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Exploring the thermal interactions in vasomotion, sudomotion and thermogenesis

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EXPLORING THE THERMAL INTERACTIONS IN VASOMOTION, SUDOMOTION AND THERMOGENESIS

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CERTIFICATION

I, Joanne N. Caldwell Odgers, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Medicine, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

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ABSTRACT

Humans regulate body temperature across a wide range of environmental conditions. This is achieved through specialised control pathways that enable the body to detect, integrate and respond to variations in internal and external temperatures. Thermoreceptors, located both centrally and peripherally, detect these temperature changes and provide thermoafferent feedback which is integrated by the hypothalamus and results in the modulation of three thermoeffector mechanisms: vasomotion, sudomotion and thermogenesis. Vasomotion primarily regulates body temperature in thermoneutral environments, while sudomotion and thermogenesis are only activated when skin blood flow can no longer defend mean body temperature. Interestingly, the control of each of these thermoeffectors is not only influenced by core temperature, but also by peripheral (skin) temperature. Indeed, it is generally understood that an approximate ratio of 9:1 (core:skin) exists in the heat. However, the interactions of core and local skin temperature, and the determinants of each of these thermoeffectors (vasomotion, sudomotion and thermogenesis) remains to be more clearly elucidated.

Accordingly, the current series of investigations was focussed upon understanding how these thermoeffectors respond and interact during exposure to a range of environmental conditions, but with a particular emphasis upon skin blood flow in the acral and non-acral skin regions. Firstly, we investigated methods for cooling hyperthermic individuals through whole-body water immersion, and the physiological mechanisms associated with this treatment (Chapters 2 and 3). Secondly, we explored the interactions among central and peripheral thermoreceptor feedback, and direct thermal effects on skin blood flow in both acral and non-acral skin regions (Chapter 4 and 5). Finally, we investigated how thermoeffector activation was modified following deliberate modification of the steady-state, pre-exposure mean body temperatures (Chapter 6).

The first phase of these investigations was designed to provide an understanding of the physiological mechanisms associated with cooling hyperthermic individuals using water immersion. In the first experiment (Chapter 2), we explored three different cooling methods to reduce body core temperature of hyperthermic individuals: air (20-22^{o}C); cold-water

immersion (14°C); temperate-water immersion (26°C). In the second experiment (Chapter 3), we investigated whether a more powerful vasoconstriction was present during cold-water immersion, as determined by measuring forearm blood flow. It was also hypothesised that a more powerful vasoconstrictor response would occur during cold-water immersion compared to temperate-water immersion, and that this would occur in both normothermic and hyperthermic states.

For experiment one, eight males participated in three trials, and were heated to an oesophageal temperature of 39.5°C by exercising in the heat (36°C, 50% relative humidity) whilst wearing a water-perfusion garment (40°C). Subjects were cooled using each of three methods: air (20-22°C); cold-water immersion (14°C); temperate-water immersion (26°C). The time to reach an oesophageal temperature of 37.5°C averaged 22.81 min (air), 2.16 min (cold) and 2.91 min (temperate). While each of the between-trial comparisons were statistically significant ($P<0.05$), cooling in temperate water took only marginally longer than in cold water. For experiment two, eight male subjects completed four trials; two control trials (Trial A and Trial B) and two experimental trials (Trial C and Trial D). Each trial consisted of a pre-immersion phase followed by a water-immersion phase. The pre-immersion phase was from either a normothermic (control) or profoundly hyperthermic (experimental) state, while the water immersion phase was in either 14°C (cold immersion) or 26°C (temperate immersion) water. While there were no significant differences in forearm or cutaneous blood flow between either of the control trials (Trial A and Trial B; $P>0.05$), a significantly greater reduction in forearm and cutaneous blood flow occurred during the cold-water immersion (Trial C) at each time point within the first 4 min of immersion following hyperthermia compared to temperate-water immersion. These observations have not previously been described, and have considerable practical significance. It was concluded that, for hyperthermic, but asymptomatic individuals, temperate-water immersion more than adequately facilitated brain cooling, due to the maintenance of a greater peripheral blood flow.

The purpose of phase two of this experimental series was firstly to design, construct and validate water displacement plethysmographs (Part A) for the forearm, hand and foot that could clamp segmental skin temperature whilst simultaneously measuring cutaneous blood

flow (Chapter 4), and secondly (Part B) to investigate the interactions of the central (core) drive and peripheral (cutaneous) feedback on the control of skin blood flow in acral and non-acral skin regions (Chapter 5). For this phase, it was hypothesised that forearm vascular conductance, measured with either a mercury-in-silastic strain-gauge plethysmograph or a water-filled (displacement) plethysmograph, would not differ across these measurement techniques and that core temperature would exert the greatest neural influence on skin blood flow within all body regions (forearm, hand, calf and foot), but for a given core temperature, skin blood flow would change in proportion to changes in local skin temperature for each site.

For part A, two experiments were performed. In the first, the forearm plethysmograph was validated against a mercury-in-silastic plethysmograph under thermoneutral conditions, with and without forearm heating. Cutaneous vascular conductance was elevated almost three-fold by this treatment, however, there were no significant differences between the two forms of plethysmography in either state ($P>0.05$). In study two, hand and foot blood flows were measured under clamped thermoneutral conditions, but with three local skin temperature treatments (5º, 25º, 40ºC). The hand had significantly higher blood flows than the foot at both 25ºC (4.07 versus 2.20 mL.100 $mL^{-1}.min^{-1}$; $P<0.05$) and 40ºC (8.20 versus 16 4.47 mL.100 $mL^{-1}.min^{-1}$; $P<0.05$). The foot was maximally constricted during the two lower temperatures, yet the cutaneous thermal sensitivity of the hand was almost two-fold greater ($P<0.05$). This research highlighted not only the significance of measuring limb segment blood flow though venous-occlusion plethysmography, but also demonstrated that minimal variation exists between the strain-gauge and water-filled plethysmograph.

For Part B of this research phase, subjects completed three trials where segmental blood flow was measured using four water-filled plethysmographs (forearm, hand, calf and foot) under three separate thermal states, induced using whole-body water immersion and five local skin temperatures. Core temperature for each state was either hypothermic (36.07ºC ±0.37), normothermic (37.04ºC ±0.27) or hyperthermic (38.47ºC ±0.34). During each trial, five local skin temperatures (5º, 15º, 25º, 33º and 40ºC) were induced at each of four treatment sites (forearm, hand, calf and foot). The lowest recorded skin blood flow, during whole-body hypothermia and local cooling (5ºC), was 0.27 mL.100 $mL^{-1}.min^{-1}$ in the foot,

and the highest recorded skin blood flow, during whole-body hyperthermia and local heating (40^{o}C), was in the hand (27.6 mL.100 mL^{-1}.min^{-1}). For each of the four measurement sites (forearm, hand, calf and foot) local skin temperature had little to no effect on skin blood flow during the cold exposure. Indeed, the thermosensitivity remained close to zero for each site: forearm (0.04 mL.100 mL^{-1}.min^{-1}.^{o}C ±0.02); hand (0.04 mL.100mL^{-1}.min^{-1}.^{o}C ±0.02); calf (0.02 mL.100 mL^{-1}.min^{-1}.^{o}C ±0.01); and foot (0.05 mL.100mL^{-1}.min^{-1}.^{o}C ±0.05). However, the rate of increase in skin blood flow with increased local skin temperature (sensitivity) was more than double that observed during the cold exposure at each site. This study provided further supporting evidence that local skin temperature has minimal influence on skin blood flow across either acral or non-acral skin regions within hypothermic individuals, due to the presence of very powerful, centrally driven, vasoconstriction under these conditions. In contrast, the influence of local skin temperature became more pronounced in normothermic individuals. When individuals were hyperthermic, the graded changes in local skin temperature augmented skin blood flow to a greater extent than during both the hypothermic and normothermic conditions across all sites.

Finally, to understand how each of the thermoeffectors respond to changes in mean body temperature, an investigation of the effects of slight deviations in the steady-state, pre-exposure mean body temperature upon the vasomotor, sweating and shivering thresholds was completed. This was achieved by altering the pre-exposure mean body temperature through either whole-body heating or cooling, and then by driving body temperature in the opposite direction whilst measuring the thermoeffector thresholds. It was hypothesised that pre-cooling and pre-heating would shift the mean body temperature thermoeffector thresholds for sweating and shivering by a magnitude equal to that of the pre-exposure displacement of mean body temperature, and that the mean body temperature for vasodilatation and sweating thresholds would occur simultaneously during heating, while the vasoconstrictor threshold would always precede the shivering thresholds during cooling. Each trial consisted of a pre-experimental whole-body water immersion (28-23^{o}C, 35^{o}C or 39^{o}C) phase followed by an experimental phase of either passive heating or passive cooling. During the experimental phase, subjects were passively warmed or cooled, with the use of a water-perfusion garment in the opposite direction to the pre-treatment exposure temperature.

Following whole-body cooling, the threshold for precursor forearm sweating was elevated by 0.18ºC (±0.03; P=0.07), whilst that for discharged sweat was raised by 0.19ºC (±0.04; *P*<0.05). Conversely, the vasomotor threshold was reduced by 0.36ºC (±0.11; P<0.05) for the same site. This change in vasomotor threshold was not significantly different from the calf (0.40º ±0.13ºC) or finger thresholds (0.45º ±0.11ºC). For this thermoeffector, the change in the pre-heating mean body temperature (0.39±0.10ºC), relative to the control trial, did not differ significantly from its threshold change (*P*>0.05). Conversely, the thresholds for shivering and forearm skin blood flow (vasoconstriction) were 32.9ºC (±0.1) and 33.0ºC (±0.2), respectively (*P*>0.05) following whole-body heating. The change in pre-cooling temperature was 0.82ºC (±0.2) and was not significantly different from the change in temperature for the shivering threshold, which was increased by 0.67 (±0.2; *P*>0.05). The vasomotor zone between vasoconstriction and vasodilatation was 3.7ºC and 3.2ºC for the control and treatment trials, respectively. The most significant finding from this study was that, following pre-treatment, the threshold for vasodilatation and shivering were shifted in equal proportions to the change in mean body temperature induced by each of the two pre-treatments, and this supported our hypotheses. Interestingly, the sudomotor threshold was shifted to a higher mean body temperature following whole-body pre-cooling, and this outcome could indicate that the two thermoeffectors might work independently in response to heat loss requirements, with sudomotor activation being initiated only when vasodilatation fails to dissipate sufficient heat from the skin to the surrounding environment.

In summary, the present series of investigations provided a comprehensive examination on the interactions of vasomotion, sudomotion and thermogenesis, both separately and inter-dependently, across a wide range of environmental conditions with emphases on the physiological mechanisms associated with the treatment of exertional heat illness, the mapping of skin blood flow across a range of core and local skin temperatures, the regional differences in skin blood flow and on the interactions between mean body temperature displacement and the thermoeffector thresholds. The resulting observations have significantly increased our understanding of thermoeffector activation across a range of thermal states.

MANUSCRIPTS PUBLISHED DURING DOCTORAL STUDIES

Taylor, N.A.S., Caldwell, J.N., and Dyer, R. (2008). The physiological demands of horseback mustering when wearing an equestrian helmet. *European Journal of Applied Physiology*. 104(2):289-296.

Machado-Moreira, C.A., Caldwell, J.N., Mekjavic, I.B., and Taylor, N.A.S. (2008). Sweat secretion from palmar and dorsal surfaces of the hands during passive and active heating. *Aviation, Space and Environmental Medicine*. 79(11):1034-1040.

Taylor, N.A.S., Caldwell, J.N., van den Heuvel, A.M.J., and Patterson, M.J. (2008). To cool, but not too cool: that is the question: immersion cooling for hyperthermia. *Medicine and Science in Sports and Exercise*. 40(11):1962-1969.

Caldwell, J.N., Engelen, L., van der Henst, C., Patterson, M.J., and Taylor, N.A.S. (2011). The interaction of body armour, low-intensity exercise, and hot-humid conditions on physiological strain and cognitive function. *Military Medicine*. 176(5):488-493.

Machado-Moreira, C.A., McLennan, P.L., Lillioja, S., van Dijk, W., Caldwell, J.N., and Taylor, N.A.S. (2012). The cholinergic blockade of both thermally and non-thermally induced human eccrine sweating. *Experimental Physiology*. 97(8):930-942.

Caldwell, J.N., Patterson, M.J., and Taylor, N.A.S. (2012). Auxiliary cooling during an exercising heat stress: physiological and cognitive functions. *European Journal of Applied Physiology*. 112(10):3597-3606.

Caldwell, J.N., Matsuda-Nakamura, M., and Taylor, N.A.S. (2014). Three-dimensional interactions of mean body and local skin temperatures in the control of hand and foot blood flows. *European Journal of Applied Physiology*. 114:1679-1689.

Caldwell, J.N., and Taylor, N.A.S. (2014). Water-displacement plethysmography: a technique for the simultaneous thermal manipulation and measurement of whole-hand and whole-foot blood flows. *Physiological Measurement*.35:1781-1795.

Taylor, N.A.S., Machado-Moreira, C.A., van den Heuvel, A.M.J., and Caldwell, J.N. (2014). Hands and feet: physiological insulators, radiators and evaporators. *European Journal of Applied Physiology*. Epub ahead of print. DOI: 10.1007/s00421-014-2940-8

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CHAPTER 1: INTRODUCTION

1.1 BACKGROUND:

To maintain homeostasis of the internal environment, humans regulate body temperature across a wide range of environmental conditions. This is achieved not only through passive heat transfer but also through integration of many physiological regulatory systems that are controlled by negative feedback. Temperature regulation is achieved through modifications in cutaneous vasomotion, sudomotion and thermogenesis. Of these, vasomotor function continually changes in response to a wide range of internal and external conditions with the capacity to enhance or reduce heat loss through changes in blood vessel diameter (Brengelmann *et al*., 1977; Rowell *et al*., 1970). When sufficient heat loss can no longer be achieved solely through passive vasodilatation (withdrawal of vasoconstrictor tone), active vasodilation and sweating are activated (Smiles *et al*., 1976; Boulant, 1981). Conversely, shivering thermogenesis is only activated when body temperature drops to a point where cutaneous vasoconstriction is insufficient to defend core temperature (Morrison *et al.*, 2008; Werner, 2010). By investigating the interactions of each of these thermoeffectors, both separately and inter-dependently, across a wide range of environmental conditions we aim to firstly, understand the physiological mechanisms associated with the cold-water immersion of hyperthermic individuals, and hence recommend appropriate treatment strategies for exertional heat illness. Secondly, we aim to understand the interactions among the central and peripheral thermoreceptor feedback and direct local effects on skin blood flow in both acral (non-hairy) and non-acral (hairy) skin regions. Finally, we aim to understand how each of the three thermoeffector thresholds (vasomotion, sudomotion and thermogenesis) respond and interact to deviations in the steady-state, pre-exposure mean body temperature changes. These broad aims form the primary *foci* for this series of research investigations.

1.1.1 Body temperature regulation: From thermoreception to effector mechanisms

Humans are homeothermic and are able to maintain a stable internal

temperature across a wide range of external temperatures (Bernard, 1876; Cannon, 1929). This occurs because humans are constantly exchanging thermal energy with the environment. The exchange in thermal energy, known as thermodynamics, is a result of well developed mechanisms that evolved into extremely powerful and efficient pathways for modifying heat balance. This is best described by the heat balance equation, which is dictated by the avenues through which heat is exchanged between the environment and the individual. It is comprised of two components: dry heat exchange (radiation, conduction and convection) and evaporative heat loss.

$$S = M - (\pm W) - E \pm R \pm K \pm C \ [\text{W.m}^{-2}] \qquad \text{Equation 1.1}$$

where:

S = stored thermal energy [W.m^{-2}]

M = metabolic rate or energy transformation [W.m^{-2}]

W = mechanical work [W.m^{-2}]

E = evaporative heat transfer [W.m^{-2}]

R = radiative heat transfer [W.m^{-2}]

K = conductive heat transfer [W.m^{-2}]

C = convective heat transfer [W.m^{-2}]

Body temperature regulation is centred around heat balance and is reliant on negative feedback from a passive system and an active (controlling) system (Werner, 2008). Within the passive system, modification of body temperature is achieved through passive heat transfer, down a thermal gradient, between the body and its surroundings. The amount of heat energy transferred or stored by all anatomical structures within the body is dependent upon body composition, physical dimensions and the surrounding environment (Havenith *et al*., 1998). The active system is composed of sensors (thermoreceptors) that detect and respond to changes in the passive system. That is, neural feedback received from these thermoreceptors continually responds to changes in core and skin temperature (Hellon, 2011). This feedback is then integrated centrally, within the preoptic posterior and anterior hypothalamus, to initiate one or more effector responses (Smiles *et al*., 1976; Boulant, 1981; Werner *et al*., 2008). These include either behavioural changes (posture,

clothing, physical activity and climate control) or autonomic functions (vasomotion, sudomotion and thermogensis). Effector mechanisms are activated as a results of an integrated response to both core and skin temperature (Hellon, 2011; Boulant, 1996).

1.1.1.1 Contributions of core and skin temperature in thermoregulation

While the sensing of body temperature is essential to its control mechanisms, this does not occur uniformly across the entire body. Detection of body temperature occurs through activation of a range of temperature-sensitive receptors located in the skin (shell) and central nervous system (Hellon, 2011; Boulant, 1996). That is, although the thermal feedback from the body core and shell tissues is important, the thermosensitivity of these regions to the hypothalamus is not in equal proportions, where the relative importance of peripheral and deep body temperature sensors is dependent upon the circumstances in which each is dominant (Hellon, 2011). In general, it is believed that a dominant role of body core temperature in central integration and autonomic functions exists, with a range of ratios used including approximately 8:2 (core:shell) in comfortable conditions and 9:1 in the heat (Bligh, 1973). However, this ratio is an approximation and is not reflected across all environmental conditions (Bligh, 1973). Whilst core temperature plays a major role in the activation of sweat glands, variations in local skin temperature have also been shown to elicit appreciable changes on the rate of sweat secretion (Cotter and Taylor, 2005).

Furthermore, deep tissue temperature is known to have the greatest influence upon cutaneous blood flow when both core and skin temperature are changing (Wyss *et al.*, 1974), and local skin temperature, via mechanisms independent of the central sympathetic activation, is also known to greatly influence circulation through the skin (Spealman, 1945; Pérgola *et al*., 1993; Durand *et al*., 2004). Although these interactions between core and skin temperature on the control of skin blood flow have been extensively investigated (Spealman, 1945; Johnson and Park, 1979; Wilson *et al*., 2002), there is a lack of quantitative data on the interactions between central drive, peripheral thermoreceptor feedback and the direct local skin temperature effects on skin blood flow, over a wide range of thermal states. Accordingly, this interaction

forms one focus of this series of investigations, which will provide useful information pertaining to the control mechanisms of skin blood flow in response to variations in both central and peripheral temperature.

1.1.1.2 Thermoreception

In addition to the varying contributions of core and skin temperature to central integration, thermoreceptors are sensitive to warmth or coldness (Hensel *et al.*, 1974), and are activated as a result of constant (static) stimulation, when temperature remains stable, or in response to a change in temperature (dynamic), with some overlap with the frequency of firing between the dynamic and static responses (Hensel, 1981). Both warm and cold thermoreceptors respond with an overshoot when suddenly warmed or cooled and are inhibited when the opposite stimulus is applied (Hensel *et al.*, 1974). For example, when heat is applied to the body, the warm receptors become active while the cold receptors are inhibited. Collectively, the thermoreceptor location, abundance and type play a significant role in the thermoeffector responses associated with exposure to different environmental conditions and thermal states. We will explore the physiological mechanisms associated with static and dynamic activation of cold and warm thermoreceptors.

It has been shown that the thermoresponsiveness of cutaneous-cold thermoreceptors is greater than that of warm thermoreceptors (Bazett *et al.*, 1930; Hensel, 1971; Hensel *et al.*, 1974). This is supported by research conducted over the past century where the location, structure and function of thermoreceptors has been investigated (Bazett *et al.*, 1930; Hensel *et al.*, 1974). In a study performed on cat tongues, the latency measured from cold impulse to reaction time was only a few hundredths of a second at a depth of 0.18-0.22 mm (Hensel, 1971). This was supported by the results of a study conducted on human subjects where stimulation occurred at an intracutaneous depth of 0.15-0.17 mm (Bazett *et al*., 1930), indicating that nerve endings are located in the superficial sub-dermal layer of the skin. Warm receptors however, have been shown to exist deeper within the skin (Bazett *et al.*, 1930; Hensel *et al.* 1974) and to be less abundant than cold receptors (Hensel, 1981). In addition to location and abundance of receptors, the type of thermoreceptor

activation is also important in the central integration within the hypothalamus to initiate the effector response.

Peripheral thermoreceptors are classified as temperature-activated transient receptor potential ion channels (Patapoutian *et al.,* 2003). These receptors are distinctly different to other thermodynamic ion channels as they are activated alone by temperature and are individually categorised to detect a specific range of temperatures (hot or cold), enabling the human body to be regulated over a wide range (Hellon, 2011). Noxious (unpleasant) hot temperatures are detected by two types of thermoreceptors that are either activated by temperatures that are equal to or higher than 42^{o}C and show a much higher sensitivity than most ion channels (Caterina *et al*., 1997) or are activated at noxious temperatures above 52^{o}C (Caterina *et al*., 1999). Innocuous (pleasant) warmth in the range of 34-42^{o}C is served by a subset of specialised neurons that can be divided into two groups. *In vitro* tests have shown these thermoreceptors to be active at temperatures in the range of 34-38^{o}C (Smith *et al*., 2002), with a progressive decline in activation at temperatures greater than 42^{o}C (Guler *et al.*, 2002; Watanabe *et al.,* 2002).

The exact mechanisms involved in the excitation of cold sensory neurons are not as well understood as those of hot stimulation (Patapoutian *et al.,* 2003). However, it is believed that unmyelinated C and thinly myelinated delta-A afferent fibres respond to innocuous cold temperatures (Hensel and Zotterman, 1951) without responses to warming or non-thermal stimuli (Hensel and Iggo, 1971). Similarly, a sub-population of these fibre types is activated in response to noxious cold (<15^{o}C), but the frequency of firing is dependent upon the intensity of the stimulus (Cain *et al.*, 2001). When investigated *in vitro,* studies isolating the cold receptor, have shown neural activity in response to temperatures from <25-28^{o}C to as low as 8^{o}C (McKemy *et al.*, 2002). Temperatures below this range are thought to be detected by noxious cold receptors that have a lower activation threshold (<17^{o}C; Story *et al*., 2003).

One combination of thermal state and environmental conditions that is of particular interest is rapidly cooling a hyperthermic (thermal state) individual in either

ice-cold or cold water (environmental condition). Not surprisingly, immersion in ice-cold water (0-2^{o}C) has been shown to rapidly reduce body core temperature (Proulx *et al*., 2003, 2006; Casa *et al*., 2012). Although ice- and cold-water immersions maximise the thermal gradient between the skin and water, thereby rapidly enhancing the rate of cooling through conductive heat loss, it does not account for physiological mechanisms associated with rapidly activating peripheral cold thermoreceptors. In addition, when individuals are hyperthermic central and peripheral warm-receptors are activated at a constant rate. In this case, and immediately following water immersion, information is received simultaneously by activated central warm thermoreceptors and dynamically activated peripheral cold thermoreceptors. It remains unknown whether the thermoeffector response (in this instance vasoconstriction) from rapid activation of cold thermoreceptors will override that of the hot stimulus. Accordingly, this forms another focus of this series of research investigations, which will provide an understanding of the physiological mechanisms (skin blood flow) associated with cooling hyperthermic individuals who are immersed in water as part of treatment of exertional heat illness.

For body temperature to be regulated during such environmental conditions, the actions of various effector mechanisms (vasomotion, sudomotion and thermogenesis) enable heat to be lost, gained or conserved to and from the environment. This is initiated as a result of central integration and evaluation of thermoafferent information received from the central and peripheral thermoreceptors and occurs primarily in the pre-optic anterior hypothalamus (Hellon, 2011). Whilst measuring hypothalamic function in humans is limited, a plethora of *in vivo* human research exists (Wyndham, 1965; Mekjavic and Eiken 2006). These experiments have investigated the output (effector) responses following independent and combined manipulation of both central and peripheral thermal inputs (sensors). The hypothalamus receives thermoafferent flow from the entire body and integrates all of these inputs to control the effector response. However, there are still gaps in our understanding of the physiological mechanisms that make up these models and this will be explored in the current research series. It is also believed that reciprocal cross inhibition exists within the hypothalamus (Werner *et al*., 2008). This theory is based

on the identification of two nuclei foci within the hypothalamus, one responsible for heat production and the other for heat loss (Boulant, 1981). When activation of either occurs, inhibition of the other is apparent (Boulant, 1981). For example, firing of warm thermoreceptors above a certain threshold will initiate sweating, while activation of cold fibres will suppress this thermoeffector response (Boulant, 1981). These are complex interactions, and their integrated influences form another focal area within this research; the interactions of vasomotion, sudomotion and thermogenesis during passive heating and cooling.

1.1.1.3 The control of human skin blood flow

Human skin blood flow is controlled via two separate sympathetic pathways. Within acral skin regions (nose, ears, lips, palms and soles), blood vessels are subjected to high levels of vasoconstrictor tone (when thermoneutral), modulated via adrenergic vasoconstrictor fibres, the tone of which is passively released with increases in core temperature (Blair *et al*., 1960; Fox *et al*., 1963). However, in non-acral regions skin blood flow is controlled by two separate sympathetic pathways. In these hairy regions, minimal adrenergic vasoconstriction tone is present in a thermoneutral environment, therefore further increases in skin blood flow, as occurs with heating, is explained by activation of vasodilatory nerves (Roddie *et al*., 1957). Although there are a number of theories presented in the literature (Charkoudian, 2003; Johnson *et al*., 2014), the neurotransmitter involved in this second active vasodilatation remains unknown (Roddie, 2003; Kellogg, 2006).

Under thermoneutral conditions, active vasoconstriction is mediated through the innervation of the sympathetic nervous system to most of the cutaneous circulation, including the arteriovenous anastomoses (Roddie, 1983). The central control mechanisms having the greatest influence upon skin blood flow are activated through a noradrenergic pathway. Noradrenaline, being the active neurotransmitter in this pathway, binds to vascular *alpha* adrenergic receptors eliciting contraction of the vascular smooth muscle of the arterioles and venules, leading respectively to vasoconstriction and venoconstriction (Rowell, 1983; Johnson and Proppe, 1996). Further noradrenergic constriction is induced during cooling where, in some regions,

skin blood flow is very low. Active vasodilatory mechanisms are also involved in the control of skin blood flow during hyperthermia. In this state, the arterioles found in non-acral skin are actively dilated, enabling greater blood flow than would be possible through passively removing vasoconstrictor tone in this region.

Although skin blood flow can be controlled via these two mechanisms, acral (non-hairy) regions are unique, as they are only controlled via an active adrenergic mechanism. Thus, vasoconstriction is active and vasodilatation is passive. By simultaneously investigating forearm, calf (non-acral), hand and foot blood (acral) flows, we are, to some extent, able to tease out these separate influences on skin blood flow. This can be accomplished across a range of thermal steady states. In addition, local tissue temperatures also modulate skin blood flow. For example, local vascular smooth muscle activity continuously responds to changes in vascular pressure resulting in altered vessel diameters (Rowell, 1983; Johnson and Proppe, 1996; Durand *et al*., 2004). Also, local tissue temperature changes affect skin blood flow via mechanisms independently of central sympathetic activation (Spealman, 1945; Pérgola, 1993). These are well known interactions, but these have not been explored in detail, and certainly not for both acral and non-acral skin regions. Therefore, a secondary focus of these experiments was to investigate the interaction of the central (core) and peripheral (cutaneous) feedback on the control of skin blood flow. One outcome of this research will be the production of a thermal map for human skin blood flow for acral and non-acral skin regions, and these blood flow maps will form an essential building block upon which subsequent experiments will be overlayed.

1.1.1.4 Activation of sudomotor function

When rises in body temperature can no longer be maintained through changes in vasomotor tone, sweating is activated. There are two types of sweat glands, apocrine and eccrine, with evidence of mixed apoeccrine existing (Sato *et al.,* 1987). Although there are some similarities between the two glands, apocrine and eccrine sweat glands differ in the size, location and neurotransmission. Eccrine sweat glands are smaller in size, located more superficially in the dermis, and are neurally controlled by acetylcholine as the neurotransmitter (Groscurth, 2002). In addition,

these glands are primarily responsible for thermal sweat secretions in humans. Sweat produced from eccrine glands is composed mostly of water, but also containing some electrolytes (Sato *et al.*, 1989). This is important in enabling extremely effective means for cooling the skin through the evaporation of sweat.

It is well known that the onset of thermally driven sweating is a result of the activation of the eccrine sweat glands that is usually apparent at 0.2-0.5^{o}C above the thermoneutral state (Mekjavic *et al.*, 1991). This activation is controlled by the hypothalamus, which is innervated by pre-ganglionic myelinated fibres that pass through the medulla and into the lateral horn of the spinal cord (Sato *et al.*, 1989; Groscurth, 2002; Boulant, 1996). Sweat gland activation is achieved by the cholinergic (acetylcholine) pathway of the sympathetic nervous system (Dale and Feldberg, 1934; Chalmers and Keele, 1952; Landis, 1990). While some researchers believed activation of eccrine sweat glands was also achieved by other neural pathways (adrenergic; Robertshaw, 1977; Sato and Sato, 1981) recent experiments have shown no evidence for significant participation of neurotransmitters other than acetylcholine in both glabrous and non-glabrous skin regions (Machado-Moreira and Taylor, 2012a; Machado-Moreira and Taylor, 2012b; Machado-Moreira *et al*., 2012).

In addition to these control mechanisms for sudomotor function, it was previously shown that cutaneous active vasodilatation and the onset of sweating began at about the same time in a resting person during heat stress (Grant and Holling, 1938). This led to the hypothesis that a common neural pathway between the onset of sweating and active vasodilatation existed (Brengelmann *et al*., 1981; Love and Shanks, 1962; Fox and Edholm, 1963). This causal theory was based upon the release of kallerkrein from activated eccrine sweat glands used in the formation of bradykinin believed to cause active vasodilatation (Fox and Edholm, 1963; Fox and Hilton, 1958). However, more recent research has revealed no link between the onset of active vasodilatation and sweating during heating (Kellogg *et al*., 2002) and although it has been shown that the threshold for discharged sweat occurs at a mean body temperature of approximately 0.5^{o}C above the threshold for precursor sweat threshold (Machado-Moreira *et al*., 2014), the precise relationship between these two

thermoeffectors remains poorly define (Johnson *et al.*, 2014). In addition,. The interactions between the onset of active vasodilatation and sweating (thermoeffector thresholds) will also be explored in the current series of research.

1.1.1.5 Activation of shivering thermogenesis

At the opposite end of the thermoregulatory spectrum, and during cold exposure, shivering thermogenesis is activated when body temperature drops to a point where vasoconstriction can no longer defend core temperature. This is achieved from involuntary and synchronous activation of both extensor and flexor muscles, enabling muscle contraction to increase heat production (Mahmood and Zweifler, 2007). The onset of shivering occurs when afferent input is generated by peripheral and visceral thermoreceptors and received by the pre-optic hypothalamus to initiate shivering. This causes an increase in muscle tone, particularly that of the torso and upper limbs, in response to reduced mean body temperature (Meigal *et al*., 1998; Pozos *et al.,* 1987). Thermogenesis is controlled in each of these tissues by parallel networks in the central nervous system, which respond to feedback (afferent) signals from cutaneous and deep body thermoreceptors. This feedback activates the appropriate sympathetic and some somatic efferents (Morrison *et al.,* 2008), thereby initiating shivering. It is believed that some organisation of the efferent pathways involved in cold shivering occur in the pre-optic anterior hypothalamus and septal regions that pass caudally to the ventral posterior hypothalamus (Boulant, 1996). In particular, several studies have shown that both central (Hammel *et al.,* 1968) and peripheral (Lim, 1960) control mechanisms exist in the activation of shivering and in this series of experiments, we will simultaneously evaluate these feedback pathways.

It is evident that integration of central and peripheral thermoafferent signals is complex. For some time, it was believed that the central integration and the thermoeffector responses (vasomotion, sudomotion and thermogenesis) centred around a predetermined core temperature value or set-point (Cabanac and Massonnet, 1977). However, this concept was disproved by Mekjavic *et al*. (1991) who showed the existence of a core thermoregulatory interthreshold, thermoneutral, vasomotor or null zone of 0.57-0.59°C, where neither sweating nor shivering were activated. This

concept forms another emphasis for this research, and as we aim to increase our understanding of the control of the thermoeffector responses.

1.2 FOCUS OF THE CURRENT RESEARCH

The principal focus of the current series of research investigations centred upon exploring the thermal interactions in the control of vasomotion, sudomotion and thermogenesis over a diverse range of thermal states and environmental conditions. Accordingly, three research phases were conducted and included six experiments.

The *foci* of this investigative series was centred upon furthering our understanding the thermoeffector mechanisms in response to various environmental conditions. To understand their interaction, each of the thermoeffector responses make up various thermoregulatory zones (Figure 1.1). Fine regulation of heat loss is provided by vasomotor activity, which, in neutral thermal environments, can maintain a constant body temperature within a narrow temperature range (zone 1: the interthreshold zone), bound by the lower and upper critical temperatures (lines C and B, respectively). These are essentially the threshold temperatures at which the effectors of endogenous heat production (thermogenesis), non-evaporative (vasomotion) and evaporative heat loss (sudomotion) are activated. As mean body temperature increases, vasodilatation and sweating are activated, though not necessarily simultaneously. Conversely, reductions in temperature first elicit vasoconstriction and then thermogenesis.

Firstly, in these series of research, we investigated the vasomotor responses when the internal and environmental temperature is driven above the interthreshold zone (above line B) and was then rapidly reduced to well below the interthreshold zone (below line C; Chapter 2 and 3). This research was designed to provide an understanding of the interactions between the passive (thermal gradient) and active (vasomotion) systems involved in the regulation of body temperature. Secondly, although it is often convenient to think about these critical and threshold temperatures in terms of ambient temperature, mammalian species regulate mean body temperature, which is a weighted composite of deep body (core) and superficial (skin) temperatures

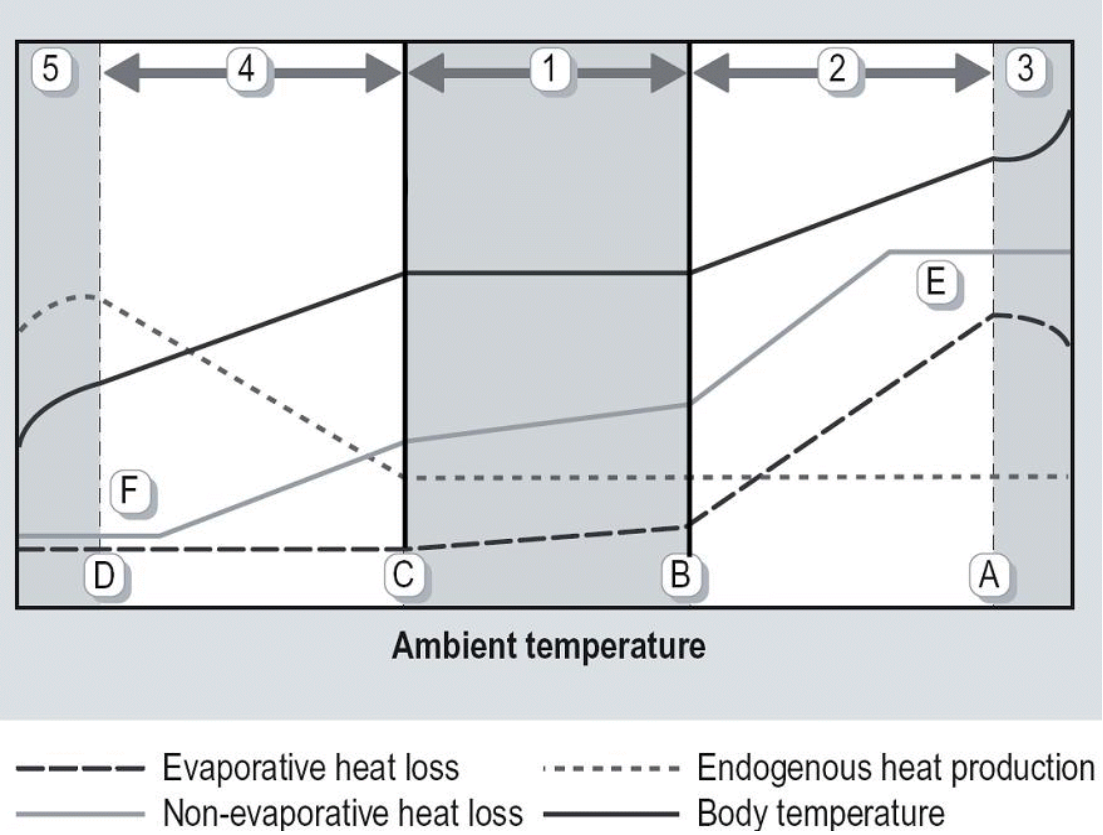

Figure 1.1: Thermoeffector responses (vasomotion, sudomotion and thermogenesis) across a wide range of air temperatures as activated in response to changes in body temperature. This is defined by five thermoregulatory zones (1-5): The interthreshold zone (1) also defined as vasomotor, comfort, thermoneutral, indifference or null zone; the sudomotor regulatory zone (2); the upper thermoregulatory failure zone (3); metabolic regulatory zone (4) and the lower thermoregulatory failure zone (5). Each zone is bound by peak sweat secretion (**A**) and maximal shivering thermogenesis (**D**) and the thermoeffector thresholds are illustrated by **B** (upper critical temperature) and **C** (lower critical temperature). Maximal vasodilatation (E) and vasoconstriction are also presented. From Werner *et al*. (2008).

(Werner *et al.*, 2008). Therefore, in Chapter 4 and 5, we explored vasomotor function across the full range of thermoregulatory zones including variations in both central and peripheral temperature and in both acral and non-acral skin regions. Finally, the focus of Chapter 6 shifted to the thermoeffector thresholds that bound either side of the interthreshold zone. In this chapter, our focus was centred upon what determines the mean body temperature at which these thermoeffectors (sweating and shivering) are activated and their relationship to vasomotor function.

1.2.1 Phase 1: Vasomotor function of hyperthermic individuals during temperate- and cold-water immersion:

Exercise-induced hyperthermia is commonly experienced by individuals required to perform work in extreme environmental conditions. Rapid patient cooling and effective treatment can impact upon the prognosis of heat illness. Currently, there are a number of methods shown to be effective treatments for hyperthermia (Barwood *et al*., 2009; Proulx *et al*., 2003, 2006; Armstrong *et al*., 1996), however, cold-water immersion is the most rapid treatment due to its thermal conductivity and specific heat capacity. While ice-cold water immersion will rapidly extract heat, some have concerns regarding the sudden cold-water immersion of hyperthermic individuals (Makranz, *et al*., 2011), while others believe that cutaneous vasoconstriction may reduce convective heat transfer from the core (Taylor *et al*., 2008). Since the rate of heat removal from the body is not only determined by the medium in which an individual is surrounded, but also by the rate that heat is delivered from the core to the periphery, through alterations in skin blood flow, it was essential to evaluate the impact of water temperature on the rate of reducing body core temperature and its relationship with vasoconstriction.

Accordingly, the first experiment was designed to provide an understanding of the physiological mechanisms associated with cooling hyperthermic individuals using water immersion. By knowing how much hot blood is flowing to the skin, we can determine how much heat is extracted in the different water temperatures. The rationale is that cold-water immersion will rapidly activate noxious cold receptors, thereby initiating a more powerful thermoefferent response and hence a greater

reduction in cutaneous blood flow. This study is designed to help build our understanding of the control of skin blood flow from both central and peripheral sites.

In the first experiment we explored three different cooling methods to reduce body core temperature of hyperthermic individuals: air (20-22ºC); cold-water immersion (14ºC); temperate-water immersion (26ºC). However, in the second experiment we investigated whether a more powerful vasoconstriction was present during cold-water immersion. In this experiment, forearm skin blood flow was measured using venous-occlusion plethysmography with a mercury-in-silastic strain gauge.

The first phase of this research was comprised of two experiments to increase our understanding of the physiological mechanisms associated with cooling hyperthermic individuals with the use of whole-body water-immersion (Chapters 2 and 3). Therefore, the aim was to evaluate the effectiveness of two water-immersion temperatures (14º and 26ºC) on the rate of body core cooling and its relationship with cutaneous vasoconstriction. For these experiments the following hypotheses were tested:

Hypothesis one: Rapid and effective deep-body heat loss, as reflected within oesophageal temperature, could be achieved in hyperthermic individuals during a temperate-water immersion (26ºC).
Hypothesis two: The core temperature (oesophageal) cooling rate during water immersion following profound hyperthermia will not differ significantly between cold (14ºC) and temperate water (26ºC).
Hypothesis three: A more powerful vasoconstrictor response will occur during cold-water immersion compared to temperate-water immersion, and this will occur in both normothermic and hyperthermic states.

To test these hypotheses, oesophageal temperature was monitored during water immersion in two different temperatures (14º and 26ºC) following either a normothermic state or exercise-induced hyperthermia. In one study (Chapter 2), the

rate of oesophageal temperature reduction, in both water-immersion temperatures (14^{o} and 26^{o}C) was compared to cooling in an air conditioned laboratory (control: 22^{o}C). This condition was not evaluated in the second study within this phase, however forearm blood flow was measured during water immersion to understand cutaneous vascular responses to cold- (14^{o}C) and temperate-water immersion (26^{o}C) following normothermic and hyperthermic states.

1.2.2 Phase 2: Part A: A method for measuring cutaneous vasomotor function

Although the strain gauge was an appropriate method for measuring skin blood flow in phase one, it is only one of many methods currently used to measure cutaneous blood flow and does not go without limitations. One particular limitation is the difficult in measuring the influence of local skin temperature changes on cutaneous blood flow. Due to this limitation, water-filled plethysmography was used for the next series of experiments in order to further explore the influence of peripheral temperature on the control of skin blood flow. Measuring cutaneous blood flow forms an essential building block for understanding thermoeffector responses to changes in central and peripheral temperatures. While there are a number of invasive and noninvasive methods available to measure cutaneous blood flow in humans, the most common method, which has been used for over a century (Schäfer, and Moore, 1896), is venous occlusion plethysmography (Joyner *et al*., 2001). This simple, noninvasive technique measures changes in organ volume over time, during venous occlusion, but without disruption to arterial flow. Whitney (1953) developed the mercury-in-silastic strain-gauge plethysmograph which indirectly measures limb volume changes from the corresponding alterations in limb circumference. Although this method continues to be used widely in the study of peripheral blood flow (Hokanson *et al*., 1975), it does have limitations when examining the influence of local temperature changes on blood flow. However, water-filled plethysmographs are advantageous (Raine and Sneddon, 2002), since one can easily alter the temperature surrounding the limb segment in which blood flow is measured.

Due to the design of the water-filled plethysmograph, in which a water compartment surrounds the measurement site, it is possible that local tissue

temperature could be altered without influencing core or mean skin temperature. While thermoreception is known to influence efferent thermoregulatory responses, employing the use of a water-perfusion garment to clamp mean skin temperature has been shown to remove the autonomic influence that may normally occur in untreated tissue sites. This is achieved by opening up feedback loops, thereby minimising afferent feedback from sites remote from the assessed sites (Cotter and Taylor, 2005). Thus, across a wide range of core temperatures, the effect of local tissue temperature on cutaneous blood flow can be explored independently of the central sympathetic nervous system. Indeed, one can explore the interactions of central (core) and peripheral (cutaneous) feedback on the control of skin blood flow, and a water-filled plethysmograph will allow for both acral (hands and feet) and non-acral (forearm and calf) skin regions to be investigated. Therefore, to facilitate these measurements of blood flow, four water-filled displacement plethysmographs (forearm, hand, calf and foot) were developed and built (Chapter 4), and these also enabled the evaluation of local skin temperature influences (as determined by changes in water temperature) on skin blood flow. For the first study, blood flow in the forearm was measured using both the newly developed water-filled and the strain-gauge plethysmographs in neutral and locally heated conditions in order to validate the water-filled plethysmographs. This was essential to ensure the newly developed displacement plethysmographs measured accurate skin blood flow responses. However, the second study was designed to collect hand and foot blood flows using two purpose-built (displacement) plethysmographs under clamped thermoneutral conditions, and during three local (hand and foot) skin temperature treatments. This approach would enable an evaluation of the capacity of these plethysmographs to be used in a larger project during which the role of local skin temperature would be investigated under three whole-body thermal states. While we know that there are both central and local influences upon skin blood flow, the extent of our knowledge is limited. Therefore, the following experiment (part B) was designed to increase our knowledge in this area using these four water-filled plethysmographs.

1.2.3 Phase 2: Part B: The interaction of core and local skin temperature on cutaneous vasomotor function

The second phase of this research involved further investigation into the relationship between central and peripheral temperatures upon cutaneous blood flow. This included mapping of cutaneous blood flow across a range of thermal states and regions, comprising of two parts. The first part consisted of the design, construction and validation of four water-displacement plethysmographs for the forearm, hand and foot that could clamp segmental skin temperature whilst simultaneously measuring cutaneous blood flow. The second part of this phase consisted of an investigation into the interactions of the central (core) drive and peripheral (cutaneous) feedback on the control of skin blood flow in acral (hand and foot) and non-acral (forearm and calf) skin regions.

Measuring cutaneous blood flow is important for understanding thermoeffector responses to changes in central and peripheral temperatures. This is important because cutaneous blood flow, under the control of sympathetic drive, has the capacity to enhance or reduce heat loss through changes in blood vessel diameter. Although changes in deep tissue temperature generally have the greatest influence upon skin blood flow for both acral and non-acral skin regions, local tissue temperatures also modulate skin blood flow via mechanisms independent of central sympathetic activation (Spealman, 1945; Pérgola, 1993). For example, local vascular smooth muscle activity continuously responds to changes in vascular pressure resulting in altered vessel diameters (Rowell, 1983; Johnson and Proppe, 1996; Durand *et al.*, 2004). These separate and combined influences upon skin blood flow are well known, but have not been explored in detail, and certainly not simultaneously for the acral and non-acral skin regions. By concurrently investigating forearm, calf, hand and foot blood flows, across a range of core and skin temperatures, we are, to some extent, able to tease out these separate influences on skin blood flow. Therefore, one outcome of this research was to produce of a three-dimensional thermal map for human skin blood flow across a broad range of thermal states and local skin temperatures for acral (hand and foot) and non-acral (forearm and calf) skin regions. For the three studies in phase 2, it was hypothesised that:

Hypothesis one: Forearm vascular conductance, measured with either a mercury-in-silastic strain-gauge plethysmograph or a water-filled (displacement) plethysmograph, would not differ across these measurement techniques.
Hypothesis two: Local heating alone cannot evoke maximal cutaneous vasodilatation.
Hypothesis three: Core temperature will exert the greatest neural influence on skin blood flow within all regions (forearm, hand, calf and foot), but for a given core temperature, skin blood flow will change in proportion to changes in local skin temperature for each site.
Hypothesis four: Of the four regions (forearm, hand, calf and foot), skin blood flow will be the highest in the hand during hyperthermia, when local skin temperature is 40^{o}C.
Hypothesis five: Skin blood flow will be greater in the hand than in the foot across all combinations of core and local skin temperatures.

To evaluate hypotheses one and two, two experiments were performed for the validation of the water-filled plethysmographs. In study one, the forearm plethysmograph was validated against a mercury-in-silastic plethysmograph under thermoneutral conditions, with and without forearm heating. In study two, hand and foot blood flows were measured under clamped thermoneutral conditions, but with three local skin temperature treatments (5^{o}, 25^{o}, 40^{o}C). However, hypotheses three, four and five were tested by simultaneously measuring skin blood flow of the forearm, calf, hand and foot, using venous-occlusion plethysmography, across a range of steady-state core temperatures from mild hypothermia to mild hyperthermia, and during controlled changes in local skin temperature at each steady state. Thermal strain was induced using passive heating and cooling.

Although this study provides missing quantitative data across a range of thermal states, it does not account for broadening our understanding of the interactions of vasomotor function with sudomotion and thermogenesis. Therefore, to more completely understand these complex interactions, the final phase of this research series was to investigate how altering the pre-exposure core temperature

affects the threshold for which skin blood flow occurs and its relationship to the sweating and shivering thresholds.

1.2.4 Phase 3: The effect of pre-exposure core temperature on thermoeffector thresholds

Whilst much is known about the mechanisms that determine the thermoregulatory thresholds (Figure 1.1), there are still areas for which our understanding is only partial. Recent experiments from the current laboratory have shown that the sweating threshold during heat exposure moves to a lower body temperature following both heat adaptation (Patterson, 1999), and pre-exposure cooling (Booth, 2001). Indeed, these displacements were equal in size to the reduction in the pre-exposure body temperature. Thus, it appeared that the magnitude of the change in body temperature provided more important feedback than did the absolute body temperature. Furthermore, in other pre-cooling experiments, the threshold for cutaneous vasodilatation was simultaneously displaced upwards (MacDonald *et al.*, 2000). Thus, the effector thresholds had moved in opposite directions. These are novel and exciting observations. While we are currently unable to explain some of those observations, they provided the stimulus for another set of experiments; the last research phase of this research.

Therefore, this project (Chapter 6) was designed to evaluate the possibility that the magnitude of the change in body temperature might provide important feedback and is perhaps of greater importance than absolute body temperature. This was achieved by inducing deliberate, yet precisely controlled displacements of body temperature prior to separate heating and cooling stimuli. Accordingly, it was hypothesised that:

Hypothesis one: The pre-cooling and pre-heating of subjects would shift the mean body temperature thermoeffector thresholds for sweating and shivering by a magnitude equal to that of the pre-exposure displacement of mean body temperature.
Hypothesis two: The mean body temperature for vasodilatation and sweating thresholds would occur simultaneously during heating, while the vasoconstrictor

threshold would always precede the shivering thresholds during cooling.

These hypotheses were tested by altering mean body temperature through either whole-body heating or cooling, and then driving body temperature in the opposite direction to determine the thermoeffector thresholds. Sweating, skin blood flow and oxygen consumption were measured in order to determine the mean body temperature that corresponded to the onset of each of these thermoeffectors.

1.3 SUMMARY OF EXPERIMENTS:

As a consequence of these experimental design considerations, six experiments were developed and are presented in five chapters (2-6). In Chapter 2, we investigated the rate of cooling oesophageal temperature in cold- and temperate-water where in Chapter 3 we investigated the vasomotor responses of subjects during water immersion following exercise-induced hyperthermia. In Chapter 4 and 5, we explored vasomotor function in response to central and peripheral temperature and in both acral and non-acral skin regions using four purpose built water-filled (displacement) plethysmographs (forearm, hand, calf and foot). Finally, in Chapter 6 we investigated the dependent and inter-dependent influences of the thermoeffector thresholds. In this chapter, our focus was centred upon what determines the mean body temperature at which these thermoeffectors (sweating and shivering) are activated and their relationship to vasomotor function.

1.4 REFERENCES

Armstrong, L.E., Crago, A.E., Adams, R., Roberst, W.O., and Maresh, C.M. (1996). Whole-body cooling of hyperthermic runners: comparison of two field therapies. *American Journal of Emergency Medicine. 14:355-358.*

Barwood, M.J., Davey, S., House, J.R., and Tipton, M.J. (2009). Post-exercise cooling techniques in hot, humid conditions. *European Journal of Applied Physiology.* 107:385-396.

Bazett, H.C., McGlone, B., and Brocklehurst, R.J. (1930). The temperature in the tissues which accompany temperature sensations. *Journal of Physiology.* 69: 88-112.

Bernard, C. (1876). *Leçons sur la chaleur animale, sur les effets de la chaleur et sur la 32 fièvre.* J.-B. Baillière et Fils, Paris.

Blair, D.A., Glover, W.E., and Roddie, I.C. (1960). Vasomotor fibres to skin in the upper arm, calf and thigh. *Journal of Physiology.* 153:232-238.

Bligh, J. (1973). Temperature regulation in mammals and other vertebrates. *North-Holland Publishing Company.* Holland.

Booth, J.D. (2001). Metabolic and thermal consequences of heat exposure following pre-exposure whole-body cooling. *Doctor of Philosophy.* University of Wollongong.

Boulant, J.A., (1981). Hypothalamic mechanisms in thermoregulation. *Federation Proceedings.* 306:553-565.

Boulant, J.A. (1996). Hypothalamic neurons regulating body temperature. *Comprehensive Physiology 2011, Supplement 14: Handbook of Physiology, Environmental Physiology*: 105-126. First published in print 1996.

Brengelmann, G.L., Johnson, J.M., Hermansen, L., and Rowell, L.B. (1977). Altered control of skin blood flow during high internal temperatures. *Journal of Applied Physiology: Respiration, Environmental and Exercise Physiology.* 43:790-794.

Brengelmann, G.L., Freund, P.R., Rowell, L.B., Olerud, J.E., and Kraning, K.K. (1981). Absence of active vasodilatation associated with congenital absence of sweat glands in humans. *American Journal of Physiology.* 240:H571-H575.

Cabanac, M., and Massonnet, B. (1977). Thermoregulatory responses as a function of

core temperature in humans. *Journal of Physiology*. 265: 587-596.

Cain, D.M., Khasabov, S.G., and Simone, D.A. (2001). Response properties of mechanoreceptors and nociceptors in mouse glabrous skin: An in vivo study. *Journal of Neurophysiology.* 85:1561-1574.

Cannon, W.B. (1929). Organisation for physiological homeostasis. *Physiological Reviews*. 9: 399-431.

Casa, D.J., Armstrong, L.E., Kenny, G.P., O'Connor, F.G., and Huggins, R.A. (2012). Exertional heat stroke: New concepts regarding cause and care. *Current Sports Medicine Reports.* 11(3):115-123.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., and Julius, D. (1997). The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*. 389: 816-824.

Caterina, M.J., Rosen, T.A., Tominaga, M., Brake, A.J., and Julius, D. (1999). A capsaicin-receptor homology with a high threshold for noxious heat. *Nature.* 398: 436-441.

Chalmers, T.M., and Keele, C.A. (1952). The nervous and chemical control of sweating. British Journal of Dermatology. 64:43-54.

Charkoudian, N. (2003). Skin blood flow in adult human thermoregulation: How it works, when it does not, and why. *Mayo Clinic Proceedings.* 78:603-612.

Cotter, J.D., and Taylor, N.A.S. (2005). The distribution of cutaneous sudomotor and alliesthesail thermosensitivity in mildly heat stressed humans: an open-loops approach. *Journal of Physiology.* 565:335-345.

Dale, H.H., and Feldberg, W. (1934). The chemical transmission of secretory impulses to the sweat glands of the cat. *Journal of Physiology.* 82:121-128.

De Whitte, J., and Sessler, D.I. (2002). Perioperative shivering: physiology and pharmacology. *Anesthesiology.* 96:467-484.

Durand, S., Zhang, R., Cui, J., Wilson, T.E., and Crandall, C.G. (2004). Evidence of myogenic response in vasomotor control of forearm and palm cutaneous microcirculations. *Journal of Applied Physiology.* 97:535-539.

Fox, R.H., and Edholm, O.G. (1963). Nervous control of the cutaneous circulation. *British Medical Bulletin.* 19:110-114.

Fox, R.H., Goldsmith, D.J., Kidd, D.J., and Lewis, H.E. (1963). Blood flow and other

thermoregulatory changes with acclimatization to heat. *Journal of Physiology.* 166:548-562.

Fox, R.H., and Hilton, S.M. (1958). Bradykinin formation in human skin as a factor in heat vasodilatation. *Journal of Physiology.* 142:219-232.

Grant, R.T., and Holling, H.E. (1938). Further observations on the vascular responses of the human limb to body warming: evidence for sympathetic vasodilator nerves in the normal subject. *Clinical Science.* 3:273-285.

Groscurth, P. (2002). Anatomy of sweat glands. *Current Problems in Dermatology.* 30: 1-9.

Guler, A.D., Lee, H., Iida, T., Shimizu, I., Tominaga, M., and Caterina, M. (2002). Heat-evoked activation of the ion channel, TRPV4. *Journal of Neuroscience.* 22: 6408-6814.

Hammel, H.T. (1968). Regulation of internal body temperature. *Annual Review of Physiology.* 30:641-710.

Havenith, G., Coenen, J.M., Kistemaker, L., and Kenney, W.L. (1998). Relevance of individual characteristics for human heat stress response is dependent on exercise intensity and climate type. *European Journal of Applied Physiology and Occupational Phsysiology.* 77:231-241.

Hellon, R. (2011). Thermoreceptors. *Comprehensive Physiology*. 659-673. First published in print 1996.

Hensel, H., and Zotterman, Y. (1951). The effect of menthol on the thermoreceptors. *Acta Physiologica Scandinavica.* 24: 27-34.

Hensel, H., and Iggo, A. (1971). Analysis of cutaneous warm and cold fibres in primates. *Pflugers Archive*. 329: 1-8.

Hensel, H., and Schäfer, K. (1974). Effects of calcium on warm and cold receptors. *Pflügers Archive.* 352:87-90.

Hensel, H. (1981). Thermoreception and temperature regulation. *Monographs of the Physiological Society.* 38: 1-321.

Hokanson, D.E., Sumner, D.S., and Strandness, D.R. Jr. (1975). An electrically calibrated plethysmograph for direct measurement of limb blood flow. *IEEE transactions on biomedical engineering.* 22:25-29.

Johnson, J.M., and Park, M.K. (1979). Reflec control of skin blood flow by skin

temperature: role of core temperature. *Journal of Applied Physiology.* 47:1188-1193.

Johnson, J.M., and Proppe, D.W. (1996). Cardiovascular adjustments to heat stress. In: *Handbook of Physiology*. Fregely, M.J., Blatteis, C.M, eds. Section 4: Environmental Physiology. Vol 1. New York, NY: Oxford University Press; 215-243.

Johnson, J.M., Minson, C.T., and Kellogg, D. L. (2014). Cutaneous vasodilator and vasoconstrictor mechanisms in temperature regulation. *Comprehensive Physiology.* 4:33-89.

Joyner, M.J., Dietz, N.M., and Shepherd, J.T. (2001). From Belfast to Mayo and beyond: the use and future of plethysmography to study blood flow in human limbs. *Journal of Applied Physiology*. 91:2431-2441.

Kellogg, D.L., Liu, Y., McAllister, K., Friel, C., and Pérgola, P.E. (2002). Bradykinin does not mediate cutaneous active vasodilatation during heat stress in humans. *Journal of Applied Physiology.* 93:1215-1221.

Kellogg, D.L. (2006). In vivo mechanisms of cutaneous vasodilation and vasoconstriction in humans during thermoregulatory challenges. *Journal of Applied Physiology.* 100:1709-1718.

Landis, S.C. (1990). Target regulation of neurotransmitter phenotype. *Trends in Neuroscience.* 13:344-350.

Lim, T.P. (1960). Central and peripheral control mechanisms of shivering and its effects on respiration. *Journal of Applied Physiology.* 15:567-574.

Love, A.H. G., and Shanks, R.G. (1962. The relationship between the onset of sweating and vasodilatation in the forearm during body heating. *Journal of Physiology.* 162: 121-128.

MacDonald, A.D., Groeller, H., Fogarty, A.L., Armstrong, K.A., Booth, J.D., Hahn, A., & Taylor, N.A.S. (2000). Exercise in the heat: cardiovascular consequences of whole-body and head-torso pre-cooling. *Abstracts of the Ninth International Conference on Environmental Ergonomics*. July 30th-August 4 th, 2000. Dortmund, Germany. P. 3.

Machado-Moreira, C.A., and Taylor, N.A.S. (2012*a*). Sudomotor responses from glaborous and non-glaborous skin during cognitive and painful stimulations

following passive heating. *Acta Physiologica*. 204:571-581.

Machado-Moreira, C.A., and Taylor, N.A.S. (2012*b*). Psychological sweating from glaborous and non-glaborous skin surfaces under thermoneutral conditions. *Psychophysiology.* 49:369-374.

Machado-Moreira, C.A., McLennan, P.L., Lillioja, S., van Dijk, W., Caldwell, J.N., and Taylor, N.A.S. (2012). The cholinergic blockade of both thermally and non-thermally incduced human eccrine sweating. *Experimental Physiology.* 97:930-942.

Machado-Moreira, C.A., Barry, R.J., Vosselman, M.J., Ruest, R.M., and Taylor, N.A.S. (2014). Temporal and thermal variations in site-specific thermoregulatory sudomotor thresholds: Precursor versus discharged sweat production. *Psychophysiology.* In print.

McKemy, D.D., Neuhausser, W.M., and Julius, D. (2002). Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature.* 416: 52-58.

Mahmood, M.A., and Zweifler, R.M. (2007). Progress in shivering control. *Journal of Neurological Science.* 261:47-54.

Makranz, C., Heled, Y., and Moran, D.S. (2011). Hypothermia following exertional heat stroke treatment. *European Journal of Applied Physiology.* 111:2359-2362.

Meigal, A.Y., Oksa, J., Hohtola, E., Lupandin, Y.V., and Rintamaki, H. (1998). Influence of cold shivering on fine motor control in the upper limb. *Acta Physiologica Scandinavica.* 163:41-47.

Mekjavic, I.B., Sundberg, C.J., and Linnarsson, D. (1991). Core temperature “null zone”. *Journal of Applied Physiology.* 71: 1289-1295.

Mekjavic, I.B., and Eiken, O. (2006). Contribution of thermal and nonthermal factors to the regulation of body temperature in humans. *Journal of Applied Physiology.* 100: 2065-2072.

Morrison, S.F., Nakamura, K., and Madden, C.J. (2008). Central control of thermogenesis in mammals. *Experimental Physiology.* 93:773-797.

Patapoutian, A., Peier, A.M., Story, G.M., Viswanath, V. (2003). ThermoTRP channels and beyond: mechanisms of temperature sensation. *Nature Reviews*

Neuroscience. 4: 529-539.

Patterson, M.J. (1999). The regulation of human body fluids during heat adaptation. *Doctor of Philosophy.* University of Wollongong.

Pérgola, P.E., Kellogg, D.L., Johnson, J.M., Kosiba, W.A., and Soloman, D.E. (1993). Role of sympathetic nerves in the vascular effects of local temperature in human forearm skin. *American Journal of Physiology.* 265:H785-H792.

Pozos, R.S., Israel, D., McCutcheon, R., Wittmers Jr., L.E., and Sessler, D. (1987). Human studies concerning thermal-induced shivering, postoperative "Shivering", and cold-induced vasodilatation. *Annals of Emergency.* 16:1037-1041.

Proulx, C.I., Ducharme, M.B., and Kenny, G.P. (2003). Effect of water temperature on cooling efficiency during hyperthermia in humans. *Journal of Applied Physiology.* 94:1317-1323.

Proulx, C.I., Ducharme, M.B., and Kenny, G.P. (2006). Safe cooling limits from exercise-induced hyperthermia. *European Journal of Applied Physiology.* 96:434-445.

Raine, N.M., and Sneddon, J.C. (2002). A simple water-filled plethysmograph for measurement of limb blood flow in humans. *Advances in Physiological Education.* 26:120-128.

Robertshaw, D. (1977). Neuroendocrine control of sweat glands. *Journal of Investigative Dermatology.* 69:121-129.

Roddie, I.C., Shepherd, J.T., and Whelan, R.F. (1957). The contribution of constrictor and dilator nerves to the skin vasodilatation during body heating. *Journal of Physiology.* 136:489-497.

Roddie, I.C. (1983). Circulation to skin and adipose tissue. In: Shepherd, J.T., Abboud, F.M. (eds). The cardiovascular system. Volume III. Peripheral circulation and organ blood flow Part 1. *Handbook of Physiology*. American Physiological Society. Bethesda, MD, 285-317.

Roddie, I.C. (2003). Sympathetic vasodilatation in human skin. *Journal of Physiology (London).* 548:336-337.

Rowell, L.B., Brengelmann, G.L., Blackmon, J.R., and Murray, J.A. (1970). Redistribution of blood flow during sustained high skin temperature in resting

man. *Journal of Applied Physiology*. 28:415-420.

Rowell, L.B. (1983). Cardiovascular adjustments to thermal stress. In: *Handbook of Physiology.* Shephard, J.T., Abboud, F.M., eds. Section 2: Cardiovascular System. Vol 3, pt 2, Behesda, Md: American Physiological Society. 967-1023.

Sato, K., and Sato, F. (1981). Pharmacologic responsiveness of isolated single eccrine sweat glands. *American Journal of Regulatory, Integrative and Comparative Physiology.* 240:R44-R51.

Sato, K., Leidal, R., and Sato, F. (1987). Morphology and development of an apoeccrine sweat gland in human axillae. *American Physiological Society.* 252: R166-T180.

Sato, K., Kanf. W.H., Saga, K., and Sato, K.T. (1989). Biology of sweat glands and their disorders. 1. Normal sweat gland function. *Journal of the American Academy of Dermatology.* 20: 537-563.

Schäfer, E.A., and Moore, B. (1896). On the contractility and innervation of the spleen. *Journal of Physiology.* 20:1-50.

Smiles, K.A., Elizondo, R.S., and Barney, C.C. (1976). Sweating responses during changes of hypothalamic temperature in the rhesus monkey. *Journal of Applied Physiology.* 40:653-657.

Smith, G.D., Gunthorpel, M. J., Kelsell, R. E., Hayes, P. D., Reilly, P., Facer, P., Wright, J. E., Jerman, J. C., Walhin, J.-P., Ooil, L., Egerton, J., Charles, K. J., Smart, D., Randall, A. D., Anand, P., and Davis, J. B. (2002). TRPV3 is a temperature-sensitive vanilloid receptor-like protein. *Nature.* 418: 186-190.

Spealman, C.R. (1945). Effect of ambient air temperature and of hand temperature on blood flow in hands. *American Journal of Physiology.* 145:218-222.

Story, G.M., Peier, A.M., Reeve, A.J., Eid, S.R., Mosbacher, J., Hricik, T.R., Earlet, T.J., Hergarden, A.C., Andersson, D.A., Hwang, S.W., McIntyre, P., Jelga, T., Bevan, S., and Patapoutian, A. (2003). ANKTM1, a TRP-like channel expressed in nocioceptive neurons is activated by cold temperatures. *Cell*. 112: 819-829.

Taylor, N.A.S., Caldwell, J.N., van den Heuvel, A.M.J., Patterson, M.J. (2008). To cool, but not too cool: that is the question: immersion cooling for hyperthermia. *Medicine and Science in Sport and Exercise.* 40(11):1962-1969.

Watanabe, H., Vriens, J., Suh, S.H., Benham, C.D., Droogmans, G., and Nilius, B. (2002). Heat-evoked activation of TRPV4 channels in a HEK293 cell expression system and in native mouse aorta endothelial cells. *Journal of Biological Chemistry.* 277: 47044-47051.

Werner, J., Mekjavic, I.B., and Taylor, N.A.S. (2008). Concepts in physiological regulation: a thermoregulatory perspective. In: Taylor, N.A.S., and Groeller, H. (Editors). *Physiological bases of human performance during work and exercise.* Churchill Livingstone Elsevier, Edinburgh. Pp. 325-340.

Werner, J. (2010). System properties, feedback control and effector coordination of human temperature regulation. *European Journal of Applied Physiology.* 109:13-25.

Whitney, R.J. (1953). The measurement of volume changes in human limbs. *Journal of Physiology.* 121:1-27.

Wilson, T.E., Cui, J., and Crandall, C.G. (2002). Effect of whole-body and local heating on cutaneous vasoconstrictor responses in humans. *Autonomic Neuroscience: Basic and Clinical.* 97:122-128.

Wyndham, C.H. (1965). Role of skin and core temperatures in man's temperature regulation. *Journal of Applied Physiology.* 20: 31-36.

Wyss, C.R., Brengelmann, G.L., Johnson, J.M., Rowell, L.B., and Niederberger, M. (1974). Control of skin blood flow, sweating, and heart rate: role of skin vs core temperature. *Journal of Applied Physiology.* 36:726-733.

CHAPTER 2: THE EFFECT OF WATER TEMPERATURE ON THE REDUCTION IN BODY CORE TEMPERATURE AFTER WORK IN A HOT ENVIRONMENT.

2.1 INTRODUCTION

Cold-water immersion is a very effective way in which to extract heat. However, some have urged caution concerning its implementation in hyperthermic people. Recently, Casa *et al*. (2007) presented a rebuttal to some arguments raised against this treatment for exertional heatstroke. One emphasis was to contest the possibility that cold-induced thermogenesis elevates heat production and storage, thereby delaying cooling. Certainly, shivering will occur, *albeit* with a reduced sensitivity in pre-heated people (Benzinger, 1970), so its contribution to heat production is not very powerful. Furthermore, such low-intensity shivering will accelerate heat loss due to its affect on the insulating boundary layer (Golden and Tipton, 2002; Stocks *et al*., 2004). If shivering is only moderate, as is the case when hyperthermic individuals are immersed in cold water, the impaired formation, or subsequent disturbance of an established boundary layer can increase heat loss beyond the heat production associated with shivering. The second major emphasis of Casa *et al*. (2007) was to challenge the possibility that an acute, cold-induced cutaneous vasoconstriction might delay heat loss, or even transiently elevate heat storage, as evident from the paradoxical, and often protracted elevation in core temperature frequently observed during the cold immersion of thermoneutral individuals. This study focussed more on the latter aspect of this argument. However, it is believed that the more significant physiological aspect may be the dramatic decrease in heat loss associated with the powerful suppression of peripheral blood flow, and the consequential reduction in convective heat delivery from the core to the periphery. Indeed, this is a focus of the current study, in which experimental evidence will be presented to show that the immersion of hyperthermic individuals (rectal temperature ~40^{o}C) in temperate water will elicit very rapid cooling.

