Cutaneous sudomotor function in healthy individuals

ABIGAIL DOYLE

A thesis submitted in partial fulfilment of the requirements of Liverpool John Moores University for the degree of Master of Philosophy

October 2024

Abstract

Human perspiration or sweating is a vital physiological process that plays a crucial role in thermoregulation and maintaining homeostasis. Sweating is a critical response for losing heat in order to ensure appropriate maintenance of core body temperature. Sweating can also occur as part of the response to non-thermoregulatory events such as psychological stress. Sweating occurs via eccrine and apocrine sweat glands, which respond in different magnitudes to various stimuli, including heat, physical activity and mental stress. Eccrine glands, distributed widely across the skin, are primarily responsible for cooling the body through the secretion of a dilute saline solution, which evaporates and dissipates heat. In contrast, apocrine glands, located in specific areas such as the armpits and groin, mostly become active during mental stress and are associated with the production of a thicker, odoriferous secretion. The activation of these glands is regulated by the autonomic nervous system, with acetylcholine serving as the primary neurotransmitter, while adrenergic stimulation can also trigger sweating, especially in response to mental stimuli.

There are many situations and or conditions where sweating can be impaired or accentuated which can significantly affect quality of life. For example, hot flushes are the most common symptom of the female menopause; a milestone of every female's life typically between the ages of 45-55 years. Hot flushes are a sudden, intense sensation of heat causing skin reddening, flushing and profuse sweating. The mechanisms of a hot flush, especially as it pertains to the skin and the sweating that occurs, are unclear, however. Furthermore, sweating from apocrine glands can cause malodour and damage clothes. Personal hygiene products, such as antiperspirants, have become essential to combat body malodour and excessive axillae perspiration. Evaluating and analysing the effectiveness of these products is crucial for companies to ensure product quality. The knowledge of axillary sweating responses to various everyday activities such as

exercise, heat exposure and mental stress is scarce, however. Understanding the mechanisms and functions of sweating in these situations is essential for further understanding the pathophysiology and the development of strategies for hot flushes and apocrine sweating. The overall aim of this thesis is to therefore examine the control of cutaneous sudomotor responses in healthy individuals in response to common physiological conditions that affect this process. The specific objectives are 1). To develop a sound protocol, informed by information collected from the literature, to assess both function and structure of skin blood vessels and sweat glands in premenopausal and postmenopausal women both with and without hot flushes and 2). To examine the local axilla sweating responses to different forms of stress that trigger sweating in the axilla region in young healthy females. In order to achieve aim #1 a protocol was designed involving 3 distinct groups: postmenopausal women who experience hot flushes, postmenopausal women who do not experience hot flushes, and a young, healthy premenopausal group. The structure and function of skin blood vessels and sweat glands of these 3 groups will be assessed using the microdialysis and skin punch biopsy methods. Visit 1 involves the evaluation of post-ganglionic skin blood vessel and sweat gland responsiveness using transdermal/cutaneous microdialysis for the intradermal administration of pharmacological agents to assess skin blood flow and sweating Dose-response curves for skin blood flow and sweating will be created, and EC50 values will be estimated (increasing doses of acetylcholine, sodium nitroprusside, or CGRP). For Visit 2, a skin punch biopsy will be performed from the same site to assess the structure of skin blood vessels and sweat glands. Samples will be sectioned, stained for blood vessels and sweat glands, will be analysed using confocal microscopy to evaluate the number and size of blood vessels and sweat gland secretory coils. .By comparing the three groups, the study hopes to identify any differences in cutaneous structure and function that may contribute to our understanding of how menopause, and the presence or absence of hot flushes, affects skin physiology.

In order to achieve aim #2 the sweating responses of female participants across three conditions; exercise, passive heating, and mental stress were investigated. Visit 1 involved a 2-hour exercise protocol at room temperature (~18°C, ~40% rh), Visit 2 consisted of passive heat exposure in a heated room (~40°C, ~30% rh), and Visit 3 involved a battery of mental stress tests with cognitive challenges. 30 females took part in the three trials (27±7 years, 63±8 kg, 161±7 cm). Two participants were postmenopausal, and two were using oral contraceptives. Local axillary sweating responses were measured using two methods. The SweatSENSE method involved placing small paper sensors on the left and right axilla for 5 seconds, with the colour change analyzed to index sweating activity. Continuous sweating was also measured from the left axilla using capacitance hygrometry. Significant main effects of time and condition ($P < 0.01$) were observed, along with significant time × condition interactions ($P < 0.01$), indicating distinct sweating profiles across conditions. The results indicated that sweating was highest during the exercise condition, as demonstrated by both capacitance hygrometry data and Sweatsense measurements followed by passive heating then emotional stress. Exercise elicited the most pronounced and intermittent sweating response, while passive heating and mental stress produced more gradual and consistent increases. A significant main effect of condition was observed ($P < 0.001$), indicating overall differences in core temperature across trials. Core temperature was highest during the exercise condition (37.3 °C), followed by emotional stress (37.0 °C) and passive heating (36.8 °C). While core temperature increased over time ($P = 0.006$), there was no significant time × condition interaction ($P = 0.124$). Skin temperature was highest during passive heating (34.5°C followed by emotional stress (32.1°C) then the exercise trial (31.6°C). Skin temperature remained elevated and stable during passive heating. In contrast, emotional stress induced an initial rise followed by a gradual decline across sites, while exercise led to variable responses, with increases at the thigh and calf, but decreases or plateaus at the

forearm and axilla." By comparing the sweating responses across these conditions, the study provided valuable data that could contribute to the development of more effective antiperspirants and enhance the understanding of the physiological mechanisms underlying sweating.

In summary, this thesis investigated the physiological mechanisms of sweating, focusing on the structural and functional properties of skin blood vessels and sweat glands in premenopausal and postmenopausal women, with and without hot flushes, and examines local axillary sweating responses across different stressors. Using advanced methods, including microdialysis, skin punch biopsies, the SweatSENSE technique, and capacitance hygrometry, the findings deepened our understanding of sweating mechanisms and help to inform the development of more effective antiperspirants, while also shedding light on the relationship between menopause and sweating.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

The protocol chapter is currently under review in Frontiers in Physiology and the study is still ongoing.

Table of Contents

Chapter 1. Background/Literature Review

1.1. Thermoregulation 11

1.2. The cardiovascular system 12

1.2.1. Microvasculature 13

1.3. The skin and skin blood flow 14

1.4. Sweating and sweat glands. 17

1.4.1 Eccrine Sweat Glands 20

1.4.2 Apocrine Sweat Glands 21

1.4.3 Apoeccrine Sweat Glands 21

1.4.4 Function of Eccrine vs. Apocrine Sweat Glands 22

1.4.5. Sweating responses to common daily exposures: Exercise vs. passive heating vs. mental stress 23

1.5 Assessment of Sweating 26

1.5.1. Sweat gland function. 26

1.5.2. Sweat gland structure 30

1.6 Female Vascular Health 31

1.6.1. Female Reproductive Hormones 31

1.6.2. Menopause 33

1.6.2.1. Hot Flushes 35

1.6.2.2 The physiology of a hot flush 36

1.6.2.3 Mechanisms contributing to menopausal hot flushes. 37

1.7 Objective and Aims of Thesis. 39

Chapter 2. 40

The development of a protocol to assess both function and structure of skin blood vessels and sweat glands in premenopausal and postmenopausal women both with and without hot flushes 40

2.1. Introduction 41

2.2. Methods 44

2.2.1. Experimental Design 44

2.2.2. Sampling 44

2.2.3. Study Procedures 46

2.2.4. Outcome Measures 51

2.2.5. Data Analyses.....52
2.2.6. Research governance and monitoring.....52
2.2.7. Public involvement.....54
2.2.8. Dissemination.....54
Chapter 3.....55
3.1. Introduction.....56
3.2. Methods.....59
3.2.1. Participants.....59
3.2.2. Design.....59
3.2.3. Procedures.....59
3.2.4. Instrumentation.....61
3.4. Discussion.....72
Chapter 4......78
Synthesis of Findings.....78
4.1 Aims and Objectives.....79
4.2. The development of a sound protocol, informed by information collected from the literature, to assess both function and structure of skin blood vessels and sweat glands in premenopausal and postmenopausal women both with and without hot flushes.....80
5. References.....85

Chapter 1.

Background/Literature Review

Chapter 1. *Background/Literature Review*

The purpose of this literature review is to describe and discuss the control of sweating in healthy individuals and to describe the effect of common physiological conditions that affect this process; specifically, the menopausal hot flush and common scenarios that cause sweating; exercise, passive heat stress exposure and emotional/mental stress.

1.1. Thermoregulation

Humans have extensive abilities to adjust their thermoregulatory activities when responding to changes not only in endogenous (exercise) conditions, but also exogenous (environmental) conditions (Kondo et al., 2009). It is important for the human body to regulate core body temperature, maintaining it at approximately 37ºC. An increase in core body temperature can be a consequence of exercise, clothing and/or exposure to a hot environment (Casa et al., 2019) and can lead to heat exhaustion, which may be life-threatening if cooling measures are not implemented (Barker et al., 2019). The regulation of internal body temperature is achieved through mechanisms of heat generation and conservation (metabolism, shivering, and cutaneous vasoconstriction) and heat loss (cutaneous vasodilation and sweating). These processes can be responses to both internal and external stimuli. The central control of thermoregulation occurs via temperature-sensitive and temperature-insensitive neurons within the preoptic/anterior hypothalamus (PO/AH) of the brain (Boulant, 2000, Boulant, 1996; Charkoudian and Stachenfeld, 2014, Boulant, 2006) and is operated via a closed loop negative feedback system through the autonomic nervous system (see Figure 1.1). Changes within the internal and external environment trigger thermal receptors at the skin surface, as well as in the spine and viscera, and will initiate a cascade of events to help maintain thermal homeostasis. For example, afferent information pertaining to heat gain from the thermal receptors at the skin surface (e.g., an increase in skin temperature) will induce appropriate efferent responses of cutaneous vasodilation and sweating that trigger heat loss and peripheral cooling (Boulant., 2006, Charkoudian and

Stachenfeld, 2014). The main effectors of autonomic thermoregulatory responses to heat gain are the cutaneous blood vessels and sweat glands of the skin.

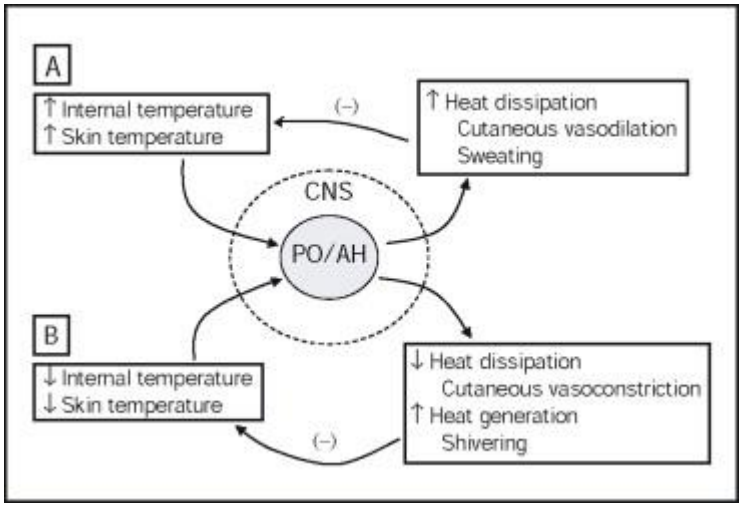

Figure 1.1. A summary of neural thermoregulatory control (Charkoudian, 2003).

1.2. The cardiovascular system

The cardiovascular system is a multifaceted network of organs and vessels, each playing critical roles in maintaining overall health within the body. Comprised of the heart, vasculature, and blood, the system performs multiple vital functions, such as transportation, homeostasis and protection. The cardiovascular system creates a fundamental delivery network for cellular nutrition and removal of metabolic waste products, gaseous exchange and hormonal transport, alongside being a key regulator of homeostasis for body temperature, pH balance, hydration and blood pressure (Smith et al., 2023). The cardiovascular system works intimately with other major systems, including the respiratory, neural, endocrine, digestive, skeletal, urinary and integumentary systems. The vascular tree, in particular the microvasculature, is paramount when contributing to a complex transport network of vessels to provide these systems with a site to enable exchange of macromolecules and fluids, along with acknowledging various mechanical forces and chemical signals concerned with homeostasis and protection (Hightower et al., 2011).

1.2.1. Microvasculature

The microvasculature includes the smallest resistance vessels (<150μm in diameter), playing a fundamental role in the maintenance of tissue health and function through a variety of mechanisms. It consists of a vast array of vessel types organised in a hierarchical pattern, including terminal arterioles, capillaries, and venules (Levy et al.,2001; Roustit et al., 2013), overall facilitating an efficient blood flow throughout tissues. These vessels are composed of multiple cell types and are intricately connected with the tissue environment. One of the primary functions of the microvasculature is to regulate blood flow, ensuring that tissues receive an adequate supply of oxygen and nutrients while removing waste products. This perfusion is essential for tissue viability and function. Additionally, the microvasculature serves as a conduit for communication between tissues, facilitating the exchange of signalling molecules involved in homeostasis, immune responses, and reparative processes. Its structural and functional adaptability allows it to meet the dynamic demands of different tissues under various physiological conditions. Overall, the microvasculature is an essential component of tissue biology, playing a vital role in maintaining tissue health, facilitating communication between cells and tissues, and supporting various physiological processes necessary for organismal homeostasis (Beare et al., 2018). Pre-capillary resistance vessels meet the local metabolic demands via the interplay between neural signalling, chemical mediators, and vascular smooth muscle function overall regulating blood blow based on metabolic demand. The tunica media comprises the vascular smooth muscle cells which can be seen within the vessel wall and play a crucial role in response to signalling molecules, contracting or relaxing when appropriate, to regulate vessel diameter and blood flow. They also work intimately with the endothelial monolayer (lining the inner surface of blood vessels) which is supplied by autonomic nerves and also responds to local chemical mediators, including nitric oxide (NO) (Woodward et al., 2018).

Optimal microvascular function is crucial for the body's organ systems to work efficiently, including the eyes, kidneys, heart, muscle, adipose tissue and the skin. The cutaneous microvasculature has received significant attention in previous research due to its accessibility and value in many physiological processes. It comprises of a specific vascular network divided into two primary stations within the superficial and deep layers of the skin known as the dermis (Johnson et al., 2011). These vessels run parallel to the surface of the skin and contain most blood vessels within the microvasculature, playing a fundamental role in functions including thermoregulation, nutrient exchange and immune responses (Deuflhard et al., 2004). Noradrenergic vasoconstrictor and cholinergic vasodilator mechanisms alongside endothelial release of NO use dual sympathetic neural control to mostly govern the non-glabrous skin of the limbs, head and trunk (Cable et al., 2006; Johnson et al., 2014).

1.3. The skin and skin blood flow

The skin plays a significant role in human thermoregulation. Within the skin exists arterioles and venules of the cutaneous microcirculation, which hold physiological importance and can be found in the dermis plexus in both superficial and deep layers of the skin (Kellogg., 2006). Most microvasculature is located within the superficial papillary dermis, approximately 1-2mm beneath the epidermal surface (see Figure 1.2), and contains high resistance terminal arterioles, papillary loops (also known as true capillaries), and post capillary venules.

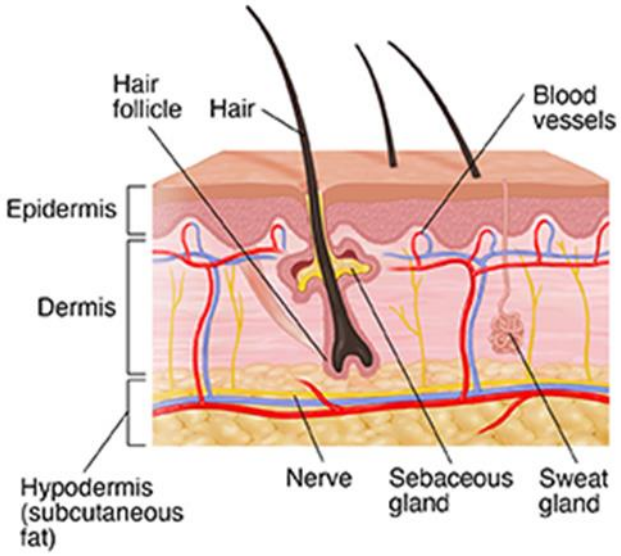

Figure 1.2. Cross sectional diagram depicting the skin (cutaneous membrane) and subcutaneous layer; comprising of a superficial, thin epidermis and a deep, thicker dermis layer. Deep within the skin is the subcutaneous layer, attaching the dermis to the beneath organs and tissues. Smooth muscle, nerves and sweat glands are found in the dermis layer. (Lawton., 2019)

The papillary loops are situated near the dermal-epidermal junction, where they contribute to a significant thermal gradient because of their extensive surface area and abundant blood flow, playing a crucial role in regulating heat exchange (Rowell., 1974; Charkoudian and Stachenfeld., 2014). Blood circulation in the papillary loop is regulated by extensively innervated arterioles composed of endothelial cells surrounded by two layers of vascular smooth muscle cells. The upper and lower plexuses are situated in both glabrous/non-hairy (plantar feet, palms, and lips) and non-glabrous/hairy skin (limbs, torso, and head), with arteriovenous anastomoses (AVAs) mainly present in glabrous skin (Johnson et al., 1986; Roddie., 2003). AVA's receive significant innervation from sympathetic adrenergic fibres (vasoconstrictor nerves) that help regulate blood flow through the AVAs. When sympathetic nerve activity has risen within the AVAs during mild/moderate heat stress, this causes vasoconstrictor tone to decline, resulting in an increase in skin blood flow. A temporary reduction in sympathetic activity within the AVAs also contribute to cold-induced vasodilation during prolonged severe cold stress (Roddie, 2003).

When skin/core temperature drops, or if skin temperature falls below core temperature, a thermoregulatory reflex-driven response is initiated, which causes a decrease in skin blood flow to ultimately conserve body heat. This response is achieved through a heightened arteriolar vasoconstrictor tone consequently leading to arteriolar vasoconstriction, and hence reduced skin blood flow. Conversely, during situations of heat exposure (where skin temperature surpasses core temperature) or during exercise-induced elevation in body temperature, vasoconstrictor activity becomes limited, allowing active vasodilator tone to act on the cutaneous arterioles. This results in cutaneous vasodilation, followed by an increase in sweat rate to help combat heat loss and skin cooling (Johnson et al., 2014).

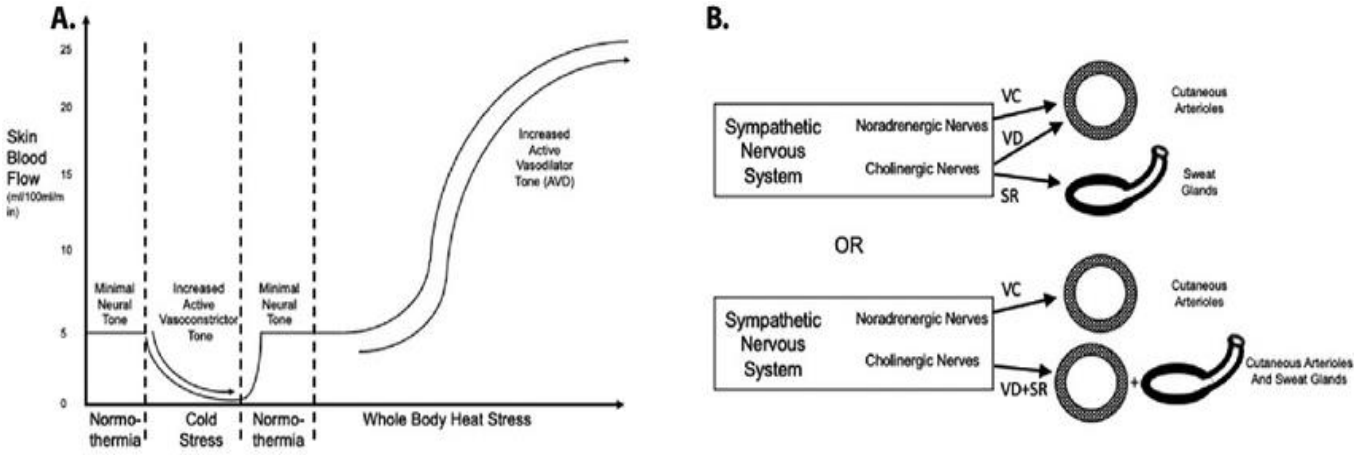

Figure 1.3. A) Variation in skin blood flow during cold stress, normal temperature, and heat stress. (B) Two possible neural control anatomical configurations for active vasodilation (VD), vasoconstriction (VC), and sudomotor responses (SR). (Trbovich et al., 2022).

Throughout the duration of whole-body heat stress, there is a gradual augmentation in skin blood flow, which is most closely associated with core body temperature (Taylor and Bishop, 1993) (Figure 1.3). On the other hand, skin temperature can influence the point at which skin blood flow starts to rise exponentially in response to internal temperature (Figure 1.4) as well as the magnitude of the elevation in skin blood flow (Brengelmann et al., 1981, Farrell and Bishop, 1995).

Subsequently, both reflex (neural) and local (non-neural) effectors play a role in modulating changes in skin blood flow during environmental stress.

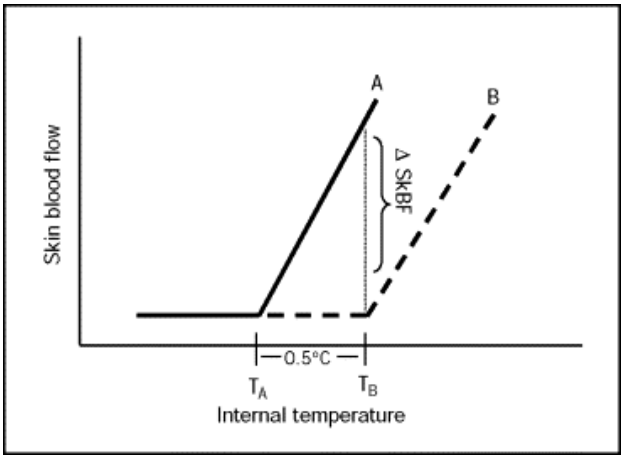

Figure 1.4. Schematic representations of skin blood flow responses during whole-body heating. TA and TB indicate internal temperature thresholds, which are 0.5°C apart, corresponding to responses A and B, respectively. The skin temperature of response A is higher than B and thus lowers the internal temperature threshold. The slope of each line beyond the threshold illustrates the sensitivity of the vasodilatory response. The vertical line is included to highlight the significant variations in skin blood flow at a specific internal temperature, despite the seemingly minor difference in threshold. Note: SkBF = change in skin blood flow (Charkoudian, 2003).

1.4. Sweating and sweat glands.

Sweating plays a crucial role in facilitating heat loss and occurs through millions of sweat glands situated all within the body's skin. Similar to the control of skin blood flow, sweating is regulated by a closed loop negative feedback system via the central nervous (CNS) and autonomic nervous (ANS) systems. In order to increase heat loss sympathetic cholinergic effector nerves release the neurotransmitter acetylcholine (ACh) that binds to muscarinic receptors on sweat glands to initiate sweating and subsequent evaporation of heat from the skin's surface (Yu et al., 2018) (Figure 1.5). Sweating can also be modified via non-neural or local factors such as changes in skin temperature (see Figure 1.6) and or other variables (e.g., Nitric Oxide, CGRP).

