WHY WET FEELS WET?
AN INVESTIGATION INTO THE NEUROPHYSIOLOGY OF HUMAN SKIN WETNESS PERCEPTION

By

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ABSTRACT

The ability to sense humidity and wetness is an important sensory attribute for many species across the animal kingdom, including humans. Although this sensory ability plays an important role in many human physiological and behavioural functions, as humans' largest sensory organ i.e. the skin seems not to be provided with specific receptors for the sensation of wetness (i.e. hygroreceptors), the neurophysiological mechanisms underlying this complex sensory experience are still poorly understood.

The aim of this Thesis was to investigate the neurophysiological mechanisms underpinning humans' remarkable ability to sense skin wetness despite the lack of specific skin hygroreceptors. It was hypothesised that humans could "learn" to perceive the wetness experienced when the skin is in contact with a wet surface or when sweat is produced through a complex multisensory integration of thermal (i.e. heat transfer) and tactile (i.e. mechanical pressure and friction) inputs generated by the interaction between skin, moisture and (if donned) clothing. Hence, as both thermal and tactile skin afferents could contribute significantly to drive the perception of skin wetness, their role in the peripheral and central sensory integration of skin wetness perception was investigated, both under conditions of skin's contact with an external (dry or wet) stimulus as well as during the active production of sweat.

A series of experimental studies were performed, aiming to isolate the contribution of each sensory cue (i.e. thermal and tactile) to the perception of skin wetness during rest and exercise, as well as under different environmental conditions. It was found that it is not the contact of the skin with moisture per se, but rather the integration of particular sensory inputs which drives the perception of skin wetness during both the contact with an external (dry or wet) surface, as well as during the active production of sweat. The role of thermal (cold) afferents appears to be of a primary importance in driving the perception of skin wetness during the contact with an external stimulus. However, when thermal cues (e.g. evaporative cooling) are limited, individuals seem to rely more on tactile cues (i.e. stickiness and skin friction) to characterise their perception of skin wetness. The central integration of conscious coldness and mechanosensation, as sub-served by peripheral cutaneous A-nerve fibers, seems therefore the primary neural process underpinning humans' ability to sense wetness. Interestingly, these mechanisms (i.e. integration of thermal and tactile sensory cues) appear to be remarkably consistent regardless of the modality for which skin wetness is experienced, i.e. whether due to passive contact with a wet stimulus or due to active production of sweat.

The novelty of the findings included in this Thesis is that, for the first time, mechanistic evidence has been provided for the neurophysiological processes which underpin humans' ability to sense wetness on their skin. Based on these findings, the first neurophysiological sensory model for human skin wetness perception has been developed. This model helps explain humans' remarkable ability to sense warm, neutral and cold skin wetness.

Keywords: skin wetness, hygrosensation, thermosensation, mechanosensation, skin, thermoreceptors, mechanoreceptors, somatosensory, sensation, perception, temperature, humidity, clothing

STATEMENT

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All the laboratory studies presented were performed solely by the author.

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1 CHAPTER ONE - Introduction and review of the literature

1.1 Introduction

Since the seminal work of Pharo Gagge at the John B. Pierce Foundation Laboratory (Gagge, 1937), the measurement of skin wetness as a physical variable has received great attention, particularly for its role in the estimation of the body's heat balance under conditions of increased metabolic heat production (e.g. resulting from exercising muscles), and decreased gradient for heat loss to the environment (e.g. resulting from high ambient temperatures) (Nadel and Stolwijk, 1973; Candas et al., 1979; Havenith, 2001a; Havenith et al., 2013). However, although much is known on the biophysical role of skin wetness in contributing to thermal homeostasis, surprisingly little has been done to elucidate how humans sense wetness on their skin and how the level of "physical" skin wetness relates to the level of "perceived" skin wetness.

The ability to sense humidity and wetness is an important attribute in the animal kingdom. For many insects, discriminating between dryness and wetness is vital for procreation and survival (Liu et al., 2007). Sensing wetness is also critical for humans, both for behavioural and autonomic adaptations. Perceiving changes in ambient humidity and skin wetness has been shown to impact thermal and clothing comfort (Fukazawa and Havenith, 2009) and thus the thermoregulatory behaviour (Schlader et al., 2010), both in healthy and clinical populations (e.g. individuals suffering from rheumatic pain) (Strusberg et al., 2002). From an autonomic perspective, decreases in ocular wetness seem to initiate the lacrimation reflex in order to maintain a tear film to protect the ocular surface (Hirata and Oshinsky, 2012). Also, tactile roughness and wetness discrimination is critical for precision grip (Augurelle et al., 2003) and object manipulation (André et al., 2010). However, although the ability to sense wetness plays an important role in many physiological and behavioural functions, the neurophysiological mechanisms underlying this complex sensory experience are still poorly understood (Montell, 2008).

In contrast with insects, in which humidity receptors sub-serving *hygrosensation* have been identified and widely described (Tichy and Kallina, 2010), humans' largest sensory organ i.e. the skin does not seem to be provided with specific receptors for the sensation of wetness (Clark and Edholm, 1985). Thus, as human beings, we seem to "*learn*" to perceive the wetness experienced when the skin is in contact with a wet surface or when sweat is produced (Bergmann Tiest et al., 2012a) through a complex multisensory integration (Driver and Spence, 2000) of thermal (i.e. heat transfer) and tactile (i.e. mechanical pressure and skin friction) inputs generated by the interaction between skin, moisture and (if donned) clothing (Fukazawa and Havenith, 2009). However, what remains unclear is the individual role of thermal and tactile cues and how these are integrated peripherally as well as centrally by our nervous system when experiencing the perception of skin wetness.

This Thesis investigates the neurophysiological and psychophysical bases of humans' ability to perceive wetness on the skin. Increasing the knowledge on how humans perceive skin wetness has both a fundamental, as well as an applied significance. On the fundamental side, this could contribute to a better understanding of how the peripheral and central nervous system interact to generate complex somatic perceptions (Craig, 2003). On the applied side, this could be useful for its potential clinical (i.e. development of diagnostic tests for patients with somatosensory disorders, e.g. Multiple Sclerosis and Diabetic Neuropathy) (Gin et al. 2011) as well as industrial applications (i.e. development of new strategies in clothing design aiming to improve thermal and clothing comfort) (Fukazawa and Havenith, 2009).

1.1.1 **Rationale**

The principal input to the experimental work presented in this Thesis was offered by the inability to provide a conclusive answer to a practical question posed by the industry co-sponsor of this PhD, i.e. Oxylane Research, the research and design department of the French sports clothing manufacturing company Decathlon. During the initial phase of this PhD, Oxylane Research was developing an evaporative cooling garment to aid exercise performance under heat stress. The concept behind this cooling garment (whose effectiveness is investigated in the first laboratory study of this thesis, see Chapter Three) was to provide additional evaporative cooling to the body through the process of water evaporation.

When wetted with water, the garment was designed to allow sustained evaporation, thereby cooling the underlying skin and improving thermal comfort during exercise in a hot environment. However, as a potential undesired effect, the garment could have generated a sensation of skin wetness in the wearer, which could have been experienced as thermally uncomfortable. Indeed, although very limited, evidence in the literature indicated that humans seem to interpret the coldness experienced during the evaporation of water from the skin as a signal of the presence of water (and thus wetness) on the skin' surface (Bergmann Tiest et al. 2012; Daanen, 2009). Therefore, as skin wetness has been repeatedly shown to play a significant role in the onset of thermal and clothing discomfort (Candas et al., 1979; Fukazawa and Havenith, 2009), it was considered essential by the sponsor to take into account the mechanisms by which skin wetness is sensed (e.g. the role of evaporative cooling in inducing this sensation) as part of the cooling garment's development. This, in order to minimise the chances that the cooling garment would induce undesired wetness perceptions which could trigger sensations of thermal discomfort in the wearer.

Due to the lack of studies specifically investigating the biophysical and neurophysiological processes which underpin the perception of skin wetness, and due to consequent inability to provide conclusive evidence on the mechanisms triggering the perception of skin wetness, it was therefore decided to perform a systematic experimental analysis of the factors involved in this complex sensory experience, both when this results from the contact with a wet surface (e.g. a wet fabric) as well as when moisture is actively produced by the body (i.e. sweating). Investigating the neurophysiology of human skin wetness perception was considered critical in order to provide basic and applied knowledge which could be used by the sponsor to improve the sport clothing design, with the aim of maximising thermal comfort in extreme exercise conditions (e.g. performance in hot and/or cold environments).
This systematic experimental analysis of human skin wetness perception represents the basis for this Thesis.

In light of the above, the following is a review of the relevant literature required for consideration when investigating the neurophysiology of human skin wetness perception.

1.2 Human temperature regulation

1.2.1 Human heat balance

As homeothermic mammals, humans need to maintain their core body temperature within a very narrow range (~36 to ~40 °C) in order to ensure optimal cellular and molecular function (Nakamura and Morrison, 2007). Due to the variable nature of our surrounding environment, we constantly face the need of autonomically and behaviorally thermoregulate, as either core overheating and overcooling can pose a major challenge to our survival (Parsons, 2003).

The human body prevents core overheating and/or overcooling by achieving thermal balance, a dynamic thermal state which sees a balance between heat gains and heat losses from the body to the environment. Deep body (core) and skin (shell) temperatures are the principal variables driving the onset of the adaptive responses that regulate the balance between heat production and heat loss from the body to the environment (McArdle et al., 2007).

In this respect, the conceptual heat balance equation summarizes the biophysical and environmental factors involved in determining the heat exchanges between the body and the surrounding environment (i.e. thermal audit) (Parsons, 2003) :

$$M - W = E + R + C + K + S$$

Where:

M = rate of metabolic energy production ($W \cdot m^{-2}$)

W = rate of mechanical work ($W \cdot m^{-2}$)

E = rate of evaporative heat loss ($W \cdot m^{-2}$)

R = rate of radiative heat loss ($W \cdot m^{-2}$)

C = rate of convective heat loss ($W \cdot m^{-2}$)

K = rate of conductive heat loss ($W \cdot m^{-2}$)

S = rate of heat storage ($W \cdot m^{-2}$)

The metabolic rate of the body (M) provides energy to perform mechanical work (W) and the net difference between the two (M – W) represents the amount of energy released by the body as heat. This value is always positive and represents the body

heat production. To achieve thermal balance (i.e. S = 0) the heat produced by the body has to be balanced by the heat released to the environment. This occurs via four main physical avenues: evaporation (E), radiation (R), convention (C) and conduction (K). Therefore, for heat balance (S = 0):

$$M - W - E - R - C - K = 0$$

From a biophysical standpoint, if the value resulting from the above equation is positive, body heat content gains occur; if negative, body heat content losses occur. From a physiological point of view, if heat gains surpass heat losses, body core temperature will rise whereas if the contrary occurs, body core temperature will drop (Parsons, 2003).

A schematic representation of the biophysical processes responsible for heat exchanges between the body and the surrounding environment is shown in figure 1. Physical factors such as air temperature, radiant temperature, relative humidity and air velocity significantly contribute to determine these processes.

Figure 1: Biophysical processes determining heat exchanges between the body and the surrounding environment (M= metabolic energy production) (Havenith 2002). *Figure removed due to copyright.*

In humans, thermal balance between heat production and heat loss is achieved by means of autonomic and behavioural thermoregulatory responses.

1.2.2 **Autonomic thermoregulation**

Autonomic thermoregulatory responses in humans are triggered by thermal stimulation of various areas of the central nervous system (e.g. medulla oblongata, pons and midbrain). Amongst these, the pre-optic area (POA) of the hypothalamus is considered as the main thermal-controller (Romanovsky, 2007). By receiving afferent information from thermally-sensitive neurons (i.e. thermoreceptors) located peripherally (i.e. skin) as well as centrally (i.e. brain, spinal cord and viscera) in our body (Schepers and Ringkamp, 2010; Nakamura, 2011), this area provides commands to peripheral thermo-effectors in order to initiate autonomic responses defending body temperature from environmental challenges (Nakamura and Morrison, 2007). According to the type of external stimuli (i.e. warm or cold) which trigger the activation of peripheral and/or central thermoreceptors, specific autonomic responses are activated. These consist primarily of changes in the vasomotor tone (i.e. vasoconstriction and vasodilation) and in the sudomotor activity (i.e. sweating) as well as of activation of shivering and non-shivering thermogenesis (Nakamura, 2011) (Fig. 2).

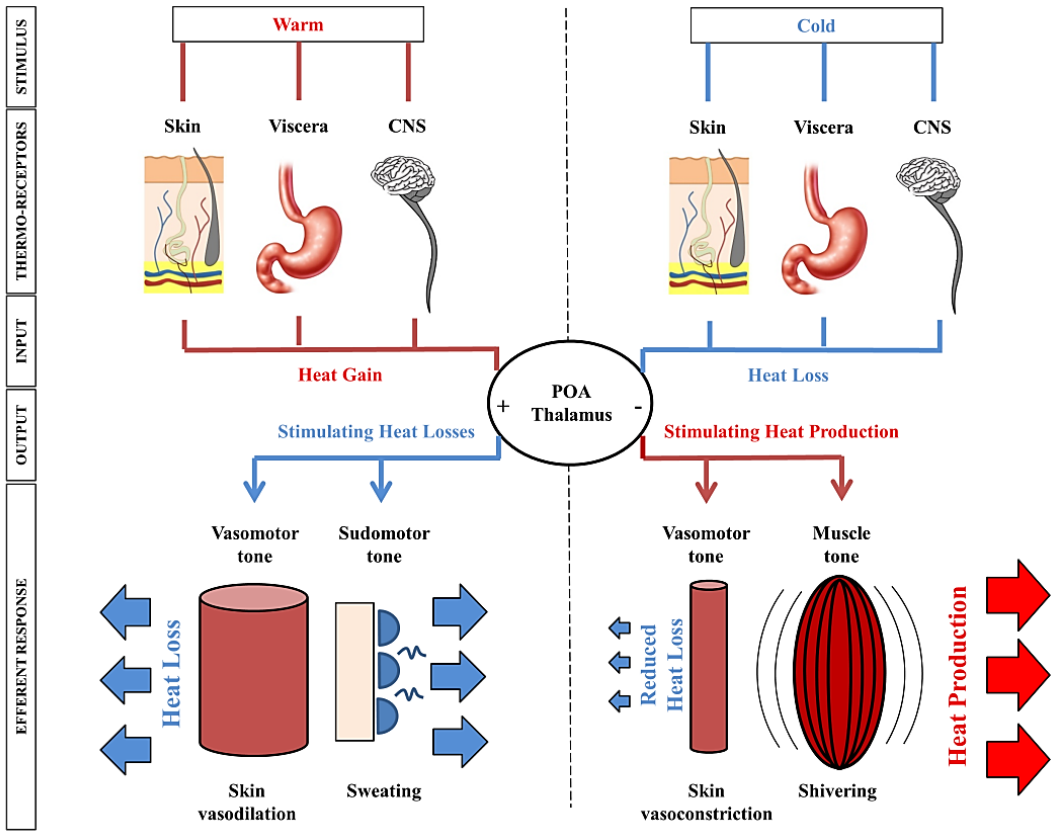

Figure 2: A schematic representation of the autonomic responses triggered by changes (i.e. increases and/or decreases) in body temperature. POA: pre-optic area of the hypothalamus. CNS: central nervous system.

In case of rises in body temperature (i.e. condition of heat gain), heat losses to the environment are initially facilitated by means of skin vasodilation and subsequently by sweating. In case of drops in body temperature (i.e. condition of heat losses), heat losses are initially limited by means of skin vasoconstriction and heat gains increased by means of shivering thermogenesis (Parsons, 2003).

The autonomic mechanisms controlled by the POA act as regulators of heat production and heat losses within the body and from the body to the environment. Aiming to maintain core temperature closely to a specific temperature (i.e. ~37 °C) (Mekjavic and Eiken, 2006), these responses are activated when this parameter rises above or drops below specific thresholds (Mekjavic and Eiken, 2006) (Fig. 3).

Figure 3: Biophysical processes (i.e. shivering and sweating responses) determining heat exchanges (i.e. heat production and heat loss) between the body and the surrounding environment as a result of changes (i.e. rises or drops as represented by right and left arrows respectively) in core temperature from its interthreshold zone (Mekjavic and Eiken, 2006). *Figure removed due to copyright.*

Although powerful, the functional capacity of human autonomic thermoregulation is however limited by physiological and biophysical constraints (Schlader et al., 2010). Maximal sweating as well as maximal vasodilation and vasoconstriction are limited by physiological (e.g. sweat gland density and output, number of capillaries) and biological factors (e.g. age) (Kenney and Munce, 2003; Martini et al., 2011). From a biophysical point of view, anthropometrical characteristic also play a role in limiting the functional ability of the autonomic thermoregulatory system. For example, body surface area to mass ratio is an important parameter for heat exchange, which can limit the ability to dissipate heat to the environment. Heat losses are indeed proportional to the gradient between the skin and environment and to the surface area available for heat exchange (Havenith, 2001b). Thus, given the same body mass, individuals with smaller body surface areas require greater increases in e.g. skin vasodilation and/or sweating than individuals with larger body surface areas, in order to dissipate the same amount of heat to the environment, and to prevent core overheating.