From a thermodynamics perspective, the greater the temperature difference between an object and its surrounding environment, the faster its temperature will change. Thus, when a heated inanimate object is immersed in cold water, its rate of heat loss will be proportional to the thermal gradient established between the surface of the object and the layer of water adjacent to that surface. When exposed to different ambient media, heat loss

will also be a function of the physical properties of each medium. For instance, differences in the thermal conductivity (26.2 versus 630.5 $mW.m^{-1}.K^{-1}$), specific heat capacity (1.007 versus 4.1885 $J.g^{-1}.K^{-1}$) and density (0.0012 versus 0.9922 $g.cm^{-3}$; Lide, 1997) between air and water (respectively) dictate the rate at which heat is lost when exposed to each medium. The product of specific heat capacity and density yields a volume-specific heat capacity, which quantifies the thermal energy necessary to raise the temperature of a given volume of water by 1 K. Across the physiologically-relevant temperatures, the volume-specific heat capacity of water is >3,400 times that of air. Without question, water does not just have a greater capacity to accept thermal energy, but this energy transfer will proceed at a much greater rate. Not surprisingly, immersion in ice-cold water (2°C) has been shown to be a most effective means of rapidly reducing rectal temperature in hyperthermic individuals (Proulx *et al*., 2003, 2006).

It is not the intention of the current study to challenge first principles in biophysics, but to evaluate how changes in physiological function (cold-induced vasoconstriction) can modify thermal energy transfer during water immersion, since less powerful vasoconstriction during an immersion may actually enhance heat loss, as it can when auxiliary cooling is used to extract heat (Cheuvront *et al.*, 2002; Stephenson *et al*., 2007). Furthermore, sudden immersion in ice-cold (5°C) water, accompanied by an initial period of breath holding, precipitates potentially lethal, cold-shock responses (Tipton, 1989), including supraventricular ectopic arrhythmias (Tipton *et al*., 1994) and reduced cerebral blood flow (Mantoni *et al*., 2007). These responses are associated with the sudden, powerful and simultaneously activated discharge from the cold-sensitive cutaneous thermoreceptors, and the inhibition of warm-sensitive receptors. The intensity of this feedback is a function of the rate and magnitude of the change in skin temperature (Pierau, 1996), resulting in a dramatic elevation of sympathetic activity (Kregel *et al*., 1992), and the cold-shock responses (Tipton, 1989). Thus, while cold-water immersion is very effective for removing heat, in some circumstances its use may result in undesirable side effects, and one such condition may relate to the immersion of hyperthermic individuals.

2.1.1 Aims and hypotheses

Therefore, we tested the hypothesis that a rapid and effective heat loss, as reflected

within oesophageal temperature, could still be achieved in hyperthermic individuals during a temperate-water immersion (26ºC), whilst simultaneously avoiding the cold-shock responses. This more conservative approach would also satisfy pragmatic issues related to the availability of cold water in the field. If effective, such immersions would perhaps be more appropriate for asymptomatic, hyperthermic individuals, and could perhaps also be efficacious for those with exertional heat illness.

2.2 METHODS

2.2.1 Subjects

Eight healthy, physically-active males participated in this study (23 years (SD 4.99); 182.5 cm (SD 7.72); 77.3 kg (SD 6.77); surface area 1.99 m^2 (SD 0.010); surface area to mass ratio 0.025 $m^2.kg^{-1}$ (SD 0.002)). Subjects were screened to eliminate those with a history of cardiovascular, respiratory or thermoregulatory problems contraindicative of participation in this experiment, and all subjects provided written, informed consent to procedures approved by the Human Research Ethics Committee (University of Wollongong).

2.2.2 Procedures

Subjects acted as their own controls and completed three trials, each consisting of 5 min of pre-heating rest, 90-min of exercise with heating, 5 min pre-cooling preparation and then a supine cooling phase (commencing at 100 min; Figure 2.1). Subjects were first heated to an oesophageal temperature of 39.5ºC using the controlled-hyperthermia technique (Fox *et al*., 1963; Patterson *et al*., 2004). This was achieved using a combination of exercise and exogenous heat (climate chamber (36ºC, 50% relative humidity) and water-perfusion garment (40ºC)). Subjects wore running shorts and shoes, and performed semi-recumbent cycling for 90 min. Exercise followed a four-phase pattern to achieve three target oesophageal temperatures (38.5ºC, 39.0ºC, 39.5ºC). Within each phase, the work durations remained constant, but the work rate was varied to achieve and hold the target temperature. Thus, the targets dictated the work rate for each individual, resulting in the following average work rates: 30 min at 147.7 W (SD 23.9: phase one), 30 min at 96.9 W (SD 14.7: phase two) and 20 min 92.4 W (SD 4.2: phase three). The final stage of exercise involved relatively low-intensity, intermittent work (10 min averaging 67.8 W, SD 13.5)

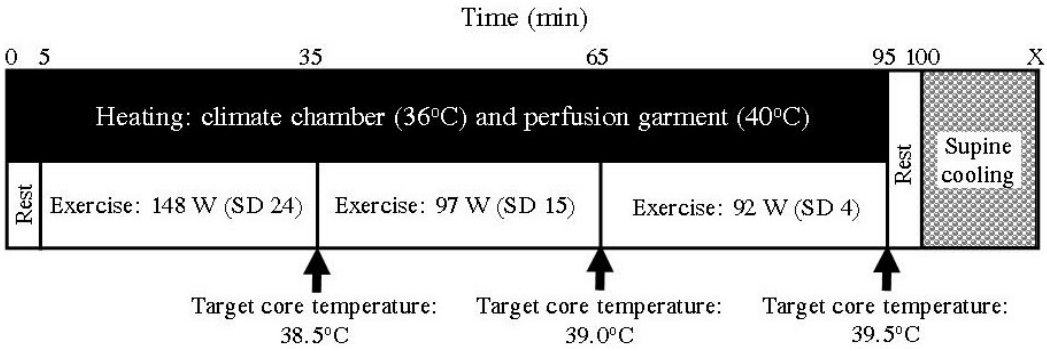

Figure 2.1: Overview of the experimental protocol.

and rest, and was designed solely to clamp oesophageal temperatures at 39.5ºC for 10 min. Iso-osmotic drinks (100 mL at chamber air temperature) were consumed at the end of the first three work phases. The work rates used in the first trial for each subject were replicated within subsequent trials. This protocol was designed to ensure a uniform heat distribution throughout the body tissues prior to cooling. This was a critical experimental objective, since comparisons among the cooling methods would be less valid if subjects commenced the cooling treatments with variations in thermal energy content.

Following heating, subjects were transferred (wheelchair) to the immersion tank (adjacent to the chamber). The time between the termination of heating and the commencement of cooling was standardised across all trials (5 min: 3.5 min in chamber (removal of water-perfusion garment), 1.5 min to transfer from chamber to supine cooling). Cooling commenced at 100 min, and was performed in a supine posture for all trials, with subjects placed on a wide-mesh litter (Figure 2.2; feet ~5º below the horizontal plane). A supine posture facilitated rapid immersion and replicated the posture of a person being treated for hyperthermia. Immersion was to chin depth, with the head raised sufficiently to enable total immersion without a breathing impediment. The three trials differed only in the form of post-exercise cooling: (1) control (non-immersion or air cooling): subjects lay supine (litter) in an air-conditioned laboratory (20-22ºC); (2) cold-water immersion cooling (water temperature = 14ºC); and (3) temperate-water immersion (water temperature = 26ºC). The former water temperature was used by Proulx *et al*. (2003, 2006), thereby providing a means for inter-laboratory data comparison, while the latter is a reasonable approximation of the temperature of water that may be available in the field in some hot climatic regions. Proulx *et al*. (2003, 2006) also used colder water temperatures (2º, 8ºC), but these temperatures were not used in the current study, since such water is unavailable in the field, and since cooling data already existed for these temperatures (Proulx *et al*., 2006). In each trial, cooling continued until an oesophageal temperature of 37.5ºC was achieved.

Testing for each subject was conducted at the same time of day, using hydrated subjects (Armstrong *et al.,* 1994), and with the trial sequence balanced across subjects. Pre-experimental urine specific gravities were: control 1.018 (SD 0.009), cold 1.013 (SD 0.006), temperate 1.021 (SD 0.006; Clinical Refractometer no. 140, Shibuya Optical Co.

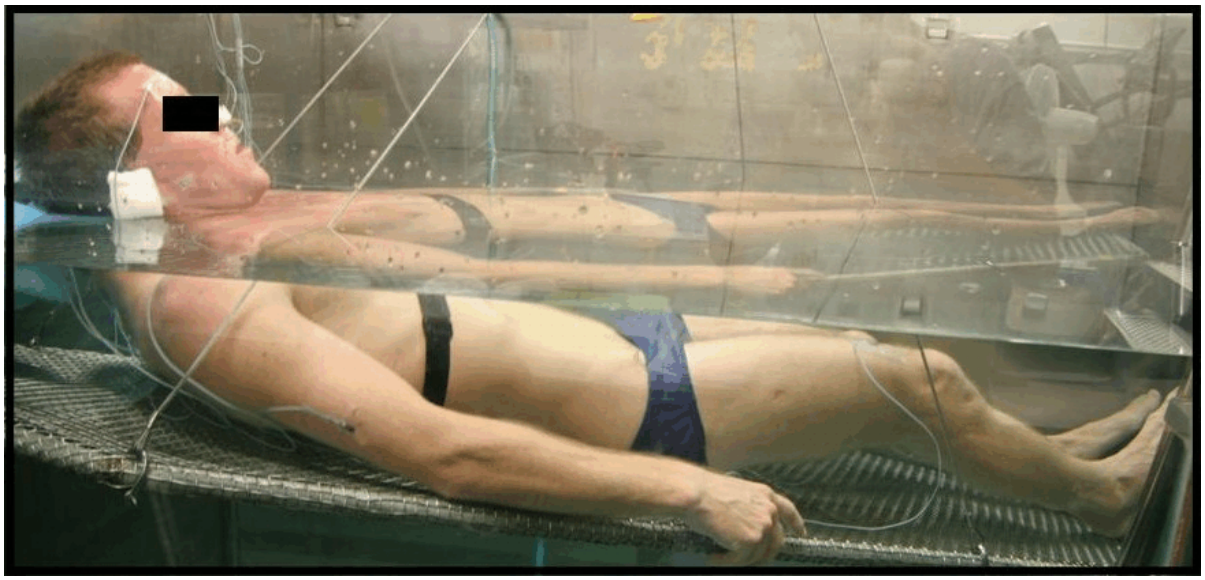

Figure 2.2: Post-exercise water immersion. The wide-mesh litter, set at a fixed tilt angle, was used for every air and immersion cooling trial.

Ltd., Tokyo, Japan). Subjects were asked to refrain from strenuous exercise, and the consumption of alcohol and tobacco during the 12 h prior to each trial. Subjects were also instructed to drink 15 $mL.kg^{-1}$ of additional water in the evening before testing, and to eat an evening meal and breakfast high in carbohydrate and low in fat. An abstinence from caffeine for 2 h prior to testing was also required. On arrival at the laboratory, subjects provided a urine sample to check hydration state, and were provided with supplementary water (10 $mL.kg^{-1}$) if not adequately hydrated (urine specific gravity >1.020; S8 in control trial). Water was also consumed during the insertion of the oesophageal probe (~400 mL). Before leaving the laboratory, subjects were rehydrated, consuming an iso-osmotic drink equivalent to 150% of the body mass change (100% in the laboratory and 50% taken away).

2.2.3 Measurements

Core temperature was measured continuously from the oesophagus (inserted transnasally; Mekjavic and Rempel (1990); Edale instruments Ltd., Cambridge, U.K.), the auditory canal (insulated to minimise auditory canal gradient effects; Edale instruments Ltd., Cambridge, U.K.) and the rectum (10 cm beyond anal sphincter; Edale instruments Ltd., Cambridge, U.K.). Data were sampled at 15-s intervals using a portable data logger (Grant Instruments Ltd., 1206 Series Squirrel, U.K.). Skin temperatures were measured (15-s intervals) using thermistors taped to eight skin sites (Type EU, Yellow Springs Instruments Co. Ltd., Yellow Springs, OH, USA): forehead, right scapula, right chest, right upper arm, left forearm, left dorsal hand, right anterior thigh and left posterior calf. Mean skin temperature was derived using standard surface area coefficients (ISO 9886, 1992; after Hardy and DuBois, 1938). All thermistors were calibrated in a stirred water bath against a certified reference thermometer (Dobros total immersion, Dobbie Instruments, Sydney, Australia). Heart rate was monitored from ventricular depolarisation throughout each trial (15-s intervals; Vantage NV Sports Tester, Polar Electro Oy, Kempele, Finland).

2.2.4 Design and analysis

This project was based upon a fully-crossed, repeated-measures experimental design, with subjects participating in all trials. Between-trial differences were analysed using two-way, repeated-measures analyses of variance (with Tukey's *HSD post hoc*

procedure) and paired *t*-tests. *Alpha* was set at the 0.05 level for all statistical comparisons. When the sphericity assumption was not satisfied, non-parametric tests were conducted (Friedman's Chi square for main effects (χ^2), and the Wilcoxon signed-rank test for paired comparisons). For multiple comparisons using the Wilcoxon signed-rank test, an adjusted *alpha* level for each comparison was computed using the Boole-Bonferroni inequality adjustment. Data are presented as means with standard errors of the means (±SEM), unless otherwise stated (standard deviations: SD). Graphs are referenced to the commencement of the resting phase of the heat exposure (0 min).

2.3 RESULTS

The heating protocol was designed to both elevate and ensure relatively homogeneous body tissue temperatures. The extent that this was achieved is evident in Figure 2.3, from which it is clear that the oesophageal temperature profiles, upon which the heating protocol was based, were faithfully reproduced across all trials. The oesophageal temperature targets (38.5º, 39.0º, 39.5ºC) were successfully achieved, and the final oesophageal temperatures did not differ significantly among conditions: control: 39.4º (SD 0.08); cold immersion: 39.3º (SD 0.05); temperate immersion: 39.3ºC (SD 0.09; *P*=0.359). The similarity of these temperatures is very important, since the rate of conductive, radiative and convective heat transfers are a function of the magnitude of the body core to ambient thermal gradient. Auditory canal temperatures peaked at 39.9º (control; SD 0.27), 39.7º (cold; SD 0.25) and 39.7ºC (temperate; SD 0.11; *P*=0.184). The corresponding rectal temperatures were higher and slightly more variable: 40.4º (SD 0.41); 40.1º (SD 0.16); 40.1ºC (SD 0.67; *P*=0.060). The final mean skin temperatures averaged 38.1º (SD 0.50: control), 37.7º (SD 0.40: cold immersion) and 37.9ºC (SD 0.40: temperate immersion), with between-trial differences also being non-significant (*P*=0.217). Over the last 10 min of heating, heart rates averaged 146.2 beats**.**min^{-1} (SD 0.78: control), 152.6 beats**.**min^{-1} (SD 1.11: cold immersion) and 151.6 beats**.**min^{-1} (SD 2.35: temperate immersion). While drinking was permitted, it was controlled and limited (Figure 2.3). Thus, subjects were progressively dehydrated to experience fluid deficits (corrected for fluid consumption) between 2.5-3%, but differences among conditions were not significant (*P*=0.334). From these data, it may be concluded that all subjects were moderately (38.5-39.5ºC) to profoundly hyperthermic (>39.5ºC), though none displayed overt signs or symptoms of

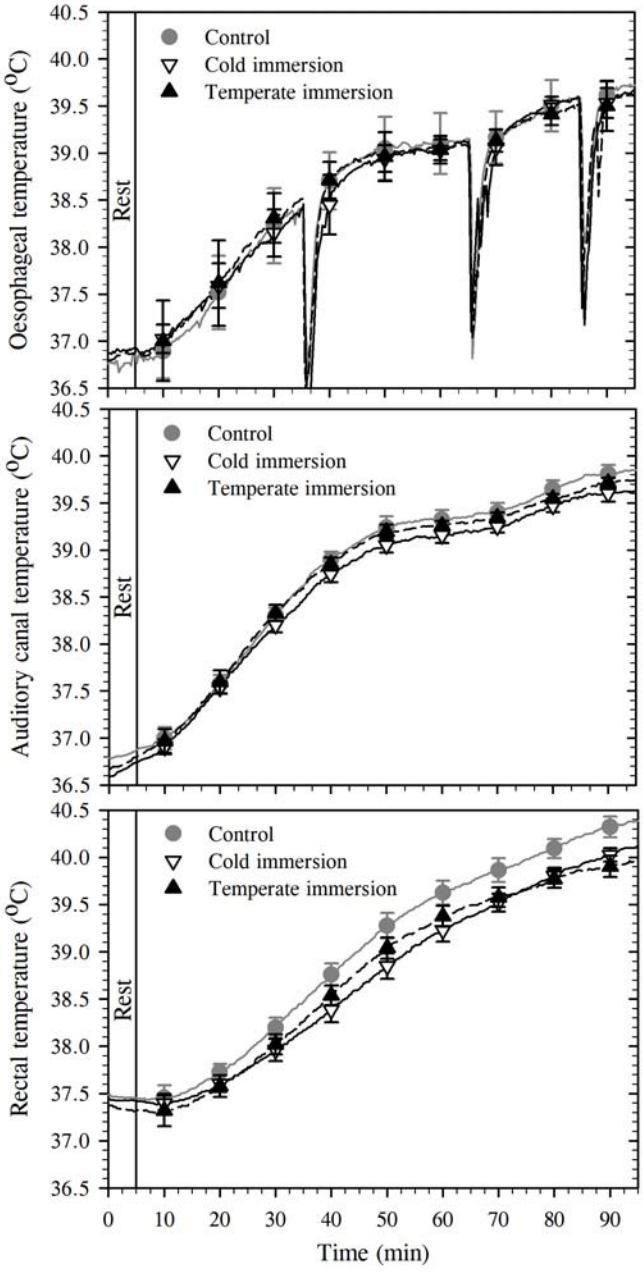

Figure 2.3: Oesophageal, auditory canal and rectal temperatures during heated exercise (36ºC, 50% relative humidity, water-perfusion garment (40ºC)) to an oesophageal temperature of ~39.5ºC (rectal temperature ~40ºC). Data are means with standard errors of the means. Each trial consisted of 5 min of pre-heating rest, 90-min of heating, 5 min pre-cooling preparation and then supine cooling (commencing at 100 min). Artefacts from the consumption of three iso-osmotic drinks are evident within the oesophageal traces. Legends refer to the three modes of cooling: control (no immersion: air 20-22ºC), cold-water immersion (14ºC) and temperate-water immersion (26ºC).

heat illness. It was also concluded that the controlled-hyperthermia technique provided an effective means for ensuring that subjects had an equivalent thermal energy content across trials, and before cooling commenced.

When analysed over the entire cooling period, the mean skin temperatures averaged 33.8^{o} (SD 1.31: control), 21.2^{o} (SD 1.20: cold immersion) and 26.8^{o}C (SD 1.33: temperate immersion). These averages all differed significantly from one another (P<0.001). When the response curves were compared, significant time by treatment interactions were evident between the control and cold trials (P=0.002), and between the control and temperate trials (P=0.001), but not between the two immersion treatments (P=0.199).

The time taken for oesophageal temperature to reach 37.5^{o}C from the start of cooling (100 min) was derived (Figure 2.4), and these data are contained in Table 2.1 for each subject, rounded to the nearest 15 s. The relationship for cooling rate (temperature over time) was assumed to be linear over the steepest part of the curve and therefore the relationship was calculated over the linear portion. There is a limitation in this assumption as a plateau in the response will reduce the reported cooling rate. However, this error would be minimal because oesophageal temperature was responding rapidly. These data did not meet the sphericity assumption, and were reanalysed using the Friedman's c^2 (c^2=14.25, P<0.05). Each comparison was statistically significant at the adjusted *alpha* level (P<0.017). Table 2.2 shows the oesophageal temperature cooling rates for each subject which averaged 0.10^{o}C**.**min^{-1} (control; ±0.04), 0.88^{o}C**.**min^{-1} (cold; ±0.06) and 0.71^{o}C**.**min^{-1} (temperate; ±0.02). These rates also differed significantly between experimental conditions (χ^2=13, P<0.05), with each of the between-trial differences being significant at the adjusted *alpha* level (P<0.017). Cooling rates for auditory canal temperatures averaged 0.10^{o}C**.**min^{-1} (control; ±0.01), 0.53^{o}C**.**min^{-1} (cold; ±0.05) and 0.31^{o}C**.**min^{-1} (temperate; ±0.01; P<0.001), while those for rectal temperatures were 0.07^{o}C**.**min^{-1} (control; ±0.01), 0.18^{o}C**.**min^{-1} (cold; ±0.04) and 0.10^{o}C**.**min^{-1} (temperate; ±0.02; P=0.016). This large difference in cooling rate between oesophageal and rectal temperatures is due to the dominance of conductive heat transfer at the rectum, as opposed to convective heat transfer at the oesophagus (Taylor *et al*., 2014). Comparisons with the non-immersed (control) condition are largely academic, since the laboratory was air conditioned, and this state may not be relevant in the field.

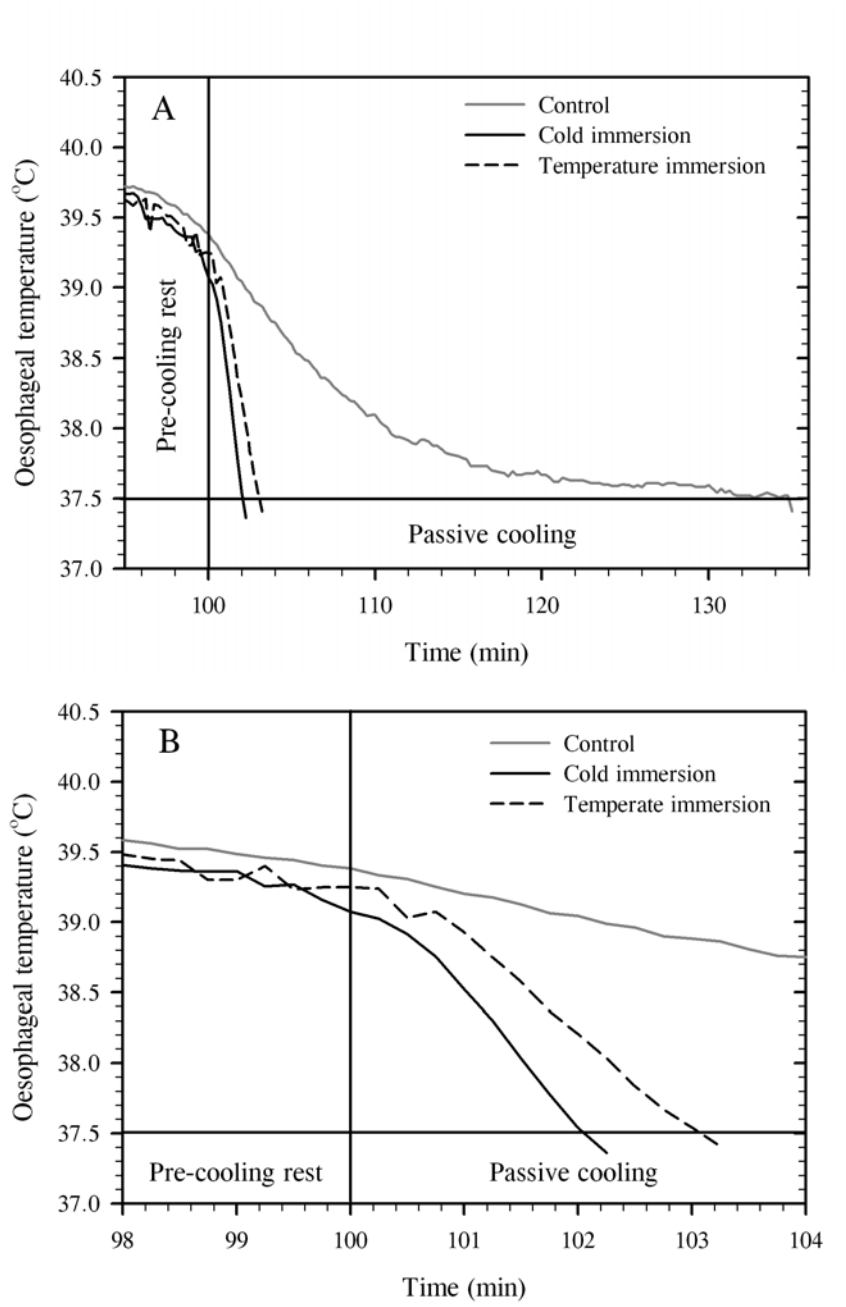

Figure 2.4: Oesophageal temperatures during cooling (control: air 20-22ºC), cold-water immersion (14ºC), temperate-water immersion (26ºC)) following heated exercise to an oesophageal temperature of ~39.5ºC (rectal temperature ~40ºC). Data are presented as means, terminating when oesophageal temperatures reached 37.5ºC (Figure 2.4A). Also shown are the first 4 min of cooling (an expanded version of A; Figure 2.4B) to facilitate a comparison between the two immersion trials. Each trial consisted of 5 min of pre-heating rest, 90-min of heating, 5 min pre-cooling preparation and then supine cooling (commencing at 100 min).

Table 2.1: Time (minutes) to reach an oesophageal temperature of 37.5ºC using three cooling procedures. To provide an indication of cooling time distributions, data are presented as means with standard deviations.

Subject	Control: air 20-22ºC (min)	Cold-water immersion: 14ºC (min)	Temperate-water immersion: 26ºC (min)
S1	17.75	2.00	2.50
S2	14.00	3.00	6.00
S3	29.25	2.25	2.75
S4	20.25	1.75	2.25
S5	18.25	1.75	2.25
S6	60.75	3.50	2.75
S7	10.25	1.50	2.00
S8	12.00	1.50	2.75
Mean	22.81	2.16	2.91*
S.D.	16.41	0.73	1.23

Cooling rates were all computed from 100 min (immersion time) after first resting for 5 min. At this time, mean oesophageal temperatures were <39.5ºC for each trial, with some inter-subject variability (minimum 39.0ºC; maximum 39.6ºC). * denotes significantly different from cold-water immersion (14ºC).

Nevertheless, cooling was still reasonably rapid, *albeit* much more variable among subjects. Notwithstanding the statistical differences across all treatments, it is clear that whole-body cooling to an oesophageal temperature of 37.5ºC in temperate water took only marginally longer to be achieved: 2.91 min versus 2.16 min (Table 2.1). Since one purpose of this experiment was to evaluate methods that may be used in the field, then one must conclude that temperate water is more than adequate to rapidly cool hyperthermic individuals. Indeed, one cannot imagine that the time difference of 0.75 min (45 s) would have any meaningful physiological or clinical implications.

2.4 DISCUSSION

The current experiment has established, on the basis of oesophageal temperatures, that rapid and effective heat removal can be achieved during a temperate-water immersion (26ºC) in moderately to profoundly hyperthermic, but asymptomatic individuals. There is little doubt that colder water immersions should elicit slightly faster cooling, but what was in doubt was the physiological and clinical significance of this difference. Since cooling during a life threatening heat illness aims to rapidly reduce central nervous system temperature, then observations based upon oesophageal temperature reductions are more pertinent to this objective than are those based upon rectal temperature, because the former provides a much closer approximation of the temperature of blood flowing to the brain (Whitby and Dunkin, 1971). If one accepts this, then one may question the need to use water cooler than 14ºC, or perhaps even 26ºC, since the respective cooling times at these water temperatures were still <4 min and 6 min across every subject (Table 2.1). These observations, whilst apparently not being previously described within the literature, have considerable practical significance, although some would suggest they were predictable on a first-principles basis.

Elementary heat transfer equations inform us that the reduction in the skin-water thermal gradient, and the corresponding lengthening of the transcutaneous conductive distance, result in the predicted total heat loss in temperate water, being only ~15% of that predicted for 14ºC water. However, these calculations assume a constant rate of convective heat delivery to the muscles and skin in both immersions. For muscle tissue, this assumption appears invalid, with muscle temperature being rapidly and powerfully

influenced by water temperature in resting, thermoneutral individuals (Kregel *et al*., 1992; Booth *et al*., 2004), presumably due to differences in local blood flow. Indeed, sympathetic discharge to skeletal muscles is unaffected at immersion temperatures >21ºC, but is elevated at temperatures <15ºC, and is particularly powerful in water <10ºC (Kregel *et al*., 1992). For the current experiment, one could predict muscle temperatures to be ~24ºC (cold) and 30ºC (temperate). From actual skin temperatures (cold: 21.2ºC; temperate: 26.8ºC), one can predict skin blood flow for each immersion using linear extrapolation, assuming a linear relationship exists and maximal flow (7.5 $L.min^{-1}$) occurs at ~42ºC (Rowell, 1974), while minimal flow occurs at about 10ºC (Roddie, 1983). Although there are limitations in assuming the relationship between skin temperature and skin blood flow is linear, these calculations are designed to demonstrate differences between each of the experimental conditions where the predictions are an approximation only. From these assumptions, one can approximate convective heat delivery (mass flow) to the skin to be about 115% greater during the temperate immersion. Thus, it is assumed that the rapid heat loss during that trial was due to less powerful peripheral vasoconstrictor responses, with greater heat being transported to the muscles and skin. If this less constricted state can be maintained in warmer individuals, and this has been established for blood flow to the extremities (Ferris *et al*., 1945; Brajkovic *et al*., 1998), then, for a given ambient temperature, heat loss will be accelerated. Hence, a sustained physiological mechanism (peripheral blood flow) appears to have countered the impact of a smaller thermal gradient, resulting in physiologically and clinically insignificant differences in the rate of heat extraction in hyperthermic individuals between trials.

It is of note that the immersion time presently required to reach a core temperature of 37.5ºC in 14ºC water differed dramatically from that reported by Proulx *et al*. (2006) for the same water temperature: 2.16 min (current study) versus 17.3 min. However, Proulx *et al*. (2006) determined cooling times from the point at which rectal temperature reached 37.5ºC. In the current study, these times were based upon oesophageal temperature, which is better at tracking dynamic changes in core temperature of the heart and major blood vessels. That is, oesophageal temperature closely tracks changes in central blood volume due to its close proximity to the heart (Taylor *et al*., 2014). While it may be correct that most clinicians can only measure rectal temperature, rectal tissue survival should not take

Table 2.2: Oesophageal temperature cooling rates ($^{o}C.min^{-1}$) during three cooling procedures. Data are means with standard errors of the means.

Subject	Control: air 20-22ºC ($^{o}C.min^{-1}$)	Cold-water immersion: 14ºC ($^{o}C.min^{-1}$)	Temperate-water immersion: 26ºC ($^{o}C.min^{-1}$)
S1	0.11	0.96	0.76
S2	0.14	0.70	0.33
S3	0.07	0.84	0.66
S4	0.08	0.91	0.72
S5	0.09	0.78	0.98
S6	0.03	0.62	0.76
S7	0.14	1.11	0.83
S8	0.16	1.08	0.61
Mean	0.10	0.88	0.71
SEM	0.04	0.06	0.02

Cooling rates were all computed from 100 min (immersion time) after first resting for 5 min. At this time, mean oesophageal temperatures were <39.5ºC for each trial, with some inter-subject variability (minimum 39.0ºC; maximum 39.6ºC).

precedence over that of the central nervous system, regardless of the tools that one possesses. Thus, a more appropriate comparison between investigations is to evaluate cooling time differences based upon oesophageal temperatures. This is because rectal temperature variations may be the result of changes in peripheral blood flow, rather than tracking whole-body temperature changes (Taylor *et al.*, 2014). Proulx *et al*. (2003) only measured rectal temperatures, while Proulx *et al*. (2006) measured three core indices, but chose only to report cooling times based on rectal temperatures. In Figure 1 of that report, cooling data for each index are shown for one individual. If one digitises these data, required cooling times of ~3 min are achieved at water temperatures of 2º and 14ºC, and ~7 min for water at 8º and 20ºC. Proulx *et al*. (2006) did, however, report cooling rates for each of three core temperatures (Table 2.2). During immersion in 14ºC water, oesophageal temperature cooled at 0.77ºC.min^{-1} (SD 0.25), and was slightly slower than the current cooling rate (0.86ºC.min^{-1} (SD 0.17); Table 2.2), despite possible differences in the total thermal energy content of the subjects across studies due to differences in the heating protocol (90 min (current project) versus 45.4 min) and body mass (9 kg heavier in this project). Indeed, this difference in methodological emphasis across studies is really quite important, since, if Proulx *et al*. (2006) had reported cooling times based on oesophageal temperatures, then others would perhaps have been less enthusiastic in the use of ice-cold water to cool hyperthermic individuals. That is, if one sees that a 17.3-min immersion at 14ºC is necessary to cool the body, then one is absolutely encouraged to explore every opportunity to shorten this time, and this could most easily be achieved by using colder water. However, if one sees that an immersion of <4 min in water at 14ºC is all that is necessary to drop oesophageal temperature to 37.5ºC, or 1.6 min to achieve 38.5ºC (Figure 2.4B), then one's enthusiasm for more uncomfortable, and even painful (Kregel *et al*., 1992) water temperatures will perhaps be viewed with less urgency. Nevertheless, these oesophageal versus rectal temperature cooling time differences do provide important information. First, rapid and effective central nervous system cooling will occur in water at both 14º and 26ºC. Second, cooling will not be uniform across body tissues, with less well-perfused tissue beds (*e.g*. the rectum) being cooled more slowly (Taylor *et al*., 2014). It must therefore be assumed that the brain, though well perfused, may not necessarily cool within 2.16 min, since blood leaving the heart will possibly gain thermal energy *en route* to the brain, and it will take some time for thermal equilibrium to be achieved between the

blood and the brain. Thus, while oesophageal temperature provides better index of brain cooling than does rectal temperature, brain cooling rates remain unknown. However, it can be assumed, based on the evidence from oesophageal temperatures, that 14ºC water will provide a more than adequate condition for rapid brain cooling to occur.

Thus far, we have considered only asymptomatic, yet moderately to profoundly hyperthermic individuals. Patients and animals suffering severe hyperthermia, heat exhaustion and heatstroke will often experience a dramatic reduction in skin blood flow, relative to the asymptomatic hyperthermic state, to defend mean arterial pressure. Indeed, some may even suffer peripheral circulatory failure (O'Donnell and Clowes, 1972; Hales, 1976). While this is widely accepted, such a failure is by no means a universal observation, with a significant number of such patients sustaining vasodilatation (Al-Khawashi *et al*., 1983; Hales, 1996), *albeit* at a reduced level. Nevertheless, it may be argued that, in the presence of circulatory failure, it is critical to use the coldest water possible. This view is not contested, particularly when it has been established that peripheral circulation is no longer viable. However, if one accepts that cutaneous venules and arterioles are likely to be maximally constricted when normotensive, normothermic individuals are immersed in water at 14ºC or colder (Barcroft and Edholm, 1943; Spealman, 1945; Cannon and Keatinge, 1960), then it is not unreasonable to assume that such subjects would possibly cool at a rate quite similar to that expected in patients with peripheral circulatory failure. Indeed, the non-significant differences in oesophageal temperature cooling rates reported by Proulx *et al*. (2006) across water temperatures from 2-20ºC, are consistent with this hypothesis. Thus, if one agrees with these assumptions and accepts the empirical evidence, then one should perhaps be less anxious over the need to use water cooler than 14ºC, since the required oesophageal temperature cooling time at this water temperature was <4 min in each of the current subjects. These observations clearly show that immersed, hyperthermic humans do not lose heat at rates equal to that predicted for inanimate objects. While the mechanisms that explain this phenomenon remain unexplored, it is assumed, when immersed in less cold water, that a sustained blood flow to the less superficial tissues of hyperthermic individuals continues to support the convective delivery of heat to the periphery, which is then transferred to the skin surface via tissue conduction.

It is concluded on the basis of oesophageal temperature measures that, for hyperthermic, but asymptomatic individuals, temperate-water immersion will more than adequately facilitate rapid brain cooling, due to the maintenance of a greater peripheral blood flow. Indeed, a case may be promoted that cold-water immersion should be avoided for such individuals, since this is not only very unpleasant, but it may result in cardiovascular failure (cold shock) in some high-risk individuals. Furthermore, for heat exhausted and heatstroke patients, it appears that water any cooler than 14^{o}C may not be required. Thus, in a true emergency situation, one must immediately immerse the patient in the most readily available cool-temperate water, and then seek cooler water. Data from the current experiment, and also from Proulx *et al*. (2006), demonstrate that rapid central heat removal will be achieved via this initial immersion, and sufficient life-saving cooling may perhaps be achieved before one has time to organise immersion within colder water.

2.5 REFERENCES:

Al-Khawashi, M.I., Mustafa, M.K.Y., Khogali, M., and El-Sayed, H. (1983). Clinical presentation of 172 heat stroke cases seen at Mina and Arafat. In: Khogali, M., and Hales, J.R.S. (eds). *Heat stroke and temperature regulation*. Academic Press, Sydney. Pp. 99-108.

Armstrong, L.E., Maresh, C.M., Castellani, J.W., Bergeron, M.F., Kenefick, R.W., LaGasse, K.E., and Riebe, D. (1994). Urinary indices of hydration status. *International Journal of Sport Nutrition.* 4:265-279.

Barcroft, H., and Edholm, O.G. (1943). The effect of temperature on blood flow and deep temperature in the human forearm. *Journal of Physiology. (Lond).* 102:5-20.

Benzinger, T.H. (1970). Peripheral cold reception and central warm reception, sensory mechanisms of behavioral and autonomic thermostasis. In: Hardy, J.D., Gagge, A.P., and Stolwijk, J.A.J. (Eds). *Physiological and behavioral temperature regulation.* Springfield, IL: CC. Thomas, Pp. 831-55.

Booth, J.D., Wilsmore, B.R., MacDonald, A.D., Zeyl, A., Storlien, L.H., and Taylor, N.A.S. (2004). Intramuscular temperatures during exercise in the heat following pre-cooling and pre-heating. *Journal of Thermal Biology*. 29(7-8):709-715.

Brajkovic, D., Ducharme, M.B., and Frim, J. (1998). Influence of localized auxiliary heated on hand comfort during cold exposure. *Journal of Applied Physiology*. 85:2054-2065.

Cannon, P., and Keatinge, W.R. (1960). The metabolic rate and heat loss of fat and thin men in heat balance in cold and warm water. *Journal of Physiology. (Lond).* 154:329-344.

Casa, D.J., McDermott, B.P., Lee, E.C., Yeargin, E.C.L., Armstrong, L.E., and Maresh, C.M. (2007). Cold water immersion: the gold standard for exertional heatstroke treatment. *Exercise and Sports Science. Review.* 35(3):141-149.

Cheuvront, S.N., Kolka, M.A., Cadarette, B.S., Montain, S.J., and Sawka, M.N. (2002). Efficacy of intermittent, regional microclimate cooling. *Journal of Applied Physiology*. 94:1841-1848.

Ferris, B.G., Forster, R.E., Pillion, E.L., and Christensen, W.R. (1945). Control of peripheral blood flow: responses in the human hand when extremities are warmed. *American Journal of Physiology*. 150:304-314.

Fox, R.H., Goldsmith, R., Kidd, D.J., and Lewis, H.E. (1963). Acclimatization to heat in man by controlled elevation of body temperature. *Journal of Physiology. (Lond).* 166:530-547.

Golden, F., and Tipton, M. (2002). *Essentials of sea survival*. Human Kinetics, Champaign IL, USA. P. 27.

Hales, J.R.S. (1976). The redistribution of cardiac output in animals during heat stress. In: Tromp, S.W. (ed). *Progress in animal biometeorology.* Volume 1, part 1. Swetz Zeitlinger, Amsterdam. Pp. 285-294.

Hales, J.R.S. (1996). Limitations to heat tolerance. In: Fregly, M.J., and Blatteis, C.M. (Eds). *Environmental physiology.* Handbook of Physiology. Volume 1. Oxford University Press, New York. Pp. 285-355.

Hardy, J.D., and DuBois, E.F. (1938). The technic of measuring radiation and convection. *Journal of Nutrition*. 15:461-475.

ISO 9886. (1992). *Evaluation of thermal strain by physiological measurements.* International Standard Organisation, Geneva. Pp. 9-11.

Kregel, K.C., Seals, D.R., and Callister, R. (1992). Sympathetic nervous system activity during skin cooling in humans: relationship to stimulus intensity and pain sensation. *Journal of Physiol*ogy. 454:359-371.

Lide, D.R. (1997). *CRC handbook of chemistry and physics.* CRC Press, New York. Section 6. Pp. 1-3.

Mekjavic, I.B., and Rempel, M.E. (1990). Determination of esophageal probe insertion length based on standing and sitting height. *Journal of Applied Physiology.* 63(1):376-379.

Mantoni, T., Belhage, B., Pedersen, L.M., and Pott, F.C. (2007). Reduced cerebral perfusion on sudden immersion in ice water: a possible cause of drowning. *Aviation Space and Environmental Medicine.* 78:374-376.

O'Donnell, T.F., and Clowes, G.H.A. (1972). The circulatory abnormalities of heatstroke. *New England Journal of Medicine*. 287:734-737.

Patterson, M.J., Stocks, J.M., and Taylor, N.A.S. (2004). Humid heat acclimation does not elicit a preferential sweat redistribution towards the limbs. *American Journal of Physiology.* 286(3):R512-R518.

Pierau, F.-K. (1996). Peripheral thermosensors. In: Fregly, M.J., and Blatteis, C.M. (Eds).

Environmental physiology. Handbook of Physiology. Volume 1. Oxford University Press, New York. Pp. 85-104.

Proulx, C.I., Ducharme, M.B., and Kenny, G.P. (2003). Effect of water temperature on cooling efficiency during hyperthermia in humans. *Journal of Applied Physiology*. 94:1317-1323.

Proulx, C.I., Ducharme, M.B., and Kenny, G.P. (2006). Safe cooling limits from exercise-induced hyperthermia. *European Journal of Applied Physiology*. 96:434-445.

Roddie, I.C. (1983). Circulation to skin and adipose tissue. In: Shepherd, J.T., and Abboud, F.M. (Eds). *The cardiovascular system. Volume III. Peripheral circulation and organ blood flow Part 1*. Handbook of Physiology. American Physiological Society. Bethesda. Pp. 285-317.

Rowell, L.B. (1974). Human cardiovascular adjustments to exercise and thermal stress. *Physiological Review*. 54:75:159.

Spealman, G.R. (1945). Effect of ambient air temperature and of hand temperature on blood flow in hands. *American Journal Physiology.* 145:218-222.

Stephenson, L.A., Vernieuw, C.R., Leammukda, W., and Kolka, M.A. (2007). Skin temperature feedback optimizes microclimate cooling. *Aviation Space Environmental Medicine.* 78:377-382.

Stocks, J.M., Taylor, N.A.S., Tipton, M.J., and Greenleaf, J.E. (2004). Human physiological responses to cold exposure. *Aviation Space and Environmental Medicine.* 75(5):444-457.

Taylor, N.A.S., Tipton, M.J., and Kenny, G.P. (2014). Considerations for the measurement of deep-body, skin and mean body temperatures. *Journal of Thermal Biology.* 46:72-101).

Tipton, M.J. (1989). The initial responses to cold-water immersion in man. *Clinical Science.* 77:581-588.

Tipton, M.J., Kelleher, P.C., and Golden, F.S. (1994). Supraventricular arrhythmias following breath-hold submersions in cold water. *Undersea and Hyperbaric Medicine*. 21(3):305-313.

Whitby, J.D., and Dunkin, L.J. (1971). Cerebral, oesophageal and nasopharyngeal temperatures. *British Journal of Anaesthesia*. 43:673-676.

CHAPTER 3: A MECHANISM FOR THE INFLUENCE OF WATER TEMPERATURE ON THE IMMERSION-INDUCED COOLING OF PROFOUNDLY HYPERTHERMIC INDIVIDUALS.

3.1 INTRODUCTION

Exertional heat illness is commonly suffered by individuals required to perform work or exercise during exposure to extreme environmental conditions. Treatment for profound hyperthermia requires immediate and rapid cooling, and one method of particular interest is cold-water immersion. Not surprisingly, immersion in ice-cold water (0-2^{o}C) has been shown to rapidly reduce body core temperature and some researchers believe this is the most effective means of cooling hyperthermic individuals (Proulx *et al*., 2003, 2006; Casa *et al*., 2012). However, we previously showed cooling rates of hyperthermic individuals to be almost identical when immersed in either cold (14^{o}C) or temperate (26^{o}C) water (Taylor *et al*., 2008). Although ice- and cold-water immersion maximises the thermal gradient between the skin and water, thereby rapidly enhancing the rate of cooling through conductive heat loss, it does not allow for physiological mechanisms associated with rapidly activating peripheral cold thermoreceptors. This response is integrated in the hypothalamus along with afferent input from central thermoreceptors. When a cold stimulus is applied to the skin of a thermoneutral individual, activated peripheral thermoreceptors cause reflex vasoconstriction, thereby reducing the amount of warm blood delivered to the skin surface and increasing transcutaneous insulation (Charkoudian, 2003). In addition, the rate of activation dictates the intensity of the reflex. That is, the colder the stimulus the more intense the sympathetic response and therefore the greater reduction in cutaneous blood flow (Hensel, 1981).

Although this vasomotor reflex is true for cold-water immersion of individuals in a normothermic state (Pergola *et al*., 1996), exercise-induced hyperthermia stimulates both central and peripheral warm thermoreceptors at a constant rate. Thus, profoundly hyperthermic individuals requiring immediate treatment are exposed to heat for long durations and therefore warm-receptor activation is static (Hensel, 1981). When these individuals are suddenly immersed in cold water, rapid activation of cold thermoreceptors occurs throughout the whole body. In this case, information processing is received

simultaneously by statically activated central warm thermoreceptors and dynamically activated peripheral cold thermoreceptors. In this instance, it is not known whether a more powerful vasoconstrictor response will override that of the hot stimulus. To our knowledge no experiments have measured peripheral vascular responses during cold or temperate whole-body water immersion following exercise-induced hyperthermia, yet it is possible that the cold-induced vasoconstriction is counterproductive to the aim of the treatment.

By measuring skin blood flow during water immersion following normothermic and hyperthermic states, we will not only provide a possible explanation for the non-significant differences in cooling rates observed between cold and temperate water (Taylor *et al.*, 2008), but also enhance our understanding of the interactions of central and peripheral, warm and cold thermoreceptors. Therefore, the purpose of this research was to examine the vasomotor response during whole-body water immersion in cold and temperate water of normothermic and hyperthermic individuals.

3.1.1 Aims and hypotheses

The aim of this experiment was to evaluate the effectiveness of two different water-immersion temperatures (14º and 26ºC) on the rate of reducing body core temperature, and its relationship with cutaneous vasoconstriction, following either normothermia or exercise-induced hyperthermia. The former water temperature was used by Proulx *et al*. (2003, 2006), while the latter is an approximation of the temperature of water that may be available in the field in hot climatic regions and evaluated by Taylor *et al* (2008). To satisfy these aims we will measure skin blood flow of the forearm while individuals are immersed in both 14ºC and 26ºC water.

It was hypothesised that:

(1) The core temperature (oesophageal) cooling rate during water immersion following profound hyperthermia will not differ significantly between cold (14ºC) and temperate (26ºC) water immersion.

(2) A more powerful cutaneous vasoconstrictor response will occur during cold-water immersion compared to temperate water immersion, and this will occur in both normothermic and hyperthermic individuals.

3.2 METHODS

3.2.1 Subjects

Eight physically active and healthy males participated in this study (Table 3.1). Each subject was screened to eliminate those with a history of contraindicative cardiovascular, respiratory or thermoregulatory problems. In addition, subjects were screened to ensure a homogenous sample, based on aerobic fitness. Using a preliminary submaximal exercise test to predict peak oxygen consumption (Åstrand, 1960) subjects had an average peak aerobic power of 58.5 $mL.kg^{-1}.min^{-1}$ (SD 9.9). The characteristics of these subjects closely matched those presented in Chapter 2 (Table 2.1). Each subject received a subject information package and completed written, informed consent prior to commencing trials. The research conducted in this experiment was approved by the Human Research Ethics Committee (University of Wollongong) under approval HE07/039.

3.2.2 Experimental methods

Each subject completed four trials; two control trials and two experimental trials. Each trial consisted of a pre-immersion phase followed by a water-immersion phase. The pre-immersion phase was from either a normothermic (control) or profoundly hyperthermic (experimental) state while the water immersion phase was in either 14ºC (cold immersion) or 26ºC (temperate immersion) water. For the two control trials (***Trial A*** and ***Trial B)***, whole-body cooling was preceded by a thermoneutral baseline (Normothermic), while the pre-immersion phase of the two experimental trials (***Trial C*** and ***Trial D***) consisted of whole-body heating with exercise (Table 3.2). All testing was conducted at the same time of day, using fully-hydrated subjects, and the trial sequence was balanced across subjects.

3.2.2.1 Whole-body pre-immersion phase

For ***Trial A and Trial B***, subjects completed seated rest for 10 min in an air-conditioned laboratory (22ºC) prior to water immersion. During this phase, oesophageal, rectal and auditory canal temperature were recorded. However, a whole-body heating phase was completed for the two experimental trials only (***Trial C*** and ***Trial D***) to induce a hyperthermic state. For the pre-immersion heating phase subjects were first heated to achieve a target core (oesophageal: T_{es}) temperature of 39.5ºC. This was achieved using a combination of exercise (metabolic heat) and exogenous heat (climate chamber (36ºC,

Table 3.1: Subject characteristics.

Subject	Age (y)	Height (m)	Mass (kg)	Surface area (m^2)	Surface area to mass ratio ($m^2.kg^{-1}$)
S1	28	1.77	80.0	1.97	0.025
S2	22	1.85	98.0	2.21	0.023
S3	37	1.78	70.5	1.87	0.027
S4	31	1.85	78.0	2.01	0.026
S5	20	1.79	70.8	1.88	0.027
S6	23	1.86	79.2	2.03	0.026
S7	20	1.80	77.2	1.96	0.025
S8	22	1.91	85.3	2.14	0.025
Mean	23.7	1.83	81.2	2.03	0.025
S.D.	4.2	4.8	8.6	0.11	0.001

Table 3.2: Experimental timeline.

Time (min)	Control trials: Trials A & B	Time (min)	Experimental trials: Trials C & D
0	Subject arrival	0	Subject arrival
0-10	Subject hydration check	0-10	Subject hydration check
10-30	Subject preparation (22ºC)	10-30	Subject preparation (22ºC)
		30-60	**Heat 1:** exercise level 1: target T_{es} = 38.5ºC
		60	100 mL iso-osmotic drink
		60-90	**Heat 2:** exercise level 2: target T_{es} = 39ºC
		90	100 mL iso-osmotic drink
		90-110	**Heat 3:** exercise level 3: target T_{es} = 39.5ºC
		110	100 mL iso-osmotic drink
		110-120	**Heat 4:** exercise level 4: clamp T_{es} at 39.5ºC
30-35	Transfer subject to the immersion tank	120-125	Transfer subject to the immersion tank
35-x	**Cooling:** supine immersion: target T_{es} = 0.5ºC below baseline	125-x	**Cooling:** supine immersion: target T_{es} = 37.5ºC
x-x+15	Terminate experiment: supervised recovery	x-x+15	Terminate experiment: supervised recovery

Note: T_{es} refers to oesophageal temperature

50% relative humidity) and water-perfusion garment (40ºC)). Subjects wore running shorts and shoes, and performed semi-recumbent cycling for 90 min (Figure 2.1). This followed a four-phase pattern (Table 3.3), with three target core temperatures (38.5ºC, 39.0ºC, 39.5ºC) dictating the work rates chosen. These work rates averaged 147.7 W (S.D. 23.9: phase one), 96.9 W (S.D. 14.7: phase two) and 92.4 W (S.D. 4.2: phase three). The final stage of exercise was of a relatively low intensity (intermittent work (67.8 W, S.D. 13.5) and rest), and was designed to clamp core temperature for 10 min, thereby ensuring a more uniform heat distribution throughout the body tissues prior to cooling, whilst also enabling a check of the subjects core temperature, relative to the trial termination criteria.

Tissue temperature uniformity was deemed to be a critical experimental objective, since comparisons among the cooling methods would be invalid unless each subject commenced cooling with the same thermal energy content. Furthermore, since heat storage is a function of all heat exchanges, then sites dominated by heat conduction (rectal temperature) require more time to equilibrate (Casa and Kenny, 2009; Taylor *et al*., 2009). Within each exercise phase, the work durations remained constant (30, 30, 20, 10 min), but the work rate varied to achieve the target core temperature. That is, the targets were both core temperature and time. If the target core temperature was exceeded, the work ceased. If the target core temperature was achieved early, the work rate was reduced to hold that target until the next stage began. The work rates used in the first trial were replicated within subsequent trials.

3.2.2.2 Whole-body water immersion phase

The four trials differed through the form of water-immersion cooling that was provided from either normothermia (without exercise) or hyperthermia (following exercise in the heat). Following either the normothermic or hyperthermic state, subjects were transferred in a wheelchair to the immersion facility (adjacent to the chamber). The time between the termination of heating and the commencement of cooling (3.5 min in chamber and 1.5 min from chamber to supine cooling) was standardised across all trials. Immersion was 5-10º below the horizontal plane (supine in a litter), to chin depth and with the head raised sufficiently to enable total immersion without a breathing impediment (Figure 2.2). For the normothermic trials, subjects adopted a supine posture in an

Table 3.3: Average work rates (W) for each of the four stages during the heating phases for the two experimental trials (***Trial C*** and ***Trial D***). Data are means with standard errors of the means.

Stage of heating protocol	Hyperthermic		Mean (Watts)
	Cold immersion: Trial C (Watts)	Temperate immersion: Trial D (Watts)	
1: 0-30 min	157.9 (6.4)	164.0 (7.0)	160.9 (4.7)
2: 30-60 min	112.9 (9.8)	113.9 (7.5)	113.4 (6.0)
3: 60-80 min	89.3 (8.4)	95.8 (7.0)	92.5 (5.4)
4: 80-90 min	67.7 (5.6)	84.4 (5.1)	76.1 (4.2)

air-conditioned laboratory (20-22ºC) for 10 min prior to water immersion. For the hyperthermic trials, subjects completed 90 min of recumbent cycling with heating (climate chamber: 36ºC, 50% relative humidity) prior to water immersion. The four trials were: normothermic with cold-water (14ºC) immersion (***Trial A***); normothermic with temperate-water (26ºC) immersion (***Trial B***); hyperthermic with cold-water (14ºC) immersion (***Trial C***); hyperthermic with temperate-water (26ºC) immersion (***Trial D***). Trials were terminated when the target core temperature of 37.5ºC was achieved (***Trial C*** or ***Trial D***) or after 30 min of immersion (***Trial A*** and ***Trial B***). The target core temperature of 37.5ºC was chosen as this was also the target temperature in Proulx *et al*. (2003, 2006).

3.2.3 Experimental Standardisation

All testing was conducted at the same time of day, using fully-hydrated subjects (Table 3.4), and the trial sequence was balanced across subjects. Subjects were required to refrain from strenuous exercise and the consumption of alcohol and tobacco during the 12 h prior to each trial. For the night preceding each trial, subjects were instructed to drink 15 $mL.kg^{-1}$ of additional water before retiring, and to eat an evening meal high in carbohydrate and low in fat. Breakfast was to be high in carbohydrate and low in fat. Subjects were asked to refrain from using caffeine for 2 h prior to each trial. On arrival at the laboratory, subjects were provided with supplementary water (10 $mL.kg^{-1}$). During ***Trial C*** and ***Trial D***, subjects consumed an iso-osmotic drink at a rate of 100 mL (at chamber air temperature) approximately every 30 min (Table 3.2). Before leaving the laboratory, subjects were rehydrated, consuming an iso-osmotic drink equivalent to 150% of the body mass change (100% in the laboratory and 50% taken away).The trial sequences were balanced across subjects.

3.2.4 Experimental measurements

Throughout the heating phase of ***Trial C*** and ***Trial D***, core temperature, skin temperature and heart rate were recorded continuously, whilst psychophysical data were collected at 10-min intervals. During the water-immersion phase and for all trials physiological data were recorded continuously, these included core temperature, skin temperature and heart rate, whilst blood pressure and psychophysical data were recorded at 5-min intervals. Forearm and cutaneous blood flow were only measured throughout the

Table 3.4: Pre-experimental hydration state (urine specific gravity).

Subject	Normothermic		Hyperthermic	
	Cold immersion (Trial A)	Temperate immersion (Trial B)	Cold immersion (Trial C)	Temperate immersion (Trial D)
S1	1.005	1.025	1.005	1.010
S2	1.010	1.015	1.015	1.020
S3	1.003	1.008	1.010	1.008
S4	1.010	1.015	1.005	1.005
S5	1.015	1.003	1.025	1.025
S6	1.020	1.015	1.015	1.015
S7	1.020	1.015	1.010	1.015
S8	1.010	1.005	1.015	1.010
Mean	1.012	1.013	1.010	1.015
S.D.	0.01	0.01	0.01	0.01

cooling phase of this study.

3.2.4.1 Hydration state

Prior to commencing each trial, urine specific gravity was measured for each subject. Gross mass changes (before and after each trial) were used to determine changes in body mass (relative dehydration: ±20 g; fw-150k, A&D scale, CA, U.S.A.) over the course of the trial. Data were corrected for fluid replacement and urine production.

3.2.4.2 Body tissue temperatures

3.2.4.2.1 Oesophageal temperature

An oesophageal thermistor (Edale Instruments Ltd, U.K.) was inserted through the nose to a depth of about 40 cm from the nares (after Mekjavic and Rempel, 1990) with data recorded throughout each trial at 15-s intervals using a portable data logger (Grant Instruments Ltd., 1206 Series Squirrel, U.K.). This measure was taken as the primary index of core temperature.

3.2.4.2.2 Auditory canal temperature

Auditory canal temperature was measured with an ear-moulded plug containing a thermistor protruding into the ear 1 cm from the mould (Edale Instruments Ltd., U.K.). A large piece of cotton wool was secured over the top of the ear to minimise the effect of the environmental temperature. Data were recorded throughout each trial at 15-s intervals using a portable data logger (Grant Instruments Ltd., 1306 Series Squirrel, U.K.)

3.2.4.2.3 Rectal canal temperature

Rectal temperature was also measured continuously (15-s intervals), at a depth of 12 cm beyond the anal sphincter (Edale Instruments Ltd, U.K.).

3.2.4.2.4 Skin temperatures

Skin temperatures were measured using skin thermistors taped (single layer) to eight sites (Type EU, Yellow Springs Instruments Co.Ltd, Yellow Springs, OH, USA). These sites were forehead, right scapula, right chest, right upper arm, left forearm, left dorsal hand, right anterior thigh and left posterior calf (ISO 9886, 1992). Data were recorded throughout each

trial at 15-s intervals using a portable data logger (Grant Instruments Ltd., 1206 Series Squirrel, U.K.). From these data, an area-weighted mean skin temperature (T_{sk} was derived using standard skin surface area weightings (ISO 9886, 1992; after Hardy and DuBois, 1938):

$$T_{sk}=0.07 \cdot T_{sk-1}+0.175 \cdot T_{sk-2}+0.175 \cdot T_{sk-3}+0.07 \cdot T_{sk-4}+0.07 \cdot T_{sk5}+0.05 \cdot T_{sk-6}+0.19 \cdot T_{sk-7}+0.2 \cdot T_{sk-8}$$

where:

T_{sk-1} = forehead

T_{sk-2} = chest

T_{sk-3} = scapula

T_{sk-4} = upper arm

T_{sk-5} = forearm

T_{sk-6} = hand

T_{sk-7} = thigh

T_{sk-8} = calf

3.2.4.2.5 Mean body temperatures

Mean body temperature (T_b) was calculated as a weighted mean of core and skin temperature. The weighting differed depending on the thermal state of the individual. The hot equation was used during the heating phase of ***Trial C*** and ***Trial D***, the neutral equation was used during the pre-immersion phase of ***Trial A*** and ***Trial B*** and the cold equation was used during cooling for all four trials. These are presented as follows (Hardy and Dubois, 1938; Vallerand *et al.* 1992):

Hot: $T_b = (0.9 \cdot T_c) + (0.1 \cdot T_{sk})$ [^oC]

Neutral: $T_b = (0.7 \cdot T_c) + (0.3 \cdot T_{sk})$ [^oC]

Cold: $T_b = (0.65 \cdot T_c) + (0.35 \cdot T_{sk})$ [^oC]

where:

T_c = Oesophageal temperature [^oC]

T_{sk} = Mean skin temperature [^oC]

3.2.4.2.6 Whole-body sweat losses

Whole-body sweat loss was determined for the two experimental trials since these trials involved exercise in the heat. This was calculated as the difference in body mass between the commencement and completion of exercise. Values are reported as percentage changes from pre-trial baseline mass.

3.2.4.3 Cardiovascular measures

3.2.4.3.1 Heart rate

Heart rate was measured at 15-s intervals throughout each trial from ventricular depolarisation (Polar Electro Sports Tester, Finland).

3.2.4.3.2 Forearm blood flow

Forearm blood flow was determined using venous-occlusion plethysmography (EC 4 Plethysmorgraph, D.E. Hokanson, Inc., U.S.A.). This measurement was only taken during the water-immersion cooling phase and the forearm was fully immersed in the water at all times. Blood flow to the hand was occluded by placing a cuff around the left wrist and inflating it to 160 mmHg, while venous return was occluded by inflating a venous-occlusion cuff, placed proximal to the left elbow, to a pressure of 50 mmHg. Detection of the rate of swelling of the forearm was determined using a mercury-filled, strain-gauge placed around the forearm at its greatest circumference. Changes in the electrical resistance of the strain-gauge were measured as an analog output, sampled at 20 Hz, that was passed via an eight-channel, 12-bit analog-to-digital converter (Computer Boards Inc., PPIO-A18, Mansfield, U.S.A) to a laptop computer. Data were collected for 2 min at rest, immediately prior to immersion then for 4 min every 5 min during immersion. The venous-occlusion cuff was automatically controlled (AG 101 cuff inflator air source, D.E. Hokanson, Inc., U.S.A.), and followed a cycle of 8 s of inflation and 12 s of deflation, for a total of 12 inflations every 5 min.

3.2.4.3.3 Cutaneous blood flow

Cutaneous blood flow was estimated using laser-Doppler flowmeter located on the dorsal surface of the left forearm at the midpoint of the ulna (TSI Laserflo BPM^2, Vasamedics Inc., St Paul, MN, U.S.A.). This site was chosen because it has previously been shown to provide the best representation of upper body segmental blood flow (Cotter *et al*.,

1993). Samples were obtained using this device, and sampled at 8.0 Hz, but averaged at 2.0 Hz and collected using an eight channel, 12-bit analog to digital converter (Computer Boards Inc., PPIO-A18, Mansfield, OH, U.S.A). Data are reported as a percentage of baseline (%).

3.2.4.3.4 Mean arterial pressure

Systolic and diastolic blood pressures were measured using the auscultatory method using a stethoscope and aneroid sphygmomanometer (Single adult stethoscope, Ultrascope Inc, Charlotte, NC). Mean arterial pressure was then derived using the equation:

$$MAP = DBP + \tfrac{1}{3}(SBP-DBP) \quad [mmHg]$$

where:

MAP = Mean arterial pressure [mmHg]

DBP = Diastolic blood pressure [mmHg]

SBP = Systolic blood pressure [mmHg]

3.2.4.3.5 Forearm vascular conductance

Forearm vascular conductance ($mL.min^{-1}.mmHg^{-1}$) was calculated by dividing blood flow ($mL.min^{-1}$) by mean arterial pressure (mmHg)**.** Since skin blood flow is a function of both mean arterial pressure and total peripheral resistance, this calculation was important to determine whether changes in skin blood flow occurred from changes in vascular conductance.

3.2.4.3.6 Heat delivery

Heat delivery from the core (oesophageal) to the skin (mean skin temperature) was calculated as a function of the thermal gradient and forearm blood flow using the equation:

$$H = 3.85 \cdot Q_f \cdot (T_c - T_{sk}) \quad [kJ.min^{-1}]$$

where:

H = heat delivery [$kJ.min^{-1}$]

3.85 = specific heat of blood [$kJ.L^{-1}.{}^oC^{-1}$]

Q_f = forearm blood flow [$L.min^{-1}$]

T_c = Oesophageal temperature [^oC]

T_{sk} = mean skin temperature [^oC]

3.2.4.4 Psychophysical measures

Psychophysical measures were recorded at the start of both the heating and cooling stages. Subjects were then asked to rate perceived work effort (exertion), thermal sensation and thermal discomfort. Subjects were provided with relevant subjective scales prior to the start of each trial, and with written and oral instructions on how to use each scale (Appendices A, B and C).

3.2.5 Design and data analysis

Trials were administered in a balanced order across subjects to eliminate any effect of trial order. This was determined using a *Latin square* design model (Table 3.5). Statistical analyses were performed using BMDP Software. Data were first analysed to provide standard descriptive parameters (means, standard errors and confidence intervals). Between-trial comparisons were performed using two-way analysis of variance. Sources of significant differences were isolated using *Tukey's HSD* statistic.

3.3 RESULTS

3.3.1 Core temperature

The overall core temperature responses during control trials (***Trial A*** and ***Trial B***) where subjects were immersed in cold (14^{o}C) or temperate (26^{o}C) water following a normothermic state, are presented in Figure 3.1. There were changes in oesophageal and rectal temperatures throughout the immersion phase of these two trials, however auditory canal temperature showed a significantly greater decrease throughout the duration of the immersion (P<0.05).

Core temperature for the experimental trials (***Trial C*** and ***Trial D***), where subjects completed exercise in the heat to induce a hyperthermic state prior to immersion in either 14^{o}C or 26^{o}C water, are presented in Figure 3.2. While there were no significant differences in any of the core temperature responses (oesophageal, auditory canal or rectal) during the heating (pre-immersion) phase between ***Trial C*** and ***Trial D*** (P>0.05), the rectal temperatures were significantly higher than both the oesophageal and auditory canal temperatures (P<0.05) during the immersion phase of both experimental trials (P<0.05). In fact, when averaged across the entire duration of the immersion phase, rectal temperature was as much

Table 3.5: Experimental order (Latin square).

Subject	TRIAL 1	TRIAL 2	TRIAL 3	TRIAL 4
S1	C	A	B	D
S2	B	A	C	D
S3	A	D	B	C
S4	A	C	D	B
S5	A	D	B	C
S6	C	D	A	B
S7	D	B	C	A
S8	C	B	A	D

Note: '*A*' refers to normothermic baseline (no exercise) with temperate-water immersion (26^{o}C); '*B*' refers to normothermic baseline (no exercise) with cold-water immersion (14^{o}C); '*C*' refers to hyperthermic baseline (heated exercise) with temperate-water immersion (26^{o}C); '*D*' refers to hyperthermic baseline (heated exercise) with cold-water immersion (14^{o}C).

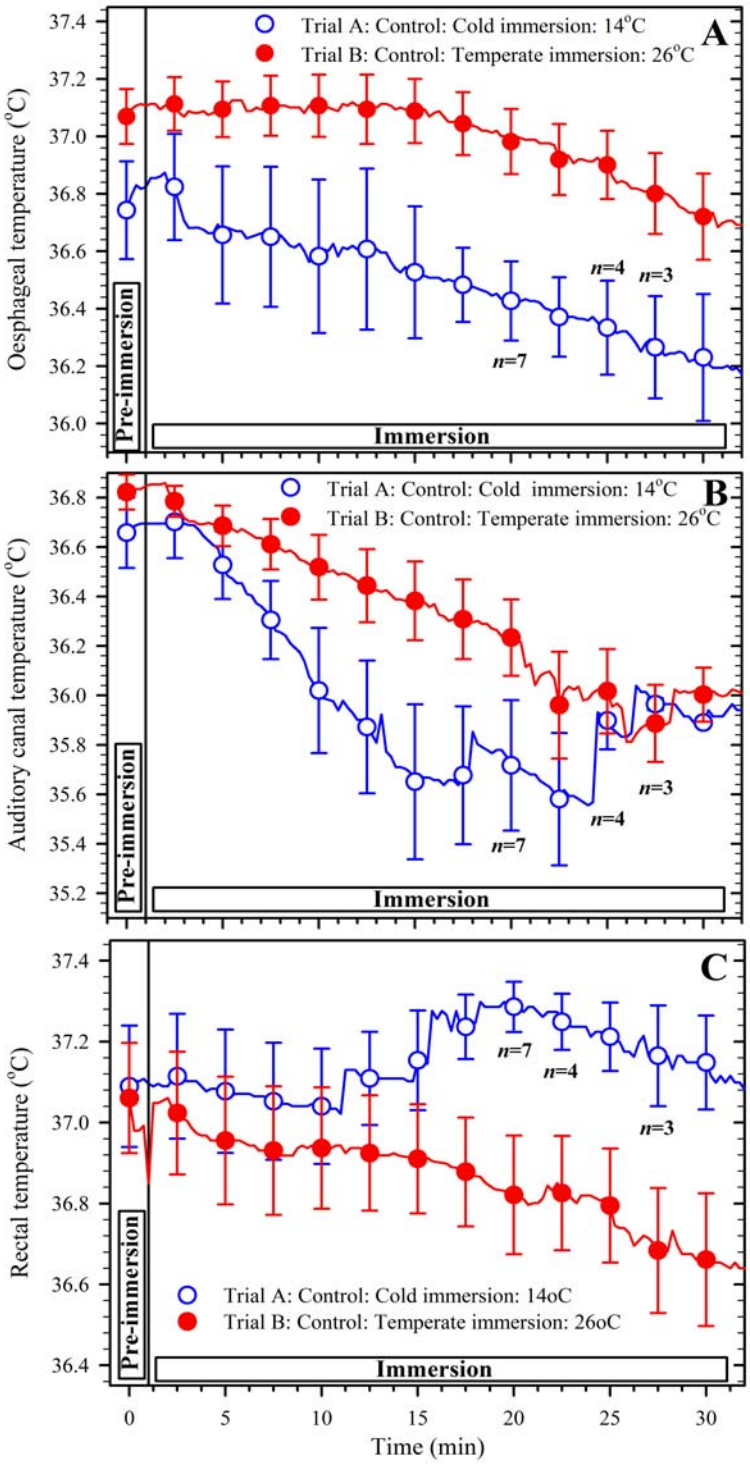

Figure 3.1: Oesophageal (**A**), auditory canal (**B**) and rectal (**C**) temperatures during each of two water immersions: cold (14ºC) and temperate (26ºC). Each immersion followed a pre-immersion normothermic state. *n* refers to the number of subjects who remained in the water until their core temperatures reached 37.5ºC.