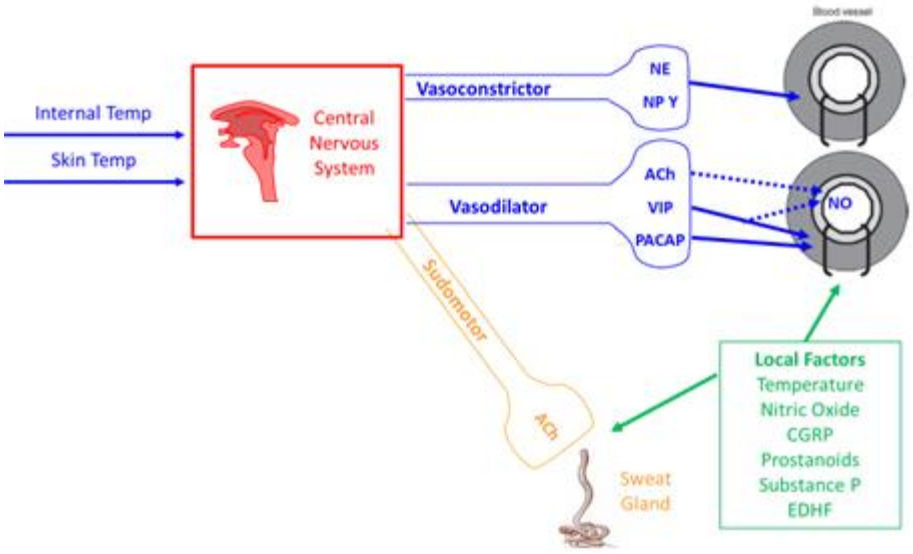

Figure 1.5. A schematic illustrating the proposed mechanisms of neural control over skin blood flow involves both active vasodilator and vasoconstrictor nerves. Changes in sweat rate are mediated by the function of the sudomotor nerves. Both the sudomotor and active vasodilator nerves are sympathetic cholinergic nerves that release acetylcholine (Ach) along with co-transmitters, likely including vasoactive intestinal peptide (VIP) and calcitonin gene-related peptide (CGRP). Acetylcholine serves as the primary neurotransmitter responsible for sweating. (Charkoudian., 2010)

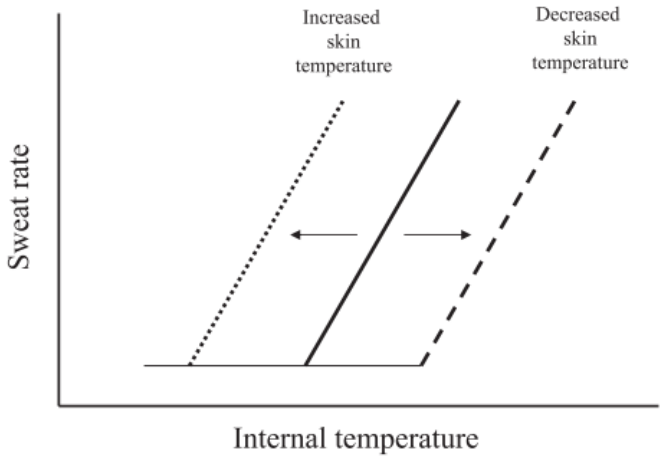

Figure 1.6. Impact of skin temperature on the relationship between sweat rate and internal temperature. At first, as internal temperature rises, sweating does not take place. However, once a certain threshold is exceeded, the increase in sweat rate aligns with the rise in internal temperature, and this threshold can be influenced by mean skin temperature (Nadel et al, 1971).

Whilst sweat glands typically respond to acetylcholine released by sympathetic cholinergic nerves, they can also be activated by sympathetic stimuli through adrenergic nerves and or the release of the hormone adrenaline. The sympathetic nerves that supply sweat glands contain a high number of cholinergic terminals but only a few adrenergic terminals (Uno, 1977). When an individual experiences emotional or mental stress, the body's fight-or-flight response is activated. This results in the release of adrenaline and other stress hormones, which can directly stimulate sweat glands (Chu et al., 2024). This type of sweating is often referred to as 'emotional sweating' and is more common in areas like the palms, forehead and the axillae. In summary, while sweating is mainly associated with heat regulation, it can also be triggered by psychological factors, showcasing the body's complex response to various stimuli.

Sweat glands can be subdivided into three groups; eccrine, apocrine and apoeccrine (Sato, 1983; see Figure 1.7). The eccrine sweat glands are the most abundant type of sweat glands and cover a significant portion of the body's surface area, including the limbs, torso, and face, producing the largest volume of sweat (Sato, 1983). In contrast, apocrine and apoeccrine glands generate less sweat due to their specific locations on the body, such as in the armpits and groin (Costill, 1997; Sato et al., 1991).

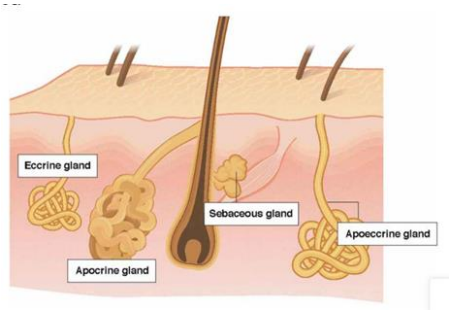

Figure 1.7. A diagram comparing the three different types of sweat glands. (Baker, 2019).

1.4.1 Eccrine Sweat Glands

The eccrine sweat gland, recognised as the first discovered type of sweat gland, is the smallest yet most prevalent gland type (Weiner and Hellman, 1960). The human body contains about 2 to 4 million eccrine sweat glands, located on glabrous (hairless) and non-glabrous skin (Kuno 1956, 1938). The distribution of these glands is uneven across the body, with the highest density found on the palms and soles, ranging from about 250 to 550 glands per square centimetre (Taylor and Machado-Moreira, 2013). A smaller number of eccrine glands are present on non-glabrous skin, but they are distributed over a larger surface area (Taylor and Machado-Moreira, 2013).

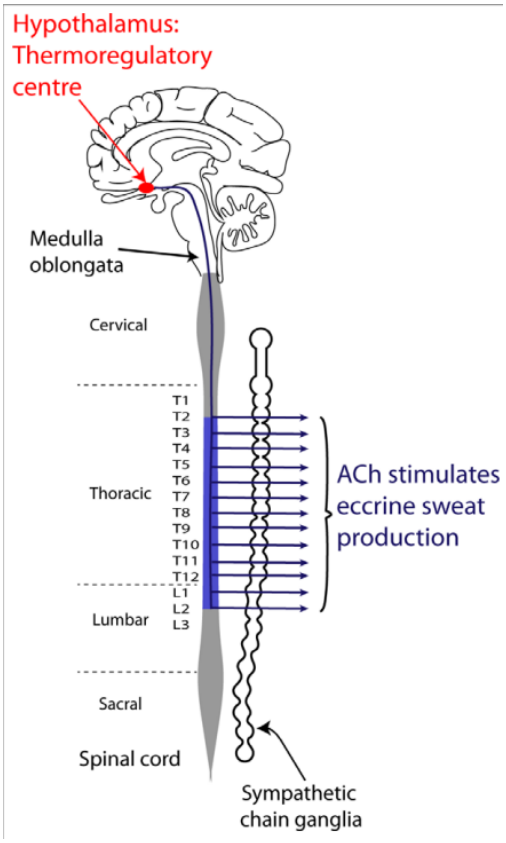

Figure 1.8. Signalling pathway for the thermoregulatory centre to activate secretion from the eccrine sweat gland. The efferent nerves travel from the thermoregulatory centre through the medulla oblongata to the spinal cord, and then synapse in the sympathetic ganglia. Upon stimulation, the postganglionic sympathetic nerves release acetylcholine (ACh), which initiates sweat secretion from the eccrine glands (Yu et al., 2018).

The eccrine glands become operational at an early stage in life, and from around 2-3 years old, the overall number of eccrine glands remains constant (Weiner and Hellman, 1960). Consequently, the density of sweat glands decreases as the skin expands during growth from infancy, generally showing an inverse relationship with the body's surface area. This leads to children having higher sweat gland densities compared to adults (Montagna and Parakkal, 1974), while larger or more obese individuals tend to have lower sweat gland densities than their smaller or leaner individuals (Bar-Or et al., 1968). It is important to note that a higher sweat gland density does not necessarily equate to a higher sweating rate. The variability in regional and whole-body sweating rate among individuals is primarily attributed to variations in sweat secretion rate per gland, rather than the total number of active sweat glands (Sato et al., 1985; Sato and Dobson, 1970). Eccrine sweat primarily consists of water and sodium chloride (NaCl), along with a blend of various chemicals sourced from the interstitial fluid and the eccrine gland.

1.4.2 Apocrine Sweat Glands

Apocrine sweat glands are in the underarms, groin, breasts, face and scalp (Robertshaw, 1983). Unlike eccrine glands, apocrine glands are larger and open into hair follicles instead of directly onto the skin's surface (Weiner and Hellman, 1960). Although being present since birth, the secretory function of apocrine glands does not start until puberty (Sato et al., 1987). These glands release a thick, lipid-rich sweat that contains proteins, sugars, and ammonia. In various species, apocrine glands are considered scent glands that generate pheromones, contributing to body odour. While the intricacies of apocrine glands are not completely understood, research has indicated that apocrine sweat glands react to both cholinergic and adrenergic stimuli (Sato et al., 1987).

1.4.3 Apoeccrine Sweat Glands

Apoeccrine glands develop from eccrine sweat glands during the ages of 8 to 14, eventually making up to 45% of the total axillary glands by the late teenage years (Sato et al., 1987). These

glands are intermediate in size and exhibit features of both eccrine and apocrine glands. Confined to the axillary area, apoeccrine glands release sweat directly onto the skin's surface through a distal duct, similar to eccrine glands. The secretion from these glands consists of a substantial amount of saltwater solution, resembling eccrine sweat (Sato et al., 1987). While the exact purpose of this type of sweating is not fully understood, it is unlikely to play a significant role in thermoregulation due to its ineffective evaporation in the underarm region. Knowledge about apoeccrine glands is still limited, but in vitro studies indicate that they may be more responsive to cholinergic stimuli than to adrenergic stimuli (Sato et al., 1987).

1.4.4 Function of Eccrine vs. Apocrine Sweat Glands

Eccrine sweat glands are important in the thermoregulatory response to exposure to hot environments and exercise. They secrete larger volumes of sweat, which contains fewer organic substances in contrast to apocrine glands. Eccrine sweat glands on the palmar and plantar surfaces have a lower rate of secretion compared to that of the remainder of the body's surface area however, meaning profuse sweating is unlikely in this area. Profuse sweating may become apparent over the general body surface area, with sweat not being very concentrated and resulting in a sweat rate of 1-2L/hr in some cases (Kuno, 1965).

There are obvious physiological variances between apocrine and eccrine glands along with the contrasting methods of sudomotor transmission. Once stimulated, the secretory coil of eccrine sweat glands releases a plasma-like precursor ultrafiltrate. The fluid undergoes changes as it travels through the sweat duct due to the active reabsorption of chloride and sodium. This process results in the formation of a hypotonic sweat solution that gathers in the lumen of the sweat gland prior to emerging on the skin's surface.

Apocrine sweat glands are present at birth; however they don’t start functioning until puberty, likely due to hormonal changes at that time (Chamberlain et al., 1999). They are associated with hair follicles in the groin and axillary region. The sweat is a viscous, protein-rich product that is

initially odourless but may develop an odour after exposure to bacteria (Hodge et al., 2022). Apocrine sweat glands receive adrenergic sympathetic innervation as well as hormonal stimulation because they respond to noradrenaline and adrenaline and they are involved in emotional sweating due to stress, fear, and pain (Hodge et al., 2022).

Although the role of sweating in thermoregulation is well recognised, many people believe that it also fulfils several other significant homeostatic functions that are not related to temperature control. For instance, sweat glands are thought to function as an excretory system, like the kidneys, by removing excess micronutrients, metabolic waste, and toxins from the body (Allan and Wilson, 1971; Robertshaw, 1977). This idea can result in individuals participating in certain activities like prolonged sauna sessions or exercising in extreme conditions to induce heavy sweating for purported health benefits. However, the efficacy of sweat glands as excretory organs for maintaining homeostasis remains uncertain due to the lack of comprehensive reviews on this subject.

1.4.5. Sweating responses to common daily exposures: Exercise vs. passive heating vs. mental stress

Many kinds of stress cause several reactions in humans, such as sweating, increases in hormones, change of behaviour and feelings etc (Kamei et al., 1997; Kita et al., 1997). Human sweating is primarily triggered by thermal stimuli (e.g., exercise or passive heat exposure) and plays a crucial role in maintaining a stable body temperature. Sweating can also be caused by psychological stimuli as well. Thermally-induced perspiration is typically regulated by the autonomic nervous system, while sweating resulting from mental or emotional stress is managed by the sympathetic nervous system. Consequently, sweat gland activity is directly controlled by the nervous system, making it a reliable indicator of bodily responses to surprise, mental stress, and similar experiences (Kamei et al., 1997).

During physical activity, contracting muscles release a significant amount of heat as a metabolic byproduct, leading to an increase in body heat. This rise in body heat content and subsequent elevations in predominantly central thermoreceptors is relayed to the preoptic hypothalamic region of the brain, which then initiates sweating and cutaneous vasodilation to help dissipate heat (Gleeson, 1998; Sawka and Wenger, 1988; Boulant, 1981). The evaporation of sweat is the main method of heat loss during exercise. As sweat forms, heat is transferred from the body to the water (sweat) on the skin's surface. Once this water absorbs enough heat, it transforms into water vapor, effectively removing heat from the body (with 580 kcal of heat lost for every 1 kg of sweat evaporated) (Wenger, 1972; Sawka et al., 2011).

When ambient temperature exceeds skin temperature, as is often the case during passive heat exposure, heat is transferred from the surrounding air to the body via radiation. Previous research has shown great similarity in thermoregulatory responses to passive heating and active heating via muscular exercise (Nielsen and Nielsen, 1965). During stationary passive heating, sweating is present as a physiological response, even when no physical activity is involved (Gonzalez-Alonso and Calbet, 2003). This process typically unfolds as skin temperature increases because of heat exposure, which is detected by the thermoreceptors in the skin and also the central nervous system. The preoptic area of the hypothalamus processes this information and triggers the body’s thermoregulatory responses (Maughan and Burke, 2012); the sweat glands begin producing sweat via the activation of sympathetic cholinergic nerves that release acetylcholine, stimulating the sweat glands to secrete sweat onto the skin's surface for evaporation. With continued exposure to heat, and attendant further elevations in core temperature the body will continue to increase sweat production, enhancing its ability to regulate temperature (Gonzalez-Alonso & Calber, 2003; Sawka et al., 1999).

Sweat production can also occur as a reaction to mental stress (Hu et al., 2018) without changes in body temperature (Harrison and MacKinnon,1996; Kobayshi et al., 2003). Mental impulses

such as mental arithmetic has shown to cause sweating (Ohhashi,1993), and the stress reaction induced by recall of words is also shown to induce sweating (Ohhashi, 1993; Tulen et al., 1989). Non-thermal (psychological) sweating was previously thought to be limited to the glabrous skin of the hands and feet (Kuno, 1956; Darrow, 1937). It was even believed to be induced by distinct efferent pathways (List and Peet, 1938; Chalmers and Keele, 1952; Iwase et al., 1997) characterised by a noradrenergic phenotype (Robertshaw, 1977; Noppen et al., 1997; Nakazato et al., 2004). However, recent studies have demonstrated that psychological sweating can occur throughout the entire body (Machado-Moreira and Taylor, 2012) but it is most noticeable on the hands, feet, face (all via eccrine sweat glands) and in the axillary region (via apocrine sweat glands; see Figure 1.9; Wilke et al., 2007).

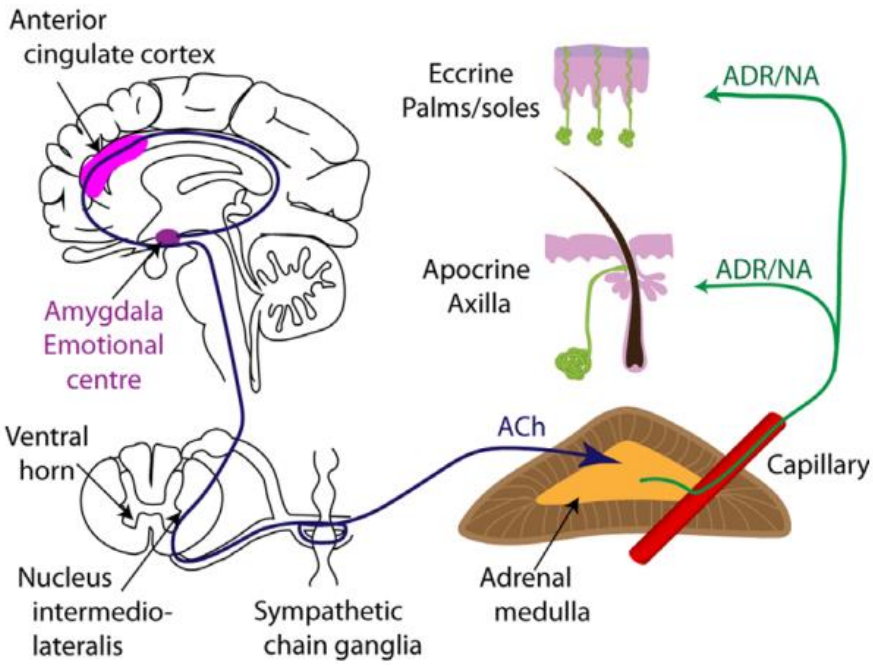

Figure 1.9. The pathway for sweating responses to emotional changes involves the central nervous system, specifically the amygdala, cingulate cortex, and medulla oblongata. These areas communicate through efferent fibres that descend through the ventral horn of the spinal cord and connect to sympathetic neurons in the nucleus intermediolateral. Sympathetic stimulation triggers the release of adrenaline (ADR) and noradrenaline (NA) from the adrenal medulla, facilitating sweat secretion from both eccrine and apocrine glands (Kritikos et al., 2021)

1.5 Assessment of Sweating

1.5.1. Sweat gland function.

Techniques for accurately measuring perspiration from the human body have been in use since as early as 1614, when Sanctorius estimated insensible perspiration by weighing subjects on a balance sensitive to 0.1 gram (Sanctorus, 1614, cited in Kuno, 1956). This method is still utilized for measuring total insensible weight loss. Currently, there are various techniques available for measuring both overall and localised sweat rates. The primary methods employed in regional sweat rate research are:

- Sweat droplet analysis
- Ventilated or unventilated capsules
- Absorbents placed inside capsules
- Absorbents applied directly to the skin

In recent decades, the predominant method in sweat research has been the use of capsules (Hertzman, 1957; Van Beaumont and Bullard, 1963; Ogawa and Bullard, 1972; Sawka et al., 1984). These capsules come in various types, including ventilated, unventilated, or absorbent, and are all attached securely to the skin during testing. The ventilated sweat capsule, known as the hygrometry technique, involves pumping dry air at a consistent flow rate through a capsule attached to the skin.

The determination of local sweat rate on the skin surface beneath the capsule (approximately 1–20 cm^2) is calculated based on the changes in temperature and water vapor content between the effluent and influent air of the capsule (Brengelmann et al., 1975; Graichen et al., 1982). The ventilated capsule technique, known for its high reliability (with a coefficient of variation of 2%) (Kenefick et al., 2012), is considered the standard method for measuring local sweat rate (Morris et al., 2013). This allows a precise measure of sweat rate and enables the continuous

measurement over time. However, it is important to note that hygrometry may sometimes overestimate sweat flow rates due to the controlled ventilation and maintenance of dry skin (which promotes sweating) under the capsule, which may not accurately reflect real-world conditions such as the microclimate under clothing or during exercise in humid or still air.

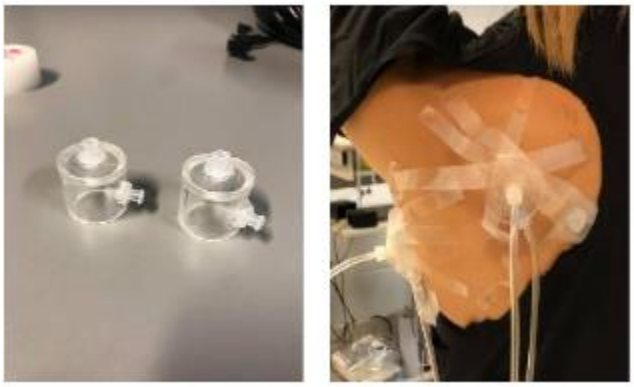

Figure 1.10. An example of acrylic capsules fixed to the skin using tape/topical skin glue that are typically used for the ventilated capsule technique of determining local sweat rate.

The technique for assessing regional sweat loss using an unventilated capsule measures the weight variation of a desiccant contained within it (Nilsson, 1977). Initially, hygroscopic salts were employed, and the water content was quantified through gravimetric analysis (Robens et al., 2004). Presently, the incorporation of absorbent materials within a sealed capsule has become the preferred approach for unventilated systems. The absorbent capsule method relies on a material that captures sweat produced on the skin's surface inside the capsule, with the weight change over time serving as the basis for calculating the sweat rate.

The drawback of employing any of the capsule techniques is the disruption of the microclimate surrounding the skin surface, which directly affects the evaporation process (Nilsson, 1977; Smith., 2009). This interference can lead to either increased humidity (with absorbents) or decreased humidity (with capsules). The primary disadvantage of using absorbents is the risk of

moisture becoming trapped on the skin's surface. This trapped moisture can impede evaporation and potentially lead to hidromeiosis, resulting in a reduction in regional sweating (Smith, 2009). Furthermore, capsule methods can be challenging to use in experiments involving intense physical activity, primarily due to the tubing needed in ventilated techniques. Moreover, glued capsules cannot be swiftly attached and removed, which may be necessary in certain situations.

Gravimetric sweat assessment techniques involve the direct collection of sweat from the skin surface (approximately 4–100 cm^2) using various materials such as filter paper (Ohara, 1968), absorbent patches (Dziedzic et al., 2014; Havenith et al., 2008), Parafilm-M® pouches (Brisson et al., 1991; Boisvert et al., 1993), cotton gloves/socks (Smith & Havenith, 2012), latex gloves, or plastic sweat collectors (Cole & Boucher, 1986). These methods determine Local Sweat Rate based on the mass change of the collection system. Gravimetric techniques, especially absorbent patches, are more practical than hygrometry for testing athletes' sweat in the field. However, a limitation of gravimetry is that the collection system can alter the local environment, affecting the sweat flow rate onto the skin surface. The lack of ventilation due to an occlusive covering can lead to increased moisture accumulation on the skin, potentially blocking sweat ducts and suppressing sweat (hidromeiosis). To mitigate hidromeiosis, it has been suggested to limit the duration of the collection system on the skin and/or use patches made of highly absorbent materials (Taylor & Machado-Moreira, 2013; Havenith et al., 2008).

A recently developed method for quantifying local sweating is the SweatSENSE method. The SweatSENSE technique is a low-cost method developed to assess local axilla sweating (Hand et al., 2024). SweatSENSE is a sweat-responsive, polydiacetylene-based chemosensor on a paper substrate that undergoes a selective blue to pink/red colourimetric change when in contact with acids and alcoholic compounds found in sweat. SweatSENSE is non-hydrochromic, in that it does not respond to water, and provides an instantaneous point measure of localised sweat rate. SweatSENSE is prepared via inkjet printing, resulting in a 5 x 5 cm patch. These sensors are

applied to the body test site for 5 seconds before being removed, and the colour change in response to sweat can be immediately observed upon removal of the sensor from the test site. Once removed, this reaction is fixed and irreversible. SweatSENSE has the ability to demonstrate an individual's sweating behaviour during real-life scenarios and activities, in real time and without the need for expensive, highly-specialised equipment.

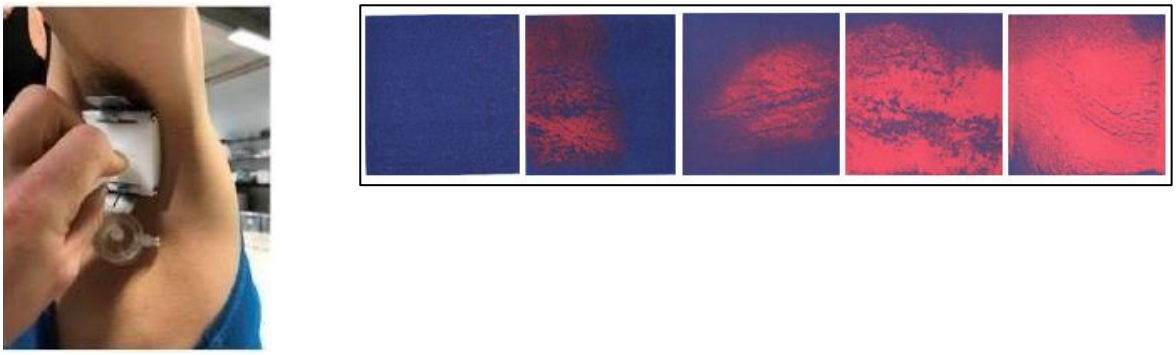

Figure 1.12. Representative SweatSENSE sensors, demonstrating the colour response in the presence of sweat at different % Total Sweat Coverage levels, from left to right 0%, 24%, 31%, 68% and 89%. SweatSENSE is a highly-sensitive, novel sweat-responsive non-hydrochronic polymeric PCDA paper-based sensor that measures localised sweating instantaneously. This is then analysed using a bespoke application to produce the % Total Sweat Coverage output.

Measuring axilla sweat rate is crucial for gaining a deeper understanding of apocrine glands and assessing the efficacy of antiperspirants. By quantifying sweat production in the axilla region, researchers and clinicians can gather valuable insights into the functioning of apocrine glands, which play a significant role in body odour and certain skin conditions. Additionally, monitoring sweat rate can help evaluate the effectiveness of antiperspirant products in reducing sweat production and managing hyperhidrosis. This data is essential for developing targeted treatments and improving overall skincare practices.

