Limitations and future perspectives

This study provides information on the effect of PMHA on metabolic, thermophysiological and cardiovascular parameters. However, with respect to the general interpretation of the study results, a few limitations need to be taken into consideration.

First of all, the study population was limited to a group of overweight elderly men, and therefore, more information on the effect of PMHA on metabolic, thermophysiological and cardiovascular parameters in other populations, and especially in women, is needed.

Furthermore, although participants were asked to not deviate from their normal lifestyle and habitual physical activity, we did not record any information with respect to diet and exercise during the study period. Therefore, future studies should include measurements of dietary intake and physical activity in order to correct for changes of behavioural patterns, which might affect insulin sensitivity.

Conclusions

This study shows that passive acclimation to mildly elevated temperatures significantly lowers FPG and FPI in an overweight elderly population. Whole-body insulin sensitivity and HSP72 expression were, however, not affected by PMHA. Core temperature was lowered by PMHA, both in a thermoneutral condition and during warming. Moreover, mean arterial pressure was lowered in thermoneutrality and during warming. This indicates beneficial health effects of heat acclimation for the specific study group, as cardiovascular diseases are commonly encountered in overweight and elderly people.

Although we did not reveal any effect of PMHA on whole-body insulin sensitivity, as measured by hyperinsulinemic-euglycemic clamps, and HSP72 in this study, preclinical data suggests that heat acclimation could affect insulin sensitivity. Since most studies on the relationship of heat acclimation, insulin sensitivity and HSP72 are carried out at cellular level or in animal models, more research is needed to test if alternative heat acclimation protocols may affect whole-body insulin sensitivity and HSP72 in humans.

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SUPPLEMENTS

Supplement 1 | M-values before and after PMHA

Participant ID	M-value [mg/kg/min] pre	M-value [mg/kg/min] post	Relative change M-value
1	1.84	2.13	13.62
2	2.19	1.61	-36.02
3	3.25	3.66	11.20
4	3.49	2.95	-18.31
5	3.63	3.79	4.22
6	2.03	2.06	1.46
7	3.44	2.07	-66.18
8	3.52	3.43	-2.62
9	2.60	3.57	26.35
10	4.59	4.18	-9.81
N=10.			

Supplement 2 | Skin temperature, skin perfusion, blood pressure, heart rate, stroke volume and cardiac output before and after PMHA during UP

	Pre PMHA	Post PMHA	Δ post-pre PMHA	P-value (pre vs. post PMHA)
Mean skin temperature [˚C]				
Baseline	33.90±0.41	33.95±0.30	0.05±0.34	0.658
T1	35.36±0.33	35.35±0.23	-0.01±0.29	0.935
T2	36.19±0.27	36.16±0.21	-0.03±0.22	0.678
T3	36.60±0.30	36.51±0.23	-0.09±0.19	0.155
Proximal skin temperature [˚C]				
Baseline	33.53±0.63	33.55±0.53	0.02±0.05	0.915
T1	35.21±0.43	35.18±0.36	-0.03±0.30	0.726
T2	36.00±0.29	35.97±0.28	-0.03±0.32	0.749

T3	36.39±0.27	36.28±0.24	-0.12±0.32	0.260
Distal skin temperature [°C]				
Baseline	33.86±0.78	34.08±0.38	0.22±0.77	0.362
T1	35.27±0.60	35.51±0.29	0.24±0.50	0.142
T2	36.24±0.50	36.32±0.27	0.08±0.42	0.531
T3	36.85±0.47	36.77±0.28	-0.08±0.55	0.626
Relative hand skin blood flow [%]				
Baseline	100±0	100±0	n/a	n/a
T1	104±37	156±168	52±145	0.267
T2	118±49	196±280	78±246	0.316
T3	115±39	127±50	12±43	0.369
Relative underarm skin blood flow [%]				
Baseline	100±00	100±00	n/a	n/a
T1	158±53	153±85	-4±124	0.911
T2	289±134	278±205	12±293	0.898
T3	508±236	468±263	40±295	0.662
Systolic blood pressure [mmHg]				
Baseline	120±15	118±12	-2.36±7.29	0.307
T1	n/a	n/a	n/a	n/a
T2	121±15	119±16	-2.58±3.88	0.052$
T3	120±12	118±14	-1.79±4.86	0.250
Diastolic blood pressure [mmHg]				
Baseline	80±7	76±7	-3.73±3.59	0.006**
T1	n/a	n/a	n/a	n/a
T2	79±7	75±8	-4.06±2.96	0.001**
T3	78±5	76±7	-2.15±4.60	0.152

Heart rate [bpm]				
Baseline	64±11	63±8	-1.26±4.31	0.356
T1	69±8	67±8	-2.25±3.45	0.107
T2	71±11	67±10	-3.82±4.05	0.011*
T3	72±11	70±10	-2.11±4.62	0.160
Stroke volume [mL/min]				
Baseline	105.43±22.23	100.74±30.47	-4.69±26.91	0.576
T1	n/a	n/a	n/a	n/a
T2	104.01±22.75	101.00±27.88	-3.02±22.67	0.668
T3	103.52±19.04	102.68±31.39	-0.86±25.97	0.915
Cardiac output [L/min]				
Baseline	6.65±1.42	6.16±1.93	-0.49±1.68	0.356
T1	n/a	n/a	n/a	
T2	7.17±1.28	6.80±1.97	-0.36±1.62	0.472
T3	7.38±1.15	7.06±2.20	-0.32±1.78	0.561

Data is presented as mean±SD. N=11. $ indicates 0.05<P<0.1 for changes post PMHA, * indicates P<0.05 for changes post PMHA, ** indicates P<0.01 for changes post PMHA.

Chapter 5

The effect of warmth acclimation on behaviour, thermophysiology and perception

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ABSTRACT

Public and commercial buildings tend to overheat, and a considerable amount of energy is consumed by air-conditioning and ventilation. However, many occupants remain unsatisfied and consequently exhibit thermoregulatory behaviour (TRB) e.g. open windows or control the air-conditioning. This, in turn, might negatively influence the building energy use. This paper hypothesises that warmth acclimation influences thermophysiology, perception and TRB in a warm environment. Therefore, the effect of warmth acclimation on TRB, physiology and perception is investigated.

Twelve participants underwent a so-called SWITCH protocol before and after warmth acclimation (7 days, 6 h/day, about 33˚C, about 22%RH). During SWITCH, participants chose between a warm (37˚C) and a cold (17˚C) condition. TRB was determined by the number of switches and the time spent in a specific condition. Mean skin temperature was recorded to assess behavioural thresholds. Thermal comfort and sensation were indicated on visual analogue scales (VAS). After acclimation, the upper critical behavioural threshold significantly increased from 35.2±0.6˚C to 35.5±0.5˚C ($P \leq 0.05$) and the range of mean skin temperatures at which no behaviour occurred significantly widened (3.6±0.7 to 4.2±0.6 ($P < 0.05$)). The total number of switches tended to decrease ($P = 0.075$). The present study is the first to show that prolonged passive exposure to warmth extends TRB thresholds.

INTRODUCTION

In temperate climate zones, public and commercial buildings tend to overheat due to, amongst other things, highly insulating construction materials, high internal heat loads and the progression of global warming (1). Approximately one-third of the primary energy supply in the Western world is used for air-conditioning and ventilation of buildings, mostly to ensure occupant comfort (2). However, a great number of building occupants remain unsatisfied with the thermal environment. People nowadays spend most of the day indoors (3) and therefore it is of particular interest to study the interactions of people and their thermal environment to create a well-balanced and energy-effective indoor ambience. The present paper focuses on the impact of prolonged exposure to relatively warm temperatures on autonomous (physiological) and conscious (behavioural) human thermoregulation and temperature perception.

Thermoregulatory behaviour in the built environment

Building occupants are mainly exposed to fixed indoor temperatures (±0.5˚C), as recommended by international standards (4). These standards are mostly intended to create a thermoneutral environment, but the application of those standards is, nevertheless, often not a guarantee for acceptable and adequate indoor temperatures. Consequently, building occupants frequently influence their thermal environment by, for example, opening a window or controlling the air-conditioning, in order to maximise thermal acceptability and to improve satisfaction. Those actions can be referred to as thermoregulatory behaviour (TRB). Importantly, the indoor climate of a building can affect the human metabolism and related biological processes and uncomfortable warm environments might cause sleepiness and restrict productivity (5). Therefore, it is important to ensure optimal indoor conditions to guarantee a healthy and stimulating work environment.

TRB might, however, in turn, affect heating, ventilation and air conditioning (HVAC) performance and thereby influence energy expenditure and energy costs of the building. Knowledge gaps regarding the mechanisms and driving forces of TRB make it a rather difficult factor to predict. The lack of possibilities to predict TRB also makes it difficult to foresee the impact of TRB on the energy use of a building. TRB might influence building energy use when occupants are able to interfere with the system, since TRB might cause a discrepancy between the predicted and the actual energy consumption of a building. More insight in mechanisms and triggers of TRB provides the opportunity to improve building energy simulation models by inclusion of user characteristics, since the current models are often lacking this information. The latter stresses the need for a better understanding of the interplay between the occupant and the respective building to prevent a wasteful use of resources and to reduce energy costs.

Thermoregulatory behaviour, thermophysiology and thermal perception

In order to comprehend the mechanisms of TRB, it is essential to understand the controlling factors. TRB is greatly determined on autonomously regulated physiological processes of the human body. Changes of core and skin temperature have previously been identified as the main driving forces of TRB (6, 7). More recently, Schlader *et al.* (8) have indicated that especially in mild thermal environments, skin temperature is the primary initiator of TRB. However, the mechanisms that actually control TRB and the respective thresholds that must be reached or exceeded before behaviour is initiated, remain ambiguous. Apart from the physiological determinates, another important factor for TRB is the subjective perception of a thermal environment, expressed by, for example, thermal sensation (TS) and thermal comfort (TC) (9, 10). When TS progresses away from neutral and/or an environment is perceived as being 'uncomfortable', TRB is likely to occur (11). The latter implies that TRB is not solely initiated and influenced by physiological reactions but is part of a complicated synergy of physiological, conscious and subconscious factors.

Thermoregulatory behaviour and heat acclimation

Due to the increasing risk of overheating of buildings it is important to study the impact of prolonged exposure to warmth on human metabolism and the thermoregulatory system (12). Heat acclimation has been studied for many years, yet there is a distinct lack of information on the effect of prolonged warmth exposure on TRB and subjective perception. Traditionally, heat acclimation studies were designed to develop active acclimation models for miners, athletes or the military (13). The majority of such studies used exercise-induced hyperthermia combined with high ambient temperatures to reach adaptations at various levels of the thermoregulatory system, but also includes non-exercise studies where hypothermia was induced by sitting in a warm bath (14-16). These adaptations include changes of core temperature, skin temperatures, evaporative heat loss, the cardiovascular system and other metabolic functions; and they result in a superior ability to dissipate heat. However, where exercise is used as an additional heat stimulus, it is difficult to distinguish between temperature- and exercise-related adaptations of the thermoregulatory system. Strikingly, information on the effects of prolonged mild passive exposure to warmth is very limited. To the best of our knowledge, no information exists on the effect of such mild warmth acclimation (neither active nor passive) on human TRB. To gain important insights for the built environment sector, it is desirable to test the effects of mild, and thus more realistic ambient conditions on the human thermoregulatory system, on both the autonomous and conscious parts.

Following the above, the present authors hypothesise that warmth acclimation might influence TRB by modulating skin temperature and thermal perception. Therefore, they investigated the

effect of mild passive warmth acclimation on thermoregulatory behaviour, skin temperature and thermal perception.

METHODS

The experiments presented in this paper are part of an extensive study designed to evaluate the effects of passive warmth acclimation on thermal physiology, thermal comfort and TRB. In the scope of this paper, we focus on the behavioural part of the experiment.

All experiments were performed at the Metabolic Research Unit of Maastricht University (MRUM) between December 2014 and August 2015. During this period, 12 young healthy male volunteers visited the MRUM for nine consecutive days. Their characteristics are provided in Table 1. Fat percentage and fat mass were determined by dual-energy x-ray absorptiometry (DEXA).

Table 1 | Participant characteristics

	Mean±SD
Age [years]	24.1±3.1
Height [m]	1.79±0.07
Weight [kg]	73.6±9.7
BMI [kg/m2]	23.0±3.0
Fat percentage [%]	19.8±2.9
Fat mass [kg]	14.9±3.4
Data is presented as mean±SD. N=12.	

All volunteers were healthy, normotensive, non-obese, non-smokers and not taking any medication that might have altered the cardiovascular system or thermoregulatory responses. The participants refrained from food, alcoholic and caffeinated beverages as of 22:00 hours the evening before being measured. For the behavioural experiment, they underwent two testing days (1 and 9) and seven days of warmth acclimation (Figure 1A). During days 1 and 9, the SWITCH protocol was performed.

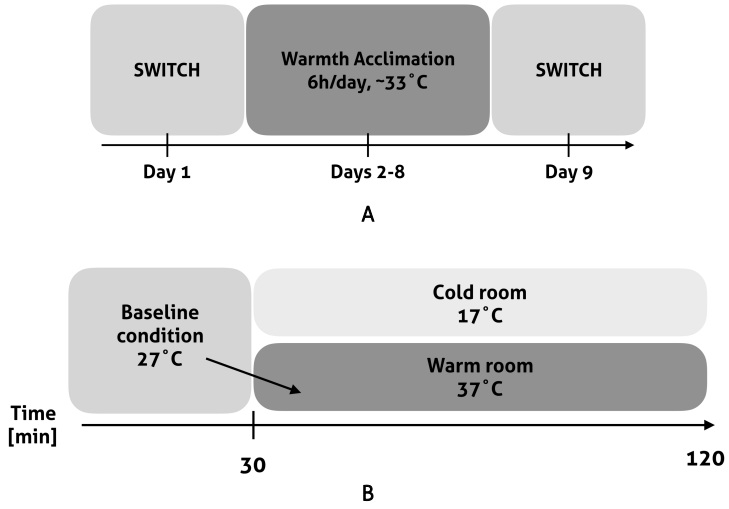


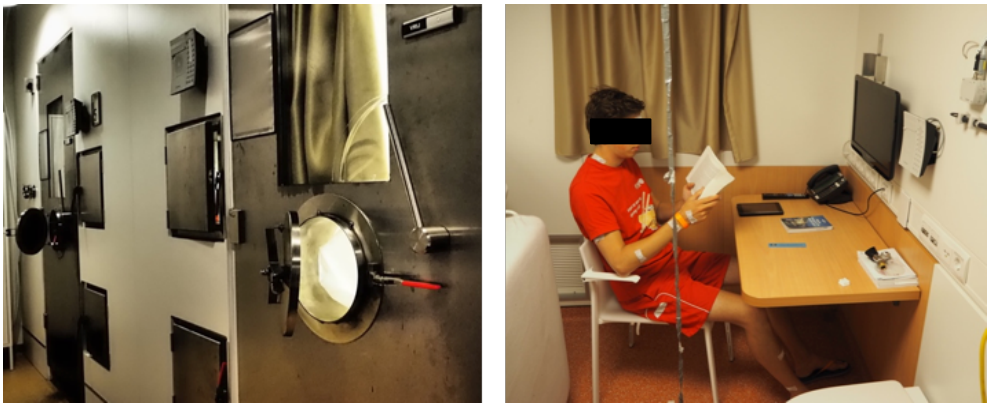
Figure 1A and 1B | *A* Time course of the study. *B* The SWITCH protocol
After 30min of baseline, participants were free to switch between a warm and a cold room; there were no limits with respect to frequency or time. The black arrow indicates the start condition.

5

The SWITCH protocol

The SWITCH protocol (Figure 1 A and B) was performed to evaluate TRB before and after warmth acclimation. SWITCH was conducted in two climate chambers of the MRUM (Figure 2A). Before commencing SWITCH, volunteers acclimatised in the baseline condition (27˚C ambient temperature) for 30 min in order to ensure a comparable starting situation. After 30 min, they were guided to a warm room (37˚C). They were instructed that as of that very moment, they were free to switch between the warm room (37˚C) and a cold room (17˚C), without any limitation to the number of switches or the time between switches. Moreover, participants were informed that they could switch between the warm and the cold condition without notifying the researcher, whenever they wanted, simply by opening the door by themselves and walking into the other room. The latter was considered to be of great importance in order to ensure that participants actually perceived the freedom to express TRB without feeling monitored. SWITCH continued for 90min. During measurements, participants were instructed to remain seated at a desk whilst remaining in one of the rooms (Figure 2B) and they were allowed to perform reading tasks (1.2 metabolic equivalent of tasks (METs)). Watching television or browsing the internet was prohibited in order to minimise the risk of distraction.

Upon arrival at the laboratory, participants were asked to change into standardised clothing, consisting of underwear, T-shirt, shorts and slippers/socks. During the experiment, they sat on a chair. The total thermal resistance of the clothing ensemble plus the desk chair added to approximately 0.41 clo (17, 18). Skin temperature was measured at 14 ISO-defined skin sites (19), to calculate mean skin temperature as well as proximal (body core) and distal (extremities) skin temperature. Skin temperatures were recorded at 1-minute intervals throughout the whole protocol (iButton, Maxim Integrated Products, USA). Air temperature and relative humidity were measured by means of wireless temperature/humidity sensors (Hygrochron iButton, DS1923, Maxim Integrated Products), according to EN-ISO 7726 (20). Moreover, participants were asked to rate their thermal environment using visual analogue scales (VAS). TS and TC votes were recorded every six minutes and an additional questionnaire was answered just before the initiation of a switch of rooms. TS was evaluated using the seven-point continuous ASHRAE thermal sensation scale ranging from -3 to 3 (-3 cold, -2 cool, -1 slightly cool, 0 neutral, 1 slightly warm, 2 warm, 3 hot). TC was indicated on a continuous five-point VAS ranging from -2 very uncomfortable to 2 very comfortable (Figure 3).



A B

Figure 2 A and B | Representative climate chambers at the MRUM

A) Two representative climate chambers at the MRUM. During SWITCH, participants commuted between two climate chambers (warm room, 37˚C and cold room, 17˚C), *B)* Participant during SWITCH.

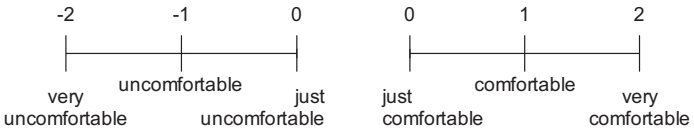


Figure 3 | Thermal comfort scale
Question: 'How do you perceive your thermal environment?'

Warmth acclimation

After the first SWITCH on day 1 of the study, the warmth acclimation period commenced (Figure 1A). Participants were exposed to 33.3±1.56˚C ambient temperature with a relative humidity of 22.3%±6.6% on seven consecutive days for six hours per day. During their stay, participants wore standardized clothing (underwear, T-shirts, shorts and beach slides, about 0.36 clo (17)) and they sat on an office chair (about 0.05 clo, (17)). They were asked to perform regular office work (1.2 METs) and were allowed to leave the room for short toilet breaks. Participants were provided with food (sandwiches and crackers/cookies; three times in six hours) and water *ad libitum*.