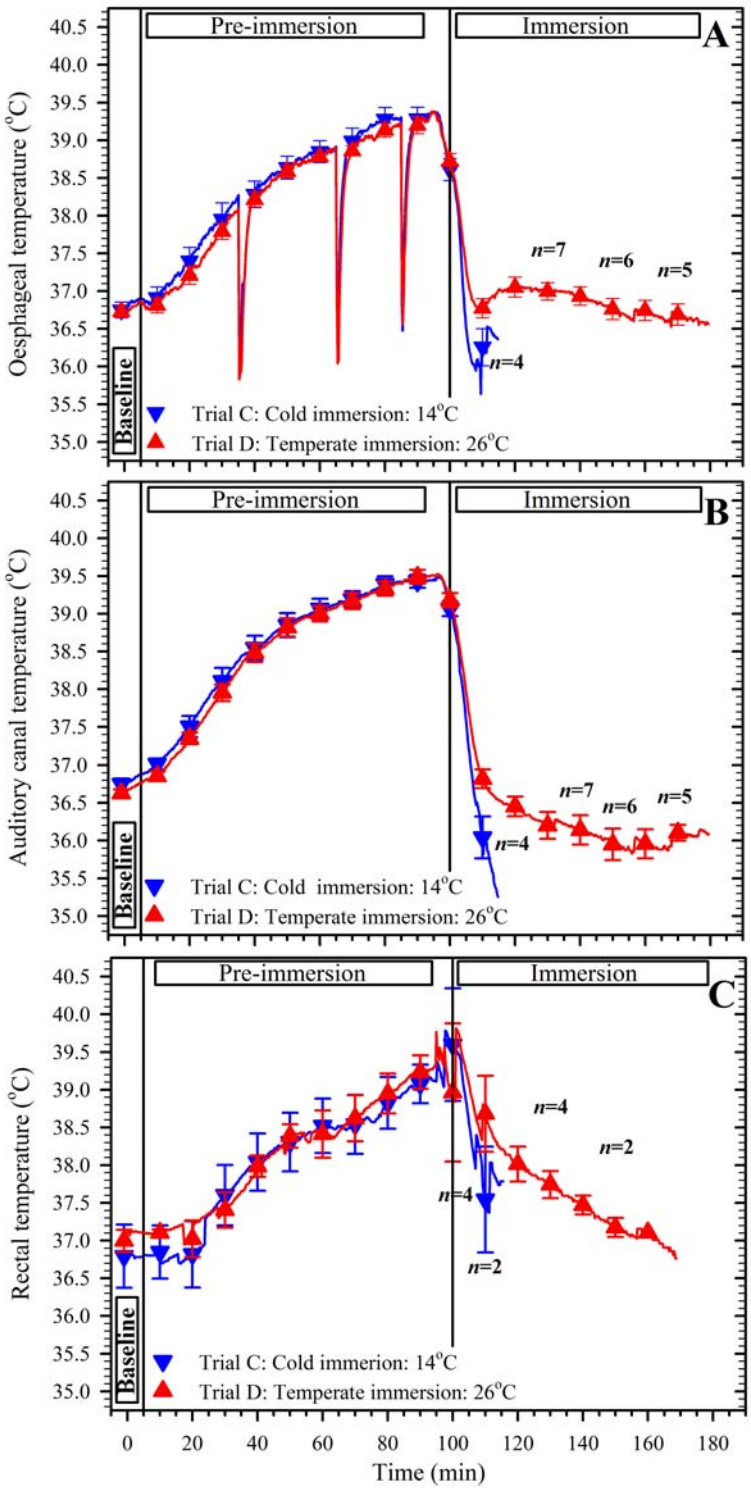

Figure 3.2: Oesophageal (**A**), auditory canal (**B**) and rectal (**C**) temperatures during each of two water immersions: cold (14ºC) and temperate (26ºC). Each immersion followed endogenous (exercise) and exogenous heating (climate chamber (36ºC, 50% relative humidity), water-perfusion garment (40ºC) to an oesophageal temperature of 39.5ºC.

as 2.2ºC and 2.1ºC higher than oesophageal temperature for the cold- and temperate-water immersions (respectively), and 1.5ºC and 2.5ºC higher than the respective auditory canal temperatures ($P<0.05$). This indicates a lag in the rectal response during immersion and is illustrated in Figure 3.3 showing the oesophageal, auditory canal and rectal temperatures during both the experimental trials. A similar pattern has been observed previously (Gagnon *et al*., 2010).

3.3.1.1 Oesophageal temperature

Prior to water immersion, there were no significant differences between the pre-immersion oesophageal temperatures for the two control trials (37.1º ± 0.1 ºC and 36.9º ± 0.2ºC for ***Trial A*** and ***Trial B*** respectively; $P>0.05$) or the pre-immersion oesophageal temperatures for the experimental trials (38.5º ºC ±0.1ºC and 38.4ºC ±0.1) for ***Trials C*** and ***Trial D*** respectively ($P>0.05$). These data are presented in Table 3.6. In addition, there was no significant difference in the rate of heating during the heating protocol for ***Trials C*** and ***Trial D*** ($P>0.05$). This was important to ensure uniform heat distribution immediately prior to cooling, so any differences that occurred during water immersion were a result of the temperature of the water itself, rather than a difference in the pre-immersion temperature. Therefore, two clearly separate pre-immersion states were achieved: normothermic (control) and hyperthermic (experimental; Table 3.6).

Figure 3.4A illustrates the oesophageal temperatures for the two control trials ***(Trial A*** and ***Trial B***) and the two experimental trials at the completion of exercise (***Trial C*** and ***Trial D***), immediately prior to immersion and during the first 5 min of immersion. During the first 5 min of cold-water immersion (***Trial A***) and temperate-water immersion (***Trial B***), oesophageal temperature did not change significantly from the pre-immersion normothermic state in either water temperature. The average cooling rates (as estimated from the portion of the oesophageal curve greater than 37.5ºC) for the control trials were 0.016ºC.min^{-1} (±0.002) and 0.035ºC.min^{-1} (±0.005) for ***Trial A*** and ***Trial B***, respectively (Table 3.7). This was a surprising outcome since the temperature gradient between the skin and water was 17.3ºC (±0.3) and 5.7ºC (±0.2) for the cold and temperate immersions, respectively. The indifferent oesophageal temperatures during 5 min of water immersion was probably due to powerful vasoconstriction in order to preserve core temperature.

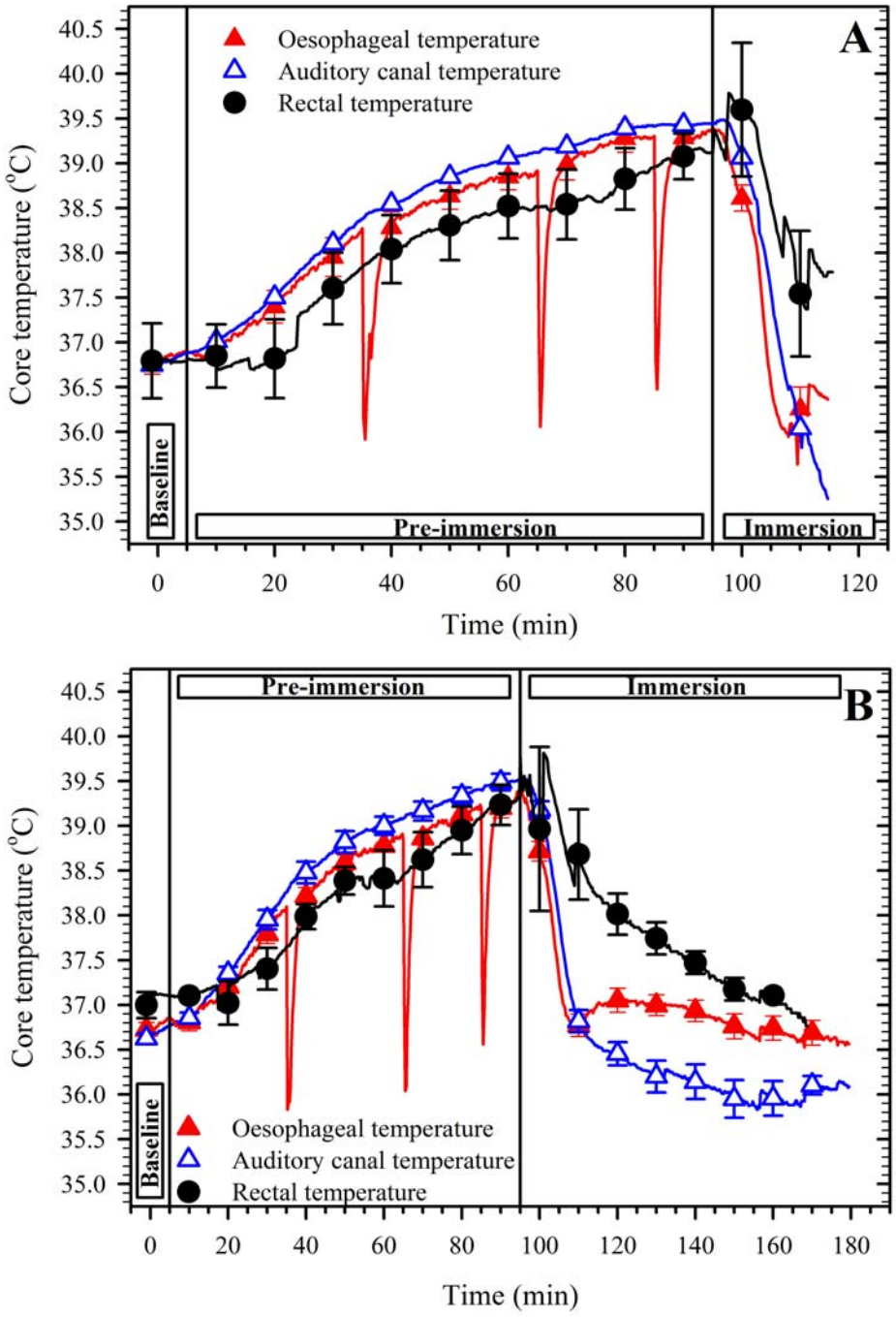

Figure 3.3: Oesophageal, auditory canal and rectal temperatures during combined endogenous (exercise) and exogenous heating (climate chamber (36ºC, 50% relative humidity), water-perfusion garment (40ºC)) to an oesophageal temperature of 39.5ºC, followed by two modes of passive cooling (supine): cold-water immersion (**A:** 14ºC) and temperate-water immersion (**B:** 26ºC).

Table 3.6: Pre-immersion oesophageal temperatures (°C)

Subject	Normothermic		Hyperthermic	
	Cold immersion (Trial A)	**Temperate immersion (Trial B)**	**Cold immersion (Trial C)**	**Temperate immersion (Trial D)**
S1	36.84	36.27	38.29	38.44
S2	37.14	37.50	38.98	38.25
S3	36.69	36.26	38.33	38.09
S4	37.46	37.31	38.73	38.70
S5	37.23	37.31	38.40	38.63
S6	37.42	36.62	38.44	38.68
S7	37.16	36.61	38.50	38.48
S8	36.98	36.99	38.53	38.18
Mean	37.11	36.85	38.52*	38.43*†
S.D.	0.09	0.17	0.08	0.06

Note: *denotes significantly different from ***Trial A*** (control with cold immersion); †denotes significantly differenct from ***Trial C*** (experimental with cold immersion).

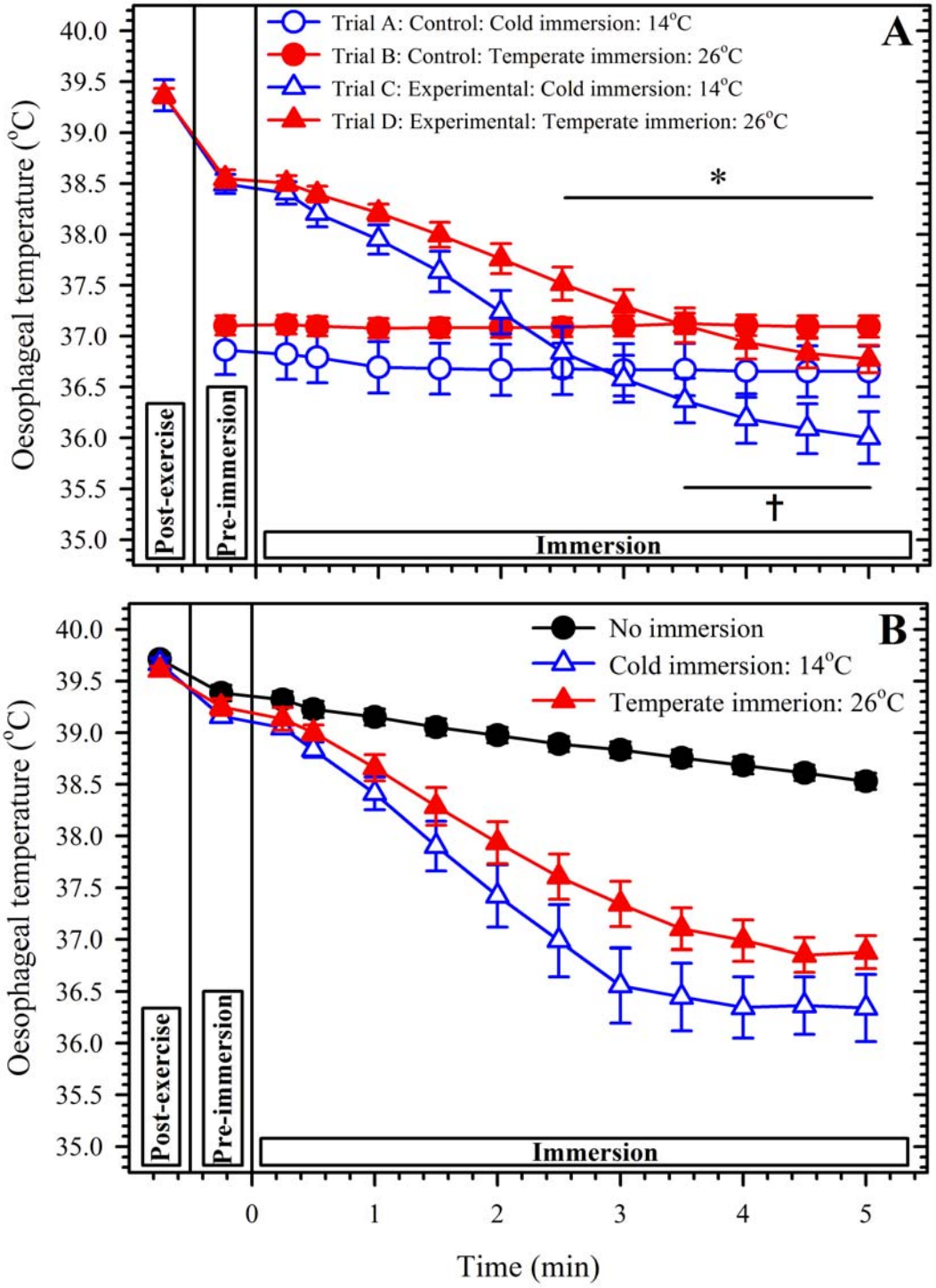

Figure 3.4: **A:** Oesophageal temperatures following heating (post-exercise), followed by two methods of cooling (supine): cold-water immersion (14ºC) and temperate-water immersion (26ºC). * significantly different from ***Trial C***; † significantly different from ***Trial B***. **B**: Oesophageal temperatures following heating (post-exercise) during three modes of cooling: air (22ºC); cold-water immersion (14ºC) and temperate-water immersion (26ºC). These data are from results presented in Chapter 2.

Table 3.7: Oesophageal temperature cooling rates ($^{o}C.min^{-1}$).

Subject	Normothermic						Hyperthermic					
	Cold immersion (Trial A)			Temperate immersion (Trial B)			Cold immersion (Trial C)			Temperate immersion (Trial D)		
	T_{es}	T_{au}	T_{re}	T_{es}	T_{au}	T_{re}	T_{es}	T_{au}	T_{re}	T_{es}	T_{au}	T_{re}
S1	0.024	0.044	0.012	0.047	0.019	0.018	0.352	0.276	0.317	0.941	0.192	0.254
S2	0.013	-	0.010	0.015	0.015	0.022	0.394	0.205	0.258	0.428	0.207	0.327
S3	0.021	0.027	0.100	0.054	0.018	0.027	0.415	0.262	0.167	0.394	0.234	0.062
S4	0.018	0.047	0.013	0.022	0.016	0.030	0.447	0.231	0.161	0.478	0.235	0.181
S5	0.008	0.036	0.011	0.034	0.013	0.019	0.449	0.289	0.337	1.129	0.239	0.326
S6	0.014	0.121	0.100	0.028	0.068	0.022	0.418	0.261	0.352	0.589	0.243	0.365
S7	0.020	0.044	0.010	0.054	0.048	0.013	0.307	0.193	0.083	0.435	0.176	0.040
S8	0.009	0.021	0.015	0.023	0.018	0.017	0.457	0.108	0.133	0.545	0.223	0.314
Mean	0.016	0.049	0.013	0.035	0.027	0.021	0.405*	0.228	0.226	0.617*†	0.219	0.234
SEM	0.002	0.012	0.01	0.005	0.01	0.003	0.052	0.059	0.103	0.27	0.024	0.126

Note: * denotes significantly different from ***Trial A*** (control with cold immersion); †denotes significantly different from ***Trial C*** (experimental with cold immersion; $P<0.05$)

However, this was not observed for the experimental trials. During the first 5 min of cold-water immersion (***Trial C***) and temperate-water immersion (***Trial D***) of slightly hyperthermic individuals who had performed exercise in the heat, oesophageal temperature decreased rapidly at a mean cooling rate of 0.62^{o}C.min^{-1} (±0.27) and 0.40^{o}C.min^{-1} (0.05) during cold-water and temperate-water immersions respectively (Table 3.7). These cooling rates closely matched those from our previous investigation (Taylor *et al*., 2008) during water immersion in the same two water temperatures (14^{o}C or 26^{o}C) following exercise in the heat using an identical protocol (Figure 3.4B). The target oesophageal temperature of 37.5^{o}C was achieved in 1.66 min (±0.20) for the cold-water immersion trial and 2.56 min (±0.22) for the temperate-water immersion (Table 3.8). Although these differences were significant (P<0.05) the time difference was only 55 s and did not differ significantly from the time to cool to 37.5^{o}C in previous investigations of 45 s (P>0.05; Taylor *et al*., 2008).

3.3.1.2 Auditory canal temperature

For the experimental (hyperthermic) trials (***Trials C*** and ***Trial D***), the auditory canal temperatures followed a similar pattern to that of oesophageal temperature (Figure 3.1). In addition, the rate of cooling for auditory canal temperatures during cold-water immersion (0.87^{o}C.min^{-1} (±0.36) and temperate-water immersion (0.61^{o}C.min^{-1} (±0.21)) were not different to that of the oesophageal temperatures (P>0.05). Although the heating and cooling patterns for the auditory canal measurements closely matched the oesophageal temperatures, the cooling pattern following the two control (normothermic) trials was quite different. Following a normothermic state, subjects experienced a significant reduction in auditory canal temperature during the cold-water (14^{o}C) immersion compared to the temperate-water immersion (26^{o}C). This was probably due to the environmental conditions as this measurement is easily influenced by external factors (Greenleaf and Castle, 1972).

3.3.1.3 Rectal temperature

At the completion of exercise in the heat, rectal temperature was 39.4^{o}C (± 0.1) and 39.4^{o}C (± 0.2) for the cold-water immersion (***Trial C***) and the temperate-water immersion trials (***Trial D***), respectively. These were not significantly different (P>0.05). Immediately prior to the immersion phase for both ***Trial C*** and ***Trial D*** rectal temperature dropped slightly (Figure 3.2), however this decrease was not significant with subjects maintaining a

Table 3.8: Time (min) to reach an oesophageal temperature of 37.5ºC during cooling in either cold (14ºC) or temperate (26ºC) water, following a hyperthermic state.

Subject	Hyperthermic	
	Cold immersion (Trial C)	Temperate immersion (Trial D)
S1	1.00	2.25
S2	1.75	3.75
S3	1.50	2.00
S4	2.00	2.75
S5	1.00	2.00
S6	2.00	2.25
S7	2.25	3.25
S8	1.25	2.20
Mean	1.66	2.56*
SEM	0.57	0.64

Note: * denotes significantly different from ***Trial C*** ($P<0.05$)

moderately hyperthermic state immediately prior to water immersion (P>0.05; cold: 39.0^{o} ±0.2^{o}C) and temperate: 39.1^{o} ±0.2^{o}C). Once immersed, rectal temperature dropped at a rate of 0.23^{o}C.min^{-1} (± 0.1) and 0.19^{o}C.min^{-1} (± 0.1) for the cold- and temperate-water immersions, respectively. These did not differ from the cooling rates observed in previous investigations (P>0.05; Taylor *et al*., 2008).

For ***Trial A*** and ***Trial B***, (Figure 3.1C), rectal temperature during cold-water immersion was not different to rectal temperature during temperate-water immersion. However, following 15 min of immersion, rectal temperature increased slightly during cold-water immersion, but decreased during temperate water immersion (P<0.05). This observation can be explained by powerful vasoconstrictor response from static activation of the cold peripheral thermoreceptors causing warm blood to be conserved within the central compartment.

3.3.2 Skin temperatures

3.3.2.1 Mean skin temperature

Mean skin temperature changes during the first 5 min of water immersion are shown in Figure 3.5. Prior to cooling, mean skin temperature for the two control trials did not differ significantly (P>0.05) and averaged 31.3^{o}C (±0.3) for the cold immersion and 31.7^{o}C (±0.2) for the temperate immersion. Similarly, there were no significant differences in the pre-immersion mean skin temperature for the experimental trials (***Trial C*** and ***Trial D***) which were 34.2^{o}C (±0.4) for the cold immersion, and 34.1^{o}C (±0.6) for the temperate immersion (P>0.05). Following immersion, mean skin temperature rapidly dropped and the rate of decrease was proportional to the temperature of the water. That is, the cooler the water the more rapid was the skin temperature reduction, and therefore the lower the mean skin temperature.

For the control and experimental trials, where subjects were immersed in cold water (14^{o}C; ***Trial A*** and ***Trial C***), mean skin temperature rapidly approached 14^{o}C within the first minute of immersion. At this time point and for these trials, mean skin temperature did not differ significantly (24.9^{o} ±0.7^{o}C and 23.7^{o} ± 0.7^{o}C, respectively; P>0.05). Similarly, for the remaining two trials (***Trial B*** and ***Trial D***), where subjects were immersed in temperate water

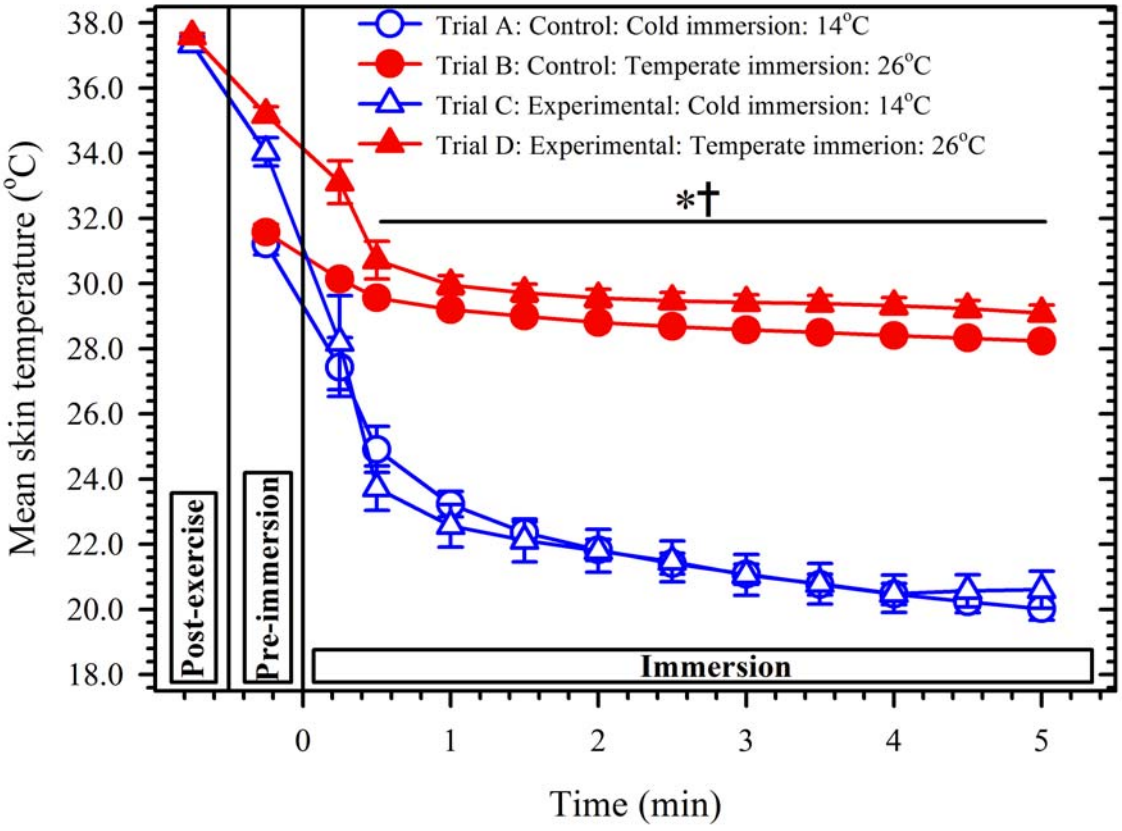

Figure 3.5: Mean skin temperature during two modes of passive, supine cooling (14ºC and 26ºC), following either a normothermic or hyperthermic state. The hyperthermic states were achieved by exercise in the heat (climate chamber (36ºC, 50% relative humidity), water-perfusion garment (40ºC). * denotes significantly different from ***Trial A*** (control with cold immersion); †denotes significantly differenct from ***Trial C*** (experimental with cold immersion; $P<0.05$)

(26ºC), mean skin temperature did not differ significantly beyond 30 s (29.6º ± 0.1ºC and 30.7º ± 0.6ºC, respectively; $P>0.05$). Although there were no differences in mean skin temperature between the control and experimental trials for each immersion temperature, mean skin temperatures during both temperate-water immersions were significantly higher than during the cold trials ($P<0.05$). When averaged across just the first 5-min cooling period, these temperatures were 22.1ºC (±5.3: ***Trial A***), 27.9º (±1.6: ***Trial B***), and 22.6ºC (±7.4: ***Trial C***), 29.9ºC (±3.2: ***Trial D***). Therefore, even though subjects were immersed at different body temperatures, it was the water temperature that dictated skin temperature within each trial.

3.3.2.2 Forearm skin temperature

While there was a rapid decrease in forearm skin temperature for all trials during immersion, the change in skin temperature during the first 5 min of immersion did not reflect that of mean skin temperature (Figure 3.6). The reduction in forearm skin temperature was significantly different between the two cold-immersion trials (***Trial A*** and ***Trial C***) for the first 2.5 min ($P<0.05$). During this time period, forearm skin temperature was higher for ***Trial A*** (normothermia) than ***Trial C*** (Hyperthermia). However, after 4 min of immersion these temperatures were equal ($P<0.05$). For the two trials where immersion was in temperate water (***Trial B*** and ***Trial D***) forearm skin temperature was significantly lower following the normothermic state compared to the hyperthermic state during the entire first 5 min of immersion ($P<0.05$).

When comparisons were made between the two hyperthermic trials (***Trial C*** and ***Trial D***), forearm skin temperature was significantly lower during the cold-water immersion (14ºC) than during temperate-water immersion (26ºC; $P<0.05$). This difference was, on average, 7.4ºC lower (±0.5) for the entire first 5 min of immersion. The initial drop in forearm skin temperature from pre-immersion to within the first 25 s of immersion was only 1.9ºC (±0.6) for ***Trial D*** (26ºC). This change was significantly less than that of the ***Trial C*** (14ºC) which was 6.8ºC (±1.2; $P<0.05$). At this site, the initial change in skin temperature reflected the temperature gradient.

3.3.3 Whole-body sweat rate

Whole-body sweat loss was only calculated for the experimental trials (***Trial C*** and

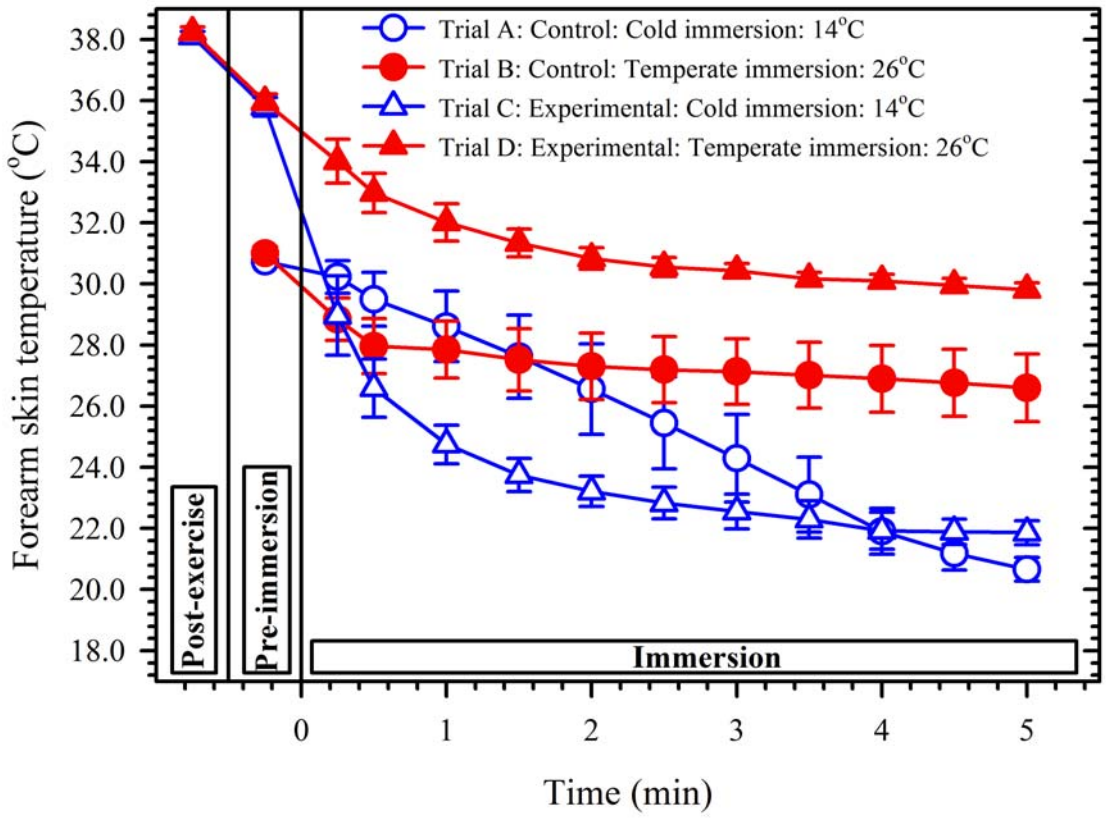

Figure 3.6: Forearm skin temperature during water immersion in either cold or temperate water following a normothermic state or hyperthermic state. (The hyperthermic states were achieved by exercise in the heat (climate chamber (36ºC, 50% relative humidity), water-perfusion garment (40ºC). * denotes significantly different from ***Trial A*** (control with cold immersion); †denotes significantly different from ***Trial C*** (experimental with cold immersion; $P<0.05$).

Trial D) since these trials involved exercise in the heat for 90 min. It was therefore important to account for any sweat losses that may have occurred during these conditions as it has been shown that dehydration can have significant influences of peripheral circulation (Kenney *et al*., 1990). Although subjects were required to drink at set intervals throughout the whole-body heating phase of the experimental trials (***Trial C*** and ***Trial D***), subjects experienced fluid deficits of 2-3% (Table 3.9) and were thus progressively dehydrated. However, the whole-body sweat losses for the cold-immersion trial (***Trial C***) was not significantly different to that of the temperate-immersion trial (***Trial D***; $P>0.05$). In addition, these values were not different to the whole-body sweat rates presented previously, for the same conditions (Taylor *et al*., 2008).

Since dehydration is associated with impaired thermoregulation through reductions in circulating blood volume (Nadel *et al.,* 1980) and elevations in plasma osmolality (Nadel *et al.,* 1980), it is possible that dehydration could reduce forearm blood flow in the current study, since dehydration has been shown to reduce forearm blood flow during prolonged heat exposures (González-Alonso *et al*., 1998). However, subjects only experienced 2-3% dehydration in the current study and it has been shown that levels greater than 3% are required to elicit a significant reduction in forearm blood flow (Hortsman and Horvath, 1972). Furthermore, the decrements in hydration were not different between the two hyperthermic trials meaning that the reductions in blood flow during cold water immersion are reflective of cold thermoreceptor activation rather than dehydration *per se*. Therefore, it is unlikely that the dehydration experienced during the experimental trials had any significant impact on the outcome of the forearm blood flow results.

3.3.4 Cardiovascular responses

3.3.4.1 Heart rate

The heart rate data for the two control trials (***Trial A*** and ***Trial B***) are presented in Figure 3.7A. For ***Trial A***, where subjects were immersed in cold water (14^{o}C), mean heart rate rapidly increased within the first 2 min of immersion. This increase was 18.8 (±7.5) beats.min^{-1} higher than ***Trial B*** (temperate immersion) at the same time point. This increased heart rate response to sudden cold water immersion is known as the cold-shock response (Tipton, 1989) and was not evident during the temperate-water immersion. These results are

Table 3.9: Whole-body sweat losses, as indicated by mass changes (%) during the pre-immersion phase of two experimental trials (***Trial C*** and ***Trial D***) which involved exercise in the heat: climate chamber (36ºC, 50% relative humidity) and water-perfusion garment (40ºC).

Subject	Cold immersion (Trial C)	Temperate immersion (Trial D)
1	1.54%	2.36%
2	2.04%	2.14%
3	3.16%	2.37%
4	3.75%	1.89%
5	2.10%	2.52%
6	2.13%	2.03%
7	3.62%	3.52%
8	2.67%	2.41%
Mean	2.63%	2.40%
S.D.	0.29%	0.18%

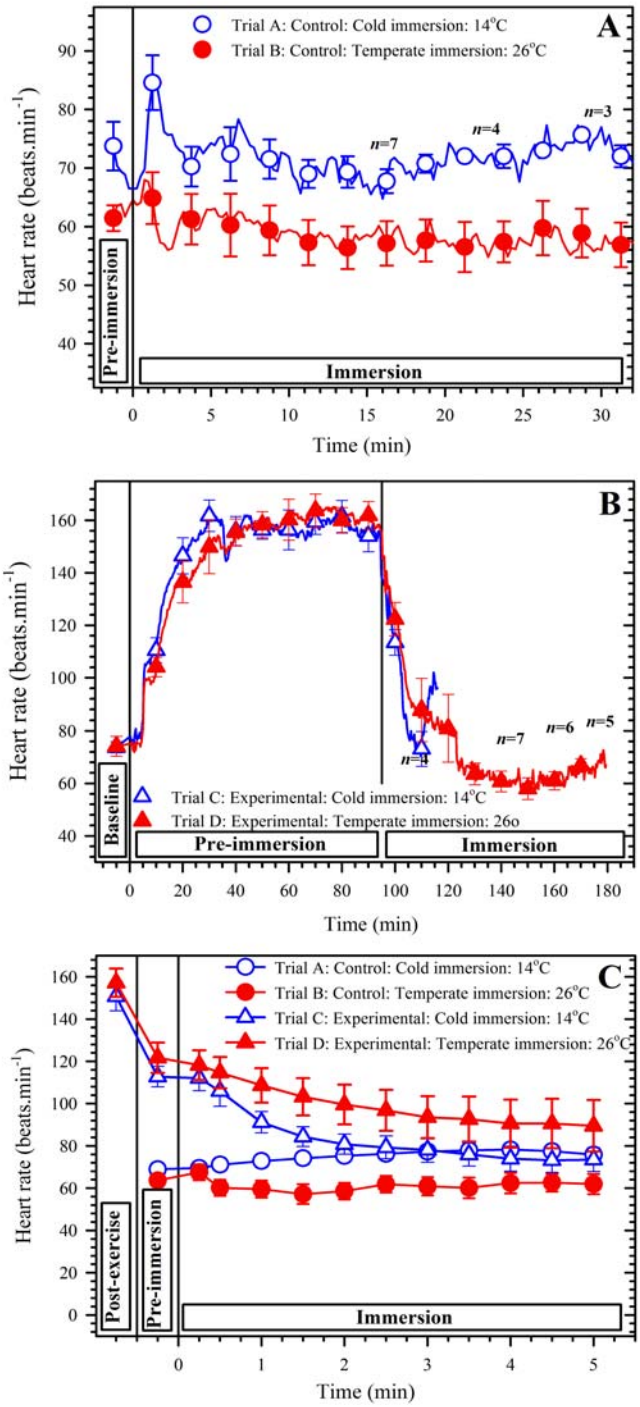

Figure 3.7: Heart rate during water immersion in either cold or temperate water following a normothermic state (**A**) or hyperthermic state (**B**). The hyperthermic states were achieved by exercise in the heat (climate chamber (36ºC, 50% relative humidity) and water-perfusion garment (40ºC). * denotes significantly different from ***Trial A*** (control with cold immersion); †denotes significantly different from ***Trial C*** (experimental with cold immersion; $P<0.05$).

consistent with previous findings from individuals suddenly immersed in cold (10°C) and temperate (27°C) water (Tipton, 1989). Although a stronger sympathetic response was evident during cold immersion following a normothermic state (***Trial A***), the same did not occur for either of the experimental trials (***Trial C*** and ***Trial D***). In fact, the mean heart rate for the entire 5 min of immersion in cold water following hyperthermia (***Trial C***) was reduced to 84.5 beats.min^{-1} (±5.1) compared to the temperate-water immersion (***Trial D***) which was 99.8 beats.min^{-1} (±9.5). This difference was significant (P<0.05).

3.3.4.2 Mean arterial pressure

The mean arterial pressure responses immediately prior to, and during, water immersion for all trials are presented in Figure 3.8. Prior to immersion mean arterial pressure (Figure 3.8A), for both experimental trials (***Trials C***: 70.5 mmHg (±1.6) and ***Trial D***: 76.4 mmHg (±6.7) where subjects were hyperthermic, were significantly lower than the control trials (***Trial A***: 94.6 ±2.2) and ***Trial B***: 97.9 ±3.9; P<0.05). This response is explained by the lower diastolic blood pressure as occurred for both experimental trials following exercise in the heat (Figure 3.8C). For these trials mean arterial blood pressure responses were not significantly different from each other (P>0.05) nor were they different during the first 5 min of immersion in cold water (87.0 ± 4.5) and temperate water (81.2 mmHg ±1.9).

Although there were no differences in blood pressure between the cold and temperate experimental trials (***Trial C*** and ***Trial D***), the blood pressure responses during water immersion for the control trials (normothermia) were different (P<0.05). For the cold-water immersion trial (***Trial A***), mean arterial pressure increased to 115.0 mmHg (±2.7) in the first minute of immersion. This was significantly higher than 99.4 mmHg (±3.2) for the temperate-water immersion (P<0.05). This response was also reflected for systolic and diastolic blood pressures and is a sign of a stronger sympathetic response (Figure 3.8B and Figure 3.8C). Throughout the cold-water immersion, mean arterial blood pressure remained significantly higher than during the temperate-water immersion. The mean arterial pressure averaged across the entire duration of cold-water immersion was 109.3 mmHg (±2.8) and 99.0 mmHg (±3.4) for the temperate-water immersion (P<0.05).

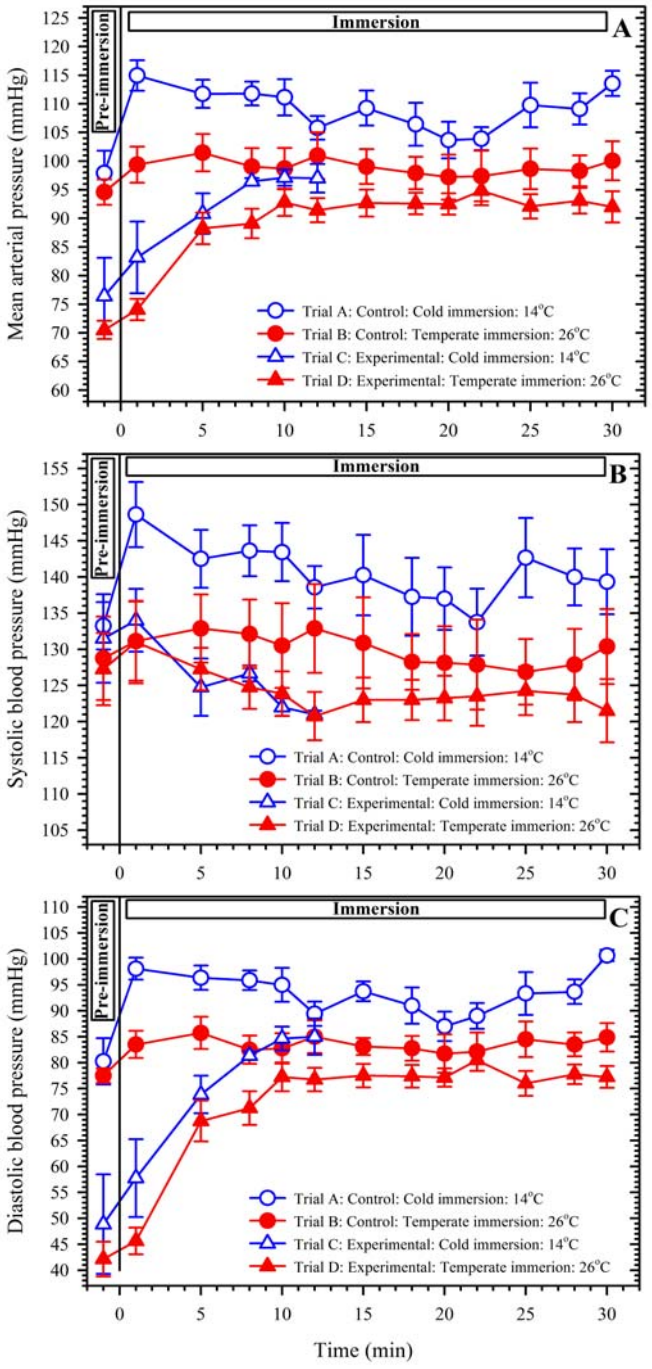

Figure 3.8: Mean arterial (**A**), systolic (**B**), and diastolic (**C**) blood pressures measured during cold-water immersion (14°C) and temperate-water immersion (26°C) following exercise in the heat or a thermoneutral baseline. The hyperthermic state was achieved by endogenous (exercise) and exogenous heating (climate chamber (36°C, 50% relative humidity), water-perfusion garment (40°C). * denotes significantly different from ***Trial A*** (control with cold immersion); †denotes significantly different from ***Trial C*** (experimental with cold immersion; $P<0.05$).

3.3.4.3 Forearm and cutaneous blood flow

There were no significant differences in forearm (Figure 3.9A) or cutaneous (Figure 3.9B) blood flow between either of the control trials during immersion (***Trial A*** and ***Trial B***; $P>0.05$). Although no differences were apparent, water immersion in both cold and temperate water elicited a reduction in forearm blood from the pre-immersion normorthermic state ($P>0.05$; Figure 3.10). Similarly, a significant reduction in forearm (Figure 3.9C) and cutaneous (Figure 3.9D) blood flow occurred for the experimental trials (***Trial C*** and ***Trial D***), when subjects were immersed in either cold (14^{o}C) or temperate (26^{o}C) water ($P<0.05$).

The greatest reduction in forearm and cutaneous blood flow occurred during the cold-water immersion (***Trial C***) and this was significantly lower than forearm and cutaneous blood flow during ***Trial D*** at each time point within the first 4 min of immersion ($P<0.05$). That is, significantly more powerful vasoconstriction was evident within the cooler water ($P<0.05$), even though core temperatures did not differ significantly between these trials prior to immersion ($P>0.05$). Therefore, it appeared that the cutaneous vascular responses were attributable to changes in local tissue temperature, and the 12^{o}C difference in water temperature did therefore modify the strength of this vasoconstrictor response.

3.3.4.4 Forearm vascular conductance

Since forearm and cutaneous blood flow showed similar patterns for all trials, only forearm vascular conductance is reported. When the influence of blood pressure on blood flow was removed through calculating vascular conductance, a much greater reduction in vascular conductance was observed during the colder-water immersion ($P<0.05$; Figure 3.11). This showed a significantly greater resistance to blood flow in the cold-water immersion trials following exercise induced hyperthermia than occurred during the temperate-water immersion (26^{o}C) therefore significantly less blood was delivered to the skin during immersion in 14^{o}C water ($P<0.05$). Although regional differences in cutaneous vascular responses exist, as occurs with sweating, forearm blood flow provides a good reflection of most non-acral tissues (Whitney, 1953), then it is assumed these data provide valid representation of the cutaneous vascular response for the majority of the body.

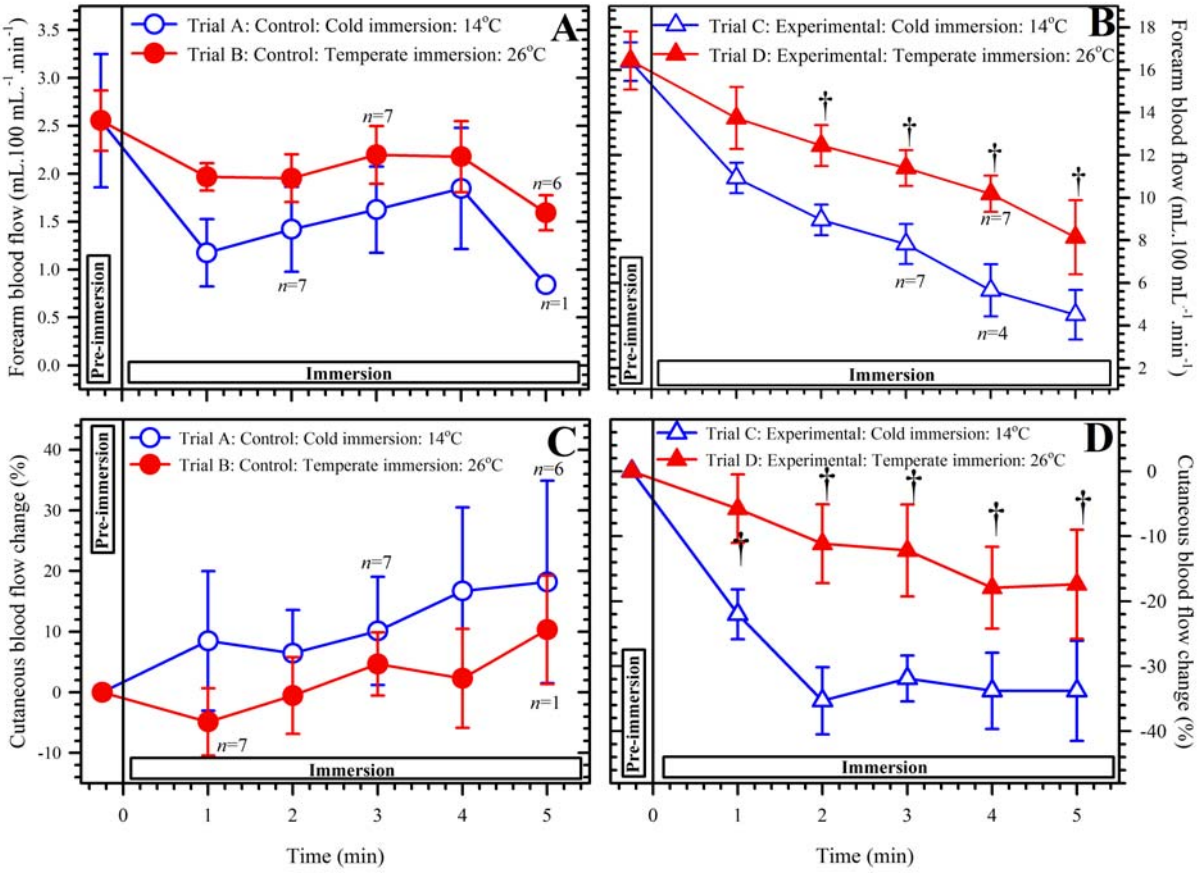

Figure 3.9: Forearm blood flow (venous-occlusion plethysmography) and the change in cutaneous blood flow (laser-Doppler flowmetry) during the first 5 min of cold-water immersion (14ºC) and temperate-water immersion (26ºC) following either a normothermic or hyperthermic state. The hyperthermic state was achieved by exercise in the heat (climate chamber (36ºC, 50% relative humidity) and water-perfusion garment (40ºC). * denotes significantly different from ***Trial A*** (control with cold immersion); †denotes significantly different from ***Trial C*** (experimental with cold immersion; $P<0.05$).

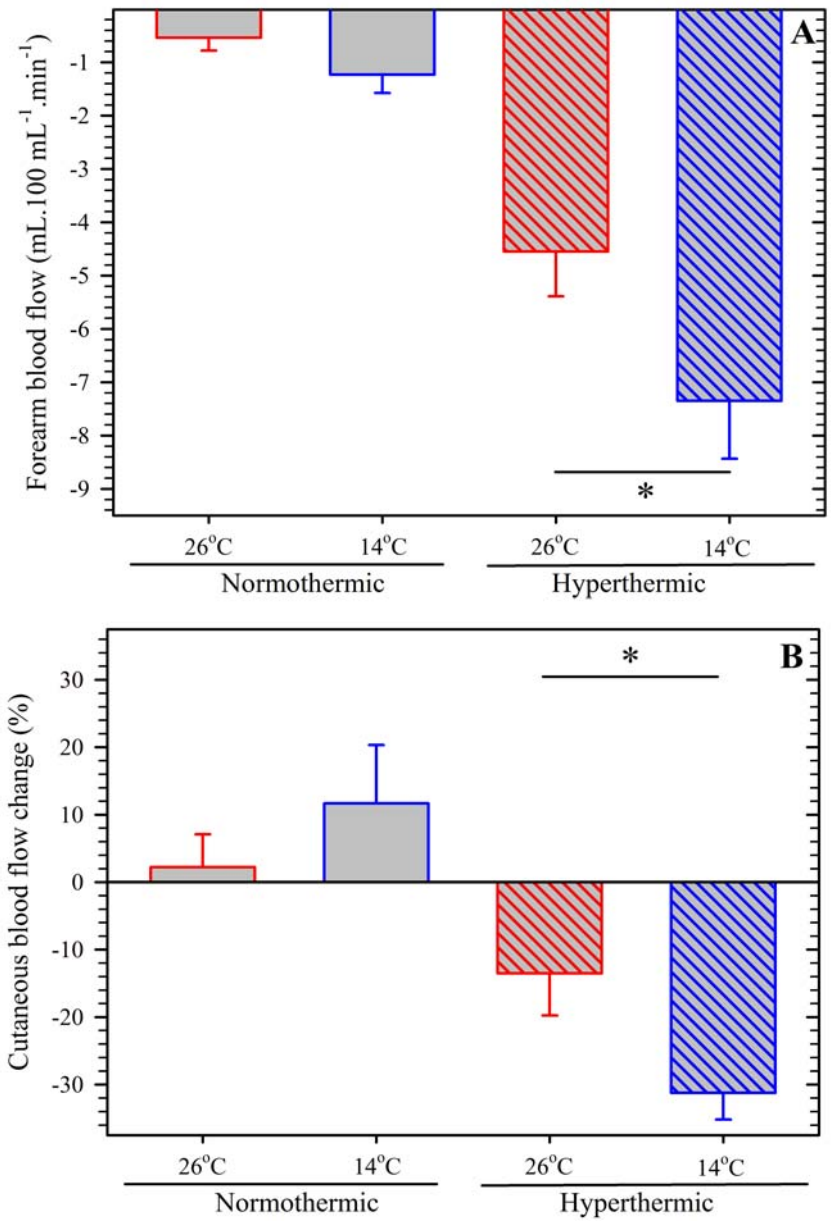

Figure 3.10: Changes in forearm (**A**) and cutaneous (**B**) blood flow during water immersion in either cold or temperate water following a normothermic state or hyperthermic state. The hyperthermic state was achieved by exercise in the heat (climate chamber (36ºC, 50% relative humidity) and water-perfusion garment (40ºC). * denotes significantly different from ***Trial A*** (control with cold immersion); †denotes significantly different from ***Trial C*** (experimental with cold immersion; $P<0.05$).

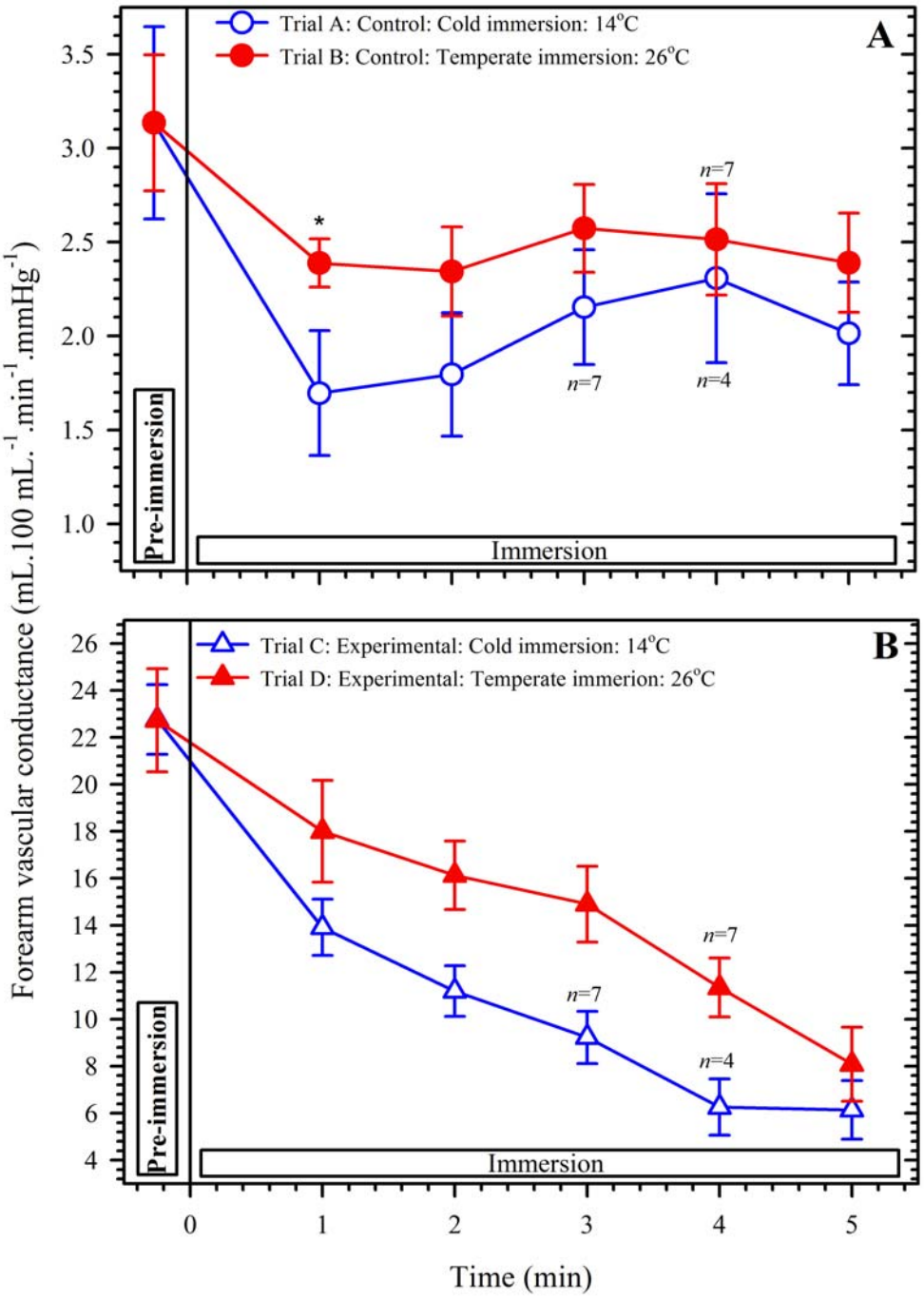

Figure 3.11: Forearm vascular conductance during the first 5 min of cold-water immersion (14ºC) and temperate-water immersion (26ºC) following either a normothermic or hyperthermic state. The hyperthermic state was achieved by exercise in the heat (climate chamber (36ºC, 50% relative humidity) and water-perfusion garment (40ºC). * denotes significantly different from ***Trial A*** (control with cold immersion); †denotes significantly different from ***Trial C*** (experimental with cold immersion; $P<0.05$).

3.3.4.5 Heat delivery from the core to the skin

When the thermal gradient between the body core (oesophageal) and skin (mean) surfaces, and skin blood flow were simultaneously considered, it was possible to approximate heat delivery to the skin (Figure 3.12). This was significantly higher during the first minute of water immersion for the immersion phase of ***Trial C*** ($P<0.05$; Figure 3.12). However, following 2 min of immersion, the rate of heat delivery to the skin was reduced substantially, and was not significantly different from heat delivery during ***Trial D*** (26^{o}C immersion; $P>0.05$). Therefore, the reduced heat delivery to the skin is a reflection of the more powerful vasoconstriction rather than being a simple function of the temperature gradient. For the normothermic trials (***Trial A*** and ***Trial B***), although not significant, the changes in heat delivery represented a reduction in the thermal gradient rather than changes in blood flow, since the changes in forearm blood flow at the same time points did not differ ($P>0.05$).

3.3.5 Psychophysical responses

Within the first five minutes of cold immersion (***Trial A***), subjects felt "very cold" to "cold" and this was significantly lower than during the temperate immersion (***Trial B***) where subjects felt "cool" ($P<0.05$; Figure 3.13). Subjects perceived both cold and temperate water immersions to be "slightly uncomfortable' to "uncomfortable" ($P>0.05$). Interestingly, for the experimental trials, subjects felt "uncomfortable" during the first 5 min of immersion in cold (14^{o}C) compared to feeling "comfortable" during immersion in temperate (26^{o}C) water ($P<0.05$). In fact, subjects felt more comfortable in 26^{o}C water following hyperthermia than they did from the normorthermic state ($P<0.05$). This is because when immersed in the same water temperature(26^{o}C) but hyperthermic (***Trial D***), subjects felt "slightly cool" to "neutral" which was warmer than when they were normothermic ($P<0.05$). These differences were not apparent for the control and experimental trials in 14^{o}C water ($P>0.05$).

3.3.6 Summary of results

Table 3.10 provides a summary of the physiological and psychophysical responses for the pre-immersion states (thermoneutral and hyperthermic) and during the first 5 min of immersion. It shows no difference within the pre-immersion states for the control trials (***Trial A*** and ***Trial B***) and experimental trials (***Trial C*** and ***Trial D***). There were no

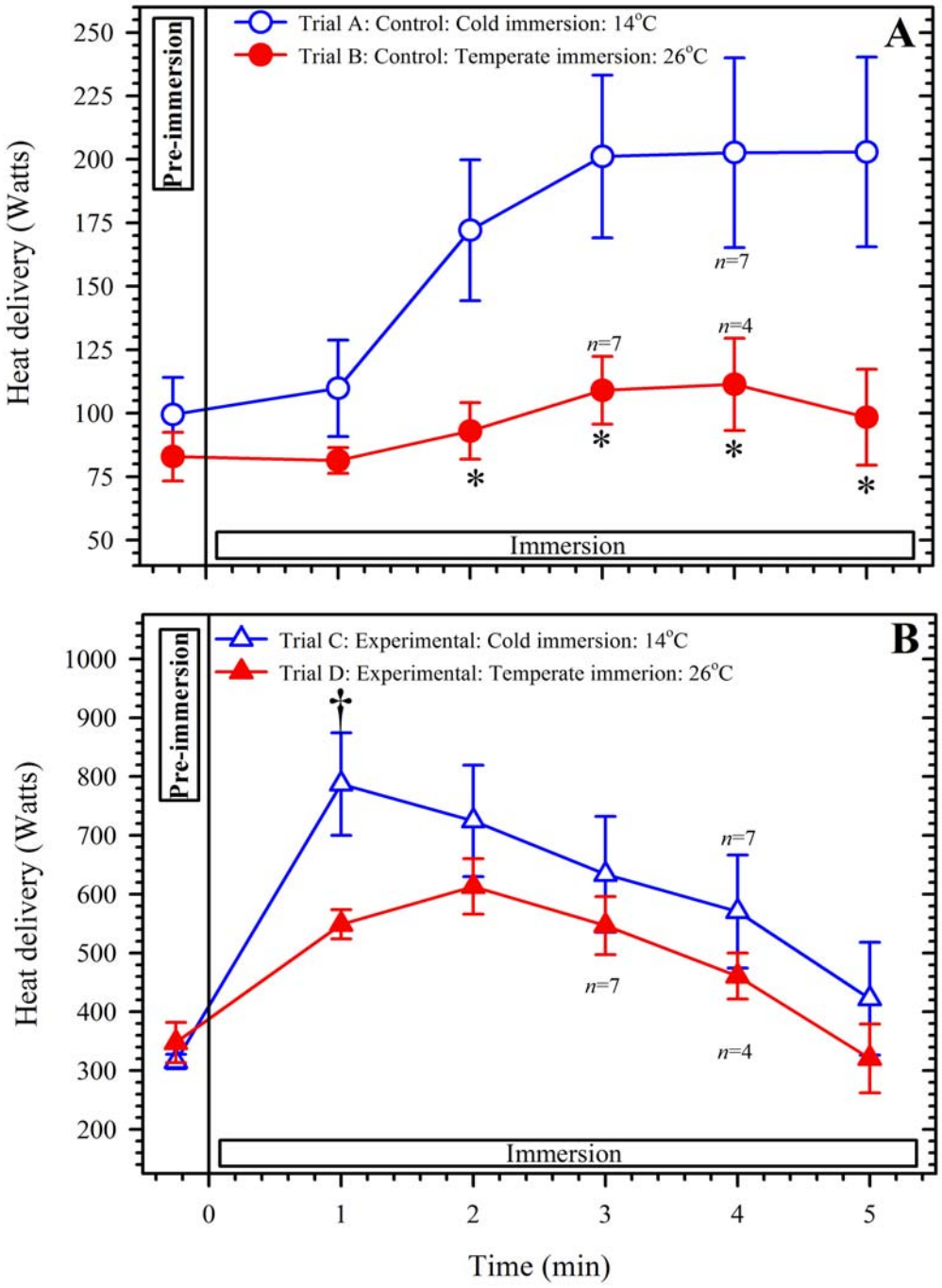

Figure 3.12: Heat delivery during water immersion in either cold or temperate water following a normothermic state (**A**) or hyperthermic state (**B**). The hyperthermic states was achieved by exercise in the heat (climate chamber (36ºC, 50% relative humidity), water-perfusion garment (40ºC). * denotes significantly different from ***Trial A*** (control with cold immersion); †denotes significantly different from ***Trial C*** (experimental with cold immersion; $P<0.05$).

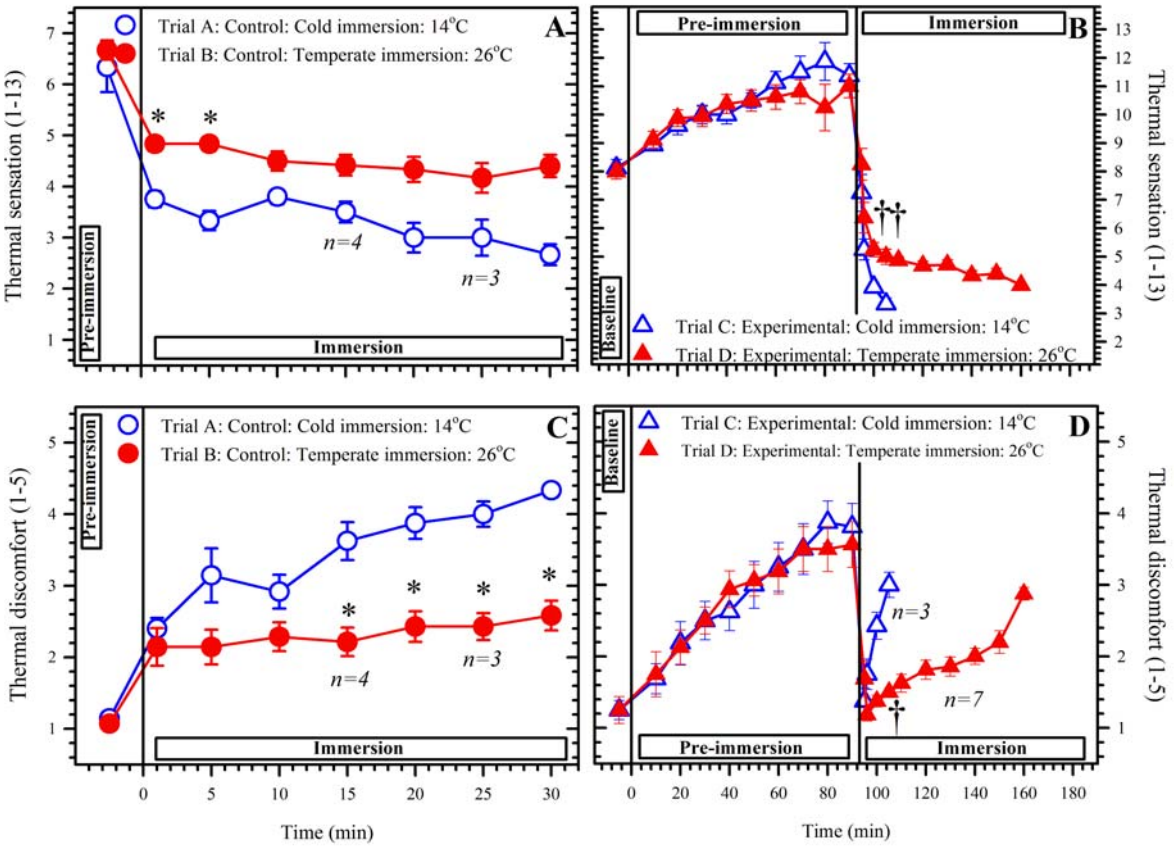

Figure 3.13: Thermal sensation (**A** and **B**) and thermal discomfort **(C** and **D**) responses during water immersion in either cold or temperate water following a normothermic state or hyperthermic state. The hyperthermic states was achieved by exercise in the heat (climate chamber (36°C, 50% relative humidity), water-perfusion garment (40°C). * denotes significantly different from ***Trial A*** (control with cold immersion); †denotes significantly different from ***Trial C*** (experimental with cold immersion; $P<0.05$).

Table 3.10: Physiological and psychophysical variables for normothermic and hyperthermic individuals immersed in cold (14ºC) or temperate water (26ºC). Data are presented as pre-immersion means (with standard errors of the means) and averaged across the first 5 min of immersion.

Physiological variable	Normothermic				Hyperthermic			
	Temperate immersion (Trial B)		Cold immersion (Trial A)		Temperate immersion (Trial D)		Cold immersion (Trial C)	
	Base	5 min	Base	5 min	Base	5 min	Base	5 min
Core temperature (ºC)	37.1 (0.1)	37.1 (0.1)	36.9 (0.2)	36.7 (0.2)	38.5† (0.1)	37.6*(0.1)	38.4† (0.1)	37.1*(0.2)
Cooling rate (ºC.min^{-1})		0.02 (0.0)		0.03 (0.0)		0.19† (0.01)		0.27*§† (0.03)
Mean skin temperature(ºC)	31.6 (0.3)	27.9 (1.0)	31.2 (0.3)	22.2*§ (0.3)	35.2† (0.2)	29.9* (0.3)	34.7† (0.2)	22.6*§ (0.7)
Heart rate (b.min^{-1})	63.7 (2.9)	61.2 (4.1)	68.9 (3.1)	75.7*§ (4.1)	121.7† (7.1)	99.8*† (9.5)	112.7† (4.9)	84.5*§† (5.1)
Mean arterial pressure (mmHg)	94.6 (2.2)	100.4* (3.1)	97.9 (3.9)	113.4*§ (2.5)	70.5† (1.6)	81.2*† (1.9)	76.4† (6.7)	87.0*§† (4.5)
Forearm blood flow (mL.100mL tissue^{-1}.min^{-1})	2.6 (0.3)	2.0 (0.2)	3.3 (0.6)	2.1 (0.4)	16.4† (1.4)	11.9*† (0.8)	16.6† (0.9)	9.3*§† (0.8)
Heat delivery (watts)	83.0 (9.6)	93.4* (10.7)	99.5 (14.6)	164.7*§ (26.9)	347.8† (34.2)	511.0*† (34.4)	315.5† (12.4)	664.5*† (76.7)
Thermal sensation (0-13)	6.7 (0.2)	4.8* (0.1)	6.3 (0.5)	3.3*§ (0.2)	8.3† (0.6)	5.3* (0.3)	8.4† (0.3)	3.9*§ (0.2)
Thermal discomfort (1-5)	1.1 (0.1)	2.1 (0.2)	1.1 (0.1)	3.1*§ (0.4)	1.7 (0.2)	1.4 (0.3)	1.4 (0.2)	2.4*§ (0.2

* significantly different from pre-immersion baseline (P<0.05). § significantly different from temperate immersion (P<0.05). † significantly different from thermoneutral (P<0.05).

significant differences between the control trials for forearm blood flow between the two water-immersion temperatures (14ºC or 26ºC) or following 5 min of immersion (P>0.05). However, for the experimental trials, forearm blood flow was significantly higher prior to immersion than when averaged across the duration of 5 min of immersion (P<0.05) and this was significantly lower during the first 5 min of cold water immersion (14ºC) than in 26ºC water (P<0.05). This difference is reflected by a significantly lower forearm blood flow in cold water immersion than temperate water immersion (P<0.05). Since the thermal gradient was higher in cold water than in temperate water, but forearm blood flow was less, heat delivery from the core to the skin was not significantly different between the two water immersion temperatures (P>0.05). In addition, subjects felt significantly cooler and more uncomfortable during the cold-water immersion compared to temperate-water immersion for both normothermic and hyperthermic states (P<0.05).