1.5.2. Sweat gland structure

The structure of a sweat gland has been well described. Methods to easily and readily assess the morphology of a sweat glad are rare however due to the size and position of the sweat glands. The skin punch biopsy technique (see Figure 1.13) can be used to assess the structure of sweat glands (as well as microvessels). This method is typically used as a diagnostic clinical investigation of skin vessels, sweat glands, and nerves that uses minimal invasiveness (Lauria et al., 2007) and international consensus guidelines have been established (Lauria et al., 2010; Schindelin et al., 2015). Typically, a very small biopsy sample is taken from the skin surface using a punch biopsy tool. Once sampled, the sample is treated and cryo-protected prior to being frozen and samples are subsequently stained to mark key structures (Walker, 1985) with a purpose to interrogate these key sites.

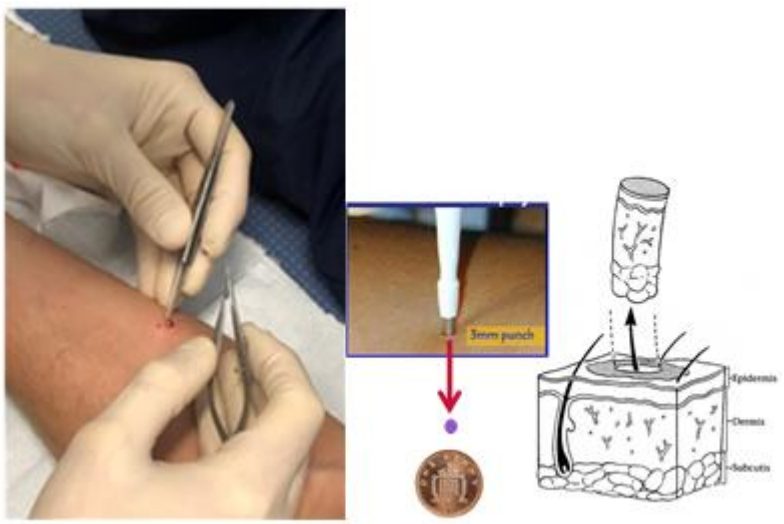

Figure 1.13. Example of the skin punch biopsy technique, and a size comparison of the sample taken.

Confocal microscopic imaging of the samples is then conducted and analysed (Figure 1.14) (Schindelin et al., 2015) to define the number and size of the key structures, e.g., blood vessels, size of the secretory coils of the sweat glands and nerve fibres around the sweat gland.

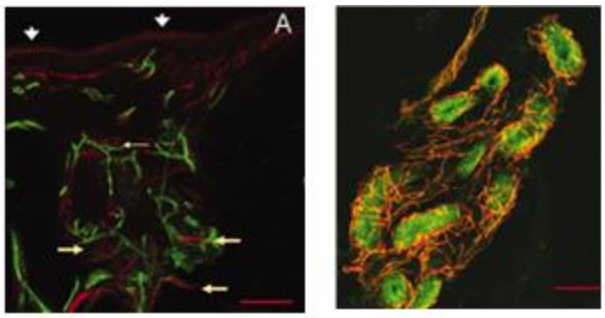

Figure 1.14 Example of confocal images of the skin blood vessels (green in left panel; skin surface is at the top of the image) and sweat glands (green in right panel) from the skin biopsy samples.

1.6 Female Vascular Health

1.6.1. Female Reproductive Hormones

Females have a reproductive lifespan of approximately 36 years, until they reach menopause (Park et al., 2002; Figure 1.15). The female hormonal lifespan consists of three milestones: puberty, pregnancy and menopause. The average menstrual cycle of a young, healthy fertile woman is considered to be 28 days; however, this is individual to every woman and anything between 21 and 40 days is deemed normal. Menstrual cycle can be classified into different stages (menstruation, follicular phase, ovulation and luteal phase) consisting of a coactive interaction between the hypothalamus, pituitary gland, ovaries and uterus to begin repetitive hormonal changes which are important for human reproduction. The phase of the cycle is orchestrated by the triggering and inhibition of follicle stimulating hormone (FSH), luteinising hormone (LH), oestrogen and progesterone.

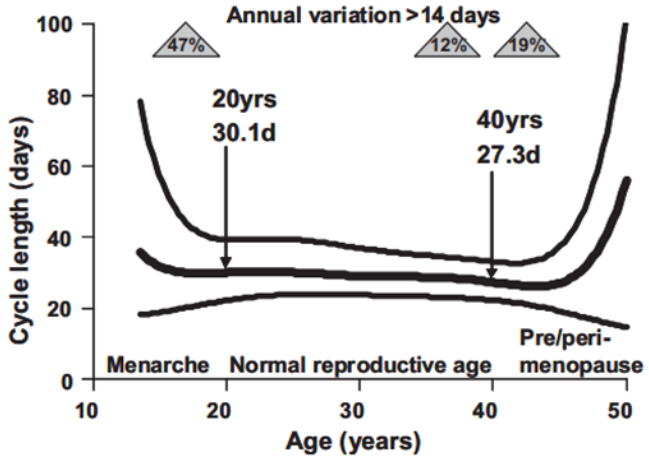

Figure 1.15. The difference in the duration of a woman's menstrual cycle in relation to their age. The above graph shows the average cycle length and range shown in 4 studies portrayed by Harlow (2000). Yrs.= years, d=days. Triangles imply the age group in which the indicated percentage of women experience more than 14 days variation in cycle length per annum.

The cycle begins at the age of menarche and stops when a female reaches menopause. Gonadotropin (a hypothalamic releasing factor) triggers the release of FSH and LH from the anterior pituitary which produces the growth of a dominant ovarian follicle. Progesterone and oestrogen are released from the ovary because of rising levels of FSH an LH. Throughout the follicular phase, oestrogen is released from the ovarian follicles which inhibits the creation of LH until a threshold is met and a later peak in LH is secreted to get the body ready for ovulation. The luteal phase is when FSH and LH cause the main follicle to move into the corpus luteum, releasing progesterone and subsequently triggering the making of oestrogen. FSH and LH production is inhibited by rising levels of oestrogen, this results in the corpus luteum to revert due to dropping levels of progesterone- causing menstruation to commence (Dawson and Reilly, 2009; Figure 1.16). When observing all the hormones that participate in the organisation of menstrual function, oestrogen is the primary hormone (Mendelshohn and Karas, 1999).

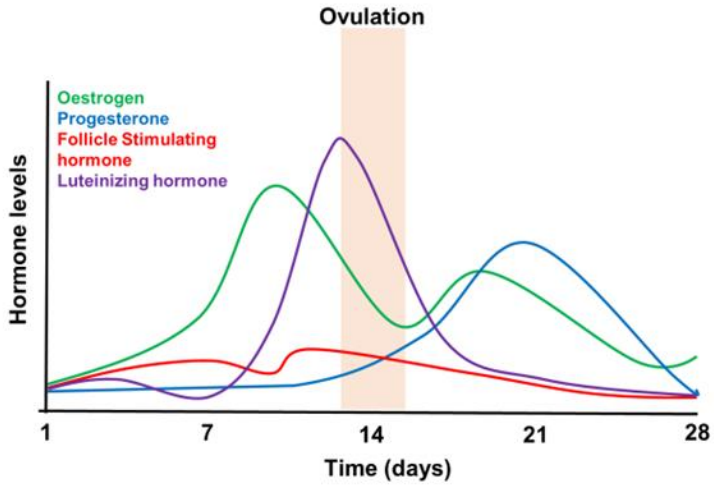

Figure 1.16. Representation of hormonal fluctuations throughout the menstrual cycle (Reed and Carr., 2015).

1.6.2. Menopause

Throughout a woman's life sudden and acute changes in sex hormones occur in relation to their menstrual cycles. Chronic changes will appear once a female has reached the onset of menopause. Menopause is a significant, physiological life event, typically between the ages of 45-55, and is defined as the ceasing of regular menstruation and reproductive capability (WHO,1996) when oestrogen production is diminished, impacting a female's organs and quality of life (Freeman et al., 2007). Perimenopause is the transition to menopause; this can last up to 8 years, and this is when menstruation becomes infrequent because of the ovarian response to changing hormone levels (Roberts and Hickey, 2016). Menopause can begin to display itself as a woman finding difficulty in navigating the complexities of her daily life, where their usual day to day activities, i.e., career responsibilities, family dynamics, and personal aspirations, become significantly affected (George et al., 2002). Women will also notice irregular periods, hot flushes, mood swings and shifts in mood including confusion, anxiety, depression and fatigue (Menopause, 2010). Menopause itself is the point at which a woman has not had a menstrual

period for 12 months and is associated with lower hormone levels, as opposed to fluctuating levels experienced during perimenopause.

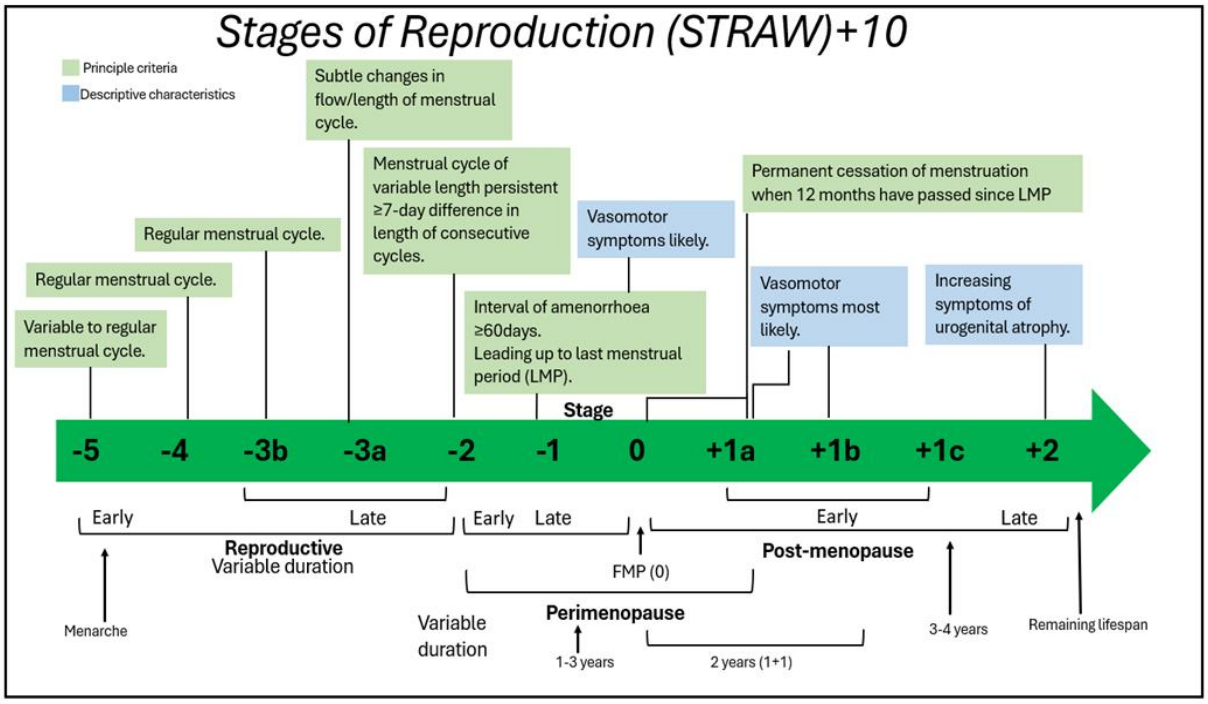

Figure 1.17 A summary of the stages of reproductive ageing (Harlow et al., 2012).

The chronic changes that occur within a female's reproductive hormones during menopause have a huge impact on the individual's health creating stress on many systems, including the cardiovascular system (Taddei, et al., 1996, Moreau, 2012), ultimately increasing the risk of developing cardiovascular disease through a variety of mechanisms (Eaker, 1993). Oestrogen is one of the primary female reproductive hormones that has a high impact on various functions of the body, including cardiovascular function (Hellsten and Gilemann, 2018). Endogenous oestrogen released from the ovaries improves endothelial function through the secretion of the vasodilator NO (Holm and Nilsson., 2013), helping arterial vasodilation, vascular tone, detection of free radicals and obstructing vasoconstrictors. These outcomes result in the prevention of vascular dysfunction (Chackrabarti et al., 2014., Forstermann and Sessa., 2012). The termination

of oestrogen production can also decrease levels of HDL cholesterol (Mandrup et al., 2017), increase body fat and diastolic blood pressure (Nyberg et al., 2014).

1.6.2.1. Hot Flushes

The major pathophysiological symptom of the female menopause is the hot flush, which is experienced by approximately 80% of women for up to 5 years (Thurston et al., 2011). Hot flushes (HFs) are a thermoregulatory event characterised by a sudden and instant increase in SkBF and an enhanced core body temperature, consequently leading to skin flushing and profuse sweating (Low et al., 2008). As there is currently an incomplete understanding of the mechanisms underlying the hot flush there are limited therapies that are suitable for all, therefore resulting in many women who find it difficult to cope with the debilitating symptoms of HFs.

Vascular (dys)function can possibly be influenced by hot flush severity (Mendelsohn and Karas, 1999; Bechlioulis et al., 2010; Bechlioulis et al., 2012; Sassarini et al., 2012; Sassarini et al., 2014). Postmenopausal women display a reduction in flow mediated dilation (FMD) (an indicator of large artery nitric oxide function) in comparison to males and premenopausal females (Thurston et al, 2008; Moreau et al, 2012) and have higher levels of vascular inflammatory markers suggestive of vascular dysfunction compared to premenopausal females (Nyberg et al., 2014). In addition, Bechlioulis et al. (2010) concluded women who experience more intense hot flushes displayed a significantly lower FMD compared to menopausal women with none to mild hot flushes. It has been mentioned women with severe hot flushes may have negatively altered vascular regulatory mechanisms (Najjar et al., 2005) or an increased sympathetic tone (Ganong, 2001). Additionally, more severe and prolonged vasomotor symptoms are linked to an 18% increased risk of type 2 diabetes mellitus (T2DM) (Gray et al., 2018). Collectively, the occurrence of hot flushes may serve as a significant and independent indicator of health.

1.6.2.2 The physiology of a hot flush

Research indicates that during a hot flush, there is an increase in skin blood flow by 80%, a rise in sweating by 500%, and a decrease in cerebral blood flow by 5% (Sloan et al., 2001; Low et al., 2008; Lucas et al., 2013). Previous research using techniques such as laser Doppler flowmetry (LDF) and capacitance hygrometry to assess physiological hot flushes has demonstrated that increases in skin temperature and blood flow occur before sweating begins (See Figure 1.18) (Low et al., 2008; Hubing et al., 2010; Low et al., 2011; Lucas et al., 2013; Bailey et al., 2016a). Blood is redistributed from central to peripheral regions, which can result in significant increases in systemic vascular conductance, increases in heart rate and decreases in blood pressure (Crandall et al., 2008; Low et al., 2008).

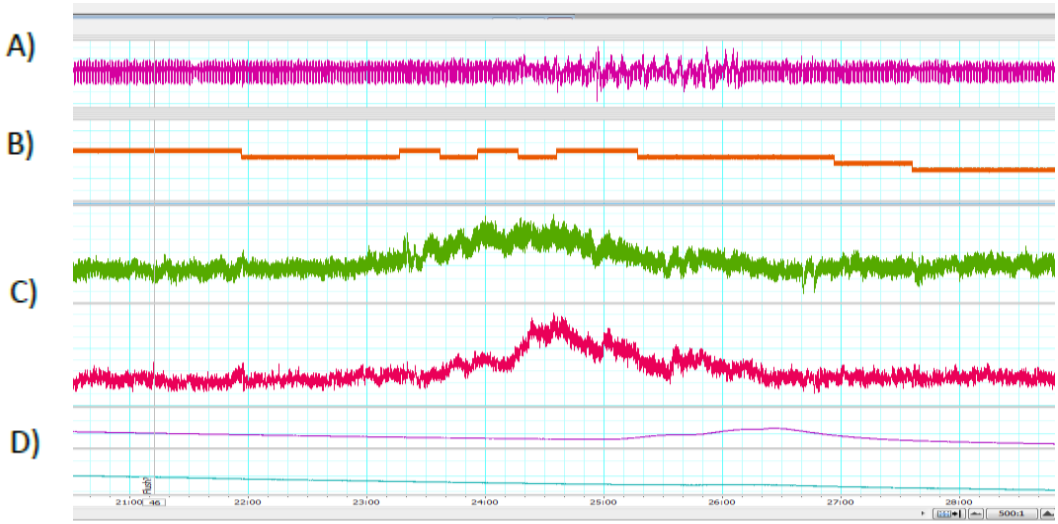

Figure 1.18. An illustration of the thermoregulatory response during a hot flush: A) the pink trace represents ECG, B) the orange trace indicates core temperature, C) the green and red traces correspond to skin blood flow in the chest and forearm, respectively, and D) the purple and blue traces depict the sweat rate in the chest and forearm, respectively.

1.6.2.3 Mechanisms contributing to menopausal hot flushes.

The physiological mechanisms underpinning the menopausal hot flush remain unclear. One potential explanation for a hot flush is a rise in core body temperature (Freedman and Woodward, 1996; Freedman, 2014). Research has shown a rise in core body temperature of 0.03-0.05°C occurred in the 30 minutes leading up to a hot flush in 65% of cases among 8 symptomatic postmenopausal women (Freedman and Krell, 1999). Subsequent research indicated that core body temperature alternates by 0.035°C over a 24-hour period between symptomatic and asymptomatic postmenopausal women, and importantly, these fluctuations do not stimulate hot flushes (Freedman, 2002). Alternative mechanisms have been suggested, including variances in brain neurotransmitters, such as an increase in hypothalamic neurokinin B (NKB) during menopause. An intravenous infusion of hypothalamic NKB led to hot flushes in 8 out of 10 women, as measured by sternal skin conductance (Jayasena et al., 2015). Additionally, changes in the sympathetic nervous system may be linked to hot flushes. A study by Low et al. (2011) discovered that the increases in skin blood flow and cutaneous vascular conductance (CVC) during a hot flush are obstructed by botulinum toxin A (BTX), which prevents the secretion of neurotransmitters from sympathetic cholinergic nerves. Notably, even without an increase in core body temperature, heightened efferent sympathetic nerve activity to the skin was observed both prior to and during a hot flush (Low et al., 2011), likely affecting changes in sweating and skin blood flow. Furthermore, functional magnetic resonance imaging (fMRI) revealed increased activity in the brainstem before the onset of a hot flush, followed by significant activation in the insula and prefrontal cortex (Diwadkar et al., 2013). This indicates that the rise in skin blood flow and sweating may be moderated by neural mechanisms rather than being solely ‘local’ or non-neural.

The main event of a hot flush is the skin flushing and sweating which occurs at the skin. The skin is made up of an extensive complex of neural, vascular and morphological structures, subsequently playing an important role in thermoregulation (Sassarini, et al., 2015) and a number

of interconnected steps are involved with the blood vessels and sweat glands for the ultimate responses of vasodilation and sweating. For example, vasodilatory neurotransmitters and local substances bind to receptors on the skin's blood vessels and sweat glands, leading to vasodilation and the release of sweat (Johnson et al., 2014; Shibasaki et al., 2006). It remains unclear whether there are differences in the function and structure of skin blood vessels and sweat glands between postmenopausal women who experience hot flushes and those who do not, as well as between pre- and postmenopausal women with varying oestrogen levels.

Oestrogen deficiency leads to a decrease in nitric oxide and CGRP levels (Chow et al., 2015; Gangula et al., 2000), potentially impacting the sensitivity of receptors for these substances on the skin blood vessels in postmenopausal women. Postmenopausal women who experience hot flushes exhibit heightened skin blood flow responses to vasodilators, affecting both the endothelium and smooth muscle (Iliodromiti et al., 2019; Sassarini et al., 2012). Consequently, the skin blood vessels of symptomatic postmenopausal women may be excessively responsive to vasoactive substances that contribute to hot flushes. The impact of oestrogen withdrawal on sweat gland receptors and sweat gland function remains unknown. It has not yet been investigated whether menopause results in alterations in the sensitivity of sweat glands, potentially leading to more frequent and intense sweating in postmenopausal women.

The skin undergoes various morphological changes during the aging process, which are accelerated during menopause due to hypo-oestrogenism. These changes include reductions in collagen content, elasticity, water content, and thickness (Reus et al., 2020). It is unclear whether hypo-oestrogenism affects crucial vascular, sudomotor, and neural structures in the skin that also contribute to hot flushes. The number of sweat glands generally remains constant throughout a person's lifespan, but they are sensitive to repeated or lack of stimuli, resulting in a decrease in number and density due to decreased use (Abdel-Rahmen et al., 1992). Similarly, the number of skin blood vessels can decrease with aging (Gunin et al., 2014) and are also sensitive to repeated

or lack of stimuli (Green et al., 2017), leading to a decrease in both number and size when there are no recurring elevations in blood flow. Currently, it is unknown whether the structure of skin blood vessels and/or sweat glands is affected by menopause or if they contribute to the occurrence of hot flushes.

1.7 Objective and Aims of Thesis.

The overall aim of this thesis is to examine the control of cutaneous sudomotor responses in healthy individuals in response to common physiological conditions that affect this process. The specific objective are as follows:

1. To develop a sound protocol, informed by information collected from the literature, to assess both function and structure of skin blood vessels and sweat glands in premenopausal and postmenopausal women both with and without hot flushes.
2. To examine the local axilla sweating responses to different forms of stress that trigger sweating in the axilla region in young, healthy females

Chapter 2.

The development of a protocol to assess both function and structure of skin blood vessels and sweat glands in premenopausal and postmenopausal women both with and without hot flushes

2.1. Introduction

Menopause marks a crucial milestone in a woman's life, usually occurring between the ages of 45 and 55. The cessation of oestrogen production has a direct effect on various organs in the body. Symptoms such as anxiety, depression, and fatigue are commonly reported during the menopause. Furthermore, hot flushes, a prevalent side effect of the menopause, are experienced by the vast majority of menopausal women, and can lead to a diminished quality of life (Freeman et al., 2007), and are also associated with an increased risk of cardiovascular disease (CVD) (Thurston et al., 2008). Hot flushes are a thermoregulatory event, described as a sudden intense sensation of heat resulting in cutaneous vasodilation and profuse sweating (Freedman et al., 2014), with 70% of females experiencing hot flushes for 1-5 years after the onset of the menopause (Shanafelt et al., 2002).

Numerous women find it challenging to manage the distressing effects of hot flushes because of the absence of effective treatments, and ultimately, limited understanding of the mechanisms that underly a hot flush. The skin is a complex organ that plays a vital role in thermoregulation, particularly during hot flushes. These episodes involve flushing, reddening, and sweating, all of which are key events that occur at the skin. The skin is equipped with various neural, vascular, and morphological structures that work together in response to increased internal and/or environmental temperatures (Sassarini et al., 2015). This response includes a series of interconnected steps, such as vasodilation and sweating, which involve the blood vessels and sweat glands. Vasodilatory neurotransmitters and local substances bind to receptors on the skin's blood vessels and sweat glands, leading to vasodilation and the release of sweat (Johnson et al., 2014; Shibasaki et al., 2006). Research has shown that the increase in skin blood flow during a hot flush is triggered by elevated levels of vasodilator neurotransmitters (Low et al., 2011) and nitric oxide (Hubing et al., 2010). Additionally, the presence of calcitonin gene-related peptide (CGRP), a potent skin vasodilator (Gennari et al., 1985), in the blood during hot flushes further

supports the role of vasodilation in these episodes (Wyon et al., 2000). It remains unclear whether there are differences in the function and structure of skin blood vessels and sweat glands between postmenopausal women who experience hot flushes and those who do not, as well as between pre- and postmenopausal women with varying oestrogen levels.