Statistical analyses

Data is presented as mean±SD or as range between minimum and maximum. Matlab R2014a was used for data preparation and both Matlab R2014A and SPSS 22.0 for Mac (SPSS Inc.) were used for statistical data analyses. TRB was evaluated by 1) counting the total number of switches, 2) clocking the time participants remained in the warm and the cold room and 3) by evaluating the course of their mean skin temperature, as the latter can represent an important predictor for TRB. Mean skin temperature as measured just before switching from the warm room to the cold room (37˚C → 17˚C) indicated the upper critical behavioural threshold (UCBT), whereas mean skin temperature measured just before switching from the cold room to the warm room (17˚C → 37˚C) marked the lower critical behavioural threshold (LCBT). Moreover, TS and TC votes upon the initiation of a switch were analysed. Paired t-tests were applied to test for statistical differences between pre- and post-acclimation measurements of skin temperature. Wilcoxon signed-rank tests were to test statistical differences between pre- and post-acclimation measurements of TRB, TS and TC. Statistical significance was assumed if $P \leq 0.05$. A trend was assumed when $0.05 < P < 0.10$.

RESULTS

Thermoregulatory behaviour pre- and post- warmth acclimation

Before acclimation, participants switched two to six times and spent a total amount of 17-77 min in the warm room and 12-74 minutes in the cold room (Table 2, Appendix 2). After acclimation, participants switched zero to six times and the total amount of time spent in the warm room increased to 35-90 min, whereas the time spent in the cold room decreased to 0-56 min (Table 2, Appendix 2). All individual movements between the warm and the cold rooms before and after warmth acclimation are represented in Figure 4, together with the matching mean skin temperatures and mean TS votes, as measured just before switching the conditions. Effects of season on the measured variables were ruled out by testing the effect of mean day outdoor temperature for each individual participant on TRB, TS, TC and mean skin temperatures.

Table 2 | Results of SWITCH pre and post warmth acclimation

	Pre-warmth acclimation	Post-warmth acclimation	P-value
Switches	3.4±1.1	2.5±1.5	0.075
Stay in warm room [min]	50.9±16.2	56.9±14.7	0.177
Stay in cold room [min]	37.3±16.7	31.9±14.4	0.283
UCBT [˚C]	35.2±0.6	35.5±0.5	0.050*
LCBT [˚C]	31.6±0.9	31.4±0.8	0.585
TBNZ	3.6±0.7	4.2±0.6	0.027*

LCBT lower critical behavioural threshold, *UCBT* upper critical behavioural threshold, *TBNZ* thermal behaviour-neutral zone, range of mean skin temperature within which no thermoregulatory behaviour occurred. Data is presented as mean±SD. *P≤0.05. N=12.

Mean skin temperature and thermoregulatory behaviour

Before warmth acclimation, UCBTs ranged from 35.1˚C to 36.1˚C and LCBTs from 30.0˚C to 32.9˚C. After acclimation, UCBTs ranged from 34.5˚C to 36.3˚C and the LCBTs from 30.1˚C to 32.8˚C. As indicated in Table 2, the UCBT's significantly increased post acclimation, but the LCBTs were not significantly different.

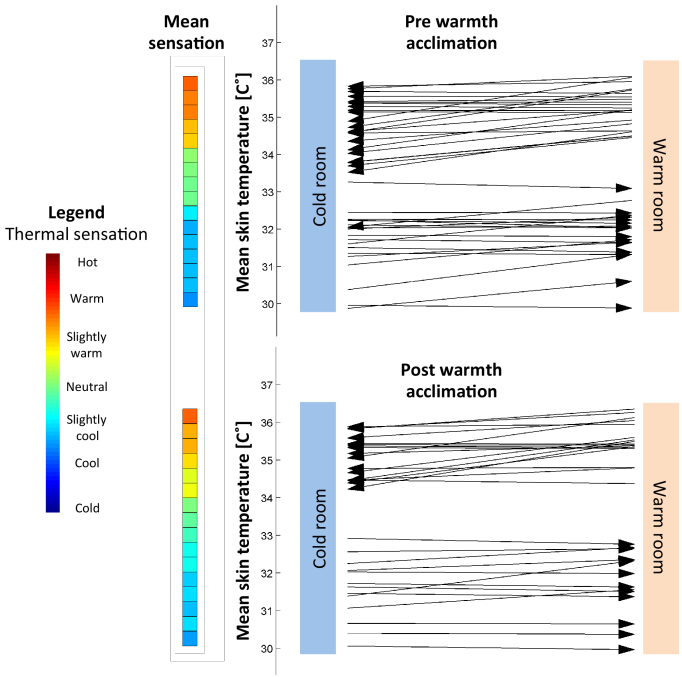


Figure 4 | Individual switches pre and post warmth acclimation

The black arrows indicate all individual switches between the warm and the cold room after baseline, before (top) and after (bottom) warmth acclimation. The arrow origins and insertions indicate mean skin temperatures as measured upon leaving the original condition and arriving at the opposite condition. Mean sensation represents the mean of the sensation votes for the respective mean skin temperature just before switching. N=12.

The range of mean skin temperatures between the two critical thresholds is described as thermoregulatory behaviour neutral zone (TBNZ), thus the range of mean skin temperature within which no switch occurred. The smallest observed range of TBNZ before acclimation was 32.3˚C – 35.3˚C (range = 3.08˚C) and 30.46˚C – 33.63˚C (range = 3.34˚C) after warmth acclimation. The largest observed range of TBNZ before acclimation was 29.97˚C – 35.14˚C (range = 5.17˚C) and 30.38˚C – 34.87˚C mean skin temperature (range = 5.09˚C) after warmth acclimation. As indicated in Table 2, the TBNZ widened significantly post-warmth acclimation and increased from averagely 31.5±0.9˚C – 35.2±0.6˚C pre-warmth acclimation to 31.4±0.8˚C – 35.5±0.5˚C post-warmth acclimation. All individual TBNZ ranges are presented in Figure 5. For participant 5, no TBNZ could be calculated as the participant did not switch post acclimation but remained in the warm room throughout the entire SWITCH period (90min after baseline). For participant 9, no LCBT could be indicated since they only switched once from the warm room to the cold room and then remained in the cold condition until the end of SWITCH.

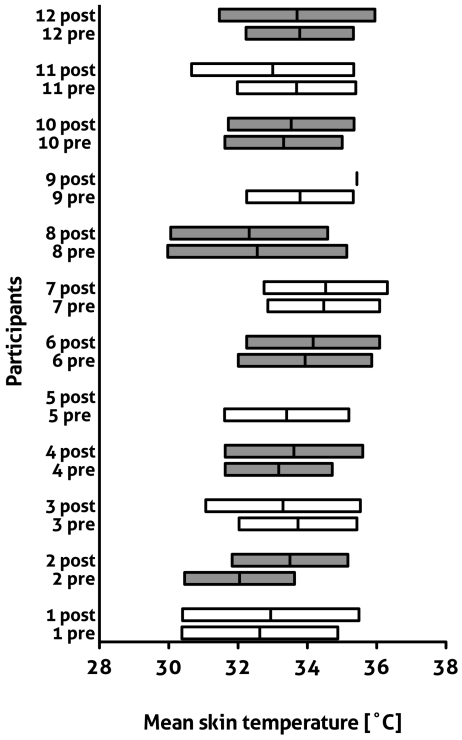


Figure 5 | Individual thermoregulatory behaviour neutral zones (TBNZ) of all participants pre and post warmth acclimation

Alternating shading is applied to illustrate the pairs of TBNZ belonging to one participant.

Thermal perception during SWITCH

The perception of the thermal environment at the UCBT and LCBT was represented by the TS and TC votes submitted by participants just before switching rooms. The minimum, maximum and range of votes at the LCBT and UCBT (within the TBNZ) before and after warmth acclimation are presented in Table 3. TS and TC votes within the TBNZ are presented in Appendix 1. TS and TC at UCBT and LCBT were not significantly influenced by warmth acclimation.

Table 3 | Thermal sensation and thermal comfort at the lower and upper critical behavioural temperature during SWITCH pre and post warmth acclimation

	Mean±SD	Minimum	Maximum
Sensation			
LCBT pre	-1.3±1.3	-2.9	2.3
UCBT pre	1.4±0.9	-0.5	2.4
LCBT post	-1.3±0.5	-2.1	-0.4
UCBT post	1.5±0.5	0.9	2.4
Comfort			
LCBT pre	0.1±0.8	-1.1	2.0
UCBT pre	-0.1±0.3	-0.6	0.3
LCBT post	0.3±0.7	-0.5	2.0
UCBT post	0.0±0.3	-0.5	0.6

LCBT lower critical behavioural threshold, UCBT upper critical behavioural threshold. No significant difference was detected between LCBT's and UCBT's pre and post warmth acclimation. N=12.

DISCUSSION AND CONCLUSIONS

The present study evaluated the effect of passive warmth acclimation on TRB, skin temperature, TS and thermal perception. In accordance with our hypothesis, we show that only seven days of passive warmth acclimation significantly influenced mean skin temperature and TRB. Post-acclimation, participants switched at significantly higher mean skin temperatures (higher UCBT), thereby broadening the range of mean skin temperatures at which no TRB occurred (TBNZ). Moreover, the total amount of switches tended to decreased post acclimation (P=0.075). TS and TC, however, did not significantly change after warmth acclimation.

It has previously been suggested that core and skin temperature are the driving forces for TRB (6, 7). More recently, Schlader *et al.* (8, 21) emphasised the importance of skin temperature in mediating behavioural thermoregulation, especially in mild thermal environments. Core temperature has been found to play a less important role, which might be due to the nature of the concept itself: the goal of both physiological and behavioural thermoregulation is to buffer

(substantial) changes in core temperature and to ensure thermal balance (22, 23). We therefore decided to focus on skin temperature as determining factor for TRB.

Generally, knowledge on the driving forces of behavioural thermoregulation in humans is very limited, which is surprising, considering the important role that TRB plays in human thermoregulation. After all, thermal physiology (e.g. vasomotion, sweating and cold-induced thermogenesis) has relatively limited capacity, whereas the capability of TRB is virtually unlimited (22, 24). From the present authors' thermophysiological studies, it is known that the individual variation in thermal responses is great: sex, age, body composition and metabolism influence the range of preferred temperatures and thereby codetermine TRB (11, 25, 26). Besides that, the state of acclimatisation is of significant importance. Our results indeed show considerable individual variation of TRB. For example, the number of total switches between warm and cold ranged from zero to six. Moreover, time spent in one of the respective conditions greatly varied between participants, and, as depicted in Figure 4, width and range of TBNZs notably differed.

Above that, sensation and comfort votes provided at the initiation of a switch remarkably varied between the participants. As indicated in Table 3, switching occurred at TS votes ranging between cold and warm for the LCBT and slightly cold and hot for the UCBT. As for TC, votes varied between uncomfortable and very comfortable for the LCBT and between uncomfortable and just uncomfortable and between just comfortable and comfortable for the UCBT (Table 3). The range of TS and TC votes within the TBNZ tended to decrease after warmth acclimation. The latter indicates that the thermal environment was perceived as less extreme and less uncomfortable, considering the tendency for fewer switches post acclimation. On average, switching to the warm room occurred when participants were feeling cool but just comfortable, whereas the switch to the cold room was initiated when the thermal environment was perceived between warm and hot and just uncomfortable to just comfortable, respectively. Participants thus tended to preserve their state of comfort and switched to the respective opposite condition, already before a distinct state of discomfort was established. The latter was even more pronounced after warmth acclimation, but the trend was not significant.

To the best of our knowledge, the present study is the first to investigate the effect of passive mild warmth acclimation on TRB. The findings indicate that prolonged passive exposure to warmth extends the behavioural threshold for warm conditions. Participants seem to tolerate higher mean skin temperatures before they feel the need to regulate their body temperature. Moreover, great individual variation for all the measured parameters was evident. Although the effects of warmth acclimation on UCBT and TBNZ were moderate on group level, the results are of statistical and physiological significance. This information could be of importance for the design of future indoor thermal environments. Building energy expenditure could easily be lowered by using a less strict air-conditioning set-point without affecting occupant satisfaction.

With respect to practical implications, the findings suggest that acclimatised occupants of an overheated building might tolerate higher skin temperatures without feeling the need to change their thermal environment. Participants tolerated approximately 0.3˚C higher mean skin temperatures after only seven days of warmth acclimation. Although the effect on mean skin temperature was relatively small, a toleration of 0.3˚C higher mean skin temperatures implies a reduced cooling demand for HVAC systems (equal to 0.3°C, assuming a constant temperature gradient between skin and air temperature to maintain heat balance and neglecting effects on evaporation either due to humidity (physical) or perspiration (physiological)). Reducing the cooling need by increasing the air-conditioning set point by only 0.4˚C may already lead to yearly savings of approximately 5% (27). Furthermore, prolongation of the acclimation period might lead to the tolerance of even higher mean skin temperatures, which might subsequently lead to even greater savings.

The present study provides an important first step towards the better understanding of physiological, subjective and behavioural changes post warmth acclimation. It was designed to develop fundamental knowledge on the interaction physiology, subjective and behavioural factors. However, with respect to the general interpretation of the study results, a few limitations need to be taken into consideration. Firstly, only healthy young men were studied, limiting the transferability of the results to the general population. Secondly, the experiments were carried out in a very controlled laboratory environment and therefore, results might not be directly transferrable to practical settings in the built environment. Temperatures applied in the SWITCH protocol, dependent on the climate zone in question, were likely to be more extreme than usually encountered in buildings. Finally, the experiment's long duration (nine months in total), which was needed to carry out all measurements, should be taken into consideration. Since the study was carried out over three different seasons (winter, spring and summer), it was possible to test the effect of season (mean day outdoor temperature 2 weeks before commencing the measurement for each individual) on the measured parameters TS, TC and mean skin temperature. None of the parameters was significantly affected, minimising bias by seasonal temperature differences. Although it has been tested and concluded that season did not influence the results, the long study period might have alleviated the effects.

Future research should focus on the evaluation of TRB and prolonged warmth exposure in a larger, more diverse population of both men and women. Moreover, field studies are needed to establish and verify the results under realistic, less controlled indoor conditions.

ACKNOWLEDGEMENTS

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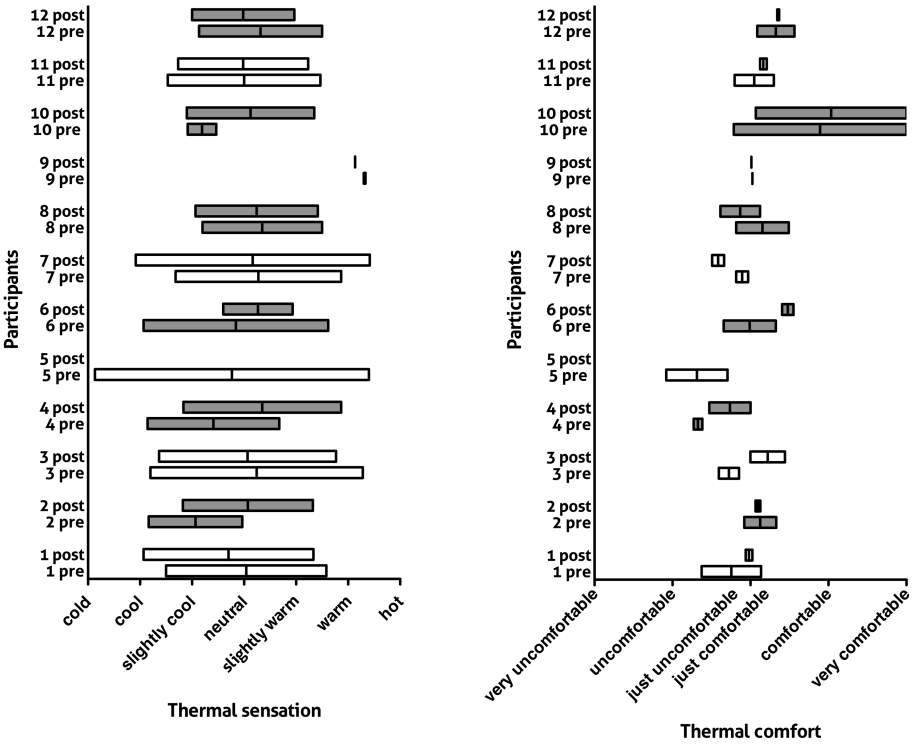
APPENDICES



A B

Appendix 1 | A Thermal sensation votes within the TBNZ of all participants pre and post warmth acclimation. B Thermal comfort votes within the TBNZ of all participants pre and post warmth acclimation

Alternating shading is applied to illustrate the pairs of TBNZ belonging to one participant.

Appendix 2 | Number of SWITCHes pre and post warmth acclimation per individual

	Switches pre	Switches post
PP01	3	2
PP02	4	6
PP03	3	2
PP04	6	2
PP05	2	0
PP06	3	3
PP07	4	4
PP08	3	3
PP09	3	1
PP10	4	3
PP11	4	2
PP12	2	2
N=12.		

Chapter 6

Local cooling in a warm environment

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ABSTRACT

Public and commercial buildings tend to overheat. Recent studies indicate individual comfort systems based on local climatisation can improve occupant satisfaction and simultaneously decrease the energy load of buildings. This study evaluated the effect of local cooling in both women and men on indicators of occupant satisfaction: thermal sensation, thermal comfort and skin temperatures.

All measurements were conducted in a climate chamber (Priva, the Netherlands) with an ambient temperature of 32.3±0.3˚C (mean±SD). In total, 16 healthy young men and women were exposed to different local cooling conditions for 45 min: face cooling, back cooling, underarm cooling, foot sole cooling and 30 min of combined face-underarm cooling. The cooling conditions were separated by 30 min of 'no cooling'. Thermal sensation and thermal comfort were evaluated with visual analogue scales. Skin temperatures (26 sites) were measured using wireless temperature sensors. 'Face cooling' and combined 'face-underarm cooling' significantly improved thermal sensation and comfort compared with 'no cooling' for both women and men. Women had significantly higher skin temperatures compared with men.

Local cooling of the face alone and face and underarms combined are effective ways to improve thermal sensation and thermal comfort in a warm thermal environment.

INTRODUCTION

One third of the primary energy supply is used for the ventilation and air-conditioning of commercial and public buildings; mainly to achieve occupant satisfaction (1). Nevertheless, thermal comfort is often not achieved. In addition, the overheating of buildings became a hot topic in the Western world in the recent years (2, 3). The risk of overheating is a consequence of a combination of very high insulating construction materials and the heat load of occupants and equipment. Moreover, global warming worsens the scenarios. As a result of the overheating problem, the energy demand of buildings rises to ensure thermal comfort for the occupants. To prevent a further rise of energy costs and simultaneously keeping occupants satisfied, efficient and creative low-energy cooling techniques are needed. It has been suggested that individualised comfort systems might be promising alternatives to overall air conditioning, especially for those buildings/building areas that host largely sedentary occupants (e.g. offices and open plan offices) (4-8).

Today, many buildings have a tightly controlled indoor climate as determined by the ASHRAE Standard 55 and ISO Standard 7730, based on Fanger's predicted mean vote model (PMV) (9-12). Creating a thermal environment conforming with these standards means that very little ambient temperature variation is tolerated. Since a large number of building occupants report thermal discomfort, even though the recommendations of the standards are met, the question is whether these (PMV-) standards are actually suitable.

The large level of perceived discomfort (especially in summer (13)) might be due to significant inter-individual differences in the thermal sensation and thermal comfort of building occupants: physiological parameters such as sex, age, body composition, metabolic rate, insulation, acclimation, behavioural parameters such as physical activity and clothing behaviour, and individual preferences for ambient temperature, might have a considerable effect on an individual's perception of the thermal environment (14, 15). A study among young Europeans indicated that the preferred ambient temperature may vary by as much as 10˚C (16).