3.4 DISCUSSION

The primary purpose of the current study was to quantify skin blood flow during whole-body water immersion in two different temperatures (14ºC and 26ºC) following either normothermic or hyperthermic pre-conditioning. This is apparently the first study designed to explore the physiological mechanisms associated with similar cooling rates previously observed for cooling hyperthermic individuals in cold (14ºC) and temperate (26ºC) water (Taylor *et al*., 2008). Although a larger thermal gradient existed during cold immersion, the results indicate more powerful vasoconstriction occurred during cold-water immersion following hyperthermia. Hence reduced skin blood flow accounted for a reduction in the amount of heat delivered from the core to the skin, therefore cooling oesophageal temperature at a rate similar to immersion in temperate water following hyperthermia. This is in agreement with both hypotheses, however there was no difference in forearm blood flow between cold- and temperate-water immersions following normothermia. Therefore, cooling hyperthermic individuals in temperate water is just as effective as cooling in cold water and this is due to more powerful vasoconstriction, but more comfortable.

3.4.1 Thermoneutral and hyperthermic pre-immersion state

In order to determine if more powerful vasoconstriction occurred during cold-water immersion following hyperthermia, it was important that each of the control and experimental trials induced the appropriate pre-immersion thermal state. Firstly, this was

essential to ensure the control trials reflected a thermoneutral state as defined by a narrow range of core and skin temperatures where body temperature was only regulated through changes in vasomotor tone (Savage and Brengelmann, 1996), and this was achieved in the current study. Secondly, it was also important to ensure subjects were moderately to profoundly hyperthermic prior to immersion for the experimental trials and that this state represented environmental and physiological conditions as may be expected in the field. This was successfully achieved, as evident by an elevated mean body temperature, increased heart rate, decreased mean arterial pressure, increased skin blood flow and the onset of sweating immediately prior to immersion (Coyle and González-Alonso, 2001; Charkoudian, 2003).

In the normothermic state body temperature is regulated by changes in vasomotor tone so that the rate of heat transfer to and from the skin maintains body temperature within a neutral range without the onset of sweating or shivering. Although exact bounds of this zone are plastic, as will be shown in Chapter 6, a range of core temperatures (36^{o}-37^{o}C) and associated skin temperature (33^{o}-35^{o}C) have previously been shown (Hardy and DuBois, 1938) and this was reflected in the current study. In addition, sweating and shivering were absent. In this neutral state, skin blood flow is controlled by sympathetically driven vasoconstriction as a result of afferent signals from overlap of both cold- and warm-thermoreceptor activation. Cold thermoreceptors have been shown to discharge statically over a temperature range of -5^{o} to 43^{o}C, with a decline in impulse frequency above 25^{o}C,while warm thermoreceptors show static discharge above 30^{o}C (Hensel, 1981).The resultant forearm blood flow in this neutral state, has been reported to range from 2-6 mL.100 mL^{-1} min^{-1} and this is consistent with the blood flow results in the current study (Love and Shanks, 1962; Caldwell and Taylor, 2014) .

However, when subjects were hyperthermic, and core and skin temperatures were driven outside the thermoneutral zone, changes in skin blood flow alone were not sufficient enough to regulate body temperature. As a result of the increased heat stimulus, an increase in impulse frequency of the warm thermoreceptors occurred along with decreased firing of cold thermoreceptors (Hensel *et al.,* 1974). These signals are processed in the preoptic anterior hypothalamus enabling the generation of autonomic responses to increase vasodilatation (passive and active) and sweating (Wyndham, 1965; Mekjavic and Eiken,

2006). The physiological mechanisms associated with these thermoregulatory and cardiovascular variables within the normothermic and hyperthermic states greatly influence the resultant mechanisms during the immersion phase of all trials. Therefore, understanding these two states was important in the exploration of the physiological responses during immersion.

3.4.2 Physiological response during water immersion from thermoneutral pre-conditioning

Sudden activation of cold thermoreceptors, as occurred during water immersion in the current study, causes an overshoot in receptor firing, where the magnitude of the response is dependent on the rate of local temperature change (Hensel *et al.*, 1974; Braun *et al*., 1980). That is, the greater the change in local temperature, the more intense the response. It is no surprise then that the impulse frequency of cold thermoreceptor firing in the 14ºC water immersion was likely to be greater than during the 26ºC water immersion since the change in skin temperature from the normothermic pre-immersion state was 17ºC and 5ºC respectively. These thermal afferent signals received from dynamic activation of cold thermoreceptors are integrated in the hypothalamus and generate a large sympathetic response also known as the cold-shock response (Tipton, 1989).

The cold-shock response has been shown to begin to occur within the first 2-3 s of immersion in cold water (Tipton and Golden, 1987) and is characterised by an increase in heart rate, cardiac output, mean arterial pressure and peripheral vasoconstriction (Cooper *et al*., 1976; Keatinge *et al*., 1964; Keatinge and McCance, 1957; Barcroft and Edholm, 1943). While cardiac output was not measured in the current study, an increased heart rate of approximately 23 beats.min^{-1} occurred in the 14ºC water compared to the 26ºC water within 2 min of immersion. This is in agreement with previous findings of individuals immersed in 10ºC water, an increase of 20 beats.min^{-1} above that seen in immersion of 27ºC (Cooper *et al*., 1976). Although a slight reduction in forearm blood flow was observed in 14ºC water compared to 26ºC water following 1 min of immersion, this difference was not significant. This is possibly because skin temperature in both water temperature were well below 33ºC which has been show to induce significant reductions in skin blood flow (Barcroft and Edholm, 1943). However, when averaged across the entire 5 min of immersion, forearm blood flow was significantly lower in14ºC water than 26ºC. This would explain why

oesophageal temperature during this period was not different to the 26ºC water, as less blood was flowing to the skin, therefore reducing the amount of heat delivery, but the larger thermal gradient meant that more heat was being lost through conduction alone.

3.4.3 Physiological response during water immersion from hyperthermic pre-conditioning

When the hyperthermic individuals were immersed in both water bath temperatures, a favourable thermal gradient existed allowing heat to flow from the core to the skin, and from the skin to the water. However, even in temperate (26ºC) water a sizeable thermal gradient existed allowing for rapid heat to be removed from the individual and rapidly reduced core temperature. Although it has been shown that the larger the thermal gradient, the greater the heat exchange, when subjects were immersed in 14ºC water, giving an extra 12ºC gradient, leading to 20ºC between the skin and the water and 4ºC between the core and the skin, the difference in cooling rate was not of practical significance between the two water immersion temperatures.

When the effect of rapidly activating peripheral cold thermoreceptors during immersion in 14ºC water was considered it was difficult to predict the exact thermoregulatory response. We know that the thermoeffector response of being hot is increased vasodilatation to facilitate convective heat delivery to the skin, and this was shown in the current study as evident by high blood flow immediately prior to immersion. As soon as the cold water came in contact with the skin, rapid activation of cold-peripheral thermoreceptors occurred. In the cold water, the intensity was greater than in the temperate water since the thermoresponsiveness is dictated by the magnitude of the change in temperature rather than the absolute temperature *per se* (Hensel, 1981). In this instance, two different afferent input signals were delivered to the hypothalamus, firstly deep tissue warm thermoreceptors were activated due to the heat within the body, but the rapid activation of cold receptors was also generated. That is, afferent flow from warm receptors acts to maintain thermoregulatory mechanisms in order to facilitate heat loss through increased vasodilatation and sweating. However, the signalling from dynamic activation of cold thermoreceptors within the skin is integrated to cause thermoefferent increased vasoconstriction. So does one input override the other?

In the current study, forearm blood flow decreased rapidly when averaged across the duration of the first five minutes of immersion and this reduction was greatest in cold water. This suggests that, although the core temperature remained elevated, the fast activation of cold thermoreceptors was more powerful than the constant activation of warm receptors. That is, the cold water stimulated fast and powerful activation of cold sensors on the skin and initiated smooth muscle activation within peripheral arteries to actively vasoconstrict, thereby reducing blood flow to the skin. Heat delivery was the same during cold- and temperate-water immersion, as a result of reduced skin blood flow during cold-water immersion. Although the impulse frequency was high during the initial stages of cold-water immersion, there was no evidence of cold shock following exercise-induced hyperthermia.

Since subjects acted as their own controls, it was assumed that any changes observed between each trial in tissue insulation were purely indicative of blood flow. In addition, changes in insulation are likely to be the result of changes in blood flow. It was also assumed that forearm blood flow was reflective of cutaneous blood flow over the entire body. However, we are well aware that the distribution of cutaneous blood flow is not uniform around the entire body. In fact, non-acral and acral skin regions have different mechanisms for the control of blood flow. This concept is explored in later chapters (Chapter 4 and Chapter 5).

3.5 CONCLUSION

The current study provided mechanistic evidence for choosing temperate water over cold water for the rapid cooling of individuals experiencing profound hyperthermia. It is evident that a more powerful vasoconstriction occurred in response to cold-water immersion compared to temperate water. It is possible that the afferent stimulus from dynamic activation of peripheral cold thermoreceptors was more powerful than that from the constantly activated warm deep tissue receptors. This increased vasoconstriction reduced convective heat delivery from the core to the skin, and during the first few minutes of immersion, led to almost identical reductions in core temperature during the remainder of the immersion. To obtain a complete understanding of these interactions we will now explore the interactions between core and local skin temperature on cutaneous blood flow in both acral and non-acral skin regions. This will involve further investigation into the relationship between central and peripheral temperatures upon cutaneous blood flow and included

mapping of cutaneous blood flow across a range of thermal states and regions.

3.6 REFERENCES

Astrand, I. (1960). Aerobic work capacity in men and women with special reference to age. *Acta Physiologica Scandinavica Supplementum.* 49:1-92.

Barcroft, H., and Edholm, O.G. (1943). The effect of temperature on blood flow and deep temperature in the human forearm. *Journal of Physiology (London).* 102:5-20.

Braun, H.A., Bade, H., and Hense, H. (1980). Static and dynamic patterns of bursting cold fibers related to hypothetical receptor mechanisms. *Pflugers Archiv.* 386:1-9.

Caldwell, J.N., and Taylor, N.A.S. (2014). Water-displacement plethysmography: a technique for simultaneous thermal manipulation and measurement of whole-hand and whole-foot blood flows. *Phusiological Measurement.* In print.

Casa, D.J., and Kenny, G.P. (2009). In defense of cold water. *Medicine and Science in Sports and Exercise.* 41:1164

Casa, D.J., Armstrong, L.E., Kenny, G.P., O'Connor, F.G., and Huggins, R.A. (2012). Exertional heat stroke: New concepts regarding cause and care. *Current Sports Medicine Reports.* 11(3):115-123.

Charkoudian, N. (2003). Skin blood flow in adult human thermoregulation: how it works, when it does not, and why. *Mayo Clinic Proceedings.* 78:603-612.

Cooper, K.E., Martin, S., and Riben, P. (1976). Respiratory and other responses in subjects immersed in cold water. *Journal of Applied Physiology.* 40:903-910.

Cotter, J.D., Mark, A.J., Regan, J.M., and Taylor, N.A.S. (1993). Optimal sites for the measurement of human skin blood flow using laser Doppler velocimetry. *Proceedings of the Australian Physiological and Pharmacological Society*. 24:184.

Coyle, E.F., and González-Alonso, J. (2001). Cardiovascular drift during prolonged exercise: New perspectives. *Exercise and Sport Science Reviews.* 29:88-92.

Gagnon, D., Lemire, B.B., Jay, O., and Kenny, G.P. (2010). Aural canal, esophageal, and rectal temperatures during exertional heat stress and the subsequent recovery period. *Journal of Athletic Training.* 45:157-163.

González-Alonso, J., Calbet, J.A.L., and Nielsen, B. (1998). Muscle blood flow is reduced with dehydration during prolonged exercise in humans. *Journal of Physiology.* 513:895-905.

Greenleaf, J.E., and Castle, B.L. (1972). External auditory canal temperature as an estimate of core temperature. *Journal of Applied Physiology.* 32:194-198.

Hardy, J.D., and DuBois, E.F. (1938). Basal metabolism, radiation, convection and

vaporization at temperatures of 22 to 35ºC. *The Journal of Nutrition.* 15:477-497.

Hensel, H., Andres, K.H., and Düring, M.v. (1974). Structure and function of cold receptors. *Pfl*ü*gers Archive*. 352: 1-10.

Hensel, H. (1981). Thermoreception and temperature regulation. *Monographs of the Physiological Society.* 38:1-321.

Horstman, D.H., and Horvath, S.M. (1972). Cardiovascular and temperature regulatory changes during progressive dehydration and euhydration. *Journal of Applied Physiology.* 33:446-450.

ISO 9886. (1992). *Evaluation of thermal strain by physiological measurements.* International Standard Organisation, Geneva. Pp. 9-11.

Keatinge, W.R., and McCance, R.A. (1957). Increase in venous and arterial pressure during sudden exposure to cold. *Lancet.* Ii:208-209.

Keatinge, W.R., McIlroy, M.B., and Goldfien, A. (1964). Cardiovascular responses to ice-cold showers. *Journal of Applied Physiology.* 19:1145-1150.

Kenney, W.L., Tankersley, C.G., Newswangers, D.L., Hyde, D.E., Puhl, S.M., and Turner, N.L. (1990). Age and hypohydration independently influence the peripheral vascular response to heat stress. *Journal of Applied Physiology.* 68:1902-1908.

Love, A.H.G., and Shanks, R.G. (1962). The relationship between the onset of sweating and vasodilatation in the forearm during body heating. *Journal of Physiology*. 162:121-128.

Mekjavic, I.B., and Eiken, O. (2006). Contribution of thermal and nonthermal factors to the regulation of body temperature in humans. *Journal of Applied Physiology.* 100: 2065-2072.

Mekjavic, I.B., and Rempel, M.E. (1990). Determination of esophageal probe insertion length based on standing and sitting height. *Journal of Applied Physiology.* 69:376-379.

Nadel, E.R., Fortney, S.M., and Wenger, C.B. (1980). Effect of hydration state on circulatory and thermal regulations. *Journal of Applied Physiology.* 49:715-721.

Pergola, P.E., Johnson, J.M., Kellogg, D.L., and Kosiba, W.A. (1996). Control of skin blood flow by whole body and local skin cooling in humans. *American Journal of Physiology.* 270:H208-H215.

Proulx, C.I., Ducharme, M.B., and Kenny, G.P. (2003). Effect of water temperature on cooling efficiency during hyperthermia in humans. *Journal of Applied Physiology.*

94:1317-1323.

Proulx, C.I., Ducharme, M.B., and Kenny, G.P. (2006). Safe cooling limits from exercise-induced hyperthermia. *European Journal of Applied Physiology.* 96:434-445.

Savage, M.V., and Brengelmann, G.L. (1996). Control of skin blood flow in the neutral zone of human body temperature regulation. *Journal of Applied Physiology.* 80: 1249-1257.

Taylor, N.A.S., Caldwell, J.N., van den Heuvel, A.M.J., Patterson, M.J. (2008). To cool, but not too cool: that is the question: immersion cooling for hyperthermia. *Medicine and Science in Sport and Exercise.* 40(11):1962-1969.

Taylor, N.A.S., Caldwell, J.N., van den Heuvel, A.M.J., Patterson, M.J. (2009). Response. *Medicine and Science in Sport and Exercise.* 41:1165.

Tipton, M.J., and Golden, F. St C. (1987). The influence of regional insulation on the initial responses to cold immersion. *Aviation, Space and Environmental Medicine.* 58:1192-1196.

Tipton, M. J. (1989). The initial response to cold-water immersion in man. *Clinical Science.* 77:581-588.

Vallerand, A.L., Savourey, G., Hanniquet, A., Bittel, J.H.M. (1992). How should body heat storage be determined in humans: by thermometry or calorimetry? *European Journal of Applied Physiology.* 65:286-294.

Whitney, R.J. (1953). The measurement of volume changes in human limbs. *Journal of Physiology.* 121:1-27.

Wyndham, C.H. (1965). Role of skin and core temperatures in man's temperature regulation. *Journal of Applied Physiology.* 20: 31-36.

CHAPTER 4: WATER-DISPLACEMENT PLETHYSMOGRAPHY: A TECHNIQUE FOR THE SIMULTANEOUS THERMAL MANIPULATION AND MEASUREMENT OF WHOLE-HAND AND WHOLE-FOOT BLOOD FLOWS

4.1 INTRODUCTION

Research from the current laboratory has recently been directed towards better understanding the roles played by the hands and feet in whole-body temperature regulation (Taylor *et al*., 2009). Although often not appreciated, these appendages, like elephant's ears (Phillips and Heath, 2001) and toucan's bills (Tattersall *et al*., 2009), are powerful participants in the autonomic control of heat loss and conservation. To this end, detailed evaluations of sweat secretion from these segments have been reported (Taylor *et al*., 2006; Babic *et al*., 2008; Machado-Moreira *et al*., 2008a; Smith *et al*., 2013). These, in combination with data from other body regions (Machado-Moreira *et al*., 2008b, 2008c), have led to a more complete appreciation of whole-body and segmental evaporative cooling (Taylor and Machado-Moreira, 2013), yet missing from these descriptions is information concerning regional differences in heat delivery from the core to the shell tissues. One reason for this is the difficulty involved with measuring blood flow within hands and feet. Therefore, in this communication, attention is directed to the measurement of limb-segment blood flow, and how this might also be achieved within an entire hand and foot.

Unfortunately, most contemporary techniques for measuring blood flow are ill-suited to quantifying whole-segment flows. Venous-occlusion plethysmography is a notable exception, and it has been extensively used to advance our understanding of organ blood flow (Schäfer, and Moore, 1896; Brodie, 1902; Brodie and Russell, 1905; Joyner *et al*., 2001; Roddie, 2011). The principles of plethysmography originate from the work of Angelo Mosso (1846-1910) who developed a displacement apparatus in the 1870s for measuring organ volume and perfusion de Cyon, 1876; Fleckenstein, 1984; Di Giulio, 2011). This technique has several physiological applications, and it is the method of choice for some pulmonary function measurements (Comroe *et al*., 1959; Blonshine and Goldman, 2008).

Herein, water-displacement, venous-occlusion plethysmography was used initially to determine segmental volume changes of the forearm, but then for the whole hand and the entire foot during local heating and cooling. The emphasis was upon cutaneous blood flow and, at rest, it has been established that thermally induced variations in limb blood flow are principally restricted to the cutaneous compartment (Edholm *et al*., 1956; Detry *et al*., 1972; Johnson *et al*., 1976). This is also the case during exercise when the limb in question is inactive (Johnson and Rowell, 1975). It was therefore assumed that plethysmographically determined hand and foot volume changes would track cutaneous vasomotor responses, and that muscle blood flow would remain stable during the localised manipulation of skin temperature in resting individuals.

However, one of the difficulties in measuring cutaneous blood flow is its role as a common effector arm within both the regulation of mean body temperature and mean blood pressure (Rowell, 1977; Kenney *et al*., 2013), particularly within heated individuals (Johnson and Proppe, 2011; Rowell, 2011). Thus, there are circumstances in which these integrated physiological systems will independently modulate blood flow, and this dictates a high degree of experimental control to distinguish among the various causal mechanisms. For instance, within the temperature domain, the thermoeffectors respond to feedback from thermosensitive sites within both the deep-body (core) and superficial (skin) tissues (Proppe *et al*., 1976; Werner *et al*., 2008; Jessen, 2011), with cutaneous feedback being significant when its temperature undergoes sudden changes (Libert *et al*., 1978), but also during heat adaptation (Regan *et al*., 1996; Taylor, 2014). Therefore, it is necessary to use thermal clamping techniques (Cotter and Taylor, 2005) when attempting to separate the independent roles of these core and skin thermoafferent signals. Since our long-term objective was to quantify cutaneous blood flow for the entire hand and foot across a wide range of different core and local skin temperatures as each variable was independently manipulated, and since laser-Doppler flowmetry is not adequate for this purpose, then it became necessary to construct displacement plethysmographs that permitted both blood flow measurement and independent thermal clamping. In this report, the operational characteristics, design, validation and use of such devices are described.

4.2 METHODS

4.2.1 Methodological overview

4.2.1.1 Operating principles

Venous-occlusion plethysmography relies on structural and functional differences between the veins and arteries, with the latter having thicker walls and greater perfusion pressures. The application of an appropriate external pressure (collecting cuff: 30-70 mmHg) can selectively occlude venous return whilst arterial inflow will initially remain unimpaired. This causes swelling in the limb segment distal to the occlusion with each cardiac cycle, which is proportional (at least initially) to the rate at which blood enters the segment, and a plethysmograph is ideally suited to measuring the rate of this volume change (Greek: *plethysmos* meaning "increase" and *graphein* "to write or represent"). However, blood flow measurements cannot be performed instantaneously, as would be the case with laser-Doppler flowmetry, but are instead dictated by the duration and frequency of each inflation/deflation cycle of the occlusion cuff.

During occlusion, the pressure and volume of venous blood gradually rise, reducing the arteriovenous pressure gradient and arterial flow. Eventually, a flow plateau is reached. Greenfield and Patterson (1954) demonstrated that during the initial period of occlusion, blood flow measured by a plethysmograph was identical to arterial inflow, but as this flow plateau was approached, arterial flow declined. If the occlusion is sustained, venous pressure will eventually rise to that of the occlusion cuff and blood will escape. Therefore, for blood flows to faithfully represent arterial flow, the occlusion cuff must prevent venous outflow (Landowne and Katz, 1942), and flow should only be analysed over the linear portion of the volume curve (Greenfield and Patterson, 1954) and beyond the initial artefact created by cuff inflation (Greenfield *et al*., 1963).

4.2.1.2 Plethysmograph design and construction

Several classical manuscripts were used as resources for the preliminary design specifications (Abramson *et al*., 1939; Allwood and Burry, 1954; Greenfield, 1954; Jonson *et al*., 1970). However, to provide a capacity to independently clamp segmental temperature whilst simultaneously measuring blood flow, it was necessary to construct plethysmographs with two water-filled, aluminium compartments. The inner compartment

surrounded the limb segment of interest, which was covered with a well-fitting latex membrane (sleeve, glove or sock), and was separated from the outer compartment by an aluminium wall (Figures 4.1A and 4.1B). This membrane was sealed to the plethysmograph using a flange and rubber diaphragm (Figures 4.1C and 4.1D), making a water-tight displacement compartment that was isolated from, but enclosed by, the outer water-filled compartment. This outer compartment was plumbed to water baths (type VFP, Grant Refrigeration Systems, U.K.) that were separately regulated at various temperatures. Water was continuously pumped through this compartment, the volume of which was ten-fold greater than the inner compartment, thereby enabling relatively rapid water-temperature changes of the inner, displacement compartment. This provided a capacity to change segmental temperature whilst the thermal state of the rest of the body was clamped.

The dimensions of each plethysmograph (Figure 4.1) were set to match anthropometric data from males with a broad range of sizes. In addition, the latex membranes were made using plaster moulds, constructed using plaster-of-paris bandage (150 mm) wrapped around the foot and forearm of designated (representative) individuals. When a firm shell (die) was established, it was filled with liquid plaster. After removing the shell, gaps were filled with plaster and the mould was sanded to a smooth finish, sealed with a primer undercoat and finished with two coats of gloss paint. For the hands, standard latex (medium) dishwashing gloves were used. To make the latex membranes, each mould was submerged into dipping latex (Spray/Dip latex, Dalchem Pty. Ltd., Cheltenham, Australia), then removed for partial drying (hair dryer: 3-5 min). This step was repeated 3-4 times to ensure the membrane was thick enough to prevent tearing, but delicate enough to ensure compression against the skin by the surrounding water, whilst providing minimal resistance to limb-segment swelling during the occlusion of venous return.

4.2.1.3 Clamping body-tissue temperatures

In the past, thermal clamping has become a necessary research adjunct to several mechanistic projects in the current laboratory (Patterson *et al*., 1998; Cotter and Taylor, 2005; Machado-Moreira *et al*., 2012), and this technique was again used. In combination

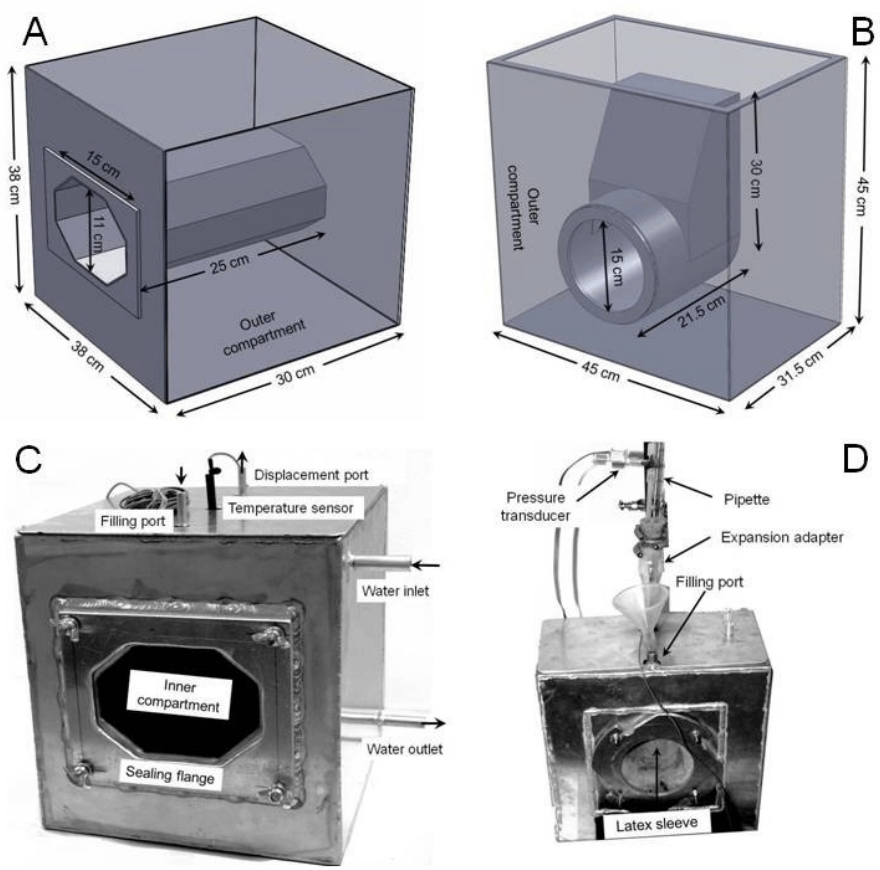

Figure 4.1: Schematic diagrams for hand (A) and foot water-displacement plethysmographs (B), with photographs of the hand plethysmograph (C) and the forearm plethysmograph (D) with its latex membrane and pressure transducer for measuring water displacement. The last plethysmograph (D) was used in the validation trials. The outer compartment was pre-heated (or pre-cooled) to the target skin temperature. Once the limb segment was in place and the sealing flange closed, the inner compartment was filled with water through the calibration port. Prior to data collection, calibration occurred via this port, with a series of known volumes injected to create a pressure-volume calibration curve.

with a climate-controlled chamber, a lattice-design, water-perfusion garment was worn that covered >90% of the skin surface, and provided a capacity to simultaneously clamp both deep-body and mean skin temperatures. This garment contained 140, 1-m long tubes, formed into a long-sleeved jacket and full-length trouser (Paul Webb Associates, Yellow Springs, U.S.A.). Water was supplied to each component from pre-heated or pre-cooled water baths (type VFP, Grant Refrigeration Systems, U.K.; type CK2, Grant Refrigeration Systems, U.K.). The head, hands and feet were not covered, nor was the suit covered with any material. Thus, whilst the garment tended to hug the body, most of the skin surface was exposed to the air (Cotter *et al*., 1995a).

4.2.1.4 Data acquisition

Arterial blood flow was measured from the volume of water displaced from each plethysmograph during venous occlusion-induced segmental swelling. To increase measurement sensitivity, the inner (displacement) compartment of each plethysmograph was filled with enough water so that it entered a narrow displacement port (chimney: Figures 4.1 and 4.2) located above the displacement chamber, thereby ensuring detection of small volume changes. Attached to these ports were glass expansion adapters (socket 29/32 cone 19/26, Livingstone International Pty. Ltd., Roseberry, NSW, Australia), each of which held a two-hole rubber stopper. One hole contained a glass pipette that was open to the atmosphere, with water from the inner compartment partially filling that pipette. Once the limb segment was sealed in position, this was the only connection from the inner chamber to the atmosphere, and it permitted the unimpeded rise and fall of the water during occlusion and recovery. The water temperatures of the inner and outer compartments were measured at 15-s intervals (YSI EU-type, Yellow Springs Instruments Co. Ltd., Yellow Springs, OH., U.S.A.; 1206 Series Squirrel, Grant Instruments Pty Ltd.,Cambridge, U.K.).

To the other hole in the stopper was attached to a rigid tube with a 3-way stopcock. One arm of the stopcock was open to the atmosphere, and was used for water filling and calibration, whilst the other led to a pressure transducer (MPS-201G, Memstech, Singapore: Figure 4.2). Prior to data acquisition, the stopcock was turned so that water from the pipette displaced all air from these tubes and was in direct contact with the

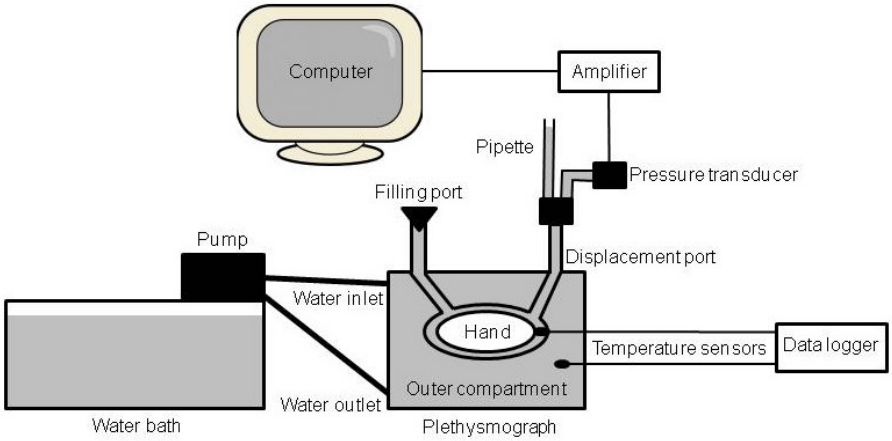

Figure 4.2: Schematic of experimental set-up for the water-displacement, hand plethysmograph showing connections to the water bath, pressure amplifier, computer and temperature data logger. See text for detailed description.

pressure transducer. In this way, the transducer responded to changes in hydrostatic pressure created by the rise and fall of water within the pipette, as its water level was always above the pressure transducer. The transducer output was amplified (four-channel amplifier, Onspot, Australia: Figure 4.2), converted to its digital equivalent (NI USB-9162, National Instruments, Hungary) and sampled at 20 Hz (Labview: version 7, National Instruments, Hungary).

4.2.1.5 Calibration

Transducer calibration was performed by connecting a modified sphygmomanometer (Yamasu 605P, Kenzmedico Co., Saitama, Japan) to the pressure transducer and applying known pressures over a physiologically relevant range. Volume calibration was performed immediately prior to data collection, and with the limb segment *in situ*. Known water volumes (3, 5, 10, 20 and 30 mL) were injected into the displacement port through the stopcock. This was performed in triplicate, enabling construction of subject-specific calibration curves: independent (volume change) versus dependent variable (transducer signal). This equation was then used to determine segmental blood flows ($mL.min^{-1}$). Calibration was conducted using a 3-mL syringe, and the device was shown to be sensitive at this volume change. The device may be sensitive to levels greater than this, however this resolution was not measured.

4.2.1.6 Calculation of segmental blood flow

Three steps were necessary for this calculation. Firstly, the volume (mL) of each participant's limb segment within the plethysmograph was derived. This was determined by measuring the volume of water added to the plethysmograph, and subtracting it from the total (known) volume of the inner compartment. Secondly, arterial inflow was calculated by measuring the slope of the relationship between pressure change and time ($mmHg.s^{-1}$) during the initial (approximately linear) phase of venous occlusion, and converting this to a volume change rate (flow: $mL.s^{-1}$) using the calibration curve for each individual. Thirdly, flows were expressed as a percentage of the limb volume, enabling the derivation of blood flow in its traditional units: mL.100 mL $tissue^{-1}.min^{-1}$. Since flow is pressure-dependent, mean arterial pressure was also measured and used to calculate vascular conductance.

4.2.2 Study one: Validation

The objective of this project was to compare forearm vascular conductance derived during two, 70-min trials. One trial used a mercury-in-silastic, strain-gauge plethysmograph (trial one; Johnson *et al*., 1984) and the other used a bespoke water-filled, displacement plethysmograph (trial two). Validation was performed under thermoneutral conditions using seated, resting subjects studied before and following local forearm heating. The working hypothesis was that vascular conductance measured using these two techniques would not be different. Procedures were approved by a Human Research Ethics Committee (University of Wollongong) in accordance with the Declaration of Helsinki.

4.2.2.1 Subjects

Eight physically active, healthy adults participated in these trials (four male and four female: aged 20-34 y, mass 66.4 kg [standard deviation (SD) 11.0], height 173.2 cm [SD 5.2]).

4.2.2.2 Standardisation

Trials were performed at the same time of day to prevent circadian influences. Subjects wore their own clothing, and were studied during thermoneutral, seated rest in an air-conditioned laboratory (21-23^{o}C). Prior to testing, subjects were asked to refrain from consuming alcohol and from strenuous exercise for 12 h, from taking caffeine within 2 h and to present in a well-hydrated state. Participants were also asked to drink 15 mL.kg^{-1} of fluid the night prior to each trial. Urine specific gravity (Refractometer No. 140, Shibuya Optical, Co. Ltd., Tokyo, Japan) was measured immediately on arrival, with individuals having values >1.013 consuming 500 mL of water before testing.

4.2.2.3 Procedural overview

Both trials followed an identical protocol, commencing with a pre-experimental preparation (20 min), during which subjects remained seated. Following a further 10 min of seated rest, baseline (thermoneutral phase) forearm blood flow was determined (left arm). Data were recorded for 2 min, followed by a 3-min rest, during which mean arterial blood pressure was measured. This sequence was repeated once. The forearm was then passively heated for 40 min (heating phase) to achieve the target local skin temperature

(~37°C). Blood flow was measured using the same data collection and rest intervals, and continued for 10 min. For trial one, heating was provided via a heat lamp (375 W.m^2) placed 1 m from the forearm, and at 45° angle. Heating was applied for 40 min before data collection commenced, however, only the dorsal surface of the forearm was heated. In trial two, the temperature of the water circulating through the outer compartment of the plethysmograph was initially set to 32°C (thermoneutral phase), and this was increased to 40°C over 10 min during the local heating phase, and then held for 30 min prior to data collection. The temperature profiles for one subject (water-filled plethysmograph) are shown in Figure 4.3.

4.2.2.4 Physiological measurements

Forearm blood flow was measured using both a mercury-in-silastic, strain-gauge plethysmograph (EC 4 Plethysmograph, D.E. Hokanson Inc., Bellevue, U.S.A.) and a water-filled plethysmograph (Figures 4.1D and 4.2). The left forearm was positioned at heart level with a downward slope from the hand to the elbow of about 45° to facilitate venous drainage during the non-occlusion periods. Hand blood flow was occluded using a cuff positioned at the wrist. This was manually inflated to 20 mmHg above systolic blood pressure (5 s) just prior to commencing venous occlusion. A second, pneumatic occlusion cuff was positioned above the elbow, and it was intermittently (automatically) inflated to a pressure of 50 mmHg (Groothuis *et al*., 2003: AG 101 Cuff inflator air source, D.E. Hokanson, Inc., Bellevue, U.S.A.). This followed a 20-s cycle consisting of 8 s of inflation and 12 s of deflation, for a total of six occlusions throughout each 2-min sampling period, with each pressure change being completed within 2 s. Artefacts associated with these pressure changes (Greenfield *et al*., 1963) were not included within the subsequent analysis.

Throughout each trial, core temperature, skin temperature and heart rate (Polar Electro Sports Tester, Finland) were recorded continuously (15-s intervals), while mean arterial blood pressure (Omron SEM-2, Omron Healthcare Inc., Kyoto, Japan) was measured during each rest period. Core temperature was approximated from the auditory canal using an ear-moulded plug containing a thermistor protruding 1 cm (Edale Instruments Ltd., U.K.). A large piece of cotton wool covered this plug and ear pinna,

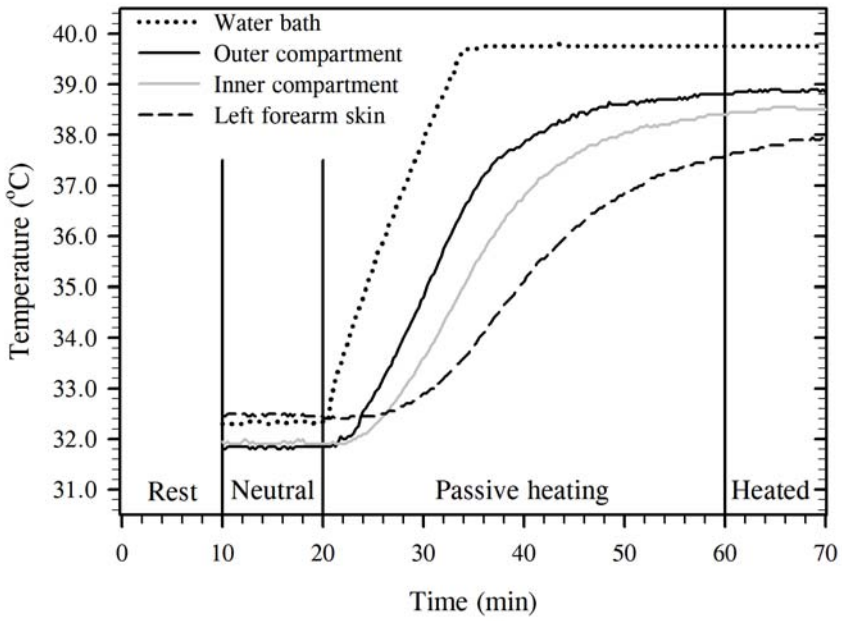

Figure 4.3: Representative temperature profiles of the water bath, outer (forearm) plethysmograph compartment, inner plethysmograph compartment and mid-dorsal forearm skin during the thermoneutral phase, 40 min of local heating and data collection when forearm temperature had stabilised.

minimising environmental artefacts (Cotter *et al*., 1995a). Skin temperature was measured from eight sites (forehead, right scapula, right chest, right upper arm, right forearm, right dorsal hand, right anterior thigh and left posterior calf: Type EU, Yellow Springs Instruments Co. Ltd, Yellow Springs, OH, U.S.A.) with mean skin temperature derived as an area-weighted average (ISO 9886: 1992). In addition, local skin temperature was measured at three sites on the treated forearm to determine its temperature distribution during blood flow measurement. These thermistors were placed on the dorsal surface of the distal, middle and proximal forearm and shielded from the heat lamp (thick cardboard). All temperatures were recorded using a portable data logger (1206 Series Squirrel, Grant Instruments Ltd., U.K.), and thermistors were calibrated against a certified reference thermometer (Dobros total immersion, Dobbie Instruments, Sydney, Australia) across the relevant temperature ranges, using a stirred water bath.

4.2.2.5 Data analysis

This trial was based upon a repeated-measures design, subjects were their own controls and participated in both trials. Between-treatment differences were analysed using one-way analysis of variance, with Tukey's *HSD post hoc* procedure used to identify sources of significant difference. Paired *t*-tests were also performed. *Alpha* was set at the 0.05 level for all comparisons, and data are presented as means with standard errors of the means (±) and standard deviations (SD) when reporting data distributions.

4.2.3 Study two: Hand and foot blood flow: local thermal influences

The aim of this study was to collect hand and foot blood flows using two purpose-built, displacement plethysmographs (Figure 4.1A and 4.1B) under clamped thermoneutral conditions, and during three local (hand and foot) skin temperature treatments. These treatments were applied by altering the water temperature of the outer compartment of each plethysmograph. This approach would enable an evaluation of the capacity of these plethysmographs to be used in a larger project during which the role of local skin temperature would be investigated under three whole-body thermal states. These procedures were also approved by the Human Research Ethics Committee.

4.2.3.1 Subjects

Eight healthy, non-smoking males participated in a single trial (age 25.4 y [SD 6.4], mass 74.5 kg [SD 8.7], height 1.71 m [SD 0.04]), wearing only swimming costumes. Subjects were not taking any medication nor did they have a history of cardiovascular or thermal illness. Identical standardisation procedures were used, and subjects were tested in a well-hydrated state, with urine specific gravity averaging 1.021 (SD 0.01) prior to testing.

4.2.3.2 Procedural overview

Following arrival, status check, preliminary experimental set-up (including donning the water-perfusion garment) and a 60-min postural control period, participants entered a preconditioned climate chamber (28^{o}C, 40% relative humidity) in a thermoneutral state and immediately adopted a supine resting posture for the final instrumentation (15 min). During preparation, the perfusion suit was fed with water at 34^{o}C, and the right hand and left foot were fitted with a latex glove and sock that were then sealed into their respective water-displacement plethysmographs (Figure 4.1). Three local thermal treatments were applied by changing the temperature of water in the outer compartment to each of the following temperatures: 5^{o}, 25^{o}, 40^{o}C. Two treatment sequences were used at each site: *sequence 1* (rising): 5^{o}, 25^{o}, 40^{o}C; *sequence 2* (falling): 40^{o}, 25^{o}, 5^{o}C. Thus, the initial water temperature of the plethysmograph was either hot or cold, and this was balanced across both subjects and limb segments. Blood-flow measurements lasted 2 min, after which the water temperature was rapidly heated or cooled to the next target temperature (~10 min). These treatments were alternated between sites. Therefore, consecutive treatments of the same segment did not occur within 15 min. Furthermore, blood flow measurement commenced only after these thermal treatments had been applied for 5 min.

4.2.3.3 Physiological measurements

Right hand and left foot blood flows were measured using the water-filled plethysmographs described above (Figures 4.1D and 4.2), and following identical methods. Similarly, skin temperatures, heart rate and mean arterial blood pressure were measured. However, in these trials, body core temperature was approximated from the

oesophagus, with a thermistor (Edale Instruments Ltd, U.K.) inserted transnasally to ~40 cm from the nares (after Mekjavic and Rempel, 1990). Local skin temperature was also measured from the dorsal hand (metacarpal surface) and foot (metatarsal surface). Calibrations were performed as described above.

4.2.3.4 Data analysis

Between-site comparisons were evaluated using multivariate analysis of variance, with three local skin temperature treatments and two measurement sites. Tukey's *HSD post hoc* procedure was used to identify sources of significant difference, with *alpha* set at the 0.05 level. Data are presented as means with standard errors of the means (±) and standard deviations (SD) when describing data distributions.

4.3 RESULTS

4.3.1 Study one

4.3.1.1 Thermoneutral conditions

There were no significant differences in either the basal (thermoneutral) auditory canal (36.9ºC [±0.08; trial one] versus 37.0ºC [±0.08]; $P>0.05$) or mean skin temperatures (31.6ºC [±0.37; trial one] versus 31.3ºC [±0.28]; $P>0.05$) between the two trials. Similarly, the dorsal-forearm skin temperatures were not different, averaging 31.2ºC (±0.54; trial one) and 31.5ºC (±0.51) across the three sites ($P>0.05$). These thermal states were matched by the baseline cardiovascular status, with mean heart rates of 70 beats.min^{-1} (±3.1; trial one) and 66 beats.min^{-1} (±1.7; $P>0.05$), and basal mean arterial pressures averaging 87.2 mmHg (±3.1; trial one) and 83.0 mmHg (±3.4; $P>0.05$). These data verify that subjects were thermoneutral and were investigated under truly resting conditions, with thermal and cardiovascular stability. This verification was essential for valid testing of the working hypothesis. Under these conditions, cutaneous vascular conductance did not differ between the two forms of plethysmography (Figure 4.4; $P>0.05$).

4.3.1.2 Passive forearm heating

When the left forearms were passively heated, the mean dorsal-forearm skin temperatures were significantly elevated relative to the thermoneutral state in both trials ($P<0.05$), but these did not differ between trials (36.9ºC [±1.24; trial one] and 36.6ºC

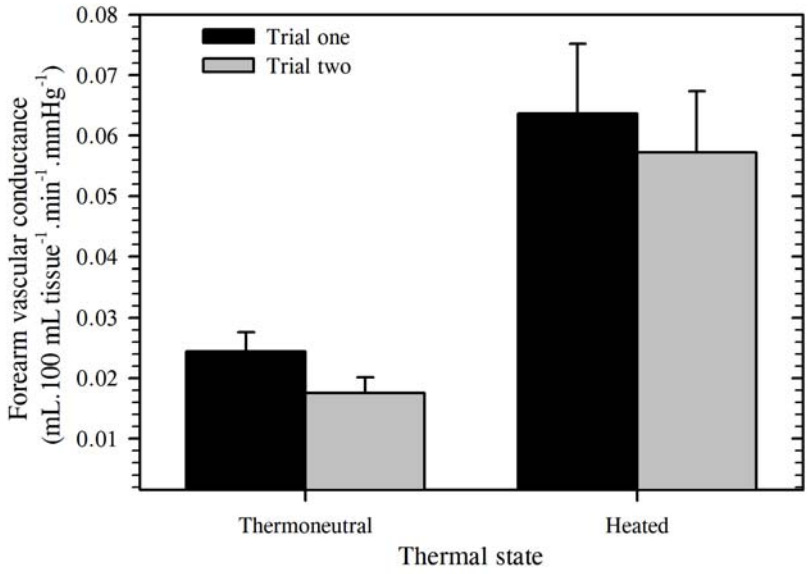

Figure 4.4: Forearm vascular conductance measured using a mercury-in-silastic, strain-gauge plethysmograph (trial one) and a bespoke water-filled, displacement plethysmograph (trial two). Deep-body and mean skin temperatures were stable, with subjects remaining in a thermoneutral state throughout. Vascular conductance was measured in each of two localised (forearm) thermal states: thermoneutral (dorsal-forearm skin temperature 31.2^{o}C [trial one] and 31.5^{o}C; P>0.05) and heated (36.9^{o}C [trial one] and 36.6^{o}C; P>0.05). Data are means with standards errors of the means (N=8).

[±1.30]; *P*>0.05).

In addition, the average forearm skin temperature observed in trial two did not differ significantly from that measured within the inner chamber of displacement plethysmograph (mean difference: 0.5ºC; *P*<0.05). Both the auditory canal (36.9ºC [±0.06; trial one] versus 37.0ºC [±0.08]; *P*>0.05) and mean skin temperatures (32.2ºC [±0.31; trial one] versus 31.9ºC [±0.24]; *P*>0.05) remained stable, and did not differ either from their thermoneutral baselines or between trials. This pattern was also evident within the cardiovascular responses (heart rate: 69.2 beats.min^{-1} [±2.6; trial one] versus 65.4 beats.min^{-1} [±3.9)]; mean arterial pressure: 87.1 mmHg [±3.7; trial one] versus 85.6 mmHg [±3.0]). Thus, the treatment-induced changes in forearm blood flow were wholly due to the localised thermal influences. Accordingly, forearm vascular conductance was elevated almost three-fold (Figure 4.4; *P*<0.05), but differences between the two forms of plethysmography were again not significant (Figure 4.4; *P*>0.05). Although the mean vascular conductance was lower in trial two under both experimental conditions, this was not a systematic difference, as only four of the eight data points were lower in each condition. These outcomes validated the blood flow measurements made using this displacement plethysmograph under these thermoneutral and locally heated states.

4.3.2 Study two

4.3.2.1 Thermal clamp and passive thermal treatments

Over the full duration of these trials, oesophageal and mean skin temperatures remained clamped, averaging 37.0ºC (±0.10) and 33.6ºC (±0.21, respectively), and they did not change significantly from the start to the end of the local temperature treatments (*P*>0.05). The cardiovascular status across these trials also remained stable, with a mean heart rate of 63 beats.min^{-1} (±6.9) and mean arterial pressures averaging 126.7 mmHg (±3.7). Whilst these thermal treatments were aimed at 5º, 25º and 40ºC, the actual hand skin temperatures obtained were 7.1º (SD 1.54), 25.4º (SD 0.29) and 40.8ºC (SD 1.35), and those of the foot were 5.7º (SD 1.13), 25.2º (SD 0.33) and 41.6ºC (SD 0.36). During these local stimulations, neither the oesophageal nor mean skin temperatures changed significantly (*P*>0.05), and so these treatments were restricted to the target body segment.

4.3.2.2 Segmental blood flow during local cooling and heating

Due to the interaction of mean arterial pressure on tissue perfusion, it is standard practice to report vascular conductance, and thereby normalise for blood pressure differences. However, since the aim of this communication was to describe a method for simultaneously stimulating and measuring hand and foot blood flows, and since mean arterial pressure remained constant throughout each trial, then only flows will be reported. In Figure 4.5, segmental blood flows for the hand and foot are illustrated for each of the three local thermal treatments. The intermediate treatment temperature (25^{o}C) was just slightly cooler than the hand and foot skin temperatures that would be observed in thermoneutral individuals (Werner and Reents 1980). However, that state was associated with significantly higher hand than foot blood flow (Figure 4.5; P<0.05), and this also occurred when these segments were heated (P<0.05). Indeed, the foot was maximally constricted when its local temperature was 25.2^{o}C, and it remained in this state when skin temperature was reduced to 5.7^{o}C (Figure 4.5; P>0.05). When heated above 25^{o}C, foot blood flow was significantly increased (P<0.05). For the hand, these local cooling and heating treatments both yielded significant flow variations (P<0.05). When analysed over treatment temperatures from 25^{o}-40^{o}C, the cutaneous thermal sensitivity of the hand (0.25 mL.100 mL^{-1}.min^{-1}.^{o}C^{-1}) significantly exceeded that of the foot (0.14 mL.100 mL^{-1}.min^{-1}.^{o}C^{-1}; P<0.05).

4.4 DISCUSSION

The design and operational characteristics of three water-displacement plethysmographs have been described. Through direct comparisons with a mercury-in-silastic, strain-gauge plethysmograph (Figure 4.4), it was possible to validate blood flow measurements made using a custom-built, forearm plethysmograph, that also provided a capability for changing and clamping segmental temperatures independently of the thermal state of the deep-body and superficial tissues. In the thermoneutral state, the average blood flow measured at the forearm (1.47 mL.100 mL^{-1}.min^{-1}), hand (6.69 mL.100 mL^{-1}.min^{-1}) and foot (2.81 mL.100 mL^{-1}.min^{-1}) with these water-displacement plethysmographs agreed with data reported within the literature: forearm (2.65 mL.100 mL^{-1}.min^{-1}; Barcroft and Edholm, 1943), hand (5.9 mL.100 mL^{-1}.min^{-1}; Spealman, 1945) and foot (2.4 mL.100 mL^{-1}.min^{-1}; Allwood and Burry, 1954). When such local temperature

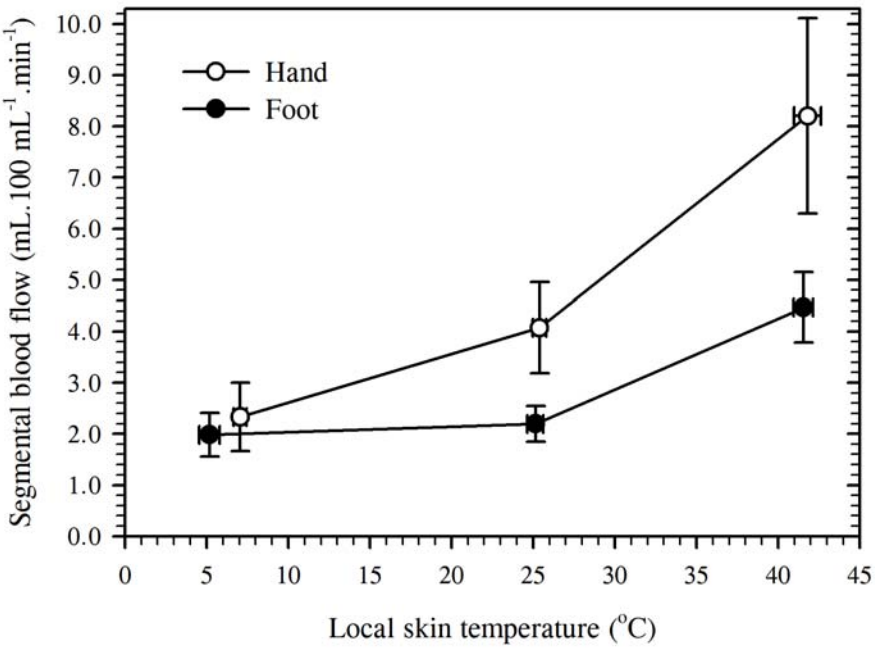

Figure 4.5: Hand and foot blood flows measured using a water-filled, displacement plethysmograph. Subjects were clamped in a stable, thermoneutral state throughout testing, which involved three localised thermal treatments (~5^{o}C, 25^{o}C and 40^{o}C) applied by manipulating the water temperature with each plethysmograph. Data are means with bidirectional standard errors of the means (*N*=8).

treatments were applied to the hand and foot (5º, 25º, 40ºC), marked segmental differences in blood flow were revealed, with the hand displaying greater absolute flows and thermal sensitivity when investigated in thermoneutral individuals (Figure 4.5). These were not novel observations, but confirmations (Kunkel *et al*., 1939; Wallin, 1990). However, since blood flow validation for the hand and foot was not feasible, then it was imperative that such data were obtained, for only then could these plethysmographs be used with confidence in subsequent research.

Given that these trials were conducted in supine and resting individuals, it can be assumed the blood flow responses were restricted to the cutaneous vascular beds (Edholm *et al*., 1956; Detry *et al*., 1972; Johnson and Rowell, 1975; Johnson *et al*., 1976). In addition, under thermoneutral conditions, skin heating elicits a biphasic, cutaneous vasodilatation (Pérgola *et al*., 1993; Charkoudian *et al*., 2002). This is brought about via both local neurogenic (C-fibre afferents; Holzer, 1998) and neurochemical mechanisms (*e.g*. nitric oxide production; Minson *et al*., 2001). The former dominates the initial reactions (3-5 min) to cutaneous heating (Pérgola *et al*., 1993; Charkoudian *et al*., 2002), and can explain almost all of the vascular responses observed during local heating in study two. Whilst the control mechanism may be identical, distinct segmental differences in these vascular responses were observed, with maximal flows favouring the hand, and these were almost two-fold greater than at the foot (Figure 4.5). Thus, over the local temperature range from 25º-40ºC, the cutaneous thermal sensitivity of the hand was significantly greater. Given the uniquely large surface area to mass ratio of the hand (Taylor *et al*., 2009), which is four-five times greater than the whole-body ratio, these appendages are ideally suited to behave as physiological conductors and radiators.

When the skin is cooled, cutaneous blood flow is minimal, particularly when combined with hypothermia (Stocks *et al*., 2004; Golden *et al*., 2013). This vasoconstriction is also of a local neural origin (Pérgola *et al*., 1993), and it occurs in combination with an elevated sensitivity of the *alpha* receptors to noradrenaline release (Flavahan *et al*., 1985). When locally cooled to 5ºC, both the hand and foot vasoconstricted to similarly low, but not minimal (Kunkel and Stead, 1938; Spealman, 1945), blood flows. However, the foot was equally vasoconstricted even when its local

temperature was 25°C. This is also a well-established phenomenon, with the vasoconstrictor tone of the acral (glabrous) skin surfaces (approximately 50% of these regions; Johnson *et al*., 1995) dominating until either the deep-body or superficial tissues are heated (Roddie, 2011). Thus, the hands, but particularly the feet, are excellent physiological insulators, especially when hypothermic, as cutaneous blood flow can fall to levels that are below the local metabolic requirement for blood (Abramson, 1965). Indeed, this characteristic makes humans ill-suited to protracted and unprotected cold exposure (Golden *et al*., 2013).

This capacity of the hands and feet to undergo such dramatic changes in cutaneous blood flow is directly related to the presence of arteriovenous anastomoses. These vessels are found almost exclusively within the glabrous skin (Sucquet, 1862; Nagasaka *et al*., 1987) and they behave as capillary by-pass vessels that, for the same pressure head, can produce a 10,000-fold blood flow elevation (Molyneux and Bryden, 1981). As a result, more blood is delivered to the deep venous plexuses with their slower moving blood, and this enhances heat exchange with the external environment (Midtgård, 1980).

4.5 CONCLUSION

In these investigations, the operation of three water-displacement plethysmographs was explored. The forearm plethysmograph was validated against a well-established technique (strain-gauge plethysmography). From trials in which hand and foot surface temperatures were modified and clamped, whilst cutaneous blood flow was simultaneously measured, evidence was found to support the proposition that these appendages, and in particular the hands, fulfill critical roles in human heat loss and conservation. Three additional experiments are planned. Firstly, the hypothesis will be tested that local heating alone (Johnson *et al*., 1986; Pérgola *et al*., 1993) cannot evoke maximal cutaneous vasodilatation. Secondly, cutaneous blood flows of the forearm, hand, calf and foot across different combinations of core and local skin temperatures will be mapped. Finally, as a companion investigation to research on cutaneous sudomotor thermosensitivity (Cotter and Taylor, 2005), these plethysmographs will be used to explore regional differences in cutaneous vasomotor sensitivity.

4.6 REFERENCES

Abramson, D.I. (1965). Pathophysiology of arteriovenous shunts in the extremities. *Journal of Cardiovascular Surgery*. 5(Suppl.):217-230.

Abramson, D.I., Zazeela, H., and Marrus, J. (1939). Plethysmographic studies of peripheral blood flow in man: I. Criteria for obtaining accurate plethysmographic data. *American Heart Journal*. 17:194-205.

Allwood, M.J., and Burry, H.S. (1954). The effect of local temperature on blood flow in the human foot. *Journal of Physiology*. 124:345-357.

Babic, M., Lenarcic, J., Zlajpah, L., Taylor, N.A.S., and Mekjavic, I.B. (2008). A device for simulating the thermoregulatory responses of the foot: estimation of footwear insulation and evaporative resistance. *Journal of Mechanical Engineering.* 54:622-638.

Barcroft, H., and Edholm, O.G. (1943). The effect of temperature on blood flow and deep temperature in the human forearm. *Journal of Physiology*. 102:5-20.

Blonshine, S., and Goldman, M.D. (2008). Optimizing performance of respiratory airflow resistance measurements. *Chest*. 134:1304-1309.

Brodie, T.G. (1902). On recording variations in volume by air-transmission. A new form of volume-recorder. *Journal of Physiology.* 27:473-487.

Brodie, T.G., and Russell, A.E. (1905). On the determination of the rate of blood-flow through an organ. *Journal of Physiology.* 32(Supplement):47-49.

Charkoudian, N. (2003). Skin blood flow in adult human thermoregulation: how it works, when it does not, and why. *Mayo Clinic Proceedings.* 78:603-612.

Charkoudian, N., Eisenach, J.H., Atkinson, J.L.D., Fealey, R.D., and Joyner, M.J. (2002). Effects of chronic sympathectomy on locally mediated cutaneous vasodilation in humans. *Journal of Applied Physiology*. 92:685-690.

Comroe, J.H., Botelho, S.Y., and DuBois, A.B. (1959). Design of a body plethysmograph for studying cardiopulmonary physiology. *Journal of Applied Physiology*. 14:439-444.

Cotter, J.D., Patterson, M.J., and Taylor, N.A.S. (1995a). A method for clamping human skin and body core temperatures. *Proceedings of the Australian Physiological and Pharmacological Society.* 26:204P.

Cotter, J.D., Patterson, M.J., and Taylor, N.A.S. (1995b). Topography of eccrine sweating

in humans during exercise. *European Journal of Applied Physiology*. 71:549-554.

Cotter, J.D., and Taylor, N.A.S. (2005). Distribution of cutaneous sudomotor and alliesthesial thermosensitivity in mildly heat-stressed humans: an open-loop approach. *Journal of Physiology*. 565:335-345.

de Cyon, E. (1876). *Atlas zur methodik der physiologischen experimente und vivisectionen*. J. Ricker'sche Buchhandlung, Giessen.

Detry, J.M., Brengelmann, G.L., and Rowell, L.B., Wyss, C. (1972). Skin and muscle components of forearm blood flow in directly heated resting man. *Journal of Applied Physiology*. 32:506-511.

Di Giulio, C. (2011). Angelo Mosso: a holistic approach to muscular fatigue. *Archives Italiennes de Biologie*. 149(Supplement):69-76.

Edholm, O.G., Fox, R.H., and Macpherson, R.K. (1956). The effect of body heating on the circulation in skin and muscle. *Journal of Physiology*. 134:612-619.

Flavahan, N.A., Lindblad, L.E., Verbeuren, T.J., Shepherd, J.T., and Vanhoutte, P.M. (1985). Cooling and alpha 1- and alpha 2-adrenergic responses in cutaneous veins: role of receptor reserve. *American Journal of Physiology*. 249:H950-H955.

Fleckenstein, K.J. (1984). The Mosso plethysmograph in 19th-century physiology. *Medical Instrumentation*. 18:330-331.

Golden, F.St.C., Francis, T.J.R., Gallimore, D., and Pethybridge, R. (2013). Lessons from history: morbidity of cold injury in the Royal Marines during the Falklands Conflict of 1982. *Extreme Physiology and Medicine*. 2:23.

Greenfield, A.D.M. (1954). A simple water filled plethysmograph for the hand or forearm with temperature control. *Journal of Physiology*. 123:62P-64P.

Greenfield, A.D.M., and Patterson, G.C. (1954). The effect of small degrees of venous distension on the apparent rate of blood inflow to the forearm. *Journal of Physiology*. 125:525-533.

Greenfield, A.D.M., Whitney, R.J., and Mowbray, J.F. (1963). Methods for the investigation of peripheral blood flow. *British Medical Bulletin*. 19:101-109.

Groothuis, J.T., van Vliet, L., Kooijman, M., and Hopman, M.T. (2003). Venous cuff pressures from 30 mmHg to diastolic pressure are recommended to measure arterial inflow by plethysmography. *Journal of Applied Physiology*. 95:342-347.

Holzer, P. (1998). Neurogenic vasodilatation and plasma leakage in the skin. *General*

Pharmacology. 30:5-11.

ISO 9886. (1992). Evaluation of thermal strain by physiological measurements. *International Standard Organisation,* Geneva.

Jessen, C. (2011). Interaction of body temperatures in control of thermoregulatory effector mechanisms. *Comprehensive Physiology, Supplement 14: Handbook of Physiology, Environmental Physiology:* 127-138. First published in print 1996.

Johnson, J.M., Brengelmann, G.L., and Rowell, L.B. (1976). Interactions between local and reflex influences on human forearm skin blood flow. *Journal of Applied Physiology*. 41:826-831.

Johnson, J.M., O'Leary, D.S., Taylor, W.F., and Kosiba, W. (1986). Effect of local warming on forearm reactive hyperaemia. *Clinical Physiology*. 6:337-346.

Johnson, J.M., Pergola, P.E., Liao, F.K., Kellogg, Jr, D.L., and Crandall, C.G. (1995). Skin of the dorsal aspect of human hands and fingers possesses an active vasodilator system. *Journal of Applied Physiology.* 78:948-954.

Johnson, J.M., and Proppe, D.W. (2011). Cardiovascular adjustments to heat stress. *Comprehensive Physiology, Supplement 14: Handbook of Physiology, Environmental Physiology:* 215-243. First published in print 1996.

Johnson, J.M., Taylor, W.F., Shepherd, A.P., and Park, M.K. (1984). Laser-Doppler measurement of skin blood flow: comparison with plethysmography. *Journal of Applied Physiology*. 56:798-803.

Johnson, J.M., and Rowell, L.B. (1975). Forearm skin and muscle vascular responses to prolonged leg exercise in man. *Journal of Applied Physiology*. 39:920-924.

Jonson, B., Dahn, I., and Nilsén, R. (1970). A plethysmographic method for determination of flow and volume pulsations in a limb. *Journal of Applied Physiology*. 28:333-336.

Joyner, M.J., Dietz, N.M., and Shepherd, J.T. (2001). From Belfast to Mayo and beyond: the use and future of plethysmography to study blood flow in human limbs. *Journal of Applied Physiology*. 91:2431-2441.

Kenney, W.L., Stanhewicz, A.E., Bruning, R.S., and Alexander, L.M. (2013). Blood pressure regulation III: What happens when one system must serve two masters: temperature and pressure regulation? *European Journal of Applied Physiology*. DOI: 10.1007/s00421-013-2652-5 *Epub ahead of print*.

Kunkel, P., and Stead, E.A. (1938). Blood flow and vasomotor reactions in the foot in health, in arteriosclerosis, and in thrombo-angiitis obliterans. *Journal of Clinical Investigation.* 17:715-723.

Kunkel, P., Stead, E.A., and Weiss, S. (1939). Blood flow and vasomotor reactions in the hand, forearm, foot and calf in response to physical and chemical stimuli. *Journal of Clinical Investigation.* 18:225-238.

Landowne, M., and Katz, L.N. (1942). A critique of the plethysmographic method of measuring blood flow in the extremities. *American Heart Journal.* 23:644-675.

Libert, J.P., Candas, V., and Vogt, J.J. (1978). Sweating response in man during transient rises of air temperature. *Journal of Applied Physiology*. 44:284-290.

Machado-Moreira, C.A., Caldwell, J.N., Mekjavic, I.B., and Taylor, N.A.S. (2008a). Sweat secretion from palmar and dorsal surfaces of the hands during passive and active heating. *Aviation, Space and Environmental Medicine.* 79:1034-1040.

Machado-Moreira, C.A., McLennan, P.L., Lillioja, S., van Dijk, W., Caldwell, J.N., and Taylor, N.A.S. (2012). The cholinergic blockade of both thermally and non-thermally induced human eccrine sweating. *Experimental Physiology.* 97:930-942.

Machado-Moreira, C.A., Smith, F.M., van den Heuvel, A.M.J., Mekjavic, I.B., and Taylor, N.A.S. (2008b). Sweat secretion from the torso during passively-induced and exercise-related hyperthermia. *European Journal of Applied Physiology.* 104:265-270.

Machado-Moreira, C.A., Wilmink, F., Meijer, A., Mekjavic, I.B., and Taylor, N.A.S. (2008c). Local differences in sweat secretion from the head during rest and exercise in the heat. *European Journal of Applied Physiology.* 104:257-264.

Mekjavic, I.B., and Rempel, M.E. (1990). Determination of esophageal probe insertion length based on standing and sitting height. *Journal of Applied Physiology*. 69:376-379.

Minson, C.T., Berry, L.T., and Joyner, M.J. (2001). Nitric oxide and neurally mediated regulation of skin blood flow during local heating. *Journal of Applied Physiology.* 91:1619-1626.

Midtgård, U. (1980). Arteriovenous anastomoses and vascularity in the feet of eiders and gulls (aves). *Zoomorphology*. 96:263-270.

Molyneux, G.S., and Bryden, M.M. (1981). Comparative aspects of arteriovenous

anastomoses. In: Harrison, R.J., and Holmes, R.L. (Editors). *Progress in anatomy*. Cambridge University Press. Pp. 207-227.

Nagasaka, T., Cabanac, M., Hirata, K., and Nunomura, T. (1987). Control of local heat gain by vasomotor response of the hand. *Journal of Applied Physiology*. 63:1335-1338.

Patterson, M.J., Cotter, J.D., and Taylor, N.A.S. (1998). Human sudomotor responses to heating and cooling upper body skin surfaces: differential cutaneous thermal sensitivity. *Acta Physiologica Scandinavica.* 163:289-296.

Phillips, P.K., and Heath, J.E., (2001). Heat loss in Dumbo: a theoretical approach. *Journal of Thermal Biology*. 26:117-120.

Pérgola, P.E., Kellogg, D.L., Johnson, J.M., Kosiba, W.A., and Solomon, D.E. (1993). Role of sympathetic nerves in the vascular effects of local temperature in human forearm skin. *American Journal of Physiology.* 265:H785-H792.

Proppe, D.W., Brengelmann, G.L., and Rowell, L.B. (1976). Control of baboon limb blood flow and heart rate - role of skin vs. core temperature. *American Journal of Physiology*. 231:1457-1465.

Regan, J.M., Macfarlane, D.J., and Taylor, N.A.S. (1996). An evaluation of the role of skin temperature during heat adaptation. *Acta Physiologica Scandinavica.* 158:365-375.

Roddie, I.C. (2011). Circulation to skin and adipose tissue. *Comprehensive Physiology, Supplement 8: Handbook of Physiology, The Cardiovascular System, Peripheral Circulation and Organ Blood Flow*: 285-317. First published in print 1983.

Rowell, L.B. (1977). Competition between skin and muscle for blood flow during exercise. In: Nadel, E.R. (Editor). *Problems with temperature regulation during exercise*. Academic Press, New York. Pp. 49-77.

Rowell, L.B. (2011). Cardiovascular adjustments to thermal stress. *Comprehensive Physiology, Supplement 8: Handbook of Physiology, The Cardiovascular System, Peripheral Circulation and Organ Blood Flow*: 967-1023. First published in print 1983.

Schäfer, E.A., and Moore, B. (1896). On the contractility and innervation of the spleen. *Journal of Physiology*. 20:1-50.

Smith, C.J., Machado-Moreira, C.A., Plant, G., Hodder, S., Havenith, G., and Taylor,

N.A.S. (2013). Design data for footwear - sweating distribution on the human foot. *International Journal of Clothing Science and Technology*. 25:43-58.

Spealman, C.R. (1945). Effect of ambient air temperature and of hand temperature on blood flow in hands. *American Journal of Physiology.* 145:218-222.

Stocks, J.M., Taylor, N.A.S., Tipton, M.J., and Greenleaf, J.E. (2004). Human physiological responses to cold exposure. *Aviation, Space and Environmental Medicine.* 75:444-457.

Sucquet, J.P. (1862). *Anatomie et physiologie. Circulation du sang. D'une circulation derivative dans les membres et dans la tête chez l'homme*. Delahaye, Paris.