Oestrogen deficiency leads to a decrease in nitric oxide and CGRP levels (Chow et al., 2015; Gangula et al., 2000), potentially impacting the sensitivity of receptors for these substances on the skin blood vessels in postmenopausal women. Postmenopausal women who experience hot flushes exhibit heightened skin blood flow responses to vasodilators, affecting both the endothelium and smooth muscle (Iliodromiti et al., 2019; Sassarini et al., 2012). Consequently, the skin blood vessels of symptomatic postmenopausal women may be excessively responsive to vasoactive substances that contribute to hot flushes. The impact of oestrogen withdrawal on sweat gland receptors and sweat gland function remains unknown. It has not yet been investigated whether menopause results in alterations in the sensitivity of sweat glands, potentially leading to more frequent and intense sweating in postmenopausal women. Furthermore, the skin undergoes various morphological changes during the aging process, which are accelerated during menopause due to hypo-oestrogenism. These changes include reductions in collagen content, elasticity, water content, and thickness (Reus et al., 2020). It is unclear whether hypo-oestrogenism affects crucial vascular, sudomotor, and neural structures in the skin that also contribute to hot flushes. The number of sweat glands generally remains constant throughout a person's lifespan, but they are sensitive to repeated or lack of stimuli, resulting in a decrease in number and density due to decreased use (Abdel-Rahmen et al., 1992). Similarly, the number of skin blood vessels can decrease with aging (Gunin et al., 2014) and are also sensitive to repeated or lack of stimuli (Green et al., 2017), leading to a decrease in both number and size when there are no recurring elevations in blood flow. Currently, it is unknown whether the structure of skin blood vessels and/or sweat glands is affected by menopause or if they

contribute to the occurrence of hot flushes. Furthermore, it remains uncertain whether there is a relationship between the function of skin blood vessels and/or sweat glands and the occurrence of hot flushes. The overall aim of this study is to, therefore, develop a sound protocol, informed by information collected from the literature, to assess both function and structure of skin blood vessels and sweat glands in premenopausal and postmenopausal women both with and without hot flushes.

It is hypothesized that women in the postmenopausal hot flush cohort will exhibit a heightened response to the drug infusion during Visit 1, resulting in a higher sweat rate and/or skin blood flow relative to their non-symptomatic counterparts. Additionally, during the skin punch biopsy in Visit 2, it is hypothesized that the postmenopausal hot flush cohort will have more and/or larger skin blood vessels and sweat glands relative to their non-symptomatic counterparts .

2.2. Methods

2.2.1. Experimental Design

This work will be a single-centre multi-cohort observational study. Participants will attend the laboratory (Tom Reilly Building, LJMU) on two individual occasions. Visit 1 consists of the recording of anthropometric measurements, before undergoing an assessment of post-ganglionic skin blood vessel and sweat gland responsiveness via transdermal/cutaneous microdialysis. 7 days later, the participants will return to the lab for visit 2 to undergo a skin punch biopsy.

2.2.2. Sampling

Recruitment (n=36) will be completed in the UK and take place over 12 months. Populations will include post-menopausal women who experience hot flushes (n=12), post-menopausal women who do not experience hot flushes (n=12) and premenopausal women (n=12). Potential participants will be recruited through providing flyers and displaying posters within high traffic areas on social media websites and around university buildings. Lead members of local menopause support groups will also be contacted to help advertise the study among their members, asking individuals to make direct contact should they be interested in participating. Individuals who reach out will be sent a participation information sheet via their preferred method e.g., post/ email. Potential participants will exclude themselves based upon the inclusion/exclusion criteria provided on the recruitment poster and participant information sheet. The investigator will personally clarify the study and address any inquiries. A designated contact point will also be made available for obtaining additional information about the trial. Potential participants will then be required to complete a health screening questionnaire to confirm they are eligible to participate in the study. Members of the research team will then review individuals for study eligibility before the final authorisation to proceed with the study by the study Physician. Once the participant has been given sufficient time to review the details, they will be requested to sign the informed consent document. The participants will receive a duplicate of the informed

consent document for their personal records, while the original will be kept in the investigator site file. If the participant wishes to withdraw from the trial, they can do so by revoking their informed consent.

Inclusion Criteria

Postmenopausal cohorts

- Female
- Aged 45-75 years
- Amenorrhoeic for >6months
- >4hot flushes per day (symptomatic cohort; quantified using a 7-day hot flush diary; Sloan et al.,2001)
- Healthy
- Non-smoker
- BMI 18-30 kg/m^2
- No history of cardiovascular or respiratory disease
- No history of metabolic diseases e.g. type II diabetes
- Drink <14 units of alcohol per week
- Not taking any medication or treatments to alleviate hot flushes

Premenopausal cohort

- Female
- Aged 18-45 years
- Eumenorrheic
- Healthy
- Non-smoker
- BMI 18-30 kg/m^2

- No history of cardiovascular or respiratory disease
- No history of metabolic diseases e.g. type II diabetes
- Drink <14 units of alcohol per week

Exclusion Criteria

- Cannot readily read and understand English
- Aged <18 years or >75 years
- Male
- Smoker
- Medical history of cardiovascular/respiratory disease
- Medical history of metabolic disease e.g. type II diabetes
- Drink >15 units of alcohol per week
- On medication or treatments to alleviate hot flushes or have taken such medication/treatments in within the previous 6 months
- BMI <18 or >30kg/m^2
- Vaccination (<1 week) due to induced systemic inflammatory reaction
- Local forearm infection
- Allergy to local anaesthetic/ Marcain/amide-group anaesthetics
- Currently pregnant, or planning on becoming pregnant
- <6months post-partum or stopped breast feeding <1month before recruitment

2.2.3. Study Procedures

Before arrival, women must follow an overnight fast (no food or drinks, only tap water), and to refrain from any caffeine, chocolate fizzy/energy drinks and alcohol 12 hours prior to testing. Also, the participants must avoid strenuous physical activity for 24 hours before testing.

Visit 1: Skin and sweat gland responsiveness (function)

Anthropometrics:

Weight and height (Seca, Birmingham, U.K) will be measured prior to the study, after following an overnight fast. Taking approximately 5 minutes.

Blood pressure:

Blood pressure (Dinamap V100, GE Healthcare, Chalfont St Giles) will be measured using an automatic blood pressure monitor around the upper arm. This will first be taken before the study starts, and then at 10-minute intervals once the study begins.

Blood test:

A blood sample will be taken before testing starts by a trained professional. This will be after following an overnight fast. Taking approximately 10 minutes.

Skin blood vessel and sweat gland function:

Upon entering the laboratory, each subject will be instructed to rest in supine position whilst they are fitted for microdialysis probe insertion for the intradermal administration of pharmacological agents to measure skin blood flow and sweating (Kimura et al.,2007) (Figure 2.1). Three microdialysis fibres (Linear 30; CMA Microdialysis Ltd, Stockholm, Sweden), containing 10mm long 6DkDa membranes will be inserted into their non- dominant forearm and perfused with saline solution (5 $\mu l.min^{-1}$) using a microinfusion pump (model 11 plus; Harvard Apparatus, Massachusetts, USA). Each probe will be separated by ≥2.5cm, and a 10mm membrane window will be used for each probe. The probes are inserted using a 25-gauge needle to pierce through the dermal space and excited 20-25mm from point of entry. Local anaesthetic will not be used for this step of the protocol. The microdialysis probe will be passed through the lumen of the needle. Once threaded through, the needle can be removed, leaving the probes in position. Although

depth of probe cannot be identified, previous studies using similar protocols were followed used a depth of 0.3-1.0mm (Kellog Jr et al., 1999). Once membranes are in place, probes will be flushed with lactated ringer solution at 2 µ1/min via an infusion pump (Pump 11, Harvard Apparatus, Natick, MA). Afterwards, custom made sweat capsules (5 x 2 cm, i.e., 10-cm^2) with combined laser doppler flowmetry probes (Perimed 413, Periflux 5001 System; Stockholm, Sweden) can be situated above the embedded microdialysis fibres sites for the measurement of skin blood flow and sweating (Cracowski et al., 2016). The bespoke chambers will use compressed nitrogen gas at a flow rate of 300ml/min. Overall humidity across each probe will be transformed to sweat rate from gas flow and recorded surface area. Placement of capsules can be indicated by markings on each probe that represents the centre point of the membrane portion of the microdialysis probe. Skin blood flow and sweat rate will be collected continuously and concurrently from the same site at a sampling rate of 50 Hz. Temperature in the laboratory will be controlled at 24°C.

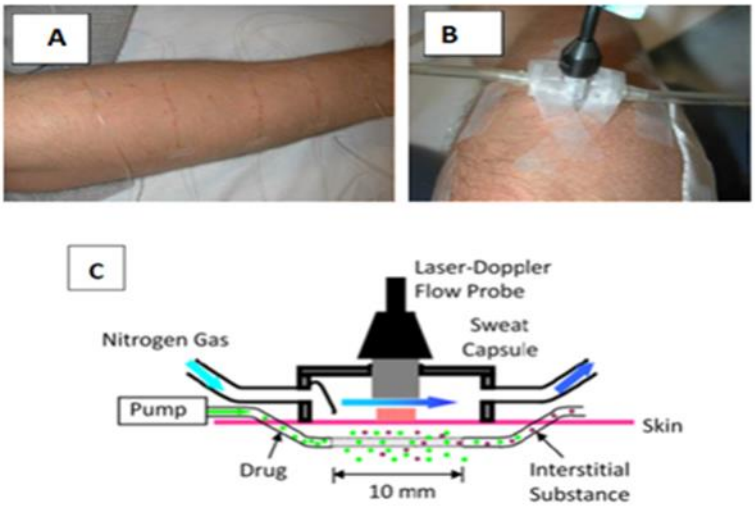

Figure 2.1. A diagram of microdialysis probe within the dermal space. The microdialysis probe is inserted into the skin (A). A bespoke sweat rate and skin blood flow measuring device is placed on top of the membrane (B). Increasing drug dosages are perfused through the probe and will exit the probe via a

semi-permeable membrane and exert an effect that can be analysed using laser doppler flowmetry (skin blood flow) or capacitance hygrometry (sweat rate) over the membrane (c).

Once microdialysis membrane placement is completed and a hyperaemic response associated with membrane placement subsides (taking approximately 90 minutes), baseline skin blood flow and sweating data will be collected for a further 5 minutes, later accompanied by perfusion of increasing dosages with one of the following at each of the 3 probes; Acetylcholine, Sodium Nitroprusside or CGRP (Kimura et al., 2007; Cracowski et al., 2016). Sodium Nitroprusside (56mM; Mayne Pharma, Royal Leamington Spa, UK) will be infused at the end of the protocol at all sites- this is to allow peak vasodilation (Minson et al.,2002; Cracowski et al.,2006). A cuff will be placed around the female's opposite arm, collecting blood pressure measurements every 5 minutes.

Visit 2: Structure and morphology of skin in symptomatic postmenopausal women

Skin blood vessel and sweat gland structure:

The skin punch biopsy technique (see Figure 2.2.) will be used to assess the structure of the skin and sweat gland. This method is a diagnostic investigation of skin vessels, sweat glands, and nerves that uses minimal invasiveness (Lauria et al., 2007). The international consensus guidelines will be used when skin biopsies are performed (Lauria et al., 2010; Schindelin et al., 2015). Upon arrival, participants will receive an intradermal injection of local anaesthetic (Marcain) upon a glabrous, non-venous area of the forearm. A 3mm diameter biopsy will be taken using a punch biopsy tool about 5mm deep. Once sampled, the biopsy will be immediately dropped into a PLP solution (paraformaldehyde, lysine, and sodium m. periodate) to be stored for up to 24 hours, then cryo-protected for 24-72 hours, prior to being frozen in liquid nitrogen. Frozen samples will then be lanced to approximately 50 microns and stained using fluorescein-labelled Ulex europaeus agglutinin I (UAE-I) and or CD31 to mark blood vessels and endothelia in the skin

biopsy, in addition to protein gene product 9.5 (1:1000; anti-PGP 9.5, Chemichon International Inc), a sweat gland antibody. CD31 is widely distributed as a pan-endothelial marker for morphological studies (Muller et al., 2002). UEA-1 is a plant lectin found in *Ulex europaeus,* which has been used as a sensitive and specific marker for endothelial cells (Walker, 1985), with a purpose to stain skin punch biopsies to determine the microvasculature.

Confocal microscopic imaging of samples (optical sections will be acquired to 2- to 4- µm intervals throughout the 50- µm section as a z-stack and will be displayed in 3D images, Zeiss-Axioplan2, Germany) will then be analysed using ImageJ software (Schindelin et al., 2015) to define the number and size of the stained blood vessels of the samples and the size of the secretory coils of the sweat glands, along with the numbers of nerve fibres around the sweat gland within the samples (Figure 2.3.). For between group analyses, data will be analysed using a one-way ANOVA.

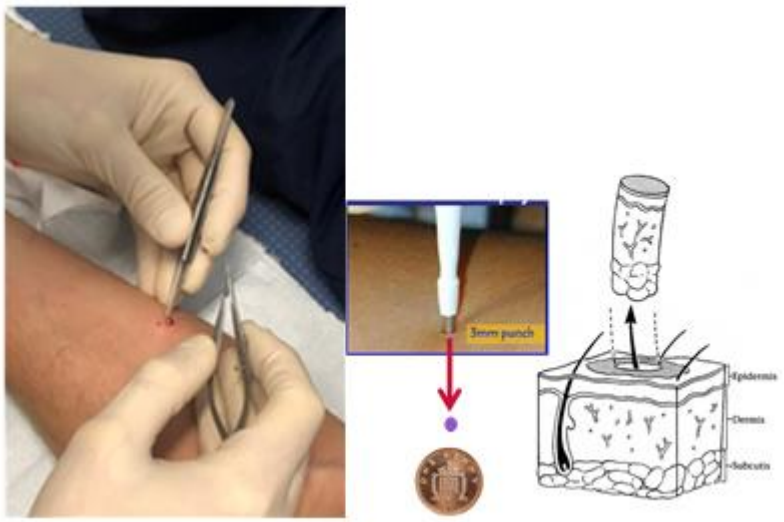

Figure 2.2 A picture represent the skin punch biopsy technique, and a size comparison of the sample taken.

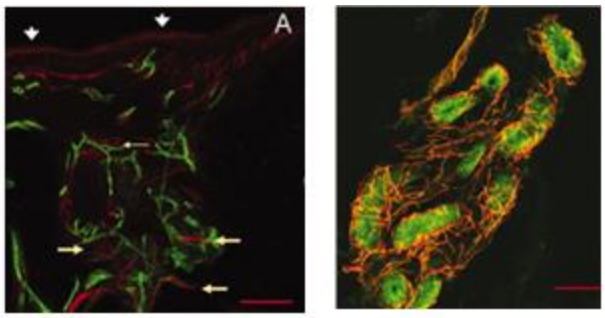

Figure 2.3 Example of confocal images of the skin blood vessels (green in left panel; skin surface is at the top of the image) and sweat glands (green in right panel) from the skin biopsy samples.

2.2.4. Outcome Measures

Primary outcomes

- Cutaneous microvascular function. The examination of cutaneous blood vessel function will be assessed using the combination of the cutaneous microdialysis and laser doppler flowmetry techniques.
- Cutaneous sudomotor function. The examination of cutaneous sweat gland function will be assessed using the combination of the cutaneous microdialysis and capacitance hygrometry techniques.
- Cutaneous blood vessel and sweat gland size. The structure of cutaneous blood vessels and sweat glands will be measured using the punch skin biopsy technique.

Secondary outcomes

- Height and weight
- Blood pressure
- Oestrogen (venous blood sample)

2.2.5. Data Analyses

The study statistician will conduct the statistical analysis in a blinded manner. This study is a comparison between cohorts of pre and postmenopausal women, meaning it is a between groups analyses. The statistical analysis plan will involve exploratory analyses using a linear mixed model with fixed effects of drug dose (cutaneous microdialysis) and or group. Dose-response curves will be created for skin blood flow and sweating at each of the three microdialysis sites using a nonlinear fitting technique allowing to identify what will be the effective concentration that will cause 50% of the maximal response (EC50). Post hoc comparisons across drug doses or between groups will be completed using Bonferroni adjusted LSD. Effect estimates with a 95% confidence interval for differences between groups, as well as changes within groups over drug doses, will be reported. Data management will abide by the governance guidelines of the study institution. All information will be inputted electronically. The research team will be responsible for managing the administrative database (participant information) and trial data. Periodic checks will be conducted to verify the accuracy of the entered data against online records. Any errors will be documented and rectified. To ensure security, all data will be stored on computers that are password-protected and encrypted. Participant files will be stored for 5 years after the trial's completion, with access limited to the research team. Our policy dictates that the research team will have exclusive access to the data for 12 months or until it is published. After this period, the data will be made publicly available through the Liverpool John Moores University (LJMU) Data Repository, under a permissive reuse license.

2.2.6. Research governance and monitoring

Approval for the study will be gained from the school ethics committee at the academic institution and the NHS Research Ethics Committee and Health Research Authority (HRA). No study activity will commence until all approvals are completed. Study conduct will adhere to the Declaration of

Helsinki (World Medical Association, 2013). Data will be collected and stored in accordance with the Data Protection Act 1998/General Data Protection Regulation 2018 in the UK.

One group will be responsible for overseeing and managing the study; the research management group who will meet weekly to discuss study progress/highlight any problems. Specifically, the research management group will focus on monitoring the study's advancement, ensuring compliance with the protocol, safeguarding participant well-being, and evaluating any new data or relevant information from other sources. Furthermore, the research management group will maintain the study master file, respond to any questions about the study, ensure data security and quality and observe data protection laws and conduct safety reporting.

The Principal Investigator will report all adverse events (AE) following the HRA (2018) safety reporting guidelines and the research management group will determine their severity (mild, moderate, severe, life-threatening, death). At each visit, all non-serious adverse events (SAEs), whether anticipated or not, will be documented and kept up to date. Any new SAEs will be reported starting from the moment of consent until the follow-up period. Within 24 hours of an event, the research management group will notify the sponsor/University ethics committee. All adverse events will be monitored until they are resolved satisfactorily. SAEs are defined as any AE at any stage in the research participation of the study which

- Results in death,
- Life threatening,
- Requires hospitalisation or extension of existing hospitalisation,
- Leads to persistent or significant disability or incapacity,
- Is a congenital anomaly/ birth defect

Life-threatening pertains to a situation where the individual was in imminent danger of losing their life during the occurrence. It does not pertain to a situation that, in theory, could have resulted in death had it been more severe.

2.2.7. Public involvement

The public will be involved in the study via providing opinions on the protocol and public-facing documentation (e.g. Participant Information Sheet) during the set-up of the study.

2.2.8. Dissemination

1. Academic dissemination: The study will be published in a high impact journal to spread the findings to a relevant audience of scientists and clinicians. The Uniform Requirements for Manuscripts Submitted to Biomedical Journals (http://wwwicmje.org/) will be respected. The findings will also be presented at international and national conferences (e.g. British Menopause Society Annual Conference, World Congress on Menopause), as well as at local LJMU or NHS meetings.

2. Public-dissemination: It is our intention to present our research findings to all our research participants in a written lay summary. Debrief meetings will be offered, whereby research findings will be discussed in a lay-friendly matter. The findings of the study will also be presented at public engagement events organised by LJMU and local charity or NHS organisations for the public and relevant healthcare professionals to attend. A news item pertaining to the research will be written and sent to relevant organisations and groups.

Chapter 3

The local axilla sweating responses to different forms of stress that trigger sweating in the axilla region

3.1. Introduction

Thermoregulation is the process of regulating the body's core temperature by managing the production and dissipation of heat (Kurz., 2008). The control of core body temperature is a result of the balance of heat gain and heat loss and is achieved through the thermoregulatory centre in the hypothalamus's preoptic area (Morrison and Nakamura., 2019). The hypothalamus is equipped with temperature sensors that receive input through both peripheral and central thermoreceptors in the skin and the viscera, spinal cord and the hypothalamus itself (Yu et al., 2018). When heat gain exceeds heat loss and internal temperature surpasses an optimal or threshold temperature, additional heat loss pathways are activated, including, dilation of peripheral blood vessels and initiation of sweating to release excess heat through radiation and evaporation from the skin, in order to cool the body down and reduce core body temperature (Charkoudian, 2003). An increase in heat gain and a rise in core body temperature can be a result of physical activity/exercise, donning of excessive or larger items of clothing or exposure to hot or humid environments (Casa et al., 2019). Sweating is therefore a key avenue for heat loss during these situations (Gangnon and Crandall., 2018). Sweating can also occur in response to other common stimuli such as emotional or mental stress, for example, anxiety or excitement (Randall., 1953).

The causes of and mechanisms of sweating in response to these varying stressors such as exercise, heat exposure and mental stress, can vary due to the range of factors that can modulate sweating, e.g., thermal and non-thermal inputs and the type of sweat gland activated (Kamei et al., 1997). During exercise there is a large increase in metabolic heat production due to muscle contractions (Racinais et al., 2017) which robustly initiates sweating alongside metaboreceptor stimulation (Amano et al., 2016). During passive heat exposure, e.g., immersion in water baths or exposure to thermal chambers, sweating is often initiated more gradually and or influenced by

the relatively larger increase skin temperature that accompanies passive heat exposure (Shibasaki and Crandall, 2010). When the body responds to an emotion, for example, anxiety, stress or excitement, sweating occurs throughout the body, but is most noticeable on the face and underarms due to the high concentration of apocrine sweat glands in these areas (Hodge et al., 2022). In situations of mental or emotional stress increases in sympathetic nerve activity/circulating catecholamines likely have a role in activating apocrine sweat glands rather than thermally induced mechanisms of sweating (Harker, 2013).

Sweat glands can be categorized into three types; eccrine, apocrine and apoeccrine (Sato, 1983). The most numerous sweat glands are the eccrine sweat glands and populate a large amount of the body surface area (e.g., limbs, torso and face) and produce the highest volume of sweat (Sato, 1983). Conversely, apocrine and apoeccrine glands produce less sweat, this is because of their location on the body (e.g., armpits, groin) (Costill, 1997, Sato et al., 1991). Eccrine sweat is mostly composed of water and NaCl, along with a mixture of various chemicals derived from the interstitial fluid and the eccrine gland (Sato et al., 1985). Apocrine glands differ from eccrine glands as they are larger and open into hair follicles rather than onto the skin surface (Weiner and Hellman, 1960). These glands produce a thick, lipid-rich sweat containing proteins, sugars, and ammonia (Sato et al., 1987). Apoeccrine glands share characteristics of both eccrine and apocrine glands and are confined to the axillary region. They release sweat directly onto the skin surface via a distal duct. The secretion from these glands largely a saltwater solution, resembling eccrine sweat (Sato et al., 1987).

The precise reasoning of sweating from apocrine/apoeccrine glands is unknown, they are unlikely to contribute to thermoregulation due to the inefficient evaporation of sweat in the underarm/groin regions (Baker., 2019). In many species apocrine glands are considered scent glands responsible for producing pheromones (body odour) (Sato et al.,1987). Sweat from apocrine/apoeccrine

glands contains fats and nutrients that create optimal conditions for the growth of various bacterial species. These bacteria possess unique enzymes that can transform non-odorous perspiration components into volatile compounds and unpleasant odours especially in specific body areas like the underarms (Sato et al., 1987). To address underarm odour, three approaches can be taken: reducing or stopping secretions from sweat glands, inhibiting bacterial growth, and absorbing body odours. Personal hygiene products, such as antiperspirants and deodorants, have become essential in daily routines to combat body malodour and excessive perspiration (Teerasumran., 2023). Commercial companies offer a variety of products for consumers to manage sweating. Evaluating and analysing the effectiveness of these products is crucial for companies to ensure product quality. To conduct studies of the efficacy of antiperspirant products, it is essential to establish baseline levels of axillary sweating without the influence of antiperspirants before assessing the products' ability to reduce sweating. The knowledge of axillary sweating responses to various everyday activities such as exercise, heat exposure and mental stress is scarce, however. The aim of the study is to compare local axilla sweating responses to different forms of typical daily stressors, passive heating, exercise and mental stress, that trigger sweating in the axilla region in young healthy females.

It is hypothesised that sweating would occur under each condition, however, sweating will be most pronounced during the exercise condition, closely followed by passive heating then the emotional stress trial.

3.2. Methods

3.2.1. Participants

This study recruited 30 females to take part in three trials. The females were healthy, free from any medical diseases or conditions, non-smokers and aged 27±7 years with a body mass of 63±8 kg and a height of 161±7 cm.

3.2.2. Design

Participants visited the laboratories in the Tom Reilly Building three times in a randomised fashion. Each session was to assess their axilla sweating responses in a different condition. Visit 1 involved an exercise protocol lasting 2 hours and 15 minutes, performed at room temperature (~18°C, ~40% rh). Visit 2 involved a passive heat protocol, where the participant sat stationary in a heated room (~40°C, ~30% rh). Visit 3 was a mental stress test, involving a series of cognitive/mental stress tests. The phase of the menstrual cycle was noted before each participant's initial trial and future trials were scheduled during a similar phase. Research has shown that menstrual cycle phase does not affect evaporative heat loss during exercise or passive heating however (Inoue et al., 2005, Notley et al., 2018). Two participants were postmenopausal and 2 were taking oral contraceptive pills.

3.2.3. Procedures

Before each visit, participants were provided with a body spray, which they had to wear for a 10-day washout period under each arm. In addition, participants had to avoid exercise for 24 hours, alcohol for 12 hours, and caffeine for 8 hours prior to the protocol. Trials were either in the morning or afternoon. Participants wore appropriate clothing, including wearing a vest, to allow easy access to the armpit area.