Despite these intrinsic physiological limits, humans successfully maintain their thermal balance while being exposed to various extreme environments (e.g. from the moon surface to the Sahara desert), in which autonomic responses alone could not guarantee survival (Romanovsky, 2007). In this respect, what assures survival to our species is the virtually unlimited power of behavioural thermoregulation.

1.2.3 **Behavioural thermoregulation**

Behavioural thermoregulation can be defined as any conscious decision taken with the aim of maintaining thermal balance and it represents an infinite resource for human body temperature regulation (Schlader et al., 2010; Flouris, 2011). Indeed, from simply looking for shade on a sunny and hot day (Parsons, 2003), to adding or removing clothing (Havenith, 2002), humans constantly adjust their thermal behaviour in order to maintain thermal comfort (Flouris, 2011).

As a conscious indicator of thermal balance, thermal comfort is defined as that condition of mind which expresses satisfaction with the surrounding thermal environment, and it is currently considered as the result of the interaction between physical, physiological and psychological factors (Vanos et al., 2010; Cheng et al., 2012). The physical factors refer to the characteristics of the environment to which individuals are exposed (e.g. ambient temperature and humidity) (Parsons, 2003). The physiological factors refer to the autonomic thermoregulatory processes used by the human body to maintain thermal homeostasis (McArdle et al., 2007). The psychological factors refer to individual sensations and to the hedonic component of the stimulus (perception) (de Dear, 2011); in this context, thermal sensation, affective judgements (how a person *would like* to feel) and personal experiences, play a fundamental role in defining thermal preference (Parsons, 2003).
The combination of such complex and dynamic psychophysiological factors produces continuous variations in individuals' satisfaction with their thermal environments, and therefore a variety of personal judgments about what is/is not perceived as thermally comfortable.

From a neuroanatomical point of view, a number of regions of the central nervous system have been identified which contribute to the central integration and processing of sensory information that are then used by humans to actively and

consciously adjust their thermal behaviour (Flouris, 2011). Interestingly, as to underline the integrative nature of human autonomic and behavioural thermoregulatory responses, some of these regions share behavioural as well as autonomic functions (Fig. 4)

Figure 4: Regions of the central and peripheral (e.g. TRP ion channels) nervous system involved in behavioural and autonomic thermoregulatory functions (Flouris, 2011). PO/AH: pre-optic/anterior hypothalamus. *Figure removed due to copyright.*

A main underlying mechanism which is essential in order to successfully adjust the thermal behaviour is that of thermal sensitivity, i.e. the ability to sense the thermal properties of the surrounding environment (Spray, 1986) as well as of one own' s body (Craig, 2003).

1.3 Thermal sensitivity

Thermal sensitivity represents an important drive of autonomic and behavioural thermoregulatory responses both in humans and in other mammalian and non-mammalian species (Spray, 1986; Gallio et al., 2011). The ability to sense the thermal properties of the surrounding environment as well as of one own' s body is made possible by the presence of thermally-sensitive neurons (i.e. thermoreceptors)

which are located peripherally (i.e. skin) as well as centrally (i.e. brain, spinal cord and viscera) in the human body (Schepers and Ringkamp, 2010; Nakamura, 2011). Whether located in the skin, viscera or brain, by responding to the thermal changes occurring in their receptive fields, these sensory neurons: a) provide afferent information regarding the thermal properties of the environment and/or of an object with which our skin is in contact (i.e. thermal sensation) (Schepers and Ringkamp, 2010); b) modulate autonomic thermal responses (e.g. suppression/increases in sweating due to thermal changes in gastro-intestinal temperature) (Morris et al., 2014).

The anatomical distribution of thermally sensitive neurons, which sees warm sensitive thermoreceptors being present in larger numbers centrally, while cold sensitive thermoreceptors are largely distributed in the periphery (Romanovsky, 2007), highlights the asymmetrical nature of our autonomic thermal physiology. Indeed, the normal core temperature (~37 °C) is closer to its upper (≥40.5 °C) than its lower survival limit (≤32 °C) (however some individuals have been reported to survive with core temperatures as low as 18-20 °C) (Parsons, 2003), indicating that rises in core temperature are more dangerous than equivalent drops in this physiological parameter (Romanovsky, 2007).

Due to their importance in providing the sensory bases for conscious thermal sensations, and in light of the topic of this Thesis (i.e. neurophysiology of a cutaneous sensation), the analysis of thermal sensitivity and of the properties of thermally sensitive neurons will focus on cutaneous thermoreceptors. As that, this will be preceded by an overview of the properties of the human skin as a biological tissue. In addition, an outline of the properties of human touch sense as well as of the characteristics of touch-sensitive neurons will be presented, in order to provide the reader with a more comprehensive overview of human cutaneous sensitivity.

1.3.1 **Human skin**

The human skin is the body's largest organ (it covers the entire body' surface) and can be considered both a protective and a sensory organ (Schepers and Ringkamp, 2010). As a protective organ, the skin provides a first barrier between the body and its surrounding environment; as a sensory organ, it mediates different sensations

through specific receptors. Finally, human skin actively participates in several physiological processes (i.e. vasomotor and sudomotor responses) aiming to maintain homeostasis (Schiffman, 2001).

The human skin is generally thin, with differences amongst body regions varying in a range of 0.5 to 3 mm (Fig. 5).

Figure 5: Regional variations in skin's thickness (Arens and Zhang, 2006). *Figure removed due to copyright.*

Externally the skin is characterised by a variety of surface qualities and extensions (hairs, grooves, pores) and it is described as glabrous (hairless) or hairy. Internally, it includes two main layers, the epidermis and dermis, the outer and inner part respectively, which are connected by an intermediate layer, the stratum basale. The skin contains vascular systems, sweat glands and cutaneous receptors which are differently distributed between the epidermis and dermis (Martini et al., 2011) (Fig. 6). Anatomical and physiological properties of each skin's layer are described below.

Figure 6: A schematic representation of human skin (Arens and Zhang, 2006). *Figure removed due to copyright.*

1.3.1.1 The epidermis

The epidermis represents the skin's outer layer. It is not vascularised, it contains specific sensory receptors (i.e. mechanoreceptors, thermal receptors and nociceptors), it is thin, mostly between 0.075 to 0.15 mm in hairy areas, whilst it is much thicker, tougher and more calloused in glabrous areas, as found on soles and palms (Arens and Zhang, 2006; Martini et al., 2011). The epidermis is composed of three layers: the stratum corneum, (the outermost layer); the statum granulosum (the intermediate layer); and the stratum spinosum, (the innermost layer) (Handler et al., 2010).

The stratum corneum represents the skin's primary barrier to water diffusion. To permit life on dry land, the presence of a barrier to prevent unregulated water loss and thus desiccation is indeed required. However, the barrier to water permeation is not absolute and a movement of water through the stratum corneum to the atmosphere (trans epidermal water loss) is considered part of the insensible water loss (Madison, 2003). The stratum corneum is 0.01 to 0.1 mm thick and it is mainly composed by an assemblage of overlapping plate-like cells, anucleated, interleaved with hydrophobic layers of lipids (Proksch et al., 2008). These plate-like cells, the corneocytes, absorb moisture and thicken as much as 25 % when immersed in water or exposed to high levels of atmospheric humidity; this adaptive ability, smoothing the outer skin surface, protects the skin from tearing when wet (Arens and Zhang, 2006). The stratum corneum is considered the most important physical barrier

against percutaneous penetration of chemicals and microbes and a key player in the regulation of water release from skin perspiration (Proksch et al., 2008).

Beneath the stratum corneum, the stratum granulosum and spinosum represent the principal components of the epidermis intermediate and innermost layer. These layers are composed by nucleated cells which, as the anucleated corneocytes, significantly contribute in the skin barrier function by preventing excessive water loss and penetration of exogenous substances (Proksch et al., 2008).
The epidermis is connected to the dermis by a basal layer of stem cells, the stratum basale, which generates epidermal cells continuously. These cells migrate upward through the epidermis where they transform themselves into the interleaved plates and lipids of the stratum corneum (Norlén and Al-Amoudi, 2004).

1.3.1.2 The dermis

The dermis represents the inner layer of the skin. It contains many specialised cells and structures such as vascular systems, sweat glands, mechanoreceptors, thermal receptors and nociceptors (Kandel et al., 2000). Beneath the dermis lies the subcutaneous fat layer, whose thickness varies according to individual's body composition (Arens and Zhang, 2006).

1.3.2 **Cutaneous thermal sensations**

In humans, non-noxious cutaneous thermal sensations are mediated by a variety of primary afferent nerve fibers that transduce, encode and transmit thermal information to the central nervous system (Schepers and Ringkamp, 2010). Fluctuations in skin temperature due to environmental stimuli (e.g. changes in ambient temperature and humidity) and the related thermal sensations have been shown to trigger autonomic (e.g. vasomotor tone and sweating/shivering response) (Kondo et al., 1997; Sendowski et al., 2000) and behavioural responses (e.g. adding or removing clothing) (Schlader et al., 2012). These responses aim to maintain thermal homeostasis and comfort (Cabanac et al., 1972; Schlader et al., 2010).

Specific temperature-activated ion channels are expressed in the terminals of A- and C-afferent nerve fibers which end as free nerve endings in the skin (Green, 2004; Schepers and Ringkamp, 2010). These encode and transmit the thermal inputs which

are then centrally integrated by the primary and secondary somatosensory cortices as well as the insular cortex (a cortical region involved in cold temperature sensation) (Craig et al., 2000) through the spino-thalamic tract and the dorsal-column medial lemniscal pathway (McGlone and Reilly, 2010).

The different levels of integration of cutaneous thermal inputs (i.e. molecular, nerve fiber, central structures) along with their anatomical and physiological properties are discussed below.

1.3.2.1 Molecular level

The recent discovery of the Transient Receptor Potential (TRP) ion-channels has opened to a better understanding of the molecular logic behind peripheral temperature sensation (Reid, 2005).

TRP(s) represent a family of ion-channels which are expressed in the cell membrane of cutaneous free nerve endings and which are activated by specific temperature ranges. When activated, these channels induce an increase in the resting membrane potential of the specific nerve ending with which they are associated, thus generating specific temperature-dependent afferent inputs (Romanovsky, 2007). Cumulatively, these ion-channels cover a wide range of temperatures (~0 to 50 °C) (Fig. 7).

Figure 7: A schematic representation of the TRP channels involved in peripheral thermo sensitivity. Blue lines refer to cold-activated channels. Red lines refer to heat-activated channels. Note: this representation is based on temperature-dependent channels' activity as measured *in vitro* (Romanovsky, 2007). *Figure removed due to copyright.*

1.3.2.2 Nerve fibers level

A number of different temperature-sensitive nerve fibers, characterized by specific anatomical and neurophysiological properties, innervate both hairy and glabrous skin, respond to non-noxious cold and warm temperature stimuli, and contribute to conscious sensations of cold and warmth (Kandel et al., 2000) (Fig. 8).

Figure 8: A schematic representation of the nerve fibers which innervate human skin: Aδ (cold-sensitive fibers), C (warm-sensitive fibers) and Aß (mechano-sensitive fibers) (Lumpkin and Caterina, 2007). DRG: Dorsal Root Ganglia. *Figure removed due to copyright.*

Myelinated Aδ-nerve fibers represent the vast majority of the so-called "cold fibers". At steady state temperatures cold fibers have a characteristic stimulus response function which is bell-shaped, with a maximal steady state activity between 20 and 30 °C and lower activity at lower and higher temperatures (Schepers and Ringkamp, 2010). At maintained temperatures above 40 °C or below 17 °C, cold fibers maintain a very low frequency discharge or become silent. Conduction velocities for these fast-responding fibers range from 5-30 $m \cdot s^{-1}$ (Campero et al., 2001). Characterized by small receptive fields, these fibers primarily sub-serve conscious cold sensations.

C-nerve fibers (i.e. polymodal afferents responding to nociceptive, warm, cool and light mechanical stimulation with conduction velocities ranging from 0.2-2 $m \cdot s^{-1}$), represent the vast majority of the afferent warmth fibers (McGlone et al., 2014).

These fibers have ongoing activity at static temperatures of 30 °C or more, and this activity vanishes upon cooling. The function of their discharge rate versus steady state stimulus temperature follows a bell-shaped curve, with maximum discharge at 40–43 °C and minimal activity at 50 °C. Characterized by small receptive fields, these fibers primarily sub-serve conscious warmth sensations.

It deserves mention that C-nerve fibers have been previously shown to respond to innocuous cold temperatures (Campero et al., 2001; Campero and Bostock, 2010). However, their contribution to conscious cold sensations has not been proven conclusively, therefore suggesting an alternative autonomic thermoregulatory function (Schepers and Ringkamp, 2010).

Table 1 summarises the properties of each class of temperature sensitive nerve fibers with their associated TRP ion-channels.

Nerve fiber	**TRP channel**	**Modality (maximal activation)**	**Axonal diameter (µm)**	**Conduction velocity ($m \cdot s^{-1}$)**
Aδ (myelinated)	TRPM8	Cold (20-30°C)	2.5	5-30
C (unmyelinated)	TRPV3 TRPV4	Warmth (30-40°C)	1	0.2-2

Table 1: Schematic summary of the two classes of cutaneous nerve afferents (and associated TRP ion-channels) which respond to non-noxious temperature stimuli and sub-serve conscious cold and warmth sensations in humans.

1.3.2.3 Central integration level

At central level, thermo-sensory information are integrated by a number of sub-cortical and cortical regions which contribute to conscious thermal sensations.

First order sensory neurons contained within Aδ and C nerve fibers synapse with second order neurons at a spinal level and project contra-laterally to the thalamus

through the spinothalamic tract (Kandel et al., 2000; McGlone and Reilly, 2010). At this level, second order sensory neurons synapse with third order neurons (i.e. thalamo-cortical) which project to different regions of the cerebral cortex (Kandel et al., 2000; McGlone and Reilly, 2010).

At cortical level, different regions are involved in integration and processing of thermo-sensory information. These are the primary and secondary somatosensory cortices, the insular cortex (a cortical region involved in cold and warm temperature sensation) (Craig et al., 2000) as well as the posterior parietal lobe (a cortical region concerned with integrating the different somatic sensory modalities necessary for perception) (McGlone and Reilly, 2010). A schematic representation of the somatosensory pathway for cutaneous temperature discrimination, including peripheral and central structures is outlined in figure 9.

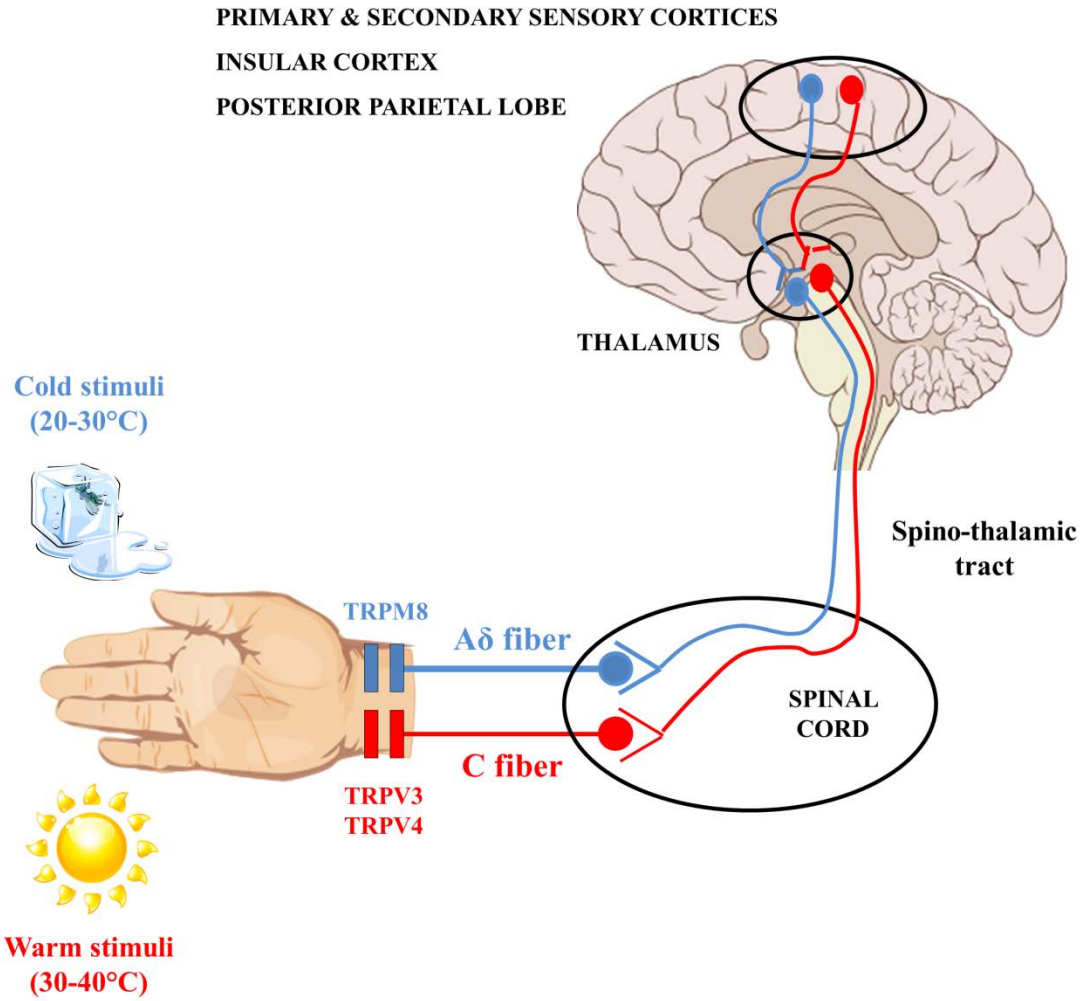

Figure 9. A schematic representation of the somatosensory pathways for cutaneous non-noxious warm and cold temperature discrimination.