Recent investigations confirmed that individually attuned comfort systems have the potential to save a significant amount of energy (up to 50% compared with overall air-conditioning), and improve individual occupant satisfaction (6). Applying individually attuned local cooling may allow an increase in overall indoor temperature without negatively affecting the occupant's thermal comfort. Moreover, individualised local cooling provides the possibility for building occupants to create their own preferred thermal environments tailored to their individual needs at a given moment.

To optimise the design of individual local cooling systems, it is necessary to evaluate the impact of different local cooling conditions and different target regions of the human body (actuators)

on thermal comfort. Furthermore, regarding the anticipated automated control of individual local cooling systems ('human in the loop' comfort systems), it is important to study possible indicators, i.e. physiological parameters (e.g. local skin temperature) that correlate to thermal comfort. Considering the overheating problem mentioned above, we especially focused on the optimisation of occupant comfort in a warm environment. It is crucial to evaluate the individual response of women and men with respect to local cooling in mild heat, since there is barely any data available. Moreover, there is lack on information on the response and effectiveness of different cooling conditions on physiological parameters and thermal comfort of women and men.

We hypothesise that individualised local cooling can effectively improve occupant thermal sensation and comfort in a warm environment. Accordingly, this study aims to:

1. Evaluate the effect of five different local cooling conditions (actuators) on whole-body thermal sensation, thermal comfort and skin temperatures of young, healthy volunteers in a warm thermal environment
2. Identify sex differences in whole-body thermal sensation, thermal comfort and skin temperatures with respect to local cooling in a warm thermal environment
3. Identify potential physiological indicators of whole-body thermal comfort in a warm thermal environment.

METHODS

Facilities

The experiment was established in a climate chamber that is located at the laboratory of the 'Priva' company (De Lier, the Netherlands). The chamber dimensions are depicted in Figure 1. During the experiments, the average ambient air temperature was 32.3±0.29˚C (mean±SD, as shown in section 2.5). Air temperature was kept stable by a combination of radiant heating and air conditioning. Floor, ceiling and three walls (Figure 1) of the climate chamber were built of water-perfused aluminium panels. Water temperature of ceiling and floor panels was set at 29˚C; wall panel water temperature was set at 32˚C and ingoing airflow was set at 35˚C. Relative humidity was not controlled in the present setting. On average, relative humidity was 29.3±3.42%.

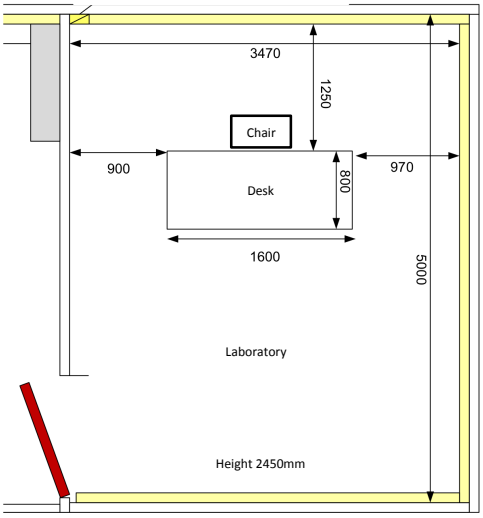

Figure 1 | Laboratory dimensions and desk position.
The yellow surfaces indicate water-perfused aluminium wall panels; the ceiling and floor consisted of water-perfused aluminium panels as well

Participants

Sixteen young, healthy volunteers, 8 men and 8 women, participated in the study. Before informed consent was obtained, the participants were provided with detailed information concerning the experimental procedures. Importantly, no information was provided about the conditions and ambient temperature they were exposed to. All participants were normotensive and non-obese. Four women were on oral contraceptives; all other participants did not take any medication that might alter their cardiovascular, hormonal or thermoregulatory responses to temperature changes. Participant characteristics are provided in Table 1.

Table 1 | Participant characteristics

	Mean±SD	Minimum	Maximum
Age [years]	23.5±3.5	20	32
Height [m]	1.79±0.11**	1.57	2.00
Weight [kg]	69.1±9.8*	56.5	92.9
BMI [kg/m2]	21.5±2.1	18.4	25.8
* P<0.05, ** P<0.001 difference between women and men.			

Experimental procedures

Participants visited the laboratory between May - July 2014. Average outside daytime temperature (8:00 AM – 8:00 PM) was 16.3˚C (17). Participants arrived at the laboratory at 8.30AM in the morning. In total, 26 wireless skin temperature sensors (iButtons, Maxim Integrated Products, CA, USA) were attached to their skin with semi-adhesive tape (Fixomull® stretch, BSN medical GmbH, Hamburg, Germany). Participants wore their own underwear and additional standard clothing, which consisted of a loose-fit cotton T-shirt, jogging pants and cotton socks (clo≈0.54) (18). After preparations were finished, participants entered the climate chamber and sat down on a chair (clo≈0.1, Figure 2) (18-20).

Figure 2 | Experimental setup
The white arrows indicate the outlet of the fan. The outlet position was adjustable in height and angle to individually direct the airflow onto the participant's face.

The experiments lasted for six hours, and in the meantime, participants were allowed to perform regular deskwork (approximately 1.2 METs). Desk and chair were individually adjustable in height to ensure comfortable sitting posture. During the experiment, participants were exposed to five different local cooling conditions that were provided randomly: 1) 'face cooling', 2) 'underarm cooling', 3) 'back cooling', 4) 'foot sole cooling' and 5) combined 'face-underarm cooling'. The different conditions were separated by a 30-min period of 'no cooling', except for the conditions 'underarm cooling' and 'face-underarm cooling'. These were executed consecutively for practical reasons (Figure 3 provides an example of the time schedule).

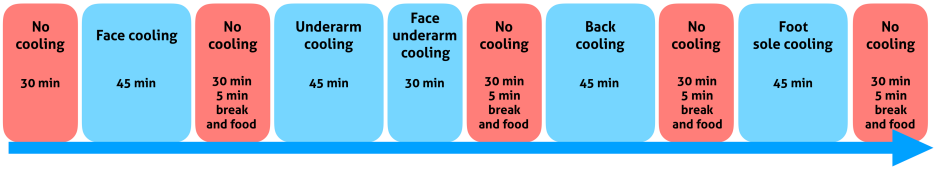

Figure 3 | Example of the time schedule
The conditions' sequence was randomly allocated. During the first 5 minutes of 'no cooling', participants were allowed to leave the climate chamber for a toilet break. After participants entered the chamber again, they were provided with a standard meal (open sandwich). Tepid water was served ad libitum.

Cooling elements and materials

'Face cooling' was conducted using a conventional fan, directed at the participant's forehead region from a distance of approximately 60cm, depending on the sitting posture of the participant. Set-up and air speed of the fan have been tested and verified in a pilot experiment prior to commencement of the present study. The custom-made fan was positioned approximately 15-20cm above the participant's head and it supplied regular ambient, non-cooled air. The airflow had a downward tendency to make it more comfortable for the participants' eyes (Figure 2). The airflow was set at the same steady state for all participants, equalling around 1.28m/s as measured by a Pitot tube (TA460-P, Airflow instruments, UK) at the outlet of the hose. During 'face cooling', participants were asked to maintain a straight sitting posture to keep their head in the airflow of the fan.

For 'underarm cooling' and 'foot sole cooling', a cooling panel with a 1mm aluminium top, a 5mm chip tray underside and a water-cooling system in between was used. The water-cooling system consisted of thin hosepipes (4mm) provided with a permanent water flow. Water temperature was controlled according to the cooling load. Temperature of the cooling panel during 'underarm cooling' and 'face-underarm cooling' was 22.7±0.81˚C while it was 21.8±0.62˚C during 'foot sole cooling' as determined by pilot testing. During 'underarm cooling', participants were asked to keep their underarms and wrists on the cooling panel while using the computer and mouse. During 'foot sole cooling', participants were asked to place their feet soles flat onto the cooling panel. During 'back cooling', participants sat on a tailor-made chair with a water-perfused seat back. The same water-cooling system as used for the cooling panel was integrated in the seat back, covered by a thin layer of fabric. Continuous water exchange in the hosepipes was maintained to prevent heat accumulation. Participants were instructed to sit straight and to keep in constant contact to the cooled seat back. For back cooling, the water supply temperature was set to 30˚C (as previously determined by pilot testing), due to the proximity of the cooling device and the large surface area covered.

Measurements

Participants evaluated the thermal environment at 10-minute intervals. Whole body thermal sensation and thermal comfort were reported on visual analogue scales (VAS) in Dutch language, using an automatic recording system on a personal desktop. We used the standard 7-point ASHRAE thermal sensation scale (-3 cold to 3 hot) and another continuous VAS scale to indicate thermal comfort (Figure 4 A). The thermal-comfort-scale was divided into two parts to urge participants to indicate whether they perceived the thermal environment as 'comfortable' or 'uncomfortable'.

Thermal preference and perceived importance to change the thermal environment were also indicated on continuous VAS scales (Figure 4 B and C). Another VAS scale was used to evaluate the subjective amount of sweating (0 no sweating, 10 sweating).

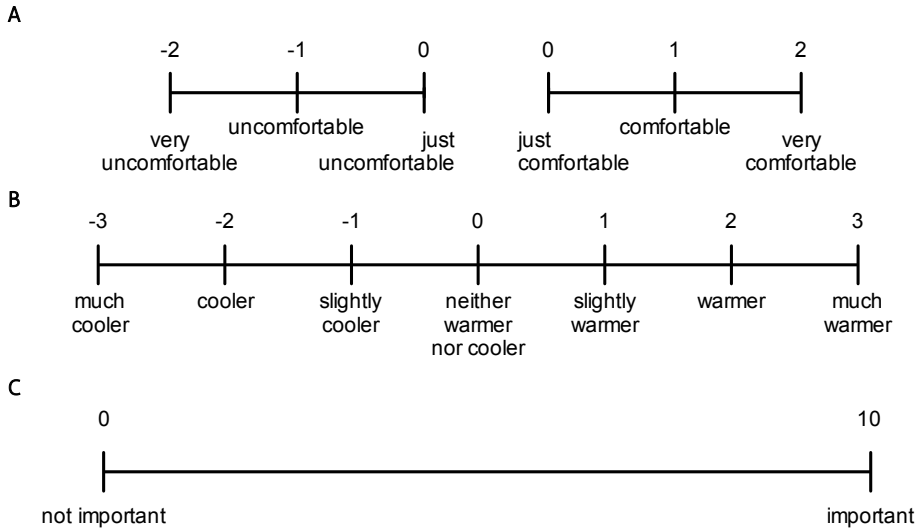

Figure 4 | English versions of the questionnaire scales

(A) Thermal comfort scale (Question: 'How do you perceive your thermal environment?'), (B) VAS for thermal preference (Question: 'What would you prefer at the moment?') and (C) VAS indicating importance to change (Question: 'How important is it for you to change the thermal environment?')

Ambient temperature and relative humidity were measured using four wireless combined temperature/humidity sensors (Hygrochron®, DS1923, Maxim Integrated Products, CA, USA). The four sensors were attached to a string, hanging next to the subject's chair-back at 10cm, 30cm, 60cm and 110cm height (from the ground). Ambient temperature measurements were performed according to EN-ISO 7726 (21) .

Skin temperatures were measured using wireless iButton® dataloggers (DS1922L, Maxim Integrated Products, California, USA). Mean skin temperature was calculated based on 14 body sites as recommended by EN-ISO 9886 (22). Twelve additional locations were added to obtain symmetrical skin temperature data from both sides of the body (Figure 5). All temperature measurements (ambient and skin temperature) were recorded at 1-min intervals.

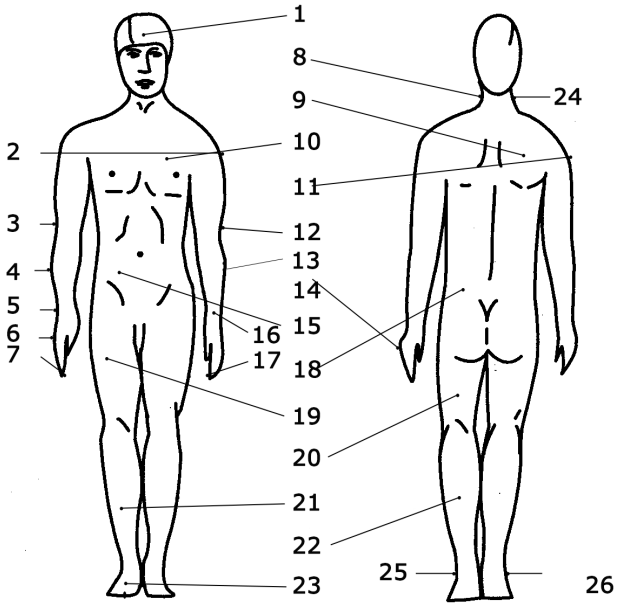

Figure 5 | iButton locations

Statistics

All results are presented as mean±standard deviation (SD). For all conditions, the average of the last five minutes of physiological data and the respective final subjective voting were used for analysis. The commercially available software package PASW Statistics 20.0 (SPSS Inc., Chicago, USA) was used to analyse the data. Analysis of conditional probability was performed using Matlab 2010a for Apple Mac computers.

Actuators

A repeated measures design was used to pairwise compare the five cooling conditions with 'no cooling' per outcome parameter. Statistical significance was assumed if $P<0.05$. If the assumption of sphericity was violated, a Bonferroni correction was performed to adjust for multiple comparisons.

Sex differences

To detect sex differences, independent sample t-tests per condition and per outcome parameter for women and men were performed. Statistical significance was assumed if $P<0.05$ and a trend was assumed if $0.05<P<0.1$.

Indicators

Physiological parameters such as skin temperature were previously identified as possible indicators of thermal sensation and thermal comfort (23). To detect physiological predictors (indicators) of whole-body thermal sensation and thermal comfort, Spearman correlation coefficients between skin temperatures and thermal sensation as well as thermal comfort were calculated. We calculated Spearman correlation coefficients for the following scenarios: 1) whole-body thermal sensation/comfort and skin temperature data of all cooling conditions combined for each participant, 2) whole-body thermal sensation/comfort and skin temperature data of all participants combined for each cooling condition. The scenarios were tested for all participants together and women and men apart.

Post-hoc power analysis

A post-hoc power calculation using G*power 3.1 software (24) was performed for the applied repeated measures ANOVA design. Using an achieved partial $\eta 2$ of 0.248, the corresponding effect size f of 0.574, an α of 0.05 and the sample size of 16, the achieved power (1-β) equals 0.92.

RESULTS

Actuators

Whole body thermal sensation and thermal comfort

Whole-body thermal sensation and thermal comfort during the cooling conditions 'face cooling', 'underarm cooling', 'back cooling', 'foot sole cooling' and 'face-underarm cooling' were compared with whole-body thermal sensation and thermal comfort during 'no cooling' periods. Within in the analyses, 'no cooling' served as baseline condition and separated the five cooling conditions (Figure 2).

The cooling conditions 'face cooling' and 'face-underarm cooling' significantly lowered ($P<0.05$, respectively $P<0.001$) whole-body thermal sensation compared with 'no cooling' (Table 2 and Figure 6). As depicted in Figure 6, 'face-underarm cooling' resulted in decreased votes of whole-body thermal sensation for the majority of the participants; some votes decreased from 'warm' or 'slightly warm' to 'neutral' during 'face-underarm cooling'. Concurrently, also whole-body thermal comfort significantly improved ($P<0.05$) during 'face cooling' and 'face-underarm cooling' (Table 2 and Figure 6). None of the other cooling conditions caused significant changes of whole-body thermal sensation and/or thermal comfort. Figure 6 shows that approximately 50% of the comfort votes during 'face-underarm cooling' raised toward the comfortable side of the scale. Importantly, no significant time effects on whole-body thermal sensation and/or thermal comfort were observed, as tested by repeated measures ANOVA.

Table 2 | Thermal sensation and thermal comfort of the final subjective voting per cooling condition

Condition	Thermal sensation	Thermal comfort
'No cooling'	1.70±0.62	-0.36±0.61
'Face cooling'	1.22±0.74*	-0.01±0.78*
'Underarm cooling'	1.54±0.92	-0.23±0.75
'Back cooling'	1.54±1.01	-0.29±0.85
'Foot sole cooling'	1.87±0.68	-0.67±0.67
'Face-underarm cooling'	1.04±0.79**	0.09±0.56*

Data is presented as mean±SD. * $P<0.05$ in comparison with no cooling, ** $P<0.001$ in comparison with no cooling, N=16.

Moreover, participants rated their 'want to' and 'importance to' change the thermal environment during all the cooling conditions (Figure 4). Again, only 'face cooling' and 'face-underarm cooling' significantly decreased their 'want' (P≤0.02 and P≤0.003) and 'importance' (P≤0.049 and P≤0.022) to change.

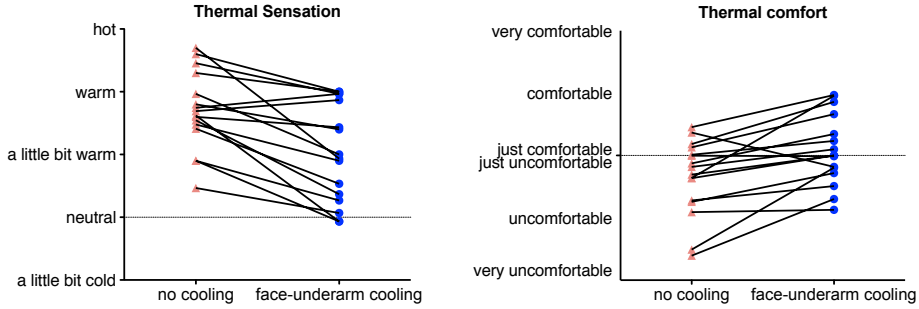

Figure 6 | Final voting's of thermal sensation and thermal comfort during 'no cooling' and 'face-underarm cooling'

N=16.

Self-reported Sweating

Self-reported sweating was significantly lower during 'face cooling' (4.80±2.60) and 'face-underarm cooling' (4.72±3.28) compared with 'no cooling' (6.63±2.15). In contrast, participants tended to report more sweating during 'foot sole cooling' (7.35±2.49), although this trend was not significant (P<0.052). 'Back cooling' and 'underarm cooling' did not significantly alter self-reported sweating compared with 'no cooling'.

Skin temperatures

There was no significant variation in mean skin temperature between the conditions (Table 3). Mean proximal skin temperature was significantly higher during 'underarm cooling', but significantly lower during 'back cooling' compared with 'no cooling'. Mean distal skin temperature was significantly higher during conditions 'face cooling', 'underarm cooling' and 'back cooling', compared with 'no cooling'.

Table 3 | Mean, proximal and distal skin temperatures of the final five minutes per condition

Conditions	No cooling	Face cooling	Underarm cooling	Back cooling	Foot sole cooling	Face-underarm cooling
	Mean skin temperature					
M+F	35.28±0.49	35.45±0.46	35.51±0.42	35.09±0.45	35.34±0.52	35.43±0.38
M	35.04±0.57	35.21±0.43	35.29±0.45	35.02±0.52	35.09±0.49	35.27±0.44
F	35.51±0.24##	35.70±0.35##	35.73±0.27##	35.15±0.38	35.59±0.45#	35.58±0.24
	Proximal skin temperature					
M+F	35.55±0.58	35.81±0.50	35.89±0.45*	34.80±0.60*	35.68±0.58	35.82±0.43
M	35.40±0.74	35.64±0.50	35.78±0.53	34.85±0.76	35.54±0.63	35.77±0.52
F	35.70±0.32	35.97±0.48	36.00±0.37	34.74±0.43	35.82±0.53	35.86±0.34
	Distal skin temperature					
M+F	34.70±0.54	35.05±0.53**	34.90±0.67*	34.98±0.54*	34.89±0.64	34.86±0.61
M	34.40±0.60	34.80±0.60	34.61±0.78	34.82±0.60	35.56±0.69	34.68±0.75
F	35.00±0.24##	35.29±0.34#	35.19±0.40#	35.14±0.45	35.22±0.41##	35.05±0.39

Data is presented as mean±SD. M = men, F = women. * P≤0.05 in comparison with 'no cooling', **P<0.001 in comparison with 'no cooling', # P<0.1 difference between women and men, ## P<0.05 difference between women and men, N=16.