Visit 1. Exercise.

The study commenced with the participant seated for a 10-minute period to establish a baseline measurement of their sweating data. The protocol included a jog on treadmill (~6-9km/hr) for 10 minutes, followed by a 5-minute seated rest. After rest, participants then performed stepping (on and off a step) broken down into 2x4 minutes with 2 minutes rest in between, and again followed by a 5-minute seated rest. Once stepping was completed, participants then cycled at a moderate pace (Lode, Excalibur, The Netherlands) at ~50 watts for 10 minutes. A further 5-minute rest was to be completed before repeating the protocol an extra two times. Participants were free to adjust the intensity of walking, cycling and running to ensure it suited their abilities.

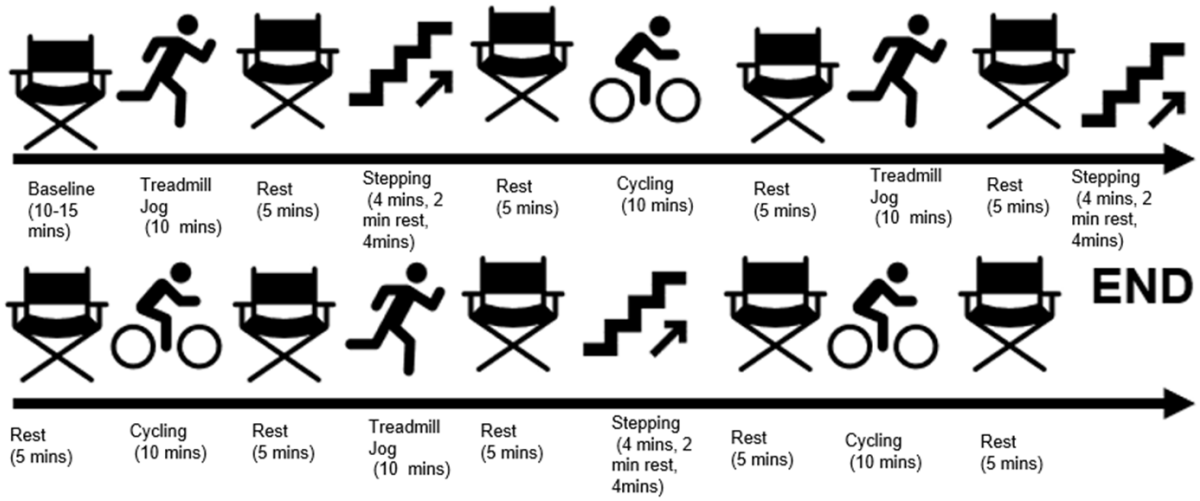

Figure 3.1. Visual schematic overviewing the breakdown of the exercise test protocol.

Visit 2. Passive heating.

For visit 2, the participant sat for 2 hours and 15 minutes in a heated room (~40ºC, ~30% rh).

Visit 3. Mental stress.

Participants took part in a mental stress test lasting 1 hour. This was made up of a series of tasks that included mental arithmetic, word recall and interview preparation activities. It was deemed unethical to expose participants to a mental stress protocol longer for 1 hour.

Task 1. Mental arithmetic. Participants conducted 3 x 5-minute tasks, which were to be completed twice through. Here the participant would continually subtract 7 from the number 2001 until the end of the 5-minute period or they answered incorrectly. An incorrect answer was followed by a loud klaxon sound.

Task 2. Reaction time. After a number appeared on a screen for 1.5 seconds the participant had to answer whether they had just seen a number which was 1 bigger than the previously displayed number by clicking a mouse button. If answered incorrectly, a loud klaxon noise was made.

Task 3. Word recall. Fifteen words would appear one by one on a screen for 3 seconds each. Following a 30 second interval, 30 words would appear on screen again, some of which would be repeated from the previous set of 15 and some would be new. The participant was to respond by pressing the letter Q if they believed a new word was presented, or the letter P if they thought it had been repeated. A loud klaxon noise was played for any incorrect responses.

Task 4. Mock interview. Upon arrival, participants were to write down their dream job and how they believe they are going to achieve this. They were then told they will have to present their goals and beliefs for this job at the end of the study in front of a camera. The interview did not take place though. The aim of this task was to invoke mental stress on the participants in addition to the acute stress induced by the other tasks.

3.2.4. Instrumentation

For all 3 trials the following measures were obtained.

Anthropometric measurements

Height was measured using a calibrated stadiometer, ensuring precise and consistent measurements. Pre- and post-testing body mass were recorded using a digital weighing scale, with participants instructed to wear minimal clothing and reduce variability. Body mass measurements were taken to the nearest 0.1 kg to ensure accuracy (Seca, Birmingham, U.K)

Heart rate

Heart rate was measured continuously throughout the testing using short-range telemetry (Polar OH1, Kempele, Finland), a widely used and validated heart rate monitor. The sensor was placed securely around the participant's upper arm, ensuring proper contact for accurate data collection.**Skin temperature**

Local skin temperatures were recorded continuously at four anatomical sites: the thigh, calf, forearm, and axilla (left and right), using thermocouples (iButtons, Maxim Integrated, Wilmington, MA, USA). The thermocouples were securely attached to the skin using medical-grade adhesive tape to ensure stable placement and minimise measurement error due to movement. Temperature data were collected in real-time, with the thermocouples providing high-precision measurements at regular intervals.

Core temperature

Core temperature was measured using an ingestible core telemetry pill (eCelsius Performance, BodyCap, Saint-Clair, France). The pill was ingested by the participant the night before each trial to allow sufficient time for the device to reach its optimal position within the gastrointestinal tract. This method provides continuous, real-time monitoring of core temperature throughout the experimental conditions. The eCelsius pill has been validated for accuracy in tracking core temperature with minimal interference from external factors, ensuring reliable data collection (Service et al., 21).

Local Sweating

SweatSENSE method

Local sweating responses were collected at regular intervals on the left and right-side axilla. This was done using the SweatSENSE methods which was made up of small paper sensors that were placed on the axilla skin for 5 seconds each time before being removed. The paper samples were then analysed for the total coverage % of colour change of paper (if sweating has occurred) as an index of sweating activity (See Figure 3.2).

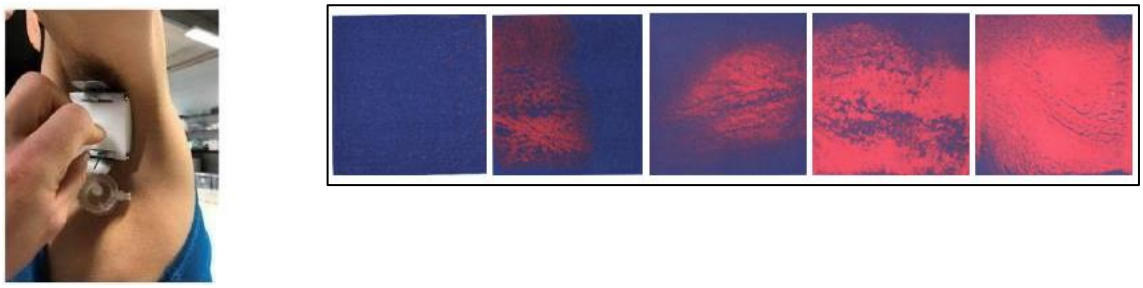

Figure 3.2. An example of the SweatSENSE method showing the application of the SweatSENSE patch to the axilla vault and examples of SweatSENSE patches showing various levels of sweating.

Capacitance hygrometry

Local sweating responses were also measured continuously via the capacitance hygrometry method. A small capsule, with a surface area of 7 cm^2, was taped to the left axilla vault using topical skin glue and hypoallergenic tape. Dry nitrogen gas was blown through the capsule at a constant flow rate (300 $ml.min^{-1}$) and any sweat produced was delivered via tubing to a humidity and temperature detector (HMT330, Vaisala, Helsinki, Finland) to index local sweating activity. Data were collected continuously using an online data acquisition software (LabChart v8, AD Instruments, Sydney), and were converted from the raw data measurement into local sweat rate ($mg.cm^2.min^{-1}$) in real time, using the capsule surface area, gas flow rate, and the humidity and temperature of the gas leaving the capsule:

Local sweat rate($mg.cm^2.min^{-1}$) = Absolute humidity(mg)*gas flow rate($ml.min^{-1}$)/capsule surface area (cm^2)

Visual analogue scales

Ratings of perceived exertion (RPE) were measured using the Borg scale (6-20 scale) at regular intervals, with participants providing a subjective assessment of their exertion levels throughout each activity (Williams., 2017). Additionally, thermal discomfort was assessed at the end of each activity and periodically during the passive heating protocol (Butera., 1998).

Statistical Analyses

Data were compared over time and between trials using a mixed ANOVA test (main effects of time and trial). Given the shorter duration of the mental stress trial, only data for the first 60 min of the exercise and passive heating trials were included in the ANOVA analyses. When any significant main effects were observed, post hoc comparisons using the Bonferroni test were carried out. All data were checked for homogeneity of variance and normality (Shapiro-Wilk test). All data are expressed as means and standard deviations and statistical significance was set at $P < 0.05$. All data were analysed using SPSS.

3.3. Results

Heart Rate

There was a significant main effect of time ($F_{14,392}$ = 152.1, P < 0.001), indicating that heart rate (HR) increased over time across all three conditions. There was also a significant main effect of condition (F(2,56) = 115.18, P < 0.001), with overall heart rate being highest during the exercise trial. Additionally, a significant interaction between time and condition ($F_{8.2,230}$ = 111.78, P < 0.001) was observed, reflecting the intermittent nature of the exercise and mental stress protocols when compared to the passive heating trial

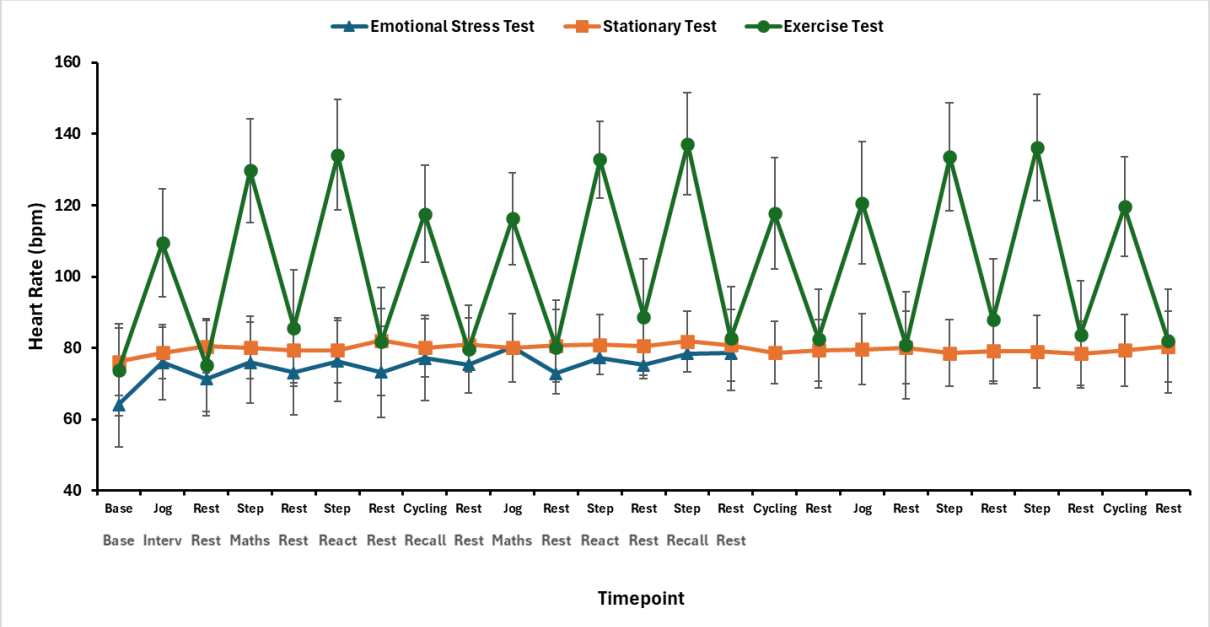

Figure 3.3. Mean (±SD) heart rate responses to the exercise, passive heating and mental stress trials. The x axis is labelled with the events of the exercise and mental stress trials.

Core Temperature

There was a significant main effect of time ($F4_{,294}$= 2.3, P = 0.006). There was a significant main effect of condition ($F_{2,42}$ = 12.4, P < 0.001), with core temperature being highest during the

exercise trial. Additionally, there was no significant interaction between time and condition ($F_{4.063,85.319}$= 1.9, P = 0.124).

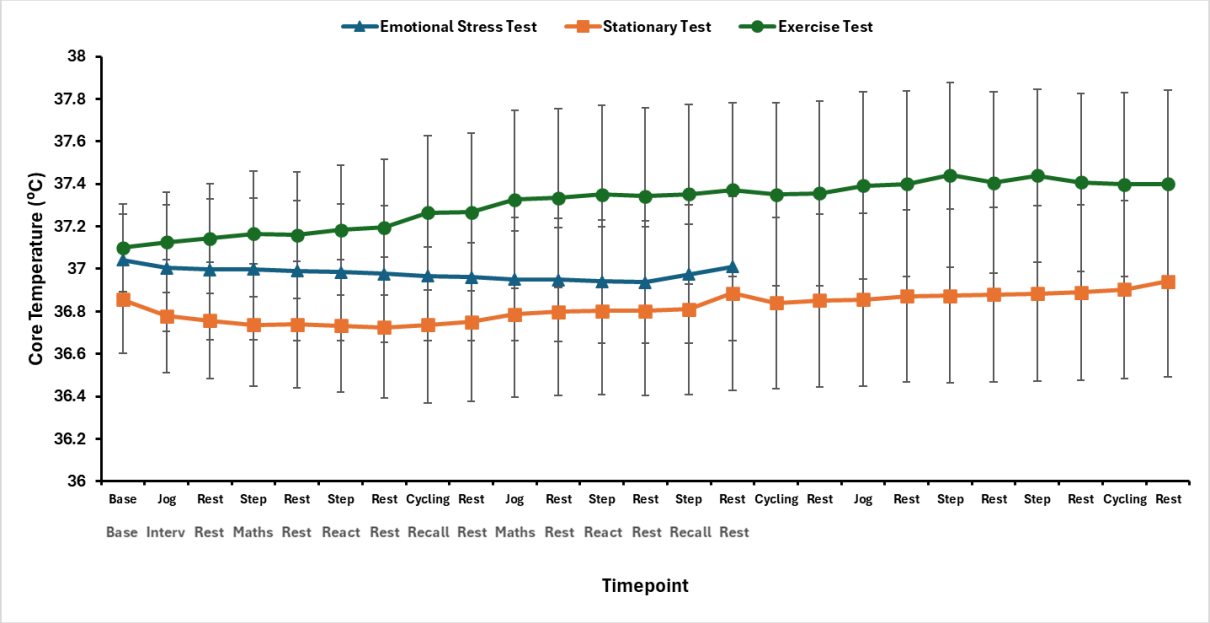

Figure 3.4. Mean (±SD) core temperature responses to the exercise, passive heating and mental stress trials. The x axis is labelled with the events of the exercise and mental stress trials.

Thermal Discomfort

There was a significant main effect of time ($F_{14,392}$ = 35.5, P < 0.001), indicating that thermal discomfort increased with the duration of the protocols. There was a significant main effect of condition ($F_{2,56}$= 45.6, P < 0.001), with thermal discomfort being highest during the passive heating trial. Additionally, there was a significant interaction between condition and time ($F_{8,233}$ = 11.7, P < 0.001). During the exercise trial, thermal discomfort generally increased, particularly during periods of exercise, whereas thermal discomfort remained relatively stable in both the passive heating and mental stress trials.

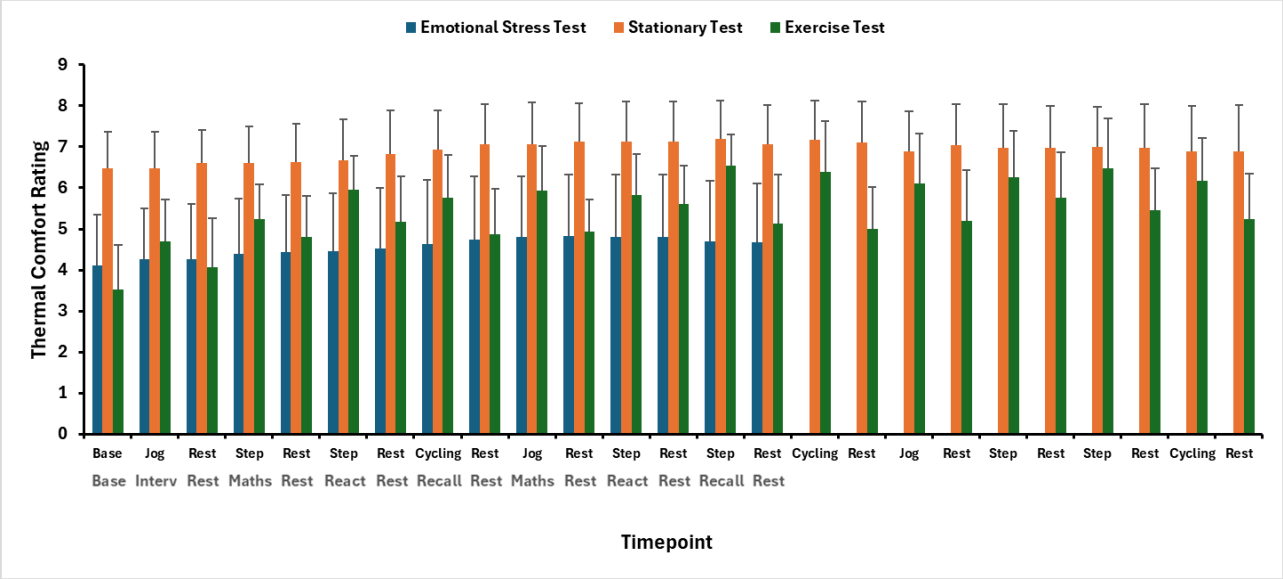

Figure 3.5. Mean (±SD) thermal discomfort responses to the exercise, passive heating and mental stress trials. The x axis is labelled with the events of the exercise and mental stress trials.

Rating of Perceived Exertion (RPE)

There was a significant main effect of time ($F_{4.83,140.12}$ = 18.83, P<0.001) as RPE increased with the increasing duration of the exercise trial.

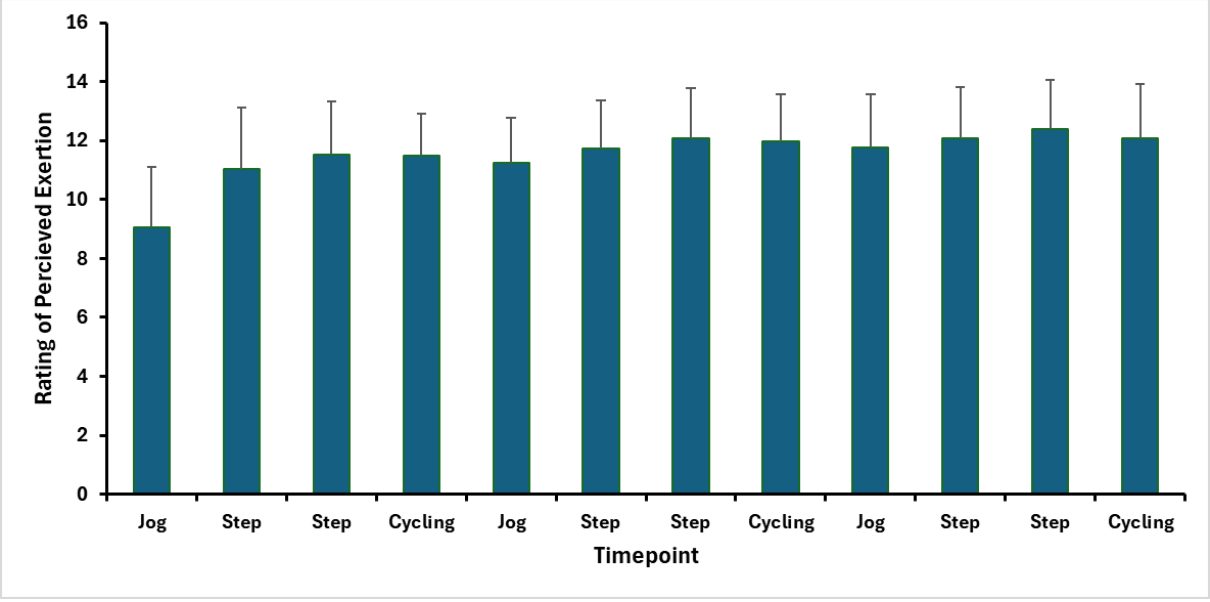

Figure 3.5. Mean (±SD) rating of perceived exertion responses to the exercise trial.

Sweat Rate

Capacitance Hygrometry

There was a significant main effect of time ($F_{9,243}$ = 21.085, P < 0.001), indicating that sweat rate increased over time in all trials. There was a significant main effect of condition ($F_{2,54}$ = 7.0, P = 0.002), with sweat rate being highest during the exercise trial and intermediate during the passive heating trial. Additionally, there was a significant interaction between time and condition ($F_{4.5,122.0}$= 7.0, P < 0.001). The increase in sweat rate was greatest during the exercise trial, showing an intermittent pattern, whereas the increase in sweat rate was more gradual during the passive heating and mental stress trials

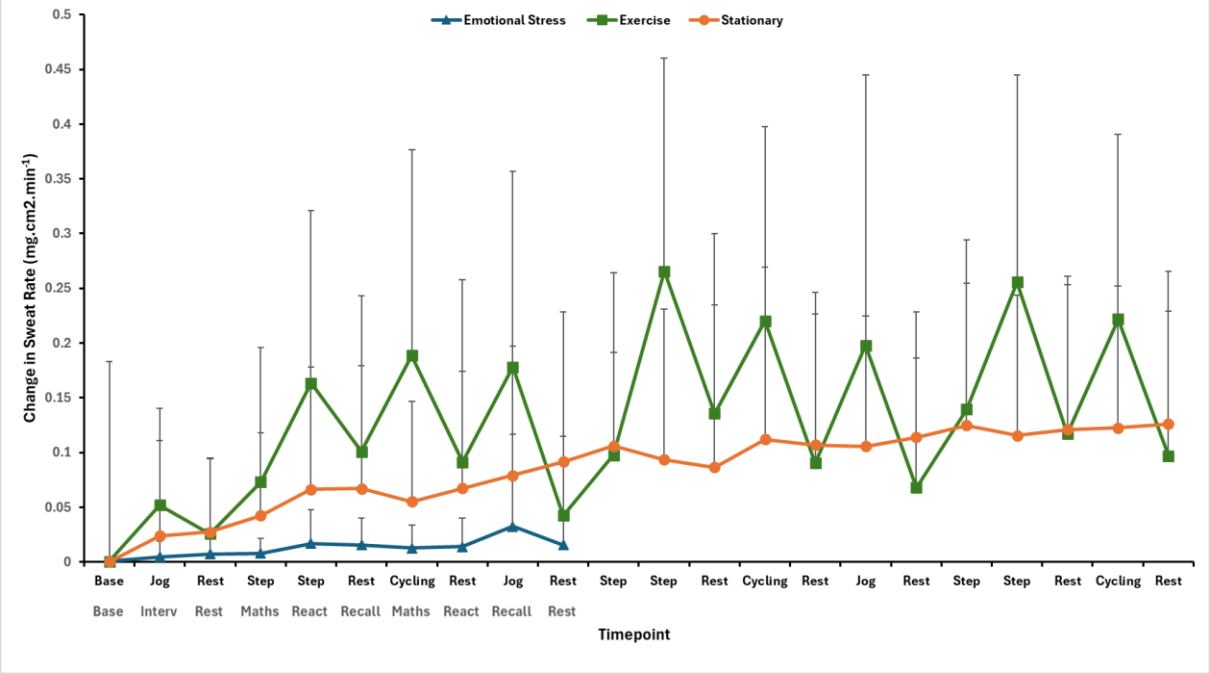

Figure 3.6. Mean (±SD) local sweat rate responses to the exercise, passive heating and mental stress trials. The x axis is labelled with the events of the exercise and mental stress trials.

SweatSENSE

There was a significant main effect of time ($F_{9,261}$ = 3.62, P<0.001). Sweat rate increased over time in all trials. There was a significant main effect of condition ($F_{2,58}$ = 7.17, P=0.002). Overall, sweat rate was highest during the exercise and passive heating trials. There was a significant time*condition interaction ($F_{6.48,187.85}$ = 2.9, P=0.008). The increase in sweat rate during the exercise trial displayed an intermittent type profile whereas the response during the passive heating trial was consistent after an initial elevation. The sweating response during the mental stress trial was similar to that during passive heating with a steady profile after an initial rise at the start of the protocol.