1.3.3 **Cutaneous touch sensations**

In humans, non-noxious cutaneous touch sensations are mediated by a variety of primary afferent nerve fibers that transduce, encode and transmit tactile information to the central nervous system (Serino and Haggard, 2010). Mechanical forces applied to the skin, resulting from external stimuli which generate pressure and/or vibrations at the skin' surface, trigger the activation of specific cutaneous mechano-receptors, collectively known as low-threshold mechano-receptors (McGlone and Reilly, 2010).

Specific touch-activated ion channels are expressed in the terminals of A- and C-afferent nerve fibers which end both as free nerve endings and with specific corpuscles (i.e. specialised cells) in the skin (Abraira and Ginty, 2013). These encode and transmit the tactile inputs which are then centrally integrated by the primary and secondary somatosensory cortices as well as the insular cortex and the posterior parietal cortex through the spino-thalamic tract and the dorsal-column medial lemniscal pathway (McGlone and Reilly, 2010).

The different levels of integration of cutaneous tactile inputs (i.e. molecular, nerve fiber, central structures) along with their anatomical and physiological properties are discussed below.

1.3.3.1 Molecular level

In recent years, numerous mechano-sensitive molecules and ion channels have been identified, which could contribute in gating and initiating mechanotransduction and touch sensations in mammals (Tsunozaki and Bautista, 2009). Candidate channels are Degenerin/Epithelial sodium channels (DEG/ENaC), TRP channels and two-pore potassium (KCNK) channels.

Several hypotheses are currently proposed on how cell-membrane ion channels activated by mechanical stimuli could gate and initiate mechanotransduction and touch sensations (Lumpkin and Caterina, 2007) (Fig. 10).

Figure 10: A schematic representation of the potential gating mechanisms of mechanotransduction: a) stretch-activated gating model; b) tethered gating model; c) indirect gating model (Lumpkin and Caterina, 2007). *Figure removed due to copyright.*

Ion channels could be stretch-activated when force in the lipid bilayer cell membrane change (Fig. 10a); alternatively, these channels could be tethered to the cytoskeleton or extracellular matrix, and could be opened by changes in the tension in the linkages between the channel and the cytoskeleton (Fig. 10b); finally, the transduction channels could be coupled to mechanically sensitive proteins through signalling intermediates (Fig. 10c). However, as the molecular bases of tactile and mechano sensations have only recently started to be unveiled, and as the vast majority of the literature is based on in vitro and/or in vivo animal studies, these hypotheses still

require further testing. Hence, still little is known on the molecular mechanisms behind human cutaneous mechanotransduction.

1.3.3.2 Nerve fibers level

Somatosensory neurons with mechano-sensitive properties lie in the dorsal root and trigeminal ganglia of the spinal cord, from which they extend sensory afferents to the skin. These are classified into three broad groups (i.e. C, Aß, and Aδ fibers) and end in the skin both in the form of free nerve endings and with specific corpuscles (i.e. specialised cells) (Tsunozaki and Bautista, 2009) (Fig. 11).

Figure 11: A schematic representation of the nerve fibers (i.e. C, Aß, and Aδ fibers) and respective corpuscles which innervate human hairy and glabrous skin (Abraira and Ginty, 2013). *Figure removed due to copyright.*

In general, weak, innocuous mechanical force applied to the skin activates the so-called low-threshold mechanoreceptors (LTMR), namely Pacinian corpuscles, Meissner's corpuscles, Merkel's disks and Ruffini endings (Fig. 11). These LTMR are associated to Aß nerve fibers, present conduction velocities in the range of 16-100 $m \cdot s^{-1}$, and differ between each other in terms of the stimuli they respond to as well as in terms of their receptive fields.

Pacinian and Meissner's corpuscles respond to the initial and final contact of a mechanical stimulus on the skin and are classified as fast adapting (FA) LTMR,

whereas Merkel's disks and Ruffini endings continue to fire during a constant mechanical stimulus and are classified as slowly adapting (SA) LTMR. With regards to their receptive fields, Meissner's corpuscles and Merkel's disks possess small receptive fields, whereas Pacinian corpuscles and Ruffini have large receptive fields (Fig. 12).

Figure 12: A schematic representation of fast (FAI and FAII) and slowly adapting (SAI and SAII) mechanoreceptors which innervate the glabrous skin of the hand, with related adaptation properties, receptive fields and innervation density. The black dots in the left panel show the receptive fields of Type I (top) and Type II (bottom) afferents. The right panel shows the average density of Type I (top) and Type II (bottom) afferents with darker areas depicting higher densities (McGlone and Reilly, 2010). *Figure removed due to copyright.*

Table 2 summarises the properties of each class of mechano sensitive nerve fibers with their associated ion channels.

Mechanoreceptor subtype	Nerve fiber	Corpuscles	Modality	Axonal diameter (µm)	Conduction velocity ($m \cdot s^{-1}$)
SAI-LTMR	Aß (myelinated)	Merkel cell	Indentation	10	16-96
SAII-LTMR	Aß (myelinated)	Ruffini	Stretch	10	20-100
FAI-LTMR	Aß (myelinated)	Meissner	Skin movement / hair follicle deflection	10	26-91
FAII-LTMR	Aß (myelinated)	Panician	Vibration	10	30-90
Aδ-LTMR	Aδ (myelinated)	Longitudinal lanceolate endings	Hair follicle deflection	2.5	5-30
C-LTMR	C (unmyelinated)	Longitudinal lanceolate endings	Hair follicle deflection	1	0.2-2
HTMR	Aß/Aδ/C	Free nerve endings	Noxious mechanical	1-10	0.5-100

Table 2: Schematic summary of the different classes of cutaneous mechanosensitive afferents which respond to tactile stimuli and sub-serve conscious touch sensations in humans (modified from Abraira and Ginty 2013).

1.3.3.3 Central integration level

At central level, tactile information is integrated by a number of sub-cortical and cortical regions which contribute to conscious touch sensations.

First order sensory neurons contained within Aß, Aδ and C nerve fibers synapse with second order neurons at a spinal level and project contra-laterally to the thalamus (Kandel et al., 2000). Tactile somatosensory paths are primarily located in the dorsal

columns, with axons transmitting tactile, pressure and vibration inputs (McGlone and Reilly, 2010).

At cortical level, different regions are involved in integration and processing of tactile information. These are the primary and secondary somatosensory cortices, the insular cortex (a cortical region involved in cold and warm temperature sensation, as well as in touch and pain) (Craig et al., 2000) as well as the posterior parietal lobe (a cortical region concerned with integrating the different somatic sensory modalities necessary for perception) (McGlone and Reilly, 2010).

1.3.4 **Differences between hairy and glabrous skin**

Hairy and glabrous skin sites differ in terms of innervation and particularly in terms of density of thermo- and mechano-sensory nerve fibers as well as in their biophysical properties. For example, the hairy skin seems to be more sensitive to thermal stimuli than the glabrous skin, which on the contrary presents higher spatial acuity (Norrsell et al., 1999). From the receptors point of view, this could be due to the fact that, although both glabrous and hairy skin sites are innervated with slowly adapting type I mechano-sensory afferents, also known as Merkel cells (low threshold mechanoreceptors transmitting acute spatial images of tactile stimuli with remarkably high spatial resolution), glabrous skin presents a higher density of these specialized organs for tactile discrimination, a fact which could explain the higher spatial acuity to mechanical stimuli of this type of skin (Abraira and Ginty, 2013). From a biophysical point of view, the presence of a thicker stratum corneum (i.e. the outermost layer of the skin) on glabrous skin, resulting in a greater thermal insulation of this type of skin, contributes to the reduced thermal conductance of the finger pad (Rushmer et al., 1966) and therefore to the lower thermosensitivity of glabrous as opposed to hairy skin during short contact cooling and/or heating. This, as a result of the longer time that is needed for a given change in temperature of glabrous skin' superficial layers to penetrate to the underlying tissues (e.g. stratum granulosum) where the thermoreceptors lay (McGlone and Reilly, 2010) (for an overview of the differences between hairy and glabrous skin see Figure 11).

1.4 Skin wetness

Despite the critical role of thermosensitivity, sensing temperature is not the only factor amongst the cutaneous sensory afferent to contribute to autonomic and behavioural thermoregulatory responses in humans. Sensing cutaneous wetness is also critical both for behavioural and autonomic adaptations. Perceiving changes in both ambient humidity and skin wetness have been shown to impact thermal comfort (Fukazawa and Havenith, 2009) and thus the thermoregulatory behaviour (Schlader et al., 2010), both in healthy and clinical populations (e.g. individuals suffering from rheumatic pain) (Strusberg et al., 2002). From an autonomic perspective, the degree of skin wetness influences sweat gland function through a progressive suppression of the sweat output (i.e. hidromeiosis) in the presence of wetted skin (Nadel and Stolwijk, 1973). This results in a reduced ability to lose heat to the environment via evaporative cooling, potentially affecting the thermal balance of the body (Candas et al., 1979). However, although the ability to sense skin wetness plays an important role in several behavioural and thermophysiological functions, little it is known on how skin wetness is sensed in humans (Montell, 2008).

1.4.1 Skin wetness as a physical variable

As a physical variable, skin wetness was first introduced by Gagge (1937) who recognized its critical role in the heat balance of the body.

Whether due to increases in metabolic heat production (e.g. as a result of exercise) or exposure to hot environments, core overheating is prevented, and heat balance maintained, by means of sweating (Candas et al., 1979). Evaporative heat loss through sweating plays a critical role in cooling the skin, thus maintaining a favourable core to skin gradient for heat losses from the body to the environment (Kondo et al., 1997). Therefore, within environmental conditions that allow full evaporation, the level of skin wetness represents an important parameter to ensure the evaporative efficiency of sweating (Candas et al., 1979). As such, skin wetness is defined as the fraction of the body covered by liquid at skin temperature (e.g. sweat), and it represents a physical measure of the degree of wetness involved in the process of evaporation (Gagge, 1937). Skin wetness is usually expressed as a decimal fraction, with 1 representing the upper limit for a fully wet skin and 0.06

representing the minimal value due to insensible perspiration through the skin (Nishi and Gagge, 1977).

Since Gagge' seminal work, the measurement of skin wetness has received great attention, particularly in the context of predicting the body's heat balance during conditions of increased metabolic heat production and decreased gradient for heat loss to the environment (e.g. resulting from high ambient temperatures) (Nadel and Stolwijk, 1973; Candas et al., 1979; Havenith, 2001a; Havenith et al., 2013). However, although much is known on the biophysical role of skin wetness in contributing to thermal homeostasis, surprisingly little has been done to elucidate how humans sense wetness on their skin and how the level of *physical* skin wetness relates to the level of *perceived* skin wetness.

1.4.2 **Skin wetness as a perceptual variable**

Investigating the neurophysiological and psychophysical bases of human skin wetness perception represents a challenge which has attracted the interest of many scientists since the early days of the 20th century. To our knowledge, the first scientist who has attempted to explain the basis of this perception was Bentley, who in 1900, with his famous "synthetic experiment", tested the perception of dipping a sheath-covered finger into warm, lukewarm and cold water in blindfolded participants. The results indicated that, despite no actual contact with moisture occurred, the participants experienced a clear perception of wetness, which was more pronounced when the water was cold than when it was warm. When informed about the characteristics of the experiment (i.e. no direct contact with water), at first participants refused to believe that the finger was not actually wet (Bentley, 1900). Based on these early observations, Bentley proposed a sensory-blending hypothesis which suggests the blend of pressure and coldness as responsible for evoking the perception of wetness.

In contrast with insects, in which humidity receptors sub-serving *hygrosensation* have been identified and widely described (Yokohari and Tateda, 1976; Tichy and Kallina, 2010), humans' largest sensory organ i.e. the skin seems indeed not to be provided with specific humidity receptors (Clark and Edholm, 1985). Therefore, as firstly observed by Bentley, our perception of skin wetness seems to rely on the

interaction of other somatosensory sub-modalities (Bolanowski et al., 2001). Bentley identified the role of both touch and temperature sense as determinant in characterizing this particular somatosensory experience.

Following this early work on the psychophysical bases of skin wetness perception, a number of studies have investigated the perception of skin wetness (Sweeney and Branson, 1990b, 1990a; Li, 2005; Daanen, 2009; Fukazawa and Havenith, 2009; Lee et al., 2011; Ackerley et al., 2012; Bergmann Tiest et al., 2012a, 2012b; Niedermann and Rossi, 2012; Gerrett et al., 2013).

By investigating the perceptual responses to either skin's contact with wet stimuli (Sweeney and Branson, 1990a, 1990b; Li, 2005; Daanen, 2009; Ackerley et al., 2012; Bergmann Tiest et al., 2012a, 2012b; Niedermann and Rossi, 2012), or to the active production of sweat (Fukazawa and Havenith, 2009; Lee et al., 2011; Gerrett et al., 2013), these studies have provided initial insights about the potential mechanisms for which skin wetness is sensed in humans. However, most of these works have tackled the investigation of skin wetness perception with an observational rather than a mechanistic approach.

The lack of a mechanistic approach to the problem of skin wetness has therefore resulted in the same studies providing relatively limited conclusive evidence on which sensory modality (between touch and temperature sense) plays the primary input in driving the perception of skin wetness, to what extent these modalities interact, and how their sensory integration relates with the potentially secondary sensory inputs (e.g. vision) which overall contribute to characterize wetness as a synthetic perception (Li, 2005).

The following paragraphs review the main findings of the above mentioned studies, with respect to investigating skin wetness perception as a result of the contact with an external (dry or wet) stimulus as well as during the production of sweat. Furthermore, an additional paragraph reviews the current knowledge on regional differences in skin wetness perception across the body.

1.4.2.1 Skin wetness perception: contact with external (dry or wet) stimuli

Most of the literature investigating skin wetness perception as a result of the contact with an external (dry or wet) stimulus has focused on investigating the minimum

amounts of wetness that individuals are able to discriminate between (i.e. *discrimination of skin wetness*) and whether individuals are able to characterize the level of skin wetness they experience during skin-wet stimuli contacts (i.e. *magnitude estimation of skin wetness*). The majority of these studies have endorsed the use of Quantitative Sensory Testing (QST) as the preferred methodology to measure human skin wetness perception. Therefore, before reviewing the main findings of the above mentioned studies, a general overview of the characteristics of these tests will be provided.

1.4.2.1.1 Quantitative Sensory Testing

Quantitative Sensory Testing (QST) represents a non-invasive sensory examination of somatosensory modalities such as light touch, vibration, thermal and pain sensation (Chong and Cros, 2004). The basic psychophysical paradigm on which this test is based is that of *stimulus-response*: by exposing the participant to a stimulus with pre-specified physical properties (e.g. temperature), the resulting sensory response (i.e. perception/absence of any perception of the stimulus; estimation of the intensity of the stimulus) is measured in order to investigate the target somatosensory function (e.g. thermal sensitivity) (Walk et al., 2009).

QST can be divided into Threshold Detection tests and Stimulus Intensity ratings. Threshold Detection tests use a graded series of stimuli of increasing and decreasing intensities in order to determine the sensory threshold at which the participant detects or no longer detects a particular somatosensory stimulus. Stimulus Intensity tests use a fixed standard stimulus of known properties in order to determine the participant's ability to provide a quantitative rating of the stimulus' intensity (Chong and Cros, 2004; Walk et al., 2009).