Sex differences

The present study did not reveal significant differences in whole-body thermal sensation and thermal comfort between women and men in any of the conditions.

Skin temperatures

Women had significantly higher mean skin temperature in condition 'no cooling', 'face cooling', 'underarm cooling' and foot sole cooling (P≤0.05) compared with men (Table 3 and Figure 7). Furthermore, women had significantly higher distal skin temperatures during 'no cooling', 'face

cooling', 'underarm cooling' and 'foot sole cooling' compared with men. Proximal skin temperature was not significantly different between women and men. Skin temperature was not significantly related to whole-body thermal sensation or thermal comfort in neither of the scenarios or groups described in the statistics section.

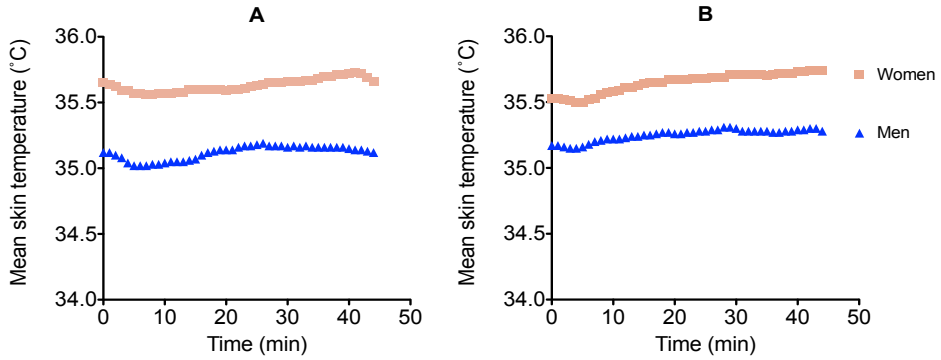

Figure 7 | Mean skin temperature trends of women and men during condition 'face cooling' (A) and 'underarm cooling' (B)
N=16, 8 men and 8 women.

DISCUSSION

The present study shows that local convective cooling of the face combined with local conductive cooling of the underarms significantly improved thermal sensation and comfort in a warm environment of 32.2˚C in contrast to the other four local cooling conditions that were tested. Although no significant differences in thermal comfort between men and women were detected, women had significantly higher mean skin temperature throughout the majority of the measured conditions. The results of the present study may have important practical implications and demonstrate the advantages of using individual comfort systems.

Actuators

The combined face-underarm cooling was the only cooling condition that encouraged the participants to rate the thermal environment as being 'just comfortable' (0.09±0.56). None of the other cooling conditions ('underarm cooling', 'back cooling' and 'foot sole cooling') significantly improved thermal sensation or thermal comfort compared with 'no cooling'. 'Foot sole cooling' was de facto the only condition causing participants to perceive the thermal environment as warmer and less comfortable compared with 'no cooling' ($P \leq 0.08$).

Earlier studies indicated cooling of proximal body regions had the highest impact on (overall) thermal sensation and thermal comfort. For example, Zhang et al. (25) reported that for convective local cooling, especially the back, chest and pelvis would be the best target regions to influence overall thermal comfort. Interestingly, the backseat-cooled chair used in the present study did not improve whole-body thermal comfort and sensation, even though it very effectively lowered the proximal body temperatures of the participants (-0.75˚C). However, cooling of the back raised complaints in some of the female participants (headache and/or dizziness), indicating that the conductive cooling technique used in the present study might have been too intense, causing a strong imbalance between proximal and distal body temperatures.

In contrast, a recent study by Pasut et al. (6) showed that chair cooling combined with the application of a desk fan very positively influenced thermal comfort in a warm condition of 29˚C. However, the authors do not report any data on the effect of the cooled chair alone without the combination of the desk fan, which makes it difficult to examine the effect of the chair cooling alone. As a matter of fact, the head, which usually represents the target of a fan, plays a very important role in thermoregulation. Alongside to thermoregulatory advantages caused by increased airflow and fresh air supply around the head region, it also improves perceived air quality, sick building symptoms and general occupant comfort (5, 26, 27). Hence, cooling of the head region together with the back might have blurred the effects of back cooling alone in the study of Pasut et al. (6).

In addition to the positive effects on occupant comfort and energy savings, individual local cooling systems might as well have beneficial health effects. It has recently been hypothesised that spending too much time in a constant climate can cause vulnerability to temperature fluctuations (28). In a warm thermal environment, human physiological thermoregulation (e.g. vasomotion and evaporation) is stimulated more compared with a thermo-neutral ambient condition, to dissipate excessive body heat. Moreover, more calories are burned in warm conditions compared with thermo-neutral conditions (29-31). Since the Western world is facing a global problem of obesity, leaving the TNZ every now and then may contribute to a healthier lifestyle (32).

Given the fact that the combined cooling technique investigated in the present study was most effective, it would be relevant to evaluate the effect of other combinations of conductive and convective local cooling on whole-body thermal comfort in a warm environment. It has been decided to apply an ambient temperature of 32.2˚C to intensify and clarify the effect of local cooling. However, it is relevant to verify the present results in moderate conditions as more regularly encountered in temperate climates. Moreover, future investigations should focus on optimisation of cooling methods, for example with respect to the level of airflow and eye dryness.

Sex differences

Although no substantial sex differences with respect to thermal sensation or thermal comfort were identified, differences in thermal physiology of women and men were evident. In three of the five provided conditions, women had significantly higher mean skin temperatures (approximately +0.5˚C) and distal skin temperatures (approximately +0.6˚C) compared with men. Congruently, Hardy and Du Bois (33) had already established various sex differences in thermoregulation in 1940. They found that women had up to 1.7˚C higher skin temperatures compared with men in warm dry ambient conditions up to 36˚C. Ever since, these findings have been confirmed by various studies (34-36). Moreover, higher evaporation rate and earlier sweat onset are evident in men, which is likely to contribute to lower skin temperatures in warm ambient conditions (34, 37). Correspondingly, a study by Schellen et al. (14) indicated women had lower mean skin temperatures and were more likely to report discomfort, when situated in a mild cold environment. This may be explained by the sex-related differences in the thermoneutral zone, which seems to be shifted to higher temperatures in women compared with men (31). The thermoneutral zone is defined as "the range of (ambient) temperatures at which temperature regulation is achieved only by control of sensible (dry) heat loss, i.e. without regulatory changes in metabolic heat production or evaporative heat loss. The thermoneutral zone (TNZ) will therefore be different when insulation, posture or basal metabolism vary" (38). Generally, women have a lower resting metabolic rate, less muscle mass and more fat mass compared with men, which is why their TNZ is expected to be different. As a matter of fact, Hardy and Du Bois (33) observed that semi-nude women increased metabolic rate at temperatures below 31°C, whereas men increased metabolic rate below 28.5°C. In practice, it is very important to consider that women and men may react differently upon the same thermal environment, and this is likely to influence individual thermal comfort and sensation.

Possible indicators

To evaluate potential physiological indicators of thermal comfort, the relations between skin temperature and whole-body thermal sensation and thermal comfort were examined. No statistically significant correlations were observed.

Next to a correlation analysis, conditional probability analysis (conform the Bayesian method (39)) was performed to calculate the probability of perceived whole-body thermal comfort at a given local skin temperature. 'Comfortable' was defined as being the entire thermal comfort VAS range from 'just comfortable' to 'very comfortable' (Figure 3A). Analysis was performed using skin temperature data and the respective whole-body thermal comfort data of the final 5 min of all conditions.

The percentage of participants perceiving thermal comfort did not change significantly with changing local skin temperatures. However, when performing conditional probability analysis for women and men separately, women were more likely to perceive thermal comfort given higher local skin temperatures at the hands and the underarms (Figure 8A and 8B). The opposite was true for men: the probability of perceiving thermal comfort decreased given higher skin temperatures at hands and underarms. For example, only 24% of the females were comfortable at 34˚C underarm skin temperature, whereas 97% of the men reported thermal comfort. In contrast, 46% of the women but only 21% of the men felt comfortable at a 36˚C hand skin temperature. The same trend appeared for various other body sites, e.g. neck, shoulder region (scapula, deltoid region), fingers and lower legs. However, the trends described above were not consistent over all body sites. Therefore, it would be necessary to perform additional studies with more participants in order to be able to generalise the results.

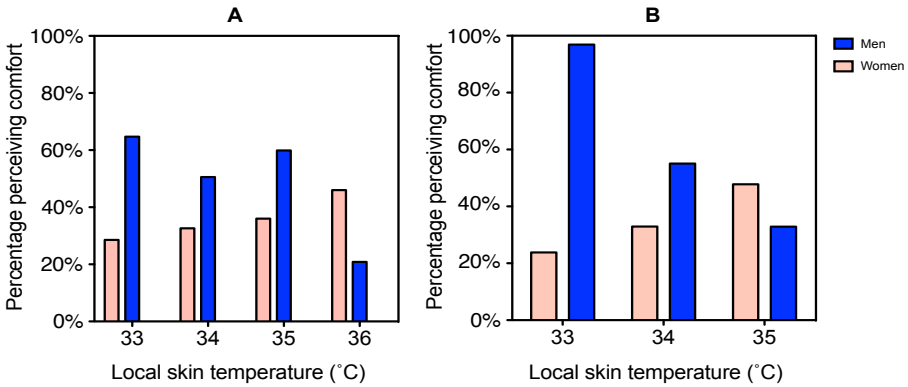

Figure 8 | Results of the conditional probability analysis of the hands (A) and the underarms (B)

The y-axis represents the percentage of participants that felt comfortable at the given local skin temperature (x-axis). N=16, 8 women and 8 men.

Although differences between women and men with respect to thermal comfort and skin temperatures were established much earlier, many models for thermal comfort, such as the PMV model, do not incorporate these differences. Interestingly, Fanger (9) indicated women and men would prefer similar temperatures, so the same boundary conditions would be needed to create thermal comfort. However, as indicated by a biophysical study by our group, thermo-neutrality and thermal comfort are not necessarily equal, and are significantly influenced by physiological differences in metabolism and tissue insulation (40). Furthermore, Karjalainen (41) underpinned the need to incorporate differences between women and men to configure indoor environments, which is in line with the results of this study.

Conclusion

Overall, the present study concludes that face cooling by means of increased airflow in combination with conductive underarm cooling is an effective way to improve thermal sensation and thermal comfort in a warm environment. Furthermore, we confirm earlier studies, which show that in warm conditions, women had higher mean and distal skin temperatures compared with men. However, no direct relationship between skin temperatures and whole-body sensation or comfort was identified.

Individualised local cooling seems to be an effective method to comply with the varying needs and preferences of female and male individuals with respect to overall thermal comfort in warm thermal environments. In practice, the application of individualised cooling may allow for increasing occupant satisfaction and simultaneously help to save energy due to a reduction of ventilation and air-conditioning of the entire office space.

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Chapter 7

General discussion and conclusion

GENERAL DISCUSSION AND CONCLUSION

Humans and their thermal environments – a hot topic

Thermoregulation has always played an important role in human life and evolution. Today, especially in industrialised countries, humans spend approximately 90% of their time indoors (1). Due to this almost permanent exposure to indoor environments, the latter has become an increasingly important factor regarding human health and wellbeing. Nowadays, an increasing amount of buildings in developed countries are equipped with heating, ventilating and air-conditioning (HVAC) systems to create a comfortable indoor climate. The great majority of modern buildings are regulated to supposedly thermo-neutral temperatures, as this is anticipated to be comfortable for most people (2, 3).

It has, however, recently been challenged if such neutral and uniform thermal conditions are actually desirable and healthy. Is has been hypothesised that, for example, less calories might be burned for thermoregulatory purposes due to the invariable thermal conditions, which might contribute to the 'weighty' tipping of the delicate energy balance (4-8). Since we are currently facing a so-called 'obesity epidemic' in virtually all parts of the World, it is of great importance to uncover possible unfavourable and unhealthy (lifestyle-) factors in our environment. Interestingly, it has been suggested that together with a healthy diet and sufficient physical activity, exposure to more varying temperatures might be a potential contributor to a healthy metabolic profile (7, 8). In a recent publication on the interplay between ambient temperature and obesity, the authors suggest: "As a return to varied, natural ingredient diets is proposed for health, so too exposure to varied, natural range of ambient temperatures may just be what we need" (7). Moreover, more frequent exposure to variable temperatures might bring along other beneficial health effects, such as improved glucose metabolism and metabolic health, cardiovascular function as well as a generally superior temperature resilience. Hence, we argue that the design and configuration of thermal indoor environments should be regarded as another important lifestyle factor, next to healthy diet and physical activity, and thus has to be taken into consideration for a healthy, balanced life.

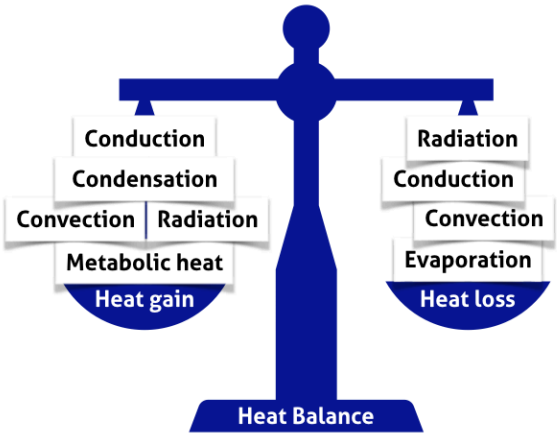
In the context of global warming, which is evidently affecting the outdoor climate and thereby also our thermal indoor environments, it is furthermore important to assess possible implications of the increasing temperatures on human metabolism and health. Although high temperatures in summer, i.e. above 30˚C, are relatively normal in subtropical and tropical climate zones, they are not (yet) regularly encountered in the Central European humid oceanic and humid continental climatic zones. Importantly, due to anticipated longer-lasting and more frequent summer heat waves, as well as the modern air-tight construction style and high internal heat load of buildings, people in Western and Central Europe will have to face overheated indoor spaces and higher

outdoor temperatures more often in the future. Therefore, it is of great importance to evaluate physiological and behavioural coping with high ambient temperatures, to test whether prolonged exposure induces (advantageous) adaptive processes and to test the impact on important parameters of human health. Moreover, it is necessary to evaluate strategies to potentially restore and retain thermal comfort in warm environments, by, for example, the application of personal cooling systems.

From this thesis, various new insights with respect to the interplay of warm thermal environments and human thermophysiology, health, behaviour and perception have arisen. In this final chapter, the main findings will be discussed and interpreted.

What is thermal neutrality?

Since the existence of thermal comfort research, it has been the main goal to pinpoint optimal thermal conditions for people dwelling in indoor environments. The term 'thermal comfort' has been used synonymously with 'thermal neutrality', which demonstrates the strong correlation that is assumed between the two concepts. Interestingly, one should note that thermal comfort refers to a subjective entity regarding the perception of an individual, whereas thermal neutrality indicates a straightforward physical approach, namely the balance between heat production and heat loss: thermal balance (Figure 1).



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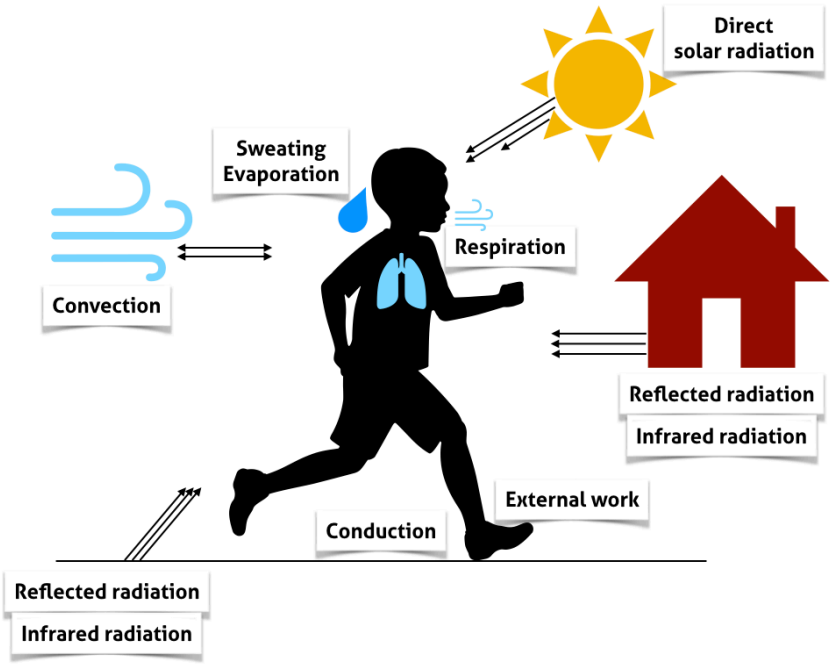

Figure 1A and B | Human heat balance
Representation of the heat balance equation *(A)* and modalities of heat transfer for a person in a thermal environment *(B)*. The conceptual heat balance equation is: Metabolic rate - Work = Evaporation + Radiation + Conduction + Convection + Heat Storage (M-W = E+R+K+C+S). Here, M-W is always a positive number. If the body is in heat balance (zero heat storage, S=0), the equation is as follows: 0 = M-W-E-R-C-K, where M-W is always positive, and E, R, C and K are rates of heat loss from body. i.e. positive value = heat loss, negative value = heat gain. Fanger (9) provided a practical heat balance equation which helped to identify the specific avenues of heat production and exchange for the human body and ensured measurability of the terms in the equation. Fanger's heat balance equation is: Metabolic heat production – Heat loss by vapour diffusion through the skin – Sweat rate – Latent respiration heat loss – Dry respiration heat loss = Radiation + Convection (H – Edif – Esw – Eres – Cres = R + C). The figures are derived from Parsons (10).

Creating thermal comfort – or thermal neutrality – for the great majority of building occupants is one of the important goals of HVAC engineers. Indoor climate regulations are based on a model derived from empirical research, the PMV model, which has been introduced by Fanger (2, 9, 11). The metabolic equivalent (MET) is one of the standard values in this model (next to clothing insulation, air temperature, radiant temperature, air speed and humidity) and still widely used in many different settings. MET is a commonly used physiological concept considered to be a simple procedure for expressing the energy cost of physical activities as a multiple of the basic metabolic

rate. For example, sleeping is defined as 0.9 MET, being in a resting fasted state equals to 1.0 MET, light office work is 1.2 MET and heavy exercise can account for up to 10 MET (12-14). Strikingly, although it is not exactly clear where it is originally derived from, the definition of 1 MET (3.5ml oxygen/kg/min or 4.184kj/kg/h which is ≈58W/m^2) is based on measurement of only one single 'average' white male person aged 40 years with a bodyweight of 70kg (14, 15). Today, the PMV model is still the basis for a wide range of indoor environmental designs, but the assumed standard value used in the model is evidently not representative for the great diversity of people usually mixing and mingling in all types of buildings. Literature demonstrates that demographical variables such sex, age and genotype as well as body composition and acclimation state have considerable impact on metabolism and thermoregulation (14-16). Strikingly, female metabolic rate may be overestimated by as much as 35%, showing that current indoor climate standards might structurally misrepresent female thermal demand (14, 15). For other subpopulations such as children and the elderly, the mismatch might be even more pronounced.