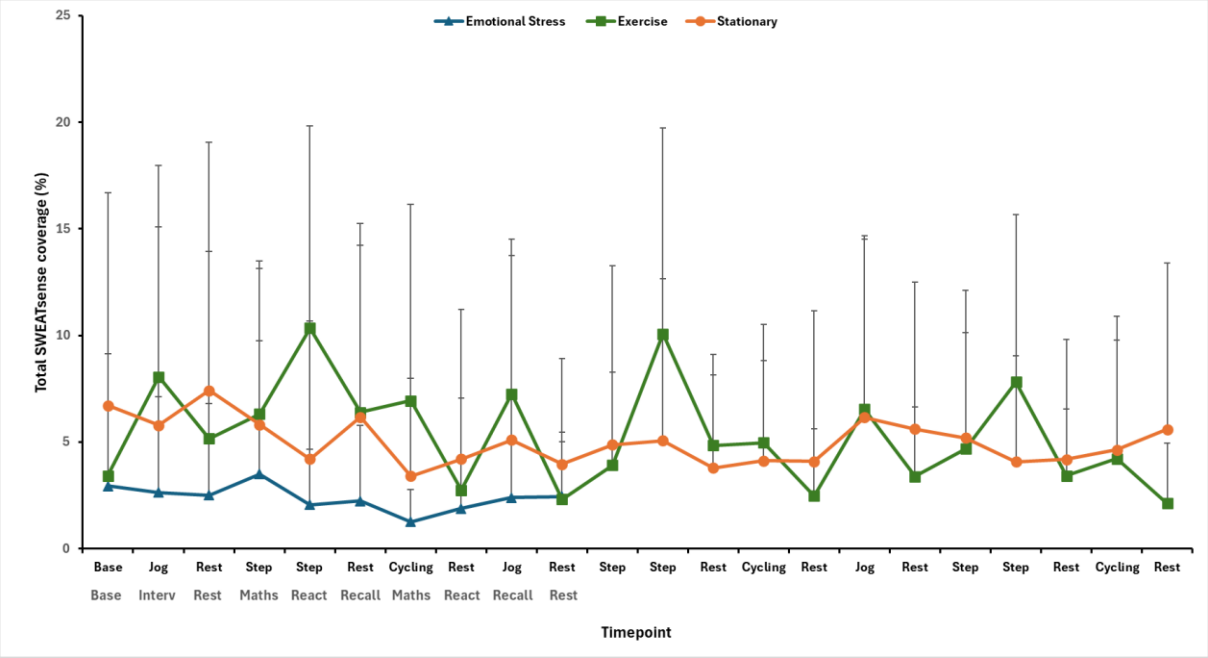

Figure 3.7. Mean (±SD) local sweat rate responses to the exercise, passive heating and mental stress trials. The x axis is labelled with the events of the exercise and mental stress trials.

Skin Temperature

The skin temperatures at the forearm, axilla, thigh and calf are presented below in Figure 3.8. ANOVA analyses were not conducted due to the multiple datapoints during the protocols (every 1 min). At all sites, skin temperature was highest in the passive heating trial and after an initial

increase remained fairly stable for the remainder of the trial in this condition. In the mental stress trial, after an initial elevation at all sites skin temperature subtly decreased until the end of the trial. During the exercise trial skin temperature generally increased on the thigh and calf. In contrast, at the forearm, after a small increase, skin temperature decreased with a plateau thereafter. Similarly, at the axilla site, after an initial elevation in skin temperature a decrease ensued for the remainder of the trial.

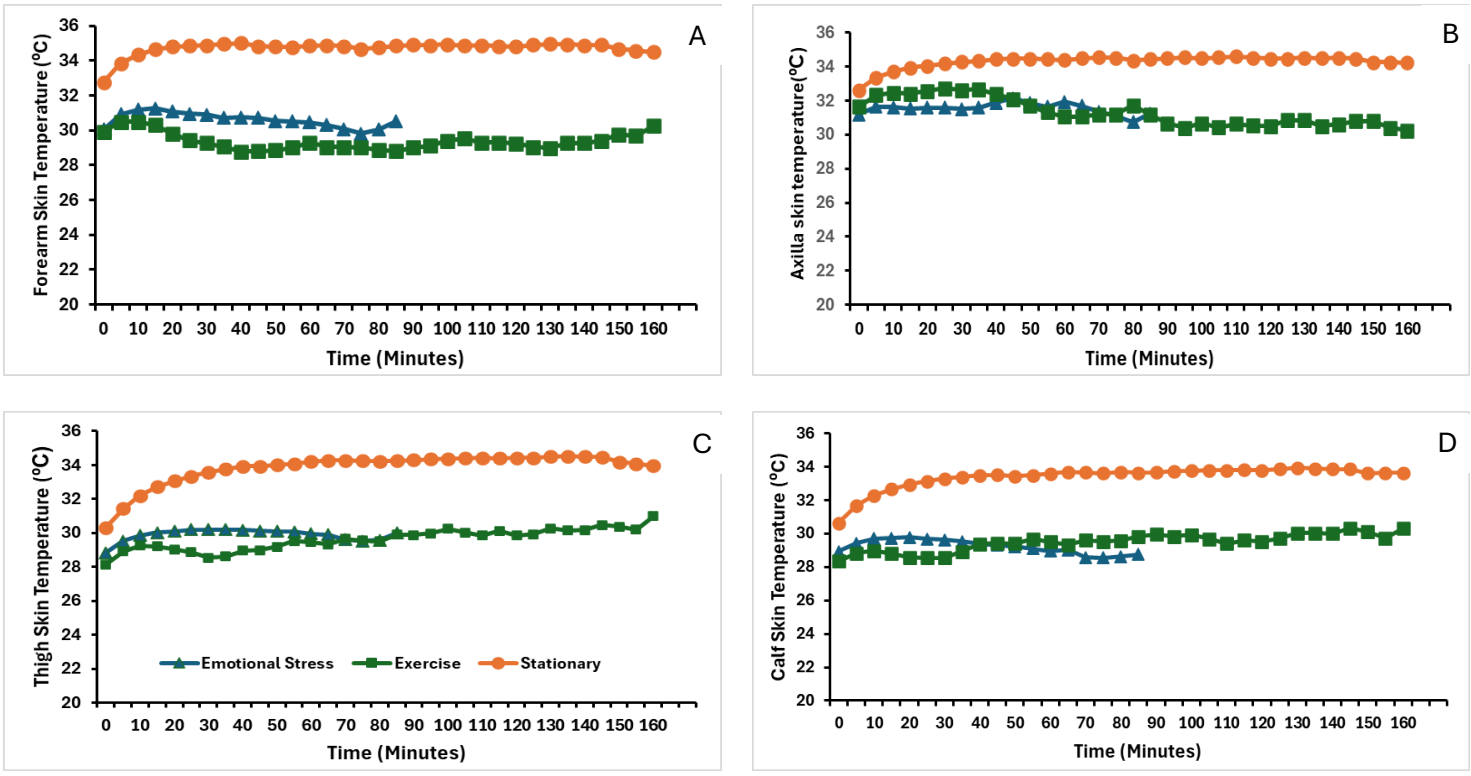

Figure 3.8. Mean (±SD) skin temperature responses to the exercise, passive heating and mental stress trials at the forearm (A), axilla (B), thigh (C) and calf (D). Error bars omitted for clarity.

3.4. Discussion

The aim of the study was to compare local axilla sweating responses between three different conditions that trigger sweating in the axilla region: exercise, passive heating and mental stress. The responses were measured using the ventilated sweat capsule hygrometry technique and the SweatSENSE method. The findings of the study showed an increase in local sweating responses during all conditions; however, the results show that the increase in sweating was highest during exercise and displayed an intermittent type profile. The smallest elevation in sweat rate was displayed during the mental stress test.

The relationship between sweat rate and exercise is not unexpected and aligns well with existing literature (Sawka et al., 1993). The exercise condition in this study showed the greatest increase in sweating, as it is the body's way of regulating temperature to maintain homeostasis and to keep core body temperature within safe limits through evaporation. During exercise, muscle activity generates significant heat as a byproduct of energy metabolism, which results in the increases in sweat rate, followed by declines during rest periods. Evaporation occurs in two stages: first, sweat on the skin's surface transitions from liquid to vapor at a constant temperature (a process known as latent heat transfer). Next, the vapor diffuses across the boundary layer into the surrounding air. The primary driving force behind evaporation is the vapor pressure gradient between the saturated skin surface and the ambient air. However, factors such as air velocity, skin moisture, and surface area also play significant roles in influencing the rate of evaporation.

While core temperature during exercise increases in proportion to metabolic rate (Nielsen, 1938) and is the key driver for sweating, the findings of the present study also suggest sweating can occur even when metabolic heat production is at near-resting levels, particularly in high external temperatures such as in the passive heat trial in the present study, when the heat gained from the environment exceeds the body's ability to lose heat, leading to heat accumulation. Passive heat stress is commonly experienced during extreme weather conditions characterized by elevated air

temperature and humidity. It is also frequently used in experiments to study reflex thermoregulatory and cardiovascular responses to increases in core and skin temperatures (Crandall and Wilson, 2015). While exercise resulted in the most significant increase in sweating, the pattern observed was intermittent, characterized by sharp spikes of elevated sweating followed by declines during rest periods. In contrast, passive heating exhibited a more gradual increase in sweating throughout the stationary test, with participants continuously sweating without any evidence of cooling down. This sustained sweating during passive heating coincided with elevated skin temperatures (and a minimal increase in core temperature), which were higher than those recorded during exercise. The minimal environment:skin temperature gradient during passive heating would have hindered dry heat loss magnifying the need for evaporation of sweat in order to lose heat.

Previous research shows that mental stress activates the sympathetic branch of the autonomic system, thereby triggering the ‘flight or fight response’ consequently leading to increased sweating as the body is prepared to cope with perceived threats (Schote et al., 2022). Stress can also trigger the release of hormones such as adrenaline, which can also stimulate sweat glands. This hormonal response enhances the body’s readiness to react to stressors (Harker, 2013). The context of sweating during mental stress can influence social interactions as it can be deemed unhygienic and unacceptable (Davidson et al., 2002). For example, excessive sweating in stressful situations can lead to self-consciousness, impacting confidence and social behavior (Ruchinskas, 2011). In the present study, mental stress showed a slight increase in sweating, however the increase was not of the same magnitude compared to exercise and passive heating conditions. The reasoning behind this could be down to the cognitive tests used; they may not have induced sufficient stress, therefore, resulting in lower sweating compared to the other two conditions. In future studies, mental stress testing should be enhanced to create a greater stress and subsequent sweating response, however, this is challenging due to the difficulty in mimicking

ecologically valid levels of stress as well as the ethical implications. Sweating during mental stress is a complex response that aids thermoregulation and reflects the body’s readiness to respond to challenges. Understanding this response can provide insights into the interplay between physical and mental health. Furthermore, the larger sweat responses in the exercise and passive heating trials also provide evidence that robust elevations in axilla sweating do occur and presumably via eccrine sweat glands (despite their lower abundance in the axillae) in order to serve thermoregulatory control of body temperature.

Research into axillary sweating and interventions to blunt or block these responses is scare. The use of antiperspirants is crucial for managing axillary sweating, as they are designed to reduce sweat production in the underarm area. Understanding how the axilla responds to stimuli like exercise and mental stress is essential for evaluating the efficacy of antiperspirants, particularly those containing aluminum-based compounds that temporarily block sweat glands. Variability in individual physiological responses highlights the need for tailored formulations, especially for those with hyperhidrosis or different activity levels. Excessive axillary sweating can significantly impact quality of life, leading to discomfort and social anxiety. Insights into sweating patterns can inform better management strategies and guidelines for antiperspirant use in various contexts, ultimately enhancing comfort and well-being. This understanding not only optimises antiperspirant effectiveness but also contributes to improved personal hygiene.

Sweating isn't limited to just the axillary region; individuals can also sweat from their palms and foreheads, particularly during mental stress. Palmar sweating, which affects the hands, can be particularly troublesome in social situations or during activities requiring a firm grip, while forehead sweating can be triggered by similar stimuli. In contrast to the axillae, the palms are distributed with eccrine glands, but they are typically not activated by heat stimuli but rather by deep breathing, mental stress, and localized touch. This type of sweating, known as "emotional sweating," helps prevent slipping during gripping or when performing delicate tasks with the

fingertips (Asahina et al., 2015). The forehead also contains eccrine sweat glands which can be triggered by heat stress and mental stimuli, e.g., embarrassment, as well as gustatory stimulation. Future studies of sweating and mental stress should also look to assess sweating at these sites in addition to the axillae as they may be more sensitive to mental stress protocols.

There are a number of individual characteristics that should be considered when researching the control of sweating. For example, larger individuals theoretically have a greater surface area, which suggests a higher potential for heat loss compared to smaller individuals (Cramer and Jay, 2016). However, they also possess a lower surface area-to-mass ratio, resulting in reduced evaporative heat loss per unit of body mass, which can mitigate some of the heat gained from the environment. Conversely, smaller individuals have a higher surface area-to-mass ratio, allowing for greater evaporative heat loss relative to their body mass. This can be particularly relevant in the axillary region, where antiperspirants aim to manage sweat production. Understanding these physiological differences can help tailor strategies for managing axillary sweating, especially in variant ambient conditions. Therefore, under fixed uncompensable ambient conditions, larger individuals would likely experience a greater rate of heat accumulation, and, consequently, increased axillary sweating assuming no differences in skin blood flow or metabolic heat production per unit of body mass (Cramer and Jay 2016) further emphasizing the importance of individualized approaches in sweat management strategies. Of note in this particular study was that the participants were all females who on average display differences in body size and surface area-to-mass ratios. The consideration of these factors as alluded to above when examining axilla sweating in male and female populations is therefore very important.

Other individual factors that can affect sweating are age, physical fitness and hydration levels. For example, research that typically examined eccrine gland sweating has shown that someone who is more physically fit typically displays higher sweating and is able to sweat sooner. It has not been adequately investigated whether sweating from the apocrine glands in the axillae,

palmar, groin and or forehead regions are similarly affected by age, physical fitness and hydration levels. Furthermore, long-term studies are essential to evaluate how individual responses to axillae sweating evolve over time, influenced by factors such as fitness levels, age, and health status. This could facilitate the development of more personalised sweat management strategies. Additionally, measuring sweat rates in real-world settings, such as during outdoor activities or in high-stress work environments, would enhance ecological validity and provide insights into how these factors affect sweating behavior and heat management. Moreover, exploring interventions aimed at improving thermoregulation, such as innovative clothing materials or cooling devices, could yield significant practical applications for managing axillary sweating.

Another novel aspect of the study was assessing thermal (dis)comfort of the individual at regular intervals during each condition. The results displayed an increase in thermal discomfort over time in each condition. The biggest increase was during the stationary passive heating trial. Thermal discomfort is chiefly modulated by both skin and core temperatures (Nakashima et al., 2018). During passive heating there was a subtle increase in core temperature alongside large increases in skin temperature due to the surrounding environment. In warm conditions, therefore, the body's capacity for losing heat decreases, as there is a lack of airflow and less of a temperature gradient between the body and surrounding atmosphere which can result in discomfort (Trangmar and Gonzales-Alonso, 2019). In the mental stress test trial, there was also an increase in thermal discomfort but without a clear increase in core or skin temperatures. It's possible that during the mental stress condition thermal discomfort increased because of the body triggering the ‘fight or flight’ response, subsequently resulting in an increased heart rate and blood flow which can create a sensation of warmth. Some other important perceptual indices that were not assessed in this study were the participants perceptions of wetness or odor which are important consequences of sweating as well as criteria for selecting personal hygiene products. Future studies of axilla sweating should look to examine both physiological and perceptual markers of sweating in order

to fully understand the control of thermoregulation and behavior as they pertain to sweating from the axillae.

In conclusion, the findings of this study demonstrate that exercise, passive heating, and mental stress all induce local axillary sweating to different degrees. Particularly, the exercise condition elicited the largest increase in axilla sweat rate. Some caution must be taken with the responses to the mental stress protocol due to the inherent methodological limitations of laboratory-based stress testing. Research on axilla sweating in these three conditions offers valuable insights for the development and application of antiperspirants tailored to specific activities and the optimisation of the timing and selection of antiperspirants for maximum sweat reduction but future studies should look to document and incorporate a range of outcome variables specific to the control of sweating and well-being.

Chapter 4.

Synthesis of Findings

4.1 Aims and Objectives

Sweating is a vital physiological process that plays a crucial role in thermoregulation and maintaining homeostasis. Sweating occurs via eccrine and apocrine sweat glands, which respond to various stimuli, including heat, physical activity and psychological stress. Eccrine glands, distributed widely across the skin, are primarily responsible for cooling the body through the secretion of a dilute saline solution, which evaporates and dissipates heat. In contrast, apocrine glands, located in specific areas such as the armpits and groin, become active during both heat and psychological stress and are associated with the production of a thicker, odoriferous secretion. The activation of sweat glands is regulated by the autonomic nervous system, with acetylcholine serving as the primary neurotransmitter, while adrenergic stimulation can also trigger sweating in response to psychological stimuli, especially in apocrine glands.

There are a number of conditions that can significantly impair sweating, such as spinal cord injury, skin burns, hyperhidrosis, ageing and the female menopause. The pathophysiological mechanisms of these alterations in sweating are not well understood, however. Moreover, research into sweating from the apocrine glands in the axillae, where personal hygiene products are often applied, is scarce. The overall aim of this thesis was to therefore examine the cutaneous sudomotor responses in healthy individuals in response to common physiological conditions that affect sweating. More specifically, the objectives were to 1). To develop a sound protocol, informed by information collected from the literature, to assess both function and structure of skin blood vessels and sweat glands in premenopausal and postmenopausal women both with and without hot flushes and 2). To examine the local axilla sweating responses to different forms of stress that trigger sweating in the axilla region. This chapter will aim to summarise the main findings of each study, consider the limitations of the studies as well discuss the implications and future directions for further research.

4.2. The development of a sound protocol, informed by information collected from the literature, to assess both function and structure of skin blood vessels and sweat glands in premenopausal and postmenopausal women both with and without hot flushes.

Hot flushes, experienced by ~80% of postmenopausal women, have a profoundly debilitating effect on daily activities and quality of life (Thurston & Joffe, 2011). Menopausal hot flushes are recognised as extreme thermoregulatory events with episodes of skin flushing and sweating typically lasting for several minutes and ranging from a few times per week up to several episodes per hour. Their underpinning mechanisms are not well understood however which has consequently limited scientific advances in hot flush therapies. Previous work has suggested that a narrowed core temperature null zone might be causative in hot flushes of menopausal women via a reduced temperature threshold for the onset of sweating, such that sweating will be activated and a hot flush triggered when any subtle increase in core temperature surpasses the lowered core temperature sweating threshold (Freedman et al., 1995). However, this hypothesis is disputed as a further study observed that 49% of women did not experience an increase in core temperature prior to a hot flush (Jones et al., 2019). Central thermoregulatory and endogenous pyrogen mechanisms also represent plausible alternatives. NK3R stimulates the brain's thermoregulatory centre and preclinical research suggested heightened signalling of NK3R in menopausal hot flushes (Rance et al., 2013). Subsequent trials and treatment with a NK3R antagonist (e.g., Fezolinetant) demonstrated a marked reduction in moderate-to-severe vasomotor symptoms (Fraser et al., 2019; Santoro et al., 2020), suggesting that one way of improving hot flushes is through modulating the NK3R pathway and hypothalamic-pituitary-gonadal (HPG) axis, a potential central driver of hot flushes. Endogenous pyrogens have also been implicated in hot flushes (Huang et al., 2017), although research is scant.

Collectively, these studies and hypotheses represent potential central mechanisms responsible for hot flushes in postmenopausal women.

Given that the symptoms of a hot flush are ultimately manifested in/at the skin it could be postulated that changes in the structure and or function of key morphological sites or processes in the skin could contribute to or be a consequence of hot flushes. The findings of the proposed protocol therefore hope to reveal important insight as to whether local changes in peripheral sites, such as the skin, contribute to the occurrence of hot flushes. Using a combination of intricate and advanced techniques the findings of the proposed protocol will reveal whether the menopause is associated with altered function of skin blood vessels and sweat glands, such as an overt sensitivity to vasoactive and sudorific substances that contribute to hot flushes (Iliodromiti et al., 2019; Sassarini et al., 2012). Furthermore, the study will examine whether the menopause affects the structure of skin blood vessels and/or sweat glands (Reus et al., 2020), which are sensitive to under/over activity (Abdel-Rahmen et al., 1992, Gunin et al., 2014, Green et al., 2017), e.g., more or larger skin blood vessels or sweat glands in symptomatic women, and whether a relationship exists between the function and/or structure of skin blood vessels and/or sweat glands, and the occurrence of hot flushes, e.g., an altered structure is compensated for by reciprocal changes in function. Observation of any such changes in function and/or structure would increase the understanding of the causes and side effects of hot flushes, which would have important implications for patients and clinicians by helping in the design of effective treatments. Such therapies may include interventions that can manipulate the activity of the skin blood vessels and/or sweat glands via pharmacological and non-pharmacological methods. The findings would also serve as preliminary data for further studies into cutaneous vascular and sudomotor structure and function in postmenopausal women, for example, examining structure and function in this population using a longitudinal design during the menopause transition and at various sites, e.g., limbs vs. the trunk.

4.3. The local axilla sweating responses to different forms of stress that trigger sweating in the axilla region.

The sweating response is typically considered with regards to thermoregulation, e.g., heat loss via the eccrine sweat glands. Sweating also occurs typically via apocrine glands in response to psychological stimuli such as stress. These apocrine glands are chiefly located in the groin and axillae and the sweat produced causes malodour and stains on clothing which can significantly affect a person's social interactions. Personal hygiene products are frequently used to abrogate axilla sweating and the examination of the efficacy of such products is therefore important. Research and understanding of axillae sweating responses to various and common stimuli are scarce, however. By examining the different stressors of exercise, passive heating and mental stress allowed an understanding of how each stressor influences sweat production in the armpits. Through this comparative analysis, the study aimed to provide insights into the mechanisms underlying axilla sweating, identify potential variations in responses to these stressors, and enhance our understanding of how different factors contribute to the overall sweating response in everyday life.

The findings indicated that all conditions caused an increase in sweat rate, however, the greatest increase was evident in the exercise condition and the smallest increase was displayed in the mental stress test. The larger sweat responses in the exercise and passive heating trials are not surprising given the robust thermal stress elicited in both trials, albeit via different pathways, e.g., larger elevations in core and skin temperatures in the exercise and passive heating trials, respectively. These sweat responses in the exercise and passive heating trials also provide evidence that elevations in axilla sweating do occur, presumably via eccrine sweat glands, in order to serve thermoregulatory control of body temperature. Future studies could further examine axilla sweating responses during exercise and passive heating trials with equivocal thermal

stress, e.g., similar elevations in core and skin temperatures to further isolate the independent effects of exercise vs. passive heating on axilla sweating.

The minimal variation in sweating observed during the mental stress condition may indicate that the mental stress tasks presented were not sufficiently challenging to elicit a significant sweating response. However, it is important to recognise the ethical considerations involved in designing experiments that induce stress; a balance must be struck to avoid causing excessive distress to participants. Consequently, the results obtained from the mental stress condition may not accurately reflect sweating responses in genuine high-stress environments or situations. Similarly, during the laboratory visits, participants did not report subjective assessments of their sweating. While thermal discomfort was recorded, the specific perception of whether individuals felt "sweaty" and or how it affected their wellbeing were not measured. It is important to note that a person may perceive themselves as sweaty without necessarily undergoing a large amount of sweating. Future studies should examine axillae sweating in more ecologically valid settings perhaps with mobile or ‘in situ’ sweat sensors alongside perceptual indices of sweating and associated sensations.

Many individual characteristics can affect sweating, such as body size, physical fitness and gender. For example, individuals who are more physically fit often exhibit a higher propensity to sweat and do so more quickly compared to those who are less fit, primarily due to a lower sweating threshold, which aids in maintaining optimal body temperature and preventing overheating. This phenomenon can be attributed to several physiological adaptations associated with regular exercise. These well accepted findings are evident for sweating from eccrine sweat glands on the limbs and or trunk of the body. Whether similar adaptations are also present for the apocrine sweat glands in the axillae or other dense populations of apocrine sweat glands such as the palms are not well known, however. Such information would further the understanding of

axillae sweating in different populations and aid in the design and application of personal hygiene products.

5. References

Abdel-Rahman TA, Collins KJ, Cowen T, Rustin M. Immunohistochemical, morphological and functional changes in the peripheral sudomotor neuro-effector system in elderly people. Journal of the autonomic nervous system. 1992;37(3):187-97.