Tattersall, G.J., Andrade, D.V., and Abe, A.S. (2009). Heat exchange from the toucan bill reveals a controllable vascular thermal radiator. *Science*. 325:468-472.

Taylor, N.A.S. (2014). Human heat adaptation. *Comprehensive Physiology*. 4:325-365.

Taylor, N.A.S., Caldwell, J.N., and Mekjavic, I.B. (2006). The sweating foot: local differences in sweat secretion during exercise-induced hyperthermia. *Aviation, Space and Environmental Medicine.* 77:1020-1027.

Taylor, N.A.S., and Machado-Moreira, C.A. (2013). Regional variations in transepidermal water loss, eccrine sweat gland density, sweat secretion rates and electrolyte composition in resting and exercising humans. *Extreme Physiology and Medicine.* 2:4.

Taylor, N.A.S., Machado-Moreira, C.A., van den Heuvel, A.M.J., Caldwell, J.N., Taylor, E.A., and Tipton, M.J. (2009). The roles of hands and feet in temperature regulation in hot and cold environments. *Proceedings of the Thirteenth International Conference on Environmental Ergonomics.* August 2nd-7th, Boston, U.S.A., 2009. Pp. 405-409. ***ISBN:*** 978-1-74128-178-1

Wallin, B.G. (1990). Neural control of human skin blood flow. *Journal of the Autonomic Nervous System.* 30(Supplement):S185-S190.

Werner, J., Mekjavic, I.B., and Taylor, N.A.S. (2008). Concepts in physiological regulation: a thermoregulatory perspective. In: Taylor, N.A.S., and Groeller, H. (Editors). *Physiological bases of human performance during work and exercise*. Churchill Livingstone Elsevier, Edinburgh. Pp. 325-340.

Werner, J., and Reents, T. (1980). A contribution to the topography of temperature regulation in man. *European Journal of Applied Physiology*. 45:87-94.

CHAPTER 5: THE INTERACTION OF CORE AND LOCAL SKIN TEMPERATURE ON CUTANEOUS BLOOD FLOW FOR ACRAL AND NON-ACRAL SKIN REGIONS

5.1 INTRODUCTION

Variations in cutaneous blood flow occur in response to both thermal (core and skin temperatures) and non-thermal (blood pressure, exercise and hydration) reflexes (Johnson and Kellogg, 2010; Johnson, 1986). These complex interactions are of considerable importance when understanding the physiological mechanisms associated with body temperature regulation. In particular, since cutaneous blood flow, under the control of the sympathetic nervous system, has the capacity to enhance or reduce heat loss through changes in blood vessel diameter, then understanding the thermal interactions between core and skin temperature has been of interest to many researchers (Spealman, 1945; Johnson and Park, 1979; Wilson *et al*., 2002). While deep tissue temperature is known to have the greatest influence upon cutaneous blood flow (Wyss *et al.*, 1974), local skin temperature, via mechanisms independent of the central sympathetic activation, is also known to greatly influence circulation through the skin (Spealman, 1945; Pérgola *et al*., 1993; Durand *et al*., 2004). Furthermore, differences in cutaneous blood flow exist between acral (non-hairy; hands, feet, ears, nose and lips) and non-acral (hairy) skin regions (Grant, 1930; Roddie, 1983). Within acral skin regions, blood vessels are subjected to high levels of vasoconstrictor tone (when thermoneutral) that is released with increases in core temperature (Blair *et al*., 1960; Fox *et al*., 1962). These non-hairy regions also contain arteriovenous anastomoses that facilitate the shunting of large volumes of blood from the arteries to the veins (Vanggard *et al*., 1999), but these vessels have not been shown to exist within non-acral regions. Additionally, within non-acral regions, minimal vasoconstriction is present in a thermoneutral environment, therefore further increases in skin blood flow, as occurs with heating, is explained by activation of vasodilatory nerves (Roddie *et al*., 1957). In this project, cutaneous blood flow to both acral and non-acral skin regions will be investigated, and an attempt is made to differentiate between these central and local thermal influences.

Although the interactions of core and skin temperature on the control of skin blood

flow have been extensively investigated, there is a lack of quantitative data within the literature on the interactions between central drive from the hypothalamus, peripheral thermoreceptor feedback responses from the skin and the direct local skin temperature effects on skin blood flow, over a wide range of thermal states. The majority of skin blood flow research has focussed on thermoneutral individuals with exposure to various local skin temperatures (Barcroft and Edholm, 1943; Taylor *et al*., 1984; Pérgola *et al*., 1993) or during both passive and active (exercise) whole-body heating (Brengelmann *et al*., 1977; Rowell *et al*., 1970). Moreover, very little research has focussed on the quantification of cutaneous blood flow in both hypothermic and hyperthermic individuals, and certainly not across a broad range of local skin temperatures in both acral and non-acral skin regions. An awareness of this gap in our understanding provided the motivation for the current research.

Since humans are regularly exposed to large variations in body temperature, this information is important in our continued understanding of the separate and inter-dependent interactions of both central (core) and peripheral (skin) temperature on cutaneous blood flow in both the acral and non-acral skin regions. Accordingly, this forms the primary focus of this research, which will provide useful information pertaining to the control mechanisms of skin blood flow in response to variations in both central and peripheral temperature.

5.1.1 Aims and hypotheses

The aim of this experiment was to investigate the interactions of the central (core) drive and peripheral (cutaneous) feedback on the control of skin blood flow in acral and non-acral skin regions. These aims were satisfied by simultaneously measuring skin blood flow of the forearm, hand, calf and foot. Skin blood flow was measured using venous-occlusion plethysmography across three thermal states (hypothermia, normothermia and hyperthermia), and during controlled changes in local skin temperature at each thermal state. Thermal strain was induced using passive, whole-body heating and cooling, permitting skin blood flow to be evaluated over a wide range of thermal loads. These experiments were all conducted with subjects maintained with a supine posture, which was held during water-immersion for at least 15 min prior to the first treatment.

It was hypothesised that:

(1) Core temperature will have the greatest influence on skin blood flow, within all regions, but for a given core temperature skin blood flow will change in proportion to changes in local skin temperature for each site (forearm, hand, calf and foot).

(2) Of these four regions, skin blood flow will be the highest in the hand during hyperthermia, when local skin temperature is 40ºC.

(3) Skin blood flow will be greater in the hand than in the foot across all combinations of core and local skin temperatures.

5.2 METHODS

5.2.1 Subjects

Eight physically active and healthy male subjects, aged between 20 and 39 years (Table 5.1) participated in this research project and were required for three experimental trials. Each subject received a subject information package and completed a written informed consent form prior to commencing trials. The research conducted in this experiment was approved by the Human Research Ethics Commitee (University of Wollongong) under approval HE09/314.

5.2.2 Experimental methods

Each subject, wearing only swimming trunks (nearly nude) completed three trials: a control trial (thermoneutral pre-treatment), passive pre-cooling (Target core temperature: 35ºC) or pre-heating (Target core temperature: 39ºC). Each trial differed only in the pre-experimental treatment and was designed to elicit three different states (normothermic (control), hypothermic and hyperthermic). Pre-treatment involved head-out supine water immersion before each trial: control (34ºC), cooling (15ºC) and heating (39ºC). In each immersion, subjects wore a water-perfusion garment that would subsequently be used to clamp core and skin temperatures during the experimental phase. Water immersion in the thermoneutral trials was designed to remove any hydrostatic effects on skin blood flow and to ensure subjects remained in the supine position for the same duration across all three trials. This was essential since postural changes have a dramatic effect on plasma volume which may interact with mean arterial pressure or central venous pressure regulation (Harrison, 1985).

Table 5.1: Subject characteristics.

Subject	Age (y)	Height (m)	Mass (kg)	Surface area (m^2)	Surface area to mass ratio ($m^2.kg^{-1}$)
S1	27	1.81	70.0	1.89	0.027
S2	24	1.76	89.8	2.06	0.023
S3	22	1.83	95.5	2.17	0.023
S4	22	1.82	64.4	1.83	0.028
S5	20	1.86	68.5	1.91	0.028
S6	22	1.78	78.0	1.95	0.025
S7	24	1.87	80.0	2.05	0.026
S8	39	1.78	70.6	1.87	0.027
Mean	25.0	1.81	77.1	1.97	0.026
S.D.	6.0	0.04	10.9	0.12	0.002

Once the target temperature was achieved, subjects were removed from the water-immersion tank and covered in warm or cold towels, depending on the pre-immersion target.

In addition, the water-perfusion suit was immediately fed with hot (48ºC), cold (10ºC) or thermoneutral (34ºC) water. This procedure was designed to add a layer of insulation to retain heat or prevent heat gain. Subjects were then moved by a hospital trolley into the thermal chamber, with their core temperature clamped at the pre-immersion target for further instrumentation. During the experimental phase, blood flow was to be measured at four sites (forearm, hand, calf and foot) and at five local skin temperature targets (5º, 15º, 25º, 33º and 40ºC) within each trial.

For the control trial (normothermic state), core temperature was clamped at ~37ºC using a water-perfusion suit (Cotter, 1997) where warm water (~34ºC) was delivered to the water-perfusion garment at a rate of 9 $L.min^{-1}$. For the pre-cooling trials (hypothermic state), subjects were immersed in cool water (20ºC) until a target oesophageal temperature of 35ºC was achieved (Marino and Booth, 1998). Following pre-immersion, subjects were instrumented inside the thermal chamber (15ºC, 20% relative humidity) and cold water (15ºC) was immediately delivered to the water-perfusion suit garment from a water bath (bath #2, Figure 5.1). For the pre-heated (hyperthermic state) trials, subjects were immersed in warm water (~40ºC; Booth *et al*., 2004) and heated until the target oesophageal temperature of 39ºC was achieved. Subjects were then transferred to the climate chamber (38ºC, 40% relative humidity), and hot water (48ºC) was immediately delivered to the water-perfusion garment from a water bath (bath #2, Figure 5.1).

Following pre-treatment, the experimental phase was completed in the climate chamber while core temperature was clamped at one of the target temperatures (35º, 37º or 39ºC). In this experiment, the primary index of core temperature will be oesophageal temperature. At the beginning of each experimental phase, instrumentation of one of four plethysmographs: forearm, hand, calf and foot occurred (administered in a randomised order) and blood flow was measured at each of five local skin temperatures (5º, 15º, 25º, 33º and 40ºC) at each site. The initial temperature of the water within the plethysmograph

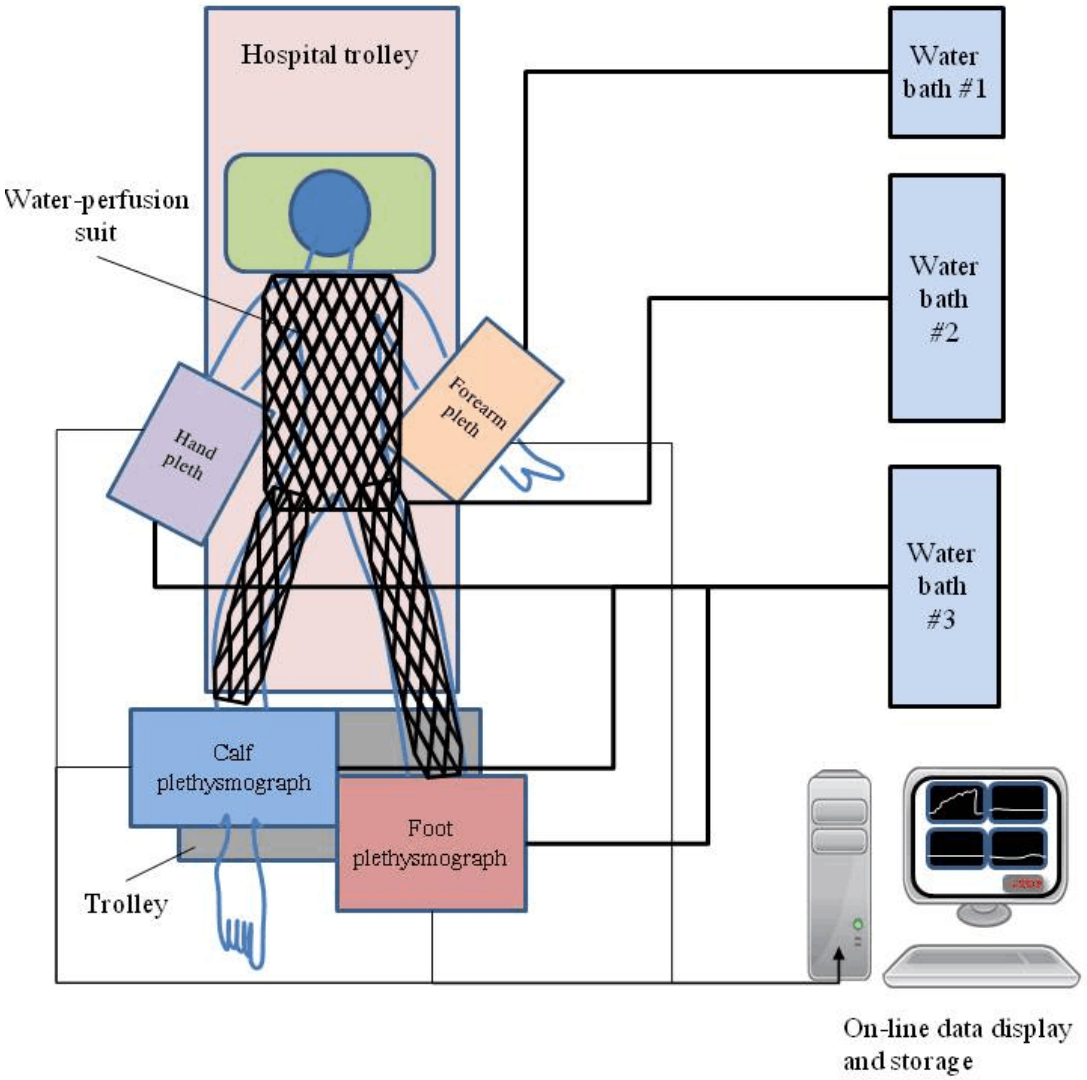

Figure 5.1: Schematic of the experimental set-up for four water-filled plethysmographs, the water-perfusion suit and the corresponding water baths. Thick lines represent tubing connected between the water bath and plethysmograph, and thin lines represent the electrical wires connected between the pressure transducer and the computer. Water bath #1 is the stimulation bath used to manipulate local skin temperature, water bath #2 is connected to the water perfusion suit and water bath #3 is connected to the other three measurement sites to maintain the temperature to be the same as the suit.

was therefore either hot (40°C) or cold (5°C). Following instrumentation, 2 min of blood flow data were recorded. At completion of the first blood flow recording, the water within the plethysmograph was rapidly heated or cooled until the next target local skin temperature was achieved. This procedure was repeated until blood flow measurements were recorded for all five targets, administered in the order: 5°, 15°, 25°, 33° and 40°C or 40°, 33°, 25°, 15°, and 5°C (Table 5.2). This stimulation occurred at one site at a time, but was cycled among the four sites in a fully randomized order (Table 5.2).

5.2.3 Experimental standardisation

Subjects were fully hydrated (Table 5.3), and were required to refrain from strenuous exercise and the consumption of alcohol and tobacco during the 12 h prior to each trial. For the night preceding each trial, subjects were instructed to drink 15 mL.kg^{-1} of additional water before retiring, and on arrival to the laboratory, subjects were provided with supplementary water (10 mL.kg^{-1}) if their urine specific gravity was greater than 1.029[1] to ensure all subjects were either well hydrated or euhydrated. This was required on six occasions, with S5 requiring additional fluid before every trial. In addition, subjects were asked to eat an evening meal high in carbohydrate and low in fat. Breakfast was also required to be high in carbohydrate and low in fat. Subjects were asked to refrain from using caffeine for 2 h prior to each trial. During each trial, subjects consumed an iso-osmotic drink *ad libitum* (approximately 200 mL every 30 min). Before leaving the laboratory, subjects were rehydrated by consuming an iso-osmotic drink equivalent to 150% of the body mass change (100% in the laboratory and 50% taken away). All testing was conducted at the same time of day within subjects, and the trial sequence was balanced across subjects.

5.2.4 Experimental measurements

Physiological measurements were recorded continuously during both the pre-treatment and experimental phases, and included body core and skin temperatures, heart rate and whole-body sweat rate. Psychophysical responses were recorded during each local skin temperature treatment and blood flow recording.

[1] Hydration classifications based upon urine specific gravity: (a) well hydrated: <1.013; (b) euhydrated: 1.013-1.029; and (c) hypohydrated: >1.029 (Armstrong *et al.*, 1994).

Table 5.2: Experimental timeline.

Time (min)	Activity summary
0-30	Subject hydration check and preparation (22°C)
30-100	**Pre-cooled**, **thermoneutral** or **pre-heated**: Target T_{es}=35°, 37° or 39°C
100-115	Prepare measurement site: **1**
115-130	**1st** Local skin temperature target: 5° or 40°C
130-145	**2nd** Local skin temperature target: 15° or 33°C
145-160	**3rd** Local skin temperature target: 25°C
160-175	**4th** Local skin temperature target: 33° or 15°C
175-190	**5th** Local skin temperature target: 40° or 5°C
190-220	Prepare measurement site: **2**
220-295	Local skin treatments: 1-5 (as above)
295-315	Prepare measurement site: **3**
315-390	Local skin treatments: 1-5 (as above)
390-410	Prepare measurement site: **4**
410-485	Local skin treatments: 1-5 (as above)
485	Terminate experiment

Table 5.3: Pre-experimental hydration state (urine specific gravity).

Subject	Hypothermia	Normothermia	Hyperthermia
S1	1.033	1.021	1.025
S2	1.020	1.022	1.029
S3	1.010	1.010	1.010
S4	1.030	1.023	1.010
S5	1.032	1.030	1.036
S6	1.020	1.015	1.035
S7	1.010	1.008	1.017
S8	1.010	1.010	1.008
Mean	1.021	1.017	1.021
S.D.	0.01	0.01	0.01

5.2.4.1 Hydration status

Prior to commencing each trial, urine specific gravity was measured for each subject to confirm a state of euhydration.

5.2.4.2 Thermal variables

5.2.4.2.1 Oesophageal temperature

An oesophageal thermistor (Edale Instruments Ltd, U.K.) was inserted through the nose to a depth of about 40 cm from the nares (after Mekjavic and Rempel, 1990) with data recorded throughout each trial at 15-s intervals using a portable data logger (Grant Instruments Ltd., 1206 Series Squirrel, U.K.). This measure was taken as the primary index of core temperature.

5.2.4.2.2 Auditory canal temperature

Auditory canal temperature was measured with an ear-moulded plug containing a thermistor protruding into the ear 1 cm from the mould (Edale Instruments Ltd., U.K.). A large piece of cotton wool was secured over the ear to minimise the effect of the environmental temperature. Data were recorded throughout each trial at 15-s intervals using a portable data logger (Grant Instruments Ltd., 1306 Series Squirrel, U.K.)

5.2.4.2.3 Rectal canal temperature

Rectal temperature was also measured continuously (15-s intervals), using a thermistor probe inserted to a depth of 12 cm beyond the anal sphincter (Edale Instruments Ltd, U.K.).

5.2.4.2.4 Skin temperatures

Skin thermistors were used to measure skin temperature (Type EU, Yellow Springs Instruments Co.Ltd, Yellow Springs, OH, USA). Skin temperatures were measured from eight sites (Edale Instruments Ltd, U.K.), with data recorded throughout each trial at 15-s intervals using a portable data logger (Grant Instruments Ltd., 1206 Series Squirrel, U.K.). From these data, an area-weighted mean skin temperature (T_{sk}) was derived (ISO 9886, 1992; after Hardy and DuBois, 1938).

$$T_{sk}=0.07\cdot T_{sk-1}+0.175\cdot T_{sk-2}+0.175\cdot T_{sk-3}+0.07\cdot T_{sk-4}+0.07\cdot T_{sk5}+0.05\cdot T_{sk-6}+0.19\cdot T_{sk-7}+0.2\cdot T_{sk-8}$$

where:

T_{sk-1} = forehead

T_{sk-2} = chest

T_{sk-3} = scapula

T_{sk-4} = upper arm

T_{sk-5} = forearm

T_{sk-6} = hand

T_{sk-7} = thigh

T_{sk-8} = calf

Local skin temperatures were not measured (and hence simulation temperatures are reported) due to the unknown influence the thermistor would have on the sensitivity of the blood flow measurement within each plethysmograph. However, pilot testing revealed a time delay of approximately 2 min from the temperature within the external chamber to that of the skin. One limitation with this prediction is this time delay was not calculated for hyperthermic and hypothermic conditions.

5.2.4.2.5 Gross mass changes

Gross mass changes (before and after each trial) were used to determine changes in hydration state (±20 g; fw-150k, A&D scale, CA, U.S.A.) over the course of the trial. Whole-body sweat losses were calculated from body mass changes and corrected for fluid replacement and urine production.

5.2.4.3 Cardiovascular variables

5.2.4.3.1 Heart rate

Heart rate was obtained at 15-s intervals throughout each trial from ventricular depolarisation using a heart rate monitor (Polar Electro Sports Tester, Finland) and was later downloaded to a computer.

5.2.4.3.2 Blood pressure

Systolic and diastolic blood pressure were measured (Omron SEM-2, Omron

Healthcare Inc., Kyoto, Japan) immediately prior to the beginning of data collection of skin blood flow from the stimulation site (see section 5.2.4.3.3). Mean arterial pressure (MAP) was calculated as a weighted mean of systolic (SBP) and diastolic blood pressure (DBP).

$$MAP = DBP + \frac{1}{3}(SBP-DBP)$$

5.2.4.3.3 Skin blood flow

Skin blood flow was measured using venous-occlusion plethysmography with a water-filled plethysmograph, positioned at each of four sites: left forearm, right hand, right calf and left foot. For the forearm and calf measurements, blood flow to the hand and foot, respectively, were occluded by placing a cuff around the left wrist or ankle and inflating it to 160 mmHg. Venous return from all sites was occluded by inflating a venous-occlusion cuff placed proximal to the elbow and knee, and inflated to a pressure of 50 mmHg. Detection of the rate of swelling of each limb was determined through water displacement within each plethysmograph. Water displacement was detected from changes in voltage within a differential pressure transducer, converted from analogue to digital data, then collected on a desktop computer (Refer to section 4.2.1.4 for full details). Data were collected for 2 min once each of the local skin temperature targets was reached. The venous-occlusion cuff was automatically controlled (AG 101 Cuff inflator air source, D.E. Hokanson, Inc., U.S.A.), and followed a cycle of 8 s of inflation and 12 s of deflating, for a total of five inflations.

5.2.4.3.4 Cutaneous vascular conductance

Cutaneous vascular conductance (CVC) was calculated as the ratio of skin blood flow to mean arterial pressure with the units expressed as mL. 100 mL tissue^{-1}. min^{-1}. mmHg^{-1}. Since skin blood flow is a function of both mean arterial pressure and total peripheral resistance, this calculation was important to determine whether changes in skin blood flow occurred from changes in vascular conductance.

5.2.4.4 Psychophysical responses

Throughout each pre-treatment, subjects were asked to rate whole-body thermal sensation, thermal discomfort and thermal pleasantness (Appendix **A**, **B**, and **D**). During the

experimental phase, in addition to whole-body thermal sensation, thermal discomfort and thermal pleasantness, these psychophysical questions were recorded for each site (forearm, hand, calf and foot) during each of the local temperature stimulations (5^{o}, 15^{o}, 25^{o}, 33^{o} and 40^{o}C). These were only recorded once the steady-state temperature for each of the five local skin temperatures was established.

5.2.5 Design and data analysis

Experimental trials were based on a repeated-measures design, where all subjects acted as their own controls and completed all three trials. Trials were administered in a randomised, but balanced order across all subjects to eliminate any effect of trial order. In addition, the order of the blood flow measurement site (forearm, hand, calf and foot) was randomised to also eliminate order effects. Also, the order of skin temperature stimulation was either from cold to hot or hot to cold, again designed to eliminate any effect of the pre-exposure temperature. This was determined using a *Latin square* design model (Table 5.4). Segmental blood flow data for each of four sites (forearm, hand, calf and foot) was analysed for each individual separately and within each thermal state across each local stimulation temperature. To produce the relationship of skin blood flow across each stimulation temperature, the resulting intercepts, slopes, and correlation parameters were averaged across each subject.

Statistical analyses was performed using BMDP Software. Data were first analysed to provide standard descriptive parameters (means, standard errors and confidence intervals). These data were used to provide X (core temperature), Y (skin blood flow) and Z (local skin temperature) co-ordinates for three levels of core temperature and five levels of skin temperature. These co-ordinates were used to construct a three-dimension surface that would provide the first complete description of the interactions of core and local skin temperature on skin blood flow for each of these four sites. Between-trial comparisons were performed using multivariate analysis of variance, with three levels of core temperature, five levels of local skin temperature and four levels of measurement sites. In addition, least squares, best-fit linear regression was performed for each of three thermal states (hypothermia, normothermia and hyperthermia). Sources of significant differences were

Table 5.4: Experimental order for each subject (*Latin square*).

SUBJECT	TRIAL 1						TRIAL 2						TRIAL 3					
S1	NEUTRAL		*A*	*H*	*C*	*F*	HOT		*H*	*C*	*F*	*A*	COLD		*C*	*F*	*A*	*H*
	T_{skl}	5	15	25	33	40	T_{skl}	5	15	25	33	40	T_{skl}	40	33	25	15	5
S2	COLD		*F*	*A*	*H*	*C*	NEUTRAL		*A*	*C*	*F*	*H*	HOT		*H*	*F*	*A*	*C*
	T_{skl}	40	33	25	15	5	T_{skl}	5	15	25	33	40	T_{skl}	5	15	25	33	40
S3	COLD		*C*	*A*	*H*	*F*	HOT		*F*	*H*	*C*	*A*	NEUTRAL		*A*	*F*	*H*	*C*
	T_{skl}	5	15	25	33	40	T_{skl}	40	33	25	15	5	T_{skl}	40	33	25	15	5
S4	NEUTRAL		*H*	*A*	*C*	*F*	COLD		*C*	*H*	*F*	*A*	HOT		*F*	*C*	*A*	*H*
	T_{skl}	40	33	25	15	5	T_{skl}	40	33	25	15	5	T_{skl}	5	15	25	33	40
S5	HOT		*A*	*H*	*F*	*C*	NEUTRAL		*H*	*C*	*A*	*F*	COLD		*C*	*F*	*H*	*A*
	T_{skl}	5	15	25	33	40	T_{skl}	5	15	25	33	40	T_{skl}	40	33	25	15	5
S6	COLD		*F*	*A*	*C*	*H*	NEUTRAL		*A*	*C*	*H*	*F*	HOT		*H*	*F*	*C*	*A*
	T_{skl}	40	33	25	15	5	T_{skl}	5	15	25	33	40	T_{skl}	5	15	25	33	40
S7	HOT		*C*	*A*	*F*	*H*	COLD		*F*	*H*	*A*	*H*	NEUTRAL		*A*	*H*	*C*	*F*
	T_{skl}	5	15	25	33	40	T_{skl}	40	33	25	15	5	T_{skl}	40	33	25	15	5
S8	HOT		*H*	*C*	*F*	*A*	COLD		*C*	*F*	*A*	*H*	NEUTRAL		*F*	*A*	*H*	*C*
	T_{skl}	40	33	25	15	5	T_{skl}	40	33	25	15	5	T_{skl}	5	15	25	33	40

Note: Letters '*A*', '*H*', '*C*' and '*F*' represent each of the four blood flow measurements sites (forearm, hand, calf, and foot, respectively). In addition, 'Cold', 'Neutral' and 'Hot' represent each of the three thermal states (target oesophageal temperatures of 35º, 37º and 39ºC respectively), and 'T_{skl}' represents the order of the local skin temperature stimulations.

isolated using *Tukey's HSD* statistic. Data are presented as means with standard errors of the mean (±), unless otherwise noted as standard deviation (SD).

5.3 RESULTS

5.3.1 Thermal variables

5.3.1.1 Oesophageal temperature

Following water immersion, three clearly separate thermal states were achieved (Table 5.5a and 5.5b). Although the target oesophageal temperature for the hypothermic state was 35ºC, this was only achieved in two subjects. Table 5.5a indicates the oesophageal temperature immediately after immersion. When compared to Table 5.5b, this demonstrates that although oesophageal temperature changed by 0.5º-0.7ºC during the transfer from the immersion tank to the climate chamber. However, three clearly separate trials were achieved. Furthermore, maintaining such a low core temperature throughout the duration of the experimental phase of this condition proved to be extremely difficult and an oesophageal temperature of 36.07ºC (±0.37) was achieved when data were averaged across the entire duration of the hypothermic trial (Figure 5.2A). This was higher than the initial target, however, a clear separation between the hypothermic and normothermic (37.04ºC) conditions was achieved, and this was the primary purpose of the pre-treatment. Similarly, following hot-water immersion, the target oesophageal temperature (39.22ºC) was achieved across all subjects, however, this was not maintained for the duration of the hyperthermic trials, and averaged 38.47ºC (±0.34) across the entire hyperthermic trial. This was significantly higher than both the hypothermic (36.1ºC) and normothermic (37.0ºC) trials ($P<0.05$). These changes from the post-immersion state to the experimental phase are contained within Table 5.5a and 5.5b. Based on these outcomes, and for the simplicity of communication, the core temperature of each of these trials will hereafter be referred to as: 36.1ºC (cold), 37.0ºC (thermoneutral) and 38.5ºC (hot).

Although the oesophageal temperature targets were not achieved for the duration of the experimental phase of the cold and hot trials for this study, the measured oesophageal temperatures did not change significantly during the course of each of the local thermal stimulation for each of the blood flow measurement sites (Figure 5.3; $P>0.05$). This outcome was important since clamping core temperature meant that all changes in skin

Table 5.5a: Post-immersion oesophageal temperatures (°C). These data were averaged across the first 2 min immediately after subjects were removed from the water.

Subject	Hypothermia	Normothermia	Hyperthermia
S1	34.68	36.91	39.18
S2	35.62	37.41	39.32
S3	35.42	36.90	39.34
S4	35.47	37.42	39.35
S5	35.14	37.07	39.27
S6	35.68	36.93	38.99
S7	35.86	37.05	39.06
S8	35.67	36.65	39.26
Mean	35.44	37.04	39.22
S.D.	0.38	0.27	0.13

Table 5.5b: Oesophageal temperatures (°C) averaged across the entire duration of each trial for each subject.

Subject	Hypothermia	Normothermia	Hyperthermia
S1	35.57	36.65	38.19
S2	36.47	37.29	38.72
S3	36.39	37.26	38.26
S4	35.59	37.10	38.58
S5	36.05	37.14	39.12
S6	36.34	36.98	38.56
S7	36.33	39.72	38.07
S8	35.85	36.64	38.27
Mean	36.07	36.97	38.47
S.D.	0.37	0.27	0.34

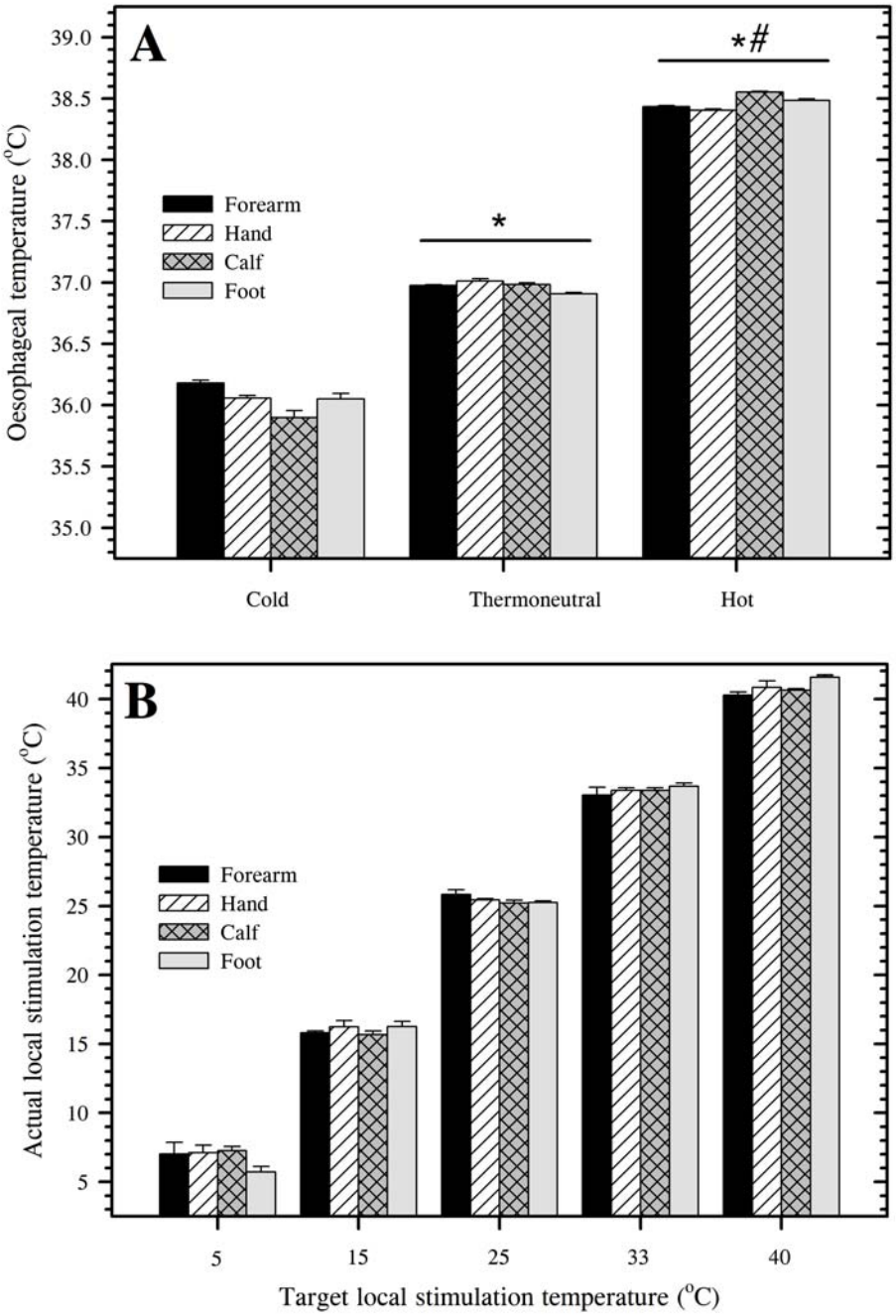

Figure 5.2: Three experimental thermal states (cold, thermoneutral or hot) as indicated by oesophageal temperature. Data are averaged across five stimulation temperatures for each stimulation site (forearm, hand, calf and foot; **A**). * = significantly different from oesophageal temperature during the cold trial (P<0.05). # = significantly different from oesophageal temperature during the thermoneutral trial (P<0.05). The five levels of local stimulation temperature for each site are illustrated (**B**) where the target temperatures were 5°, 15°, 25°, 33° and 40°C. Data shown are means with standard errors of the means (±).

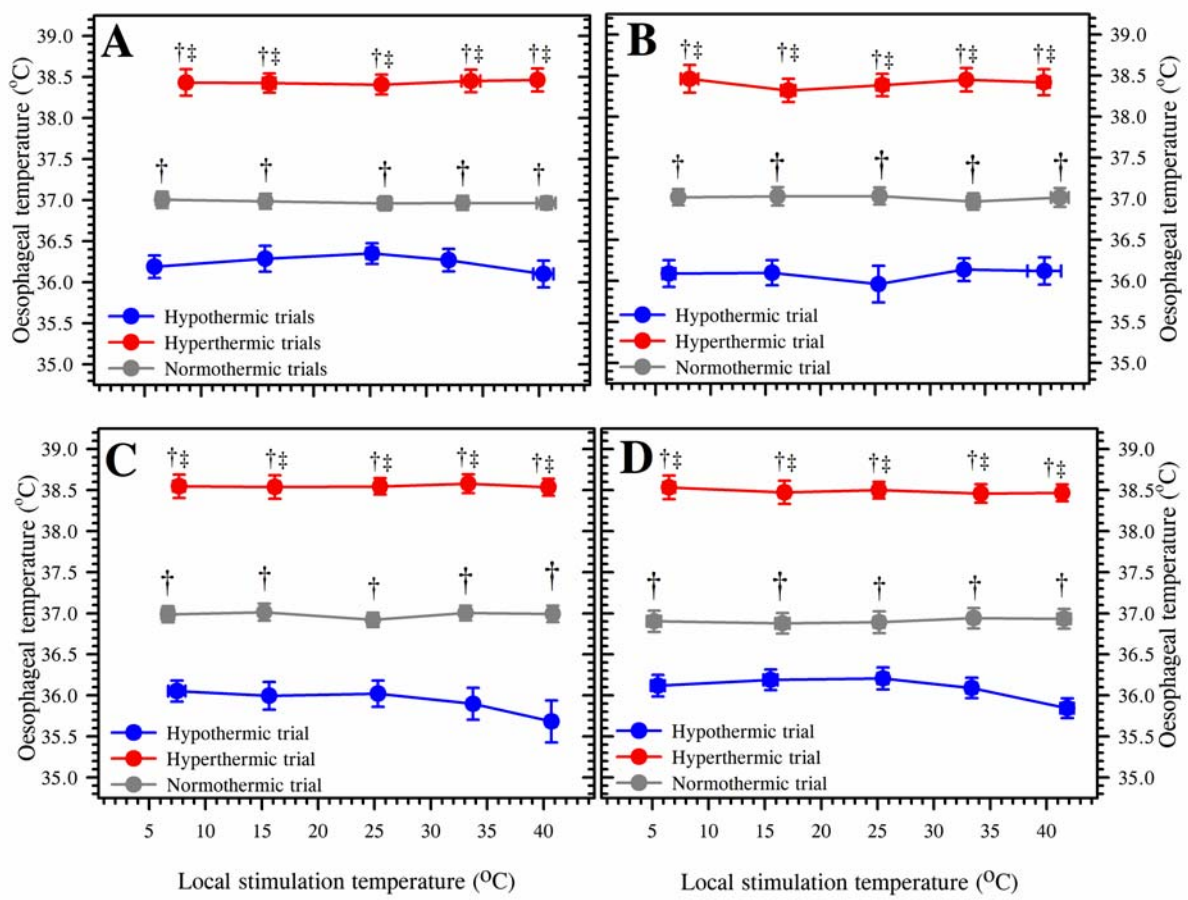

Figure 5.3: Oesophageal temperature (°C) during supine rest for each local skin stimulation temperature for subjects exposed to three thermal states (hypothermia, normothermia and hyperthermia), illustrated for each of the blood flow measurement sites: forearm (**A**), hand (**B**), calf (**C**) and foot (**D**). Data were averaged during each measurement period and are presented as means with standard errors of the means (±). † = significantly different from oesophageal temperature during the cold trial (P<0.05). ‡ = significantly different from oesophageal temperature during the thermoneutral trial (P<0.05).

blood flow throughout each trial could be ascribed solely to the local skin temperature changes, and not to a variation in core temperature.

5.3.1.2 Auditory and rectal temperature

When averaged across the entire trial, auditory canal temperature was 35.80º (±0.1), 36.89º (±0.04) and 38.55ºC (±0.18) for the cold, thermoneutral and hot trials respectively. These were each significantly different from one another ($P<0.05$), but did not differ significantly within each trial for each local stimulation temperature at each of the four sites (forearm, hand, calf and foot; $P>0.05$). Similarly, when rectal temperature was averaged across the entire trial, values were significantly different from each other ($P<0.05$). These were 36.48º (±0.21), 37.31º (±0.05), 39.00º (±0.11) for each of the cold, thermoneutral and hot trials respectively. On average, rectal temperature was approximately 0.5ºC higher than both oesophageal and auditory canal temperatures. While oesophageal temperature provides the best site for measuring core temperature, it was also important to record auditory and rectal temperatures to ensure the three different thermal states (hypothermic, normothermic and hyperthermic) were achieved across the whole of the body core for each subject.

5.3.1.3 Local stimulation temperature

Five local stimulation temperatures were achieved in the current study. Each of the five target temperatures was significantly different from the other (Figure 5.2B; $P<0.05$), but did not differ within each target for any of the measurement sites (forearm, hand, calf and foot; $P>0.05$). The changes in local skin temperature, for one subject during the hyperthermic trial, are shown in Figure 5.4. This figure shows the changes in local stimulation temperature for each of the four measurement sites (forearm, hand, calf and foot).

5.3.1.4 Mean skin temperature

When averaged across the entire trial mean skin temperatures were 22.2º (±0.69), 33.6º (±0.50) and 37.7ºC (±0.21) for the cold, thermoneutral and hot trials respectively. These were all significantly different from each other (Figure 5.5; $P<0.05$). In addition, there were no significant differences in mean skin temperature during any of the local temperature stimulations or between any of the four skin blood flow measurement sites. This thermal clamping was an important outcome, since the aim of this experiment was to investigated the

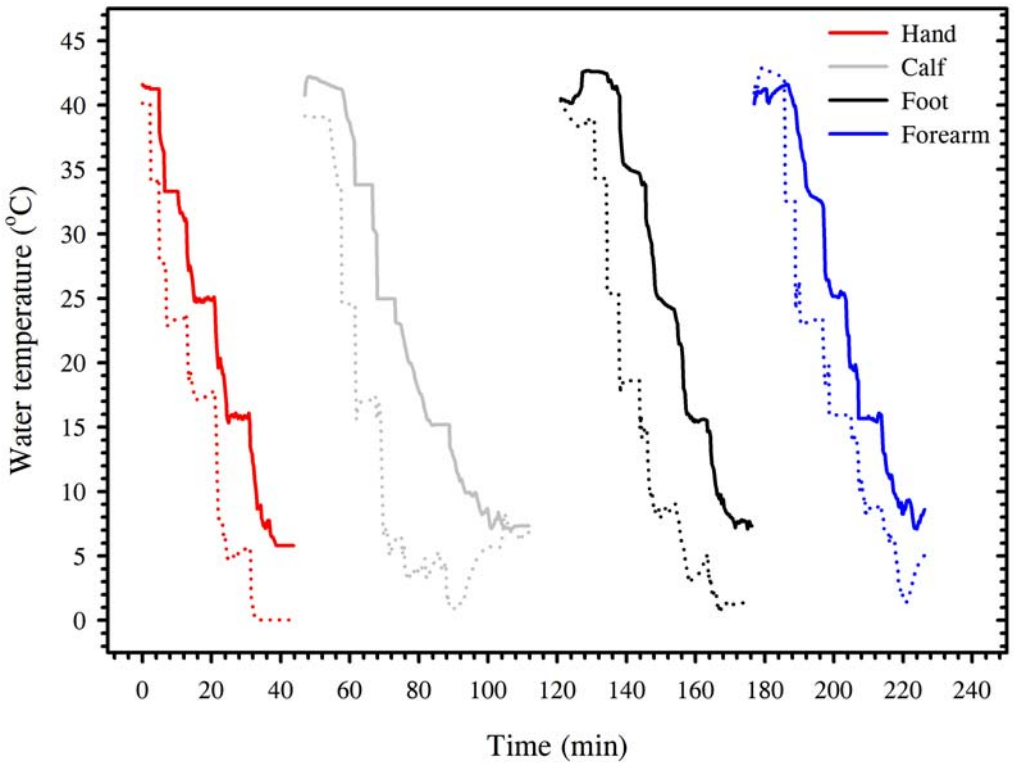

Figure 5.4: The temperature of water within the external (dotted lines) and internal (solid lines) chambers of each of four water-filled plethysmographs (hand, calf, foot and forearm) during hyperthermia for one subject over time. Skin blood flow was measured at each site during each 2-min period when the water temperature within the internal chamber achieved steady-state (5°, 15°, 25°, 33° and 40°C).

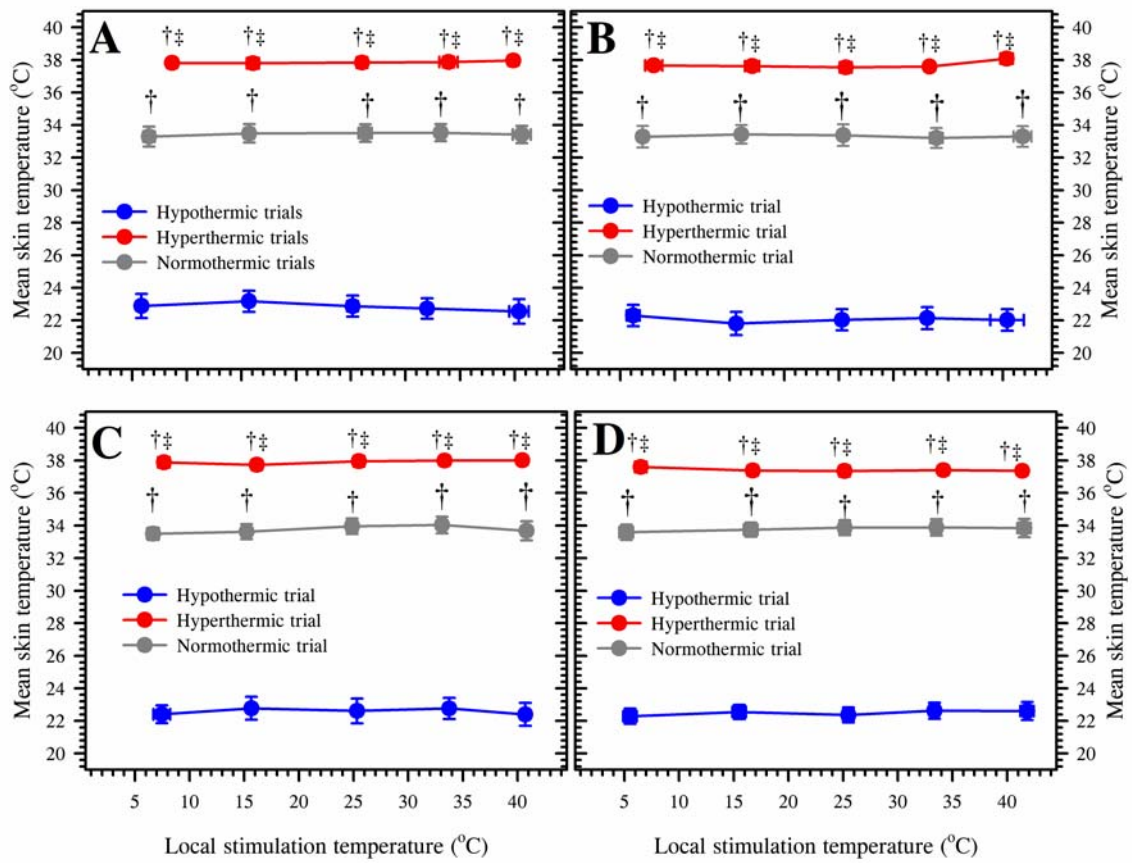

Figure 5.5: Mean skin temperature (°C) of subjects exposed to three different thermal states (hypothermia, normothermia and hyperthermia), during five local stimulation temperatures at four different skin sites (**A:** forearm, **B**: hand, **C**: calf, and **D**: foot). Subjects were in the supine position and at rest during each measurement. Data were averaged during each measurement period and are presented as means with standard errors of the mean (n=8). † = significantly different from mean skin temperature during the cold trial (P<0.05). ‡ = significantly different from mean skin temperature during the thermoneutral trial (P<0.05).

effect of local skin temperature on skin blood flow rather than whole-body skin temperature. For the purpose of these experiments, this core and mean skin temperature clamping is physiologically analogous to denervation. That is, whilst thermoafferent flow still remained, clamping ensured that this flow remained stable. Thus, the only sites from which thermoafferent information was obtained were the four measurement sites during local stimulation. Hence, we will use the term selective physiological denervation to describe this outcome.

5.3.2 Cardiovascular responses

5.3.2.1 Heart rate

The heart rate responses for each of the three thermal states, across the five local stimulation temperatures and during each of the treatment sites are presented in Figure 5.6. When averaged across the duration of the trial, there was a significantly higher heart rate response during the hyperthermic trials (98.8 ±18.3 beats.min^{-1}) compared to both the hypothermic (65.3 ±14.0 beats.min^{-1}) and normothermic (62.7±6.9 beats.min^{-1}) conditions (P<0.05). This increased heart rate during passive heating is the result of redistribution of blood to the periphery to facilitate increased convective heat delivery to the skin allowing for increased heat dissipation (Rowell *et al*., 1969).

Another interesting outcome was the slight elevation in heart rate when local heat (33^{o} and 40^{o}C) was applied to the hand of hyperthermic individuals. This showed an increase in heart rate from 98.9 beats.min^{-1} (5^{o}C exposure) to 104.8 beats.min^{-1} (40^{o}C exposure). This cardiovascular drift, independent of exercise, is associated with progressive increases in cutaneous blood flow due to the elevated core temperature (Coyle and Gonzalez-Alonso, 2001).

5.3.2.2 Blood pressure

There were no significant differences in systolic blood pressure during any of the local stimulation temperatures for any of the stimulation sites (Figure 5.7; P>0.05). However, when averaged across each of the stimulation temperatures, hand treatment elicited a significantly higher systolic blood pressure in the hypothermic trials (138.2 ± 3.2 mmHg) than the normothermic trials (124.4 ± 4.1 mmHg; P<0.05), but was not significantly different from the hyperthermic trials (126.7 ± 4.17 mmHg; P>0.05). There were no other significant differences

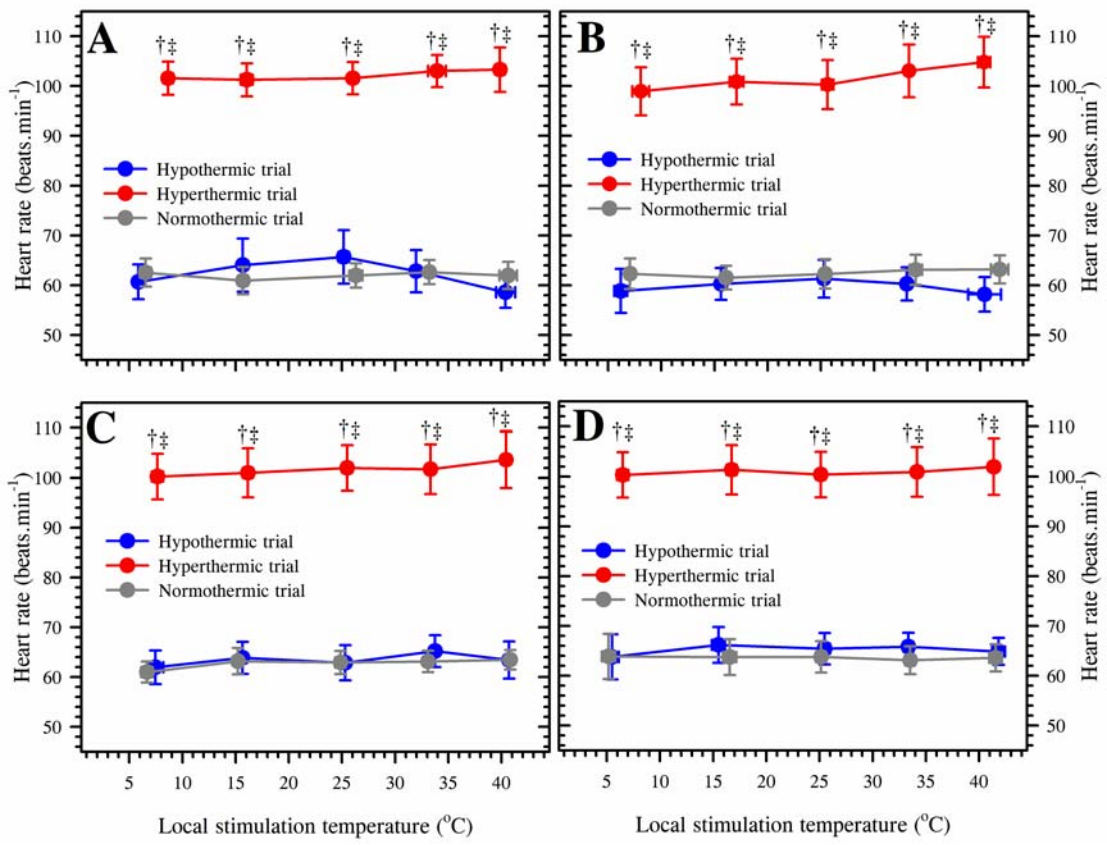

Figure 5.6: Heart rate responses during supine rest for each local stimulation temperature for subjects exposed to three thermal states (hypothermia, normothermia and hyperthermia), illustrated for each of the blood flow measurement sites: forearm (**A**), hand (**B**), calf (**C**) and foot (**D**). Values were averaged during each measurement period and are presented as means with standard errors of the means (n=8). † = significantly different from heart rate during the cold trial (P<0.05). ‡ = significantly different from heart rate during the thermoneutral trial (P<0.05).

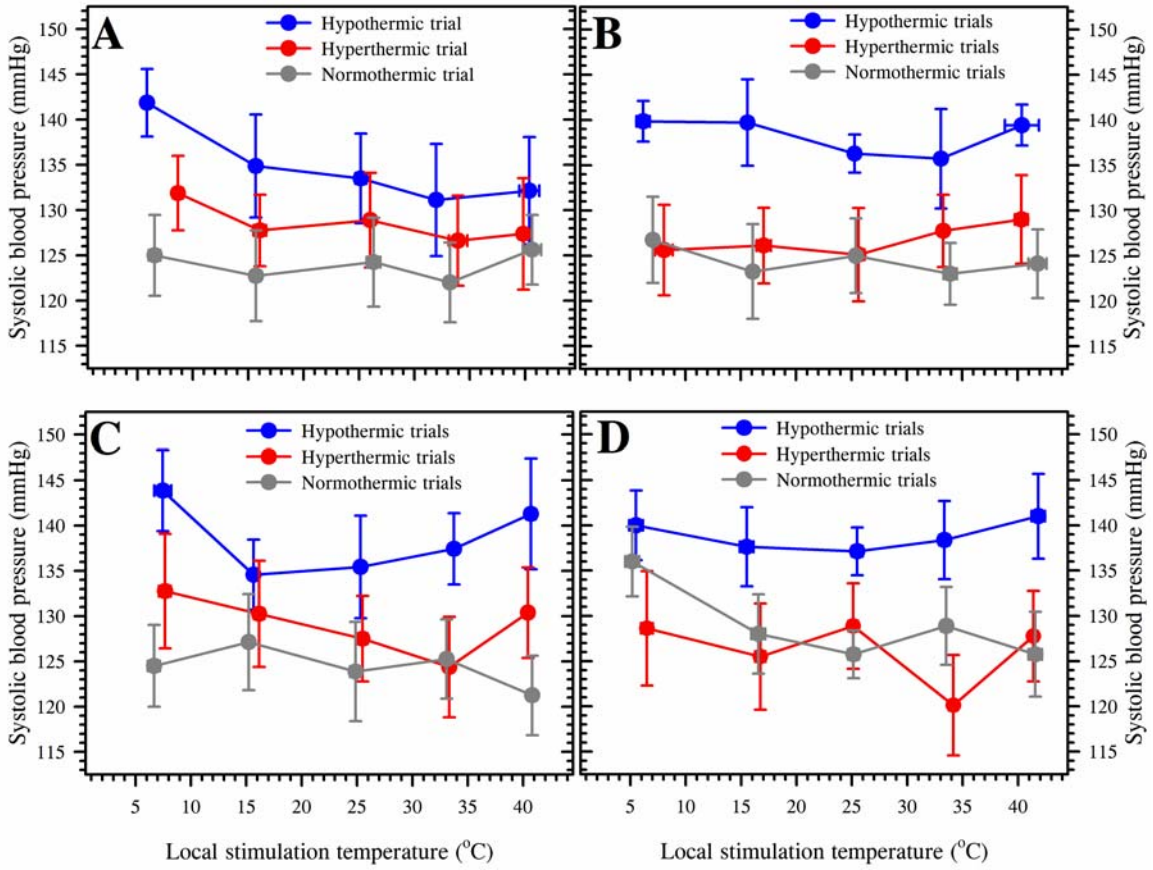

Figure 5.7: Systolic blood pressure (mmHg) responses for individuals exposed to three different thermal states (hypothermia, normothermia and hyperthermia) across five different local skin stimulation temperatures measured at four different sites (**A**: forearm, **B**: hand, **C**: calf and **D**: foot). Values are expressed as means and standard errors of the means (*n*=8).

in systolic blood pressure. Although systolic and mean arterial blood pressures remained largely unaffected during hyperthermia, diastolic blood pressure showed clear separation across the three thermal states which averaged 87.3 mmHg (±0.9), 74.9 mmHg (±0.7) and 66.6 mmHg (±0.3) for the hypothermic, normothermic and hyperthermic trials, respectively (Figure 5.8; $P<0.05$). Mean arterial pressure was well maintained during heat stress, presumably as the result of increased cardiac output (Rowell *et al*., 1969) as indicated by the elevated heart rate, but was significantly increased during hypothermia (Figure 5.9; $P<0.05$). These changes match those predicted for each thermal state.

5.3.2.3 Skin blood flow

The skin blood flow responses during each of the three thermal states (hypothermia, normothermia and hyperthermia) across five different local skin temperatures for the forearm, hand, calf and foot are presented in Figure 5.10. The lowest recorded skin blood flow, during whole-body hypothermia and local cooling (5^{o}C), was 0.27 mL.100 mL^{-1}.min^{-1} in the foot (subject number 8) and this is consistent with previous observations (Allwood and Burry, 1954; Savage *et al*., 1985). However, in the current study the highest recorded skin blood flow, during whole-body hyperthermia and local heating (40^{o}C), was in the hand (subject number 3) (27.6 mL.100 mL^{-1}.min^{-1}), with previous studies reporting an average maximal of between 18.7 and 54.4 mL.100 mL^{-1}.min^{-1} following local heating in warm subjects (Kunkel and Stead, 1939). This large variation in maximal flow is well known in acral skin regions (hands and feet) and is possibly due to fluctuations in the opening and closing of the arteriovenous anastomoses (Lossius *et al*., 1993).

Following regression analysis (Table 5.6) of local skin stimulation temperature upon skin blood flow for each of three thermal states (hypothermia, normothermia and hyperthermia) and for each of the four measurement sites (forearm, hand, calf and foot) it was found that a change in local skin temperature had little to no affect on skin blood flow during the cold exposure. That is, although the analysis revealed a strong correlation between local stimulation temperature and skin blood flow, the slope of the line remained close to zero for each site: forearm (0.04 mL.100 mL^{-1}.min^{-1}.^{o}C ±0.02); hand (0.04 mL.100 mL^{-1}.min^{-1}.^{o}C ±0.02); calf (0.02 mL.100 mL^{-1}.min^{-1}.^{o}C ±0.01); and foot (0.05 mL.100 mL^{-1}.min^{-1}.^{o}C ±0.05). Therefore, in

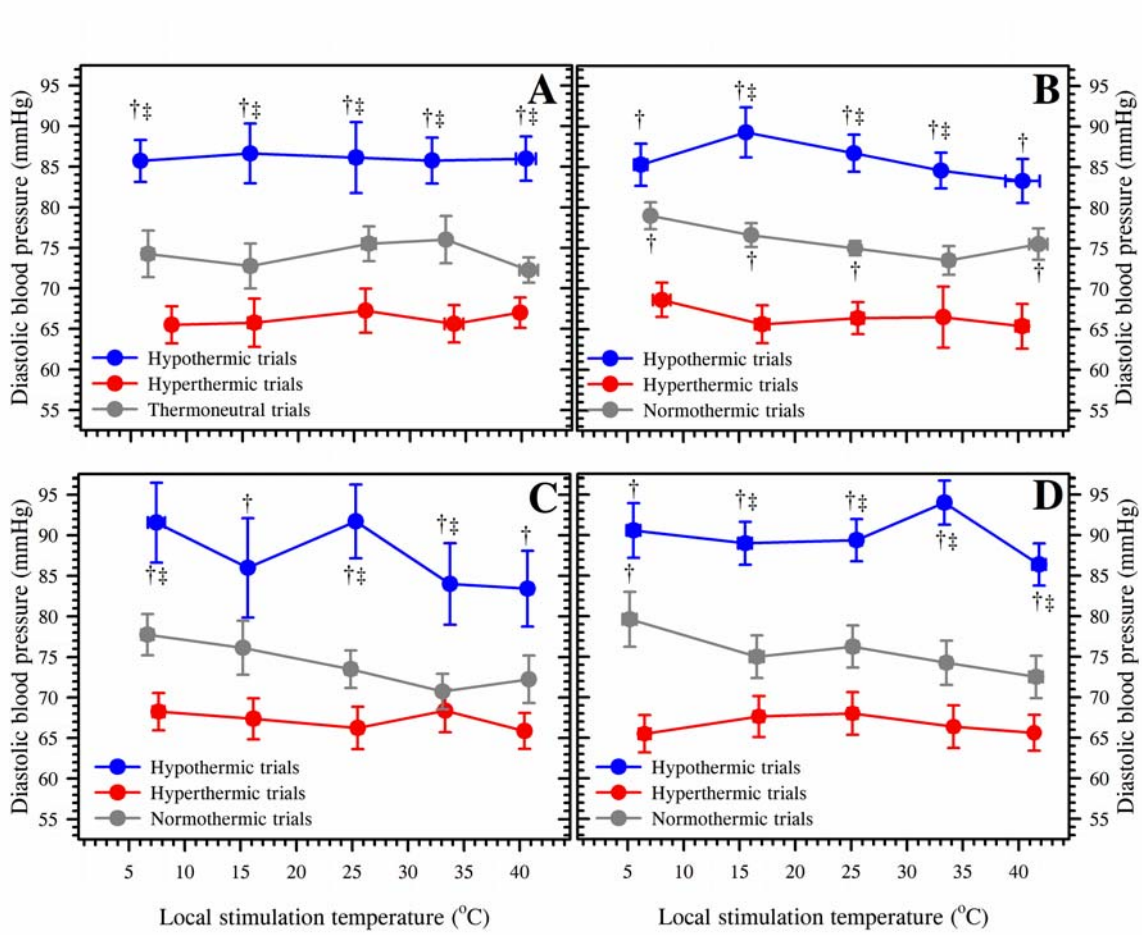

Figure 5.8: Diastolic blood pressure (mmHg) measured across each of the five local skin stimulation temperatures during three different thermal states (hypothermia, normothermia and hyperthermia), for each of the four measurement sites (**A**: forearm, **B**: hand, **C**: calf and **D**: foot). Values are expressed as means and standard errors of the means (n=8). † = significantly different diastolic blood pressure during the hyperthermic trial (P<0.05). ‡ = significantly different from diastolic blood pressure during the thermoneutral trial (P<0.05).

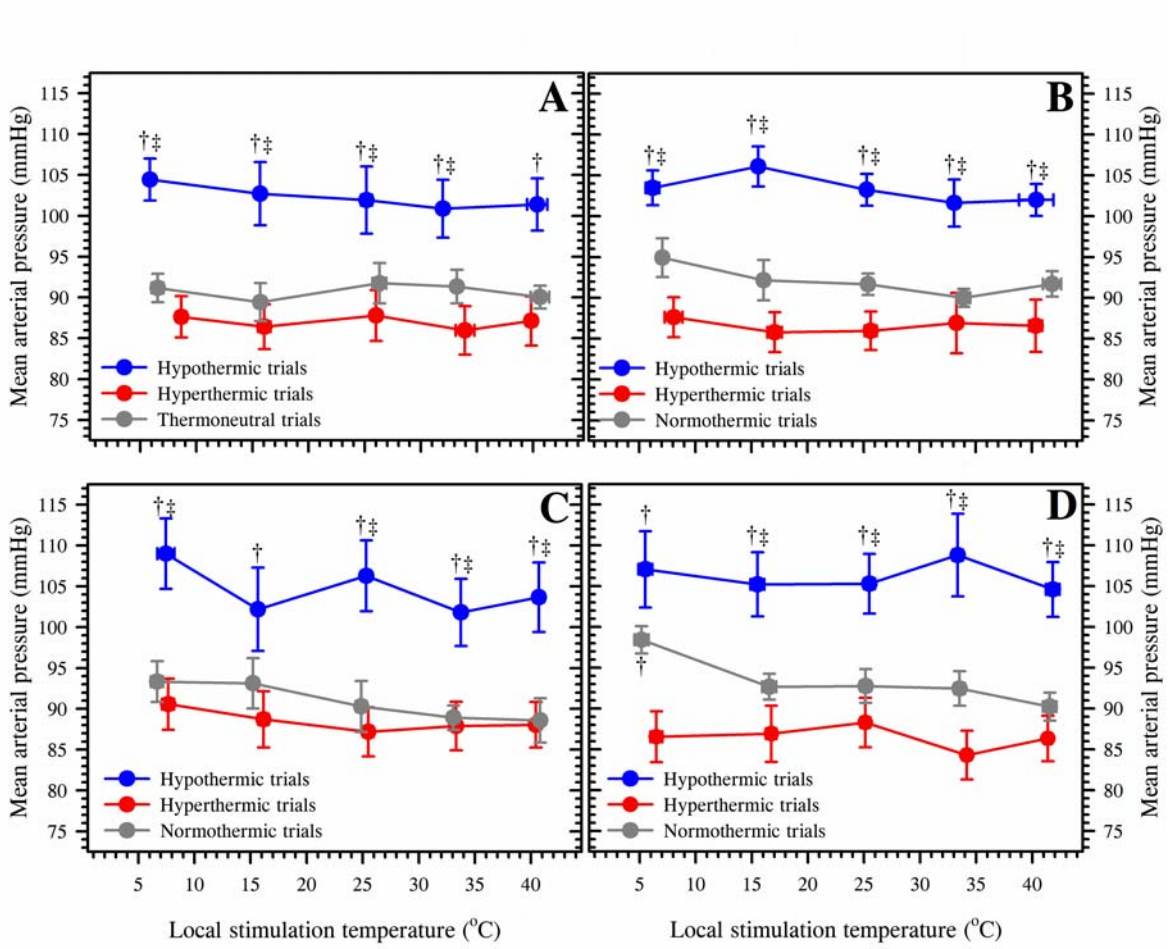

Figure 5.9: Mean arterial pressure (mmHg) during supine rest for each of five local skin stimulation temperatures for subjects exposed to three thermal states (hypothermia, normothermia and hyperthermia), illustrated for each of the blood flow measurement sites: forearm (**A**), hand (**B**), calf (**C**) and foot (**D**). Values are expressed as means and standard errors of the means (n=8). † = significantly different mean arterial blood pressure during the hyperthermic trial (P<0.05). ‡ = significantly different from mean arterial blood pressure during the thermoneutral trial (P<0.05).

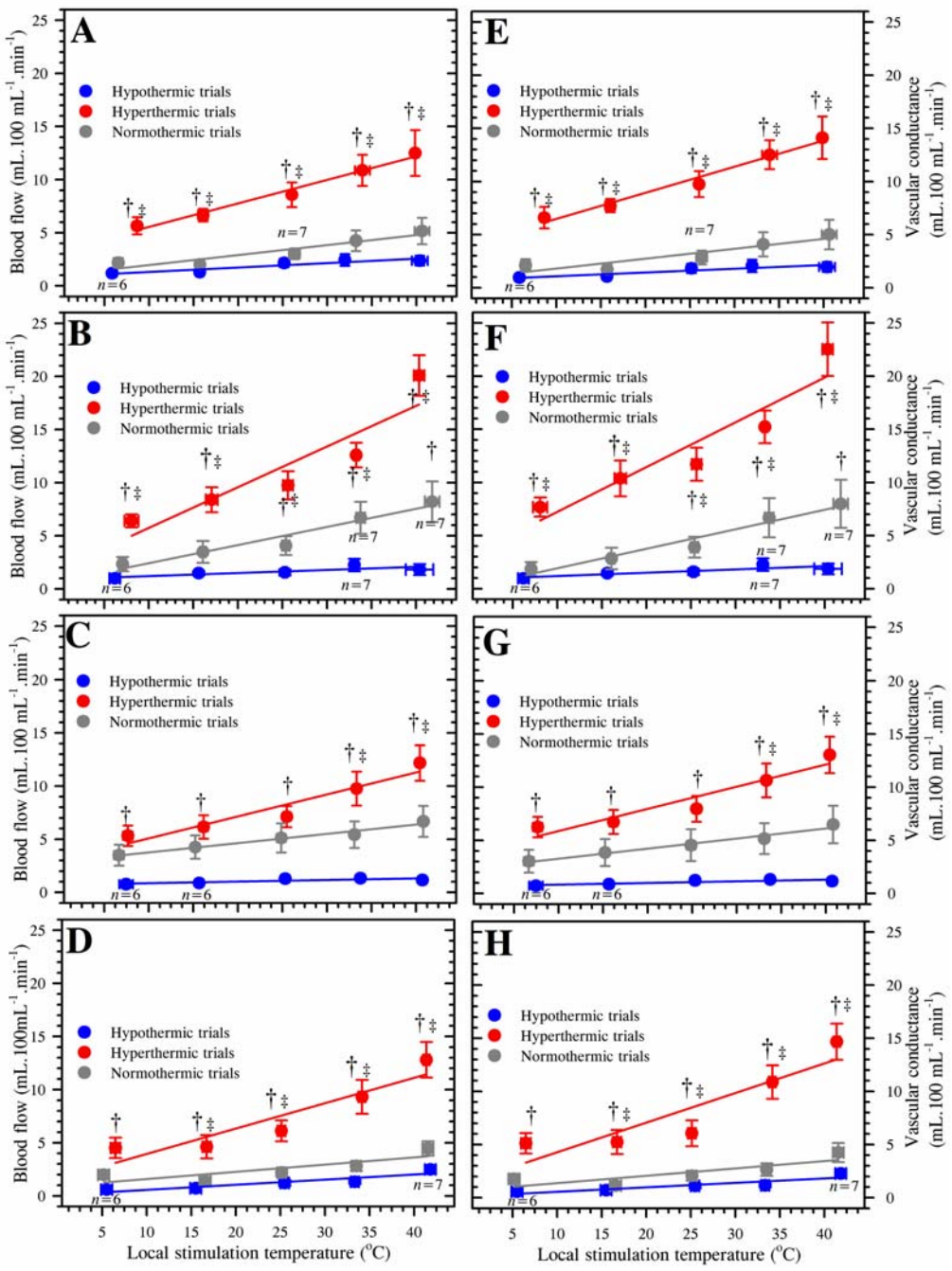

Figure 5.10: Blood flow and vascular conductance through the forearm (**A**) and (**E**), hand (**B**) and (**F**), calf **(C**) and (**G**) and foot (**D**) and (**H**) measured using venous-occlusion plethysmography, in hypothermic, normothermic and hyperthermic individuals with each limb enclosed within a water bladder, the temperature of which was changed to each of five different water temperatures. Values are expressed as means and standard errors of the means (*n*=8, unless otherwise indicated). † = significantly different during the hyperthermic trial (P<0.05). ‡ = significantly different during the thermoneutral trial (P<0.05).