Interestingly, to date, the individual human thermoneutral zone (TNZ) remains largely unknown, which is to a certain extent due to the practical difficulties considering the quantitative assessment of the TNZ. It might sound ironic that so much energy, literally and figuratively, has been invested into the design and maintenance of thermally neutral indoor environments, when the (individual) thermoneutral zone has not actually been quantified. As already mentioned in the general introduction of this thesis, it is challenging to determine the TNZ, because of the need to statically measure energy expenditure specific to a (range of) ambient air temperatures. Due to the great thermal mass of the human body it might take hours to reach thermal equilibrium in a specific thermal condition. Although one might consider measuring the human TNZ in a medium other than ambient air, for example water, due to its superior conductivity and hence better temperature transfer, the results of such examination would not realistically reflect conditions as found in buildings and daily living circumstances, where people are usually exposed to ambient air.

In order to overcome this issue, we explored a new dynamic approach to determine the human TNZ in **CHAPTER 2**. In this study, we focussed on the *metabolic* TNZ, indicated by a rise of the metabolic rate when ambient temperatures increase or decrease. Therefore, energy expenditure data of 11 young men has been assessed during temperature ramp protocols. The measurements were carried out twice, before and after passive acclimation to mild heat.

Our results show that the dynamic protocol is feasible and useful to determine the human (dynamic) LCT. We demonstrate significant inter-individual variation regarding the positioning of the LCT, denoted by the wide range of LCTs between the participants (19.2˚C – 28.9˚C). Interestingly, acclimation to mild heat did not lead to significant changes of the positioning of the metabolic LCT.

With respect to the metabolic UCT, we observed, at best, a slight gradual to none metabolic change when temperature increased during warming. Therefore, it was not possible to indicate metabolic UCTs for the great majority of participants. This result is opposed to earlier animal studies, where a clear UCT, characterised by a clear increase of metabolic rate, was identified (17). The latter might, amongst other things, be due to substantially different physiological and behavioural cooling mechanisms of non-primate animals (e.g. grooming with saliva and panting) in comparison with humans.

Comparing our data with the available literature, the LCTs determined by our dynamic protocol clearly exceeded the assumed LCT for a resting person in ambient air of approximately 28˚C by far in most cases. This might be due to the dynamic nature of our protocol, implying that participants were exposed to a specific temperature for only a short time span. A human metabolic UCT of approximately 32˚C, which has earlier been indicated in the literature, could not be confirmed by the results of the study presented in **CHAPTER 2.**

We therefore conclude that more research, employing broader and possibly less steep ramp protocols, is warranted to adequately measure both LCT and especially UCT of the human TNZ. A measurement of evaporative water loss additionally to the assessment of energy expenditure is needed to allow for a two-factor evaluation of the human UCT. It is important to further optimise and enhance the presented protocol, amongst other things due to the fact that several participants did not reach their metabolic critical temperature(s) within the given range of ambient temperatures. Ultimately, it should be desired to develop a tool that can be used as a practical and feasible standard measure for the human TNZ. The latter would be useful for the design of studies on metabolism, thermoregulation and cardiovascular aspects, as it is relevant to control for the ambient temperature as a factor of influence in such researches. Moreover, as stated in the above, it is important for the design and control range of (modern) indoor environments, as it can help to attune the thermal environment to specific thermal preferences and needs of its occupants more appropriately.

How to cope with the heat – comparing physiological adaptations to passive mild heat acclimation between young healthy and overweight elderly men

In the context of global warming and the increasing risk for overheating of indoor spaces, the primary goal of the present thesis was to evaluate the capacity for physiological adaptation to relatively mild and passively induced heat acclimation in different study groups. A lot of information is available regarding physiological adaptation to intense, exercise-induced heat acclimation, as the yielded results of those studies are meaningful for optimal training and mission preparation of, for example, miners, the military and elite athletes (for example (18-25)). Additionally, a number of studies also evaluated different modes of passive heat acclimation,

which were, however, usually provided at very high ambient temperatures or even by the immersion in hot water (26). Earlier active and passive heat acclimation studies typically report several physiological parameters indicating successful heat acclimation; and the key concepts of those are summarized in Figure 2. In the present thesis, we evaluated whether passive exposure to relatively mild heat, reflecting realistic temperature challenges in daily living circumstances, evokes similar physiological adaptations and improved resilience to heat, as more intense protocols revealed previously.

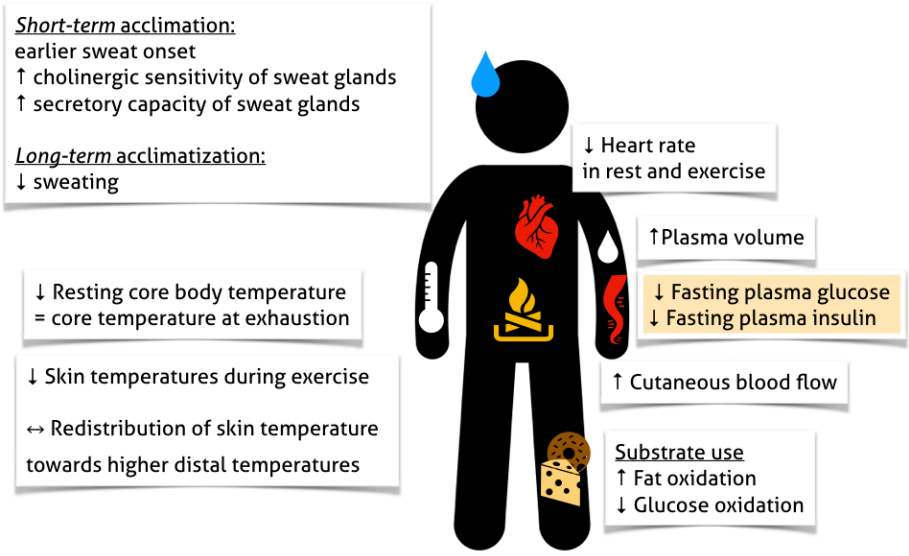

Figure 2 | Key concepts of short-term and long-term heat adaptation
The commonly reported key concepts of short-term and long-term as well as passive and active heat acclimation are shown in this figure. The yellow box (decreased fasting plasma glucose and fasting plasma insulin) denotes new information derived from the present thesis.

In **CHAPTER 3** and **4**, two passive mild heat acclimation (PMHA) studies, in young healthy men (**CHAPTER 3**) and in overweight and obese elderly men (**CHAPTER 4**) are presented. In the healthy population, participants were acclimatised to approximately 33˚C for 4-6h a day at 7 consecutive days, which amounts to 40h of PMHA. The overweight elderly group endured a slightly longer and more intense protocol with an acclimation temperature of approximately 34.5˚C, which lasted for 4-6h at 10 consecutive days, summing up to a total of 56h of PMHA.

After PMHA, both study groups exhibited significant thermophysiological changes. Most importantly, both study groups exhibited a reduction of body core temperature (Tcore) post

PMHA, both in thermal neutrality and during warming, which is the most crucial factor of successful heat acclimation (Figure 1). In the young healthy population (**CHAPTER 3**), the decrease of Tcore was approximately -0.14˚C, whereas the overweight elderly group displayed a slightly more pronounced decrease of approximately -0.19˚C (both measured at approximately 35˚C) (**CHAPTER 4**). The observed change of core temperature also resulted in a decreased core-distal skin gradient post PMHA in both groups, indicating more evenly distributed body temperatures. The latter has been shown to be beneficial when exposed to high temperatures, as a more uniform body temperature seems to decelerate warming of the body (27-29).

Earlier researches have previously described lower resting and exercising mean skin temperatures after heat acclimation, which were, however, not found in the present two studies (24, 25). In the healthy population, we observed a shift of skin temperature distribution towards warmer distal and cooler proximal body parts after PMHA, but in the overweight elderly group, this modification was not evident. Possibly, the difference of body fat content between the two study groups might have played a role here (healthy BMI ~23kg/m^2 vs. ~30kg/m^2 in the overweight elderly group). A thicker subcutaneous fat layer is known to effectively isolate thermal exchange between the body and the surrounding environment and might therefore have impaired or attenuated heat loss via the skin in the overweight elderly population. Moreover, age has been shown to cause a reduced cutaneous circulatory response in the heat (30, 31).

Another crucial factor regarding successful thermoregulation in the heat is evaporation or sweating. Upon heat acclimation, the sudomotor system has been shown to enhance in multiple ways: earlier sweat onset, more sensitive sweat glands, and increased secretory capacity are reported (18, 24, 29). In the present studies in **CHAPTER 3** and **4**, we measured total sweat loss, expressed as the change of body weight before and after the UP protocol, before and after PMHA. The decrease of body weight indicated the loss of water through sweating and respiration during the respective measured period.

Interestingly, in the healthy population, total sweat loss *decreased* significantly after PMHA, but no change was observed in the overweight elderly population. In **CHAPTER 4,** local sweat rate at the ventral side of the underarm was recorded additionally to indicate and compare the moment of sweat onset pre- and post-PMHA, but no shift of sweat onset was evident in the overweight elderly group.

A decrease of sweating, as observed in the healthy young population, has earlier been reported particularly in indigenous people from hot and tropical countries, but also as a mechanism of long-term heat adaptation and habituation (29). Here, evaporation is optimal and sweat dripping minimal (29). As an organism adapts to the typical thermal conditions of a certain geographical region, the actual exogenous thermal load becomes relatively less: a decrease of core temperature generates a certain thermoregulatory buffer. Hence, less sweating is needed to keep the body cool.

Considering the steady heat load during our experiments, namely a constant exposure to ~33˚C respectively ~34.5˚C ambient temperature, the decreased amount of total sweat loss in the young healthy population can be explained by this phenomenon. In the overweight and elderly group, the unchanged amount of total sweat loss and sweat onset combined with the more pronounced decrease of Tcore might hint towards the fact that these two mechanisms compensated for an assumed impaired capacity of heat loss via the skin, which might be due to a thicker subcutaneous fat layer and less ability for vasodilation.

Combining the results presented in the above, we have clearly established that even mild and passively administered heat acclimation induces advantageous thermophysiological changes in both young healthy as well as overweight and elderly individuals.

Healthy heat?

Previous active and passive heat acclimation studies have demonstrated that next to thermophysiological adaptations, prolonged heat exposure also goes along with favourable health effects: many studies report a decrease of resting and exercise heart rat as well as improved endothelial function, decreased arterial stiffness and lowered blood pressure (18, 21, 25, 32-34). Moreover, it has recently been indicated that repeated heat exposure, both short-term and long-term, might even improve glucose metabolism and insulin sensitivity (35-37).

In the context of global warming, we sought to understand the effect of passive mild heat acclimation on human cardiovascular and metabolic health in the present thesis. Therefore, in **CHAPTER 3** and **4**, we tested the effect of a relatively mild and passively administered heat acclimation on cardiovascular parameters in a healthy young and an overweight and obese elderly population. Additionally, in **CHAPTER 4**, we also tested the effect of PMHA on glucose metabolism and HSP72 levels in overweight and obese elderly men.

PMHA and cardiovascular functioning

In both healthy young (**CHAPTER 3**) and overweight and obese elderly (**CHAPTER 4**) individuals, a distinct effect of PMHA on blood pressure was evident in both thermoneutral conditions as well as during warming. In both cases, blood pressure, particularly diastolic blood pressure, was lowered after PMHA. Moreover, in **CHAPTER 4**, we show that heart rate significantly decreased upon PMHA in the overweight elderly group.

Considering the fact that cardiovascular diseases are commonly encountered in overweight and obese individuals, it is particularly remarkable to see that prolonged exposure to mildly elevated ambient temperatures clearly decreased blood pressure and heart rate in the respective population (**CHAPTER 4**). As earlier shown in the papers of Brunt *et al.* (33, 34), heat treatment might be a

promising approach for the improvement of cardiovascular health in a variety of target populations, especially for those individuals with limited tolerance for other treatments such as exercise or medication. In the present thesis, we support these findings and we show that exposure to mildly elevated ambient temperatures is a viable method and induces beneficial effects on blood pressure.

The effect of PMHA on intracellular HSP72 levels in human muscle and insulin sensitivity
The global incidence of obesity-induced insulin resistance and Type 2 Diabetes Mellitus (T2DM) has doubled in the last 30 years and is constantly rising, particularly in developed nations. Current therapy standards aim at the promotion of a generally healthy lifestyle, including a healthy diet and regular physical exercise, and have been shown to be very effective in the prevention and treatment of metabolic diseases. However, long-term adherence is often low and thus, long-term treatment targets are frequently not met. Therefore, new alternative treatment options are needed; and additional healthy lifestyle parameters must be identified in order to facilitate the aspired therapy success, and to support the long-term sustainability.

It has earlier been suggested that ambient temperature might play a significant role with respect to a healthy human metabolism. For example, a very recent study from our lab demonstrated that acclimation to mild cold increased insulin sensitivity in diabetic individuals (38). Interestingly, not only cold but also heat has been suggested to affect glucose metabolism in humans: already in 1999, a study by Hooper (39) showed that glucose handling improved significantly in T2DM patients after daily hot baths over the course of 3 weeks. Moreover, literature has also previously suggested an improvement of diabetes status in the warmer months of the year (40-45). However, the underlying mechanisms remained unclear for several years, until recently a number of publications indicated the link between repeated heat exposure, increased HSP72 expression and insulin sensitivity in human cell experiments and animal studies (35, 46-50).

The present study presented in **CHAPTER 4** was the first to study whole-body effects of heat acclimation on glucose metabolism and insulin sensitivity and the potential link with HSP72 levels in human muscle. We show that the relatively mild, passively administered heat acclimation evoked significant changes of glucose metabolism (lowered fasting plasma glucose *FPG*, fasting plasma insulin *FPI*), but insulin sensitivity and HSP72 expression were not affected by PMHA. We hypothesise that the decreased FPG and FPI post PMHA might be an indicator for improved balance between hepatic glucose output and insulin secretion in a basal state (51) and based on the latter, it might be cautiously suggested that PMHA improved hepatic insulin sensitivity but not peripheral insulin sensitivity. Importantly, additional measurements and analyses are required to verify this hypothesis.

Additionally, we also evaluated substrate oxidation in a basal and insulin stimulated state, and we found that PMHA induces a significant decrease of the respiratory quotient and a shift of substrate

use towards more fat oxidation and lowered glucose oxidation. Interestingly, earlier studies applying *active* exercise-induced heat acclimation found decreased muscle glycogen use post intervention as well, suggesting that heat acclimation leads to a reduced use of carbohydrate as a fuel during exercise (52-54). Here, we did not apply physical activity to induce heat acclimation, which suggests that the switch of substrate use is, at least in our studies, a direct temperature effect rather than an exercise effect.

Does heat acclimation change human behaviour?

CHAPTERS **2** to **4** of this thesis mainly focus on the evaluation of *physiological thermoregulation*, i.e. autonomic processes needed to maintain thermal equilibrium of the human body, and adaptive processes of thermophysiology to prolonged mild heat exposure. Another important aspect of thermoregulation is the *conscious behavioural regulation* of our thermal environment.

To the best of our knowledge, there is yet no information available regarding the effect of heat acclimation on thermoregulatory behaviour (TRB) and thermal perception in humans. In CHAPTER **5**, we therefore evaluated the impact of our PMHA protocol on TRB and thermal perception in eleven healthy young men. We applied a so-called SWITCH protocol, where participants were free to go back and forth between a hot and a cold room whenever they desired to change their thermal environment, over the course of 90 minutes. We measured skin temperature, which has earlier been identified as potential important catalyst of TRB (55, 56), as well as thermal sensation and thermal comfort during the test. For the first time, we show that acclimation to heat evokes significant changes of TRB: post PMHA, participants switched from the hot to the cold room at higher mean skin temperatures and their stay in the hot room tended to be longer than before the intervention. Hence, participants seemed to tolerate higher skin temperatures before they felt the need to regulate their body temperature, implying greater resilience to heat due to PMHA. Interestingly, participants seemed to strive towards maintenance of their state of thermal comfort during the SWITCH protocol, as they left the respective hot or cold room when they rated the environment as still being 'just comfortable' or 'just uncomfortable' but avoided the development of actual discomfort. We hereby confirm earlier studies suggesting that TRB is likely to be linked to both thermophysiological autonomic and subjective triggers and is exhibited when a state of thermal imbalance and/or discomfort is impending (55, 56).

How to keep a cool head in a warm environment

CHAPTER **3** to **5** of the present thesis focus on adaptive capacities of the human physiological system and thermoregulatory behaviour to warm thermal environments. Despite the acquisition

of physiological adjustments and superior resilience to heat due to heat acclimation, warm thermal environments are often perceived as uncomfortable. Moreover, individual preferences for air temperature and air movement have been shown to differ vastly between individuals, which is why it is not surprising that thermal discomfort is often reported even though recommendations for indoor thermal environments are met (57, 58). A recent study suggests a great potential for personalised comfort systems to improve thermal comfort and increase productivity, for example in (open-plan) offices, while simultaneously saving up to 50% of energy compared with overall air-conditioning (59). We therefore investigated several desk-level local cooling strategies to identify which target body sites are most suitable and most effective with respect to the retention and optimisation of thermal comfort.

In **CHAPTER 6**, we tested the application of face cooling (by means of a fan), back cooling, underarm cooling, feet cooling (all by means of a water-perfused cooling plate) and combined face-underarm cooling in a simulated office environment. 16 healthy young men and women were exposed to the different cooling techniques in order to evaluate the effectiveness with respect to thermal sensation and thermal comfort. Cooling of the face alone as well as combined face-underarm cooling significantly improved both thermal sensation and thermal comfort in the study population.

In conclusion, local cooling seems to be an effective method to comply with the varying needs and preferences of individuals and can help to obtain and maintain a state of thermal satisfaction in warm environments.

Concluding remarks and future perspectives

The present thesis aimed to evaluate different aspects of the interaction between warm thermal environments and human physiology, health and behaviour. It has clearly been established that prolonged passive exposure to mildly elevated ambient temperatures elicits adaptive processes of the thermoregulatory system. Both healthy young as well as overweight elderly individuals exhibited significant physiological adaptations upon repeated exposure to mild heat. Passive mild heat acclimation elicited favourable health effects such as a reduction of blood pressure; and in the overweight elderly group, also heart rate was lowered. Moreover, significant improvement of glucose metabolism in the form of reduced fasting plasma glucose and fasting plasma insulin levels was evident in an overweight and obese elderly population, after passive mild heat acclimation. Furthermore, it has been demonstrated that thermoregulatory behaviour alters upon acclimation, showing that next to *physiological autonomic* adaptations, also *conscious behavioural* thermoregulation changes as a result of repeated exposure to mild heat. Lastly, in this thesis, cooling of the face and combined face-underarm cooling have been identified as the most efficient methods to induce thermal comfort in a warm environment.

Future research is warranted to further elucidate the effects of passive mild heat acclimation on females, since the present experiments in **CHAPTERS 3** and **4** only included males. More research is also needed to test the in-vivo relationship between heat exposure, heat shock protein 72 expression and insulin sensitivity in the human body, both on cellular and on whole-body level. In order to identify important parameters for the design of heat therapies, future human in-vivo studies should focus on the optimisation of intensity and duration of the applied heat acclimation model to evoke the desired changes of HSP72 expression and improved insulin sensitivity.