During QST, and in response to the stimuli, participants are usually instructed to either report the presence or absence of a particular sensation with a *Yes-No* method (Chong and Cros, 2004) or to report the intensity of the perceived stimulus on psychometric scales.

1.4.2.1.1.1 Psychometric Scales

Two main types of psychometric scales are commonly used when QST is administered with a Stimulus Intensity paradigm: Likert scales and Visual Analogue Scales.

Likert scales (or categorical scales) are psychometric scales which are characterised by a number of points (typically 4 to11) with designated verbal descriptors and anchor points at the extremes of the scale which define the range of sensations/perceptions specifically tested within the construct of the scale (Likert, 1932). Visual Analogue Scales are psychometric scales which are characterized by a straight line whose extreme points represent the anchor points for the sensation/perception specifically tested (Scott and Huskisson, 1976). Examples of both types of scales, as used in thermal physiology research, are presented in figure 13.

Figure 13: An example of Visual Analogue and Likert scales as used in thermal physiology research (modified from Lee et al. 2010b). *Figure removed due to copyright.*

With regards to the specificity of each type of scale, and how appropriate their use is according to the experimental conditions designed, it is generally accepted that Likert scales are preferable for the benefits that the presence of verbal descriptors provides in helping individuals to describe their sensations. This is particularly true when external noise or distractors can influence the subjective ability to define one's own sensations (Lee et al., 2010b). With regards to Visual Analogue Scales, these are generally considered as preferable when a higher sensitivity in the measurement of a particular sensation is needed. Also, by not restricting individuals' ability to rate their sensation based on specific verbal descriptors, these scales are thought to

provide individuals with a greater flexibility and thus accuracy in their sensation discrimination (Lee et al., 2010b).

With regards to the use of QST in the investigation of skin wetness perception, this method has been widely used according to both a *discrimination paradigm* as well as *magnitude estimation paradigm*. In the light of this, the following paragraphs present an overview of the most representative studies which have endorsed the use of QST with discrimination or magnitude estimation paradigms, to investigate skin wetness perception as a result of the contact with an external (wet or a dry) stimulus.

1.4.2.1.2 Discrimination studies

The studies that have investigated skin wetness perception as a result of the contact with an external (dry or wet) stimulus using QST with a discrimination paradigm, have indicated that individuals seem to readily and accurately discriminate between higher and lower wetness levels.

During a discrimination experiment, Sweeney & Branson (1990b) showed that, when cotton test fabrics (25 cm^2) with different water content were applied to the upper back of 13 blindfolded female participants, these discriminated between moisture content with a discrimination threshold of 1.6 $\mu l \cdot cm^{-2}$ against a reference stimulus of 3.6 $\mu l \cdot cm^{-2}$ (Sweeney and Branson, 1990b).

In line with this approach, Jeon et al. (2011) applied four 100 cm^2 specimens of different types of fabric (i.e. cotton, regular polyester and two types of so-called high-performance polyester) with a range of moisture contents (1 to 21 $\mu l \cdot cm^{-2}$) to the right and left inner forearm of 10 blindfolded female participants (duration: 5 s). Test fabrics were applied simultaneously to one of two reference fabrics (with amounts of water of 5 and 15 $\mu l \cdot cm^{-2}$) and participants judged which stimulus caused greater wetness perception (Fig. 14). This study found average discrimination thresholds which differed between the different materials (higher for e.g. high-performance polyester) in range of 1.9 to 2.6 $\mu l \cdot cm^{-2}$ against the 5 $\mu l \cdot cm^{-2}$ reference stimulus, and from 3.6 to 5.4 µl against the 15 $\mu l \cdot cm^{-2}$ reference stimulus.

Figure 14: The procedure for determining discrimination of skin wetness as used by Jeon et al. (2011). *Figure removed due to copyright.*

Similarly, in a study in which 6 males and 6 females (blindfolded) interacted with 3 different types of wet materials (i.e. 19.6 cm^2 thin and thick viscose and cotton wool), in two ways of exploring (i.e. the samples were either touched statically, flat on the table, in which case only thermal cues were available; or they were touched dynamically, picked up and manipulated, in which case both thermal and mechanical cues were available), Bergmann Tiest et al. (2012a) found that discrimination thresholds ranged from ~25 to ~400 $\mu l \cdot cm^{-2}$ according to the type of contact with the stimuli (static vs. dynamic) (Fig. 15).

Figure 15: Discrimination thresholds during static and dynamic manual exploration of wet materials (from Bergmann Tiest et al. 2012a). *Figure removed due to copyright.*

In summary, the above mentioned studies have provided evidence for the fact that individuals seem to readily discriminate between higher and lower wetness levels.
However, although endorsing the use of QST, by approaching the assessment of skin wetness perception with a *discrimination paradigm* (i.e. a forced choice between two options), these studies have provided limited evidence on the potential sensory mechanisms involved in the subjects' ability to sense and discriminate skin wetness. In this respect, the studies which have approached the assessment of skin wetness with a *magnitude estimation paradigm* have provided more detailed insights on the potential sensory inputs underlying human's ability to sense wetness on the skin, thus indicating this approach (i.e. QST with a magnitude estimation paradigm) as a potentially more effective method to assess skin wetness perception.

1.4.2.1.3 Magnitude estimation studies

1.4.2.1.3.1 Thermal sense in the perception of skin wetness

The studies that have investigated what sensory inputs contribute to skin wetness perception as a result of the contact with an external (dry or wet) stimulus using QST with a magnitude estimation paradigm, have indicated that the thermal sense (and specifically cold sensations) could be the key player in driving the perception of skin wetness (Daanen, 2009; Ackerley et al., 2012; Bergmann Tiest et al., 2012b). In support of this hypothesis, it has been proposed that, as we *learn* to perceive skin wetness, we tend to associate the cold sensations evoked by the drop in skin temperature occurring during the evaporation of moisture from the skin, as a signal of the presence of moisture, and thus wetness, on the skin surface (Daanen, 2009). Therefore, cold stimuli able to reproduce such skin cooling rates are suggested to suffice in evoking the perception of skin wetness (Bergmann Tiest et al., 2012b).
In this respect, Daanen (2009) measured the temperature course of the skin (i.e. temperature' s drop of 1 to 5°C with a 0.05 to 0.2°C·s^{-1} cooling rate) when this was wetted with drops of water with volumes in a range of 10 to 100 µl (Fig. 16). The author suggested that the cold sensations experienced when such skin cooling occurs can contribute to the perception of skin wetness. Therefore, exposing the skin to a cold-dry stimulus producing such skin cooling was hypothesised and tested to be effective in evoking an illusory perception of skin wetness.

Figure 16: A schematic representation of the temperature course of the skin when this was wetted with drops of water with volumes in a range of 10 to 100 µl as patented by Daanen (2009). Skin temperature is observed to drop between 1 and 5°C with a 0.05 to 0.2°C·s^{-1} cooling rate during the initial exponential phase of evaporative skin cooling. *Figure removed due to copyright.*

The critical role of cold sensations in inducing the perception of wetness had been previously observed by Yamakawa & Isaji (1987) during a magnitude estimation experiment performed with six different textiles in three wetness conditions and at three different temperatures (Yamakawa and Isaji, 1987). In this study the authors found that subjects' ratings in terms of perceived wetness correlated to the initial cooling rates occurring during the contact between the subjects' fingers and the test fabrics: a greater initial temperature drop was linked to a greater sensation of clamminess (Fig. 17).

Figure 17: Subjects' ratings of perceived skin wetness in relation to wetness condition (i.e. dry, moist and wet) and temperature of the test fabrics (i.e. warm, medium and cold) (from Yamakawa and Isaji 1987). *Figure removed due to copyright.*

The key role of experiencing coldness in the ability to sense skin wetness has been further confirmed by Bergmann Tiest et al. (2012b), who have shown that, when manipulating dry phase-change materials which induced cool sensations, participants perceived these as being wetter than non-treated dry fabrics.

In support of the role that thermal (cold) sensations play in driving the perception of skin wetness, Niedermann and Rossi (2012) have recently shown that blindfolded individuals could discriminate between different drying states (i.e. 0, 5, 50, 95 and 100% dry) of different fabrics (e.g. 260 cm^2 cotton and polyester samples) applied to their inner forearm, only when the different drying states (e.g. 0 and 100%) induced significantly different thermal sensations [e.g. the 0% dry fabric (i.e. fully wet) was experienced as significantly wetter than the 100% dry as the 0% dry fabric induced significantly colder thermal sensations than the 100% dry].

Finally, Ackerley et al. (2012) have recently shown that 9 blindfolded females could readily discriminate between very small amount of moisture (in the range of 1.6 $\mu l \cdot cm^{-2}$) applied with a tactile stimulator over different regions of the body. Although in the mentioned study no recordings of local skin temperature and thermal sensations were performed, the authors hypothesised that participants distinguished the greater from the smaller levels of moisture due to the greater evaporative cooling resulting from the residual moisture on the skin, which induced colder thermal sensations and thus wetter perceptions (Fig. 18).

Figure 18: Subjects' ratings of perceived skin wetness in relation to wetness level of the stimulus (range: 0.8-6.6 $\mu l \cdot cm^{-2}$) (from Ackerley et al. 2012). *Figure removed due to copyright.*

In summary, the studies that have investigated what sensory inputs contribute to skin wetness perception with a magnitude estimation approach, have provided more structured evidence on the sensory inputs which could significantly contribute to drive the perception of skin wetness during the contact with an external (dry or wet) stimulus. In this respect, due to potential learning factors, thermal (cold) sensations seems to play a primary role in driving the perception of skin wetness. Furthermore, these studies have demonstrated that, assessing the psychophysical processes involved in the perception of skin wetness by using QST with a magnitude estimation paradigm can provide reliable quantitative data about the neurophysiological mechanisms underlying this complex somatosensory experience. However, as no measurements of the physiological changes occurring locally at the skin during the application of the stimuli were performed, the outcomes of the above presented studies have provided only limited evidence on the potential link between the biophysical effects of the stimuli applied (e.g. variations in skin temperature), the resulting afferent sensory inputs (e.g. cold sensations) and the way these inputs were used by the participants to characterize their perception of skin wetness. Monitoring these mechanisms is indeed critical in order to provide mechanistic evidence in support of the neurophysiological bases of skin wetness perception.

A fact which highlights the complexity of skin wetness as a perception, and thus the need for mechanistic studies, is that the role of the cold sensitive afferents in

characterizing the perception of wetness might vary largely according to the location of the thermoreceptors. For example, the augmented activation of cold sensitive thermoreceptors located on the human cornea recorded during evaporation-induced ocular surface cooling, seems responsible for the perception of ocular dryness (Belmonte and Gallar, 2011). The same physical process (cooling) encoded by the same type of thermoreceptors (cold sensitive) might be therefore responsible for two completely opposite perceptions: (ocular) dryness and (skin) wetness. This fact highlights the need for a mechanistic approach to the study of skin wetness perception, in order to develop a specific sensory model which could explain the neurophysiological and psychophysical processes behind this complex perception (Jousmäki and Hari, 1998; Guest et al., 2002).

1.4.2.1.3.2 Tactile sense in the perception of skin wetness

With regards to the potential contribution of other somatosensory modalities to the perception of skin wetness during the contact with an external (dry or wet) stimulus, the tactile sense could represent an important source of sensory information for sensing and discriminating skin wetness.

When the skin is exposed to external stimuli, surface's textures and properties (e.g. wetness or roughness) are usually discriminated based on the type and amount of tactile inputs resulting from the skin displacement as well as the rate of movement of the stimuli across the skin (Yoshioka et al., 2011). For example, when in contact with fabrics, the level of skin wetness has been shown to increase the amount of friction within the skin-clothing system, a fact which in turn may alter the tactile sensations arising from the skin's mechanical contact with the fabric (Gwosdow et al., 1986). Gwosdow et al. (1986) have observed that increases in physical skin wetness result in increases in the frictional force required to pull a fabric across the skin, with this being positively correlated with the level of subjective displeasure experienced. Increases in tactile stimulation (in the form of greater skin friction) resulting from the interaction with wet materials could therefore contribute to inducing and/or increases in the perception skin wetness.

In line with the above, Bergmann Tiest et al. (2012a) have recently provided evidence for the role of tactile inputs in the haptic perception of wetness. In their study, the authors observed that, during the interaction with wet materials (i.e. 19.6 cm^2 thin and thick viscose and cotton wool), Weber fractions (i.e. psychophysical

indicator of the just-noticeable difference between two stimuli, which is proportional to the magnitude of the stimuli) (Kandel et al., 2000) for wetness discrimination thresholds decreased significantly when individuals were allowed dynamic as opposed to the static touching (Fig. 19). This indicated that individuals' skin wetness perception was increased by a higher availability of tactile information, as occurring during the dynamic exploration as opposed to the static contact with the wet materials. The authors concluded that, when thermal cues (e.g. thermal conductance of a wet material) provide insufficient sensory inputs, individuals seem to use mechanical cues (e.g. stickiness resulting from the adhesion of a wet material to the skin) to aid them in the perception of wetness.

Figure 19: Weber fractions for wetness discrimination thresholds during static and dynamic manual exploration of wet materials (from Bergmann Tiest et al. 2012a). *Figure removed due to copyright.*

These recent findings have provided evidence for the potential role of other sensory cues than thermal in inducing the perception of skin wetness during the skin's contact with external stimuli. However, as the study of Bergmann Tiest et al. (2012a) is the only one to our knowledge to have specifically investigated how tactile inputs can influence the haptic perception of skin wetness, still little is known on how the cutaneous thermal and tactile sensory inputs are peripherally and then centrally integrated by the nervous system to give raise to the perception of skin wetness.

In order draw a more comprehensive picture on the mechanisms which allow humans to sense wetness on their skin, the psychophysical studies presented so far need to be integrated with neurophysiological studies which, by investigating the peripheral and central neural mechanisms involved in sensing skin wetness, could ultimately

contribute to the development of a specific sensory model for this complex perception. Indeed, the way we perceive "feelings" from our body results from complex integrations between the activity of the exteroceptive and interoceptive systems (Craig, 2002). Furthermore, converging evidence suggests a phylogenetically new system, (which integrates information about the overall homeostatic condition of the body) as one of the principal neuroanatomical structures that differentiates humans from non-human primates (Craig, 2003). Hence, this hypothesis confirms the multimodal approach (i.e. linking the biophysical and psychophysical factors of a sensory percept to the neurophysiology of the somatosensory system) as one of the most appropriate methods to investigate the mechanism of human sensory integration.

As the perception of skin wetness represents one of the numerous somatosensory experiences that allow us to sense and perceive our immediate environment, and eventually to interact with it (McGlone and Reilly, 2010), it is reasonable to hypothesize that other sensory inputs than e.g. temperature (i.e. touch), as well as other factors such as the environmental conditions and activity performed (rest or exercise) might significantly influence the way we experience this complex perception.

1.4.2.2 Skin wetness perception: sweat production

To our knowledge, only few studies have investigated how the level of physical skin wetness relates to the level of perceived skin wetness under conditions of sweat-induced skin wetness.

Fukazawa and Havenith (2009) investigated thermal comfort sensitivity in relation to locally manipulated skin wetness as resulting from exercise-induced sweat production. Similarly, Gerrett et al. (2013) investigated thermal comfort sensitivity in relation to sweat-induced skin wetness, however in a non-manipulated condition (natural sweat distribution across the torso during exercise). Finally, Lee et al. (2011) investigated regional differences in sweat-induced perceived skin wetness during rest and moderate exercise in 25 and 32 °C ambient temperature and 50 % relative humidity.

Interestingly, in all these studies, skin temperature was always observed to increase significantly during the exercise protocols, suggesting that participants were able to

both sense and regionally discriminate sweat-induced skin wetness, despite not experiencing any cold sensations (Fig. 20).

Figure 20: Relationship between mean skin temperature and frequency of perceived (sweat-induced) skin wetness during resting and exercising conditions (from Lee et al. 2011). *Figure removed due to copyright.*

It could be therefore suggested that in those conditions, participants relied more on tactile (i.e. stickiness of their clothing) than on thermal inputs (i.e. thermal sensations) to characterize their wetness perception.

This hypothesis could be in line with what previously shown for the skin's contact with an external stimulus (i.e. manual exploration of a wet material) by Bergmann Tiest et al. (2012a), who reported that, when thermal cues (e.g. thermal conductance of a wet material) provide insufficient sensory inputs, individuals seem to use mechanical cues (e.g. stickiness resulting from the adhesion of a wet material to the skin) to aid them in the perception of wetness (Bergmann Tiest et al., 2012a).