Table 5.6: Linear regression analysis showing the slopes, intercepts and the squares of correlation coefficients (r^2) for skin blood flow measured at the forearm, hand, calf and foot in hypothermic, normothermic and hyperthermic individuals across five levels of local skin treatment (5°, 15°, 25°, 33° and 40°C) at each of four measurement sites. Data are means with standard errors of the means in parentheses.

Variable	Measurement site	Hypothermia			Normothermia			Hyperthermia		
		Intercept	Slope	r^2	Intercept	Slope	r^2	Intercept	Slope	r^2
Skin blood flow	**Forearm**	0.88 (0.28)	0.04 (0.02)	0.86	0.56 (0.32)	0.09 (0.03)	0.88	3.02 (1.24)	0.22 (0.08)	0.98
	Hand	0.99 (0.18)	0.04 (0.02)	0.72	0.63 (0.47)	0.15 (0.04)	0.95	1.73 (1.03)	0.36 (0.07)	0.85
	Calf	0.71 (0.24)	0.02 (0.01)	0.71	2.46 (0.96)	0.08 (0.02)	0.97	3.09 (1.40)	0.18 (0.04)	0.92
	Foot	0.10 (0.19)	0.05 (0.02)	0.84	0.96 (0.63)	0.08 (0.03)	0.68	1.77 (1.34)	0.26 (0.04)	0.86
Vascular conductance	**Forearm**	0.83 (0.27)	0.04 (0.02)	0.84	0.58 (0.33)	0.13 (0.04)	0.85	3.52 (0.27)	0.27 (0.07)	0.98
	Hand	0.96 (0.18)	0.04 (0.02)	0.76	0.60 (0.48)	0.17 (0.04)	0.96	1.94 (1.01)	0.42 (0.08)	0.88
	Calf	0.67 (0.27)	0.02 (0.01)	0.73	1.97 (0.93)	0.10 (0.03)	0.97	3.54 (1.73)	0.24 (0.05)	0.91
	Foot	0.07 (0.19)	0.04 (0.01)	0.81	0.91 (0.64)	0.08 (0.03)	0.67	1.76 (1.45)	0.32 (0.04)	0.82

the hypothermic state, and as local skin temperature was increased there was a very small proportional increase in skin blood flow, and this was evident at each site (Figure 5.11).

When individuals were exposed to the thermoneutral condition, the rate of increase in skin blood with increased local skin temperature (slope) was more than double that observed during the cold exposure at each site (Forearm: $P>0.05$; Hand $P<0.05$; Calf: $P<0.05$; Foot: $P>0.05$). In fact, in this thermoneutral state, the rate of increase in hand blood flow was 89.8% greater than that during the cold condition (Figure 5.10B), but only 57.0%, 68.9% and 53.9% greater for forearm, calf and foot blood flow, respectively. This reflects the relaxation of vascular smooth muscle of the arteriovenous anastomoses within the hand. Similarly, in the hyperthermic condition, the increase in slope from thermoneutral was greatest in the foot and indicated a 74.7% increase ($P<0.05$). This was higher than the forearm ($P<0.05$), hand ($P<0.05$) and calf ($P>0.05$) that showed an increase of 44.3%, 40.1% and 66.2%, respectively.

5.3.2.4 Three-dimensional analyses of skin blood flow

Using the X, Y and Z coordinates defined above (section 5.2.5), the skin blood flow responses over a range of core and local stimulation temperatures can be explored simultaneously. The resulting three-dimensional surfaces describe the change in skin blood flow of hypothermic (36.1^{o}C), normothermic (37.0^{o}C) and hyperthermic (38.5^{o}C) individuals when local skin was cooled or heated over a range of temperatures (5^{o}, 15^{o}, 25^{o}, 33^{o} and 40^{o}C). These surfaces are shown for each measurement site: forearm (Figure 5.12), hand (Figure 5.13), calf (Figure 5.14) and foot (Figure 5.15). Each three-dimensional surface is colour coded with respect to skin blood flow to each region along the spectrum between blue (minimal blood flow) to orange (maximal blood flow).

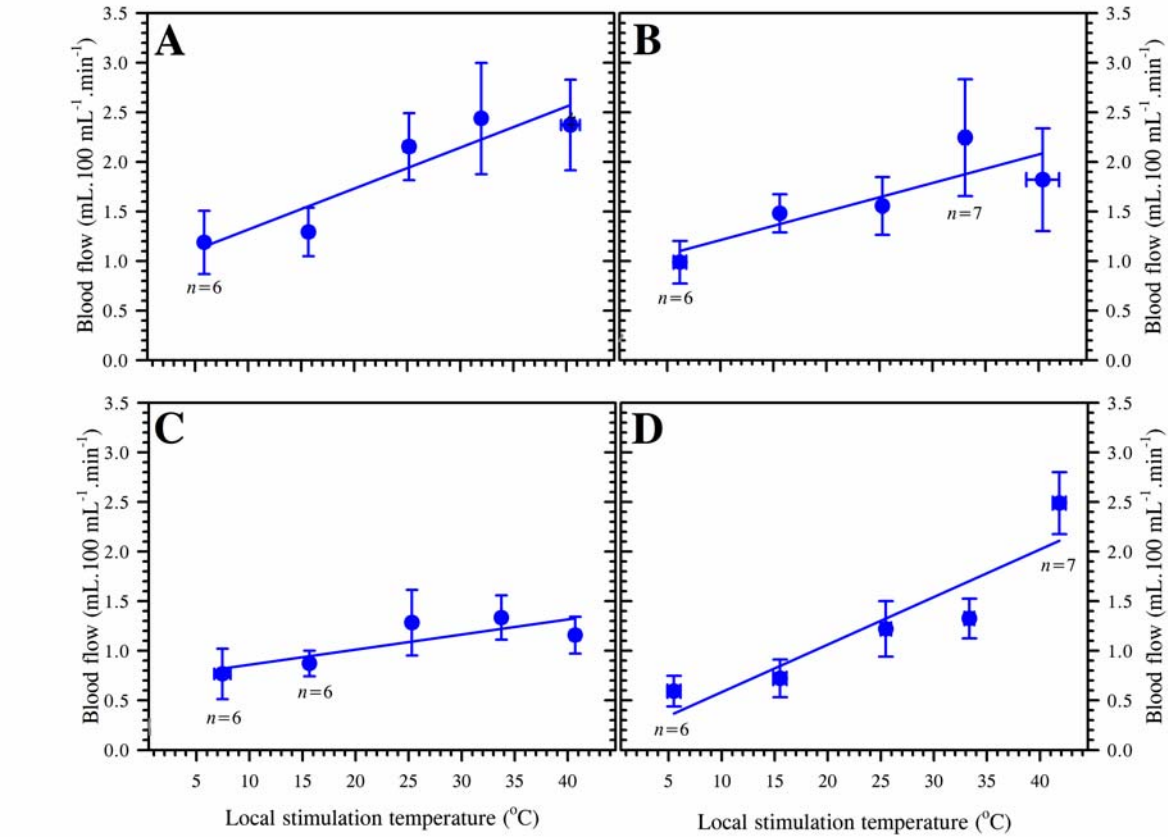

Figure 5.11: Skin blood flow from forearm (**A**), hand (**B**), calf (**C**) and foot (**D**) measured using venous-occlusion plethysmography, in hypothermic individuals with each limb enclosed within a water bladder, the temperature of which was changed to each of five different water temperatures. Values are expressed as means and standard errors of the means (*n*=8, unless otherwise indicated).

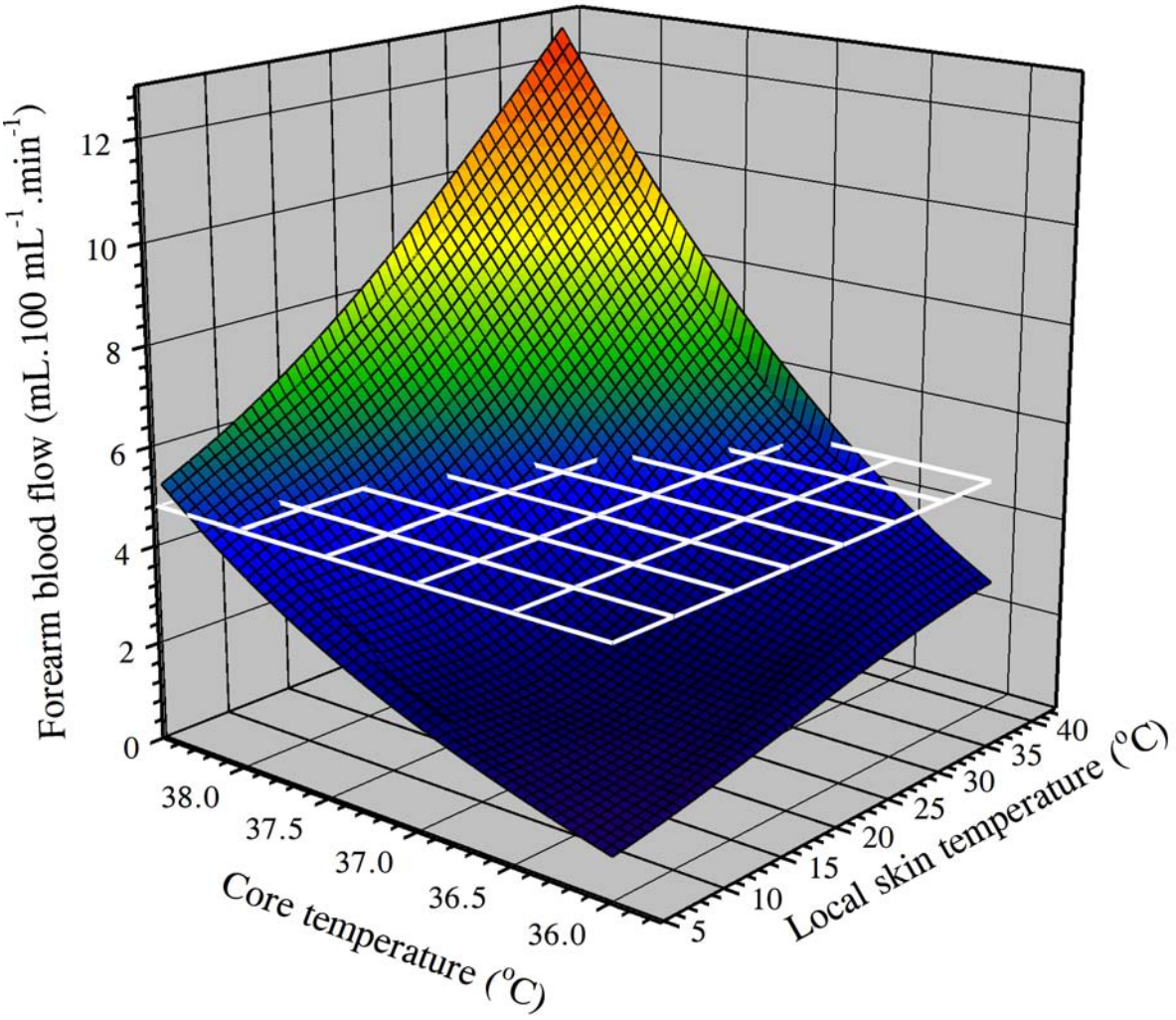

Figure 5.12: Three-dimensional surface for forearm blood flow across a range of core (oesophageal) temperatures and local skin temperatures of nearly nude individuals at rest. Subjects (*n*=8) were studied in three thermal states (hypothermic: 36.1°C; normothermic: 37.0°C; hyperthermic: 38.5°C) with five local skin temperature changes made with each of these states (5°, 15°, 25°, 33° and 40°C). While the surface shows a resolution of 0.2°C this precision is artificial and has been achieved through extrapolation. The white transparent plane represents the axis at 4.79 mL.100 $mL^{-1}.min^{-1}$ of forearm blood flow.

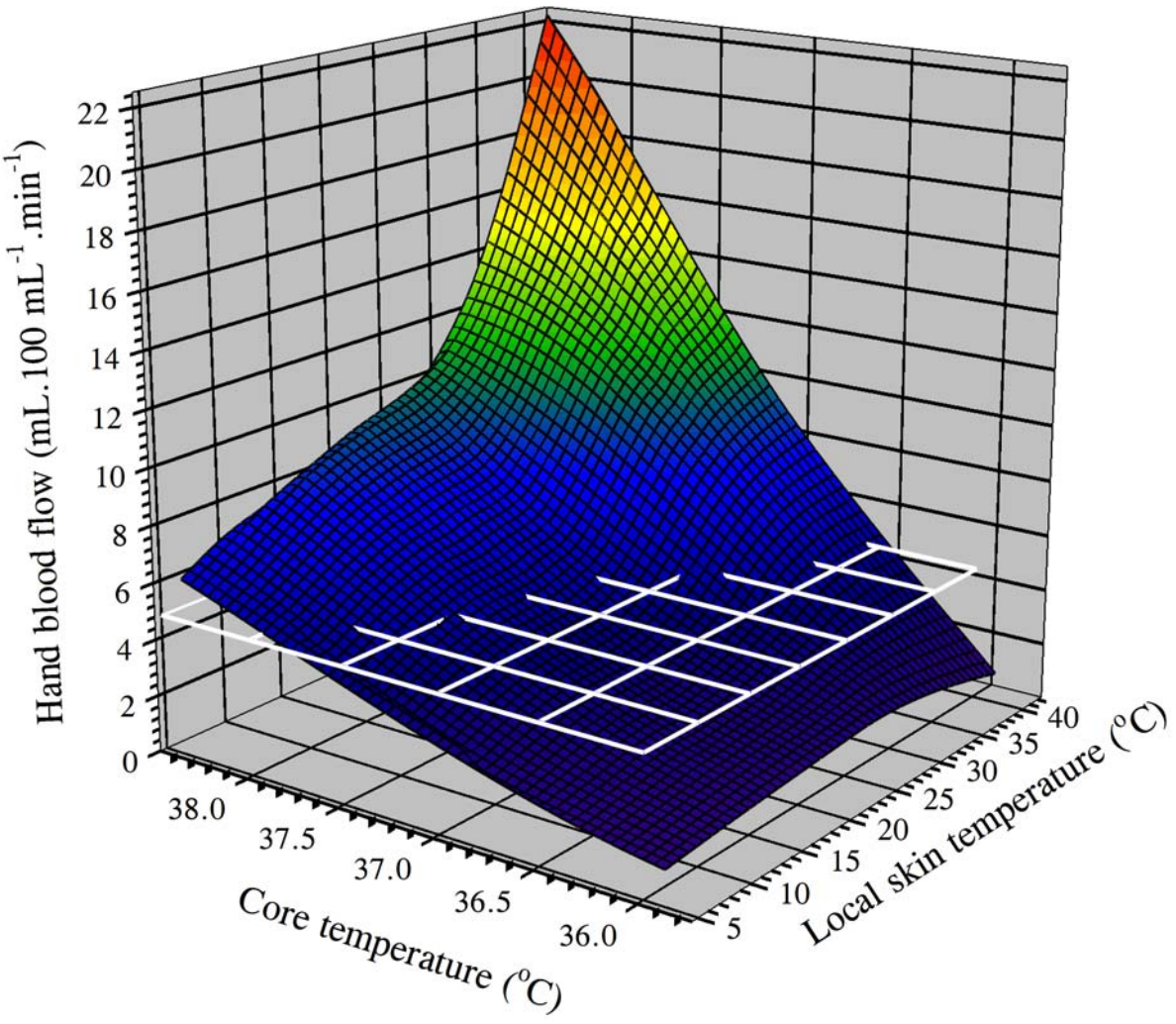

Figure 5.13: Three-dimensional surface for hand blood flow across a range of core (oesophageal) temperatures and local skin temperatures of nearly nude individuals at rest. Subjects (*n*=8) were studied in three thermal states (hypothermic: 36.1°C; normothermic: 37.0°C; hyperthermic: 38.5°C) with five local skin temperature changes made with each of these states (5°, 15°, 25°, 33° and 40°C). While the surface shows a resolution of 0.2°C this precision is artificial and has been achieved through extrapolation. The white transparent plane represents the axis at 4.79 mL.100 $mL^{-1}.min^{-1}$ of hand blood flow.

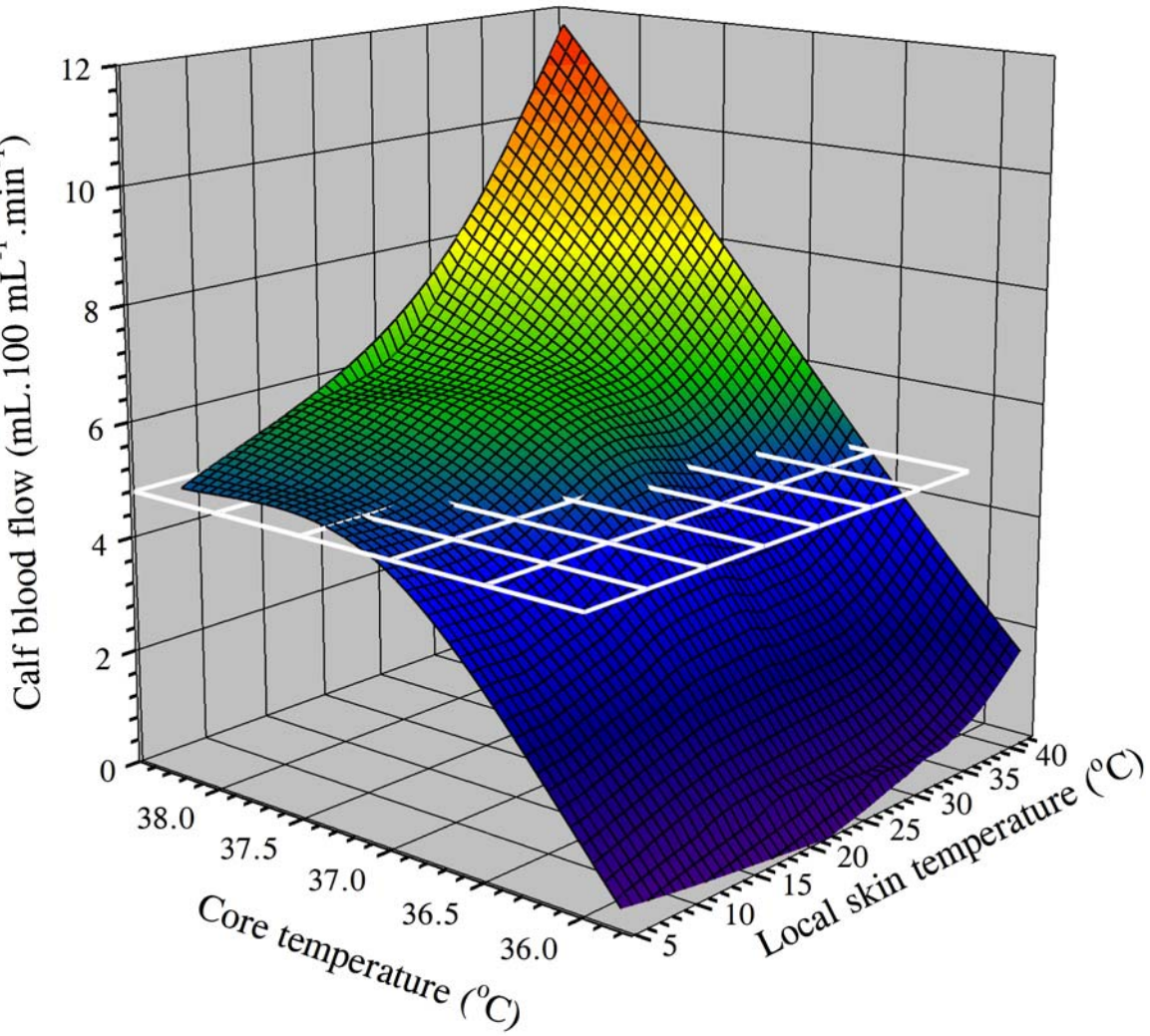

Figure 5.14: Three-dimensional surface for calf blood flow across a range of core (oesophageal) temperatures and local skin temperatures of nearly nude individuals at rest. Subjects (*n*=8) were studied in three thermal states (hypothermic: 36.1°C; normothermic: 37.0°C; hyperthermic: 38.5°C) with five local skin temperature changes made with each of these states (5°, 15°, 25°, 33° and 40°C). While the surface shows a resolution of 0.2°C this precision is artificial and has been achieved through extrapolation. The white transparent plane represents the axis at 4.79 mL.100 $mL^{-1}.min^{-1}$ of calf blood flow.

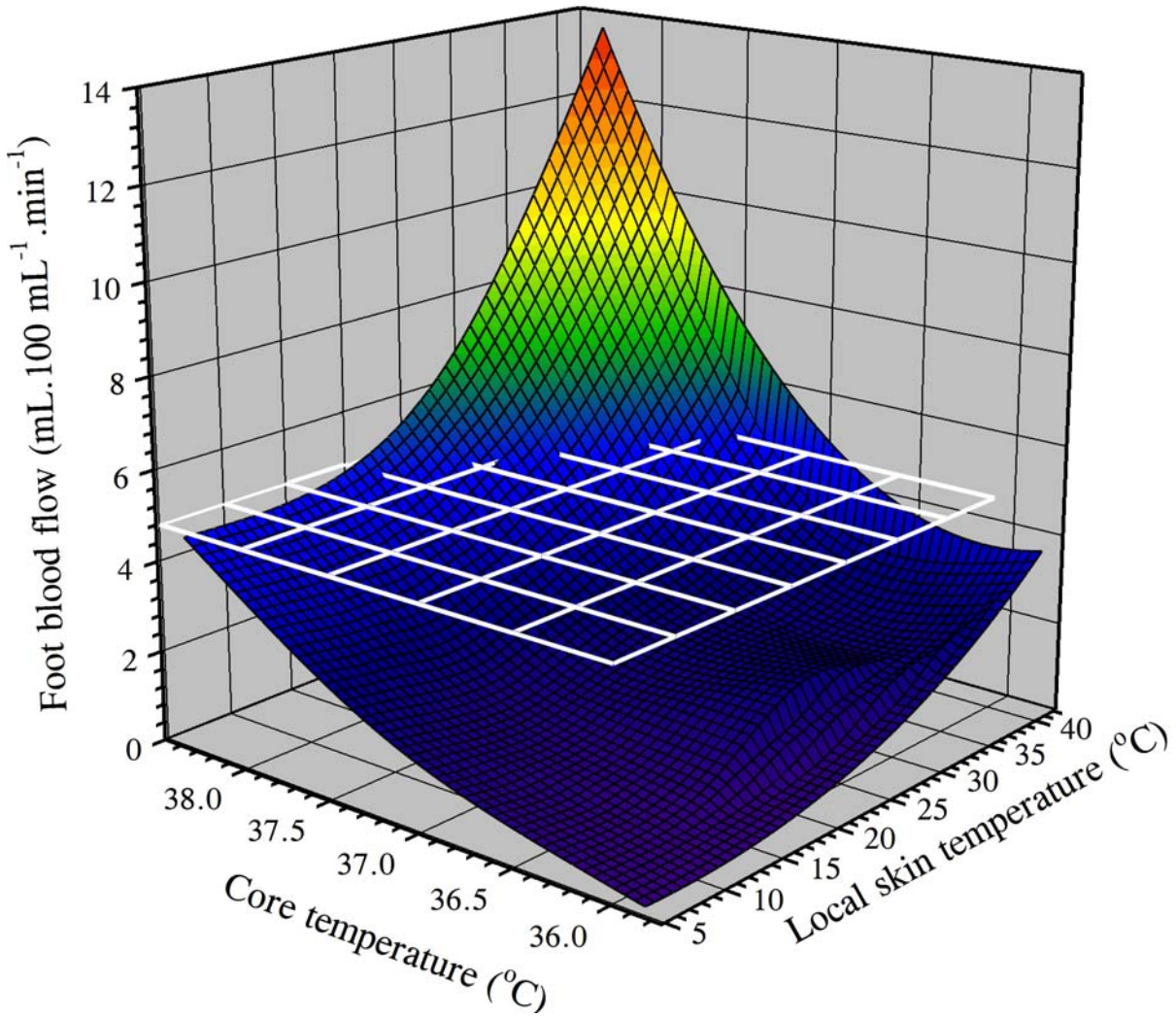

Figure 5.15: Three-dimensional surface for foot blood flow across a range of core (oesophageal) temperatures and local skin temperatures of nearly nude individuals at rest. Subjects (*n*=8) were studied in three thermal states (hypothermic: 36.1°C; normothermic: 37.0°C; hyperthermic: 38.5°C) with five local skin temperature changes made with each of these states (5°, 15°, 25°, 33° and 40°C). While the surface shows a resolution of 0.2°C this precision is artificial and has been achieved through extrapolation. The white transparent plane represents the axis at 4.79 mL.100 $mL^{-1}.min^{-1}$ of foot blood flow.

An additional feature on these Figures is blood flow along the 4.79 mL.100 mL^{-1}.min^{-1} axis; transparent white surface. This line represents the mean skin blood flow across all for sites during the thermoneutral condition with a local stimulation temperature of 33^{o}C. For the forearm, calf and foot figures this line marks the lowest third of maximal blood flow over the entire range of core and skin temperatures measured in this experiment. However, for hand blood flow (Figure 5.13) this axis crosses only one fifth of maximal blood flow in that region. Interestingly, 71.8% of blood flow readings across the entire range of core and skin temperatures presented in this study, were less than 4.79 mL.100 mL^{-1}.min^{-1} in the foot and this is indicative of very powerful vasoconstrictor tone in this region. That is, even when local heating was applied to the foot, it did not appear to abolish the strong sympathetic drive to these blood vessels. This was larger than that of the forearm (53.5%), hand (47.0%) and calf (51.3%).

5.3.3 Whole-body mass changes

Since hydration state is known to influence skin blood flow (Montain and Coyle, 1992) it was important, for the current study, that subjects remained hydrated for the duration of each trial. However, during hyperthermia subjects did experience mild dehydration (Table 5.7) and this was significantly different from the hypothermic and normothermic trials (P<0.05). This level of dehydration (~2%) may account for lower than expected maximal skin blood flows for each skin region. Although some dehydration was experienced during these trials, it is possible that this had minimal impact on the skin blood flow results since previous studies have reported dehydration of greater than 3% to cause significant reductions in skin blood flow during hyperthermia (Horstman and Horvath, 1972; Montain and Coyle, 1992).

5.3.4 Psychophysical responses

As expected, subjects on average felt ‘very cold’ and ‘uncomfortable’ throughout each of the cold trials, ‘neutral’ and ‘comfortable’ during the thermoneutral trials and ‘hot’ to ‘very hot’ and ‘uncomfortable’ during the hot trials. However, whole-body thermal sensation, thermal discomfort and pleasantness were not significantly different from each other for each of the local stimulation temperatures at each of the four stimulation sites (forearm, hand, calf or foot; P>0.05). That is, changing the local skin temperature did not influence whole-body thermal sensation, thermal discomfort or pleasantness.

Table 5.7: Whole-body sweat losses as indicated by mass changes (%) during each trial.

Subject	Hypothermia	Normothermia	Hyperthermia
S1	0.69%	0.03%	2.25%
S2	0.60%	0.53%	2.00%
S3	0.21%	0.31%	2.16%
S4	0.08%	0.85%	2.64%
S5	0.37%	1.54%	2.45%
S6	0.87%	0.30%	1.17%
S7	0.78%	0.27%	2.88%
S8	0.37%	0.06%	3.50%
Mean	0.50%	0.49%	2.38%†
S.D.	0.10%	0.18%	0.24%

† significantly different from hypothermia and normothermia ($P<0.05$).

When asked how the temperature of the hand and foot felt during local heating in the hypothermic state, subjects felt the local temperature to be warmer than during the same local stimulation on the forearm and calf (Figure 5.16). That is, when the hand and foot were heated to 33^{o} and 40^{o}C subjects sensed the local skin temperature to be 'slightly warm' to 'warm'. This was significantly warmer than when the forearm and the calf were heated to the same local skin temperature and subjects felt each site to be 'cool' to 'slightly cool' (P<0.05). Although the local temperature felt warmer at the hand and at the foot, this did not have a significant impact on thermal comfort or pleasantness and each of the four sites during whole-body cooling progressively felt more comfortable with local heating (P>0.05).

5.4 DISCUSSION

The primary focus of the current study was to not only investigate the combined influences of core (hypothermia, normothermia and hyperthermia) and local skin temperature upon cutaneous blood flow, but to also examine cutaneous blood flow differences, if they existed, between acral (hand and foot) and non-acral (forearm and calf) skin regions across a wide range of thermal loads. The most significant findings from this study were that local heating of these four sites (forearm, hand, calf and foot) in hypothermic, normothermic and hyperthermic individuals, caused skin blood flow to increase proportionally with increasing local skin temperature and this was even more pronounced during hyperthermia. Furthermore, when skin blood flow was compared across four different skin regions, blood flow to the hand was the highest in the hyperthermic state with the warmest local stimulation temperature. These observations pertain only to the supine state.

5.4.1 The effect of core and mean skin temperature on skin blood flow

It is well known that the thermal reflexes associated with the control of skin blood flow are dominated by core temperature (Spealman, 1945; Pérgola *et al*., 1993). This was evident in the current study, but most obvious when subjects were hypothermic, since changing local skin temperature had minimal impact on skin blood flow during this condition. Since small regions of skin were heated, during the highest local stimulation temperature (40^{o}C) rapid reductions in core temperature were avoided. Thermal feedback is received by the pre-optic hypothalamus, from activation of both central and peripheral thermoreceptors (Hensel, 1981). This relayed information is integrated within the hypothalamus to initiate the effector response, in this case, through sympathetic vasoconstrictor nerves and the release of

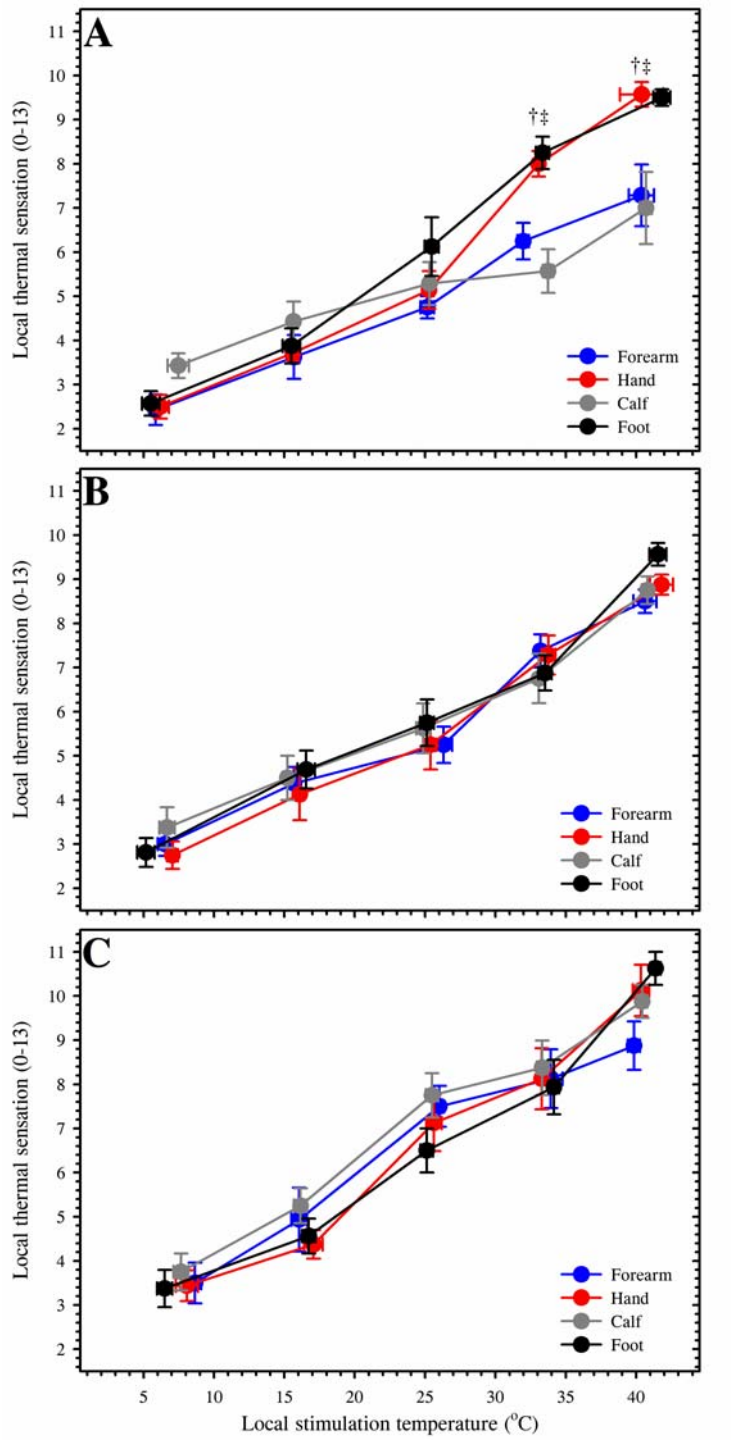

Figure 5.16: Local thermal sensation at four different skin sites (forearm, hand, calf and foot) during three different thermal states: hypothermia (**A**), normothermia (**B**) and hyperthermia (**C**) across five local skin stimulation temperatures. Data were averaged during each measurement period and are presented as means with standard errors of the mean (±). † = significantly different from local thermal sensation of the forearm. ‡ = significantly different from local thermal sensation of the calf.

noradrenaline. The binding of noradrenaline to $alpha_1$ and $alpha_2$ adrenergic-receptors mediates smooth muscle contraction, thereby reducing blood vessel diameter and increasing vascular resistance, thus reducing cutaneous vascular conductance (Keller *et al*., 2010). When individuals are cold, this vasoconstrictor response, found in both acral and non-acral skin, is very powerful and protects the core from large reductions in temperature (Johnson and Proppe, 1996). This was evident in the current study, where mean skin temperature (~22ºC) was as much as 14ºC lower than core temperature (~36ºC) during hypothermic trials.

However, when subjects were exposed to a thermoneutral environment, skin blood flow was slightly higher across all measurement sites, than during hypothermia, but was still smallest in the foot. It would appear that in the thermoneutral zone, where body temperature is regulated solely by changes in vasomotor tone (Werner *et al*., 2008), vasoconstrictor tone is not completely abolished, but is less powerful than during the cold exposure (Roddie, 1983). Blocking or cutting these vasoconstrictor nerves has been shown to cause large increases in blood flow through these blood vessels (Blair *et al*., 1960). Active vasodilatory nerves also exist in these non-acral skin regions, however, these are often not tonically active in the normothermic state (Barcroft and Edholm, 1943). Therefore, in the current study, when subjects were slightly warmer, as occurred during normothermia, the observed increases in skin blood flow, across all sites were likely to be a result of reduced, but not absent, symapathetic drive through vasoconstrictor fibres, which was initiated from reduced efferent drive from the hypothalamus. Specifically, forearm blood flow showed slight increases in the thermoneutral condition with the hot (40ºC) stimulation temperature. These values were not dissimilar to those reported by Johnson *et al*. (1996) who reported forearm blood flow to be approximately 5 mL.100 $mL^{-1}.min^{-1}$ in conditions where oesophageal temperature was 36.5ºC and local skin temperature was 32ºC.

Large increases in skin blood flow were evident in the current study following whole-body heating. This facilitated convective heat delivery to the skin, thus increasing heat dissipation. In this state, mean skin temperature (37.7ºC) was much closer to oesophageal temperature (38.5ºC) than during the cold exposure. In this condition, forearm blood flow was lower by 5-15 mL.100 $mL^{-1}.min^{-1}$ during the hottest stimulation temperature (40ºC) than what may be expected based on previous studies (Johnson *et al*., 1976). However a possible explanation for these differences in forearm blood flow responses at these higher temperatures

was the difference in mean skin temperature. Johnson *et al*. (1976) reported mean skin temperatures of 38-42ºC. In addition, the heating protocol was incremental rather than clamped and this could also affect forearm blood flow since dynamic activation of thermoreception has been shown to cause a greater sympathetic drive than during static activation (as was the case in the current study).

Nonetheless, this profound increase in peripheral circulation is primarily mediated by active vasodilatation within hairy (non-acral) skin regions and in the current study included the forearm, calf as well and dorsal surfaces of the hands and feet (Blair *et al*., 1960). These fibres are believed to not be present in acral skin, where increases in blood flow occur exclusively due to the release of vasoconstrictor tone (Roddie, 1983). While the neurotransmitter for active vasodilatation remains to be elucidated, it has been shown that vasodilatory nerves are cholinergically activated (Kellogg *et al*., 1995) and are associated with sweat gland activity (Blair *et al*., 1960; Fox *et al*., 1963; Love and Shanks, 1962). More recent evidence suggests that co-transmission occurs during cutaneous active vasodilatation where acetylcholine (primarily responsible for sweating) and one or more unknown vasodilators are co-released (Kellogg *et al*., 2006).

Peripheral vasodilatation, as occurs when individuals are hot, allows for large volumes of blood to be redistributed from central circulation and other organs to facilitate heat delivery to the skin (Rowell, 1974; Johnson and Proppe, 1996). In the current study, profound increases in skin blood flow occurred during the hyperthermic condition and caused significant changes in cardiovascular function, as indicated by an increased heart rate and reduced diastolic blood pressure. However, mean arterial pressure was well maintained during heating, suggesting that cardiac output was increased to maintain mean arterial pressure (Rowell, 1974). This would explain the higher than normal heart rates, and is consistent with other studies reporting a rise in heart rate by approximately 30 beats per minute per 1ºC elevation in core temperature (Johnson and Proppe, 1996). Previous studies have also reported that stroke volume remains unchanged or only slightly elevated, during exposure to heat, indicating the rise in cardiac output is largely driven by heart rate (Rowell *et al*., 1969; Wilson *et al*., 2007). This would also account for the fact that reduced diastolic blood pressure had little to no effect on mean arterial pressure, since it is well known that central venous pressure is reduced during heating (Peters *et al*., 2000; Crandall *et al*., 2008). Without these cardiovascular adjustments, thermoregulation

would be compromised, as a result of reduced skin blood flow and therefore reduced convective and conductive heat loss to the environment.

5.4.2 The interactions of core and local skin temperature on skin blood flow

The direct application of local heating or cooling to an area of skin also contributes to the control of skin blood flow, and this occurs independently of central sympathetic vasoconstrictor and vasodilator mechanisms (Johnson *et al*., 1976; Vanhoutte, 1980). However, the magnitude of these local affects on skin blood flow is dependent on the existing centrally mediated sympathetic drive (Spealman, 1945, Charkoudian *et al*., 2002). This theory was tested in the current experiment by simultaneously measuring skin blood flow and applying a range of local temperatures to four different skin regions across three different thermal states.

In the current study, when subjects were in the normothermic state, and when local heat was applied to the skin, increased vasodilatation occurred. This has been shown previously to occur biphasically (Pérgola *et al*., 1993; Charkoudian *et al*., 2002), and also independently of the sympathetic nervous system (Minson *et al*., 2002). That is, following local heating (>40^{o}C), within the first 3-5 minutes there is an initial rapid increase in blood flow, then a moderate decrease, then a slower (~30 min) continuous rise until a plateau is achieved (Pérgola *et al*., 1993; Brunt and Minson, 2011). The initial rapid-phase of vasodilatation and also the local sensation of heat, is through activation of sensory nerves, primarily C-fibre afferents (Charkoudian, 2003). The secondary phase of vasodilatation in response to local stimulation occurs independently of sensory nerve activation, where the plateau response is caused mostly by nitric oxide, released from the vascular endothelium (Dietz *et al*., 1994; Minson *et al*; 2001). However, maximal vasodilatation is usually higher in the second phase than in the first, but the response is dependent on the strength of the stimulus and the existing sympathetic drive (Pérgola *et al*., 1993). Since, in the current study the local stimulus lasted approximately 5 min, it is likely that vasodilatation, caused by local heating, occurred in the initial rapid-phase and would explain why maximal blood flow was slightly lower than previous reports of blood flows recorded in the same experimental conditions, but during longer exposure times (Barcroft and Edholm, 1943).

The finding in the present study that local heating did not elicit large increases in skin

blood flow across each measurement site during low core temperatures is a direct reflection of powerful, centrally mediated, sympathetic vasoconstriction. Even when the local thermal stimulus was strong (~40^{o}C), the rise in skin blood flow was minimal (<3 mL.100 mL^{-1}.min^{-1}). That is, the strong sympathetic vasoconstriction acted to limit the effect of local temperature changes, and this was apparent across all skin regions. However, when this centrally mediated smooth muscle activation is reduced, as occurs in normothermia and even more in hyperthermia, the local influences on smooth muscle contraction were more fully expressed. These interactions between sympathetic drive and local effects upon vascular smooth muscle are shown in Figure 5.17.

The mechanisms of local cooling are slightly different from those accompanying local heating. Local cooling exerts its effects through modifications of the adrenergic branch of the sympathetic nervous system (Lindblad, 1990; Pérgola *et al*., 1993). This is achieved by enhancing the release of noradrenaline from the pre-synaptic nerve terminal (Pérgola *et al*., 1993) and increases the affinity of noradrenaline binding to alpha$_2$-adrenergic receptors (Flavahan *et al*., 1985). This explains the near zero skin blood flows observed when subjects were hypothermic and the local skin stimulus was 15^{o}C or below.

5.4.3 Acral verses non-acral skin regions

It was evident from the current study that the response patterns for blood flow were different between the acral and non-acral skin regions. This is in agreement with our hypotheses that skin blood flow would be highest in the hand during hyperthermia, when local skin temperature is 40^{o}C. This observation can be explained by two main differences that exist in the control of skin blood flow within these regions. The first is the difference in the neural control of vascular smooth muscle in the non-acral skin regions, where increases in skin blood flow are due partly to the release of vasoconstrictor tone, but also by neurally mediated active vasodilator mechanisms (Roddie, 1983). In the palms of the hands and soles of the feet, active vasodilatation does not seem to occur in response to heating (Lundberg *et al*., 1989; Johnson *et al*., 1976; Johnson and Park., 1979). Secondly, acral skin regions possess arteriovenous anastomoses that, when open, allow large volumes of blood to be shunted from the arteries to the veins bypassing the capillaries and these are not present in hairy skin (Hales *et al*., 1978).

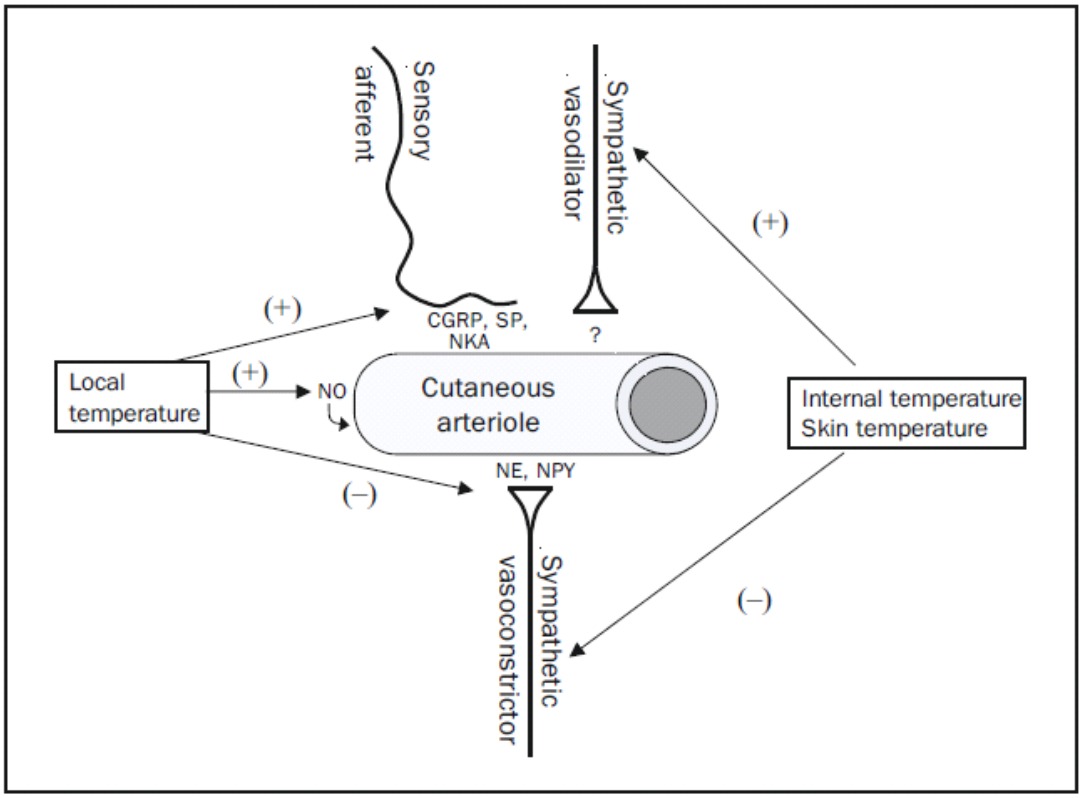

Figure 5.17: The interactions of neural mechanisms and the direct local factors that control vascular smooth muscle. Internal and skin temperature mediate either active vasoconstriction (-), through the release of noradrenaline (NE) and co-transmitter neuropeptide Y (NPY) or active vasodilatation (+), through the release of an unknown neurotransmitter (?) as part of the sympathetic nervous system. Local heating stimulates sensory nerves, to release neuropeptides, and the release of nitric oxide (NO) to cause vasodilatation. Local decreases in temperature stimulate the noradrenergic nerves to cause localised vasoconstriction. From Charkoudian (2003).

In addition to the different neural control of cutaneous blood vessels between acral and non-acral skin regions, acral regions possess arteriovenous anastomoses. These are thick-walled blood vessels, allowing blood to flow directly from arterioles to venules, bypassing high resistance capillaries (Clark, 1938) and are located in the dermis of the hands, feet, and ears, but most numerous in nail beds (Grant and Bland, 1931). The muscular walls are densely innervated by sympathetic vasoconstrictor nerves and have inner diameters of 25-125*u*m compared to 10*u*m diameters of the capillaries (Hales *et al.*, 1978). When opened, arteriovenous anastomoses have been reported to be responsible for pronounced local vasodilatation, as occurs during heating (Hales *et al*., 1978), and would explain the large shifts in skin blood flow to the hands and feet following local heating during the hyperthermic condition in the current study. That is, when the powerful centrally mediated vasoconstrictor drive from the hypothalamus was reduced as individuals were warmed, passive vasodilatation of the arteriovenous anastomoses occurred and allowed a substantial increase in blood to flow to the hands and feet, thereby facilitating heat loss. The effectiveness of the opening of arteriovenous anastomoses in shifting large volumes of blood to the peripheral circulation has been shown previously (Clark, 1938; Lossius *et al*., 1993; Krogstad *et al*., 1995).

5.4.4 Differences between hand and foot blood flow

Although the hands and feet are believed to possess the same sympathetic neural activation in active vasoconstriction with passive vasodilatation, differences in their skin blood flow response to central and local skin temperatures existed in the current study, and these have been reported previously (Kunkel and Stead, 1938; Tsuchida, 1987). The most obvious difference was maximal blood flow following local heating of the skin during hyperthermia, where the change in skin blood flow in the hand was almost double that of the foot. Previous reports have shown hand blood flow to be 50-75% greater than in the foot at the same exposure temperatures (Kunkel and Stead, 1938). It has been speculated that these differences are due, in part, to the number of arteriovenous anastomoses. However, Grant and Bland (1931) have shown a greater density of arteriovenous anastomoses within the sole of the foot than in the palm of the hand. Therefore, it is more likely that a greater vasoconstrictor tone was present in the foot than the hand (Allwood and Burry, 1954). This theory is also supported by Wallin (1990) who showed the skin temperature at which vasomotor response shifted from vasoconstriction to vasodilatation was 28^{o}C in the hand, and 33^{o}C in the foot. It is also possible that the proximity of the lower limb to the core may affect heat transfer within the body. That

is, warm blood has further to travel to the lower extremities. Thus, tissue temperatures in the lower extremities are lower and heat distribution during cold conditions is closest to the core. This would explain the observation that during the conditions tested in the current study, apart from during hyperthermia, foot blood flow was mostly less than the mean skin blood flow across all for sites during the thermoneutral condition with a local stimulation temperature of 33ºC (4.79 mL.100 mL^{-1}.min^{-1}).

5.4.5 The effect of core and local skin temperature on thermal sensation

A secondary outcome from the current study was the finding of significant differences in local thermal sensation in the hands and feet (acral skin) compared to the forearm and calf (non-acral skin) during hypothermia, when exposed to the same local skin temperature (33º and 40ºC). This is a result of the large sensory representation within the hands and feet that provide feedback to a large portion of the somatosensory cortex (Penfield and Rasmussen, 1952). Nakamura *et al* (2012) found a similar response, where slightly warming the hands of slightly cool individuals, felt warmer than similar treatment applied to the neck and sole of the foot. Although local thermal sensation was stronger in the hands and feet than the calf and forearm in the current study, this had little impact on whole body thermal sensation and this is supported by other studies (Nakamura *et al*., 2012).

Interestingly, although the sensation of temperature was warmer in the hands and feet than the calf and forearm, this had little impact on skin blood flow at 33º and 40ºC local temperatures. It is possible, since cutaneous thermoreceptors have been shown to have a greater impact on behavioural responses (Cabanac *et al*., 1972), that the local temperature stimulus was strong enough to activate the warm sensory afferents within the skin, hence eliciting the feeling of warmth, but the activation of cold thermoreceptors from the rest of the body elicited a stronger and more powerful sympathetic efferent response. Hence, the local stimulus appeared not to be able to override the powerful vasoconstriction mediated by the cold thermoreceptors when exposed to the condition.

5.5 CONCLUSION

The current investigation provides the first complete description of the interactions between core and local skin temperature upon skin blood flow for both acral and non-acral skin regions across a range of thermal states. The results of this study pertain only to the supine

state. It was hypothesised that during each thermal state, increases in local skin temperature would elicit proportional increases in skin blood flow across all four skin regions. While a positive correlation was found to exist between local skin temperature and skin blood flow across all thermal states, the current study provides further supporting evidence that local skin temperature has little to no effect on skin blood flow across acral and non-acral skin regions within hypothermic individuals. This is due to the presence of very powerful, centrally driven, vasoconstriction under these conditions. In contrast, it was found that the influence of local skin temperature upon skin blood flow became more pronounced in normothermic individuals. Whilst this was minimal in the foot it was largest in the hand. When individuals were hyperthermic, the graded changes in local skin temperature augmented skin blood flow to a greater extent than during both the hypothermic and normothermic conditions across all sites, but this influence was greatest in the foot. The fact that the hand and the foot experienced the greatest shifts in blood flow with release of vasoconstrictor tone during heating is believed to be the result of the presence of arteriovenous anastomoses within these regions. Although this study provided missing quantitative data across a range of thermal states, it did not account for broadening our understanding of the interactions of vasomotor function with sudomotion and thermogenesis. This will be explored in the final phase of this research series.

5.6 REFERENCES

Abramson, D.I., Herman, Z., and Marrus, J. (1938). Plethysmographic studies of peripheral blood flow in man. *American Heart Journal.* 17:194-205.

Abramson, D.I., and Fierst, A.M. (1942). Resting blood flow and peripheral vascular responses in different portions of the extremities. *American Heart Journal.* 8:328-334.

Allwood, M.J., and Burry, H.S. (1954). The effect of local temperature on blood flow in the human foot. *Journal of Physiology.* 124:345-357.

Armstrong, L.E., Maresh, C.M., Castellani, J.W., Bergeron, M.F., Kenefick, R.W., LaGasse, K.E., and Riebe, D. (1994). Urinary indices of hydration status. *International Journal of Sport Nutrition.* 4:265-279.

Barcroft, H., and Edholm, O.G. (1943). The effect of temperature on blood flow and deep temperature in the human forearm. *Journal of Physiology.* 102: 5-20.

Blair, D.A., Glover, W.E., and Roddie, I.C. (1960). Vasomotor fibres to skin in the upper arm, calf and thigh. *Journal of Physiology.* 153:232-238.

Booth, J.D., Wilsmore, B.R., MacDonald, A.D., Zeyl, A., Storlien, L.H., and Taylor, N.A.S. (2004). *Journal of Thermal Biology*. 29: 709-715.

Brengelmann, G.L., Johnson, J.M., Hermansen, L., and Rowell, L.B. (1977). Altered control foskin blood flow during high internal temperatures. *Journal of Applied Physiology: Respiration, Environmental and Exercise Physiology.* 43:790-794.

Brothers, R.M., Bhella, P.S., Shibata, S., Wingo, J.E., Levine, B.D., and Crandall, C.G. (2009). Cardiac systolic and diastolic function during whole body heat stress. *American Journal of Physiology - Heart and Circulation Physiology.* 296:H1150-H1156.

Brunt, V.E., and Minson, C.T. (2011). Cutaneous thermal hyperemia: more than skin deep. *Journal of Applied Physiology.* 111:5-7.

Cabanac, M., Massonnet, B., and Belaiche, R. (1972). Preferred skin temperature as a function of internal and mean skin temperature. *Journal of Applied Physiology.* 33:699-703.

Charkoudian, N., Eisenach, J.H., Atkinson, J.L.D., Fealey, R.D., and Joyner, M.J. (2002). Effects of chronic sympathectomy on locally mediated cutaneous vasodilation in humans. *Journal of Applied Physiology*. 92:685-690.

Charkoudian, N. (2003). Skin blood flow in adult human thermoregulation: How it works, when it does not, and why. *Mayo Clinic Proceedings.* 78:603-612.

Clark, E.R. (1938). Arterio-venous anastomoses. *Physiological Reviews.* 18:229-247.

Cotter, J.D. (1997). The role of regional skin temperatures in thermoregulatory control during heat stress. Doctor of Philosophy, University of Wollongong.

Coyle, E.F., and Gonzalez-Alonson, J. (2001). Cardiovascular drift during prolonged exercise: new perspectives. *Exercise and Sports Science Reviews.* 29:88-92.

Crandall, C.G., Wilson, T.E., Marving, J., Vogelsang, T.W., Kjaer, A., Hesse, B., and Secher, N.H. (2008). Effects of passive heating on central blood volume and ventricular dimensions in humans. *Journal of Physiology.* 586:293-301.

Dietz, N.M., Rivera, J.M., Warner, D.O., and Joyner, M.J. (1994). Is nitric oxide involved in cutaneous vasodilation during body heating in humans. *Journal of Applied Physiology.* 76:2047-2053.

Durand, S., Zhang, R., Cui, J., Wilson, T.E., and Crandall, C.G. (2004). Evidence of myogenic response in vasomotor control of forearm and palm cutaneous microcirculations. *Journal of Applied Physiology.* 97:535-539.

Edholm, O.G., Fox, R.H., and Macpherson, R.K. (1957). Vasomotor control of the cutaneous blood vessels in the human forearm. *Journal of Physiology.* 139:455-465.

Flavahan, N.A., Lindblad, L.E., Verbeuren, T.J., Shepherd, J.T., and Vanhoutte, P.M. (1985). Cooling and alpha1- and alpha$_2$-adrenergic responses in cutaneous veins: role of receptor reserve. *American Journal of Physiology- Heart and Circulatory Physiology.* 249:H950-H955.

Fox, R.H., Goldsmith, D.J., Kidd, D.J., and Lewis, H.E. (1963). Blood flow and other thermoregulatory changes with acclimatization to heat. *Journal of Physiology.* 166:548-562.

Grant, R.T. (1930). Observations on direct communications between arteries and veins in the rabbit's ear. *Heart.* 15:281-303.

Grant, R.T., and Bland, E.F. (1931). Observations on arteriovenous anastomoses in human skin and in the bird's foot with special reference to the reaction of cold. *Heart.* 15:385-407.

Grant, R.T., and Pearson, R.S. (1938). The blood circulation in the human limb; observations on the differences between proximal and distal parts and remarks on the regulation of body temperature. *Clinical Science.* 3:119-139.

Hales, J.R.S., Iriki, M., Tsuchiya, K., and Kozawa, E. (1978). Thermally-induced cutaneous sympathetic activity related to blood flow through capillaries and arteriovenous

anastomoses. *Pflügers Archiv European Journal of Physiology*. 375:17-24.

Hardy, J.D., and DuBois, E.F. (1938). The technic of measuring radiation and convection. *Journal of Nutrition.* 15:461-475.

Harrison, M.H. (1985). Effects of thermal stress and exercise on blood volume in humans. *Physiological Review.* 66:149-209.

Hensel, H. (1981). Thermoreception and temperature regulation. *Monographs of the Physiological Society.* 38:1-321.

Horstman, D.H., and Horvath, S.M. (1972). Cardiovascular and temperature regulatory changes during progressive dehydration and euhydration. *Journal of Applied Physiology.* 33:446-450.

ISO 7243 (1989). *Hot environments - estimation of the heat stress on working man, based on the WBGT-index (wet bulb globe temperature)*. International Standard Organisation, Geneva.

ISO 9886. (1992). *Evaluation of thermal strain by physiological measurements*. International Standard Organisation, Geneva.

Johnson, J.M., Brengelmann, G.L., and Rowell, L.B. (1976). Interactions between local and refelx influences on human forearm skin blood flow. *Journal of Applied Physiology.* 41:826-831.

Johnson, J.M., and Park, M.K. (1979). Reflex control of skin blood flow by skin temperature: role of core temperature. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology.* 47:1188-1193.

Johnson, J.M. (1986). Nonthermoregulatory control of human skin blood flow. *Journal of Applied Physiology.* 61:1613-1622.

Johnson, J.M., and Proppe, D.W. (1996). Cardiovascular adjustments to heat stress. In: *Handbook of Physiology*. Fregely, M.J., Blatteis, C.M, eds. Section 4: Environmental Physiology. Vol 1. New York, NY: Oxford University Press; 215-243.

Johnson, J.M., and Kellogg, D.L. (2010). Local thermal control of the human circulation. *Journal of Applied Physiology.* 109:1229-1238

Keller, D.M., Sander, M., Stallknecht, B., and Crandall, C.G. (2010). Alpha-Adrenergic vasoconstrictor responsiveness is preserved in the heated human leg. *Journal of Physiology.* 588:3799:3808.

Kellogg, D.L. Jr., Pérgola, P.E., Piest, K.L., Kosiba, W.A., Crandall, C.G., Grossmann, M., and Johnson, J.M. (1995). Cutaneous active vasodilation in humans is mediated by

cholinergic nerve cotransmission. *Circulation Research.* 77:1222-1228.

Kellogg, D.L. Jr. (2006). In vivo mechanisms of cutaneous vasodilataion and vasoconstriction in humans during thermoregulatory challenges. *Journal of Applied Physiology.* 100:1709-1718.

Krogstad, A.L., Elam, M., Karlsson, T., and Wallin, B.G. (1995). Arteriovenous anastomoses and the thermoregulatory shift between cutaneous vasoconstrictor and vasodilator reflexes. *Journal of the Autonomic Nervous System.* 53:215-222.

Kunkel, P., Stead, E.A., and Weiss, S. (1938). Blood flow and vasomotor reactions in the hand, forearm, foot and calf in response to physical and chemical stimuli. *The Journal of Clinical Investigation.* 17:715-723.

Kunkel, P., and Stead, E.A. (1938). Blood flow and vasomotor reactions in the foot in health, in arteriosclerosis, and in thrombo-angiitis obliterans. *Journal of Clinical Investigation.* 17:715-723.

Lindblad, L.E., Ekenvall, L., and Klingstedt. (1990). Neural regulation of vascular tone and cold induced vasoconstriction in human finger skin. *Journal of the Autonomic Nervous System.* 30:169-174.

Lossius, K., Eriksen, M., and Walløe, L. (1993). Fluctuations in blood flow to acral skin in humans: connections with heart rate and blood pressure variability. *Journal of Physiology.* 460:641-655.

Love, A.H.G., and Shanks, R.G. (1962). The relationship between the onset of sweating and vasodilatation in the forearm during body heating. *Journal of Physiology*. 162:121-128.

Lundberg, J., Norgren, L,. Ribbe, E., Rosén, I., Steen, S., Thörne, J.., and Wallin, B.G. (1989). Direct evidence of active sympathetic vasodilatation in the skin of the human foot. *Journal of Physiology.* 417:437-446.

Marino, F., and Booth, J.D. (1998). Whole body cooling by immersion in water at moderate temperatures. *Journal of Science and Medicine in Sport.* 1:72-81.

Medow, M.S., Bamji, N., Clarke, D., Ocon, A.J., and Stewart, J.M. (2011). Reactive oxygen species (ROS) from NADPH and xanthine oxidase modulate the cutaneous local heating response in healthy humans. *Journal of Applied Physiology.*

Mekjavic, I.B., and Rempel, M.E. (1990). Determination of esophageal probe insertion length based on standing and sitting height. *Journal of Applied Physiology*. 69(1):376-9.

Minson, C.T., Berry, L.T., and Joyner, M.J. (2001). Nitric oxide and neurally mediated

regulation of skin blood flow during local heating. *Journal of Applied Physiology.* 91:1619-1626.

Montain, S.J., and Coyle, E.F. (1992). Influence of graded dehydration on hyperthermia and cardiovascular drift during exercise. *Journal of Applied Physiology.* 73:1340-1350,

Nakamura, M., Yoda, T., Crawshaw,L.I., Kasuga, M., Uchida, Y., Tokizawa, K., Nagashima, K., and Kanosue, K. (2012). Relative importance of different surface regions for thermal comfort in humans. *European Journal of Applied Physiology. In press*. DOI 10.1007/s00421-012-2406-9. [*Epub aheahd of print*].

Penfield, W., and Rasmussen, T. (1952). *The cerebral cortex of man*. MacMillan Press, New York.

Pérgola, P.E., Kellogg, D.L., Johnson, J.M., Kosiba, W.A., and Soloman, D.E. (1993). Role of sympathetic nerves in the vascular effects of local temperature in human forearm skin. *American Journal of Physiology.* 265:H785-H792.

Peters, J.K., Nishiyasu, T., and Mack, G.W. (2000). Reflex control of the cutaneous circulation during passive body core heating in humans. *Journal of Applied Physiology.* 88:1756-1764.

Roddie, I.C., Shepherd, J.T., and Whelan, R.F. (1957). The contribution of constrictor and dilator nerves to the skin vasodilatation during body heating. *Journal of Physiology.* 136:489-497.

Roddie, I.C. (1983). Circulation to skin and adipose tissue. Comprehensive Physiology 2011, Supplement 8: Handbook of Physiology, The Cardiovascular System, Peripheral Circulation and Organ Blood Flow.: 285-317.

Rowell, L.B., Brengelmann, G.L., and Murray, J.A. (1969). Cardiovascular responses to sustained high skin temperature in resting man. *Journal of Applied Physiology.* 27:673-680.

Rowell, L.B., Brengelmann, G.L., Blackmon, J.R., and Murray, J.A. (1970). Redistribution of blood flow during sustained high skin temperature in resting man. *Journal of Applied Physiology.* 28:415-420.

Rowell, L.B. (1974). Human cardiovascular adjustments to exercise and thermal stress. *Physiological Reviews.* 54:75:159.

Rowell, L.B. (1983). Cardiovascular adjustments to thermal stress. In: *Handbook of Physiology.* Shephard, J.T., Abboud, F.M., eds. Section 2: Cardiovascular System. Vol 3, pt 2, Behesda, Md: American Physiological Society. 967-1023.

Savage, G.K., Cooper, K.E., Veale, W.L., and Malkinson, T.J. (1985). Peripheral blood flow during rewarming from mild hypothermia in humans. *Journal of Applied Physiology.* 58:4-13.

Savage, M.V., and Brengelmann, G.L. (1996). Control of skin blood flow in the neutral zone of human body temperature regulation. *Journal of Applied Physiology.* 80:1249-1257.

Spealman, C.R. (1945). Effect of ambient air temperature and of hand temperature on blood flow in hands. *American Journal of Physiology.* 145:218-222.

Taylor, W.F., Johnson, J.M., O'Leary, D., and Park, M.K. (1984). Effect of high local temperature on reflex cutaneous vasodilation. *Journal of Applied Physiology*. 57:191-196.

Tsuchida, Y. (1987). *Plastic and Reconstructive Surgery.* 80:709-710.

Vanggard, L., Eyolfson, D., Xu, X, Weseen, G., and Giesbrecht, G.G. (1999). Immersion of distal arms and legs in warm water (AVA rewarming) effectively rewarms mildly hypothermic humans. *Aviation, Space and Environmental Medicine.* 70:1081-1088.

Vanhoutte, P.M. (1980). Physical factors of regulation. In: *Handbook of Physiology.* Shepherd, J.T., Abboud, F.M., eds. Section 2: Cardiovascular System, Vascular Smooth Muscle: 443-474.

Wallin, B.G. (1990). Neural control of skin blood flow. *Journal of Autonomic Nervous System.* 30:S185:S190.

Werner, J., Mekjavic, I.B., and Taylor, N.A.S. (2008). Concepts in physiological regulation: a thermoregulatory perspective. In: Taylor, N.A.S., and Groeller, H. (eds). *Physiological bases of human performance during work and exercise.* . Elsevier, Edinburgh, pp 325-340.

Wilson, T.E., Cui, J., and Crandall, C.G. (2002). Effect of whole-body and local heating on cutaneous vasoconstrictor responses in humans. *Autonomic Neuroscience: Basic and Clinical.* 97:122-128.

Wilson, T.E., Sauder, C.L., Kearney, M.L., Kuipers, N.T., Leuenberger, U.A., Monahan, K.D., and Ray, C.A. (2007). Skin-surface cooling elicits peripheral and visceral vasoconstriction in humans. *Journal of Applied Physiology.* 103:1257-1262.

Wyss, C.R., Brengelmann, G.L., Johnson, J.M., Rowell, L.B., and Niederberger, M. (1974). Control of skin blood flow, sweating, and heart rate: role of skin vs core temperature. *Journal of Applied Physiology.* 36:726-733.

CHAPTER 6: THE EFFECT OF PRE-EXPOSURE MEAN BODY TEMPERATURE ON THE THERMOEFFECTOR THRESHOLDS

6.1 INTRODUCTION

Humans are unique in our ability to regulate body temperature within a narrow range (the inter-threshold zone). Within this zone, body temperature is regulated by changes in blood flow to the skin. However, the ability of skin blood flow to maintain body temperature is limited during exercise and exposure to extreme environmental conditions. When temperature is driven outside the inter-threshold zone, shivering and sweating are activated to conserve heat or to facilitate heat loss to the environment. Previously, we explored the interactions between shell and core temperature on skin blood flow at different sites across a range of mean body and local skin temperatures (Caldwell *et al*., 2014). However, our understanding of the determinants of these thermoeffector thresholds, in addition to the interactions between skin blood flow and the onset of shivering and sweating, remains equivocal, and these will form the primary *foci* of this research.

Historically, temperature regulation, as characterised by sweating, shivering and cutaneous blood flow, was believed to defend a single core temperature (set-point) (Hammel, 1968; Cabanac and Massonnet, 1977; Cabanac, 2006), however more recent evidence suggests a zone of core temperatures exists where body temperature is regulated by changes in vasomotor tone without activation of sweating or shivering, known as the inter-threshold or null zone (Mekjavic and Bligh, 1989; Werner, 2010; McAllen, *et al*., 2010). At either side of this zone are points (thresholds) where shivering and sweating are recruited. Our interest centres upon what determines the mean body temperature at which this recruitment occurs.

Whilst much is known about the mechanisms that determine these regulatory thresholds, there are still areas for which our understanding is only partial. However, previous experiments have shown that the sweating threshold during heat exposure moves to a lower body temperature following both heat adaptation (Patterson, 1999; Shido *et al*, 1999, Taylor, 2014), and pre-exposure cooling (Brück and Olschewski, 1987; Olschewski and Brück, 1988; Booth, 2004). Indeed, these displacements appeared equal in size to the

reduction in the pre-exposure body temperature. Thus, it seemed that the magnitude of the change in body temperature might provide important feedback, and perhaps of greater importance than did the absolute body temperature. Therefore, this project was designed to evaluate that possibility with regard to the shifts in thermoeffector thresholds by inducing deliberate, yet precisely controlled displacements of mean body temperature prior to separate heating and cooling stimuli.

6.1.1 Aims and hypotheses

The aim of this experiment was to investigate the effects of slight deviations in the steady-state, pre-exposure mean body temperature upon the subsequent vasomotor, sweating and shivering threshold temperatures. These aims were satisfied by altering mean body temperature through either whole-body heating or cooling, and then driving body temperature in the opposite direction to determine these thermoeffector thresholds.

It was hypothesised that:

(1) Pre-cooling and pre-heating would shift the mean body temperature thermoeffector thresholds for sweating and shivering by a magnitude equal to that of the pre-exposure displacement of mean body temperature.

(2) The mean body temperature for vasodilatation and sweating thresholds would occur simultaneously during heating, while the vasoconstrictor threshold would always precede the shivering thresholds during cooling.

6.2 METHODS

6.2.1 Subjects and overview

6.2.1.1 Subjects

Eight physically active and healthy male subjects, aged between 20 and 33 years (Table 6.1) participated in this research project, and were required for four experimental trials. Each subject received a subject information package and provided written, informed consent prior to commencing trials. This experiment was approved by the Human Research Ethics Committee (University of Wollongong) under approval HE09/314.

Table 6.1: Subject characteristics.