The study described in **CHAPTER 5** provides an important first step towards the understanding of physiological subjective and behavioural alterations post heat acclimation, but more knowledge is needed to identify the driving forces and mechanisms of TRB in a less controlled environment. Moreover, the results need to be verified in a more diverse population of both men and women and in different age categories.

Lastly, with respect to local and personalised comfort systems, future studies should focus on the development of energy-efficient personal desk-level cooling systems targeted to face and underarm areas in order to increase thermal comfort while simultaneously lowering energy costs and contributing a better CO2 footprint.

Our results illustrate the great sensibility and plasticity of the human thermophysiological system even during passive and moderate thermal challenges as encountered in everyday life.

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Chapter 8

Appendices

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ENGLISH SUMMARY

Humans and Warm Environments – Physiology, Health and Behaviour

Nowadays, people in the Western World are rarely exposed to the variation of outdoor conditions, as they spend the greatest part of their day (on average roughly 90%) indoors. The design of our indoor spaces has developed and changed tremendously over the past decades, to make them as comfortable and optimally tempered as possible. We can heat, cool, humidify, dry and replace the indoor air to our liking, and thus, create a total 'weatherlessness'.

Based on Fanger's research published in the 1970s and the subsequently developed standards for indoor environments according to the American Society for Heating, Refrigerating and Air Conditioning Engineers (ASHRAE), the goal became to strive for a thermally *neutral* environment in buildings. Thermal neutrality was assumed to be the most *comfortable* for the majority of building occupants. In order to comply with these indoor environment standards, the ambient temperature of a building is allowed to deviate from the optimal set-point as little as ±0.5˚C, independent of the outdoor temperature and season. Up until today these standards are retained, although maintaining a thermoneutral climate costs a lot of energy for heating and air conditioning of buildings. Importantly, it has also been questioned if such uniform indoor environments are actually healthy. Based on earlier research, it has been suggested that the omnipresence of thermoneutral environments may contribute to the high prevalence and further increasing numbers of obesity and metabolic diseases in developed countries. Furthermore, field studies have shown that people accept a much wider range of ambient temperature than required by the conventional models, which subsequently led to the introduction of the Adaptive Comfort Standard. The latter allows for more variation of the indoor temperature in relation to outdoor temperatures and the different seasons and has been added to the latest ASHRAE Standard 55-2013.

Measures taken to comply with the classical strict indoor air guidelines described in the above, combined with the endeavour to reduce energy costs, have led to the use of highly isolating construction materials. As a consequence, many modern buildings are nearly air tight, and the hermetic construction style paired with a high internal heat load (due to technical equipment and occupants) puts them at risk for overheating. It becomes particularly difficult to maintain a stable indoor temperature in such airtight buildings when the outside temperature rises and radiation of the sun increases. However, even in cooler winter months, more and more buildings are nowadays at risk for overheating. Due to climate change and global warming, it is expected that people living in Western and Central Europe gradually will be exposed to warm environments more often and more frequently – both indoors and outdoors.

There has been a lot of scientific interest in studying the impact of extreme temperature conditions on human physiology and health. A vast amount of studies previously investigated the effect of intense, mostly exercise-induced heat acclimation programs on a variety of health-related outcomes and performance parameters. The results of those studies are particularly important to enhance performance and safety of athletes or the military. However, the influence of passive exposure to only moderately increased temperatures, which everybody might encounter in day-to-day situations, is largely unknown. Therefore, the experiments described in this thesis cover effects of acute and longer-term exposure to mild heat on human physiology, behaviour and health.

Metabolic responses of mammals to environmental temperatures can be described in the context of the so-called thermoneutral zone (TNZ). Per definition, within the TNZ, temperature regulation is achieved without changes of energy metabolism or evaporative heat loss. In large mammals such as humans, the determination of the TNZ is compromised due to the need of static conditions in combination with the large heat sink of the body. Therefore, a new dynamic approach to study the TNZ is explored in this thesis (**CHAPTER 2**). Here, the focus lies on the investigation of the *metabolic* TNZ, thus the range of ambient temperatures at which the metabolic rate remains stable. The study protocol consisted of an upward (approximately 29˚C to 38°C) and a downward temperature drift (approximately 29˚C to 18˚C), during which physiological responses of the human body were assessed. The results show that the dynamic protocol is practically feasible and suitable to determine the lower critical temperature (LCT) of the human (dynamic) TNZ, which denotes the critical point at which heat production increases due to decreasing ambient temperatures. Significant inter-individual variation regarding the positioning of the LCT is demonstrated in this thesis, denoted by the wide range of LCTs between the participants (19.2˚C – 28.9˚C). With respect to the metabolic upper critical temperature (UCT), if at all a slight gradual increase to no metabolic change was observed when temperature increased during warming. Therefore, it was not possible to indicate metabolic UCTs for the great majority of participants. A human metabolic UCT of approximately 32˚C, which has earlier been indicated in the literature, could not be confirmed by the results of this study.

In the context of global warming and the increasing risk for overheating of (modern) indoor spaces, one goal of the present thesis was to evaluate the human capacity for physiological adaptation to relatively mild and passively induced heat acclimation in different study groups. In **CHAPTERS 3** and **4,** the effect of passive exposure to *mild* heat (passive mild heat acclimation, PMHA) was studied, simulating realistic everyday life temperature challenges, in a healthy young (**CHAPTER 3**) and an overweight elderly population (**CHAPTER 4**). In the healthy population, participants were acclimatised to approximately 33˚C for 4-6h a day at 7 consecutive days. The overweight elderly group endured a slightly longer and more intense protocol with a mean acclimation temperature of 34.5˚C, which lasted for 4-6h at 10 consecutive days. During acclimation, thermophysiological parameters such as core and skin temperatures and sweating as

well as cardiovascular outcomes (e.g. blood pressure and heart rate) were measured. After PMHA, both study groups exhibited significant thermophysiological changes. Most importantly, the results show a significant reduction of core body temperature (Tcore) post PMHA, both in thermal neutrality and during warming. In the healthy population (**CHAPTER 3**), the decrease of Tcore was approximately 0.14˚C, whereas the overweight elderly group (**CHAPTER 4**) displayed a slightly more pronounced decrease of approximately 0.19˚C. Total sweat loss, measured as the change of body weight, decreased significantly in the healthy population after PMHA, but no change was observed in the overweight elderly population.

Previous active and passive heat acclimation studies have demonstrated that next to thermophysiological adaptations, prolonged heat exposure goes along with favourable health effects. Many studies report a decrease of resting and exercise heart rate as well as improved endothelial function, decreased arterial stiffness and lowered blood pressure. In both healthy young (**CHAPTER 3**) and overweight and obese elderly (**CHAPTER 4**) individuals, a distinct effect of PMHA on blood pressure was evident in thermoneutral conditions as well as during warming. Particularly diastolic blood pressure was lowered after PMHA. Our studies confirm that mild heat treatment might be a promising approach for improving cardiovascular health in a variety of target populations, especially for individuals with limited tolerance for other treatments such as exercise or medication.

In addition to a positive effect on cardiovascular health, it has recently been indicated that repeated exposure to heat might improve glucose metabolism and insulin sensitivity. Regarding the very high prevalence of overweight, obesity, obesity-induced insulin resistance and Type 2 Diabetes Mellitus (T2DM) we are facing today in the Western World, and the failure of current therapy standards to effectively tackle the increasing numbers, new alternative treatment options are needed to facilitate the aspired therapy success and to support long-term sustainability. An earlier study indicated that glucose handling improved in T2DM patients after taking hot baths over the course of 3 weeks, and others suggested a link between repeated heat exposure, increased heat shock protein (HSP) 72 expression and improved insulin sensitivity in human cell experiments and animal studies.

In the study described in **CHAPTER 4**, the effect of PMHA on glucose metabolism and HSP72 levels in overweight elderly men was assessed. This study is the first to investigate whole-body effects of heat acclimation on glucose metabolism and insulin sensitivity and the potential link with HSP72 levels in human muscle. The results show that PMHA evoked significant changes of glucose metabolism (lowered fasting plasma glucose and fasting plasma insulin), but insulin sensitivity measured by means of hyperinsulinemic-euglycemic clamps and HSP72 expression were not affected by PMHA. Additionally, substrate oxidation in a basal and insulin- stimulated state was evaluated, and a significant decrease of the respiratory quotient and a shift of substrate use towards more fat oxidation and lowered glucose oxidation was measured post-PMHA. Similar

results have been found after active, exercise-induced heat acclimation. This indicates that both active and passive heat acclimation can cause a substrate switch towards less glucose oxidation but increased fat oxidation in human muscle.

CHAPTERS 2 to **4** of this thesis mainly focus on the evaluation of *physiological thermoregulation*, i.e. autonomic processes needed to maintain thermal equilibrium of the human body, and adaptive processes of thermophysiology, to prolonged mild heat exposure. Another important aspect of thermoregulation is the *conscious behavioural regulation* of our thermal environment. In **CHAPTER 5,** the impact of PMHA on thermoregulatory behaviour and thermal perception in humans was studied. A so-called SWITCH protocol was applied, during which participants were free to go back and forth between a hot (37˚C) and a cold (17˚C) room whenever they desired to change their thermal environment. Skin temperature, thermal sensation and thermal comfort were assessed during SWITCH. For the first time, it is shown that PMHA evokes significant changes of thermoregulatory behaviour: post acclimation, participants switched from the hot to the cold room at higher mean skin temperatures and their stay in the hot room tended to be longer than before the intervention. Hence, participants seemed to tolerate higher skin temperatures before they felt the need to regulate their body temperature, implying greater resilience to heat. This also corresponds to the changes in physiological parameters described in **CHAPTER 3** and **4**.

The last study described in this thesis (**CHAPTER 6**) focused on the evaluation of cooling strategies for optimal thermal comfort in warm environments. Despite the acquisition of physiological adjustments and improved resilience to heat upon heat acclimation (as described in **CHAPTERS 3** to **5**), warm thermal environments are often perceived as uncomfortable. Therefore, several cooling strategies were tested to identify which body sites are the most suitable and effective to target for the restoration and optimisation of thermal comfort. In a simulated office environment, the application of face cooling (by means of a fan), back cooling (water-perfused seatback), underarm cooling, feet cooling (both by means of a water-perfused cooling plate) and combined face-underarm cooling was tested. Cooling of the face alone as well as combined face-underarm cooling significantly improved both thermal sensation and thermal comfort, representing two relatively easy-to-implement strategies to improve comfort in an office environment. Cooling of the back, the feet, or the underarms alone, did not significantly affect thermal comfort.

The overall goal of this thesis was to evaluate the effect of acute and longer-term mild heat exposure on human physiology, health and behaviour. The results clearly illustrate significant responses of the human thermophysiological system, even during moderate thermal challenges as encountered in everyday life.

Future research is warranted to further elucidate the effects of passive heat exposure in females, since the experiments described in **CHAPTERS 2** to **5** only included males. Moreover, further research is needed to test the in-vivo relationships between heat exposure, HSP72 expression and

insulin sensitivity in humans, both at cellular and at whole-body level. This knowledge may lead to the design of heat therapies and the development of lifestyle interventions as well as more variable, healthy and energy-efficient indoor climate profiles.

NEDERLANDSE SAMENVATTING

Mens in een warme omgeving - fysiologie, gezondheid en gedrag

Tegenwoordig worden mensen in de westerse Wereld nog maar zelden blootgesteld aan de variatie van buitenomstandigheden, omdat ze het grootste deel van hun tijd (gemiddeld ongeveer 90%) binnenshuis doorbrengen. Het ontwerp van onze binnenruimten heeft zich de afgelopen decennia enorm ontwikkeld, met het doel om deze zo comfortabel en efficiënt mogelijk te maken. We kunnen de binnenlucht naar onze wensen verwarmen, koelen, bevochtigen, drogen en ventileren en zo een grote mate van 'weerloosheid' creëren. Op basis van het onderzoek van Fanger dat in de jaren zeventig werd gepubliceerd en de later ontwikkelde normen voor binnenomgevingen volgens de 'American Society for Heating, Refrigerating and Air Conditioning Engineers' (ASHRAE), werd het doel te streven naar een thermisch *neutrale* omgeving in gebouwen. Daarbij werd verondersteld dat thermische neutraliteit het meest *comfortabel* zou zijn voor de grote meerderheid van de mensen. Om te voldoen aan deze normen mag de omgevingstemperatuur in een gebouw maar met slechts ±0.5˚C afwijken van de setpoint, onafhankelijk van de buitentemperatuur en het seizoen. Tot op heden worden deze normen meestal gehandhaafd, hoewel het behoud van een thermisch neutraal klimaat veel energie kost voor verwarming en airconditioning van gebouwen. Daarboven bestaat ook twijfel of dergelijke uniforme binnenomgevingen echt gezond zijn. Op basis van eerder onderzoek is namelijk geconstateerd dat voortdurende blootstelling aan thermoneutrale omgevingen bij zou kunnen dragen aan de hoge prevalentie van obesitas en metabole ziekten in geïndustrialiseerde landen. Veldstudies hebben bovendien aangetoond dat mensen een veel breder spectrum aan omgevingstemperatuur accepteren dan vereist door de klassieke modellen, wat vervolgens leidde tot de introductie van het Adaptief Comfort Model. Het Adaptief Comfort Model tolereert meer variatie van de binnentemperatuur in relatie tot de buitentemperatuur en het seizoen, en het werd recentelijk toegevoegd aan de laatste ASHRAE-Standard 55-2013.

Maatregelen die zijn getroffen om gebouwen te kunnen laten voldoen aan de klassieke strenge richtlijnen voor binnenlucht zoals hierboven beschreven, in combinatie met het streven om energiekosten te verlagen, hebben geleid tot het gebruik van sterk isolerende bouwmaterialen. Als gevolg hiervan zijn veel moderne gebouwen bijna luchtdicht. Daarboven hebben moderne gebouwen vaak een hoge interne warmtelast (door technisch equipment en mensen in het pand), en gepaard met een nagenoeg hermetische bouwwijze verhoogt dit het risico op oververhitting. Het wordt dan ook vaak moeilijk om in dergelijke gebouwen een stabiele binnentemperatuur te handhaven, vooral wanneer de buitentemperatuur stijgt en de straling van de zon toeneemt. Meer en meer gebouwen lopen zelfs in de koelere wintermaanden risico te overhitten. Vanwege de klimaatverandering en het broeikaseffect wordt verwacht dat mensen die in West- en Midden-

Europa wonen steeds vaker zullen worden blootgesteld aan warme omgevingen - zowel binnen alsook buiten.

Er is veel wetenschappelijke belangstelling voor het bestuderen van de invloed van extreme temperatuursomstandigheden op de menselijke fysiologie en gezondheid. Een groot aantal studies onderzocht eerder het effect van intense, vaak door hoge temperaturen in combinatie met inspanning geïnduceerde warmteacclimatisatie (actieve warmteacclimatisatie) op verschillende gezondheids-gerelateerde uitkomstmaten en prestatieparameters. De resultaten van deze studies zijn vooral van belang voor sporters of militairen, om de veiligheid en prestatie in omgevingen met hoge temperaturen te kunnen waarborgen. De invloed van passieve blootstelling aan slechts matig verhoogde temperaturen, zoals in het dagelijkse leven, op onze fysiologie en gezondheid, is echter grotendeels onbekend. Daarom is in dit proefschrift onderzocht wat het effect is van acute en langdurige blootstelling aan warmte op de menselijke fysiologie, gedrag en gezondheid.

De metabole respons van zoogdieren op de omgevingstemperatuur kan worden beschreven in de context van de zogenaamde thermoneutrale zone (TNZ). Per definitie wordt temperatuurregulatie binnen de TNZ bereikt zonder veranderingen van het energiemetabolisme en zonder zweten. Bij grote zoogdieren, zoals de mens, is de bepaling van de TNZ ingewikkeld. Het lichaam moet hiervoor over een relatief lange tijd bij een constante omgevingstemperatuur in een metabool equilibrium gebracht worden. Vervolgens moet dit voor elke temperatuur herhaald worden om de grenswaarden van de TNZ te kunnen opsporen. Dit is praktisch heel lastig uitvoerbaar en is zeer tijdrovend. Daarom wordt in dit proefschrift een nieuwe dynamische benadering om de TNZ te bestuderen verkend (**HOOFDSTUK 2**). Hierbij lag de focus op het onderzoek van de metabole TNZ, dus het bereik van omgevingstemperaturen waarbij de stofwisselingssnelheid stabiel blijft. Het onderzoeksprotocol bestond uit een opwaarts (ongeveer 29°C tot 38°C) en een neerwaarts temperatuur verloop (ongeveer 29°C tot 18°C), gedurende welke de fysiologische reacties van het lichaam werden gemeten. De resultaten laten zien dat het dynamische protocol praktisch haalbaar en goed toepasbaar is om de lagere kritische temperatuur (lower critical temperature, LCT) van de dynamische TNZ te bepalen. Deze geeft dus het kritieke punt aan waarop de warmteproductie toeneemt als gevolg van de afnemende omgevingstemperatuur. We vonden significante interindividuele variatie met betrekking tot de LCT. De spreiding van LCT's was aanzienlijk: van 19.2˚C tot 28.9˚C. Met betrekking tot de metabole bovenste kritische temperatuur (upper critical temperature, UCT), zagen we slechts een kleine geleidelijke of zelfs geen veranderingen wanneer de temperatuur steeg. Daarom was het niet mogelijk om voor de meerderheid van de deelnemers de metabole UCT te bepalen. Een metabole UCT van ongeveer 32˚C, die eerder in de literatuur voor de mens werd aangegeven, kon niet worden bevestigd door de resultaten van dit onderzoek. Dit kan zijn veroorzaakt doordat we een dynamisch protocol gebruikten en onder die omstandigheden ook hogere temperaturen hadden moeten testen.

In de context van klimaatveranderingen en het toenemende risico op oververhitting van (moderne) gebouwen, was één doel van dit proefschrift om te evalueren in hoeverre het lichaam zich fysiologisch kan aanpassen aan relatief milde en passief geïnduceerde warmte. In tegenstelling tot eerdere actieve warmteacclimatisatie-studies was dus het doel van dit proefschrift vooral de lichamelijke reacties op meer realistische, alledaagse thermische uitdagingen te evalueren. In **HOOFDSTUKKEN 3** en **4** is het effect van passieve blootstelling aan milde warmte (passive mild heat acclimation, PMHA) beschreven in twee verschillende studiegroepen, namelijk enerzijds bij gezonde jonge mannen (**HOOFDSTUK 3**) en anderzijds bij een groep oudere mannen met overgewicht (**HOOFDSTUK 4**).

In de eerste acclimatisatiestudie werden gezonde jongvolwassen deelnemers gedurende 7 opeenvolgende dagen gedurende 4-6 uur per dag blootgesteld aan ongeveer 33°C. De tweede studie vond plaats bij een oudere groep met overgewicht. Deze proefpersoenen werden blootgesteld aan een iets intensiever protocol, namelijk 10 opeenvolgende dagen, met een gemiddelde acclimatisatietemperatuur van 34.5˚C, eveneens voor 4-6 uur per dag. Tijdens PMHA werden thermofysiologische parameters zoals kerntemperatuur, zweten en cardiovasculaire uitkomsten (bijvoorbeeld bloeddruk en hartslag) gemeten. Na acclimatisatie vertoonden beide onderzoeksgroepen significante thermofysiologische veranderingen. Het belangrijkste resultaat was hierbij de significante verlaging van de lichaamskerntemperatuur (Tcore) na PMHA, zowel in thermische neutraliteit als tijdens opwarming. Dergelijke veranderingen zijn in lijn met de resultaten van meer intensieve hitte acclimatisatie. In de gezonde populatie (**HOOFDSTUK 3**) was de afname van Tcore ongeveer 0.14°C, terwijl de groep met overgewicht (**HOOFDSTUK 4**) een iets grotere daling van ongeveer 0.19°C vertoonde. Totaal zweetverlies, gemeten als de verandering van het lichaamsgewicht, nam eveneens significant af in de gezonde populatie na acclimatisatie, maar er werd geen verandering gemeten in de oudere populatie met overgewicht.