In line with the above, it could also be speculated that the greater role that tactile inputs could have played in driving the perception of skin wetness during the above mention studies, could be the result of an increased skin' sensitivity to tactile stimuli. This, as when sweat is produced, the internal sweat production and duct filling activates the cutaneous mechanoreceptors surrounding the sweat glands (Shibasaki et al., 2004) which, by inducing the typical *"sensation of tingling"* which is often experienced at the onset of sweating, could ultimately contribute to the sensation of a

change in the skin hydration status, and thus to an increased sensitivity to skin wetness perception at the onset of sweating under warm skin temperatures. However, as in the above mentioned studies (Fukazawa and Havenith, 2009; Lee et al., 2011; Gerrett et al., 2013) the mechanical interaction at the skin as well as the skin hydration was neither manipulated nor controlled, these cannot provide conclusive evidence on the potential link between the thermal and mechanical changes occurring locally at the skin's surface when this was wet (due to sweating) and the resulting sensory inputs used by the participants to characterize their perception of skin wetness.

1.4.2.3 Skin wetness perception: regional differences across the body

1.4.2.3.1 Hairy skin

The distribution of cutaneous sensitivity to cold (as well as to warmth, see e.g. Gerrett et al., 2014) has been repeatedly shown to vary significantly across different regions of the body (Keatinge and Nadel, 1965; Burke and Mekjavic, 1991; Nakamura et al., 2008) as well as within the same body region (Ouzzahra et al., 2012). For example, the torso is suggested as amongst the most sensitive regions to cold (Keatinge and Nadel, 1965; Burke and Mekjavic, 1991; Nakamura et al., 2008). In this regard, the recent work of Ouzzahra et al. (2012) has provided evidence for the presence of an uneven distribution of cold sensitivity across the front and back torso (Fig. 21).

Figure 21: Body map of mean thermal sensations at rest and during exercise, in response to a 20 °C cold stimulus delivered by a 25 cm^2 thermal probe. Cold

sensation scale ranged from 0 (not cold) to 10 (extremely cold) (from Ouzzahra et al. 2012). *Figure removed due to copyright.*

In light of the above, if we accept the hypothesis that sensing skin wetness could be potentially and primarily driven by the level of coldness experienced, it would be reasonable to hypothesise that skin wetness perception could vary significantly across the body. To our knowledge, only few studies have investigated whether humans present regional differences in cutaneous wetness perception (Fukazawa and Havenith, 2009; Lee et al., 2011; Ackerley et al., 2012).

In a study in which thermal comfort sensitivity was investigated in relation to locally manipulated skin wetness (as resulting from exercise-induced sweat production), Fukazawa and Havenith (2009) found that the torso seems to have a lower sensitivity to wetness than the limbs. Similar findings were also reported by Gerrett et al. (2013) in a non-manipulated condition (natural sweat distribution across the torso during exercise). Lee et al. (2011) (Lee et al., 2011) showed that when asked, individuals reported the torso (i.e. chest and back) to be the region more often perceived as wet during rest and moderate exercise in 25 and 32 °C T_{air} and 50 % humidity (Fig. 22).

Figure 22: The initially perceived as wet areas (a) and most frequently perceived as wet skin areas (b) (from Lee et al. 2011). *Figure removed due to copyright.*

Ackerley et al. (2012) (Ackerley et al., 2012) have recently shown that when wet stimuli with different moisture contents (range: 0.8-6.6 $\mu l \cdot cm^{-2}$) were applied to different body regions, individuals were able to differentiate between moisture levels, with a tendency of the back as being amongst the most sensitive region to wetness (Fig. 23).

Figure 23: Subjects' ratings of perceived skin wetness in relation to the body region stimulated with different wet stimuli (range: 0.8-6.6 $\mu l \cdot cm^{-2}$) delivered by a tactile stimulator with a 24 cm2 contact surface (from Ackerley et al. 2012). *Figure removed due to copyright.*

The outcomes of these studies have provided initial insights about the hairy regions of the body on which skin wetness might be perceived to a larger extent (e.g. the torso). However, by only measuring the physical wetness (whether due to sweat production or to contact with a wet surface) these studies have provided only limited evidence on the potential link between the thermal changes occurring locally at the skin's surface when this is wet (e.g. variation in local skin temperature) and how these are perceived in terms of thermal sensations and perception of skin wetness.

1.4.2.3.2 Hairy vs. Glabrous skin

With regards to the potential differences in skin wetness perception between hairy and glabrous skin, to our knowledge, only one study has specifically addressed this topic (Ackerley et al., 2012). In their study, Ackerley et al. (2012) found no differences between the palm of the hand (i.e. glabrous skin) and the rest of the body

(i.e. hairy skin) in the ability to discriminate between externally applied stimuli with different moisture contents (range: 20-160 µl over a 24 cm^2 surface). Both hairy and glabrous skin sites were indeed observed to present the same level of skin wetness sensitivity, despite the anatomical and physiological differences between these types of skin (see paragraph *1.3.2.2.1 Differences between hairy and glabrous skin* of this Thesis), would have suggested a potential difference in the ability to sense wetness across these skin sites.

In support of the hypothesis that hairy and glabrous skin sites could present differences in skin wetness sensitivity, studies which have investigated discriminative (Ackerley et al., 2014b; Mancini et al., 2014) and pleasant touch (Löken et al., 2011), as well as temperature (Norrsell et al., 1999; Granovsky et al., 2005) and pain sensitivity (Davis, 1998; Iannetti et al., 2006) in hairy and glabrous skin have repeatedly demonstrated the existence of somatosensory differences based on the type of skin investigated and due to their different biophysical (e.g. skin thickness and thermal resistance) and physiological properties (density of specific receptors). Hence, it would be reasonable to hypothesise that according to the same principle, similar differences would be present for skin wetness perception. However, the presence of only one study specifically addressing this topic makes any hypothesis and/or conclusion purely speculative.

1.5 Summary of literature on skin wetness perception and Conclusions

From the literature review the following conclusions can be made:

1. As a physical variable, skin wetness is a fundamental parameter for the body's thermal homeostasis due to its role in facilitating heat losses via evaporation of sweat from the skin.

2. As a perceptual variable, skin wetness is an important determinant of autonomic and behavioural responses.

3. Although much is known on the biophysical role of skin wetness in contributing to thermal homeostasis, surprisingly little has been done to elucidate how humans sense wetness on their skin and how the level of *physical* skin wetness relates to the level of *perceived* skin wetness.

4. In contrast with insects, in which humidity receptors sub-serving *hygrosensation* have been identified and widely described, humans' largest sensory organ i.e. the skin seems not to be provided with specific receptors for the sensation of wetness.

5. As human beings, we seem to *learn* to perceive the wetness experienced when the skin is in contact with a wet surface or when sweat is produced through a complex multisensory integration of thermal (i.e. heat transfer) and tactile (i.e. mechanical pressure and skin friction) inputs generated by the interaction between skin, moisture and (if donned) clothing.

6. What remains unclear is the individual role of thermal and tactile cues and how these are integrated peripherally as well as centrally by our nervous system when experiencing the perception of skin wetness.

5. The first scientist who has attempted to explain the basis of this perception was Bentley, who in 1900 proposed a sensory-blending hypothesis which suggests the blend of pressure and coldness as responsible for evoking the perception of wetness.

6. Since Bentley's study, a number of researchers have investigated the perceptual responses to either: a) skin's contact with external (dry or wet) stimuli; b) the active production of sweat.

7. Studies that have investigated the perceptual responses to skin's contact with external (dry or wet) stimuli using Quantitative Sensory Testing (QST) with *a discrimination paradigm* have shown that humans readily discriminate between higher and lower wetness levels. However, these studies have provided limited evidence on the potential sensory mechanisms underpinning the ability to sense and discriminate skin wetness.

8. Studies that have investigated the perceptual responses to skin's contact with external (dry or wet) stimuli using QST with *a magnitude estimation paradigms* have shown that thermal (cold) sensory inputs play a primary role in driving the perception of skin wetness.

9. It has been proposed that we tend to associate the cold sensations evoked by the drop in skin temperature occurring during the evaporation of moisture from the skin, as a signal of the presence of moisture, and thus wetness, on the skin surface.

10. Cold stimuli able to reproduce such skin cooling rates are suggested to suffice in evoking the perception of wetness. However, limited evidence is available in support of this hypothesis.

11. Although indications of the key role of thermal cues in the perception of skin wetness have emerged, limited mechanistic evidence has been provided on the potential link between the biophysical effects of the stimuli applied (e.g. variations in skin temperature), the resulting physiologically responses (afferent sensory inputs) and the way these were used by the participants to characterize their perception of skin wetness.

12. Only one study has provided evidence on how thermal (cold) and tactile sensory cues could be integrated to aid the discrimination of skin wetness during the contact with an external (dry or wet) stimulus. However, as well as for previous studies,

limited physiological measurements were performed, eventually limiting the possibility to define a sensory model for skin wetness perception.

13. Only few studies have investigated how the level of physical skin wetness relates to the level of perceived skin wetness under conditions of sweat-induced whole-body skin wetness.

14. In all these studies, skin temperature was observed to significantly increase during the exercise protocols, thus indicating that participants were able to sense as well as to regionally discriminate skin wetness despite no cold sensations were experienced.

15. It could be hypothesised that in conditions of sweat-induced skin wetness, individuals rely more on tactile (i.e. stickiness) than on thermal inputs (i.e. thermal sensations) to characterise their skin wetness perception. However, to date, this hypothesis remains purely speculative.

16. Only few studies have investigated whether regional variations in wetness perception across the body exist. These studies have provided initial insights about the hairy regions of the body on which skin wetness might be perceived to a larger extent (e.g. the torso).

17. Only one study has investigated whether skin wetness perception varies between hairy and glabrous skin and found no differences in skin wetness sensitivity between these types of skin. However, due to the presence of a body of literature which indicates the existence of somatosensory differences (i.e. discriminative and pleasant touch, temperature and pain) between hairy and glabrous skin, it seems reasonable to hypothesise that the same would apply to skin wetness perception.

18. Overall, no studies have been found to specifically endorse a mechanistic approach (i.e. combining psychophysical and neurophysiological methods) to the investigation of the neural bases of human skin wetness perception. As that, the knowledge on how humans sense warm, neutral and cold wetness on their skin is still lacking.

1.6 General Aims

1. The principal aim of this Thesis is to investigate the neurophysiological and psychophysical bases of humans' ability to perceive wetness on the skin.

2. As both thermal (cold) and tactile skin afferents seem to significantly contribute to drive the perception of skin wetness, their role in the peripheral and central sensory integration of skin wetness perception will be investigated.

3. The sensory cues underpinning human skin wetness perception will be investigated both under conditions of skin's contact with an external (dry or wet) stimulus as well as during the active production of sweat.

4. As this appears to be lacking in the literature, a mechanistic approach to the investigation of skin wetness perception will be adopted. It will be attempted to isolate each sensory cue contributing to skin wetness perception (i.e. thermal and tactile) and to investigate these under resting and exercising conditions, as well as during exposure to different environmental conditions.

5. All the above will be performed with the overall aim of developing a neurophysiological sensory model for human's ability to sense skin wetness. This will be ultimately useful for its fundamental as well as its applied significance.

2 CHAPTER TWO – Experimental methodology

2.1 Introduction

The aim of the experimental work presented in this Thesis is to investigate the neurophysiological and psychophysical bases of humans' ability to perceive wetness on the skin. Both the contact with an external stimulus and sweat production were considered as scenarios in which the perception of skin wetness can be experienced. Specifically, it was aimed to elucidate, from a mechanistic standpoint, the individual contribution as well as the interaction between the sensory cues which seem to drive the perception of skin wetness (i.e. thermal and tactile inputs).
In order to address this aim, first it was considered necessary to investigate the sensory integration underlying skin wetness perception during the contact with an external stimulus. This would allow the design of experimental conditions which can be tightly controlled, in order to isolate the individual contribution of each of the sensory modalities involved in this perception.

During the first part of the experimental work, skin wetness perception as a result of the contact with an external stimulus was investigated using Quantitative Sensory Testing (QST) with a magnitude estimation paradigm. This has been previously shown to be more appropriate than a discrimination paradigm when investigating the sensory cues involved in the perception of skin wetness. A number of external stimuli, with different properties (i.e. temperature, pressure, level of wetness) were applied to different body regions (i.e. hairy and glabrous skin sites), during different activities (i.e. rest and exercise), during different environmental conditions (i.e. thermo-neutral and warm) and during different sensory states (i.e. presence or not of a selective reduction in the activity of specific cutaneous nerve fibers). These studies aimed to provide evidence for the development of a specific neurophysiological model for human skin wetness perception.

At this point, the model of skin wetness perception was sought to be tested under conditions in which skin wetness results from sweat production in order to elucidate whether the neurophysiological mechanisms for which skin wetness is sensed in

humans are similar when skin wetness is induced by the contact with an external stimulus or by the production of sweat.

In light of the above, this chapter presents an overview of the experimental methodology used and developed throughout this PhD, with specific regards to the methods used for those studies investigating skin wetness perception as a result of the contact with an external stimulus. A detailed description of the methods used to investigate skin wetness perception as a result of sweating is presented in Chapter Ten as part of the Laboratory study 7.

2.2 Ethical clearance

The laboratory methods for all experiments undertaken are described under generic experimental protocols and were approved by Loughborough University's Ethical Committee:

- G01/P2: Determination of the physiological and subjective (thermal sensation, discomfort, pain) response of humans when touching cold surfaces of different materials

- G10/P10: Regional sensitivity to a cold and warm stimulus over the body surface

- G03/P10: Determination of the physiological response of humans during whole body or local cooling under restricted extremity blood flow conditions

- G03/P13: Thermoregulatory effects of warming in air

2.2.1 Informed consent and health screen questionnaire

All participants gave their informed consent for participation. The test procedure and the conditions were explained to each participant. Each study design had been approved by the Loughborough University Ethics Committee and testing procedures were in accordance with the tenets of the Declaration of Helsinki.

Following familiarization with testing procedures and laboratory equipment, participants signed and informed consent form (Appendix A). A generic health screen questionnaire (Appendix B) was completed by every participant to ensure suitability for each specific study.

2.2.2 **Participant recruitment**

Participants of both sexes were recruited from the staff and student population of Loughborough University. The age range was set between 18-30 years to reduce any systemic errors due to age-related differences in thermoregulatory responses, skin properties and thermal and tactile sensitivity. Selection criteria consisted of no history of cardio-vascular diseases and sensory-related disorders; no history of muscle-skeletal injuries in the previous 12 months to the study; being physically active (i.e. performing at least 4 to 6 h of regular exercise per week for at least the last 12 months).

All the experimental studies and testing were conducted at the Environmental Ergonomics Research Centre at Loughborough University.

2.3 **Skin wetness perception: contact with external (dry or wet) stimuli**

Five experimental studies were conducted to investigate skin wetness perception during the contact with an external (dry or wet) stimulus. These were performed with the aim of isolating the individual contribution of thermal and tactile cues to the perception of wetness so that a sensory model for wetness could be developed.

In this respect, as other sensory modalities than thermal and tactile were considered as potential confounding factors in the investigation of skin wetness perception, specific set-ups were designed. Particularly, we wanted to limit the contribution of vision to perceptual experience of skin wetness, thus focusing on the somatosensory components of this perception. For this reason, in all these studies, participants were unaware of the type of stimuli used and were blind to the site of stimulation. This approach was considered effective in reducing the contribution of any expectation effect as well as of any confounding factor.

The set ups designed for these studies are described below.

2.3.1 **Experimental set ups**

For the first study investigating skin wetness during the contact with an external stimulus (see Chapter Four), the forearm was chosen as preferred site for stimulation. In this respect, participants were informed only about the body region subjected to the stimulation. No information was provided on the type and magnitude of the stimulation to limit any expectation effects. Water spray bottles were introduced as part of the set-up, to suggest wetness could be real. To blind the participants to the site of stimulation, an S-shaped wooden panel (width: 81 cm; length: 74 cm; height: 60 cm) was placed on a table. A hole (width: 12 cm; height: 13 cm) in the panel allowed participants to enter their left forearm and lay it down with the palm facing upward. This setup did not allow the participants to see the stimuli that were applied on their forearm (Fig. 1).

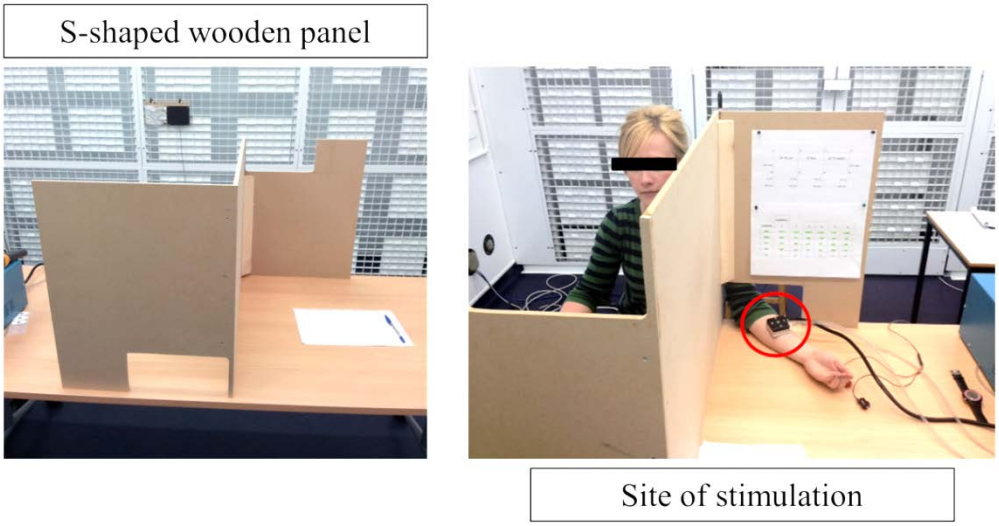

Figure 1: The S-shaped wooden panel used to blind the participants to the site of stimulation (see Chapter Four).