Subject	Age (y)	Height (m)	Mass (kg)	Surface area (m^2)	Surface area to mass ratio ($m^2.kg^{-1}$)
S1	22	1.73	68.5	1.81	0.026
S2	20	1.85	88.5	2.12	0.024
S3	33	1.80	78.0	1.97	0.025
S4	22	1.78	81.3	1.99	0.024
S5	25	1.78	70.1	1.87	0.027
S6	22	1.78	74.4	1.92	0.026
S7	23	1.84	75.1	1.97	0.026
S8	22	1.75	63.4	1.77	0.028
Mean	23.6	1.79	74.9	1.93	0.026
S.D.	4.0	0.04	7.9	0.11	0.001

6.2.1.2 Overview of experimental protocol

Each subject participated in four trials, administered in a balanced order. Each trial consisted of a pre-experimental, whole-body water immersion phase (28-23^{o}C, 35^{o}C or 39^{o}C) followed by an experimental phase of either passive heating or passive cooling. The trials differed in the pre-experimental exposure treatment where subjects were either mildly pre-cooled, pre-heated (water immersion) or in a thermoneutral state (control). The purpose of these treatments was to elicit controlled displacements of mean body temperature prior to the experimental phase. Following the pre-experimental conditions, subjects were wrapped in heavily insulated clothing, to preserve the effects of the pre-treatment, and transferred to the thermal chamber. Once in the chamber and instrumented, the water-perfusion garment was used to stabilise mean body temperature at the desired level. Once the target baseline core temperature was achieved, the temperature of the water-perfusion garment was either increased or decreased until the thermoeffector thresholds were established.

Subjects were passively warmed or cooled, with the use of a water-perfusion garment (Cotter *et al*., 1997). This change was always in the opposite direction to the pre-experimental exposure temperature. That is, for a pre-cooled trial, subjects were passively warmed until the vasomotor and sudomotor thresholds were achieved. For the pre-heating trials, subjects were passively cooled until shivering was established, and this continued for 10 min. Two control trials were completed with each commencing from a thermoneutral state. For these trials, pre-treatment involved whole-body water immersion in thermoneutral water (34^{o}C) to ensure subjects began the experimental phase from a common thermoneutral baseline, and to control for possible influences of hydrostatic pressure on these physiological responses (Risch *et al*., 1978). One of the control trials was followed by passive heating, while the other control trial was followed by passive cooling.

6.2.2 Experimental methods

6.2.2.1 Pilot testing

Prior to commencing the experimental trials, pilot tests were performed to determine the optimal water-immersion temperatures to elicit the desired pre-treatment conditions. In addition, pilot tests were performed to determine optimal climate chamber

conditions to ensure heating and cooling occurred at similar rates so as to eliminate the dynamic effect of activation of the peripheral thermoreceptors. This was essential, because dynamic activation of peripheral thermoreceptors would influence the thermoeffector responses and this was undesirable in the current experiment.

6.2.2.1.1 Determination of water immersion temperature for pre-treatment

The whole-body pre-cooling treatment was based on the protocol used by Marino and Booth (1998). Subjects were immersed in the supine position to the neck in 28ºC water. Once immersed, the thermostat on the water immersion tank was set to 24ºC. After 25 min of cooling, ice was added every 5 min thereafter, and the water was stirred to ensure the water temperature remained even throughout the trial. This protocol was pilot tested using 10 thermistors placed evenly throughout the water immersion tank (Figure 6.1).

Pilot testing was completed to ensure the heating and cooling stimuli were sufficient to induce rapid yet controlled changes in mean body temperature for the experimental phase. For the heating protocol, this was achieved through increasing the ambient air temperature within the climate chamber to 36ºC, increasing the radiant heat load through heat lamps and increasing the temperature of the water circulating through the water-perfusion garment. Figure 6.2 demonstrates the typical chain of events to increase body temperature until the sweating threshold was achieved. For the cooling trials, ambient air temperature was progressively reduced to 17ºC while the water circulating the water-perfusion suit was progressively reduced to 10ºC. This is demonstrated in Figure 6.3.

6.2.2.2 Experimental protocol

On arrival to the laboratory, subjects were instrumented with measurement equipment then dressed in the water-perfusion suit. Once dressed, subjects moved to the water-immersion tank where they were either pre-cooled or pre-heated in a supine position and immersed in water to the level of the neck (Table 6.2). During the final 10 min of immersion, the water bath connected to the water-perfusion suit, was switched on so that water circulated through the suit. This was to ensure that mean body temperature of each subject remained above (pre-heating trial) or below (pre-cooling trial) their thermoneutral baseline. The water temperature circulating the suit was 15ºC during pre-cooling and 40ºC

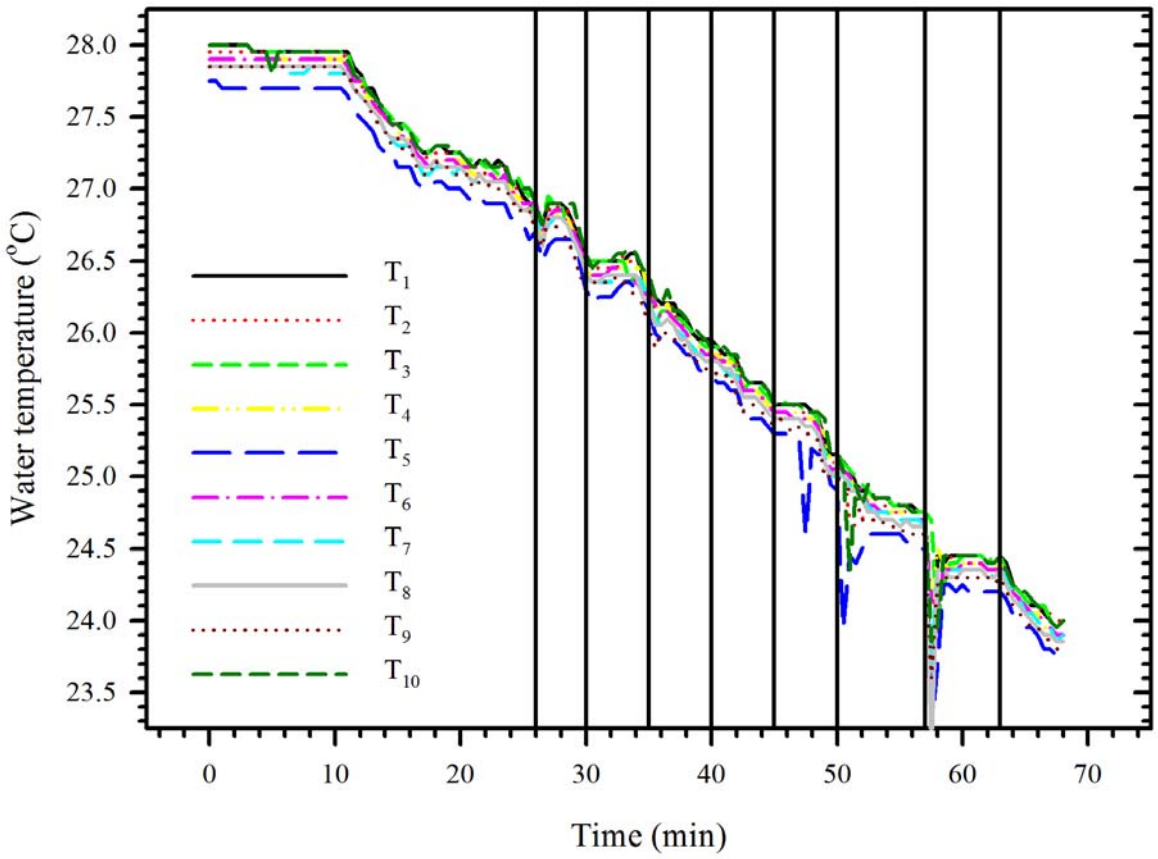

Figure 6.1: Water immersion temperature changes during pilot testing of pre-cooling protocol. T1-T10 indicated the temperature of 10 thermistors located throughout the water tank. These were placed at a range of depths from shallow to almost touching the bottom of the tank (9000 L).The solid lines represent the time point where buckets of ice were added to the tank to increase the cooling rate of the water, and these transient effects can bee seen at 45, 52 and 57 min.

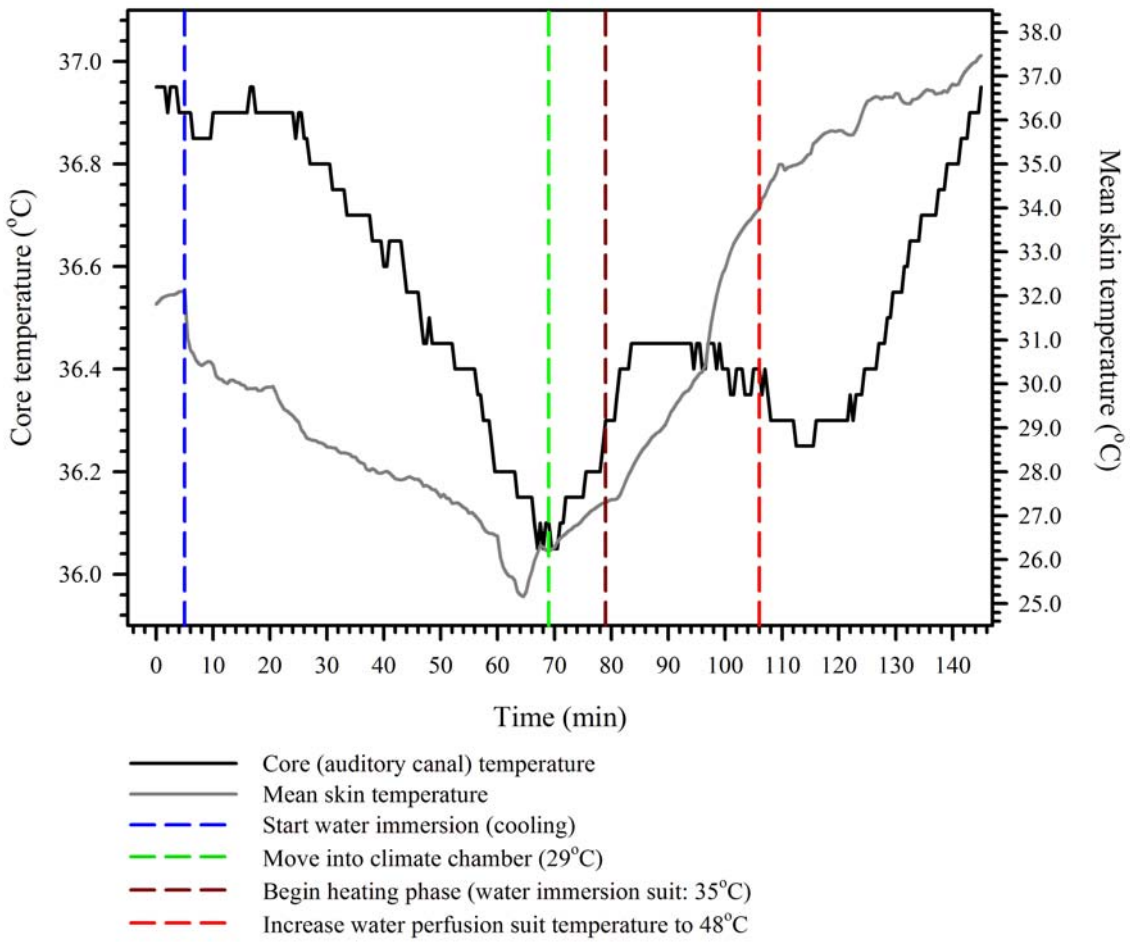

Figure 6.2: Auditory canal and mean skin temperature changes during a water immersion (pre-cooling: 24-28ºC water) phase, followed by a whole-body heating phase (climate chamber: 29ºC; water perfusion suit: 33º-48ºC) to initiate vasodilatation and sweating. Each of the broken lines demonstrates the typical chain of events to increase mean body temperature at a constant rate.

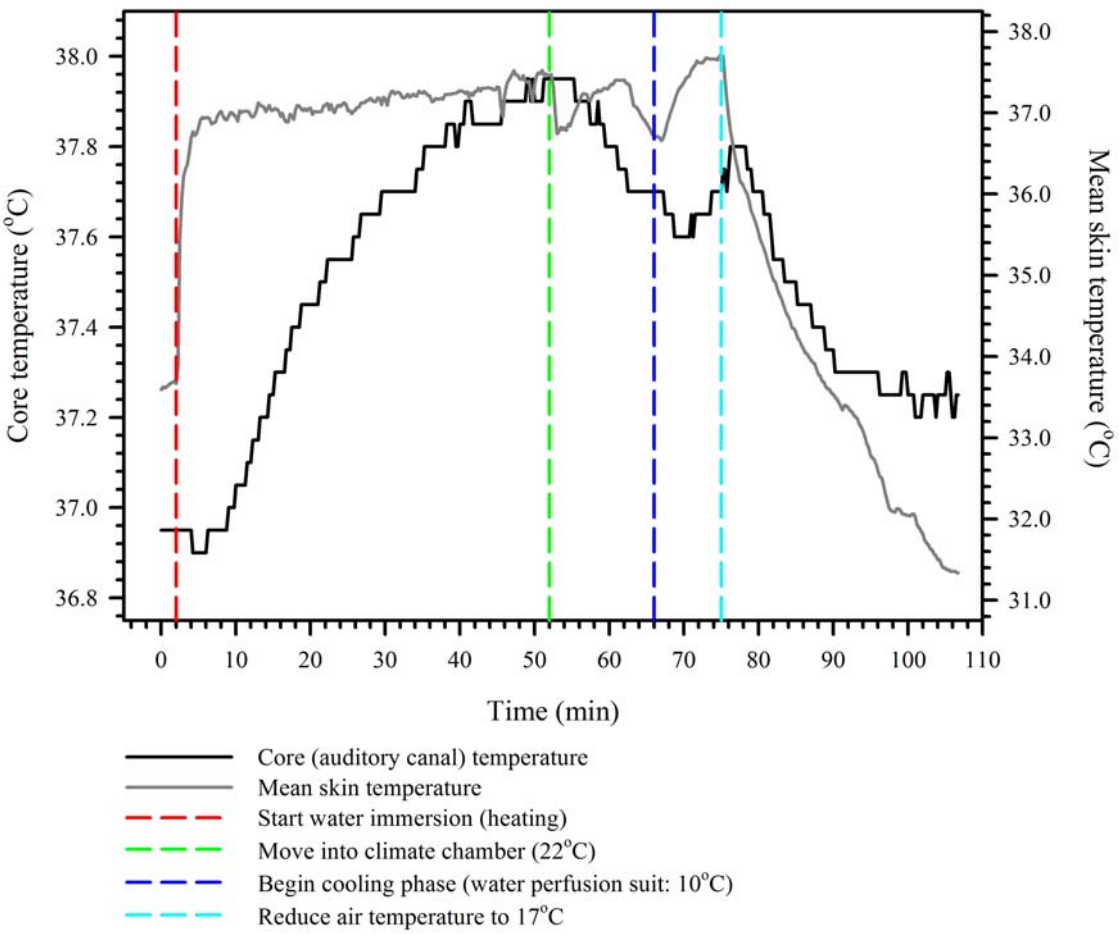

Figure 6.3: Auditory canal and mean skin temperature changes during a water immersion (pre-heating: 39ºC water) phase, followed by a whole-body cooling phase (climate chamber: 22ºC; water perfusion suit: 10º-20ºC) to initiate vasoconstriction and shivering. Each of the broken lines demonstrates the typical chain of events to decrease mean body temperature at a constant rate.

Table 6.2: Experimental timeline.

Time (min)	Activity summary
0	Subject hydration check and preparation (22ºC)
30-100	**Pre-cooled**, **thermoneutral** or **pre-heated**: Target T_{es}=35º, 37º or 39ºC
100-115	Move into climate chamber (30º or 22ºC)
115-120	Experimental phase: Baseline recording
120-210	Passive heating or cooling until established thermoeffector response
145-210	Terminate experiment

during pre-heating. Water circulated the suit until subjects were moved into the climate chamber. At this point the suit was temporarily disconnected until well inside the chamber and the suit could be connected to an additional water bath.

For the experimental phase, the average air temperature and relative humidity within the climate chamber for the experimental phase of the passive heating trials were 29.6ºC and 46.9% (control), 29.8ºC and 48.4% (whole-body cooling), 22.7ºC and 44.5% (control) and 22.3ºC and 46.0% (whole-body heating). The air temperature for the passive cooling trials are presented in Figure 6.4. The experimental set up for passive heating trials is shown Figure 6.5.

6.2.3 Experimental standardisation

Subjects were fully hydrated (Table 6.3), and were required to refrain from strenuous exercise and the consumption of alcohol and tobacco during the 12 h prior to each trial. For the night preceding each trial, subjects were instructed to drink 15 $mL.kg^{-1}$ of additional water before retiring, and on arrival to the laboratory, subjects were provided with supplementary water (10 $mL.kg^{-1}$) if their urine specific gravity was greater than 1.029[1] to ensure all subjects were either well hydrated or euhydrated. This was required on six occasions, with S5 requiring additional fluid before every trial. In addition, subjects were asked to eat an evening meal high in carbohydrate and low in fat. Breakfast was also required to be high in carbohydrate and low in fat. Subjects were asked to refrain from using caffeine for 2 h prior to each trial.

6.2.4 Experimental measurements

Physiological measurements were recorded continuously during both the pre-treatment and experimental phases, and included body core and skin temperatures, heart rate and whole-body sweat rate. Psychophysical responses were recorded throughout the immersion and experimental phases.

[1] Hydration classifications based upon urine specific gravity: (a) well hydrated: <1.013; (b) euhydrated: 1.013-1.029; and (c) hypohydrated: >1.029 (Armstrong *et al.*, 1994).

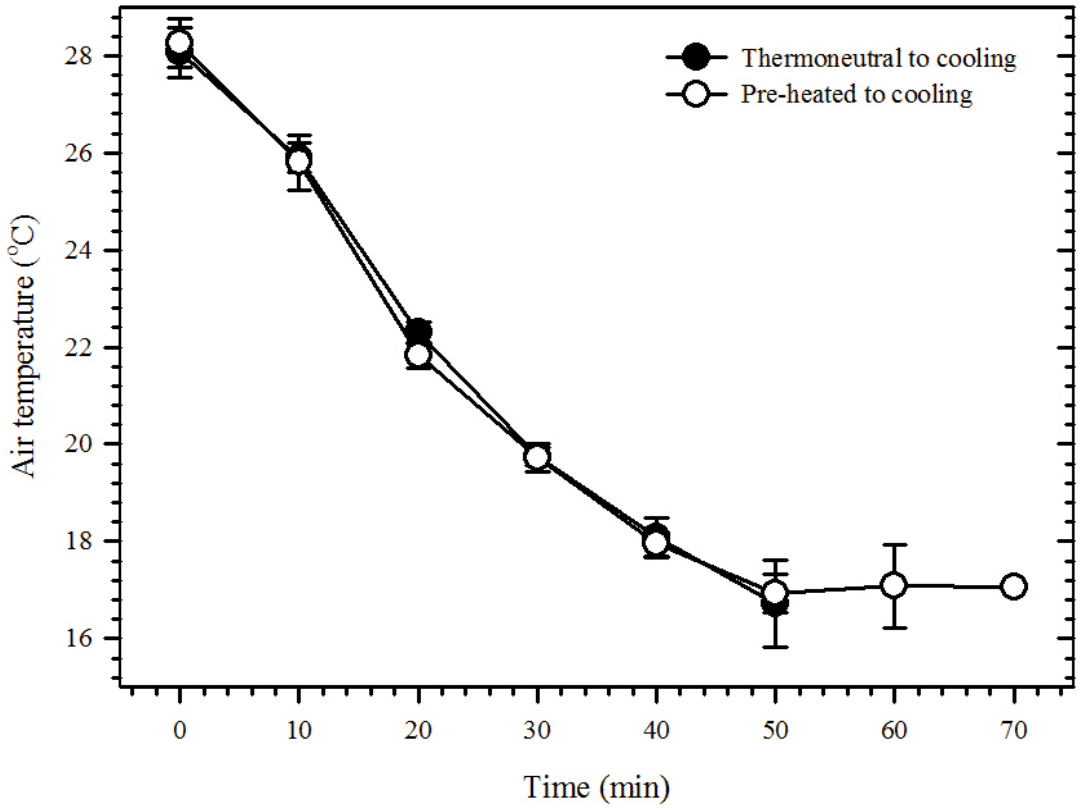

Figure 6.4: Air temperature (°C) changes within a climate chamber during the cooling phase to determine shivering a skin blood flow thresholds on rested individuals.

Table 6.3: Pre-experimental hydration state (urine specific gravity).

Subject	Trial A	Trial B	Trial C	Trial D
S1	1.020	1.025	1.020	1.030
S2	1.015	1.025	1.025	1.025
S3	1.006	1.006	1.007	1.008
S4	1.015	1.010	1.008	1.010
S5	1.008	1.016	1.030	1.030
S6	1.010	1.020	1.016	1.023
S7	1.024	1.030	1.020	1.025
S8	1.028	1.021	1.020	1.022
Mean	1.016	1.019	1.018	1.022
S.D.	0.01	0.01	0.01	0.01

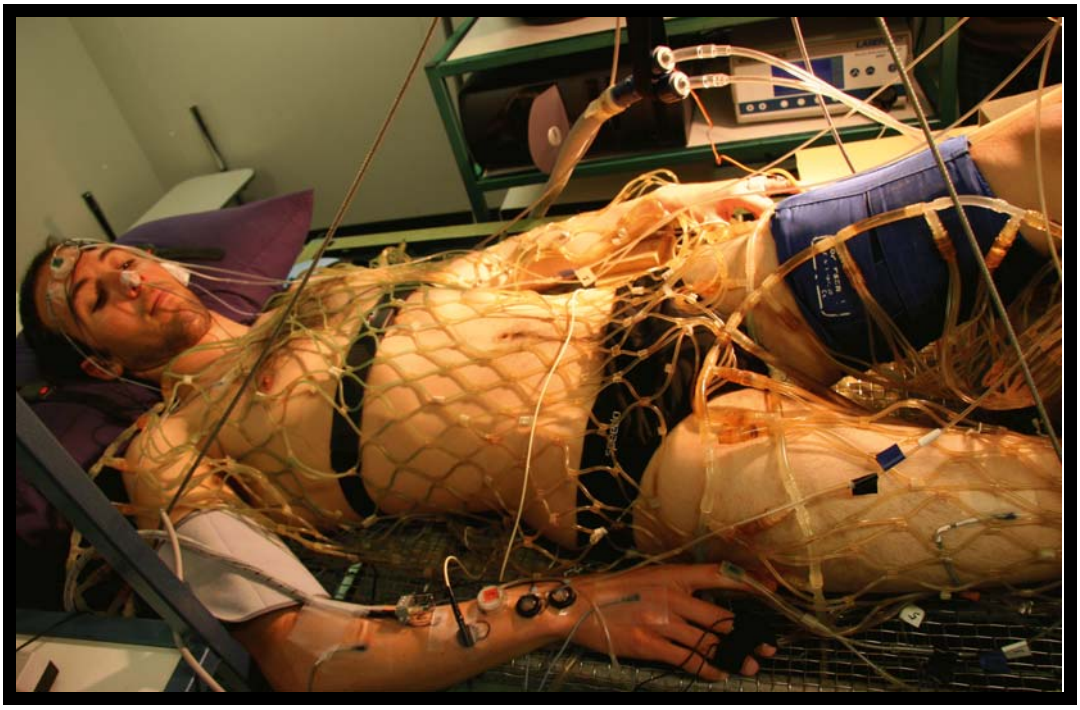

Figure 6.5: Photograph of the experimental set-up for the passive heating trials in the climate chamber for one resting subject wearing the water-perfusion garment. Sweat capsules and skin conductance electrodes are located on the dorsal surface of the right forearm, dorsal surface of the right fingers and the forehead. Cutaneous blood flow was measured using laser-Doppler flowmetry on the dorsal surface of the right forearm and on the left finger and left calf using venous-occlusion plethysmography.

6.2.4.1 Hydration status

Prior to commencing each trial, urine specific gravity was measured for each subject to confirm a state of euhydration**.**

6.2.4.2 Thermal variables

6.2.4.2.1 Oesophageal temperature

An oesophageal thermistor (Edale Instruments Ltd, U.K.) was inserted through the nose to a depth of about 40 cm from the nares (after Mekjavic and Rempel, 1990) with data recorded throughout each trial at 15-s intervals using a portable data logger (Grant Instruments Ltd., 1206 Series Squirrel, U.K.). This measure was taken as the primary index of core temperature.

6.2.4.2.2 Auditory canal temperature

Auditory canal temperature was measured with an ear-moulded plug containing a thermistor protruding into the ear 1 cm from the mould (Edale Instruments Ltd., U.K.). A large piece of cotton wool was secured over the ear to minimise the effect of the environmental temperature. Data were recorded throughout each trial at 15-s intervals using a portable data logger (Grant Instruments Ltd., 1306 Series Squirrel, U.K.)

6.2.4.2.3 Rectal canal temperature

Rectal temperature was also measured continuously (15-s intervals), using a thermistor probe inserted to a depth of 12 cm beyond the anal sphincter (Edale Instruments Ltd, U.K.).

6.2.4.2.4 Skin temperatures

Skin thermistors were used to measure skin temperature (Type EU, Yellow Springs Instruments Co.Ltd, Yellow Springs, OH, USA). Skin temperatures were measured from eight sites (Edale Instruments Ltd, U.K.), with data recorded throughout each trial at 15-s intervals using a portable data logger (Grant Instruments Ltd., 1206 Series Squirrel, U.K.). From these data, an area-weighted mean skin temperature (T_{sk}) was derived (ISO 9886, 1992; after Hardy and DuBois, 1938).

$$T_{sk}=0.07 \cdot T_{sk\text{-}1}+0.175 \cdot T_{sk\text{-}2}+0.175 \cdot T_{sk\text{-}3}+0.07 \cdot T_{sk\text{-}4}+0.07 \cdot T_{sk5}+0.05 \cdot T_{sk\text{-}6}+0.19 \cdot T_{sk\text{-}7}+0.2 \cdot T_{sk\text{-}8}$$

where:

T_{sk-1} = forehead

T_{sk-2} = chest

T_{sk-3} = scapula

T_{sk-4} = upper arm

T_{sk-5} = forearm

T_{sk-6} = hand

T_{sk-7} = thigh

T_{sk-8} = calf

6.2.4.2.5 Mean body temperatures

Mean body temperature (T_b) was calculated as a weighted mean of core and skin temperature. The weighting differed depending on the thermal state of the individual. The hot equation was used during the heating phase of ***Trial C*** and ***Trial D***, the neutral equation was used during the pre-immersion phase of ***Trial A*** and ***Trial B*** and the cold equation was used during cooling for all four trials. These are presented as follows:

Hot: $T_b = (0.9 \cdot T_c) + (0.1 \cdot T_{sk})$ [^oC]

Neutral: $T_b = (0.7 \cdot T_c) + (0.3 \cdot T_{sk})$ [^oC]

Cold: $T_b = (0.65 \cdot T_c) + (0.35 \cdot T_{sk})$ [^oC]

where:

T_c = Oesophageal temperature [^oC]

6.2.4.2.6 Gross mass changes

Gross mass changes (before and after each trial) were used to determine changes in hydration state (±20 g; fw-150k, A&D scale, CA, U.S.A.) over the course of the trial. Whole-body sweat losses were calculated from body mass changes and corrected for fluid replacement and urine production.

6.2.4.2.7 Precursor sweating (skin conductance)

Skin conductance was measured to determine the precursor sweating threshold. That is, activation of the sweat glands occurs through activation of the cholinergic pathway

before sweat reaches the skin surface (Machado-Moreira *et al*., 2014). The production of precursor sweat was evaluated from changes in the electrodermal response from the dorsal aspects of the second and third fingers of the left hand as well as the dorsal surface of the left forearm and forehead. (skin conductance: Darrow, 1934). A pair of Ag/AgCl surface electrodes (1081 FG) were attached to the digits (0.05M sodium chloride in an inert ointment base). A constant voltage of 0.5V was applied across the electrode pair, and data were collected at 10 Hz (UFI Bioderm model 2701-SC Simple Scope and SCL/SCR Data Collection System, UFI, Morrow Bay, CA, U.S.A.).

6.2.4.2.8 Sweating

Sweat capsules (1.40 cm2 and 3.16 cm2) were used to measure local sweat rates from 3 sites (dorsal surface of the left forearm, dorsal surface of the right index finger and forehead). All capsules were glued to the skin to prevent pressure artefacts (Collodion U.S.P., Mavidon Medical Products, FL, U.S.A.). The pre-capsular airflow to each capsule was independently regulated at 600 $ml.min^{-1}$(large capsules), with inlet humidity maintained at 12% by passing room air for all capsules over a common, saturated lithium chloride solution. The humidity of the post-capsular air was measured using capacitance hygrometers, which form part of a sweat monitor system (Clinical Engineering Solutions, NSW, Australia), with inlet and exhaust air temperatures and humidities being sampled at 1-s intervals from six channels (DAS1602, Keithley Instruments, Inc., Cleveland, OH, U.S.A.), and used to compute local sweat rates (Taylor *et al*., 1997). Hygrometer calibration, using three saturated salt solution standards, preceded experimentation.

6.2.4.3 Cardiovascular variables

6.2.4.3.1 Heart rate

Heart rate was obtained at 15-s intervals throughout each trial from ventricular depolarisation using a heart rate monitor (Polar Electro Sports Tester, Finland) and was later downloaded to a computer.

6.2.4.3.2 Blood pressure

Systolic and diastolic blood pressure were measured (Omron SEM-2, Omron Healthcare Inc., Kyoto, Japan) immediately prior to the beginning of data collection of skin

blood flow from the stimulation site (see section 5.2.4.3.3). Mean arterial pressure (MAP) was calculated as a weighted mean of systolic (SBP) and diastolic blood pressure (DBP).

$$MAP = DBP + \frac{1}{3}(SBP-DBP)$$

6.2.4.3.3 Skin blood flow

Skin blood flow was measured using venous-occlusion plethysmography with a strain-gauge plethysmograph, positioned at each of two sites: left finger and left calf. For the calf measurements, blood flow to the foot, was occluded by placing a cuff around the left ankle and inflating it to 160 mmHg. Venous return from both sites was occluded by inflating a venous-occlusion (collection) cuff placed proximal to the elbow and knee, and inflated to a pressure of 50 mmHg. Detection of the rate of swelling of each limb was determined through voltage changes within the strain gauge. Voltage changes were converted from analogue to digital data, then collected on a desktop computer. Data were collected for 2 min every 5 min until established sweating or shivering occurred. The venous-occlusion cuff was automatically controlled (AG 101 Cuff inflator air source, D.E. Hokanson, Inc., U.S.A.), and followed a cycle of 8 s of inflation and 12 s of deflating, for a total of five inflations.

Skin blood flow was also estimated using single-point, laser-Doppler flowmetry (TSI Laserflo BPM2 with a P-435 laser fibre optic probe, Vasamedics Inc., St. Paul, MN, U.S.A.; 37 internal refresh rate ~7 Hz). The probe was attached to the right dorsal forearm and remained in position throughout the experiment. Skin blood flow data were also collected during the experimental phase of all four trials (passive heating and passive cooling) for 2 min every 5 min until established sweating or shivering occurred. These data were collected at 20 Hz using an eight channel, 12-bit analog to digital converter (Computer Boards Inc., PPIOA18, Mansfield, OH, U.S.A.) and passed to a computer for storage.

6.2.4.3.4 Cutaneous vascular conductance

Cutaneous vascular conductance (CVC) was calculated as the ratio of skin blood flow to mean arterial pressure with the units expressed as mL. 100 mL tissue^{-1}.min^{-1}.mmHg^{-1}. Since skin blood flow is a function of both mean arterial pressure and total peripheral

resistance, this calculation was important to determine whether changes in skin blood flow occurred from changes in vascular conductance.

6.2.4.4 Psychophysical responses

Throughout each pre-treatment (thermoneutral, cold or hot water-immersion) and during the experimental phase of each trial, subjects were asked to rate whole-body thermal sensation and thermal discomfort (Appendix **A**, **B**, and **D**). During the experimental phase, these psychophysical questions were recorded for every 10 min and designed to establish whether the thermal comfort zone remained the same for each trial.

6.2.5 Design and data analysis

This experiment was based on a repeated-measures design, where all subjects acted as their own controls and completed all four trials. Trials were administered in a randomised, but balanced order across all subjects to eliminate any effect of trial order. This was determined using a *Latin square* design model (Table 6.4). One-way Analysis of Variance was used to evaluate local differences in vasomotor, sudomotor and shivering thresholds for pre-heated, pre-cooled and thermoneutral pre-experimental treatments. Tukeys *HSD post hoc* tests were used to isolate sources of significant differences. *Alpha* will be set at the 0.05 level for all analyses.

6.2.5.1 Calculation of thermoeffector thresholds

Since afferent inputs are received from both external and deep tissue thermoreceptors, the thermoeffector thresholds were described in response to mean body temperature. An example of the method of calculating the thermoeffector threshold for shivering of one subject is presented in Figure 6.6 which shows the threshold for thermogenesis. Each of the thermoeffector thresholds were calculated using the same method. To determine the thermoeffector thresholds for sweating, shivering and cutaneous blood flow during either passive heating or cooling, two lines were fitted to each data set for each trial per subject. The first line was fitted to the data prior to the visual inflection point, while the second line was fitted to the rising data and only included the steepest part of the data set. These lines corresponded to one intersecting point which was deemed to be the thermoeffector threshold. Using simultaneous equations by substitution, the threshold

Table 6.4: Experimental order for each subject (*Latin square).*

SUBJECT	TRIAL 1	TRIAL 2	TRIAL 3	TRIAL 4
S1	A	B	D	C
S2	B	D	C	A
S3	D	B	A	C
S4	C	D	B	A
S5	D	A	C	B
S6	A	C	B	D
S7	B	C	A	D
S8	C	A	D	B

Note: Letters represent each of the four thermal states: Pre-heated to cooled (**A**); Thermoneutral to cooled (**B**); Pre-cooled to heated (**C**) and Thermoneutral to heated (**D**).

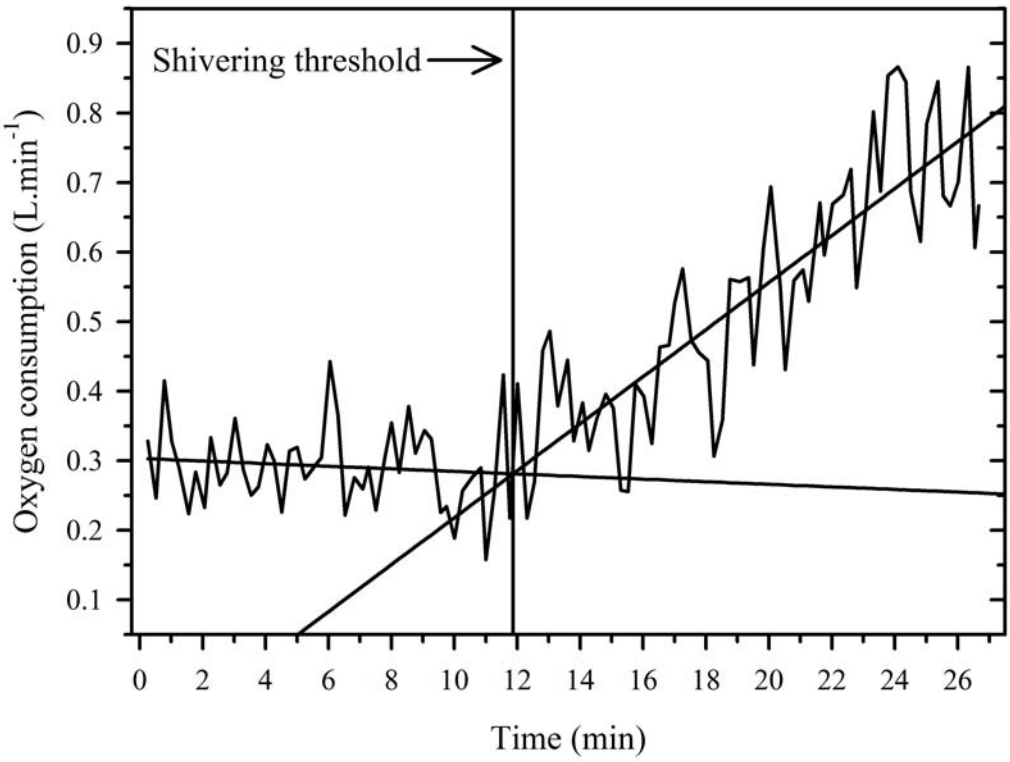

Figure 6.6: Oxygen consumption ($L.min^{-1}$) during passive cooling following a thermoneutral baseline for one subject. The vertical line indicates the shivering threshold as determine by the point of intersection of two lines (baseline and visual linear increase) were fitted to the data.

was determined as the point of intersection between the two lines (Regan *et al*., 1996; Patterson *et al*., 2004; Cheuvront *et al*., 2009). For the sudomotor thresholds, precursor sweating (as measured by skin conductance) was used to determine the onset of neural activation of the efferent pathway for sweating. This has previously been shown as a valid method since discharged sweat may not always reflect this activation (Machado-Moreira *et al*., 2014).

The interthreshold zone was calculated as the temperature difference between the onset of vasodilatation from the heating trials and the onset of vasoconstriction from the cooling trials. A calculation of the temperature range between the sweating and shivering thresholds was also performed to make comparisons of this zone with other research (Mekjavic *et al*., 1991; Lopez *et al*., 1994)

6.3 RESULTS

6.3.1 Pre-experimental treatment

Since the primary focus of this investigation was upon the importance of mean body temperature change, relative to its absolute magnitude, it was essential to elicit the described level of pre-experimental control. As a crude indication of this, oesophageal temperature recorded immediately after subjects were removed from the immersion tank are presented in Table 6.5. For the two control trials, there was no difference in oesophageal temperature ($P>0.05$). For the heating pretreatment, oesophageal temperature was 1.5^{o}C (±0.34) above that for the control trial and for the whole-body cooling pretreatment oesophageal temperature was 0.41^{o}C (±0.33). These data show three distinct thermal states immediately prior to the experimental phase of each trial ($P<0.05$). Pilot research revealed that this three-fold difference in oesophageal temperature offset was necessary to establish three distinct thermal states. Similarly, these distinct thermal states were also reflected in auditory canal temperatures and rectal temperatures.

Once subjects were transferred to the chamber, and immediately prior to the experimental phase, the respective oesophageal temperatures (Table 6.6) were 36.9^{o}C (±0.2), 36.8^{o}C (±0.2), 36.4^{o}C (±0.3) and 37.7^{o}C (±0.3) for the two control trials (passive heating, passive cooling), whole-body cooling (passive heating) trial and whole-

Table 6.5: Post-immersion oesophageal temperatures (°C). These data were averaged across the first 2 min immediately after subjects were removed from the water.

	Experimental trial: Heating		Experimental trial: Cooling	
Subject	**Control**	**Pre-cooling**	**Control**	**Pre-heating**
S1	36.75	35.90	36.84	37.82
S2	36.51	36.59	36.41	37.96
S3	36.71	36.45	36.79	38.56
S4	36.82	36.62	36.63	37.67
S5	36.69	36.47	36.64	38.61
S6	36.81	36.47	36.90	38.35
S7	36.56	35.85	36.89	38.59
S8	36.76	36.02	36.62	38.14
Mean	36.7	36.29	36.71	38.21
S.D.	0.11	0.32	0.17	0.3

Table 6.6: Oesophageal temperatures (ºC) immediately prior to the experimental phase. These data were averaged across the first 5 min immediately before the experimental phase.

	Experimental trial: Heating		Experimental trial: Cooling	
Subject	**Control**	**Pre-cooling**	**Control**	**Pre-heating**
S1	36.82	36.05	36.78	37.65
S2	36.70	36.68	36.76	37.24
S3	37.16	36.52	37.09	38.17
S4	37.07	36.74	36.63	37.31
S5	36.95	36.55	36.87	37.92
S6	36.85	36.71	36.91	37.73
S7	36.62	36.06	36.81	38.09
S8	36.87	36.22	36.65	37.62
Mean	36.88	36.43	36.81	37.72
S.D.	0.18	0.28	0.15	0.34

body heating (passive of thermogenesis as a result of decreased body temperature. Auditory canal temperatures, immediately following the thermoneutral and cooling treatments, prior to the passive heating phase were 36.5ºC (±0.1) and 36.1ºC (±0.1; $P<0.05$), respectively. For the two cooling experimental trials the respective mean body temperatures, immediately following the thermoneutral and heating treatments were 36.6ºC (±0.1) and 37.8ºC (±0.2). The respective rectal temperatures, immediately following the thermoneutral and cooling treatments, prior to the passive heating phase were 37.1ºC (±0.2) and 36.6ºC (±0.2; $P<0.05$).

For the two cooling experimental trials the respective mean body temperatures, immediately following the thermoneutral and heating treatments were 35.7ºC (±0.1) and 38.1ºC (±0.1) where the difference in the experimental baseline between these two trials was 2.4ºC (±0.1). The respective mean body temperatures, immediately following the thermoneutral and cooling whole-body water immersion treatments, prior to the passive heating phase were 36.3ºC (±0.1) and 35.7ºC (±0.10; $P<0.05$) where the difference in the experimental baseline between the two trials was 0.7ºC (±0.1). For the experimental phase for each of the four trials, this was deemed the baseline. Although a shift in temperature measured at each core and skin site occurred in the direction of thermoneutral, each of the three thermal states remained significantly different where the two control trials did not differ. This reflected a shift in mean body temperature as was desired. It is possible that using different weighting factors to calculate mean body temperature during each condition introduced slight errors in the comparisons between each condition. However, this difference is likely to be minimal as it is known that site-specific differences in thermoeffector control exist and therefore require different weighting factors in the calculation of mean body temperature for hot, thermoneutral and control conditions (Taylor *et al*., 2014). In addition, during warm conditions, there is minimal intra-site variability where the thermal gradient between the core and skin is small (Olsen, 1984). This means that skin temperature closely tracks that of core temperature in these conditions and the weighting for mean body temperature calculation favours core temperature. However, this becomes less apparent in thermoneutral and cold conditions. In fact, in cold conditions, intra-site variability is even more pronounced. To account for these differences in peripheral temperature, a greater weighting is applied to mean skin temperature in the estimation of mean body temperature.

Table 6.7: Mean body temperature rate of change ($^{o}C.min^{-1}$).

	Experimental phase: Heating		Experimental phase: Cooling	
	Control	**Pre-cooling**	**Control**	**Pre-heating**
Temperature change (^{o}C)	0.38 (0.1)	0.73 (0.1)	-1.47 (0.3)	-1.98 (0.3)
Trial duration (min)	48.8 (3.6)	88.1 (5.6)	38.1 (2.8)	53.8 (2.6)
Rate of change ($^{o}C.h^{-1}$)	0.47 (0.1)	0.52 (0.1)	-2.16 (0.31)	-2.15 (0.21)

Table 6.8: Oesophageal temperature rate of change ($^{o}C.min^{-1}$).

	Experimental phase: Heating		Experimental phase: Cooling	
	Control	**Pre-cooling**	**Control**	**Pre-heating**
Temperature change (^{o}C)	0.22 (0.07)	0.27 (0.05)	-0.71 (0.21)	-0.95 (0.16)
Trial duration (min)	48.8 (3.6)	88.1 (5.6)	38.1 (2.8)	53.8 (2.6)
Rate of change ($^{o}C.h^{-1}$)	0.27 (0.09)	0.21 (0.06)	-1.05 (0.30)	-1.09 (0.20)

Table 6.9: Mean skin temperature rate of change ($^{o}C.min^{-1}$).

	Experimental phase: Heating		Experimental phase: Cooling	
	Control	**Pre-cooling**	**Control**	**Pre-heating**
Temperature change (^{o}C)	3.19 (0.33)	5.80 (0.34)	-5.61 (0.36)	-7.83 (0.56)
Trial duration (min)	48.8 (3.6)	88.1 (5.6)	38.1 (2.8)	53.8 (2.6)
Rate of change ($^{o}C.h^{-1}$)	4.05 (0.54)	4.15 (0.49)	-8.77 (0.98)	-8.97 (0.87)

6.3.2 Characterising the experimental treatments

Another important consideration in the design of these experiments was the rate of passive heating and cooling across the passive heating trials and passive cooling trials. To eliminate the influence of the dynamic firing of the thermoreceptors, and therefore their influence on the thermoeffector thresholds, it was essential that the rate of heating and cooling during the experimental phases were identical. This was successfully achieved. Indeed, no significant differences occurred between either passive heating or passive cooling trials ($P>0.05$). There was, however, a significant difference between the passive cooling rate and passive heating rate ($P<0.05$), but this difference did not affect the outcome of this research, because thermoeffector threshold comparisons were only made between the cooling or heating trials (Table 6.7). These changes in mean body, oesophageal and mean skin temperature across all four trials are presented in Figures 6.7, 6.8 and 6.9.

The heart rate responses for each of the three thermal states, across the five local stimulation temperatures and during each of the treatment sites are presented in Figure 6.8. At the cessation of water immersion there was a significantly higher heart rate between the control trial (58.6 ± 2.4 beats.min^{-1}) and whole-body heating (84.8 ± 3.2 beats.min^{-1}; $P<0.05$) as well as the control trial (54.5 ± 2.1 beats.min^{-1}) and whole-body cooling (66.4 ± 3.3 beats.min^{-1}; $P<0.05$). This was an expected outcome since it is well known that during heating at rest the increased heart rate during passive heating is the result of redistribution of blood to the periphery to facilitate increased convective heat delivery to the skin allowing for increased heat dissipation (Rowell *et al*., 1969). Interestingly, heart rate was also elevated at the completion of whole-body cooling. This is likely to be the result of the onset thermogenesis as a result of decreased body temperature.

Mean arterial pressure was slightly elevated immediately following whole-body cold water immersion (92.6 ±2.2 mmHg). This was not surprising since immersion in cold water increases peripheral vasoconstriction, therefore increasing central venous pressure (Figure 6.9). Ordinarily, blood pressure would be regulated to normal values through a decreased heart rate. However, in this instance, with the addition of shivering and increased metabolic rate, mean arterial pressure was elevated. Once passive heating occurred during the experimental phase, mean arterial pressure returned to match that of the control trial (86.2

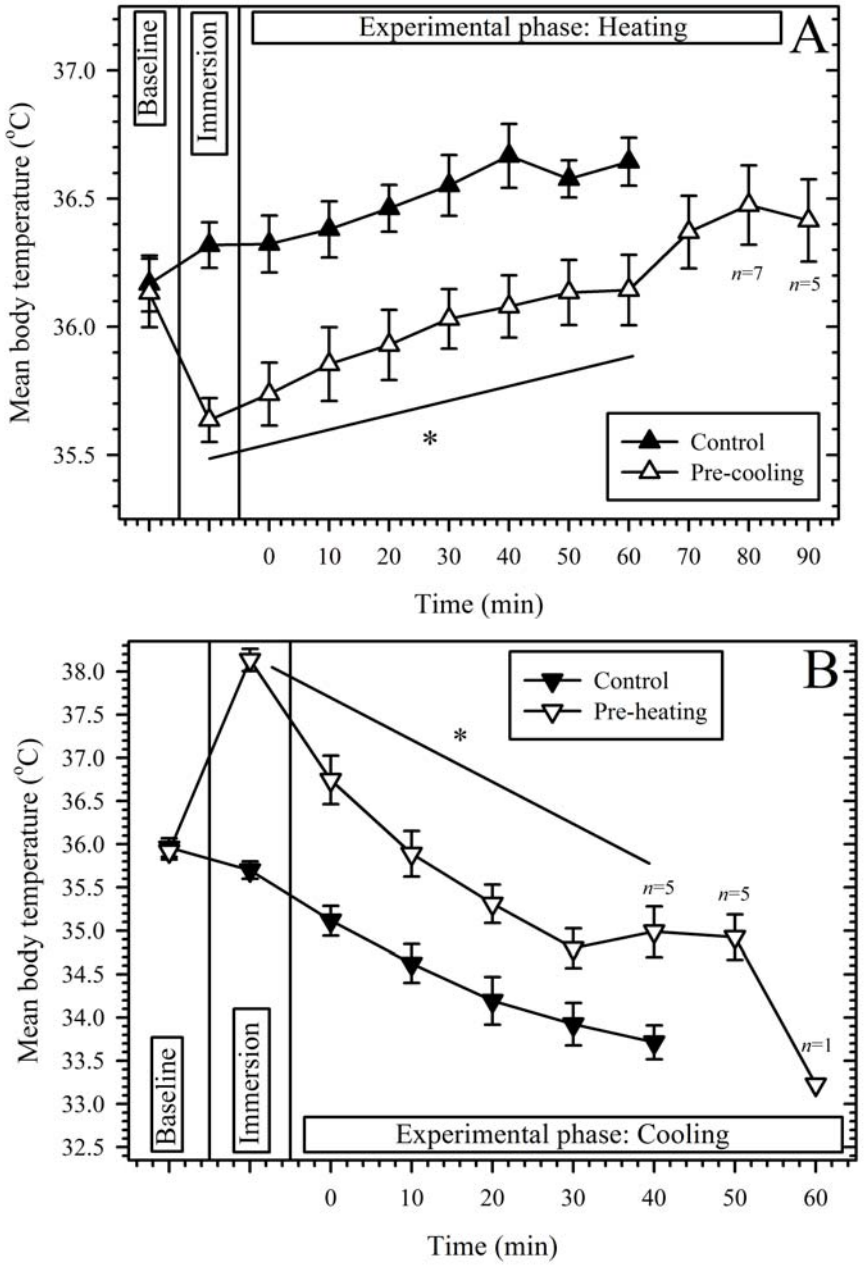

Figure 6.7: Mean body temperature (°C) **A**: during passive (supine) heating, preceded by 45 min of immersion in either thermoneutral (control: 33°C) or gradually cooled water (28-23°C) and **B**: during passive (supine) cooling, preceded by immersion in either thermoneutral (control: 33°C) or heated water (39°C). The time scale is different between these figures as trials were terminated once each thermoeffector (vasomotion, sudomotion and thermogenesis) was established* = significant between-treatment difference (P<0.05). N=8 unless otherwise stated.

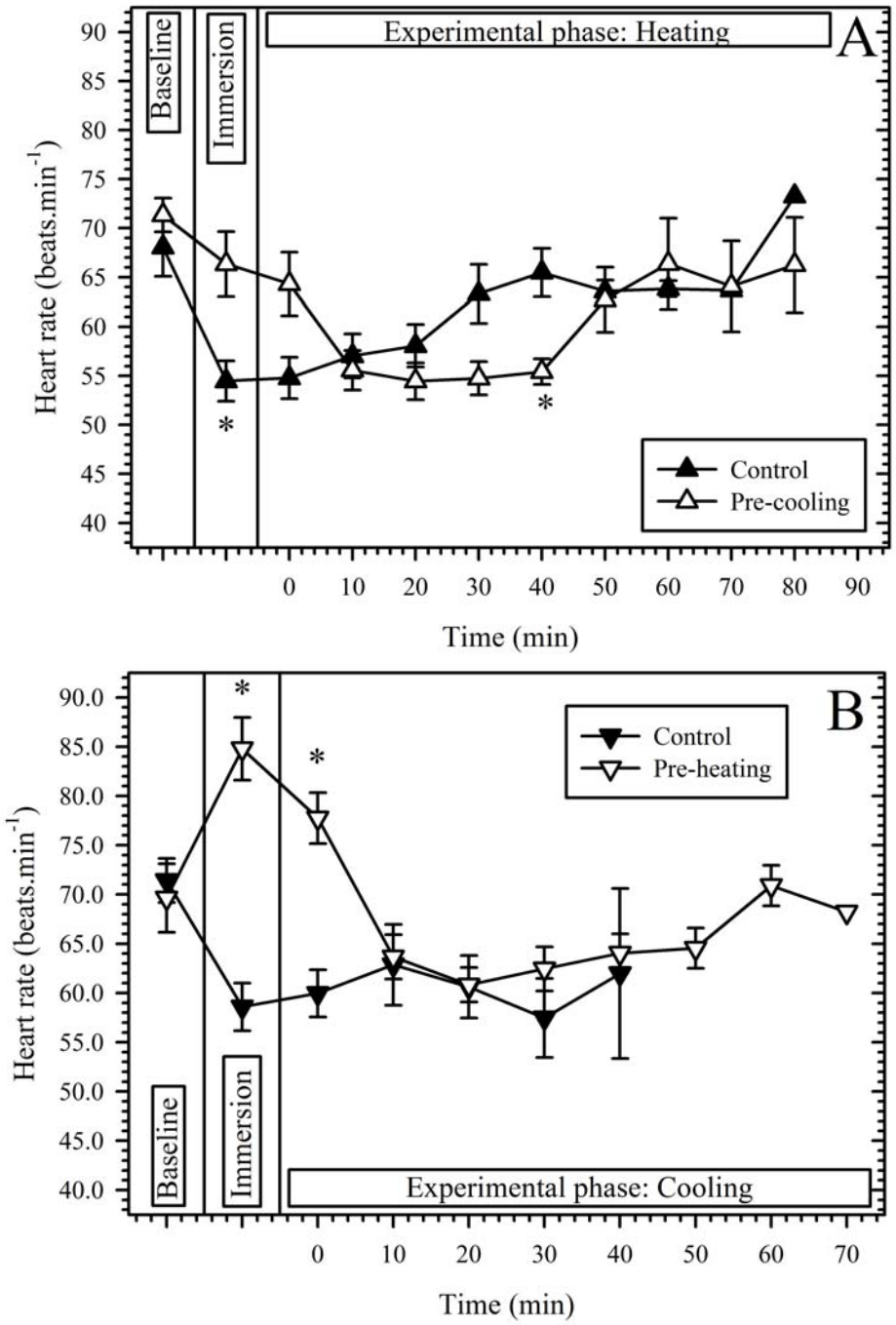

Figure 6.8: Heart rate (beats.min^{-1}) **A**: during passive (supine) heating, preceded by 45 min of immersion in either thermoneutral (control: 33ºC) or gradually cooled water (28-23ºC) and **B**: during passive (supine) cooling, preceded by immersion in either thermoneutral (control: 33ºC) or heated water (39ºC). * = significant between-treatment difference (P<0.05). N=8 unless otherwise stated.

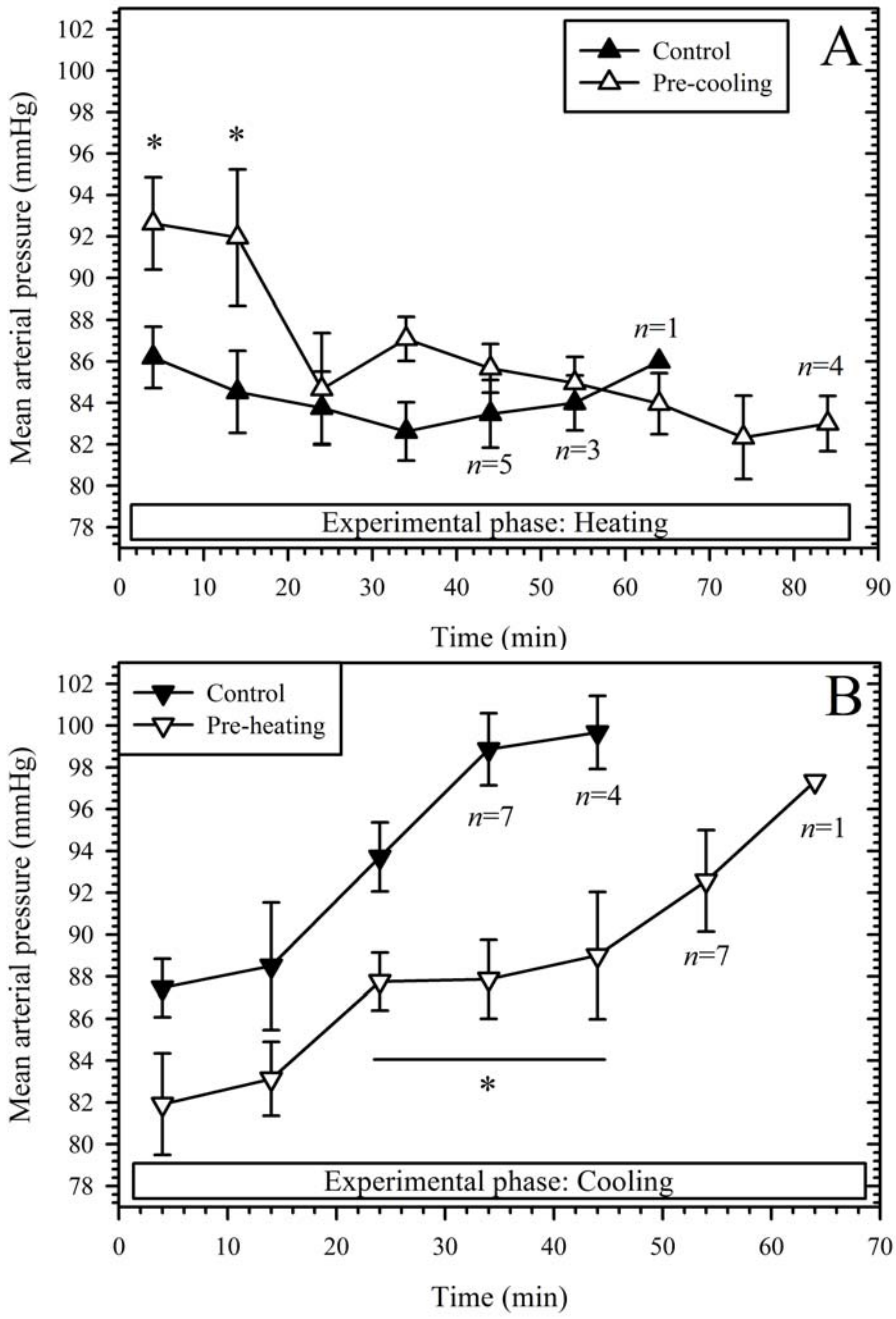

Figure 6.9: Mean arterial pressure (mmHg) **A**: during passive (supine) heating, preceded by 45 min of immersion in either thermoneutral (control: 33ºC) or gradually cooled water (28-23ºC) and **B**: during passive (supine) cooling, preceded by immersion in either thermoneutral (control: 33ºC) or heated water (39ºC). * = significant between-treatment difference (P<0.05). N=8 unless otherwise stated.

±1.5 mmHg). The mean arterial pressure pattern for the passive cooling trials was however slightly different. While there was no difference in mean arterial pressure following water immersion (P>0.05), a slight linear increase in mean arterial pressure was observed during passive cooling for both the control (0.35 mmHg.min^{-1}) and whole-body heating (0.24 mmHg.min^{-1}) trials. Again, this would be attributed to the increased muscle activation at the onset of thermogenesis.

6.3.3 Thermoeffector thresholds

For the passive-heating control trial, the mean body temperature thresholds for each thermoeffector (vasodilatation and sweating) was numerically, but not significantly different (Figure 6.8; P>0.05). However, following whole-body cooling, the threshold for precursor forearm sweat was elevated by 0.18ºC (±0.03; P=0.07), whilst that for discharged sweat was raised by 0.19ºC (±0.04; P<0.05). Conversely, the vasodilatation threshold was reduced by 0.36ºC (±0.11; P<0.05) for the same site. The change in vasomotor threshold was not significantly different for the calf (0.40º ±0.13ºC) or finger thresholds (0.45º ±0.11ºC). For this thermoeffector, the change in the pre-heating mean body temperature (0.39±0.10ºC), relative to the control trial, did not differ significantly from its threshold change (P>0.05).

Conversely, when subjects were passively cooled, for the control trial, the mean body temperature thresholds for shivering and forearm skin blood flow (vasoconstriction) were 32.2ºC (±0.2) and 32.8ºC (±0.1), respectively (Figure 6.9; P<0.05). Following whole-body pre-heating the threshold for shivering and forearm skin blood flow (vasoconstriction) were 32.9ºC (±0.1) and 33.0ºC (±0.2), respectively (P>0.05). For this thermoeffector the change in pre-cooling temperature was 0.82ºC (±0.2) and this was not significantly different from the change in temperature for the shivering threshold which was increased by 0.67 (±0.2; P>0.05). Although the threshold temperature for finger blood flow (33.3º ±0.2ºC) was higher than that of the forearm, this was not significant (Figure 6.10; P>0.05).

The interthreshold zones for both the control and experimental trials are demonstrated in Figure 6.13 and Figure 6.14. These figures show that both the shivering and sweating thresholds were moved to a higher mean body temperature. In comparison, the

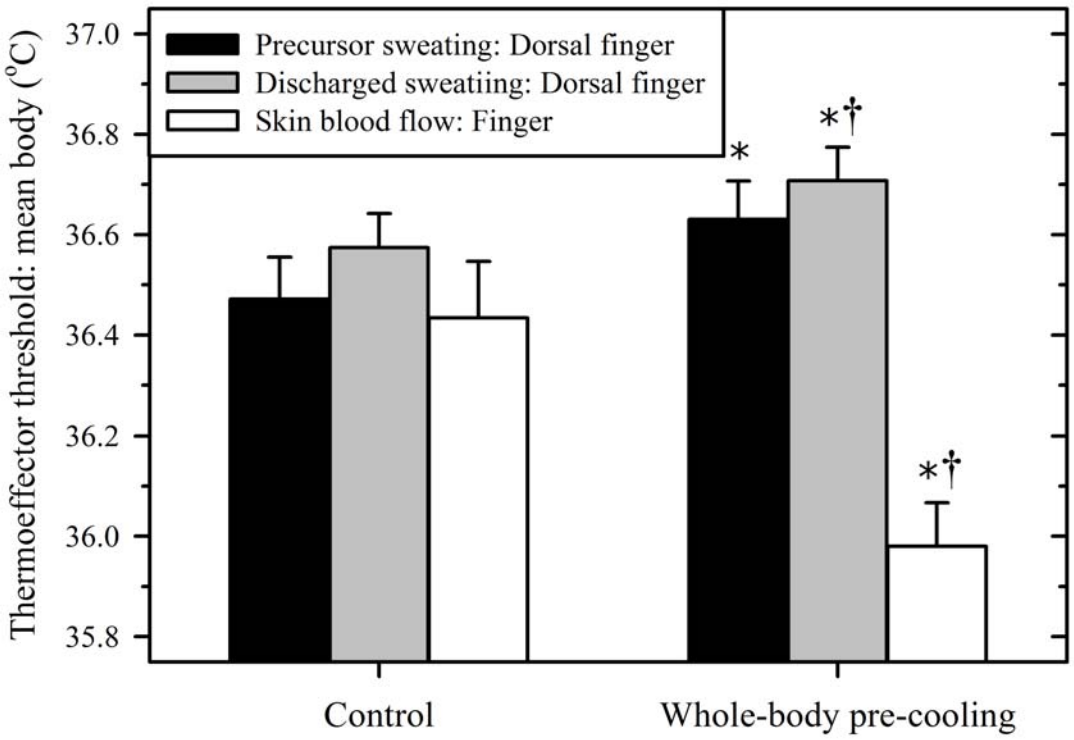

Figure 6.10: Sudmotor and vasomotor thresholds (mean body temperature) of the forearm (**A**) and finger (**B**) during passive (supine) heating, preceded by immersion in either thermoneutral (control: 33°C) or gradually cooled water (28-23°C). * = significant between-treatment difference (P<0.05); † = significant difference between the within-treatment thresholds (P<0.05).

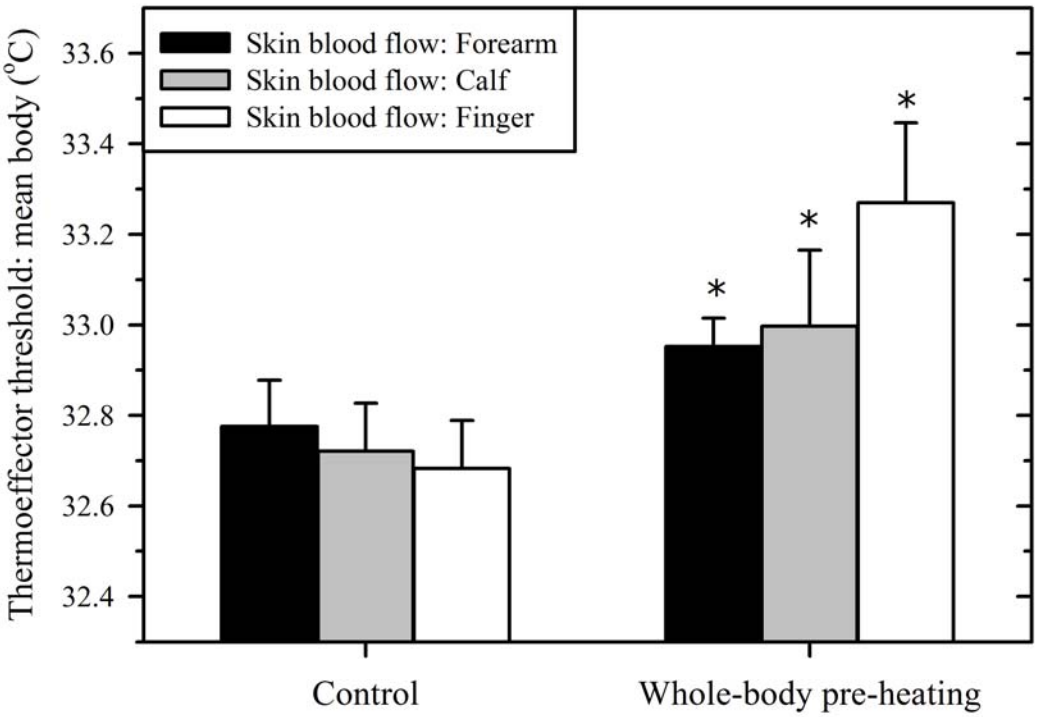

Figure 6.11: Whole-body shivering and vasomotor thresholds (mean body temperature) of the forearm (**A**), finger (**B**), and calf (**C**) during passive (supine) cooling, preceded by 45 min of immersion in either thermoneutral (control: 33ºC) or heated water (39ºC). * = significant between-treatment difference (P<0.05); † = significant difference between the within-treatment thresholds (P<0.05).

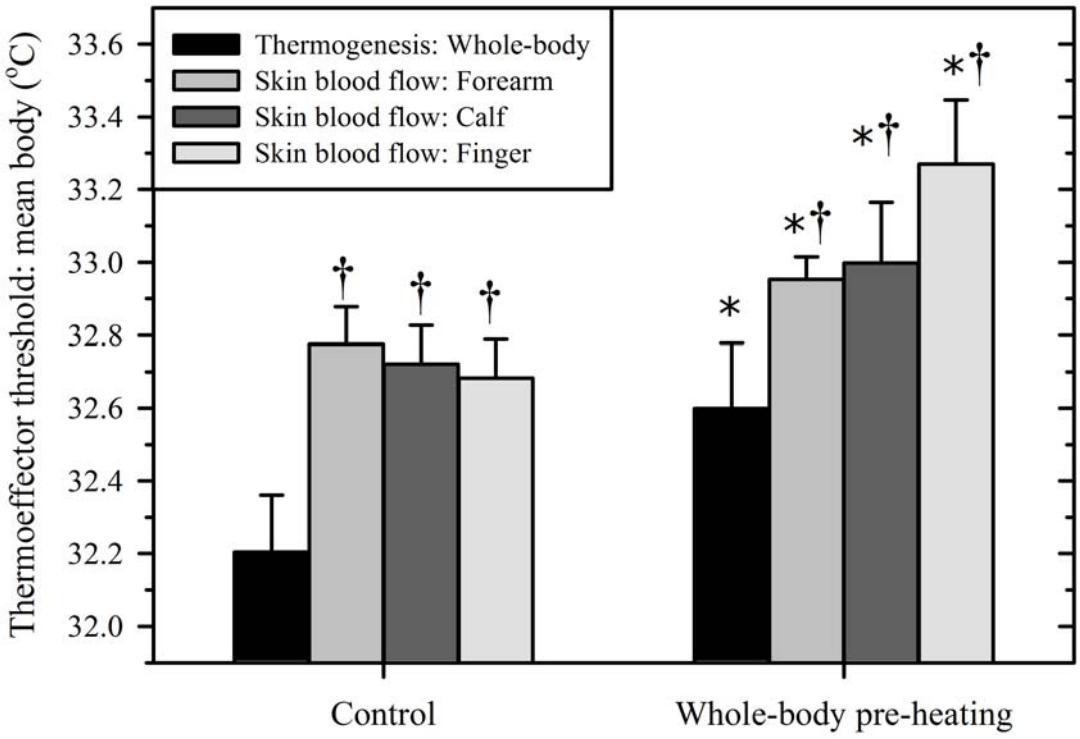

Figure 6.12: Vasomotor thresholds (mean body temperature) of the forearm (A), finger (B), and calf (C) during passive (supine) cooling, preceded by immersion in either thermoneutral (control: 33ºC) or heated water (39ºC). * = significant between-treatment difference (P<0.05); † = significant difference between the within-treatment thresholds (P<0.05). N=8 unless otherwise stated.