Allan, J.R. and Wilson, C.G., 1971. Influence of acclimatization on sweat sodium concentration. *Journal of Applied Physiology*, *30*(5), pp.708-712.

Amano, T., Gerrett, N., Inoue, Y., Nishiyasu, T., Havenith, G. and Kondo, N., 2016. Determination of the maximum rate of eccrine sweat glands' ion reabsorption using the galvanic skin conductance to local sweat rate relationship. *European journal of applied physiology*, *116*, pp.281-290.

Armstrong, L.E., 2007. Assessing hydration status: the elusive gold standard. *Journal of the American College of Nutrition*, *26*(sup5), pp.575S-584S.

Asahina, M., Poudel, A. and Hirano, S., 2015. Sweating on the palm and sole: physiological and clinical relevance. *Clinical Autonomic Research*, *25*, pp.153-159.

Bailey, T.G., Cable, N.T., Aziz, N., Atkinson, G., Cuthbertson, D.J., Low, D.A. and Jones, H. (2016) Exercise training reduces the acute physiological severity of post-menopausal hot flushes. J Physiol, 594 (3), 657-667.

Bain, A.R., Deren, T.M. and Jay, O., 2011. Describing individual variation in local sweating during exercise in a temperate environment. *European journal of applied physiology*, *111*(8), pp.1599-1607.

Baker, L.B., 2019. Physiology of sweat gland function: The roles of sweating and sweat composition in human health. *Temperature*, *6*(3), pp.211-259.

Baker, L.B., Stofan, J.R., Hamilton, A.A. and Horswill, C.A., 2009. Comparison of regional patch collection vs. whole body washdown for measuring sweat sodium and potassium loss during exercise. *Journal of Applied Physiology*.

Baker LB. Sweating rate and sweat sodium concentration in athletes: a review of methodology and intra/interindividual variability. Sports Med 47: 111–128, 2017.

Bakos, O., Lundkvist, Ö., Wide, L. and Bergh, T., 1994. Ultrasonographical and hormonal description of the normal ovulatory menstrual cycle. *Acta obstetricia et gynecologica Scandinavica*, *73*(10), pp.790-796

Bar-Or O, Magnusson LI, Buskirk ER. Distribution of heat-activated sweat glands in obese and lean men and women. Hum Biol. 1968;40(2):235–248.

Beare, J.E., Curtis-Whitchurch, L., LeBlanc, A.J. and Hoying, J.B., 2018. Microvasculature in health and disease.

Bechlioulis, A., Kalantaridou, S.N., Naka, K.K., Chatzikyriakidou, A., Calis, K.A., Makrigiannakis, A., Papanikolaou, O., Kaponis, A., Katsouras, C., Georgiou, I., Chrousos, G.P. and Michalis, L.K. (2010) Endothelial Function, But Not Carotid Intima-Media Thickness, Is Affected Early in Menopause and Is Associated with Severity of Hot Flushes. The Journal of Clinical Endocrinology & Metabolism, 95 (3), 1199-1206. Bechlioulis, A., Naka, K.K., Kalantaridou, S.N., Kaponis, A., Papanikolaou, O., Vezyraki, P., Kolettis, T.M., Vlahos, A.P., Gartzonika, K. and Mavridis, A. (2012) Increased vascular inflammation in early menopausal women is associated with hot flush severity. Obstetrical & Gynecological Survey, 67 (11), 706-707.

Boisvert, P., Desruelle, A.V. and Candas, V., 1997. Comparison of sweat rate measured by a pouch collector and a hygrometric technique during exercise. *Canadian journal of applied physiology*, *22*(2), pp.161-170.

Boisvert, P., Nakamura, K., Shimai, S., Brisson, G.R. and Tanaka, M., 1993. A modified, local sweat collector for warm and humid conditions. *European journal of applied physiology and occupational physiology*, *66*, pp.547-551.

Boulant, J.A., 1981, December. Hypothalamic mechanisms in thermoregulation. In *Federation proceedings* (Vol. 40, No. 14, pp. 2843-2850).

Boulant, J.A., 1998. Hypothalamic neurons: Mechanisms of sensitivity to temperature a. *Annals of the New York Academy of Sciences*, *856*(1), pp.108-115.

Boulant, J.A., 2000. Role of the preoptic-anterior hypothalamus in thermoregulation and fever. *Clinical infectious diseases*, *31*(Supplement_5), pp.S157-S161.

Boulant, J.A., 2006. Neuronal basis of Hammel's model for set-point thermoregulation. *Journal of Applied Physiology*, *100*(4), pp.1347-1354.

Boulant, J.A. and Gonzalez, R.R., 1977. The effect of skin temperature on the hypothalamic control of heat loss and heat production. *Brain research*, *120*(2), pp.367-372.

Brebner, D.F. and Kerslake, D.M., 1964. The time course of the decline in sweating produced by wetting the skin. *The Journal of physiology*, *175*(2), p.295.

Brengelmann, G.L., Freund, P.R., Rowell, L.B., Olerud, J.E. and Kraning, K.K., 1981. Absence of active cutaneous vasodilation associated with congenital absence of sweat glands in humans. *American Journal of Physiology-Heart and Circulatory Physiology*, *240*(4), pp.H571-H575.

Brengelmann, G.L., McKeag, M. and Rowell, L.B., 1975. Use of dew-point detection for quantitative measurement of sweating rate. *Journal of applied physiology*, *39*(3), pp.498-500.

Briant, L.J.B., Charkoudian, N. and Hart, E.C., 2016. Sympathetic regulation of blood pressure in normotension and hypertension: when sex matters. *Experimental physiology*, *101*(2), pp.219-229.

Brislane, Á., 2019. *Peripheral and Cerebrovascular Function during the Female Lifespan*. Liverpool John Moores University (United Kingdom)

Brisson, G.R., Boisvert, P., Peronnet, F., Perrault, H., Boisvert, D. and Lafond, J.S., 1991. A simple and disposable sweat collector. *European journal of applied physiology and occupational physiology*, *63*, pp.269-272.

Butera, F.M., 1998. —Principles of thermal comfort. *Renewable and Sustainable Energy Reviews*, *2*(1-2), pp.39-66.

Cabanac, M. and Massonnet, B., 1977. Thermoregulatory responses as a function of core temperature in humans. *The Journal of physiology*, *265*(3), pp.587-596.

Cable, N.T., 2006. Unlocking the secrets of skin blood flow. *The Journal of Physiology*, *572*(Pt 3), p.613.

Casa DJ, Cheuvront SN, Galloway SD, et al. Fluid needs for training, competition, and recovery in track-and-field athletes. *Int J Sport Nutr Exerc Metab*. 2019;29(2):175–180.

Candas, V., Libert, J.P. and Vogt, J.J., 1983. Sweating and sweat decline of resting men in hot humid environments. *European journal of applied physiology and occupational physiology*, *50*, pp.223-234.

Chakrabarti, S., Morton, J.S. and Davidge, S.T., 2014. Mechanisms of estrogen effects on the endothelium: an overview. *Canadian Journal of cardiology*, *30*(7), pp.705-712.

Chalmers, T.M. and Keele, C.A., 1952. The nervous and chemical control of sweating. *British Journal of Dermatology*, *64*(2), pp.43-54.

Chamberlain, R.S., Huber, K., White, J.C. and Travaglino-Parda, R., 1999. Apocrine gland carcinoma of the axilla: review of the literature and recommendations for treatment. *American journal of clinical oncology*, *22*(2), pp.131-135.

Charkoudian, N., 2003, May. Skin blood flow in adult human thermoregulation: how it works, when it does not, and why. In *Mayo clinic proceedings* (Vol. 78, No. 5, pp. 603-612). Elsevier.

Charkoudian, N., 2010. Mechanisms and modifiers of reflex induced cutaneous vasodilation and vasoconstriction in humans. *Journal of applied physiology*, *109*(4), pp.1221-1228.

Charkoudian, N. and Stachenfeld, N.S., 2014. Reproductive hormone influences on thermoregulation in women. *Compr Physiol*, *4*(2), pp.793-804.

Cheuvront, S.N., Haymes, E.M. and Sawka, M.N., 2002. Comparison of sweat loss estimates for women during prolonged high-intensity running. *Medicine and science in sports and exercise*, *34*(8), pp.1344-1350.

Chow RWY, Handelsman DJ, Ng MKC. Minireview: Rapid Actions of Sex Steroids in the Endothelium. Endocrinology. 2010;151(6):2411-22.

Chu, B., Marwaha, K., Sanvictores, T., Awosika, A.O. and Ayers, D., 2024. Physiology, stress reaction. In *StatPearls [Internet]*. StatPearls Publishing.

Cole, D.E. and Boucher, M.J., 1986. Use of a new sample-collection device (Macroduct) in anion analysis of human sweat. *Clinical chemistry*, *32*(7), pp.1375-1378.

Collins, K.J. and Weiner, J.S., 1962. Observations on arm-bag suppression of sweating and its relationship to thermal sweat-gland fatigue. *The Journal of Physiology*, *161*(3), p.538.

Costill DL. Sweating: its composition and effects on body fluids. *Ann N Y Acad Sci*. 1977;301:160–174.

Cracowski, J.L., Minson, C.T., Salvat-Melis, M. and Halliwill, J.R., 2006. Methodological issues in the assessment of skin microvascular endothelial function in humans. *Trends in pharmacological sciences*, *27*(9), pp.503-508.

Cracowski, J.L. and Roustit, M., 2016. Current methods to assess human cutaneous blood flow: an updated focus on laser-based-techniques. *Microcirculation*, *23*(5), pp.337-344.

Cramer, M.N. and Jay, O., 2016. Biophysical aspects of human thermoregulation during heat stress. *Autonomic Neuroscience*, *196*, pp.3-13.

Crandall, C.G. and Wilson, T.E., 2015. Human cardiovascular responses to passive heat stress. *Comprehensive Physiology*, *5*(1), p.17.

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. J Physiol, 586 (Pt 1), 293-301.

Crawshaw, L., Grahn, D., Wollmuth, L. and Simpson, L., 1985. Central nervous regulation of body temperature in vertebrates: comparative aspects. *Pharmacology & therapeutics*, *30*(1), pp.19-30.

Darrow, C.W., 1937. Neural mechanisms controlling the palmar galvanic skin reflex and palmar sweating: a consideration of available literature. *Archives of Neurology & Psychiatry*, *37*(3), pp.641-663.

Davidson, J.R., Foa, E.B., Connor, K.M. and Churchill, L.E., 2002. Hyperhidrosis in social anxiety disorder. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *26*(7-8), pp.1327-1331.

Davis, S.L., Shibasaki, M., Low, D.A., Cui, J., Keller, D.M., Purdue, G.F., Hunt, J.L., Arnoldo, B.D., Kowalske, K.J. and Crandall, C.G., 2007. Skin grafting impairs postsynaptic cutaneous vasodilator and sweating responses. *Journal of burn care & research*, *28*(3), pp.435-441.

Dawson, E.A. and Reilly, T., 2009. Menstrual cycle, exercise and health. *Biological Rhythm Research*, *40*(1), pp.99-119.

Deecher, D.C., 2005. Physiology of thermoregulatory dysfunction and current approaches to the treatment of vasomotor symptoms. *Expert Opinion on Investigational Drugs*, *14*(4), pp.435-448.

Deuflhard, P. and Hochmuth, R., 2004. Multiscale analysis of thermoregulation in the human microvascular system. *Mathematical methods in the applied sciences*, *27*(8), pp.971-989.

Diwadkar, V.A., Murphy, E.R. and Freedman, R.R. (2013) Temporal sequencing of brain activations during naturally occurring thermoregulatory events. Cerebral Cortex, 24 (11), 3006-3013.

Drummond, P.D. and Lance, J.W., 1987. Facial flushing and sweating mediated by the sympathetic nervous system. *Brain*, *110*(3), pp.793-803.

Dziedzic, C.E., Ross, M.L., Slater, G.J. and Burke, L.M., 2014. Variability of measurements of sweat sodium using the regional absorbent-patch method. *International journal of sports physiology and performance*, *9*(5), pp.832-838.

Eaker, E.D., Chesebro, J.H., Sacks, F.M., Wenger, N.K., Whisnant, J.P. and Winston, M., 1993. Cardiovascular disease in women. *Circulation*, *88*(4), pp.1999-2009.

Ecochard, R. and Gougeon, A., 2000. Side of ovulation and cycle characteristics in normally fertile women. *Human Reproduction*, *15*(4), pp.752-755

Farrell, D.M. and Bishop, V.S., 1995. Permissive role for nitric oxide in active thermoregulatory vasodilation in rabbit ear. *American Journal of Physiology-Heart and Circulatory Physiology*, *269*(5), pp.H1613-H1618.

Fogarty, A., Bartlett, R., Ventenat, V. and Havenith, G., 2007. Regional foot sweat rates during a 65 minute uphill walk with a backpack.

Foster, K.G. and Weiner, J.S., 1970. Effects of cholinergic and adrenergic blocking agents on the activity of the eccrine sweat glands. *The Journal of Physiology*, *210*(4), p.883.

Förstermann, U. and Sessa, W.C., 2012. Nitric oxide synthases: regulation and function. *European heart journal*, *33*(7), pp.829-837.

Frank, S.M., Raja, S.N., Bulcao, C.F. and Goldstein, D.S., 1999. Relative contribution of core and cutaneous temperatures to thermal comfort and autonomic responses in humans. *Journal of Applied physiology*, *86*(5), pp.1588-1593.

Freedman, R.R. (2002) Core body temperature variation in symptomatic and asymptomatic postmenopausal women: brief report. Menopause, 9 (6), 399-401.

Freedman, R.R., 2014. Menopausal hot flashes: mechanisms, endocrinology, treatment. *The Journal of steroid biochemistry and molecular biology*, *142*, pp.115-120.

Freedman, R.R. and Krell, W. (1999) Reduced thermoregulatory null zone in postmenopausal women with hot flashes. American Journal of Obstetrics & Gynecology, 181 (1), 66-70.

Freeman, E. and Sherif, K., 2007. Prevalence of hot flushes and night sweats around the world: a systematic review. *Climacteric*, *10*(3), pp.197-214..

Freedman, R.R. and Woodward, S. (1996) Core body temperature during menopausal hot flushes. Fertil Steril, 65 (6), 1141-1144.

Fritel, X., Ringa, V., Quiboeuf, E. and Fauconnier, A., 2012. Female urinary incontinence, from pregnancy to menopause: a review of epidemiological and pathophysiological findings. *Acta obstetricia et gynecologica Scandinavica*, *91*(8), pp.901-910.

Gangula PRR, Lanlua P, Wimalawansa S, Supowit S, DiPette D, Yallampalli C. Regulation of Calcitonin Gene-Related Peptide Expression in Dorsal Root Ganglia of Rats by Female Sex Steroid Hormones1. Biology of Reproduction. 2000;62(4):1033-9.

Ganong, W. (2001) Review of medical physiology. New York (NY): McGraw, Hill.

Gagnon, D. and Crandall, C.G., 2018. Sweating as a heat loss thermoeffector. *Handbook of clinical neurology*, *156*, pp.211-232.

Gonzalez-Alonso, J., & Calbet, J. A. L. (2003). "Reductions in systemic and cutaneous blood flow during passive heating in humans." *Journal of Physiology*, 552(1), 305-313. doi:10.1113/jphysiol.2003.048198.

George, S.A., 2002. The menopause experience: a woman's perspective. *Journal of Obstetric, Gynecologic, & Neonatal Nursing*, *31*(1), pp.77-85.

Gisolfi CV and Wenger CB. Temperature regulation during exercise: old concepts, new ideas. Exerc Sport Sci Rev 12: 339–372, 1984

Gleeson, M., 1998. Temperature regulation during exercise. *International Journal of Sports Medicine*, *19*(S 2), pp.S96-S99.

Graichen, H., Rascati, R. and Gonzalez, R.R., 1982. Automatic dew-point temperature sensor. *Journal of applied physiology*, *52*(6), pp.1658-1660.

Gray, K.E., Katon, J.G., LeBlanc, E.S., Woods, N.F., Bastian, L.A., Reiber, G.E., Weitlauf, J.C., Nelson, K.M. and LaCroix, A.Z. (2018) Vasomotor symptom characteristics: are they risk factors for incident diabetes? Menopause

Green DJ, Hopman MT, Padilla J, Laughlin MH, Thijssen DH. Vascular Adaptation to Exercise in Humans: Role of Hemodynamic Stimuli. Physiol Rev. 2017;97(2):495-528.

Griffin, J.D., Kaple, M.L., Chow, A.R. and Boulant, J.A., 1996. Cellular mechanisms for neuronal thermosensitivity in the rat hypothalamus. *The Journal of physiology*, *492*(1), pp.231-242.

Gundlah, C., Pecins-Thompson, M., Schutzer, W.E. and Bethea, C.L., 1999. Ovarian steroid effects on serotonin 1A, 2A and 2C receptor mRNA in macaque hypothalamus. *Molecular brain research*, *63*(2), pp.325-339.

Gunin AG, Petrov VV, Vasil'eva OV, Golubtsova NN. [Blood vessels in human dermis during aging]. Adv Gerontol. 2014;27(1):54-61.

Harker, M., 2013. Psychological sweating: a systematic review focused on aetiology and cutaneous response. *Skin pharmacology and physiology*, *26*(2), pp.92-100.

Harlow, S.D., Gass, M., Hall, J.E., Lobo, R., Maki, P., Rebar, R.W., Sherman, S., Sluss, P.M., De Villiers, T.J. and STRAW+ 10 Collaborative Group, 2012. Executive summary of the Stages of Reproductive Aging Workshop+ 10: addressing the unfinished agenda of staging reproductive aging. *The Journal of Clinical Endocrinology & Metabolism*, *97*(4), pp.1159-1168.

Harrison, J. and MacKinnon, P.C., 1966. Physiological role of the adrenal medulla in the palmar anhidrotic response to stress. *Journal of Applied Physiology*, *21*(1), pp.88-92.

Havenith, G., Fogarty, A., Bartlett, R., Smith, C.J. and Ventenat, V., 2008. Male and female upper body sweat distribution during running measured with technical absorbents. *European journal of applied physiology*, *104*(2), pp.245-255.

Havenith, G., Fogarty, A, Bartlett, R., Smith, C., and Ventenat, V (2008) Upper body sweat distribution during and after a 60 minute training run in male and female runners Eur J Appl. Physiol I 04:245-255

Hellsten, Y. and Gliemann, L., 2018. Limb vascular function in women—Effects of female sex hormones and physical activity. *Translational Sports Medicine*, *1*(1), pp.14-24.

Hensel, H., 1973. Neural processes in thermoregulation. *Physiological reviews*, *53*(4), pp.948-1017.

Hertzman, A.B (1957). Individual differences m regional sweating J. Appl. Physiol. I 0(2) 242-248

Hodge, B.D., Sanvictores, T. and Brodell, R.T., 2018. Anatomy, skin sweat glands.

Holm, A. and Nilsson, B.O., 2013. Identification and characterization of new mechanisms in vascular oestrogen signalling. *Basic & clinical pharmacology & toxicology*, *113*(5), pp.287-293.

Hu, Y., Converse, C., Lyons, M.C. and Hsu, W.H., 2018. Neural control of sweat secretion: a review. *British Journal of Dermatology*, *178*(6), pp.1246-1256.

Hubing KA, Wingo JE, Brothers RM, Del Coso J, Low DA, Crandall CG. Nitric oxide synthase inhibition attenuates cutaneous vasodilation during postmenopausal hot flash episodes. Menopause. 2010;17(5):978-82.

Hymczak H, Gołąb A, Mendrala K, Plicner D, Darocha T, Podsiadło P, Hudziak D, Gocoł R, Kosiński S. Core Temperature Measurement-Principles of Correct Measurement, Problems, and Complications. Int J Environ Res Public Health. 2021 Oct 10;18(20

Iliodromiti S, Sattar N, Delles C, Nelson SM, Gill JMR, Lumsden M. Menopausal hot flashing and endothelial function in two vascular beds: findings from a cross-sectional study of postmenopausal women. Menopause. 2019;26(9):1002-9.

Inoue Y, Tanaka Y, Omori K, Kuwahara T, Ogura Y, Ueda H. Sex- and menstrual cycle-related differences in sweating and cutaneous blood flow in response to passive heat exposure. Eur J Appl Physiol 94: 323–332, 2005.

Iwase, S., Ikeda, T., Kitazawa, H., Hakusui, S., Sugenoya, J. and Mano, T., 1997. Altered response in cutaneous sympathetic outflow to mental and thermal stimuli in primary palmoplantar hyperhidrosis. *Journal of the autonomic nervous system*, *64*(2-3), pp.65-73.

Jayasena, C.N., Comninos, A.N., Stefanopoulou, E., Buckley, A., Narayanaswamy, S., Izzi-Engbeaya, C., Abbara, A., Ratnasabapathy, R., Mogford, J. and Ng, N. (2015) Neurokinin B administration induces hot flushes in women. Scientific reports, 5, 8466.

Johnson, J.M., 1996. Cardiovascular adjustments to heat stress. *Environmental physiology*, pp.215-243.

Johnson, J.M., Brengelmann, G.L., Hales, J.R., Vanhoutte, P.M. and Wenger, C.B., 1986, December. Regulation of the cutaneous circulation. In *Federation proceedings* (Vol. 45, No. 13, pp. 2841-2850).

Johnson, J.M., Minson, C.T. and Kellogg Jr, D.L., 2011. Cutaneous vasodilator and vasoconstrictor mechanisms in temperature regulation. *Comprehensive physiology*, *4*(1), pp.33-89.

Johnson JM, Minson CT, Kellogg DL, Jr. Cutaneous vasodilator and vasoconstrictor mechanisms in temperature regulation. Comprehensive Physiology. 2014;4(1):33-89.

Kahn, R.H., 1904. Über die Erwärmung des Carotidenblutes. *Arch. Anat. Physiol. Suppl*, *1904*, pp.81-134.

Kamei, T., Naitoh, K., Nakashima, K., Ohhashi, T., Kitagawa, S. and Tsuda, T., 1997. Physical stimuli and emotional stress-induced sweat secretions in the human palm and forehead, Japan J. *Perspiration Research*, *4*, pp.11-15.

Karpinski, Christine, and Christine A. Rosenbloom. *Sports nutrition: a handbook for professionals*. Academy of Nutrition and Dietetics, 2017.

Kellogg Jr, D.L., 2006. In vivo mechanisms of cutaneous vasodilation and vasoconstriction in humans during thermoregulatory challenges. *Journal of applied physiology*, *100*(5), pp.1709-1718.

Kellogg Jr, D.L., Liu, Y., Kosiba, I.F. and O'Donnell, D., 1999. Role of nitric oxide in the vascular effects of local warming of the skin in humans. *Journal of Applied Physiology*, *86*(4), pp.1185-1190.

Kenefick, R.W., Cheuvront, S.N., Elliott, L.D., Ely, B.R. and Sawka, M.N., 2012. Biological and analytical variation of the human sweating response: implications for study design and analysis. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *302*(2), pp.R252-R258.

Kenney, W.L. and Johnson, J.M., 1992. Control of skin blood flow during exercise. *Medicine and science in sports and exercise*, *24*(3), pp.303-312.

Kimura, K., Low, D.A., Keller, D.M., Davis, S.L. and Crandall, C.G., 2007. Cutaneous blood flow and sweat rate responses to exogenous administration of acetylcholine and methacholine. *Journal of applied physiology*, *102*(5), pp.1856-1861.

K. Kita, K. Hirayama, I. Moroo, A. Hatakeyama, N. Kawaguchi, T. Hattori

J. Perspiration Res., 4 (1) (1997), pp. 31-33

Kobayashi, M., Tomioka, N., Ushiyama, Y. and Ohhashi, T., 2003. Arithmetic calculation, deep inspiration or handgrip exercise-mediated pre-operational active palmar sweating responses in humans. *Autonomic Neuroscience*, *104*(1), pp.58-65.

Kolka, M.A. and Stephenson, L.A., 1987. Cutaneous blood flow and local sweating after systemic atropine administration. *Pflügers Archiv-European Journal of Physiology*, *410*(4), pp.524-529.

Kondo, N., Taylor, N.A., Shibasaki, M., Aoki, K. and Che Muhamed, A.M., 2009. Thermoregulatory adaptation in humans and its modifying factors. *Glob Environ Res Glob*, *13*, pp.35-41.

Kritikos, J., Alevizopoulos, G. and Koutsouris, D., 2021. Personalized virtual reality human-computer interaction for psychiatric and neurological illnesses: a dynamically adaptive virtual reality environment that changes according to real-time feedback from electrophysiological signal responses. *Frontiers in Human Neuroscience*, *15*, p.596980.

Kuno Y. Human perspiration. Springfield (IL): Charles C. Thomas Publisher; 1956.