For the second and fourth studies investigating skin wetness during the contact with an external stimulus (see Chapter FIVE And SEVEN), the upper and lower back were chosen as preferred sites for stimulation. In this respect, participants were informed only about the body region subjected to the stimulation. No information was provided on the type and magnitude of the stimulation to limit any expectation

effects. Being this their back, participants were naturally blind to the site of stimulation (Fig. 2).

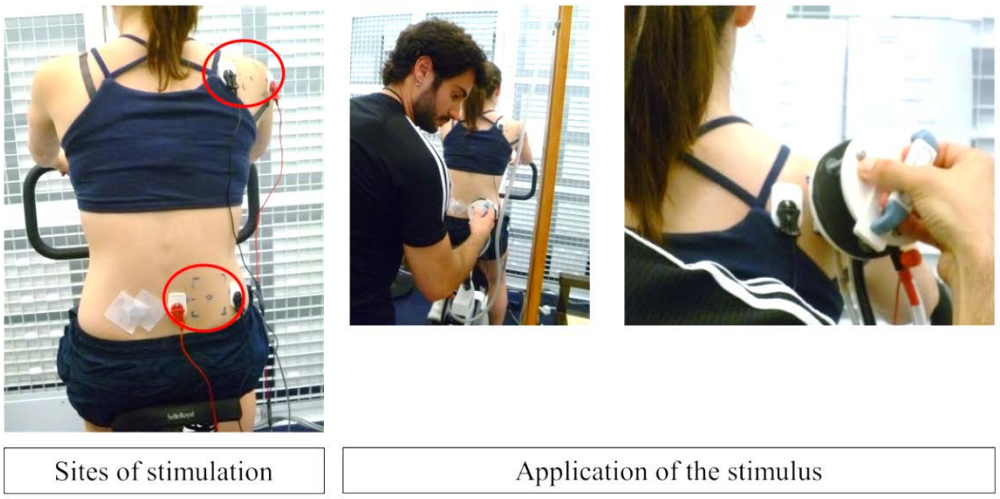

Figure 2: The experimental set-up adopted for the Laboratory study 3 and 5 (see Chapter Five and Seven).

For the third study investigating skin wetness during the contact with an external stimulus (see Chapter Six), 12 regions of the front and back of the torso were chosen as preferred sites for stimulation (Fig. 3). In this respect, participants were informed only about the body region subjected to the stimulation. No information was provided on the type and magnitude of the stimulation to limit any expectation effects.

Figure 3: The 12 skin sites of the front and back of the torso chosen for stimulation in the Laboratory study 4 (see Chapter Six).

To blind the participants to the sites of stimulation, the following set up was designed (Fig. 4). When the front torso was stimulated, participants were asked to lie on a bench on their back, with their arms alongside the body and a rectangular-shaped textile screen (length: 81cm; height: 67cm) was placed above participants' neck. The screen was adjusted until each participant confirmed that they could not see either their front torso or the investigator. When the back torso was stimulated, participants were asked to lie on their front, with their arms alongside the body, and to face towards the left, while the investigator was standing on their right hand side.

Figure 4: The experimental set-up adopted for the Laboratory study 4 (see Chapter Six).

For the fifth and last study investigating skin wetness during the contact with an external stimulus (see Chapter Eight), the forearm and index finger pad were chosen as preferred site for stimulation (Fig. 5). In this respect, participants were informed only about the body region subjected to the stimulation. No information was provided on the type and magnitude of the stimulation to limit any expectation effects. Water spray bottles were introduced as part of the set-up, to suggest wetness could be real.

Figure 5: The 2 skin sites chosen for stimulation in the Laboratory study 6 (see Chapter Eight).

As for the Laboratory study 2 (see Chapter Four), to blind the participants to the site of stimulation, an S-shaped wooden panel (width: 81 cm; length: 74 cm; height: 60 cm) was placed on a table. A hole (width: 12 cm; height: 13 cm) in the panel allowed participants to enter their left forearm so that they could interact with the stimuli. This setup did not allow the participants to see the stimuli that were applied on their forearm. Furthermore, as in this study a compression ischemia protocol was used (see Chapter Eight), during the experimental tests in which this protocol was performed, a blood pressure cuff was applied on participants' forearm (Fig. 6).

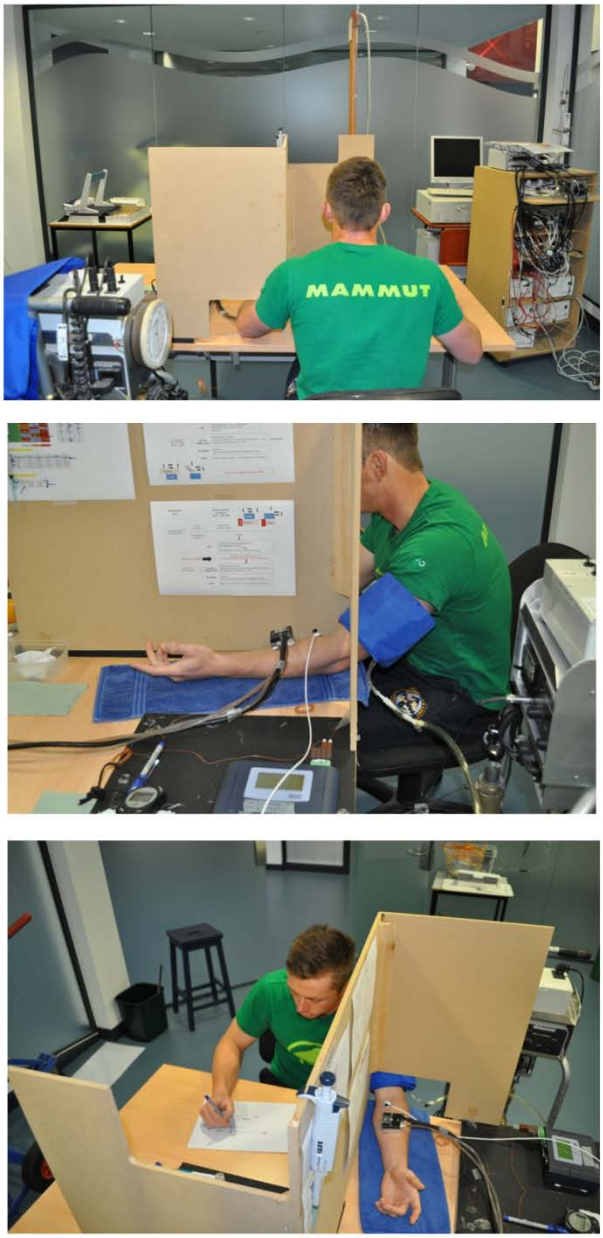

Figure 6: The S-shaped wooden panel used to blind the participants to the site of stimulation in Laboratory study 6 (see Chapter Eight).

2.3.2 **Stimulator**

With regards to the type stimulator to be used to induce a perception of skin wetness, this had to satisfy specific criteria which were essential to effectively investigate the thermal and tactile components involved in the perception of wetness. Hence, this stimulator had to be:

- Controllable in terms of its temperature, in terms of the mechanical interaction it could generate on the skin, and in terms of its wetness level.

- Relatively small and easily applicable to different parts of the body, during different conditions (i.e. rest and exercise).

These criteria were found to be satisfied by the Physitemp thermal probe (Physitemp Instruments Inc., USA). This thermal stimulator presents a thermal probe with a contact metallic surface of 25 cm^2 and a weight of 269 g (Fig. 7a). The thermal probe is driven by a thermoelectric (Peltier effect) module. The system is composed of a Controller (read out unit) to which the thermal probe is connected. For stable operation, the thermoelectric module requires a trickle of cooling water. This is supplied by a Pump and Tank unit connected to the Controller (Fig. 7b).

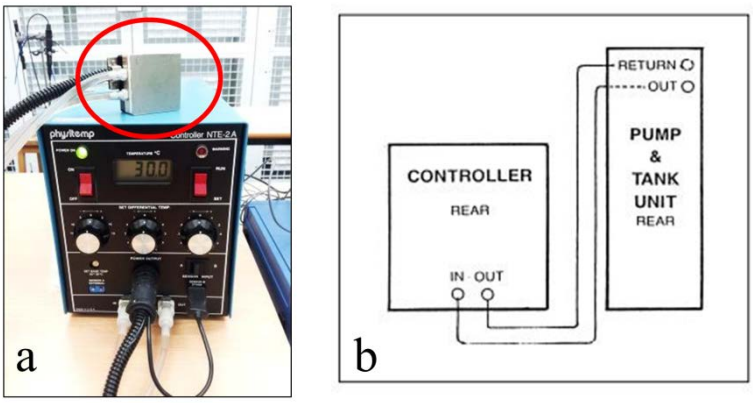

Figure 7: The thermal probe used for the application of the external stimuli. Panel a shows the Control unit with the 3 dials allowing control of the probe's temperature (step changes of ± 5 °C, ± 1 °C or ± 0.1 °C) and the thermal probe with the contact metallic surface of 25 cm^2 (in the red circle). Panel b shows a schematic diagram of how the Control unit was connected to the Pump and Tank unit.

The thermal probe had a base adjustable temperature range of 20-30 °C. According to the base temperature, a temperature control range of ± 20.5 °C is allowed. The thermal probe has a response time of <4 s in heating and cooling.

As it stood, the thermal probe assured that some of the requirements needed (i.e. having a controllable temperature as well as being relatively small and easily applicable to different parts of the body) were met. However, specific modifications were needed to assure that the same stimulator could allow the application of stimuli with different levels of wetness, as well as to control the mechanical pressure applied to the skin.

With regards to the first requirement, to make the contact with the probe' surface either dry or wet, test fabrics (100 % cotton) with a surface of 100 cm^2, were placed on the thermal probe and fixed by an elastic band (Fig. 8). According to the test, these were wetted with water at ambient temperature (~23 °C), using a variable volume pipettor (SciQuip LTD, Newtown, UK).

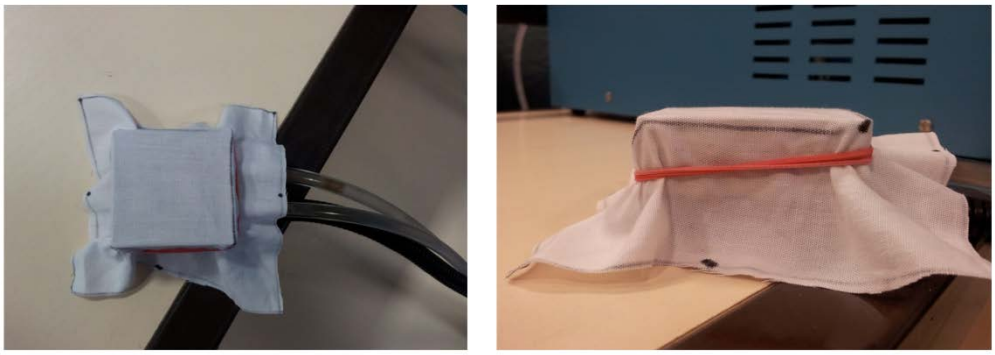

Figure 8: The test fabrics used to make the probe's contact surface either dry or wet.

With regards to the second requirement, to manipulate and control the mechanical pressures applied by the thermal probe, we designed and developed a pressure control system (Fig. 9). The system consisted of an air bladder, inserted into a frame attached to the thermal probe, which was connected to a manometer (containing water) throughout a silicon tube. The frame consisted of two wooden discs laid one upon the other and coupled by three springs which allowed the top disc to scroll down freely. A handle was attached to the top disc so that the probe could be applied to the skin. When this happened, the air bladder deformed, producing a pressure change in the system which resulted in displacing the water in the manometer from its set "null" point (no pressure applied). The point reached by the water in the tube

as a result of the pressure change was used as an indicator to control the mechanical pressure. To calibrate and standardize this last one, a digital scale (Mettler Toledo Inc., USA) was used to measure the force resulting from the application of the probe.

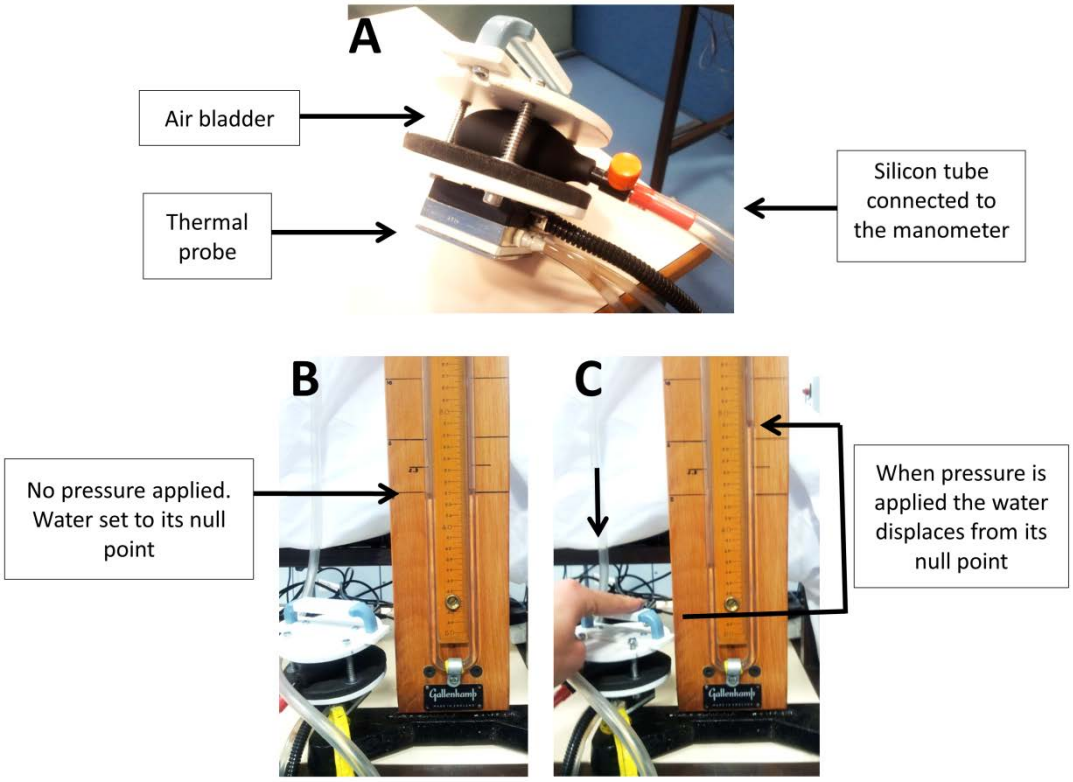

Figure 9: The pressure control system developed to manipulate and control the mechanical pressures applied by the thermal probe.

The range between the lowest and the highest pressure applicable and measurable by the system resulted in 7 to 55 kPa. Tests were performed during the development of the prototype to check the accuracy and repeatability of the nominal pressures applied with the pressure control system. 100 trials were conducted. These consisted of measuring the force resulting from the application of the probe on a digital scale (Mettler Toledo Inc., USA) while controlling that the water displacement on the manometer was the one required for the pressures selected. 95% confidence interval values were calculated for the two reference pressures (i.e. 7 and 10 kPa) and resulted as follow: 7 kPa = 7.1 kPa (lower bound) – 7.2 kPa (upper bound); 10 kPa= 10.4 kPa (lower bound) – 10.6 kPa (upper bound).

2.3.2.1 Temperature stimuli

One of the aims of the experimental studies performed was to isolate the individual contribution of thermal cues to the perception of wetness. Hence, the relationships between physical temperature and thermal sensation had to be taken into account in order to appropriately choose the characteristics of the temperature stimuli to be used [e.g. absolute vs. relative (to skin temperature) temperatures].

According to Hensel (1981), the physical correlates of thermal sensations (E_t) can be expressed as a function of the absolute skin temperature (T), the rate of change of skin temperature over time ($\Delta T/\Delta t$) and the stimulus area (F) as follow:

$$E_t \rightarrow f(T, \frac{\Delta \mathrm{T}}{\Delta \mathrm{t}}, \mathrm{F})$$

Due to the absence of a simple correlation between physical temperature and temperature sensation, these factors need to be carefully considered in the design of temperature stimuli for thermosensory investigations (Hensel, 1981).