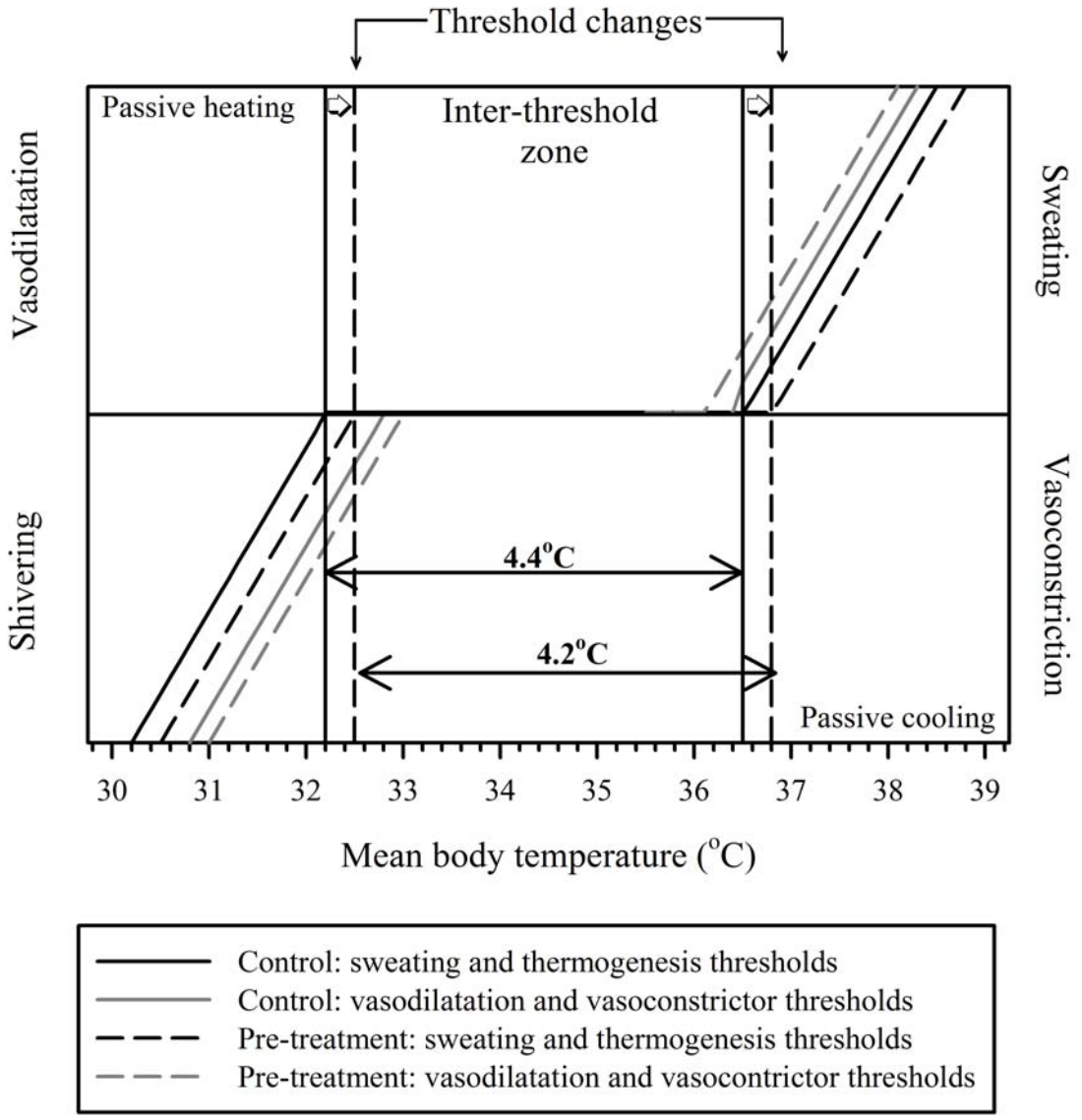

Figure 6.13: The mean body temperatures inter-threshold zone (forearm) as determined by the sweating and shivering thresholds (vertical black solid lines). Shifts in these thresholds are shown (vertical black broken lines) as a result of either whole-body pre-cooling or pre-heating. The oblique lines show each of the four thermoeffector responses (Figure 6.11 and Figure 6.12), with those for vasomotor function shown in grey (see legend).

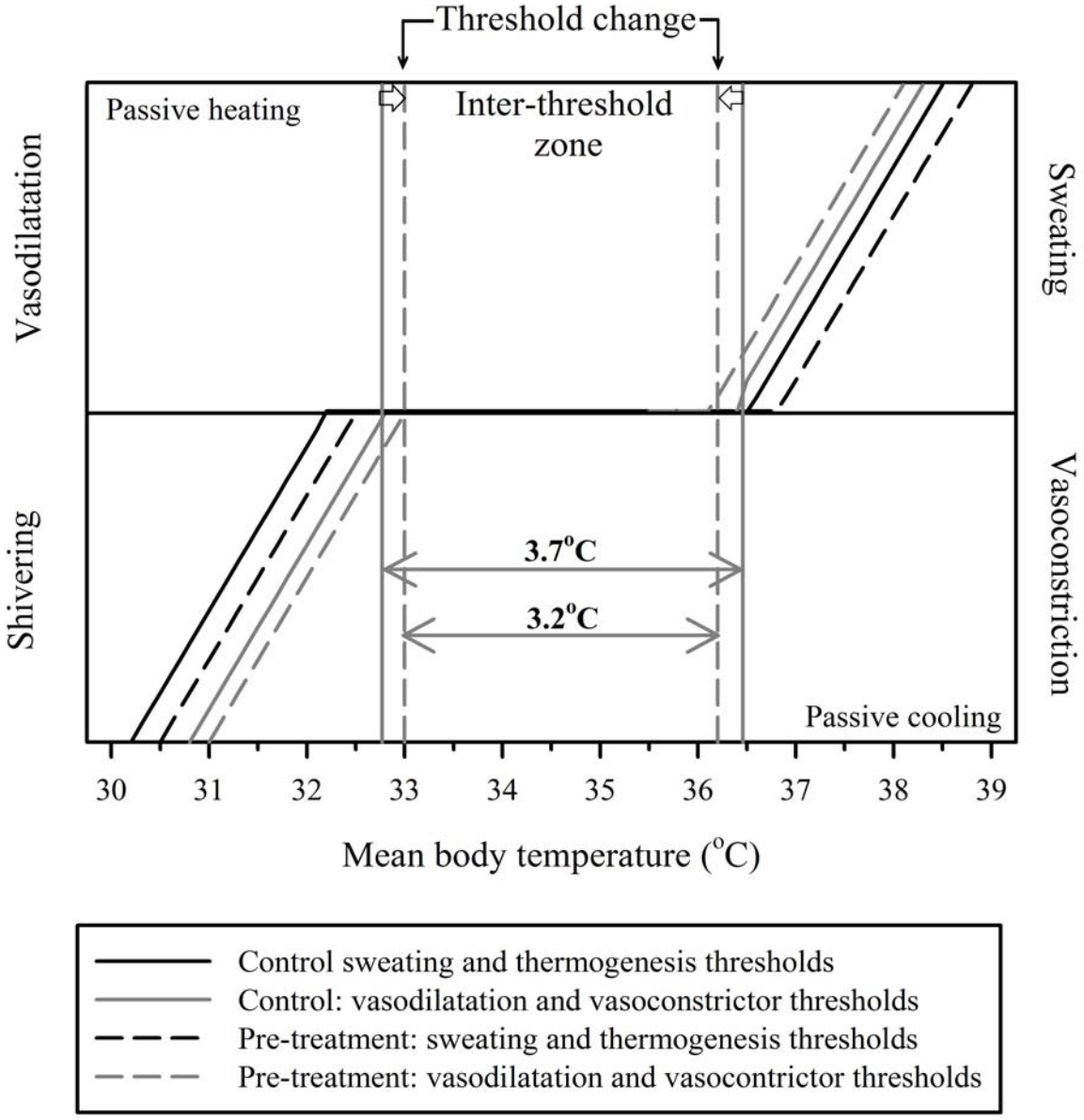

Figure 6.14: The mean body temperatures inter-threshold zone (forearm) as determined by the vasodilatation and vasoconstriction thresholds (vertical grey solid lines). Shifts in these thresholds are shown (vertical grey broken lines) as a result of either whole-body pre-cooling or pre-heating. The oblique lines show each of the four thermoeffector responses (Figure 6.11 and Figure 6.12), with those for sudomotor function and thermogenesis shown in grey (see legend).

vasomotor thresholds (Figure 6.14) demonstrate a shift in vasodilatation threshold to a lower mean body temperature following pre-cooling and a shift to a higher mean body temperature following pre-heating. These results support the hypothesis that pre-cooling and pre-heating would shift the mean body temperature thermoeffector thresholds for sweating and shivering by a magnitude equal to that of the pre-exposure displacement of mean body temperature.

6.3.4 Psychophysical responses

6.3.4.1 Thermal sensation

As expected, subjects felt 'hot' to 'very hot' at the completion of whole-body heating, 'cold' to 'very cold' at the completion of whole-body cooling and 'neutral' during the thermoneutral water immersions. Once in the chamber and at the onset of the experimental phase, subjects gradually became 'warm' to 'hot' during passive heating and 'very cold' to 'extremely cold' during passive cooling. The thermal sensation responses across the duration of the experimental phase was not significantly different (Figure 6.15; $P>0.05$). Not surprisingly, during the most extreme exposures subjects felt 'uncomfortable'(Figure 6.16).

6.4 DISCUSSION

The primary purpose of the current study was to investigate whether the thermoeffector thresholds were dependent upon the absolute or the change in mean body temperature. This was evaluated by manipulating the pre-exposure body temperature and then quantifying the thermoeffector thresholds for sweating, shivering and skin blood flow. Additionally, an investigation into the interactions between the thresholds for vasomotor and sudomotor function, and between vasomotor function and thermogenesis was explored. This allowed for determination of the interthreshold zone. Finally, we investigated whether the same patterns in thermoeffector thresholds might exist in different skin regions (forearm, calf and finger). The most significant finding from the current study was that, following pre-treatment, the threshold for vasodilatation and shivering were shifted in equal proportions to the change in mean body temperature induced by each of the two pre-treatments, and this supported our hypotheses. Interestingly, the sudomotor threshold was shifted to a higher mean body temperature following whole-body pre-cooling and could indicate that the two thermoeffectors work independently in response to heat loss requirements, with sudomotor

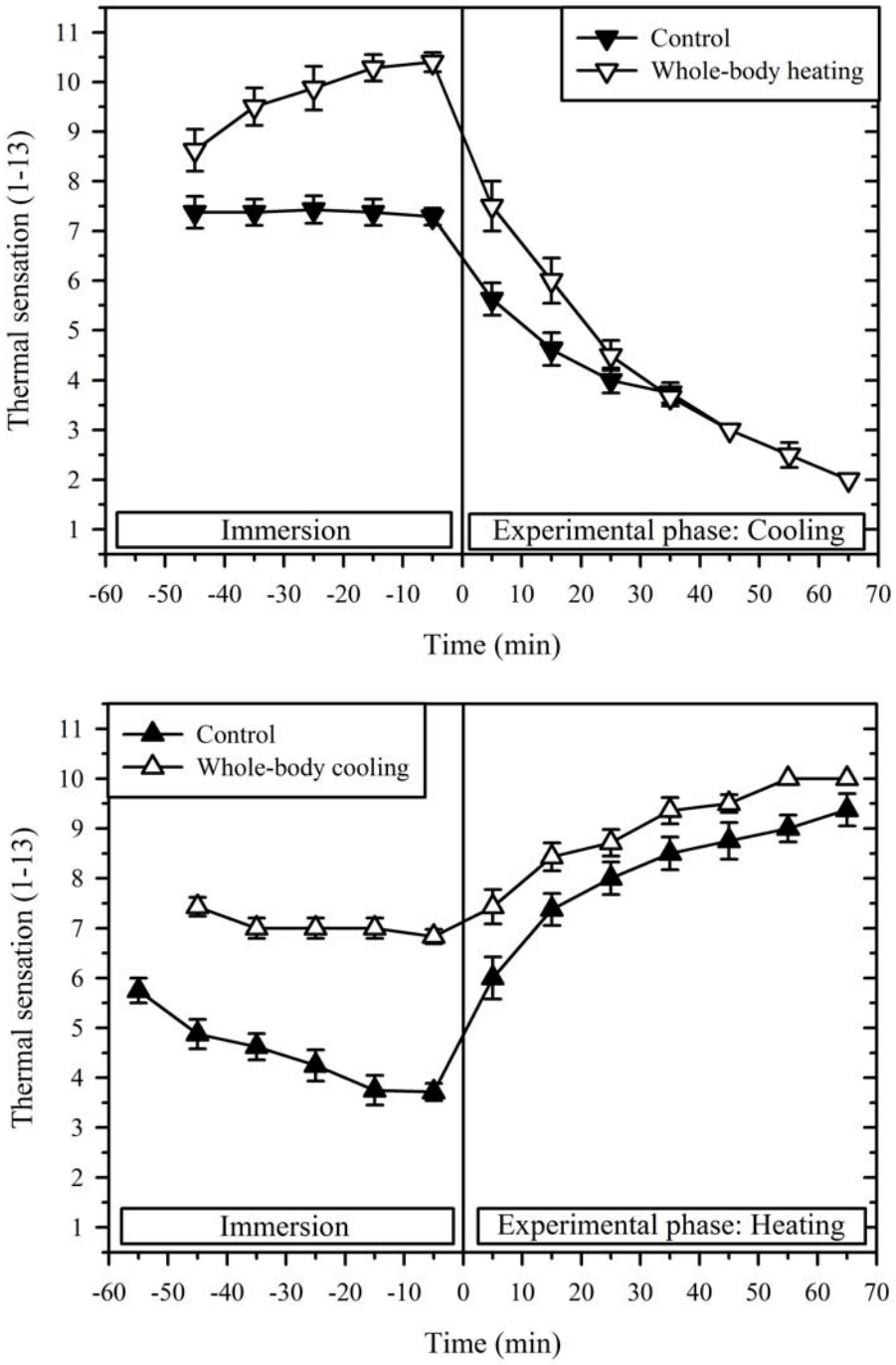

Figure 6.15: Thermal sensations (1-13) A: during passive (supine) heating, preceded by 45 min of immersion in either thermoneutral (control: 33ºC) or gradually cooled water (28-23ºC) and B: during passive (supine) cooling, preceded by immersion in either thermoneutral (control: 33ºC) or heated water (39ºC). * = significant between-treatment difference (P<0.05); † = significant difference between the within-treatment thresholds (P<0.05). N=8 unless otherwise stated.

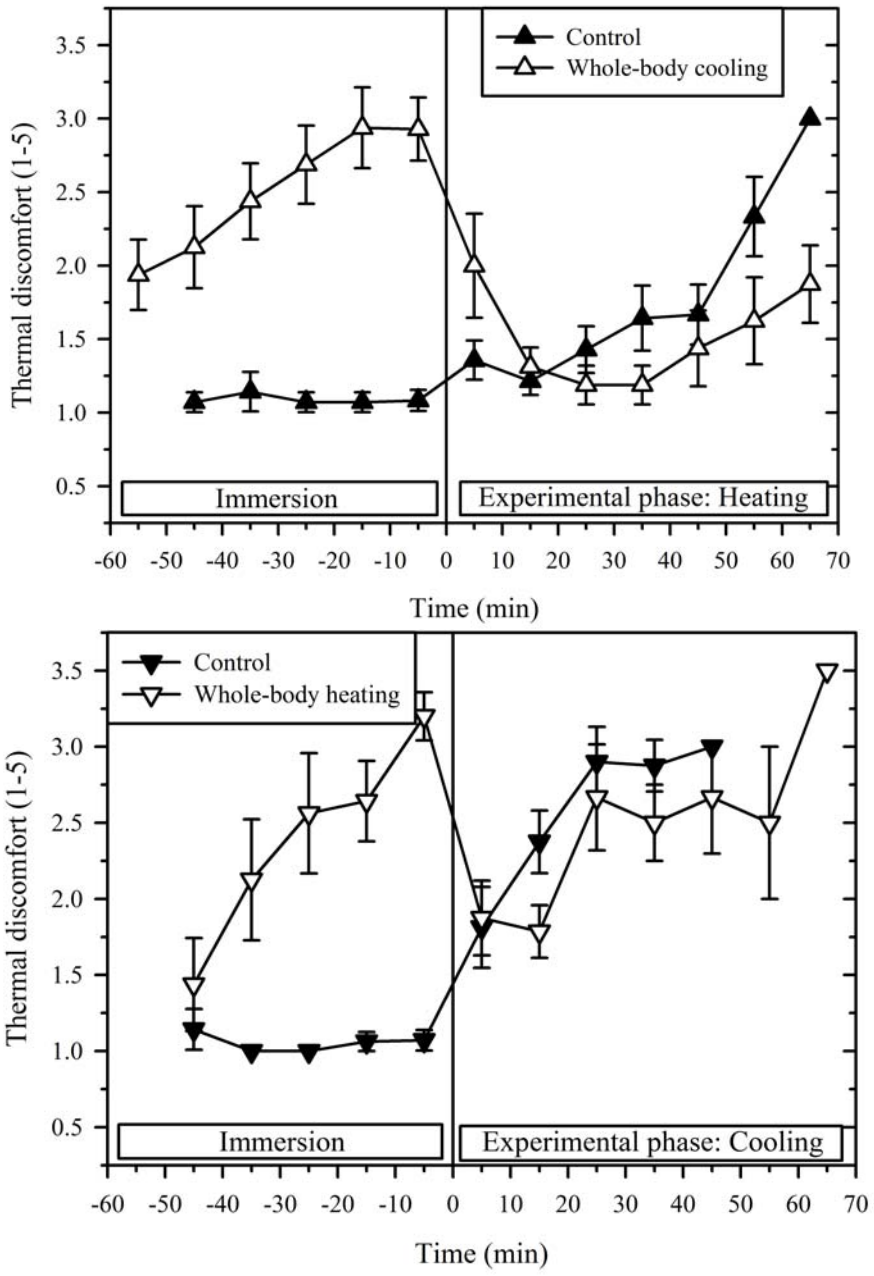

Figure 6.16: Thermal discomfort (1-5) **A**: during passive (supine) heating, preceded by 45 min of immersion in either thermoneutral (control: 33ºC) or gradually cooled water (28-23ºC) and **B**: during passive (supine) cooling, preceded by immersion in either thermoneutral (control: 33ºC) or heated water (39ºC). * = significant between-treatment difference (P<0.05); † = significant difference between the within-treatment thresholds (P<0.05). N=8 unless otherwise stated.

activation only being initiated if vasodilatation was no longer able to dissipate sufficient heat to regulate mean body temperature.

6.4.1 Influence of pre-cooling on vasomotor and sudomotor thresholds

It has previously been shown that whole-body cooling delayed vasodilatation during subsequent exercise in the heat, with the mean body temperature threshold elevated by 0.59ºC (P<0.05), while the sudomotor threshold was lowered by 0.37ºC (P>0.05; MacDonald *et al*., 2000). Thus, cooling displaced these thresholds in opposite directions, relative to control, resulting in a threshold difference of 0.85ºC (P<0.05).To our knowledge, the opposite displacement of these thermoeffector thresholds has not been described following thermal pre-treatment. In that experiment, however, subjects changed from a supine immersion to seated exercise (cycling). Notwithstanding this postural change and its effect on skin blood flow, non-thermal drives will enhance sweating and suppress skin blood flow during the early phase of an exercise transition (Kondo *et al*., 2010), and these effects are consistent with those threshold changes, and occur over similar durations. This rendered the interpretation of those observations difficult, and thereby provided a stimulus for the current research investigation.

In the current study, the opposite pattern was evident during passive, supine heating, following whole-body cooling. That is, the vasodilatation threshold occurred at a lower mean body temperature while the sudomotor threshold was elevated. This resulted in a threshold difference of 0.64ºC (P<0.05) between an earlier vasodilatation and delayed sweating. In the control state, one would anticipate vasodilatation to occur before the initiation of sweating, and this was observed in both trials, but it was significantly earlier following cooling, and was equivalent to the change in mean body temperature induced by the pre-exposure treatment. This is not the first time that we have reported an equivalence between a thermoeffector threshold change and body temperature displacement. This phenomenon was first described for the reduction in the sudomotor threshold during heated exercise after cooling (MacDonald *et al*., 2000), then following heat acclimation (Patterson *et al*., 2004), and again for a cooling experiment (Booth *et al*., 2004). It is clear that these thermoeffectors show some independence of the absolute body temperature, but appear to be coupled with the change in body temperature. The shift in vasodilatation threshold observed

in the current study may be the result of applying heat at a lower mean body temperature thereby activating thermoreceptors and initiating the removal of afferent sympathetic drive earlier (passive vasoconstriction) than during the control trials. Since skin blood flow was slightly increased following pre-cooling, vasomotion was initially a more effective mechanism for thermoregulation during whole-body heating than during the control condition. It is therefore likely that sweating was not activated until heat loss could not be achieved solely through skin blood flow.

A second significant observation is the apparent capacity of these thermoeffector thresholds to vary independently. Indeed, vasomotion, but not sudomotion, was activated earlier. It is therefore possible that these results indicate the ability to delay sudomotor activation if sufficient heat is lost through changes in skin blood flow. This was an interesting outcome because, although the relationship between vasodilatation and sweating has been extensively researched, the exact interactions between these thermoeffectors remains somewhat poorly understood (Johnson *et al*., 2014).

Grant and Holling (1938) observed both thermoeffectors to begin at the same time following intense heating, leading to the belief that a close mechanistic relationship existed between the vasodilatation and sweating thresholds (Estañol *et al*., 2004; Love and Shanks, 1962; Fox and Edholm, 1963). This theory was based upon the possibility that the release of kallerkrein from activated eccrine sweat glands, that is used to form bradykinin, and is therefore believed to cause active vasodilatation (Fox and Edholm, 1963; Fox and Hilton, 1958). In the current study, during the control trials, the interaction between vasomotor and sudomotor thresholds was at least consistent with their simultaneous activation. That is, both precursor sweating and vasodilatation thresholds occurred at the same mean body temperature. However, this did not occur during passive heating following whole-body cooling. Indeed, activation of precursor sweating was delayed while vasodilatation occurred at a lower mean body temperature. It is also likely that these shifts in skin blood flow are due to withdrawal of vasoconstrictor activity, not increases in active vasodilatory activity. Currently, evidence that a neural link exists between sudomotor and vasodilator nerves remains unknown and many researchers have demonstrated no link between the onset of sweating and increased vasodilatation (Senay *et al*., 1963; McCook *et al*., 1965; Kellogg *et*

al., 2002). Therefore, the current study supports the hypothesis that a causal link between the activation of sweating and active vasodilatation does not exist.

Rather than simultaneous activation of vasomotor and sudomotor thresholds during passive heating, it appears that activation occurred in two phases following whole-body cooling. The first phase occurred when the vasodilatation threshold was reached following a change in mean body temperature equivalent to that induced by the pre-exposure treatment. If absolute mean body temperature was the decisive determinant of this threshold, then this outcome could not have been observed since, at that time, absolute mean body temperature was lower than observed in the control trial (36.2^{o}C versus 36.5^{o}C; P<0.05) As a result, increases in skin blood flow occurred at a lower mean body temperature than observed during the control trials. This supported greater convective and conductive heat losses from the skin to the surrounding environment.

The second thermoeffector phase occurred when the sweating threshold was reached, and this was at a higher mean body temperature than in the control trial. In this case, it appears that sweating was only activated to facilitate heat loss needs rather than being due to a common neural pathway between active vasodilatation and sudomotion. That is, the increased vasodilatation, and hence skin blood flow, facilitated the requirement for heat loss and sweating was not activated until the dry heat loss was no longer sufficient. Indeed, the independent nature of vasodilatation and sudomotor thresholds observed in the current study is supported by previous experiments failing to reveal any time relationship between sweating and vasodilatation onset (Senay *et al*., 1963; Wyss, 1974; Johnson and Park, 1981).

Furthermore, exercise has been shown to disturb the strong correlation between skin blood flow and sweating, where the vasodilatation and sweating thresholds occur at different temperatures to that observed during passive heating at rest (Johnson and Park, 1981).In this case, sweating is enhanced and skin blood flow suppressed during the early phase of exercise (Kondo *et al*., 2010). The results of the current study indicate an opposite shift in vasomotor and sudomotor thresholds following whole-body cooling to that observed by others during exercise (MacDonald *et al*., 2000; Johnson and Park, 1981). This was an important outcome since most current research investigating the influence of pre-cooling on

thermoeffector thresholds was conducted during exercise (Schmidt and Brück, 1981; Booth *et al*., 2004; Mack *et al*., 1995). By removing the non-thermal influences (Kenny and Journeay, 2010; Kondo *et al*., 2010) induced by exercise we can more fully understand how the thermal influences of vasomotor and sudomotor thresholds interact and this was achieved during heated rest in the current study.

6.4.2 Influence of pre-heating on the vasomotor and thermogenic thresholds

In the current study, we also tested the hypothesis that pre-heating would shift the threshold for vasoconstriction and thermogenesis to a higher mean body temperature. The vasoconstrictor threshold preceded that of shivering for both the control trial and during passive cooling following whole-body heating and this was in agreement with the hypothesis. This is a well known interaction since shivering is only activated when behavioural responses and vasoconstriction are insufficient to defend core temperature (Mahmood and Zweifler, 2007; Werner, 2008). However, following whole-body heating the thresholds for vasoconstriction and thermogenesis moved to a higher mean body temperature during passive cooling than in the control state. This shift in shivering threshold was again proportional to the change in mean body temperature. These threshold changes can be explained by the shift in afferent signal received from activation of thermoreceptors since they respond to both absolute temperature (static) and a change in temperature (dynamic). During passive cooling following whole-body heating, cold-sensitive thermoreceptors were activated at a higher mean body temperature. This gave rise to hypothalamic processing to prevent heat loss by activating vasoconstriction and thermogenesis earlier, and therefore at a warmer mean body temperature. These differences were not caused by the rate of change in mean body temperature as these were identical in both trials.

These shifts in vasomotor and shivering thresholds to a higher mean body temperature have previously been shown during passive cooling following exercise where the shift in threshold was approximately 0.25ºC and 0.41ºC respectively (Kenny *et al*., 1998). Consistent with these findings an increase in cold tolerance in mice was found during cold exposure following exercise, however this was only attributed to the vasoconstrictor threshold and not the onset of thermogenesis (Shechtman and Talan, 1994). Since both

whole-body passive heating and exercise augment skin blood flow, one possible explanation for the changes in the vasoconstrictor threshold observed in the current study following whole-body passive heating may be increased activation of cold thermoreceptors during cold exposure which rapidly inhibits activation of heat loss mechanisms (sweating and vasodilatation) but initiates heat conservation (vasoconstriction) and heat production (shivering). This is known as the reciprocal cross inhibition theory (Mekjavic and Eiken, 2006). In contrast with the findings from the current study cold adaptation, posture, hypoxia anaesthesia and antihypertensive agents (alpha$_2$ adrenergic receptor agonist) have all shown the thresholds for vasoconstriction and shivering to move to a lower mean body temperature (Werner, 2008; Tattersall and Milsom, 2009; Nakajima *et al*., 2000; Nicolaou *et al*., 1997), while cooling speed of core temperature has been shown to have no effect on these thermoeffector thresholds (Taniguchi *et al*., 2011).

While passive heating, following whole-body cooling, showed the capacity of thermoeffector thresholds to work independently, this was not observed during passive cooling, following whole-body heating. This can be explained by the inability in this study to measure local thermogenic responses. Given the shivering threshold was determined through changes in whole-body oxygen consumption measured at the mouth, the exact local threshold could not be determined. Therefore, understanding the local relationship between the vasomotor and thermogenic thresholds was impossible in the current study. However, it is possible that an increase in regional muscular tone in response to activation of cutaneous and deep body cold thermoreceptors occurred prior to establishing the threshold for shivering and this has previously been shown to occur in muscles of the trunk region followed by shivering of the limbs (Tikuisis *et al*., 1991). Regardless of the regional differences associated with shivering, vasoconstriction has always been shown to precede shivering and the interthreshold zone can be determined as the temperature range between the onset of sweating the onset of shivering and thermogenesis as measured as oxygen consumption at the mouth.

6.4.3 Determination of the interthreshold range

Another aim of the current study was to evaluate the impact of the change in pre-exposure mean body temperature and determine the size of the interthreshold zone. The data

from these experiments allow for determination of the interthreshold range (the vasomotor or null zone) for mean body temperature. The mean body temperature between vasoconstriction to vasodilatation was 3.7^{o}C for the control trials and 3.2^{o}C for the treatment trials. When the interthreshold zone between the onset of sweating and thermogenesis was evaluated, a range of 4.4^{o}C for the control trials and 4.2^{o}C for the pre-treatment trials was observed.

These values are vastly different from those observed by Mekjavic *et al* (1991), who reported a mean body temperature interthreshold range (between sweating and shivering) of approximately 1.8^{o}C. This 2.6^{o}C difference between the interthreshold zone observed in the current study compared with that of Mekjavic *et al* (1991) can be explained by three methodological differences. Firstly, Mekjavic *et al* (1991) clamped skin temperature at 28^{o}C for both the heating and cooling phases removing any peripheral thermoreceptor influence on the thermoeffector response. Thus, their mean body temperature was an exclusively central phenomenon. Whereas in the current study, whole-body heating and cooling were achieved by either increasing or decreasing skin temperature until each threshold was established, and this perhaps more closely reflects changes that might occur naturally.

Secondly, the interthreshold zone reported in Mekjavic *et al* (1991) was measured for each subject in one trial where core temperature was firstly driven up until the onset of sweating occurred then reduced until shivering was established. However, in the current study, the sweating and shivering thresholds were determined in two separate trials. Finally, the heating phase involved exercise to elevate core temperature but the cooling phase was passive, while in the current study, subjects were passively heated and cooled without the influence of exercise. Interestingly, Lopez *et al* (1994) determined the interthreshold zone between sweating and shivering to be 1.1^{o}C. Their methods were also different to those used by Mekjavic *et al* (1991) as well as in the current study. Lopez *et al* (1994) determined the interthreshold zone by clamping skin temperature (36.8^{o}C) and increasing core temperature (passive heating) until sweating was established then subjects were cooled by central venous infusion of lactated Ringer's solution (~3^{o}C) until shivering was established. Whilst each experiment has its advantages and disadvantages, these experimental differences make direct comparisons very difficult, if not impossible.

Apart from these obvious methodology differences between Mekjavic *et al* (1991) and Lopez *et al* (1994) compared to the current study, in both of these experiments, subjects were heated to an elevated core temperature of 37.4°C (Mekjavic *et al*., 1991) 37.0°C (Lopez *et al*., 1994) prior to being cooled. In the current study, we showed that whole-body heating prior to passive cooling shifted the threshold for shivering to a higher mean body temperature. Therefore, it is possible in both experiments performed by Mekjavic *et al* (1991) and Lopez *et al* (1994) that the heating phase of their trials shifted the threshold for thermogenesis to a higher mean body temperature and would therefore partly explain their smaller interthreshold zone than that observed in the current study.

6.4.4 Regional differences in thermoeffector thresholds

The final aim of the current study was to investigate whether differences in the thermoeffector thresholds between acral and non-acral skin regions were apparent during passive heating following whole-body cooling and during passive cooling following whole-body heating. Although acral skin regions possess arteriovenous anastomoses and vasomotor function is controlled only by active vasoconstriction through the cholinergic pathway, no significant regional differences were observed in the magnitude or direction of the shift in the onset of vasodilatation during passive heating following whole-body cooling compared to the control trial. That is, although differences exist in the neural control of these regions, this did not impact the thermoeffector thresholds. In addition, regional differences exist for sudomotor function within acral skin, with previous research showing reduced sweating in acral skin compared to non-acral skin (Saad *et al*., 2001; Machado-Moreira *et al*., 2008). Interestingly, although these mechanistic differences exist between these acral and non-acral skin regions, the same shifts in the sweating threshold were observed during passive heating following whole-body cooling measured at the forearm and finger in the current study. This finding is supported by McCook *et al* (1965) who showed no consistent relationship between vasodilatation and sweating thresholds between regions.

It was not possible to measure regional differences for thermogensis thresholds during passive cooling in the current study, however regional (forearm, calf, and finger) vasoconstrictor threshold measurements were evaluated during passive cooling. There were no regional differences in the threshold for vasoconstriction during passive cooling for the

control trials. However, following whole-body heating, the onset of vasoconstriction occurred in the finger first (at a higher mean body temperature) followed by calf and forearm blood flow. Although these differences were not significant, it is possible that large reductions in finger blood flow occurred due to constriction of arteriovenous anastomoses (Hales *et al.*, 1978).

6.5 CONCLUSION

The current study showed that whole-body pre-cooling, followed by passive heating, reduced mean body temperature to below that of thermoneutral state and this caused a proportional shift in the vasodilatation threshold. The opposite pattern occurred during passive cooling after whole-body pre-heating, where the shivering threshold moved to a higher mean body temperature where the displacement was equal to the shift in pre-exposure mean body temperature. Both of these observations indicate that these thermoeffectors show some independence of the absolute body temperature, but appear to be coupled with the change in body temperature, and this is the first study to describe this pattern. A second significant outcome of the current study was the sudomotor threshold was shifted to a higher mean body temperature following whole-body pre-cooling and could indicate that the two thermoeffectors work independently in response to heat loss requirements, with sudomotor activation being initiated when vasodilatation fails to dissipate sufficient heat from the skin to the surrounding environment. However, this opposite response was not observed between the vasoconstriction and shivering thresholds. In addition, the data from these experiments allowed for determination of the interthreshold range for mean body temperature as observed in these trials. Finally, no regional differences in the thermoeffector thresholds between acral and non-acral skin regions were apparent during passive heating following whole-body cooling or during passive cooling following whole-body heating. The findings of the current experiment have not been described before and therefore should be explored in future investigations with the addition of other non-thermal factors, such as postural changes, exercise, hypoxia, and dehydration.

6.6 REFERENCES

Booth, J.D., Wilsmore, B.R., MacDonald, A.D., Zeyl, A., Storlien, L.H., and Taylor, N.A.S. (2004). Intramuscular temperatures during exercise in the heat following pre-cooling and pre-heating. *Journal of Thermal Biology*. 29: 709-715.

Brück, K., and Olschewski, H. (1987). Body temperature related factors diminishing the drive to exercise. *Canadian Journal of Physiology and Pharmacology.* 65(6):1274-1280.

Cabanac, M., and Massonnet, B. (1977). Thermoregulatory responses as a function of core temperatures in humans. *Journal of Physiology.* 265:587-596.

Cabanac, M. (2006). Adjustable set point: to honor Harold T. Hammel. *Journal of Applied Physiology.* 100:1338-1346.

Caldwell, J.N. Matsuda-Nakamura, M., and Taylor, N.A.S. (2014). Three dimensional interactions of mean body temperature and local skin temperature in the control of hand and foot blood flows. *European Journal of Applied Physiology.* DOI 10.1007/s00421-014-2894-x

Cheng, C., Matsukawa, T., Sessler, D.I., Ozaki, M., Kurz, A., Merrifield, B., Lin, H., and Olofsson, P. (1995). Increasing mean skin temperature linearly reduces the core-temperature thresholds for vasoconstriction and shivering in humans. *Anesthesiology.* 82:1160-1168.

Cheuvront, S.N., Bearden, S.E., Kenefick, R.W., Ely, B.R., DeGroot, D.W., Sawka, M.N., and Montain, S.J. (2009). A simple and valid method to determine thermoregulatory sweating threshold and sensitivity. *Journal of Applied Physiology*. 107:69-75.

Cotter, J.D., Patterson, M.J., and Taylor, N.A.S. (1997). Sweat distribution before and after repeated heat exposure. *European Journal of Applied Physiology.* 76: 181-186.

Darrow, C.W. (1934). The significance of skin resistance in the light of its relation to the amount of perspiration. *Journal of General Psychology*. 11:451-452.

Estañol, B., Corona, M.V., Elías, Y., Téllez-Zenteno, J.F., Infante, O., and García-Ramos, G. (2004). Sympathetic co-activation of skin blood vessels and sweat glands. *Clinical Autonomic Research.* 14:107-112.

Fox, R.H., and Edholm, O.G. (1963). Nervous control of the cutaneous circulation. *British Medical Bulletin.* 19:110-114.

Fox, R.H., and Hilton, S.M. (1958). Bradykinin formation in human skin as a factor in heat

vasodilation. *Journal of Physiology.* 142:219-232.

Grant, R.T., and Holling, H.E. (1938). Further observations on the vascular responses of the human limb to body warming: evidence for sympathetic vasodilator nerves in the normal subject. *Clinical Science.* 3:273-285.

Hales, J.R.S., Iriki, M., Tsuchiya, K., and Kozawa, E. (1978). Thermally-induced cutaneous sympathetic activity related to blood flow through capillaries and arteriovenous anastomoses. *Pflügers Archiv European Journal of Physiology.* 375:17-24.

Hammel, H.T. (1968). Regulation of internal body temperature. *Annual Review of Physiology.* 30:641-710.

Hardy, J.D., and DuBois, E.F. (1938). The technic of measuring radiation and convection. *Journal of Nutrition.* 15:461-475.

ISO 9886. (1992). *Evaluation of thermal strain by physiological measurements.* International Standard Organisation, Geneva.

Johnson, J.M., and Park, M.K. (1981). Effect of upright exercise on threshold for cutaneous vasodilation and sweating. *Journal of Applied Physiology: Respiration, Environmental and Exercise Physiology.* 50:814-818.

Johnson, J.M., Minson, C.T., and Kellogg, D. L. (2014). Cutaneous vasodilator and vasoconstrictor mechanisms in temperature regulation. *Comprehensive Physiology.* 4:33-89.

Kellogg, D.L., Liu, Y., McAllister, K., Friel, C., and Pérgola, P.E. (2002). Bradykinin does not mediate cutaneous active vasodilatation during heat stress in humans. *Journal of Applied Physiology.* 93:1215-1221.

Kenny, G.P., Chen, A.A., Nurbakhsh, B.A., Denis, P.M., Proulx, C.E., and Giesbrecht, G.G. (1998). Moderate exercise increases postexercise thresholds for vasoconstriction and shivering. *Journal of Applied Physiology.* 85:1357-1361.

Kenny, G.P., and Journeay, W.S. (2010). Human thermoregulation: separating thermal and nonthermal effects on heat loss. *Frontiers in Bioscience.* 15:259-290

Kenny, G.P., Gagnon, D., Shiff, DE., Armstrong, R., Journeay, W.S., and Kilby, D. (2010). Influence of nonthermal baroreceptor modulation of heat loss responses during uncompensable heat stress. *European Journal of Applied Physiology.* 108:541-548.

Kondo, N., Nishiyasu, T., Inoue, Y., and Koga, S. (2010). Non-thermal modification of heat-loss during exercise in humans. *European Journal of Applied Physiology.* 110:447-

458.

Lopez, M., Sessler, D., Walter, K., Emerick, T., and Ozaki, M. (1994). Rate and gender dependence of the sweating, vasoconstriction, and shivering thresholds in humans. *Anesthesiology.* 80:780-788.

Love, A.H. G., and Shanks, R.G. (1962. The relationship between the onset of sweating and vasodilatation in the forearm during body heating. *Journal of Physiology.* 162: 121-128.

McAllen, R.M., Tanaka, M., Ootsuka, Y, and McKinley, M.J. (2010). Multiple thermoregulatory effectors with independent central controls. *European Journal of Applied Physiology.* 109:27-33.

McCook, R.D., Wurster, R.D., and Randall, W.C. (1965). Sudomotor and vasomotor responses to changing environmental temperature. *Journal of Applied Physiology.* 20:371-378.

MacDonald, A.D., Groeller, H., Fogarty, A.L., Armstrong, K.A., Booth, J.D., Hahn, A., & Taylor, N.A.S. (2000). Exercise in the heat: cardiovascular consequences of whole-body and head-torso pre-cooling. *Abstracts of the Ninth International Conference on Environmental Ergonomics*. July 30th-August 4 th, 2000. Dortmund, Germany. P. 3.

Mack, G., Nishiyasu, T., and Shi, X. (1995). Baroreceptor modulation of cutaneous vasodilator and sudomotor responses to thermal stress in humans. *Journal of Physiology.* 483:537-547.

Marino, F., Booth, J. (1998). Whole body cooling by immersion in water at moderate temperatures. *Journal of Science and Medicine in Sport.* 1(2):72-81.

Matsukawa, T., Kurz, A., Sessler, D.I., Bjorksten, A.R., Merrifield, B., and Cheng, C. (1995). Propofol linearly reduces vasoconstriction and shivering thresholds. *Anesthesiology.* 82:1169-1180.

Mahmood, M.A., and Zweifler, R.M. (2007). Progress in shivering control. *Journal of Neurological Science.* 261:47-54.

Mekjavic, I., and Bligh, J. (1989). The core threshold for sweating. *Canadian Journal of Physiology and Pharmacology.* 67(9):1038-1044.

Mekjavic, I., and Eiken, O. (2006). Contributions of thermal and nonthermal factors to the regulation of body temperature in humans. *Journal of Applied Physiology.* 100:2065-

2072.

Mekjavic, I.B., and Rempel, M.E. (1990). Determination of esophageal probe insertion length based on standing and sitting height. *Journal of Applied Physiology.* 69:2376-379.

Mekjavic, I., Sundberg, C.J., and Linnarsson, D. (1991). Core temperature “null zone”. *Journal of Applied Physiology.* 71(4):1289-1295.

Machado-Moreira, C.A., Caldwell, J.N., Mekjavic, I.B., and Taylor, N.A.S. (2008). Sweat secretion from palmar and dorsal surfaces of the hands during passive and active heating. *Aviation, Space and Environmental Medicine.* 79:1034-1040.

Machado-Moreira, C.A., Barry, R.J., Vosselman, M.J., Ruest, R.M., and Taylor, N.A.S. (2014). Temporal and thermal variations in site-specific thermoregulatory sudomotor thresholds: precursor versus discharged sweat production. *Psychophysiology.* In print.

Nakajima, Y., Mizobe, T., Takamata, A., and Tanaka, Y. (2000). Baroreflex modulation of peripheral vasoconstriction during progressive hypothermia in anesthetized humans. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology.* 279: R1430-1436.

Nicolaou, G., Chen, A., Johnston, C., Kenny, G.P., and Bristow, G.K. (1997). Clonidine decreases vasoconstriction and shivering thresholds, without affecting the sweating threshold. *Canadian Journal of Anaestheology.* 44:636-642.

Olschewski, H., and Brück, K. (1988). Thermoregulatory, cardiovascular, and muscular factors related to exercise after precooling. *Journal of Applied Physiology.* 64(2):803-811.

Patterson, M.J., Stocks, J.M., & Taylor, N.A.S. (2004). Humid heat acclimation does not elicit a preferential sweat redistribution towards the limbs. *American Journal of Physiology*. 286: R512-R518.

Regan, J.M., Macfarlane, D.J., and Taylor, N.A.S. (1996). An evaluation of the role of skin temperature during heat adaptation. *Acta Physiologica Scandinavica.* 158:365-375.

Risch, W.D., Koubenec, H.J., Beckmann, U., Lange, S., and Gauer, O.H. (1978). The effect of graded immersion on heart volume, central nervous pressure, pulmonary blood distribution, and heart rate in man. *Pflüegers Archiv.* 374:115-118.

Saad, A.R., Stephens, D.P., Bennett, L.A., Charkoudian, N., Kosiba, W.A., and Johnson,

J.M. (2001). Influence of isometric exercise on blood flow and sweating in glabrous and nonglabrous human skin. *Journal of Applied Physiology*. 91:2487-2492.

Schmidt, V., and Brück, K. (1981). Effect of a precooling maneuver on body temperature and exercise performance. *Journal of Applied Physiology: respiratory, environmental, and exercise physiology*. 50(4):772-778.

Senay, L.C., Prokop, L.D., Cronau, L., and Hertzman, A.B. (1963). Relation of local skin temperature and local sweating to cutaneous blood flow. *Journal of Applied Physiology.* 18:781-785.

Shechtman, O., and Talan, M.I. (1994). Effect of exercise on cold tolerance and metabolic heat production in adult and aged C57BL/6J mice. *Journal of Applied Physiology.* 77:2214-2218.

Shido, O., Sugimoto, N., Tanabe, M., and Sakurada, S. (1999). Core temperature and sweating onset in humans acclimated to heat given at a fixed daily time. *American Journal of Physiology.* 26:R1095-R1101.

Taylor, N.A.S., Patterson, M.J., Cotter, J.D., and Macfarlane, D.J. (1997). Effects of artificially-induced anaemia on sudomotor and cutaneous blood flow responses to heat stress. *European Journal of Applied Physiology*. 76:380-386.

Taylor, N.A.S. (2014). Human heat adaptation. *Comprehensive Physiology.* 4:325-365.

Tattersall, G.J., and Milsom, W.K. (2009). Hypoxia reduces the hypothalamic thermogenic threshold and thermosensitivity. *Journal of Physiology.* 587:5259-5274.

Tikuisis, P., Bell, G., and Jacobs, I. (1991). Shivering onset, metabolic response, and convective heat transfer during cold exposure. *Journal of Applied Physiology.* 70:7996-2002.

Werner, J. (2008). Process- and controller- adaptations determine the physiological effects of cold acclimation. *European Journal of Applied Physiology.* 104:137-143.

Werner, J. (2010). System properties, feedback control and effector coordination of human temperature regulation. *European Journal of Applied Physiology.* 109:13-25.

Whitney, R.J. (1953). The measurement of volume changes in human limbs. *Journal of Physiology.* 121:1-27.

Wyss, C.R., Brengelmann, G.L., Johnson, J.M., Rowell, L.B., and Niederberger, M. (1974). Control of skin blood flow, sweating, and heart rate: role of skin vs core temperature. *Journal of Applied Physiology.* 36:726-733.

CHAPTER 7: CONCLUSION

7.1 CONCLUSION

The broad focus of the current research was on the interactions of vasomotion, sudomotion and thermogenesis, both separately and inter-dependently, across a wide range of environmental conditions. Firstly, we aimed to understand the physiological mechanisms associated with cold-water immersion and hence recommend appropriate emergency treatments for individuals with exercise-induced hyperthermia (Chapter 2 and 3). Secondly, we aimed to understand the interactions among central and peripheral thermoreceptor feedback, and direct thermal effects on skin blood flow in both acral and non-acral skin regions (Chapter 4 and 5). Finally, we investigated how thermoeffector activation was modified following deliberate deviations in the steady-state, pre-exposure mean body temperatures (Chapter 6).

The first phase of these investigations was designed to provide an understanding of the physiological mechanisms associated with cooling hyperthermic individuals using water immersion. This research was essential for understanding and optimising the physiological mechanisms involved in immersion cooling. By knowing the rate blood was flowing to the skin, we could determine how much heat could be extracted at different water temperatures. The rationale was that cold-water immersion would rapidly activate noxious cold receptors, thereby initiating a more powerful thermoefferent response and a greater reduction in cutaneous blood flow. In the first experiment we explored three cooling methods: air (20-22^{o}C); cold-water immersion (14^{o}C); temperate-water immersion (26^{o}C).

This experiment established, on the basis of oesophageal temperatures, that rapid and effective heat removal was achieved during a temperate-water immersion (26^{o}C) in moderately to profoundly hyperthermic, but asymptomatic individuals. There is little doubt that colder water immersions should elicit slightly faster cooling, but what was in doubt was the physiological and clinical significance of this difference. Since cooling during a life-threatening heat illness is aimed at rapidly reducing central nervous system temperature, then observations were based upon oesophageal temperature reductions

rather than upon rectal temperature, because the former provides a much closer approximation of the temperature of blood flowing to the brain (Taylor *et al*., 2014*a*; Whitby and Dunkin, 1971). Since the respective cooling times at these water temperatures were still <4 min and 6 min across every subject then one may question the need to use water cooler than 14ºC, or perhaps even 26ºC. These observations, whilst apparently not being previously described within the literature, have considerable practical significance. It was concluded that, for hyperthermic, but asymptomatic individuals, temperate-water immersion more than adequately facilitated brain cooling, due to the maintenance of a greater peripheral blood flow. It appears that, for heat exhausted and heatstroke patients, water any cooler than 14ºC may not be required. Thus, in a true emergency situation, one must immediately immerse the patient in the most readily available cool-temperate water, and then seek cooler water.

Although we hypothesised that these cooling rates were the result of more powerful vasoconstriction in 14ºC water, but not in 26ºC water, skin blood flow, was not measured in this study. Therefore, in the second experiment we investigated whether a more powerful vasoconstriction, as reflected by changes in cutaneous blood flow, was apparent during cold-water immersion. This was the first study to explore the physiological mechanisms associated with similar cooling rates previously observed for cooling hyperthermic individuals in cold (14ºC) and temperate (26ºC) water (Taylor *et al*., 2008). The results were consistent with more powerful vasoconstriction during cold-water immersion following hyperthermia, even though a larger thermal gradient existed. That study provided mechanistic evidence for choosing temperate water over cold water for rapid cooling, as it was likely that the dynamic activation of peripheral cold thermoreceptors provided a powerful vasoconstrictor drive that dominated, at least initially, vasodilatation drive activated by the warm deep tissue receptors. Thus, vasoconstriction reduced convective heat delivery from the core to the skin during the first few minutes of immersion and led to almost identical reductions in core temperature during the remainder of the immersion. Therefore, cooling hyperthermic individuals in temperate water proved to be just as effective as cooling in cold water, and this was due to more powerful vasoconstriction. In this experiment, forearm skin blood flow was measured using venous-occlusion plethysmography with a mercury-in-silastic strain-

gauge. However, this is only one of many methods currently used to measure cutaneous blood flow, and does not go without limitations. One particular limitation is the difficulty in measuring the influence of local skin temperature changes on cutaneous blood flow.

Therefore, to facilitate the measurement of blood flow through human limb segments, four water-filled plethysmographs (forearm, hand, calf and foot) were developed, and these also enabled the evaluation of local skin temperature influences (as determined by changing water temperature) on skin blood flow. The details of these were described throughout Chapter 4. For the first study, blood flow in the forearm was measured using both the newly developed water-filled and the strain-gauge plethysmographs in neutral and locally heated conditions to validate the former technique. This research highlighted not only the significance of measuring limb segment blood flow though venous-occlusion plethysmography, but also demonstrated that minimal variation exists between the strain-gauge and water-filled plethysmograph. While both of these methods accurately measured limb segment blood flow, venous-occlusion plethysmography using the water-filled plethysmograph was deemed the most suitable method for investigating changes in local skin temperature on skin blood flow of whole-limb segments. Having established this essential methodological capacity, in the following experiment we were able to utilise this technique to investigate the combined influences of core and skin temperature on skin blood flow for acral and non-acral skin regions.

The experiment in Chapter 5 provided information pertaining to the control mechanisms associated with vasomotor function and the interactions of central and peripheral control of skin blood flow. This experiment provided the first complete description of those interactions for both acral and non-acral skin regions across a range of thermal states. It was hypothesised that during each thermal state, increases in local skin temperature would elicit proportional increases in skin blood flow across all four skin regions. While a positive correlation was found between local skin temperature and skin blood flow across all thermal states, this study provided further supporting evidence that local skin temperature has little to no effect on skin blood flow across acral and non-acral skin regions within hypothermic individuals, due to the presence of very powerful,

centrally driven, vasoconstriction under these conditions. In contrast, the influence of local skin temperature became more pronounced in normothermic individuals. Whilst this was minimal in the foot, it was largest in the hand. When individuals were hyperthermic, the graded changes in local skin temperature augmented skin blood flow to a greater extent than during both the hypothermic and normothermic conditions across all sites, but this influence was now greatest in the foot. The fact that the hand and the foot experienced the greatest shifts in blood flow with released vasoconstrictor tone during heating, is believed to be the result of the presence of arteriovenous anastomoses within these regions, as the opening of the anastomoses will increase blood flow (Taylor *et al*., 2014b). Although this study provided the missing quantitative data across a range of thermal states, it did not broaden our understanding of the interactions of vasomotor function with sudomotion and thermogenesis.

Therefore, to more completely understand these complex interactions, the final phase of this research series was to investigate how altering the pre-exposure core temperature affects the threshold for which skin blood flow occurs, and its relationship to the sweating and shivering thresholds. This project (Chapter 6) was designed to evaluate the possibility that the magnitude of the change in body temperature might provide important feedback and is perhaps of greater importance than absolute body temperature in the activation of those thermoeffectors. This was achieved by inducing deliberate, yet precisely controlled displacements of body temperature prior to separate heating and cooling stimuli. The most significant finding from this study was that, following pre-treatment, the threshold for vasodilatation and shivering were shifted in equal proportions to the change in mean body temperature induced by each of the two pre-treatments, and this supported our hypotheses. Interestingly, the sudomotor threshold was shifted to a higher mean body temperature following whole-body pre-cooling, and this outcome could indicate that the two thermoeffectors work independently in response to heat loss requirements, with sudomotor activation being initiated only when vasodilatation fails to dissipate sufficient heat from the skin to the surrounding environment.

7.1.1 Concluding remarks

(i) For hyperthermic, but asymptomatic individuals, temperate-water (26^{o}C) immersion elicited rapid cooling of oesophageal temperature that did not differ significantly from the rate of cooling observed in cold-water (14^{o}C)

(ii) Further investigation into the physiological mechanisms underlying these cooling rates, revealed more powerful vasoconstriction that occurred during cold-water immersion following hyperthermia.

(iii) To investigate the relationship between core and local skin temperature, four water-filled plethysmographs were developed. These were validated against a mercury-in-silastic strain-gauge showing sufficient sensitivity for evaluating large ranges in local blood flow.

(iv) Little to no effect on skin blood flow across acral and non-acral skin regions within hypothermic individuals was apparent when local skin temperature was changed. However, the graded changes in local skin temperature augmented skin blood flow to a greater extent than observed during both the hypothermic and normothermic conditions.

(v) The hand and foot experienced the greatest shifts in blood flow during heating, and this indicates a release of vasoconstrictor tone. This is possibly the result of the presence of arteriovenous anastomoses within these regions.

(vi) Finally, our data demonstrated that the magnitude of the change in body temperature provided more important feedback than absolute body temperature in determining the thermoeffector thresholds. In addition, these observations are consistent with the possibility of these thermoeffectors to work independently.

7.2 RECOMMENDATIONS

While each of the experiments in this series of research has significantly contributed to the gaps in our understanding of the thermal interactions upon vasomotion,

sudomotion and thermogenesis, there are still areas which remain to be explored. For example, in the first phase of this research, we examined physiological mechanisms associated with rapidly cooling a hyperthermic individual in cold water. This was designed to gain an understanding of the interactions between statically activated central warm receptors, while simultaneously and rapidly activating cold-peripheral thermoreceptors. In this case and in cold water, thermoafferent input received from the hot stimulus was over-ridden by a more powerful vasoconstriction, as measured at the forearm. Although this provided useful information pertaining to the recommendations for cooling hyperthermic individuals, we assumed that the vasomotor response, at this site, was identical to peripheral blood flow in other regions in the body. However, we know that vasomotor function in acral regions is significantly different from non-acral regions, and this was demonstrated in Chapter 5. In addition, we do not know whether a similar thermoeffector response will occur when the opposite stimuli are applied. That is, it remains unclear whether during immediate warm-water immersion, following hypothermia, if rapid activation of warm thermoreceptors would elicit rapid increases in skin blood flow of a hypothermic individual. This information will not only provide recommendations for the treatment of hypothermia, but will also help us further understand the interactions between warm and cold thermoreceptors when statically or dynamically activated.

For this reason, a logical first study would be to further investigate the interactions between central and peripheral (mean-skin) temperature upon vasomotor function in acral and non-acral skin regions. In this experiment, subjects would be required for four trials. Each trial would involve two phases: a pre-treatment phase and an experimental phase. Two pre-treatments would be designed to either induce a hyperthermic or hypothermic state, where the experimental phase would include either cold- (14^{o}C), temperate- (26^{o}C) or hot- (39^{o}C) water immersion. To induce a hypothermic state, subjects would be passively cooled (climate chamber), until shivering was established, however for the hyperthermic trials, subjects would be heated using the protocol described in Chapters 2 and 3. Throughout the experimental phase of all trials, skin blood flow would be measured at the forearm, finger and calf using venous-occlusion plethysmography with a

mercury-in-silastic strain-gauge. The results of this study will help to develop a clearer understanding of the conditions that affect vasomotor functions when whole-body and mean skin temperature is rapidly changed.

Since the interactions of core and local skin temperature on skin blood flow in acral and non-acral skin regions were teased out in quite some detail in males (Chapter 5), it would seem logical to further investigate these interactions in females. This is of particular interest since gender differences appear to influence physiological responses, particularly during the luteal phase of the menstrual cycle. Gender differences exist in morphological configuration and sweat responses. Indeed, it would appear that women are better suited to vasomotor thermal adjustments, and are generally more reliant upon convective and conductive heat transfer than upon cooling through evaporation of sweat when compared to males (Thompson and Khalil, 2003).

The rationale behind this experiment is that women generally have lower skin temperatures during cool-air and thermoneutral exposures (Cunningham *et al*., 1978), which are sustained during exercise (Avellini *et al*., 1980). This is the opposite of that which is observed in the heat, with women generally showing higher skin temperatures (McArdle *et al*., 1992). These lower temperatures in the cold are most notable where subcutaneous adipose tissue provides greater tissue insulation from the conductive transfer of central heat (*i.e*. the trunk and proximal limb segments). In addition, some of this temperature difference can be attributed to a lower skin blood flow (Fox *et al*., 1969). Consequently, women display a greater resistance to cutaneous heat loss, and the skin-environment temperature gradient is generally smaller in the cold (Fox *et al*., 1969). These trends are also apparent at the hand. For example, Crooke *et al.* (1990) and Candas and Dufour (2007) have reported lower blood flow in the hands of women. These differences appear to be due to increased sympathetic tone under these conditions, rather than to local structural or functional differences in cutaneous circulation (Taylor *et al*., 2014*b*). It has also been shown that women exhibit a greater cutaneous vascular response within the acral surfaces of the hand following local cooling Cankar and Finderle (2003). Therefore, to further understand cutaneous circulation in females, the experimental

protocol from Chapter 5, using the water-filled plethysmographs described in Chapter 4, would be repeated, but this time in females.

Although the last phase of the current research explored the thermal influences that determine the thermoeffector thresholds, there are many non-thermal factors that modulate thermoeffector activation. Interestingly, understanding the determinants and interaction of these various non-thermal influences upon the thermoeffector thresholds remains largely unexplored. Although some studies have shown the thresholds to shift in response to alterations in these non-thermal stimuli (Barrera-Ramirez *et al*., 2014; Cheuvront *et al*., 2004; Golja *et al*., 2004; Kenny *et al*., 2010), little is known regarding the interactions of baroreceptor, osmoreceptor and chemoreceptor feedback on these thresholds.

Non-thermal factors may influence the control of these physiological mechanisms because the regulation of mean arterial pressure, plasma osmolality and blood gases share common effector pathways with the mechanisms that regulate body temperature (Hayward, 1977). For example, studies have shown that changes in plasma osmolality affect the rate and onset of sweating by altering the firing rate of central warm-sensitive neurons that contribute to the regulation of sweating (Silva & Boulant, 1984). In addition, increased plasma osmolality impairs sweat responses primarily by elevating the internal temperature threshold for the onset of sweating (Fortney *et al.,* 1984, Takamata, *et al.,* 1995; Shibasaki *et al.,* 2009). It is therefore quite conceivable that both central and peripheral interactions occur for these regulatory mechanisms. This was also demonstrated by Nakajima *et al*. (2000) who showed that baroreceptor loading promoted hypothermia as a result of a reduction in the vasoconstriction threshold. In this study, thermal afferent input to the hypothalamus was blocked through anaesthesia. By removing the thermal influence they were able to independently investigate the influence of baroreceptors upon vasomotor function. Furthermore, receptor feedback from the high- and low-pressure baroreceptors passes to the hypothalamus (Hori *et al.,* 1988). Finally, Tattersall and Milsom (2009) assessed hypothalamic thermosensitivity and the threshold temperature for metabolic heat production under hypoxic conditions. They measured the

integration of relevant ventilatory and cardiovascular parameters with thermoregulatory adjustments and determined that metabolic thermosensitivity was reduced when the hypothalamus was operating under hypoxic conditions.

To further investigate the influences of these non-thermal factors upon the vasomotor, sudomotor and thermogenic thresholds, three logical studies would follow. The purpose of the first study would be to investigate the separate effects of baroreceptor feedback upon the control of skin blood flow, sweating and shivering. While in the second study, we would investigate the combined interactions of thermoreceptor and baroreceptor feedback on vasomotor, sudomotor and shivering thresholds. Finally, and to more completely understand the thermal and non-thermal interactions on each of the thermoeffector thresholds, a study of the combined effects of baroreceptor, osmoreceptor and chemoreceptor function on vasomotor, sudomotor and shivering thermogenesis thresholds would be conducted. Although, both osmoreceptors and chemoreceptors have been shown to influence blood flow (Lynn *et al*., 2012; Fortney *et al.*, 1984, Aalkjaer and Poston, 1996), sweating (Fortney *et al.*, 1984) and shivering (Johnson and Proppe, 1996), the interaction of these functions on thermoeffector thresholds remains unexplored.

To investigate the interactions between thermal and non-thermal factors on the thermoeffector thresholds the first trial would be designed to explore the effects of baroreceptor modulation upon these thresholds. For this experiment, each subject would complete four trials of which lower-body positive and negative pressures would be used to load or unload the baroreceptors. The target positive and negative pressures would be +/-20 mmHg (low pressure receptors) and +/-50 mmHg (high pressure receptors). These values are based on the current literature (Mark and Mancia, 1983; Jackson and Kenny, 2003), however, pilot testing would confirm the necessary pressure targets. Each trial would differ in the pressure applied to the lower body of the subject, while core temperature would be driven up and down until the thermoeffector thresholds were established (as per the methods used in Chapter 6). In addition to modulating baroreceptor activation, in the second study we would now include shifting the pre-

exposure mean body temperature to above or below a normothermic state. For this experiment, subjects would be required for eight trials. Four trials would include whole-body pre-cooling, while the other four trials would include whole-body pre-heating, with either negative or positive pressure, targeting either the high or low baroreceptors. The final study would include a similar methodological design as studies one and two, but this time with the addition of manipulations to osmoreceptor (hyperosmolality, hyposmolality) and chemoreceptor (hypoxia, hypercapnia) function.

7.3 REFERENCES

Aalkjaer, C., and Poston, L. (1996). Effects of pH on vascular tension: which are the important mechanisms? [Review]. *Journal of Vascular Research*. 33(5):347-59.

Avellini, B.A., Kamon, E., and Krajewski, J.T. (1980). Physiological responses of physically fit men and women to acclimation to humid heat. *Journal of Applied Physiology: Respiration, Environmental and Exercise Physiology.* 49:254-261.

Barrera-Ramirez, J., McGinn, R., Carter, M.R., Franco-Lopez, H., and Kenny, G.P. (2014). Osmoreceptors do not exhibit a sex-dependent modulation of forearm skin blood flow and sweating. *Physiological Reports.* 2(2):1-13.

Candas, V., and Dufour, A. (2007a). Hand skin temperatures associated with local hand discomfort under whole-body cold exposure. *Journal of the Human-Environment. System*. 10:31-37.

Cankar, K., and Finderle, Z. (2003). Gender differences in cutaneous vascular and autonomic nervous response to local cooling. *Clinical Autonomic. Research.* 13:214-220.

Cheuvront, S.N., Carter III, R., Montain, S.J., Stephenson, L.A., and Sawka, M.N. (2004). Influence of hydration and airflow on thermoregulatory control in the heat. *Journal of Thermal Biology*. 29:471-477.

Crooke, J.P., Creager, M.A., Osmundson, P.J., and Shepherd, J.T. (1990). Sex differences in control of cutaneous blood flow. *Circulation.* 82:1607-1615.

Cunningham, D.J., Stolwijk, J.A.J., and Wenger, C.B. (1978). Comparative thermoregulatory responses of resting men and women. *Journal of Applied Physiology*. 45:908-915.

Fortney, S.M., Wenger, C.B., Bove, J.R., and Nadel, E.R. 1984. Effect of hyperosmolality on control of blood flow and sweating. *Journal of Applied Physiology.* 57(6) 1688-1695.

Fox, R.H., Lofstedt, B.E., Woodward, P.M., Friksson, F., and Werkstrom, B. (1969). Comparison of thermoregulatory functions in men and women. *Journal of Applied Physiology*. 26:444-453.

Golja, P., Kacin, A., Tipton, M.J., Eiken, O., and Mekajavic, I.B. (2004). Hypoxia increases the cutaneous threshold for the sensation of cold. *European Journal of*

Applied Physiology. 92:62-68.

Hayward, J.N. (1977). Functional and morphological aspects of hypothalamic neurons. *Physiological reviews.* 57:574-658.

Hori, T., Nakashima, T., Koga, H., Kiyohara, T., and Inoue, T. (1988). Convergence of thermal, osmotic and cardiovascular signals on preoptic and anterior hypothalamic neurons in the rat. *Brain Research Bulletin.* 20:879-885.

Jackson, D.N., and Kenny, G.P. (2003). Upright LBPP application attenuates elevated postexercise resting thresholds for cutaneous vasodilation and sweating. *Journal of Applied Physiology.* 95:121-128.

Johnson, J.M., and Proppe, D.W. (1996). Cardiovascular adjustments to heat stress. In: Fregley, M.J., and Blatteis, C.M. (Eds) *Handbook of Physiology.* Oxford University Press. New York. Pp. 215-243.

Kenny, G.P., Gagnon, D., Shiff, DE., Armstrong, R., Journeay, W.S., and Kilby, D. (2010). Influence of nonthermal baroreceptor modulation of heat loss responses during uncompensable heat stress. *European Journal of Applied Physiology.* 108:541-548.

Lynn, A.G., Gagnon, D., Binder, K., Boushel, R.C., and Kenny, G.P. (2012). Divergent roles of plasma osmolality and baroreflex on sweating and skin blood flow. *Journal of Physiology - Regulatory, Integrative and Comparative Physiology.* 302:R634-R642.

McArdle, W.D., Toner, M.M., Magel, J.R., Spina, R.J., and Pandolf, K.B. (1992). Thermal responses of men and women during cold-water immersion: influence of exercise intensity. *European Journal of Applied Physiology and Occupational Physiology.* 65:265-270.

Mark, A.L., and Mancia, G. (1983). Cardiopulmonary baroreflexes in humans. In: Shepherd, J.T., and Abboud, F.M. (Eds). *Handbook of Physiology, Section 2: The Cardiovascular System*. Bethesda, Md: American Physiological Society. Pp. 795-813.

Nakajima, Y., Mizobe, T., Takamata, A., and Tanaka, Y. (2000). Baroreflex modulation of peripheral vasoconstriction during preogressive hypothermia in anesthetized humans. *American Journal of Physiology-Regulatory Integrative and Comparative*

Physiology. 279: R1430-1436.

Shibasaki, M., Aoki, K., Morimoto, K., Johnson, J.M., and Takamata, A. (2009). Plasma hyperosmolality elevates the internal temperature threshold for active thermoregulatory vasodilation during heat stress in humans. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*. 297: R1706-R1712.

Silva, L.N., and Boulant, A.J. (1984). Effects of osmotic pressure, glucose and temperature on neurons in preoptic tissue slices. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 247:R335–R345

Takamata, A., Mack, G.W., Gillen, C.M., Jozsi, A.C., and Nadel, E.R. (1995). Osmoregulatory modulation of thermal sweating in humans: reflex effects of drinking. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*. 268: R414-422.

Tattersall, G.J., and Milsom, W.K. (2009). Hypoxia reduces the hypothalamic thermogenic threshold and thermosensitivity. *Journal of Physiology*. 587:5259-5274.

Taylor, N.A.S., Caldwell, J.N., van den Heuvel, A.M.J., Patterson, M.J. (2008). To cool, but not too cool: that is the question: immersion cooling for hyperthermia. *Medicine and Science in Sport and Exercise.* 40(11):1962-1969.

Taylor, N.A.S., Tipton, M.J., and Kenny, G.P. (2014*a*). Considerations for the measurement of deep-body, skin and mean body temperatures. *Journal of Thermal Biology*. Under review.

Taylor, N.A.S., Machado-Moreira, C.A., van den Heuvel, A.M.J., and Caldwell, J.N. (2014*b*). Hands and feet: Physiological insulators, radiators and evaporators. *European Journal of Applied Physiology*. In print.

Thompson, J., and Khalil, R.A. (2003). Gender differences in the regulation of vascular tone. *Clinical and Experimental Pharmacology and Physiology*. 30:1-2.

Whitby, J.D., and Dunkin, L.J. (1971). Cerebral, oesophageal and nasopharyngeal temperatures. *British Journal of Anaesthesia*. 43:673-676.

APPENDIX A: *Thermal sensation*

Thermal sensation was monitored using a modified version of the Gagge scale (Gagge *et al.*, 1967). The question: "*How does the temperature of your whole body feel?*":

13-point thermal sensation scale

1	Unbearably cold
2	Extremely cold
3	Very cold
4	Cold
5	Cool
6	Slightly cool
7	Neutral
8	Slightly warm
9	Warm
10	Hot
11	Very hot
12	Extremely hot
13	Unbearably hot

APPENDIX B: *Thermal discomfort*

Thermal discomfort was evaluated using another modified scale (Gagge *et al.*, 1967), and in response to the question: "*How comfortable does the temperature of your body feel?*".

The 5-point thermal discomfort scale

1.0 Comfortable

1.5

2.0 Slightly uncomfortable

2.5

3.0 Uncomfortable

3.5

4.0 Very uncomfortable

4.5

5.0 Extremely uncomfortable

APPENDIX C: *Rate of perceived exertion (effort sense)*

Perceived exertion was elevated using the 15 point Borg scale (Borg, 1962), and in response to the question: “How hard are you exercising?”

The 15-point Borg scale

6	
7	Very, very light
8	
9	Very light
10	
11	Fairly light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Very, very hard
20	

APPENDIX D: *Thermal pleasantness*

Thermal pleasantness was evaluated using another modified scale (Mower, 1976), and in response to the question: "*How pleasant does the temperature of your body feel?*".

The 9-point thermal pleasantness scale

1.0 Extremely unpleasant

1.5

2.0 Very unpleasant

2.5

3.0 Unpleasant

3.5

4.0 Slightly unpleasant

4.5

5.0 Neutral (indifferent)

5.5

6.0 Slightly pleasant

6.5

7.0 Pleasant

7.5

8.0 Very pleasant

8.5

9.0 Extremely pleasant

APPENDIX E: Dimensions of the four newly constructed plethysmographs

	Forearm	Hand	Calf	Foot
Inner compartment				
Length (cm)	15.0	15.0	20.5	21.5
Diameter (cm)	10.0	7.5/11.0	17.5	15.0
Circumference/ Perimeter (cm)	36.6	32.9	56.5	47.1
Volume (L)	1.4	1.1	5.0	7.4
Outer compartment				
Width (cm)	30.0	30.0	30.5	45.0
Height (cm)	30.0	30.0	30.5	45.0
Length (cm)	15.0	30.0	20.3	32.0
Volume (L)	13.5	27.0	18.8	64.8
Available volume (L)	11.7	25.9	13.8	57.4