KUNO, Y., 1965. The mechanism of human perspiration and sweating in exercise. *Japanese Journal of Physical Fitness and Sports Medicine*, *14*(2).

Kuno Y. Variations in secretory activity of human sweat glands. Lancet. 1938;1:299–303

Kurz, A., 2008. Physiology of thermoregulation. *Best Practice & Research Clinical Anaesthesiology*, *22*(4), pp.627-644.

Knopp, R.H., Zhu, X. and Bonet, B., 1994. Effects of estrogens on lipoprotein metabolism and cardiovascular disease in women. *Atherosclerosis*, *110*, pp.S83-S91.

Lauria, G. and Devigili, G., 2007. Skin biopsy as a diagnostic tool in peripheral neuropathy. *Nature clinical practice Neurology*, *3*(10), pp.546-557.

Lauria, G., Hsieh, S.T., Johansson, O., Kennedy, W.R., Leger, J.M., Mellgren, S.I., Nolano, M., Merkies, I.S., Polydefkis, M., Smith, A.G. and Sommer, C., 2010. European Federation of Neurological Societies/Peripheral Nerve Society Guideline on the use of skin biopsy in the diagnosis of small fiber neuropathy. Report of a joint task force of the European Fe-deration of Neurological Societies and the Peripheral Nerve Society. *European journal of neurology*, *17*(7), pp.903-e49.

Lawton, S.J.N.T., 2019. Skin 1: the structure and functions of the skin. *Nurs. times*, *115*, pp.30-33.

Lerner, D.J. and Kannel, W.B., 1986. Patterns of coronary heart disease morbidity and mortality in the sexes: a 26-year follow-up of the Framingham population. *American heart journal*, *111*(2), pp.383-390.

Levy, B.I., Ambrosio, G., Pries, A.R. and Struijker-Boudier, H.A.J., 2001. Microcirculation in hypertension: a new target for treatment?. *Circulation*, *104*(6), pp.735-740.

List, C.F. and Peet, M.M., 1938. Sweat secretion in man: I. Sweating responses in normal persons. *Archives of Neurology & Psychiatry*, *39*(6), pp.1228-1237.

Low, D.A., Davis, S.L., Keller, D.M., Shibasaki, M. and Crandall, C.G., 2008. Cutaneous and hemodynamic responses during hot flashes in symptomatic postmenopausal women. *Menopause (New York, NY)*, *15*(2), p.290.

Low DA, Hubing KA, Del Coso J, Crandall CG. Mechanisms of cutaneous vasodilation during the postmenopausal hot flash. Menopause. 2011;18(4):359-65

Nielsen, B., Hales, J.R., Strange, S., Christensen, N.J., Warberg, J. and Saltin, B., 1993. Human circulatory and thermoregulatory adaptations with heat acclimation and exercise in a hot, dry environment. *The Journal of physiology*, *460*(1), pp.467-485.

Machado-Moreira, C.A., Smith, F.M., van den Heuvel, A.M., Mekjavic, I.B. and Taylor, N.A., 2008. Sweat secretion from the torso during passively-induced and exercise-related hyperthermia. *European journal of applied physiology*, *104*, pp.265-270.

Machado-Moreira, C.A. and Taylor, N.A., 2012. Psychological sweating from glabrous and nonglabrous skin surfaces under thermoneutral conditions. *Psychophysiology*, *49*(3), pp.369-374.

Machado-Moreira, C.A. and Taylor, N.A.S., 2012. Sudomotor responses from glabrous and non-glabrous skin during cognitive and painful stimulations following passive heating. *Acta Physiologica*, *204*(4), pp.571-581.

Makena Hightower, C., Salazar Vázquez, B.Y., Woo Park, S., Sriram, K., Martini, J., Yalcin, O., Tsai, A.G., Cabrales, P., Tartakovsky, D.M., Johnson, P.C. and Intaglietta, M., 2011. Integration of cardiovascular regulation by the blood/endothelium cell-free layer. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, *3*(4), pp.458-470

Mandrup, C.M., Egelund, J., Nyberg, M., Slingsby, M.H.L., Andersen, C.B., Løgstrup, S., Bangsbo, J., Suetta, C., Stallknecht, B. and Hellsten, Y., 2017. Effects of high-intensity training on cardiovascular risk factors in premenopausal and postmenopausal women. *American journal of obstetrics and gynecology*, *216*(4), pp.384-e1.

Marcus, R., 2002. Post-menopausal osteoporosis. *Best practice & research Clinical obstetrics & gynaecology*, *16*(3), pp.309-327.

Marriott, B.M. ed., 1993. Nutritional Needs in hot environments: Applications for military personnel in field operations.

Martin GR (1994) Vascular receptors for 5-hydroxytryptamine: distribution, function and classification. Pharmacol Ther 62: 283–324

Matthews, K.A., Crawford, S.L., Chae, C.U., Everson-Rose, S.A., Sowers, M.F., Sternfeld, B. and Sutton-Tyrrell, K., 2009. Are changes in cardiovascular disease risk factors in midlife women due to chronological aging or to the menopausal transition?. *Journal of the American College of Cardiology*, *54*(25), pp.2366-2373.

Maughan, R.J., Shirreffs, S.M. and Leiper, J.B., 2007. Errors in the estimation of hydration status from changes in body mass. *Journal of sports sciences*, *25*(7), pp.797-804.

Mendelsohn, M.E. and Karas, R.H., 1999. The protective effects of estrogen on the cardiovascular system. *New England journal of medicine*, *340*(23), pp.1801-1811.

Menopause, W.C., 2010. What is menopause?. *Échographie et imagerie pelvienne en pratique gynécologique*, p.193.

Minson, C.T., Holowatz, L.A., Wong, B.J., Kenney, W.L. and Wilkins, B.W., 2002. Decreased nitric oxide-and axon reflex-mediated cutaneous vasodilation with age during local heating. *Journal of Applied Physiology*, *93*(5), pp.1644-1649.

Gonzalez, R.R., and Pandolf, K B (1984). Heat exchange during upper and lower-body exercise. J. Appl Physwl 57(4):1050-1054

Montagna W, Parakkal PF. Eccrine sweat glands. In: Montagna W, Parakkal PF, editors. The structure and function of skin. New York (NY): Academic Press, Inc.; 1974. p. 366–411

healthy women. *Advances in vascular medicine*, *2014*.

Moreau, K.L., Hildreth, K.L., Meditz, A.L., Deane, K.D. and Kohrt, W.M., 2012. Endothelial function is impaired across the stages of the menopause transition in healthy women. *The Journal of Clinical Endocrinology & Metabolism*, *97*(12),pp.4692-4700.

Moreau, K.L., Meditz, A., Deane, K.D. and Kohrt, W.M., 2012. Tetrahydrobiopterin improves endothelial function and decreases arterial stiffness in estrogen-deficient postmenopausal women. *American Journal of Physiology-Heart and Circulatory Physiology*, *302*(5), pp.H1211-H1218.

Morris, N.B., Cramer, M.N., Hodder, S.G., Havenith, G. and Jay, O., 2013. A comparison between the technical absorbent and ventilated capsule methods for measuring local sweat rate. *Journal of applied physiology*, *114*(6), pp.816-823.

Morrison, S.F. and Nakamura, K., 2019. Central mechanisms for thermoregulation. *Annual review of physiology*, *81*(1), pp.285-308.

Mosca, L., Hammond, G., Mochari-Greenberger, H., Towfighi, A. and Albert, M.A., 2013. American Heart Association Cardiovascular Disease and Stroke in Women and Special Populations Committee of the Council on Clinical Cardiology, Council on Epidemiology and Prevention, Council on Cardiovascular Nursing, Council on High Bloo. Fifteen-year trends in awareness of heart disease in women: results of a 2012 American Heart Association national survey. *Circulation*, *127*(11), pp.1254-1263.

Müller, A.M., Hermanns, M.I., Skrzynski, C., Nesslinger, M., Müller, K.M. and Kirkpatrick, C.J., 2002. Expression of the endothelial markers PECAM-1, vWf, and CD34 in vivo and in vitro. *Experimental and molecular pathology*, *72*(3), pp.221-229.

Nair, A.R., Pillai, A.J. and Nair, N., 2021. Cardiovascular changes in menopause. *Current cardiology reviews*, *17*(4).

Nadel, E.R., Bullard, R.W. and Stolwijk, J.A., 1971. Importance of skin temperature in the regulation of sweating. *Journal of applied physiology*, *31*(1), pp.80-87

Nagashima, K, Tokizawa, K. and Marui, S (2018). Thermal comfort. In *Handbook of Clinical Neurology*, (Editor Andrej A. Romanovsky). 156, 249-260. London: Elsevier.

Najjar, S.S., Scuteri, A. and Lakatta, E.G. (2005) Arterial aging: is it an immutable cardiovascular risk factor? Hypertension, 46 (3), 454-462.

Nielsen, B., Hales, J.R., Strange, S., Christensen, N.J., Warberg, J. and Saltin, B., 1993. Human circulatory and thermoregulatory adaptations with heat acclimation and exercise in a hot, dry environment. *The Journal of physiology*, *460*(1), pp.467-485.

Nielsen, B. and NIELSEN, M., 1965. Influence of passive and active heating on the temperature regulation of man. *Acta Physiologica Scandinavica*, *64*(4), pp.323-331..

Nielsen, M., 1938. Die regulation der körpertemperatur bei muskelarbeit 1. *Skandinavisches Archiv für Physiologie*, *79*(2), pp.193-230.

Nillson, G E (1977). Measurement of water exchange through skin. Med Bwl. Eng. Comput 15·209-218

Nyberg, M., Seidelin, K., Andersen, T.R., Overby, N.N., Hellsten, Y. and Bangsbo, J., 2014. Biomarkers of vascular function in premenopausal and recent postmenopausal women of similar

age: effect of exercise training. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *306*(7), pp.R510-R517.

Nakazato, Y., Tamura, N., Ohkuma, A., Yoshimaru, K. and Shimazu, K., 2004. Idiopathic pure sudomotor failure: anhidrosis due to deficits in cholinergic transmission. *Neurology*, *63*(8), pp.1476-1480.

Noppen M, Sevens C, Vincken WG: Effects of non-pharmacological sympathetic sudomotor denervation on sweating in humans with essential palmar hyperhidrosis. Clin Biochem 1997, 30:171–175.

Notley SR, Dervis S, Poirier MP, Kenny GP. Menstrual cycle phase does not modulate whole body heat loss during exercise in hot, dry conditions. J Appl Physiol (1985). 2019 Feb 1;126(2):286-293.

Ohhashi, T. and Uono, M., 1993. Palmer perspiration–measurement and clinical applications. *Life Medicom Co, Ltd., Nagoya*.

Ogawa, T., and Bullard, R.W. (1972). Charactenstlcs of subthreshold sudomotro neural impulses J Appl. Physwl 33(3):300-305

Ohara, K.O.K.I.C.H.I., 1968. Heat tolerance and sweating type. *Nagoya medical journal*, *14*(3), pp.133-144.

Park, S.J., Goldsmith, L.T. and Weiss, G., 2002. Age-related changes in the regulation of luteinizing hormone secretion by estrogen in women. *Experimental Biology and Medicine*, *227*(7), pp.455-464.

Périard, J.D., Racinais, S. and Sawka, M.N., 2015. Adaptations and mechanisms of human heat acclimation: applications for competitive athletes and sports. *Scandinavian journal of medicine & science in sports*, *25*, pp.20-38.

Périard, J.D., Travers, G.J., Racinais, S. and Sawka, M.N., 2016. Cardiovascular adaptations supporting human exercise-heat acclimation. *Autonomic Neuroscience*, *196*, pp.52-62.

Polotsky, H.N. and Polotsky, A.J., 2010, September. Metabolic implications of menopause. In *Seminars in reproductive medicine* (Vol. 28, No. 05, pp. 426-434). © Thieme Medical Publishers.

Quesada, A. and Etgen, A.M., 2000. Tyrosine kinase effects on adrenoceptor-stimulated cyclic AMP accumulation in preoptic area and hypothalamus of female rats: modulation by estradiol. *Brain research*, *861*(1), pp.117-125.

Racinais, S., Cocking, S. and Périard, J.D., 2017. Sports and environmental temperature: from warming-up to heating-up. *Temperature*, *4*(3), pp.227-257.

RANDALL, W.C., 1953. The physiology of sweating. *American Journal of Physical Medicine & Rehabilitation*, *32*(5), pp.292-318.

Rea, H. and Kirby, B., 2019. A review of cutaneous microdialysis of inflammatory dermatoses. *Acta Dermato-Venereologica*, *99*(11), pp.945-952.

Reed, B.G. and Carr, B.R., 2015. The normal menstrual cycle and the control of ovulation.

Reus TL, Brohem CA, Schuck DC, Lorencini M. Revisiting the effects of menopause on the skin: Functional changes, clinical studies, in vitro models and therapeutic alternatives. Mechanisms of Ageing and Development. 2020;185:111193.

Roberts, H. and Hickey, M., 2016. Managing the menopause: An update. *Maturitas*, *86*, pp.53-58.

Robertshaw, D., 1977. Neuroendocrine control of sweat glands. *Journal of Investigative Dermatology*, *69*(1), pp.121-129.

Robertshaw D. Apocrine sweat glands. In: Goldsmith LA, editor. Biochemistry and physiology of the skin. New York: Oxford University Press, Inc.; 1983. p. 642–653

Roddie, I.C., 2003. Sympathetic vasodilatation in human skin. *The Journal of physiology*, *548*(Pt 2), p.336.

Roger, V.L., Go, A.S., Lloyd-Jones, D.M., Benjamin, E.J., Berry, J.D., Borden, W.B., Bravata, D.M., Dai, S., Ford, E.S., Fox, C.S. and Fullerton, H.J., 2012. Executive summary: heart disease and stroke statistics-2012 update: a report from the American Heart Association. *Circulation*, *125*(1), pp.188-197.

Rosano, G.M.C., Vitale, C., Marazzi, G. and Volterrani, M., 2007. Menopause and cardiovascular disease: the evidence. *Climacteric*, *10*(sup1), pp.19-24.

Roustit, M. and Cracowski, J.L., 2013. Assessment of endothelial and neurovascular function in human skin microcirculation. *Trends in pharmacological sciences*, *34*(7), pp.373-384.

Sassarini J, Fox H, Ferrell W, Sattar N, Lumsden MA. Hot flushes, vascular reactivity and the role of the alpha-adrenergic system. Climacteric : the journal of the International Menopause Society. 2012;15(4):332-8.

Sassarini, J. and Lumsden, M.A., 2015. Vascular function and cardiovascular risk factors in women with severe flushing. *Maturitas*, *80*(4), pp.379-383.

Sato F, Owen M, Matthes R, et al. Functional and morphological changes in the eccrine sweat gland with heat acclimation. J Appl Physiol (1985). 1990;69 (1):232–236.

Sato K, Dobson RL. Regional and individual variations in the function of the human eccrine sweat gland. J Invest Dermatol. 1970;54(6):443–449.

Sato, K., Kang, W.H., Saga, K. and Sato, K.T., 1989. Biology of sweat glands and their disorders. II. Disorders of sweat gland function. *Journal of the American Academy of Dermatology*, *20*(5), pp.713-726.

Sato K. The physiology and pharmacology of the eccrine sweat gland In: Goldsmith L, editor. Biochemistry and physiology of the skin. New York: Oxford University Press; 1983. p. 596–641

Sato K, Leidal R, Sato F. Morphology and development of an apoeccrine sweat gland in human axillae. Am J Physiol. 1987;252(1 Pt 2):R166–80.

Sato K, Ohtsuyama M, Samman G. Eccrine sweat gland disorders. J Am Acad Dermatol. 1991;24(6 Pt 1):1010–1014.

SAWKA, M. and Wenger, C.B., 1988. Physiological responses to acute exercise-heat stress.

Sawka, M.N., 2007. 442. *Medicine & Science in Sports & Exercise*, *39*(5), p.66.

Sawka, M.N. and Coyle, E.F., 1999. Influence of body water and blood volume on thermoregulation and exercise performance in the heat. *Exercise and sport sciences reviews*, *27*, pp.167-218.

Sawka, M.N., Leon, L.R., Montain, S.J. and Sonna, L.A., 2011. Integrated physiological mechanisms of exercise performance, adaptation, and maladaptation to heat stress. *Comprehensive Physiology*, *1*(4), pp.1883-1928.

Sawka, M.N., Wenger, C.B., Young, A.J. and Pandolf, K.B., 1993. Physiological responses to exercise in the heat. *Nutritional needs in hot environments: applications for military personnel in field operations*, *55*.

Schindelin, J., Rueden, C.T., Hiner, M.C. and Eliceiri, K.W., 2015. The ImageJ ecosystem: An open platform for biomedical image analysis. *Molecular reproduction and development*, *82*(7-8), pp.518-529.

Schote, A.B., Dietrich, K., Linden, A.E., Dzionsko, I., Molano Moreno, L.D.L.A., Winnikes, U., Zimmer, P., Domes, G. and Meyer, J., 2022. Real sweating in a virtual stress environment:

investigation of the stress reactivity in people with primary focal hyperhidrosis. *Plos one*, *17*(8), p.e0272247.

Service, T.W., Junker, K., Service, B., Coehoorn, C.J., Harrington, M., Martin, S. and Stuart-Hill, L.A., 2023. An assessment of the validity and reliability of the P022–P version of e-Celsius core temperature capsules. *Journal of Thermal Biology*, *112*, p.103486.

Shanafelt, T.D., Barton, D.L., Adjei, A.A. and Loprinzi, C.L., 2002, November. Pathophysiology and treatment of hot flashes. In *Mayo Clinic Proceedings* (Vol. 77, No. 11, pp. 1207-1218). Elsevier.

Shibasaki, M. and Crandall, C.G., 2010. Mechanisms and controllers of eccrine sweating in humans. *Frontiers in bioscience (Scholar edition)*, *2*, p.685.

Shibasaki M, Wilson TE, Crandall CG. Neural control and mechanisms of eccrine sweating during heat stress and exercise. J Appl Physiol. 2006;100(5):1692-701.

Shirreffs SM, Sawka MN. Fluid and electrolyte needs for training, competition, and recovery. J Sports Sci. 2011;29(Suppl 1):S39–46.

Simon, E., 2000. The enigma of deep-body thermosensory specificity. *International Journal of Biometeorology*, *44*, pp.105-120.

Sloan, J.A., Loprinzi, C.L., Novotny, P.J., Barton, D.L., Lavasseur, B.I. and Windschitl, H., 2001. Methodologic lessons learned from hot flash studies. *Journal of Clinical Oncology*, *19*(23), pp.4280-4290.

Smith, C.J., 2009. *Regional sweat rates in humans* (Doctoral dissertation, Loughborough University).

Smith, C.J. and Havenith, G., 2012. Body mapping of sweating patterns in athletes: a sex comparison.

Smith, D.L. and Fernhall, B., 2023. *Advanced cardiovascular exercise physiology*. Human Kinetics.

Somani, Y.B., Moore, D.J., Kim, D.J.K., Gonzales, J.U., Barlow, M.A., Elavsky, S. and Proctor, D.N., 2019. Retrograde and oscillatory shear increase across the menopause transition. *Physiological reports*, *7*(1), p.e13965.

Stampfer, M.J. and Colditz, G.A., 1991. Estrogen replacement therapy and coronary heart disease: a quantitative assessment of the epidemiologic evidence. *Preventive medicine*, *20*(1), pp.47-63.

Steiner, D.F. and Freinkel, N., 1972. Handbook of physiology. *Endocrinology (Am. Physiol. Soc., Washington, DC), Sect*, *7*, pp.175-198.

Tabarean I, Morrison B, Marcondes MC, Bartfai T, Conti B. Hypothalamic and dietary control of temperature-mediated longevity. Ageing Res Rev. 2010 Jan;9(1):41-50

Taddei, S., Virdis, A., Ghiadoni, L., Mattei, P., Sudano, I., Bernini, G., Pinto, S. and Salvetti, A., 1996. Menopause is associated with endothelial dysfunction in women. *Hypertension*, *28*(4), pp.576-582.

Taylor, W.F. and Bishop, V.S., 1993. A role for nitric oxide in active thermoregulatory vasodilation. *American Journal of Physiology-Heart and Circulatory Physiology*, *264*(5), pp.H1355-H1359.

Taylor, C.L., and Buettner, K (1953). Influence ofevaporative forces upon skm temperature dependency of human perspiration. J. Appl Physiol. 6 113-123

Taylor, N.A. and Cotter, J.D., 2006. Heat adaptation: guidelines for the optimisation of human performance. *International SportMed Journal*, *7*(1), pp.33-57.

Taylor NA, Machado-Moreira CA. Regional variations in transepidermal water loss, eccrine sweat gland density, sweat secretion rates and electrolyte composition in resting and exercising humans. Extreme Physiol Med. 2013;2(1):4.

Taylor, N A S , Caldwell, J N , and Mekjavic, LB. (2006) The sweatingg foot: local differences in sweat secretion durmg exercise-induced hyperthermia. A vi at Space Environ Med. 77(10)·1020-1027

Teerasumran, P., Velliou, E., Bai, S. and Cai, Q., 2023. Deodorants and antiperspirants: New trends in their active agents and testing methods. *International journal of cosmetic science*, *45*(4), pp.426-443.

Thurston, R.C. and Joffe, H., 2011. Vasomotor symptoms and menopause: findings from the Study of Women's Health across the Nation. *Obstetrics and Gynecology Clinics*, *38*(3), pp.489-501.

Thurston, R.C., Sutton-Tyrrell, K., Everson-Rose, S.A., Hess, R. and Matthews, K.A., 2008. Hot flashes and subclinical cardiovascular disease: findings from the Study of Women's Health Across the Nation Heart Study. *Circulation*, *118*(12), pp.1234-1240.

Townsend, N., Nichols, M., Scarborough, P. and Rayner, M., 2015. Cardiovascular disease in Europe—epidemiological update 2015. *European heart journal*, *36*(40), pp.2696-2705.

Trangmar, S.J. and González-Alonso, J., 2019. Heat, hydration and the human brain, heart and skeletal muscles. Sports Medicine, 49(Suppl 1), pp.69-85.

Trbovich, M., Wu, B., Koek, W., Wecht, J. and Kellogg, D., 2022. Are Thermoregulatory Sweating and Active Vasodilation in Skin Controlled by Separate Nerves During Passive Heat Stress in Persons With Spinal Cord Injury?. *Topics in Spinal Cord Injury Rehabilitation*, *28*(4), pp.84-95.

Uno, H., 1977. SYMPATHETIC INNERVATION OF THE SWEAT GLANDS AND PILORRECTOR MUSCLES OF MACAQUES AND HUMAN BEINGS. *Journal of Investigative Dermatology*, *69*(1).

Van Beaumont, W, and Bullard, R W. (1965) Sweating: direct influence of skm temperature Science. 14 7 1465-1467

Walker, R.A., 1985. Ulex europeus I—peroxidase as a marker of vascular endothelium: its application in routine histopathology. *The Journal of pathology*, *146*(2), pp.123-127.

Wallace, H., 2022. *The Female Factor: Making women's health count–and what it means for you*. Hachette UK.

Weiner JS, Hellmann K. The sweat glands. Biol Rev. 1960;35:141–186.

Wilke, K., Martin, A., Terstegen, L. and Biel, S.S., 2007. A short history of sweat gland biology. *International journal of cosmetic science*, *29*(3), pp.169-179.

Williams, N., 2017. The Borg rating of perceived exertion (RPE) scale. *Occupational medicine*, *67*(5), pp.404-405.

Wissler, E.H., 2018. *Human temperature control*. New York, NY: Springer Berlin Heidelberg.

Woodward, K.A., Draijer, R., Thijssen, D.H. and Low, D.A., 2018. Polyphenols and microvascular function in humans: a systematic review. *Current pharmaceutical design*, *24*(2), pp.203-226.

World Health Organization, 1996. Research on the menopause in the 1990s: report of a WHO scientific group.

Wyon YA, Spetz AC, Theodorsson GE, Hammar ML. Concentrations of calcitonin gene-related peptide and neuropeptide Y in plasma increase during flushes in postmenopausal women. Menopause. 2000;7(1):25-30.

Zhang, Y.H., Hosono, T., Yanase-Fujiwara, M., Chen, X.M. and Kanosue, K., 1997. Effect of midbrain stimulations on thermoregulatory vasomotor responses in rats. *The Journal of Physiology*, *503*(Pt 1), p.177.

Zouboulis, C.C., Blume-Peytavi, U., Kosmadaki, M., Roó, E., Vexiau-Robert, D., Kerob, D. and Goldstein, S.R., 2022. Skin, hair and beyond: the impact of menopause. *Climacteric*, *25*(5), pp.434-442.