With regards to the experimental work presented in this Thesis, both relative temperature stimuli (i.e. stimuli with a fixed temperature difference with skin temperature, e.g. -2 °C lower than skin temperature) and absolute temperature stimuli (i.e. stimuli with an absolute temperature, independent from skin temperature, e.g. a 25 °C stimulus), characterised by the same surface area (i.e. 25 cm^2), and fixed application time (i.e. 10 to 30 s), were used.

Relative temperature stimuli were primarily chosen for those studies in which a cold-dry stimulus was used to test wetness perception (see e.g. Chapters four, five and seven), and baseline skin temperature was preferred not to be adapted to a specific initial temperature; this, as the preliminary contact with the dry thermal probe (aiming to set the initial skin temperature) could have influenced the way the actual dry stimulus (delivered with the same probe) would have been experienced in terms of wetness perception. Hence, a relative temperature (to skin temperature) was considered appropriate in order to assure that the thermal stimulus would generate potentially equal relative changes in skin temperature between and within participants, without contributing to any expectation effect.

On the other side, absolute temperature stimuli were primarily chosen for those studies in which wet stimuli were used (see e.g. Chapters nine) and adapting baseline

skin temperature was not considered to influence the way the actual stimulus would be perceived in terms of wetness. Within these conditions, the rate of change in skin temperature was accurately monitored and maintained consistent between and within participants.

It deserves mention that, when defining the characteristics of a temperature stimulus (e.g. relative vs. absolute), and when interpreting the resulting thermal sensations, the relationship between thermal sensation (i.e. phenomenal quality of thermal stimulation) and thermal sensitivity [i.e. combination of phenomenal and physical quality of thermal stimulation (e.g. change in thermal sensation for a given change in temperature)] should be carefully evaluated in light of the above mentioned parameters (i.e. skin temperature, rate of change in skin temperature over time and stimulus area).

2.3.3 **Measurement of skin temperature**

In order to overcome previous limitations as observed in the literature (i.e. absence of specific physiological measurements of local changes at the skin during the application of the external stimuli), for the experimental work presented in this Thesis it was decided to monitor the local changes in skin temperature when the skin was stimulated by the different stimuli used, as well as the whole-body changes in mean skin temperature when participants were exposed to different environmental conditions.

Local skin temperature before or after the contact with the stimuli was measured by using a single spot infrared thermometer (FLUKE 566, Fluke Corporation, USA) with a temperature range of -40 to 800 °C and an accuracy of ± 1 °C (Fig. 10). In order to maximize the accuracy of the temperature reading, during all testing the infrared thermometer was calibrated against a matt black plate whose temperature was monitored with a thermistor (Grant Instruments, Cambridge, UK).

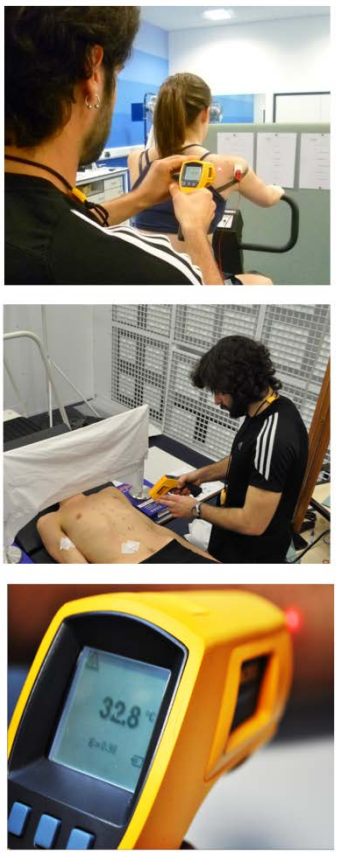

Figure 10: The infrared thermometer used to measure local skin temperature before or after the contact with the stimuli.

Local skin temperature during the contact with the stimuli was measured by using a thin thermocouple (0.08 mm wire diameter, 40 Gauge; 5SRTC-TT-TI-40-2M, Omega, Manchester, UK). This was applied either on the ventral side of the forearm or index finger pad using transpore tape (3M, Loughborough, UK), with the sensor tip touching the skin, but not covered by tape (Fig. 11). To monitor and record contact temperatures, the thermocouple was plugged in Grant Squirrel SQ2010 data logger (Grant Instruments Ltd., Cambridge, UK).

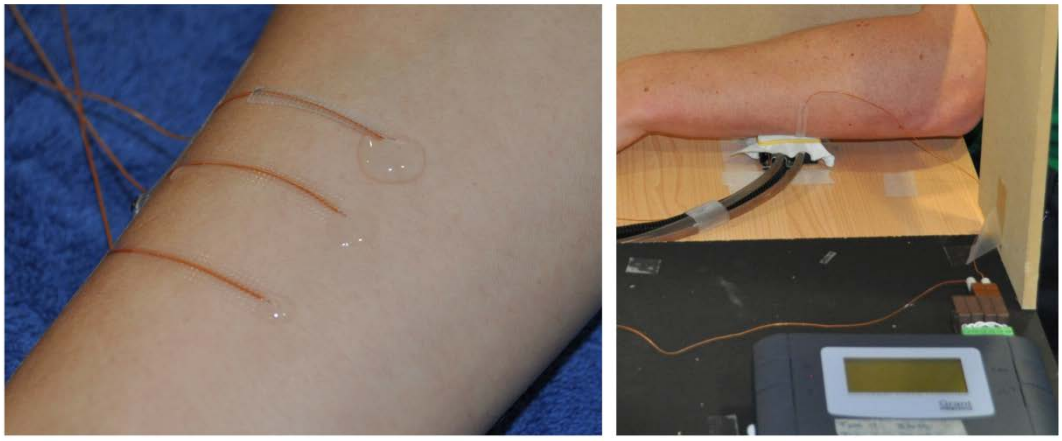

Figure 11: The thin thermocouples used to measure local skin temperature during the contact with the stimuli.

Finally, to estimate mean skin temperature, iButtons wireless temperature loggers (Maxim, San Jose, USA) with a temperature range of -55 to 100 °C , resolution of 0.5 °C and response time of 2 s were used. These were taped to five skin sites on the left side of the body (i.e. cheek, abdomen, upper arm, lower back and back lower thigh) to record local skin temperature (Fig. 12). Mean skin temperature (T_{sk}) was calculated according to the work of Houdas and Ring (1982) as follow:

$$Mean\ T_{sk} = (cheek\ \times 0.07) + (abdomen \times 0.175) + (upper\ arm \times 0.19) + (lower\ back \times 0.175) + (back\ lower\ thigh \times 0.39)$$

Figure 12: The iButtons (wireless temperature loggers) used to measure local skin temperature to be used for the estimation of mean skin temperature.

2.3.4 Measurement of perceptual responses: psychometric scales

Two main types of psychometric scales were used within the experimental work presented in this Thesis to assess thermal sensation and wetness perception (along with thermal comfort and pleasantness sensation): Likert scales and Visual Analogue scales.

With regards to the specificity of each type of scale, and how appropriate their use is according to the experimental conditions designed, it is generally accepted that Likert scales are preferable for the benefits that the presence of verbal descriptors provides in helping individuals to describe their sensations. This is particularly true when external noise or distractors can influence the subjective ability to define one's own sensations (Lee et al., 2010b). In line with this point, and with regards to this Thesis, Likert scales were mainly used for those studies in which participants were exercising (see Chapter Five) or could not mark their sensation by hand writing due a particular experimental set up (see Chapter Seven).

With regards to Visual Analogue Scales, these are generally considered as preferable when a higher sensitivity in the measurement of a particular sensation is needed. Also, by not restricting individuals' ability to rate their sensation based on specific verbal descriptors, these scales are thought to provide individuals with a greater flexibility and thus accuracy in their sensation discrimination (Lee et al., 2010b). In line with this point, Visual Analogue Scales were mainly used for those studies in which a greater accuracy in wetness discrimination was needed due to a large number of stimuli with different properties (see Chapter Four and Nine).

Figure 13 shows an overview of the Likert scales and Visual Analogue scales used for the assessment of thermal sensation and wetness perception in each of the studies investigating skin wetness perception during contact with an external stimulus.

LIKERT SCALES

CHAPTER FIVE Laboratory study 3 | CHAPTER SEVEN Laboratory study 4 | CHAPTER EIGHT Laboratory study 5

Thermal sensation

+4	Warm
+3	
+2	Slighlty warm
+1	
0	Neutral
-1	
-2	Slighlty cool
-3	
-4	Cold
-5	
-6	Very cold

Wetness perception

+4	Dry
+3	
+2	Slighlty dry
+1	
0	Neutral
-1	
-2	Slighlty wet
-3	
-4	Wet
-5	
-6	Dripping wet

VISUAL ANALOGUE SCALES

CHAPTER FOUR Laboratory study 2

Thermal sensation

0	Not cold at all
1	
2	
3	
4	
5	
6	Extremely cold
>6	Painfully cold

Wetness perception

0	Dry
1	
2	
3	
4	
5	
6	Extremely wet

CHAPTER NINE Laboratory study 6

Thermal sensation

Hot _____ Cold

Wetness perception

Completeley dry _____ Completely wet

Figure 13. Overview of the Likert scales and Visual Analogue scales used for the assessment of thermal sensation and wetness perception in each of the studies investigating skin wetness perception during contact with an external stimulus. For the Visual Analogue scales used in the Laboratory study 6, the length of the line was 100 mm.

3 CHAPTER THREE - Laboratory study 1: Mild evaporative cooling applied to the torso provides thermoregulatory benefits during running in the heat

Publication(s) based on this chapter:

Filingeri, D., Fournet, D., Hodder, S., Havenith, G. (2014) Mild evaporative cooling applied to the torso provides thermoregulatory benefits during running in the heat. *Scandinavian Journal of Medicine and Science in Sports*. In Press. (Appendix C)

3.1 Abstract

We investigated the effects of mild evaporative cooling applied to the torso, before or during running in the heat. Nine males performed 3 trials: control-no cooling (CTR), pre-exercise cooling (PRE-COOL) and during-exercise cooling (COOL). Trials consisted of 10 min neutral exposure and 50 min heat exposure (30 °C; 44 % humidity), during which a 30 min running protocol (70 % VO_{2max}) was performed. An evaporative cooling t-shirt was worn before the heat exposure (PRE-COOL) or 15 min after the exercise was started (COOL). PRE-COOL significantly lowered local skin temperature (T_{sk}) (up to -5.3 ± 0.3 °C) (p<0.001), mean T_{sk} (up to -2.0 ± 0.1 °C) (p<0.001), sweat losses (143 ± 40 g) (p=0.002) and improved thermal comfort (p=0.001). COOL suddenly lowered local T_{sk} (up to -3.8 ± 0.2 °C) (p<0.001), mean T_{sk} (up to -1.0 ± 0.1 °C) (p<0.001), heart rate (up to -11 ± 2 bpm) (p=0.03), perceived exertion (p=0.001) and improved thermal comfort (p=0.001). We conclude that the mild evaporative cooling provided significant thermoregulatory benefits during exercise in the heat. However, the timing of application was critical in inducing different thermoregulatory responses. These findings provide novel insights on the thermoregulatory role of T_{sk} during exercise in the heat.

3.2 Introduction

Human temperature regulation is challenged during exercise in the heat (Havenith, 2001). The increase in the metabolic heat production (resulting from exercising muscles), and the decrease in the gradient for heat loss to the environment (resulting from high ambient temperatures and humidity), translate into an increased rate of body heat storage (Tikuisis et al., 2002). This results into a quicker obtainment of the "critical" (i.e. ~40 °C) core temperature (T_c), suggested as one of the main limits to aerobic performance in the heat (González-Alonso & Teller, 1999). Elevated T_c can result in a decreased neural drive to muscle contraction (Nybo & Nielsen, 2001), as well as in cellular perturbations, which could disrupt metabolic and contractile processes within skeletal muscle (Febbraio, 2000). The limit that elevated T_c poses on aerobic performance is particularly evident within conditions of exercise performed at a fixed intensity and to fatigue, as opposed to self-paced exercise, in which behavioural adjustments (i.e. pacing) often prevent the obtainment of such physiological strains (Schlader et al. 2011c).

Pre-cooling strategies (i.e. cold water immersion, ice vests, ice/cold fluids ingestion) have been developed to counterbalance the effects of exercising under heat stress (Tyler et al., 2013). These methods have primarily focused on reducing T_c before exercise, in order to increase the margin for metabolic heat production, and thus the time to reach the critical temperature (Marino, 2002). However, emerging evidence suggests that the role of elevated (>35 °C) skin temperature (T_{sk}) is also critical in impairing aerobic performance under heat stress (Sawka et al., 2012). Elevated T_{sk} narrows the skin to core temperature gradient, thus increasing the skin blood flow requirements, and eventually resulting in an increased level of cardiovascular strain (Sawka et al., 2012). This is exacerbated by the competition for the available cardiac output between the blood flow required by the exercising muscles to meet the oxygen demands, and the blood flow required by the skin to meet the demands of temperature regulation (i.e. heat dissipation to the environment) (González-Alonso et al. 2008). Also, heat-induced changes in T_{sk} influence perceptual and cutaneous-sensory feedback such as thermal sensation, comfort and "sensation of fatigue" (Cheung, 2010) which have been proposed as critical determinants of pacing strategies during performance under heat stress (Schlader et al. 2011b; Tucker &

Noakes, 2009). Therefore, in order to preserve performance under heat stress, keeping the skin cool during the exercise, might be as important as a pre-exercise reduction in T_c (Schlader et al. 2011c).

Cooling methods, such as air and water cooled systems (Stephenson et al., 2007), garments made of phase change materials (House et al., 2013), as well as the use of menthol (Gillis et al., 2010), have been developed and shown to be potentially effective in preserving performance in the heat, due to their effects on T_{sk} and thermal sensation (Hasegawa & Takatori, 2005). The beneficial effects of these cooling strategies have been shown to vary largely according to the environmental conditions (i.e. the higher the heat load the more beneficial the cooling), the duration of cooling (i.e. the longer the more beneficial) and most importantly, to the type and duration of exercise performed (i.e. cooling is more beneficial for endurance exercise performed for up to 60 min as opposed to single sprint exercise) (Wegmann et al., 2012). However, due to some specific disadvantages, such as weight of the systems, wearability of the garments, duration of the cooling effect (e.g. garments made of phase change materials require large quantities of coolant to provide prolonged cooling) (Kenny et al. 2011) or side effects of menthol application (i.e. skin irritation), these methods still present numerous practical limitations (Tyler et al., 2013), and are therefore best suited to specific conditions (e.g. cooling methods with limited capacity are preferable for short duration exercise under conditions of higher heat loads) (Kenny et al., 2011).

In this respect, evaporative cooling garments have recently received attention, as they could represent a potentially effective alternative to more traditional cooling methods (Webster et al., 2005; Bogerd et al., 2010). These lightweight garments induce mild-cooling via the process of water evaporation. These are made of particular hydrophilic fabrics, which, if wetted, allow sustained water evaporation, thereby cooling the garment and underlying skin. Although using the concept of mild evaporative cooling translates into the possibility to design cooling garments which are lightweight and practical, the limited empirical evidence on their physiological as well as perceptual (i.e. thermal comfort) effects makes any conclusion on these methods difficult to draw (Tyler et al., 2013).

Developing lightweight, thermally comfortable cooling methods, which can be effective in counteracting the thermal strain, has important practical implications, not only for elite performance under heat stress, but also, in the context of amateur and recreational exercise. Individuals who enjoy outdoor sporting activities, such as running or cycling, encounter a variety of environmental conditions, some of which (e.g. heat) can significantly decrease their thermal comfort (Vanos et al., 2010). As the type and amount of physical activity performed has been shown to be influenced by the level of comfort achievable with the surrounding environment (Vanos et al., 2010), developing a practical cooling method, being able to reduce the thermal discomfort experienced while exercising in the heat, might have a positive impact on the activity levels of healthy individuals.