Eerdere actieve en passieve warmteacclimatiestudies hebben aangetoond dat naast de thermofysiologische veranderingen ook sprake kan zijn van gunstige gezondheidseffecten door langdurige blootstelling aan warmte. Veel studies rapporteren een afname van rust- en inspanningshartslag evenals verbeterde endotheelfunctie, verminderde arteriële stijfheid en verlaagde bloeddruk. In zowel de gezonde jonge groep (**HOOFDSTUK 3**) als ook in de zwaarlijvige oudere populatie (**HOOFDSTUK 4**) was er een duidelijk effect van acclimatisatie op de bloeddruk waarneembaar, in zowel thermoneutrale condities als tijdens opwarming. Vooral de diastolische bloeddruk was verlaagd na PMHA. De studies beschreven in deze proefschrift bevestigen dus dat de meer milde warmte een veelbelovende aanpak kan zijn voor het verbeteren van de cardiovasculaire gezondheid bij een verscheidenheid aan doelgroepen. Dit geldt met name ook voor mensen met een beperkte tolerantie voor andere behandelingen, zoals bijvoorbeeld lichaamsbeweging of medicatie.

Naast een positief effect op de cardiovasculaire parameters werd onlangs door studies aangetoond dat (herhaalde) blootstelling aan warmte het glucosemetabolisme en de insulinegevoeligheid zou kunnen verbeteren. Ten aanzien van de hoge prevalentie van obesitas-geïnduceerde insulineresistentie en Type 2-Diabetes Mellitus (T2DM) in de westerse wereld, en het falen van de huidige therapienormen om de nog steeds groeiende aantallen effectief aan te pakken, zijn nieuwe alternatieve behandelingsopties nodig om het beoogde therapiesucces te verbeteren en om lange-termijn resultaten te faciliteren. Een eerdere studie toonde aan dat de suikerstofwisseling bij T2DM-patiënten verbeterde na dagelijkse warm-water baden gedurende drie weken. Andere studies toonden een verband aan tussen herhaalde blootstelling aan warmte, verhoogde expressie van *heat shock protein 72* (HSP72) en verbeterde insulinegevoeligheid in experimenten met menselijke cellen en dierstudies. In **HOOFDSTUK 4** van dit proefschrift wordt het effect van PMHA op het glucosemetabolisme en HSP72 onderzocht bij oudere mannen met overgewicht. Deze studie is de eerste die de effecten van passieve warmtebehandeling op het glucosemetabolisme en de insulinegevoeligheid en de mogelijke link met HSP72-spiegels in de mens onderzocht. De resultaten tonen aan dat PMHA significante veranderingen van het glucosemetabolisme veroorzaakte (verlaagde nuchtere plasmaglucose en nuchtere plasmainsuline), maar de insulinegevoeligheid gemeten met behulp van de zogenaamde hyperinsulinemische-euglycemische clamp veranderde niet. Ook de HSP72-expressie werd niet beïnvloed door PMHA. Daarnaast evalueerden we de oxidatie van glucose en vet in basale en insuline-gestimuleerde toestand. We vonden dat PMHA een significante afname van het ademhalingsquotiënt induceert en een verschuiving van substraatgebruik naar meer vet-oxidatie en verminderde glucose-oxidatie. Vergelijkbare resultaten zijn gevonden na actieve, door inspanning geïnduceerde warmteacclimatisatie. Dit geeft aan dat zowel actieve als passieve warmteacclimatisatie een substraatomschakeling kan veroorzaken naar minder glucose-oxidatie maar verhoogde vet-oxidatie in menselijke spieren.

HOOFDSTUKKEN 2 t/m **4** van dit proefschrift concentreren zich voornamelijk op de evaluatie van *fysiologische thermoregulatie*, dus de autonome processen die nodig zijn om het thermisch evenwicht van het menselijk lichaam te handhaven, en daarnaast op de adaptieve thermoregulatoire processen na langdurige blootstelling aan warmte. Een ander belangrijk aspect van thermoregulatie is de *gedragsregulatie* van onze thermische omgeving. In **HOOFDSTUK 5** wordt de invloed van PMHA op thermo-regulerend gedrag en thermische perceptie bij mensen beschreven. We hebben een zogenaamd SWITCH-protocol toegepast, waarbij deelnemers vrij waren om heen en weer te gaan tussen een warme (37°C) en een koele (17°C) kamer wanneer ze hun thermische omgeving wilden veranderen. Huidtemperatuur, thermische sensatie en thermisch comfort werden geregistreerd tijdens SWITCH. Voor de eerste keer laten we zien dat PMHA significante veranderingen in het temperatuur-regulerend gedrag veroorzaakt: na acclimatisatie verplaatsten de deelnemers van de warme naar de koude kamer bij hogere huidtemperaturen. Bovendien duurde hun verblijf in de warme kamer meestal langer dan vóór de interventie. De proefpersonen leken hogere huidtemperaturen te verdragen voordat ze de

noodzaak voelden om hun lichaamstemperatuur actief te reguleren, wat duidt op een verbeterde resistentie voor warmte. Dit houdt ook verband met de veranderingen in fysiologische parameters beschreven in **HOOFDSTUKKEN 3** en **4**.

De laatste studie beschreven in dit proefschrift (**HOOFDSTUK 6**) richt zich op de evaluatie van koelstrategieën voor optimaal thermisch comfort in warme omgevingen. Ondanks de fysiologische aanpassingen en een verbeterde warmteresistentie na warmteacclimatisatie (zoals beschreven in **HOOFDSTUKKEN 3** t/m **5**), worden warme omgevingen nog vaak als ongemakkelijk ervaren. Daarom werden verschillende koelingsstrategieën voor gebruik op kantoor getest op geschiktheid en effectiviteit om het thermisch comfort te bevorderen. In een gesimuleerde kantooromgeving werden diverse delen van het lichaam gekoeld, te weten: het gezicht (door middel van een ventilator), de rug (met een watergekoelde stoel), onderarmen, voeten (beide met behulp van een watergekoelde koelplaat) en gecombineerde gezichts- en onderarmkoeling. Koeling van het gezicht alleen, evenals gecombineerde gezichts- en onderarmkoeling verbeterden zowel de temperatuurwaarneming als ook het thermisch comfort significant. Allebei zijn relatief eenvoudige en goedkoop te implementeren strategieën om het comfort in een kantooromgeving te verbeteren. Koeling van de rug, de voeten of de onderarmen alleen had geen significant effect op het thermisch comfort.

Het algemene doel van dit proefschrift was om het effect van acute en langdurige blootstelling warmte op de menselijke fysiologie, gezondheid en gedrag te evalueren. De resultaten illustreren duidelijk de significante reacties en adaptaties van de menselijke thermofysiologie. Zelfs milde thermische uitdagingen, die regelmatig aangetroffen kunnen worden in het dagelijks leven, kunnen dus deze aanpassingsprocessen van het lichaam initiëren en bewerkstelligen.

Vervolgonderzoek is nodig om de effecten van passieve warmteblootstelling bij vrouwen te onderzoeken, aangezien de experimenten die in **HOOFDSTUKKEN 2** t/m **5** zijn uitgevoerd bij alleen maar mannen. Bovendien is meer gedetailleerd onderzoek nodig om de *in-vivo* relaties tussen blootstelling aan warmte, HSP72-expressie en insulinegevoeligheid bij mensen te testen, zowel op cellulair niveau als op het niveau van het hele lichaam. Deze kennis kan leiden tot het ontwerp van warmtetherapieën en de ontwikkeling van leefstijlinterventies, evenals meer variabele, gezonde en energie-efficiënte binnenklimaatprofielen.

DEUTSCHE ZUSAMMENFASSUNG

Menschen und warme Umgebungen – Physiologie, Gesundheit und Verhaltensmuster

In der westlichen Welt sind die Menschen heutzutage kaum noch den natürlichen Schwankungen der Außentemperaturen ausgesetzt, da sie die meiste Zeit des Tages (durchschnittlich mehr als 90%) in Gebäuden und Innenbereichen verbringen. Entsprechend hat sich die Gestaltung des Klimas für unsere Innenräume in den letzten Jahrzehnten stark entwickelt und verändert, denn der Aufenthalt soll so komfortabel und angenehm wie möglich sein. In modernen Gebäuden ist es heutzutage möglich nach Belieben zu heizen, zu kühlen, zu belüften und die Luftfeuchtigkeit anzupassen. Somit haben wir im Prinzip eine ‚Wetterlosigkeit' unserer Innenräume erschaffen.

Basierend auf Studien aus den 1970er Jahren von Fanger, und den infolgedessen entwickelten Standards für Innenräume gemäß der Amerikanischen Gesellschaft für Heizungs-, Kühlungs- und Klimaanlageningenieure (American Society for Heating, Refridgerating and Air-Conditioning Engineers; ASHRAE), wurde angestrebt, eine thermisch *neutrale* Umgebung in Gebäuden zu schaffen. Es wurde nämlich angenommen, dass für die Mehrheit der Menschen eine Umgebung mit neutralen Temperaturen am komfortabelsten sei. Um aber die oben genannten Standards einzuhalten, darf die Innentemperatur eines Gebäudes, unabhängig von den Außentemperaturen, nicht mehr als ±0.5˚C um den angestrebten Sollwert schwanken. Diese Normen gelten auch heute noch, obwohl die Einhaltung der Richtwerte sehr viel Energie für Beheizung und Klimatisierung benötigt. Darüber hinaus wurden ebenfalls begründete Zweifel über die Gesundheit solcher uniformen Innentemperaturen geäußert. Es wird demnach vermutet, dass die Allgegenwärtigkeit jener uniformen und neutralen Innenraumtemperaturen zu den in Industrienationen stets steigenden Zahlen von Übergewicht und Stoffwechselerkrankungen beitragen könnte. Feldstudien haben außerdem gezeigt, dass Menschen tendenziell stärkere Schwankungen von Temperaturen akzeptieren, als durch die konventionellen Modelle auferlegt. Diese Erkenntnis hat folglich dazu geführt, den sogenannten 'Adaptiven Komfort Standard' zum neuesten ASHRAE Standard 55-2013 hinzuzufügen, welcher mehr Abweichung der Innentemperaturen in Relation zu den herrschenden Außenkonditionen und Jahreszeiten erlaubt.

Das Bemühen, die klassischen, strengen Raumklima-Standards umzusetzen und einzuhalten und zudem die Energiekosten zu verringern, hat dazu geführt, dass besonders stark isolierende Materialien für den Bau von neuen Gebäuden eingesetzt wurden. Infolgedessen sind viele moderne Bauten nahezu hermetisch abgeschlossen und luftdicht. Solch eine Konstruktionsweise in Kombination mit einer hohen internen Wärmelast (durch technisches Equipment und die Gebäudenutzer) führt jedoch zu erhöhtem Überhitzungsrisiko vieler Gebäude. Demnach ist es vor allem in warmen Monaten schwierig, eine stabile Innentemperatur zu halten, wenn die

Außentemperatur steigt und starke Sonneneinstrahlung hinzukommt. Mehr und mehr sind jedoch auch Gebäude in kühleren Wintermonaten von Überhitzung betroffen. Da vor allem in West- und Zentraleuropa durch Klimawandel und Erderwärmung die Durchschnittstemperaturen weiter steigen werden, ist es wahrscheinlich, dass Menschen in Zukunft häufiger wärmeren Temperaturen ausgesetzt sein werden, als sie derzeit gewohnt sind. Dies sowohl in Innenräumen als auch draußen.

Es hat stets ein großes wissenschaftliches Interesse daran bestanden, die Einflüsse extremer Temperaturen auf die Physiologie und Gesundheit des Menschen zu erforschen. Viele bestehende Studien erläutern den Effekt sehr intensiver und ‚aktiver', durch eine Kombination aus hohen Umgebungstemperaturen und körperlicher Betätigung induzierter Hitzeakklimatisierung, auf eine Palette von Gesundheitsfaktoren und Leistungsparametern. Die Ergebnisse solcher Studien können vor allem für die Leistungssteigerung und Sicherheit von Athleten oder dem Militär von Bedeutung sein. Interessanterweise ist über die Auswirkung von erhöhten, jedoch relativ milden Temperaturen auf den menschlichen Körper in alltäglichen Situationen, nur wenig bekannt. Aus diesem Grund beschreiben die Experimente, die dieser Dissertation zugrunde liegen, Akut- und Langzeiteffekte von moderater Temperaturerhöhung auf die humane Physiologie, die Gesundheit und temperaturregulierende Verhaltensmuster.

Der Grundumsatz von Säugetieren unter Einfluss von Temperaturveränderungen kann anhand der sogenannten ‚thermoneutralen Zone' (TNZ) beschrieben werden. Per Definition findet die Temperaturregulierung innerhalb der TNZ ohne Veränderungen des Energiemetabolismus und ohne aktive Verdunstungskälte (Schwitzen) statt. Bei großen Säugetieren mit viel Körpermasse, wie zum Beispiel dem Menschen, ist die Bestimmung des entsprechenden Temperaturbereichs, welcher der TNZ gleichsteht, sehr kompliziert. Für die Ermittlung der TNZ muss der entsprechende Körper über einen längeren Zeitraum bei gleichbleibender Umgebungstemperatur in thermisches Equilibrium gebracht werden. Dies muss für jede zu messende Temperatur wiederholt werden, was sich in der Vergangenheit als praktisch schwierig und sehr zeitaufwendig erwiesen hat. Daher wird in **KAPITEL 2** dieser Doktorarbeit ein neuer, dynamischer Ansatz zur Erforschung der menschlichen TNZ beschrieben. Hierbei liegt der Fokus auf der Ermittlung der *stoffwechselbedingten* TNZ, also jenem Temperaturbereich, bei dem der Grundumsatz des Menschen stabil und auf basalem Niveau bleibt. In der entsprechenden Studie wurden die physiologischen Reaktionen zu einem aufsteigenden (29˚C bis 38˚C) und einem absteigenden (29˚C bis 18˚C) Temperaturprotokoll gemessen. Die Ergebnisse zeigen, dass das dynamische Protokoll praktisch gut durchführbar und geeignet ist, um den unteren Grenzwert (lower critical temperature, LCT) der menschlichen (dynamischen) TNZ zu bestimmen. Die LCT ist jener kritische Punkt, ab welchem die Wärmeproduktion des menschlichen Körpers aufgrund sinkender Umgebungstemperaturen über das basale Niveau hinaus zunimmt, mit dem Ziel die Körperkerntemperatur stabil zu halten. Weiterhin zeigen die Resultate, dass signifikante inter-individuelle Unterschiede in Bezug auf die LCT bestehen, gekennzeichnet durch das breite

Spektrum der LCTs die bei den verschiedenen Studienteilnehmern gemessen wurden (zwischen 19.2˚C und 28.9˚C). In Bezug auf den oberen Grenzwert (upper critical temperature, UCT) der TNZ musste festgestellt werden, dass allenfalls eine kleine graduelle oder gar keine Steigerung des Grundumsatzes in Reaktion auf die steigenden Temperaturen bestand. Demnach war es bei den meisten Probanden dieser Studie nicht möglich, die stoffwechselbedingte UCT zu bestimmen. Ein oberer Grenzwert von 32˚C, welcher in der Literatur beschrieben wird, konnte in dieser Studie nicht bestätigt werden.

Im Kontext der globalen Erderwärmung und des stets steigenden Überhitzungsrisikos von (modernen) Gebäuden war eines der Ziele dieser Doktorarbeit die Kapazitäten des menschlichen Körpers sich an Wärme anzupassen zu evaluieren. Im Gegensatz zu den oben erwähnten ‚aktiven' Hitzeakklimatisierungsstudien wurden die verschiedenen Studiengruppen in den in **KAPITEL 3 UND 4** beschriebenen Experimenten recht milden Temperaturen passiv ausgesetzt, um alltägliche Herausforderungen simulieren zu können (passive mild heat acclimation, PMHA). Dies wurde einerseits in einer Gruppe junger, gesunder Personen (**KAPITEL 3**) und andererseits mit übergewichtigen, älteren Probanden (**KAPITEL 4**) durchgeführt. Die junge, gesunde Gruppe wurde an 7 aufeinanderfolgenden Tagen für 4-6 Stunden an eine Temperatur von ca. 33˚C akklimatisiert. Die übergewichtige, ältere Probandengruppe erfuhr ein geringfügig intensiveres Akklimatisierungsprotokoll, nämlich ca. 34.5˚C an 10 aufeinanderfolgenden Tagen, ebenfalls für 4-6 Stunden pro Tag. Während der Akklimatisierung wurden bei beiden Gruppen thermophysiologische Parameter wie zum Beispiel Kerntemperatur, Hauttemperatur, Schweißproduktion sowie kardiovaskuläre Faktoren (z.B. Blutdruck und Herzfrequenz) gemessen. In beiden Gruppen wurden nach der Akklimatisierung signifikante Veränderungen einiger thermophysiologischer Parameter gemessen. Am Wichtigsten war hierbei die nach der Akklimatisierung gemessene deutliche Verringerung der Körperkerntemperatur, welche sowohl in thermoneutralen Umständen als auch während steigender Temperaturen niedriger war als bei der Vormessung. In der jungen, gesunden Population betrug die Verringerung ca. 0.14˚C (**KAPITEL 3**) und in der übergewichtigen, älteren Gruppe ca. 0.19˚C (**KAPITEL 4**). Eine Verringerung der Körperkerntemperatur ist allgemein ein deutliches Zeichen für erfolgreiche Wärmeakklimatisierung. Die Schweißproduktion, gemessen anhand der Differenz des Körpergewichts vor und nach der Testung, war in der jungen, gesunden Gruppe nach Akklimatisierung signifikant geringer, wobei in der älteren übergewichtigen Gruppe keine Differenz gemessen wurde.

Bisherige aktive und passive Akklimatisierungsstudien haben gezeigt, dass neben thermophysiologischen Veränderungen häufig auch vorteilhafte Gesundheitseffekte durch Wärme erzielt werden können. Viele Studien beschreiben eine Reduzierung von Herzfrequenz sowie verbesserte Endothelfunktion, verbesserte Endothelelastizität und niedrigeren Blutdruck. In dieser Versuchsreihe wurde bei beiden Studiengruppen nach den getesteten passiven Akklimatisierungsphasen eine Senkung des Blutdrucks gemessen. Letzteres trat sowohl in

thermoneutralen Umständen auf als auch bei steigenden Temperaturen. Dies lässt die Schlussfolgerung zu, dass Wärmetherapie ein vielversprechendes Konzept zur Verbesserung kardiovaskulärer Gesundheit darstellt, vor allem in solchen Bevölkerungsgruppen, die eine begrenzte Toleranz für andere Therapiemaßnahmen (z.B. Sport oder Medikation) haben.

Andere mögliche positive Gesundheitseffekte von Wärme, beispielsweise eine Verbesserung des Glukosestoffwechsels und der Insulinsensitivität, wurden ebenfalls in früheren Studien erwähnt. Da die Zahlen von Übergewicht und Diabetes Mellitus Typ 2 (DMT2) in der westlichen Bevölkerung dramatisch hoch sind und stets weiter steigen, und darüber hinaus die heute bekannten Therapiemaßnahmen häufig wenig zufriedenstellende Langzeiteffekte erzielen, ist es wichtig, neue und alternative Behandlungsmöglichkeiten zu erkunden. Eine frühere Studie zeigte, dass der Glukosestoffwechsel bei DMT2-Patienten durch tägliches Baden in warmem Wasser über 3 Wochen hinweg effektiv verbessert werden konnte. Frühere Zell- und Tierversuche suggerierten eine Verbindung zwischen wiederholter Hitzeaussetzung, verbesserter Insulinsensitivität und vermehrter Expression des sogenannten ‚Heat Shock Proteins' (HSP) 72. In der Studie in **KAPITEL 4** wurde deshalb auch der Effekt des passiven Wärmeakklimatisierungsprotokolls auf den Glukosestoffwechsel und HSP72-Level erstmalig getestet. Diese Studie ist demnach die erste, die die potenzielle Verbindung zwischen passiver Ganzkörper-Wärmeakklimatisierung, Insulinsensitivität und HSP72-Expression in Menschen, bzw. menschlichen Muskelzellen, untersucht. Die Ergebnisse der Studie zeigen, dass Wärmeakklimatisierung signifikante Verbesserungen der Nüchternglukose und des Nüchterninsulins, gemessen in Blutplasma bei der Gruppe älterer und übergewichtiger Männer, erzielte. Die periphere Insulinsentivität, gemessen anhand der sogenannten 'hyperinsulinämischen-euglykämischen Clamp'-Technik, wie auch die HSP72-Level in Muskelzellen, blieben jedoch nach Wärmeakklimatisierung unverändert. Während des Clamps wurde auch die Substratoxidation anhand von Gasaustausch in der Atemluft analysiert, wobei festgestellt wurde, dass sowohl im basalen Zustand als auch im insulin-stimulierten Zustand eine vermehrte Fettverbrennung und Reduzierung der Glukoseverbrennung herrschte. Ähnliche Resultate wurden auch schon in früheren aktiven Hitzeakklimatisierungsstudien beschrieben. Dies deutet darauf hin, dass sowohl aktive als auch passive Hitze- bzw. Wärmeakklimatisierung einen Wechsel der Substratoxidation im Muskel bewirken kann, hin zu mehr Fettoxidation und weniger Glukoseoxidation.

In den **KAPITELN 2** bis **4** dieser Doktorarbeit steht vor allem die Untersuchung physiologischer Wärmeregulierung und adaptiven Mechanismen im Fokus. Diese werden durch das autonome Nervensystem des menschlichen Körpers gesteuert, um eine stabile Körperkerntemperatur zu garantieren. Ein weiterer wichtiger Faktor der Temperaturregulierung ist jedoch die *bewusste, verhaltensgesteuerte Regulierung* unserer thermischen Umgebung. In **KAPITEL 5** wird deshalb eine Studie beschrieben, in welcher der Effekt von passiver Wärmeakklimatisierung auf thermoregulatorisches Verhalten und Temperaturwahrnehmung in Menschen getestet wurde.

Ein sogenanntes 'SWITCH-Protokoll' wurde angewendet, bei welchem die Probanden frei zwischen Aufenthalt in einem warmen (37˚C) und einem kühlen Raum (17˚C) wählen konnten, und sooft hin- und herwechseln durften wie es ihnen beliebte. Es wurden hierbei Hauttemperaturen, Temperaturempfinden und Komfort gemessen. Diese Studie ist ebenfalls die erste, die den Effekt von passiver Wärmeakklimatisierung auf thermoregulatorisches Verhalten beschreibt. Die Ergebnisse zeigen, dass Probanden nach Akklimatisierung erst bei höheren Hauttemperaturen zum kühlen Raum wechselten, und ihr Aufenthalt im warmen Raum im Mittelwert länger dauerte als vorher. Demnach kann angenommen werden, dass nach der Wärmeakklimatisierung höhere Hauttemperaturen toleriert werden bevor verhaltensgesteuerte Thermoregulation initiiert wird, und, dass deshalb davon ausgegangen werden kann, dass die Akklimatisierung verbesserte Wärmeresistenz mit sich bringt. Dies ist ebenfalls im Einklang mit den Ergebnissen der physiologischen Tests beschrieben in **KAPITEL 3** und **4**.

Die letzte Studie in **KAPITEL 6** dieser Dissertation beschreibt die Testung verschiedener Kühlungsstrategien zur (Wieder-)Herstellung von Komfort in warmen Umgebungen. Obwohl Wärmeakklimatisierung potenziell zu Adaptierungsmechanismen und somit zu verbesserter Wärmeresistenz führt (siehe **KAPITEL 3** bis **5**), werden erhöhte Temperaturen in Innenräumen häufig trotzdem als unangenehm empfunden. Um praktische Möglichkeiten der Verbesserung und Optimierung des thermischen Komforts zu evaluieren, wurden verschiedenartige Kühlungsstrategien an unterschiedlichen Körperstellen getestet. In einer simulierten Büroumgebung wurde die Anwendung von Gesichtskühlung (anhand eines Ventilators), Rückenkühlung (wassergekühlte Rückenlehne), Unterarmkühlung, Fußkühlung (beide anhand einer wassergekühlten Metallplatte) und kombinierte Unterarm- und Gesichtskühlung evaluiert. Kühlung des Gesichts und kombinierte Unterarm-Gesichtskühlung erzielten hierbei die besten Ergebnisse. In beiden Fällen wurde Temperaturwahrnehmung und Komfort signifikant verbessert. Sowohl Gesichts- als auch Unterarmkühlung sind dabei relativ einfach zu implementierende Anwendungen, welche Komfort und Temperaturwahrnehmung in überhitzten Büros effektiv und schnell verbessern könnten. Rücken-, Fuß- und Unterarmkühlung alleine erzielten jedoch keine signifikanten Effekte in Bezug auf thermischen Komfort.

Im Großen und Ganzen war das Ziel dieser Doktorarbeit, den Effekt von akuter und längerfristiger Bloßstellung an Wärme und die Auswirkungen auf menschliche Physiologie, Gesundheit und Verhalten zu untersuchen. Die Ergebnisse der hier präsentierten Studien zeigen dabei, dass signifikante Reaktionen und Adaptionsmechanismen des Temperaturregelsystems auftreten, obschon die Temperaturkonditionen in den hier beschriebenen Experimenten im Gegensatz zu früheren Studien relativ mild waren, um realistischen Temperaturherausforderungen des alltäglichen Lebens zu entsprechen.

Zukünftige Studien sollten darauf zugeschnitten werden, Thermoreaktionen bei anderen Populationen, vor allem bei Frauen, zu verifizieren, da alle hier beschriebenen Experimente der

KAPITEL 2 bis **5** lediglich an Männern durchgeführt wurden. Des Weiteren sollten in Zukunft weitere Studien zur Erörterung der Zusammenhänge zwischen Wärme bzw. Hitze, Insulinsensitivität und HSP72-Levels, sowohl auf zellulärem als auch auf Ganzkörper-Niveau durchgeführt werden, da dies zur weiteren Entwicklung von temperaturbasierten Therapien und Lebensstilveränderungen beitragen könnte. Mehr Wissen auf diesem Gebiet könnte ebenfalls dazu beitragen gesündere und energieeffizientere Klimastandards zu entwickeln und zu fördern.

VALORISATION

The present thesis describes human physiological responses and behavioural coping to warm environments as well as the influence of prolonged exposure to elevated temperatures (passive mild heat acclimation) on human health, thermoregulatory behaviour and thermal perception. Moreover, a practical approach with respect to the management and optimisation of individual thermal comfort in warm thermal environments is presented. The valorisation potential of this thesis will be described in terms of societal and economic relevance, and implications for specific target groups, future research and possible applications for industrial development.

What is the societal and/or economical relevance of this research?

The design of indoor spaces has developed and changed a lot over the past decades, to make them, amongst other things, as comfortable and optimally tempered as possible for the occupants. This is especially true for most developed countries, where people nowadays are hardly ever exposed to the variation of outdoor conditions, as people spend the greatest part of their time (more than 90%) indoors (1). Based on earlier research, thermal neutrality was assumed to be the most *comfortable* for the majority of building occupants (2), and was thus manifested in the standards for indoor environments (ASHRAE Standard 55 (2) and ISO Standard 7730 (3)). Up until today, these standards are retained, albeit that some parts are criticized by many, and for several reasons. For example, operating a building on a tightly controlled set point, and not tolerating a reasonable amount of variation, costs a lot of energy. To date, roughly one-third of the primary energy supply in the Western World is used for heating, air-conditioning and ventilation of buildings (4). Moreover, reasonable doubt has been expressed about the healthiness of such uniform indoor environments (5-10).

Measures taken to comply with the strict indoor air guidelines described above, combined with efforts to reduce energy costs, have led to the use of highly isolating construction materials, which puts buildings at risk for overheating (11). Therefore, in the future, even more energy will have to be spent to condition our indoor spaces, especially in summer. Due to climate change and global warming, the scenario will progressively become more serious. Della-Marta *et al.* (12) have shown that between 1880 and 2005, the frequency of hot days in Western Europe has almost tripled and summer heat waves nowadays last twice as long. By the end of the 21st century, countries in central Europe are expected to experience as many hot days as are currently encountered in Southern Europe (13). Hence, events such as the extreme and unusual European summer heat wave of 2003 will likely not be as unusual any more in the near future: people living in Western and Central Europe will soon be exposed to warm environments more often and more frequently – both indoors and outdoors.

A vast amount of studies previously investigated the effect of intense, mostly exercise-induced heat acclimation programs on a variety of health-related outcomes and performance parameters (examples include (14-20)). However, the influence of passive exposure to only moderately increased temperatures, which more realistically reflects (summer) day-to-day temperature challenges, is largely unknown.

The scenario presented above clearly demonstrates the need for sophisticated information on the effect of passive and relatively mild heat stress on the human body. In the context of the above-discussed consequences of climate change and overheating of buildings, it is crucial to evaluate the impact of warm environments on human health as well as evaluate available (physiological and behavioural) coping mechanisms with elevated temperatures.

Therefore, this thesis examined several aspects of acute and longer-term exposure to mild heat on human physiology, health, thermal perception and thermoregulatory behaviour. Physiological and behavioural coping with high ambient temperatures was evaluated, as well as the impact of prolonged exposure to elevated temperatures on parameters of human metabolic and cardiovascular health. Furthermore, potential strategies to retain and restore thermal comfort in warm environments were also assessed.

For which target groups outside the scientific community are the results of this thesis interesting?

Firstly, the results yielded from the studies presented in this thesis are of great value for the general population of Western and Central Europe, particularly with respect to health and wellbeing in warm environments. We have shown that, to a certain degree, exposure to elevated temperatures elicits adaptive processes of the thermoregulatory system. Both healthy young as well as overweight elderly individuals exhibited significant physiological adaptations upon repeated exposure to mild heat. Furthermore, it has been shown that albeit the general perception of heat being a stressor, especially for the cardiovascular system and in vulnerable populations, we found that exposure to passively induced mild heat acclimation elicited favourable health effects such as a reduction of blood pressure in both healthy young and overweight elderly participants. Moreover, a significant improvement of glucose metabolism in the form of reduced fasting plasma glucose and fasting plasma insulin levels was evident in an overweight elderly population, after passive mild heat acclimation. Regular exposure to mild heat might therefore be considered as an (add-on) treatment option for high blood pressure and (onset) type 2 diabetes.

For the built environment sector, information yielded from this thesis can be of importance for the design of new (healthy and energy-efficient) indoor environments. Thermoregulatory behaviour is an important factor when considering the design and development of indoor spaces,

as occupants might, for example, adjust thermostats and operate windows, based on their thermal perception and comfort levels. The latter has significant impact on the performance of a building and on the thermal satisfaction and perceived control of its occupants. Hence, the findings of this thesis might be important for building performance assessments and estimation of energy use of a building. Furthermore, the results reveal that mild heat acclimation induces physiological adaptation, which improves resilience to heat and affects thermoregulatory behaviour. This means for example, that the temperature set-point of a building could be handled less strictly than proposed by the classical PMV model and thus drift more freely with changing outdoor conditions, which is conceptualised in the Adaptive Comfort Model (21-23). On the one hand, this has the potential to save a vast amount of energy for air-conditioning and on the other hand, it may improve metabolic health and create more resilience to heat (so-called 'temperature training' for the occupants).

Which concrete products, services, processes or activities can be translated and developed from your research?

The effect of mild heat on human physiology and health has been assessed from a variety of different perspectives. Firstly, the present thesis aimed to study the effect of heat exposure and heat acclimation on human health and wellbeing in the context of global warming, and the thereof resulting more frequent overheating of indoor spaces. The results presented in this thesis show that the human body is able to adapt relatively quickly, within the course of 7-10 days to elevated ambient temperatures, resulting in greater resilience to heat. Importantly, our data shows that this is true for a relatively mild increase of (indoor) ambient temperature up to 35˚C, which might be encountered more frequently in the coming decennia in Western and Central Europe, due to the climate change.

Secondly, considering the results of this thesis from a more practical perspective, we support earlier indications that temperature interventions, such as an individually-tailored heat therapy, might be a useful tool to improve not only cardiovascular health but also glucose metabolism. Although temperature interventions might not (yet) be regarded as stand-alone therapy for cardiovascular as well as metabolic diseases, it might be worthwhile to further investigate options to use temperature treatments as add-on therapies, additionally to the usual standard care.

Thirdly, human physiology and thermoregulatory behaviour are important factors to consider when attempting to design and develop healthy and energy-efficient indoor environments. As mentioned earlier, thermoregulatory behaviour can have significant impact on the performance of a building and on the satisfaction of its occupants. Additionally, local comfort systems can be helpful to save energy while simultaneously providing individually-tailored solutions for occupant comfort optimisation (for example with respect to open-plan office spaces). Recent investigations

suggest that individually-attuned comfort systems have the potential to not only restore thermal comfort and satisfaction, but also to save up to 50% of energy use compared with overall air-conditioning. In this thesis, a personalised cooling system had been investigated. The use of a desk fan, possibly in combination with a cooled desktop, is promising approach to efficiently (and inexpensively) improve thermal comfort and thermal sensation in overheated office spaces. The combination of such a personalised comfort system together with a drifting indoor temperature set-point has great potential to save energy while simultaneously retain occupant satisfaction and potentially improve occupant cardiovascular and metabolic health. We argue that the design and configuration of thermal indoor environments should be regarded as an important lifestyle factor, next to healthy diet and physical activity, and thus has to be taken into consideration for a wholesome, balanced life.

Planning and Realisation

Based on the results of this dissertation and on other studies from our research group regarding cold exposure and cold acclimation, two TKI project proposals were recently granted. In the scope of the first one (DYNKA, TKI Urban Energy-TEUE117001), dynamic office environments will be evaluated in the field, in real-life conditions. The second project (PERDYNKA, TKI Urban Energy-1507503) will focus on personalised control of dynamic office environments, also under 'living-lab' conditions. These projects will result in:

1. Ready-to-use dynamic climate scenario's in combination with dynamic lighting that can be implemented in offices.
2. The design of individually-controlled comfort systems for offices combining dynamic indoor temperature and dynamic lighting fixtures.
3. The development of an ICT platform for light and climate control
4. A set of building requirements for physics and installation technique for an optimally healthy, productive and acceptable indoor climate

The results are also expected to be applicable not only in offices but also in other environments such as schools, dwellings and care centres. Indoor climate and health implications are further studied in two EU projects. One project (Horizon2020-EE04-2016) focuses on the energy efficiency of Hybrid Geotabs building energy (heating and cooling) systems. The main goal is to optimise the design strategy of such systems with the explicit inclusion of health aspects. The aim of the other EU-project, (Mobistyle, Horizon2020-EE07-2016-IA) is to motivate behavioural change by raising consumer awareness and by providing attractive personalized systems. Here, the combination of pro-active knowledge services and the effect thereof on energy use, indoor environment, health and lifestyle, by ICT-based solutions will be addressed. All projects are spin-offs, which are partly derived from the studies and study ideas presented in this thesis.

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LIST OF PUBLICATIONS

Published peer-reviewed papers

Pallubinsky H, Schellen L, Kingma BRM, Dautzenberg B, van Baak MA, van Marken Lichtenbelt WD. 'Thermophysiological adaptations to passive mild heat acclimation'. Temperature (2017)

van Marken Lichtenbelt WD, Hanssen M, Pallubinsky H, Kingma B, Schellen L. 'Healthy excursions outside the thermal comfort zone'. Building Research & Information (2017)

Pallubinsky H, Kingma BRM, Schellen L, Dautzenberg B, van Baak MA, van Marken Lichtenbelt WD. 'The effect of warmth acclimation on behavior, thermophysiology and perception'. Building Research & Information (2017)

Pallubinsky H, Schellen L, Rieswijk TA, Breukel, CMGAM, Kingma BRM, van Marken Lichtenbelt WD. 'Local cooling in a warm environment'. Energy and Buildings (2016)

Pallubinsky H, Schellen L, Kingma BRM, van Marken Lichtenbelt WD. 'Human thermoneutral zone and thermal comfort zone: effects of mild heat acclimation'. Extreme Physiology & Medicine (2015)

Vosselman M, Hoeks J, Brans B, Pallubinsky H, Nascimento EBM, van der Lans AAJJ, Broeders EPM, Mottaghy FM, Schrauwen P, van Marken Lichtenbelt WD. 'Low brown adipose tissue activity in endurance trained compared to lean sedentary men'. International Journal of Obesity (2015)

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In preparation

Pallubinsky H, Dautzenberg B, Phielix E, van Baak MA, Schrauwen P, van Marken Lichtenbelt WD. 'The effect of passive mild heat acclimation on glucose metabolism in overweigth elderly men'.

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van Marken Lichtenbelt WD, Pallubinsky H, te Kulve M. 'Modulation of thermogenesis – a built environment perspective'.

ABOUT THE AUTHOR

Hannah Pallubinsky was born on the 6th of June 1988 in Aachen (Germany). Upon completing her secondary school at the Städtisches Gymnasium Würselen, she studied law at the University of Trier and the University of Münster between 2007 and 2009. In September 2009, Hannah decided to change her educational field and enrolled at Zuyd Hogeschool in Heerlen, where she joined a Bachelor of Physiotherapy program for high-achieving students, allowing her to finish her Bachelor's degree (B.Sc.) after 3 years. In September 2012, she started her Master's program, Sports and Physical Activity Interventions, at Maastricht University. During her Master's, she completed a 6-month internship at the Department of Human Biology in the research group led by Prof. Dr. Wouter van Marken Lichtenbelt. During this period, she focused on the investigation of brown adipose tissue activity and energy metabolism in endurance-trained athletes and sedentary people.

Hannah obtained her Master's degree in 2013, and immediately started working as a PhD student under the supervision of Prof. Wouter van Marken Lichtenbelt. During her PhD, she studied the role of acute exposure to heat and cold as well as passive mild heat acclimation on human thermophysiology, health and behaviour, as demonstrated in this dissertation and the publications in scientific journals. Hannah has presented her work at several national and international conferences such as the International Conference on Environmental Ergonomics 2015, 6th International Conference on the Physiology and Pharmacology of Temperature Regulation 2017 and the 8th, 9th and 10th Windsor Conference on Thermal Comfort in 2014, 2016 and 2018.

Since February 2018 she has been working as a Postdoctoral researcher at the Department of Nutrition and Movement Sciences (Maastricht University) and pursuing her research on thermophysiology, metabolic health and thermal comfort within the scope of this position.