The first aim of this study was to investigate the physiological [i.e. heart rate (HR), T_c, mean and local T_{sk}, and body sweat loss] and perceptual [thermal, wetness and comfort sensations, and (session) ratings of perceived exertion (RPE)] effects of a lightweight, short-sleeved garment which induced mild evaporative cooling of the torso, with the aim to provide thermoregulatory benefits during submaximal running in the heat [i.e. 30 °C ambient temperature (T_{air}) and 44 % relative humidity (RH)]. In this respect, we hypothesised that the mild evaporative cooling applied to the torso would significantly lower local and mean T_{sk}, thus reducing total sweat production and thermal discomfort. The second aim of this study was to investigate the impact of varying the timing of cooling (i.e. wearing the garment before or during exercise) on the above mentioned physiological and perceptual parameters. We hypothesised that applying the cooling during the exercise (i.e. when participants were already hyperthermic) would significantly lower the HR, the perceived exertion and the overall level of thermal discomfort. Rapidly cooling the skin has been indeed shown to reduce the cardiovascular strain observed during exercise in the heat, due to its effects on skin blood flow (Sawka et al., 2012). When exercising under heat stress, elevated T_c and T_{sk} pose a major challenge to the cardiovascular system, due to an increased competition for the available cardiac output between the blood flow required by the exercising muscles to meet the oxygen demands, and the blood flow required by the skin for heat dissipation to the environment (González-Alonso et al. 2008). As the increased cardiovascular strain limits aerobic performance (i.e. VO_{2max}) in the heat (i.e. due to a higher fractional VO_{2max} for any given power output)

(Kenefick et al. 2010), and as skin blood flow changes as a function of T_{sk} (Cheuvront et al. 2010), rapidly cooling the skin was hypothesised to lower the cardiovascular strain by reducing the skin blood flow requirements for heat dissipation. In terms of performance benefits, reducing the cardiovascular challenge of exercising under heat stress could be beneficial to help maintaining the adequate cardiac output required by the exercise, without a concurrent reduction in maximal aerobic power due to increased thermoregulatory demands (Kenefick et al. 2010). Finally, investigating the effects of varying the timing of cooling was considered relevant for its behavioural and perceptual effects. Reductions in T_{sk} during exercise in the heat (and the accompanying thermal sensations) have been previously shown to improve heat tolerance (Hasegawa and Takatori, 2005), and to benefit performance (Schlader et al., 2011). Also, due to the limited number of studies addressing this concept, cooling during exercise in the heat is an area which is receiving increasing attention (Tyler et al., 2013).

3.3 Materials and methods

3.3.1 Participants

Nine healthy male students [age 21 ± 2 years, height 179 ± 8 cm, body mass 80 ± 9 Kg, body fat 8 ± 3 %, estimated maximum oxygen consumption (VO_{2max}) 54 ± 5 $ml \cdot min^{-1} \cdot kg^{-1}$] volunteered to participate in this study. Inclusion criteria for this study were: 1. no history of cardiovascular disease, sensory-related disorders and muscle-skeletal injuries in the previous 12 months; 2. being physically active (i.e. performing at least 4 to 6 h of regular exercise per week for at least the last 12 months). All participants gave their informed consent for participation. The test procedure and the conditions were explained to each participant. The study design had been approved by the Loughborough University Ethics Committee and testing procedures were in accordance with the tenets of the Declaration of Helsinki. For a period of 48 h before each trial, the participants were instructed to refrain from strenuous exercise. Furthermore, the participants were asked not to consume caffeine or alcohol 24 h before each trial, and to refrain from food 2 h before each trial.

3.3.2 **Experimental design**

Participants attended one preliminary session to determine their anthropometrical characteristics and aerobic capacity. Each participant's body mass, height and skinfolds thickness (7 sites) were measured and recorded. For body composition calculations, ACSM's guidelines for exercise testing and prescription were used (Gordon, 2009). Body density was calculated using the following seven sites (chest, midaxillary, triceps, subscapular, abdomen, suprailiac and thigh) equation:

$$Body\ density = 1.112 - 0.00043499(sum\ of\ seven\ skinfolds) + 0.00000055(sum\ of\ seven\ skinfolds)^2 - 0.00028826(age)$$

A submaximal fitness test, was performed to estimate individuals' aerobic fitness level (expressed as VO_{2max}) using the Astrand-Rhyming method (Gordon, 2009). The test was completed on a treadmill (Woodway Pps Med, Woodway Incorporated, Waukesha, WI, USA) in a thermo-neutral environment (20 °C T_{air}, 40 % RH) to prevent any thermal strain.

The preliminary session was then followed by three experimental trials, performed in a counterbalanced order: pre-exercise cooling (PRE-COOL), during-exercise cooling (COOL) and control-no cooling (CTR). The cooling trials differed in terms of the timing of applying the cooling: before or during the exercise protocol (i.e. 15 min after the exercise was initiated). All experimental trials consisted of 10-min thermo-neutral exposure (22 °C T_{air}; 30 % RH; 0.4 $m \cdot s^{-1}$ environmental chamber's air velocity), followed by 50-min heat exposure (30 °C T_{air}; 44 % RH; 0.4 $m \cdot s^{-1}$ environmental chamber's air velocity). During the heat exposure, participants first rested on a chair for 10 min to familiarise with the environmental conditions in which the exercise protocol would be performed and to allow stabilisation of physiological values. Then, they performed a 5-min running warm up, at a speed corresponding to 50 % of their individual VO_{2max}. This was followed by 25-min running performed at 70 % of their individual VO_{2max}. During the exercise protocol, participants were exposed to a 2 $m \cdot s^{-1}$ frontal air velocity. At the end of the exercise protocol, participants were asked to rest on a chair for 10 min before leaving the environmental chamber. A schematic outline of the experimental design is shown in figure 1.

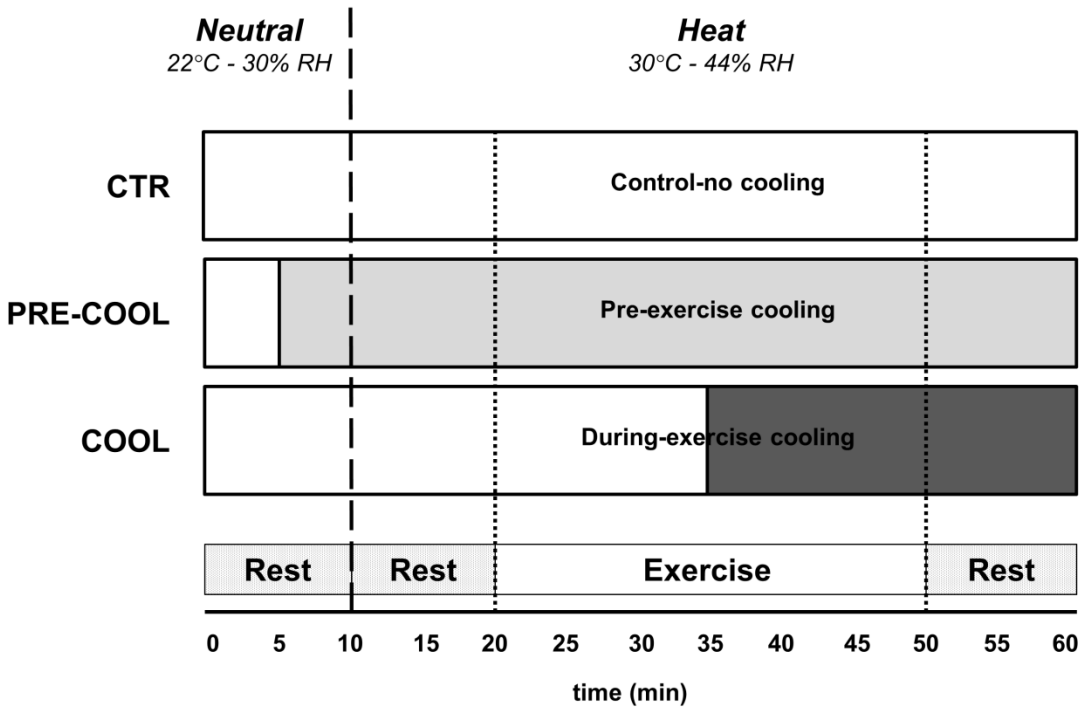

Figure 1: A summary of the experimental protocol. CTR, no-cooling; PRE-COOL, pre-exercise cooling applied after 5 min of neutral exposure and before the exercise was performed; COOL, during exercise-cooling applied 15 min after the exercise was started. During all trials participants first rested for 10 min in the neutral environment before moving to the hot environment. Here they rested for the first 10 min, then they started a 5 min running warm up performed at 50 % of their VO_{2max}, followed by a 25 min running protocol performed at 70 % of their VO_{2max}. After the exercise protocol, participants rested in the hot environment for 10 min.

3.3.2.1 Cooling garment

Cooling was applied through an evaporative cooling, short-sleeved, tight fitting t-shirt (Oxylane, Quecha Aquafreeze, France), which, when worn, covered the torso and shoulders of the participants. The garment induced mild cooling via the process of water evaporation. This was made of a hydrophilic and hydrophobic structure. The outer layer enclosed a hydrophilic fabric, and acted as a water reservoir and distributor, whereas the inner layer, which enclosed a hydrophobic fabric, prevented the wearer from being in contact with the wet outer layer. By wetting the garment, leaving the hydrophilic fabric fully wetted, water starts to evaporate, thereby cooling the garment and underlying skin. Twenty minutes before the exercise protocol was

initiated, the cooling garment was fully dampened in water at ambient temperature (~22 °C), then stored in a sealed container to limit evaporation of water to the environment, and maintained in the thermo-neutral environment until it had to be worn by participants. This procedure was repeated for all trials in order to assure consistency. As the dry and fully wet garment weighed 154 g and 425 g respectively, the corresponding water content of the wet garment was 271 g. The insulation value for the cooling garment was 0.041 $m^{2}\cdot K\cdot W^{-1}$ (0.26 clo) and was determined using a thermal torso manikin with a uniform skin temperature of 34 °C and environmental temperature of 35 °C and 30 % RH. During the CTR trail, (as well as during the first part of the COOL trial), participants wore a reference short sleeved, tight fitting t-shirt (dry mass: 101 g) made of a hydrophilic fabric only (Oxylane, Kalenji Essential, France). This covered the same areas as the cooling garment (i.e. torso and shoulders) and had an insulation value of 0.031 $m^{2}\cdot K\cdot W^{-1}$ (0.20 clo).

3.3.3 **Experimental protocol**

Participants arrived at the laboratory 30 min before the time scheduled for the test to allow preparation procedures. Before they changed into shorts, socks and running shoes, participants were asked to void their bladder and semi-nude body mass (i.e. only cotton underwear was worn) was recorded on a digital scale (Sartorius Yacoila, Sartorius AG, Gottingen, Germany; precision 0.01 g). Then, they were instructed to self-insert a rectal thermometer (Grant Instruments Ltd., Cambridge, UK) 10 cm beyond the anal sphincter for the measurement of T_c.

To estimate mean T_{sk}, five wireless temperature sensors (iButtons, Maxim, San Jose, USA) were taped to five skin sites (i.e. cheek, abdomen, upper arm, lower back and back lower thigh) on the right side of the body to record local T_{sk} (1 min intervals). These five local T_{sk} measurements were used to estimate mean T_{sk} using Houdas-5W equation (Houdas & Ring, 1982):

$$Mean\ T_{sk} = (cheek\ \times 0.07) + (abdomen \times 0.175) + (upper\ arm \times 0.19) + (lower\ back \times 0.175) + (back\ lower\ thigh \times 0.39)$$

To gain additional information on the local skin temperature changes occurring as a result of the cooling garment, the local T_{sk} of four representative skin sites directly exposed to the cooling (i.e. lateral chest, lateral abdomen, lateral upper and lower

back)was recorded with four supplementary skin thermistors (Grant Instruments, Cambridge, UK) which were taped to the left side of the body. The reason for these supplementary local measurements was to gain additional information on the local T_{sk} changes occurring as a direct result of the cooling garment, being these often localised and transient, and thus potentially underestimated when only measurements of whole-body mean T_{sk} are considered (Tyler et al. 2013).
Skin and rectal temperature thermistors were connected to an Eltek/Grant 10 bit, 1000 series data logger (Grant Instruments, Cambridge, England) recording temperature at 10 s intervals. Finally, each participant wore a Polar HR monitor (Polar Electro Oy, Kempele, Finland), which recorded HR at 10 s intervals.

After preparation, participants (who were wearing only shorts, socks and running shoes) moved into the thermo-neutral environment, where they rested on a chair for 10 min. As soon as they assumed a seated position, they were asked to rate their thermal, wetness and comfort sensations, while recording of the physiological parameters was started. Three modified rating scales were used to record individual thermal, wetness and thermal comfort sensations: a 13point thermal sensation scale (i.e. -6 very cold; -4 cold; -2 slightly cool; 0 neutral; +2 slightly warm; +4 hot; +6 very hot); a 13-point wetness perception (i.e. -6 dripping wet; -4 wet; -2 slightly wet; 0 neutral; +2 slightly dry; +4 dry; +6 very dry); a 13-point thermal comfort scale (i.e. -6 very uncomfortable; -4 uncomfortable; -2 slightly uncomfortable; 0 neutral; +2 slightly comfortable; +4 comfortable; +6 very comfortable) (Olesen & Brager, 2004). No descriptors were applied to intermediate scores (i.e. -5; -3; -1; +1; +3; +5). Participants familiarised with the scales during the preliminary session.

After 5 min of thermo-neutral exposure, depending on the experimental trial, participants wore the reference garment (CTR and COOL trials) or the evaporative cooling garment (PRE-COOL trial), and thermal, wetness and comfort sensations were immediately recorded. Upon completion of the 10-min thermo-neutral exposure, participants moved into the environmental chamber set for the heat exposure. During the first 10-min exposure, participants rested on a chair to familiarise with the environmental conditions in which the exercise protocol would be performed and to allow stabilisation of physiological values. During this time and throughout the rest of the test, individual sensations were recorded every 5 min. Following the

acclimation period, participants moved to the treadmill to perform a 5 min warm-up (50 % VO_{2max}), followed by 25 min running (70 % VO_{2max}), while exposed to a 2 $m \cdot s^{-1}$ frontal air velocity. On average, the 50 and 70 % VO_{2peak} running speeds corresponded to 7 ± 1 and 10 ± 1 $km \cdot h^{-1}$ respectively (these values represent mean ± standard deviation) . During the exercise protocol, participants were asked to rate their RPE at 5 min intervals, using the 6 to 20 Borg's scale (Borg, 1982). During the COOL trial only, after 15 min from when the exercise was initiated, participants changed from the reference to the cooling garment, which was then kept on until the end of the trial.

Upon completion of the 30-min running protocol, participants were asked to move to a chair and rest in the warm environment for 10 min. At the end of the 10 min post-exercise recovery, they were asked to score a session RPE, corresponding to the overall perceived effort the session performed had required (Foster et al., 2001). During all trials, water at room temperature was provided ad libitum and the amount consumed recorded. Finally, semi-nude body mass (i.e. only cotton underwear was worn) was recorded after each trial and body sweat loss was adjusted for water intake.

3.4 Statistical analysis

In the present study, the independent variables were the condition (i.e. CTR, PRE-COOL and COOL) and time. The dependent variables were HR, T_c, mean and local T_{sk}, body sweat loss, thermal sensation and comfort, wetness perception and RPE. Parametric statistics were used to investigate the main effects and interactions of the variables during the 30-min exercise protocol. Baseline, post-heat adaptation and post-exercise data were also analysed and reported. Data were first tested for normality of distribution and homogeneity of variance using Shapiro-Wilk and Levine's tests respectively. Then data were analysed by a 2 way repeated measure ANOVA, with condition and time as repeated measures variables. Body mass loss data were analysed by a one way repeated measure ANOVA, with condition as repeated measures variable. Huynh–Feldt or Greenhouse-Geisser corrections were undertaken to adjust the degrees of freedom for the averaged tests of significance. When a significant main effect was found, Tukey's post-hoc analyses were performed. In all analyses, $p<0.05$ was used to establish significant differences.

Estimated marginal means and 95 % confidence intervals were used to investigate the main effects and interactions of the variables. Observed power was computed using α=0.05. Data are reported as mean ± standard error. Statistical analysis was performed using IBM SPSS Statistics 19 (IBM, USA).

3.5 Results

3.5.1 Heart rate

Mean HR values for each trial are shown in figure 2a. A significant main effect of condition ($F= 4.18_{(2, 16)}$, $p= 0.03$; observed power= 0.6) and time ($F= 124_{(1.17, 9.37)}$, $p<0.001$; observed power= 1) was found on the HR values recorded during the exercise protocol. Overall, the average HR recorded during the COOL and PRE-COOL trials was respectively 7 ± 3 and 3 ± 2 bpm lower than in the CTR trial. A significant interaction between condition and time was also found ($F= 3.17_{(10, 80)}$, $p= 0.002$; observed power= 1) Post-hoc analyses indicated that during the COOL trial, 20 min after the exercise protocol was started, the HR was significantly lower than in the CTR (-11 ± 2 bpm, $p= 0.002$) and PRE-COOL trial (-7 ± 2 bpm, $p= 0.004$). Post-exercise HR values were found to differ significantly between conditions, with the values recorded during the COOL trial being 10 ± 3 ($p= 0.004$) and 6 ± 2 bpm ($p= 0.013$) significantly lower than in the CTR and PRE-COOL trial respectively.

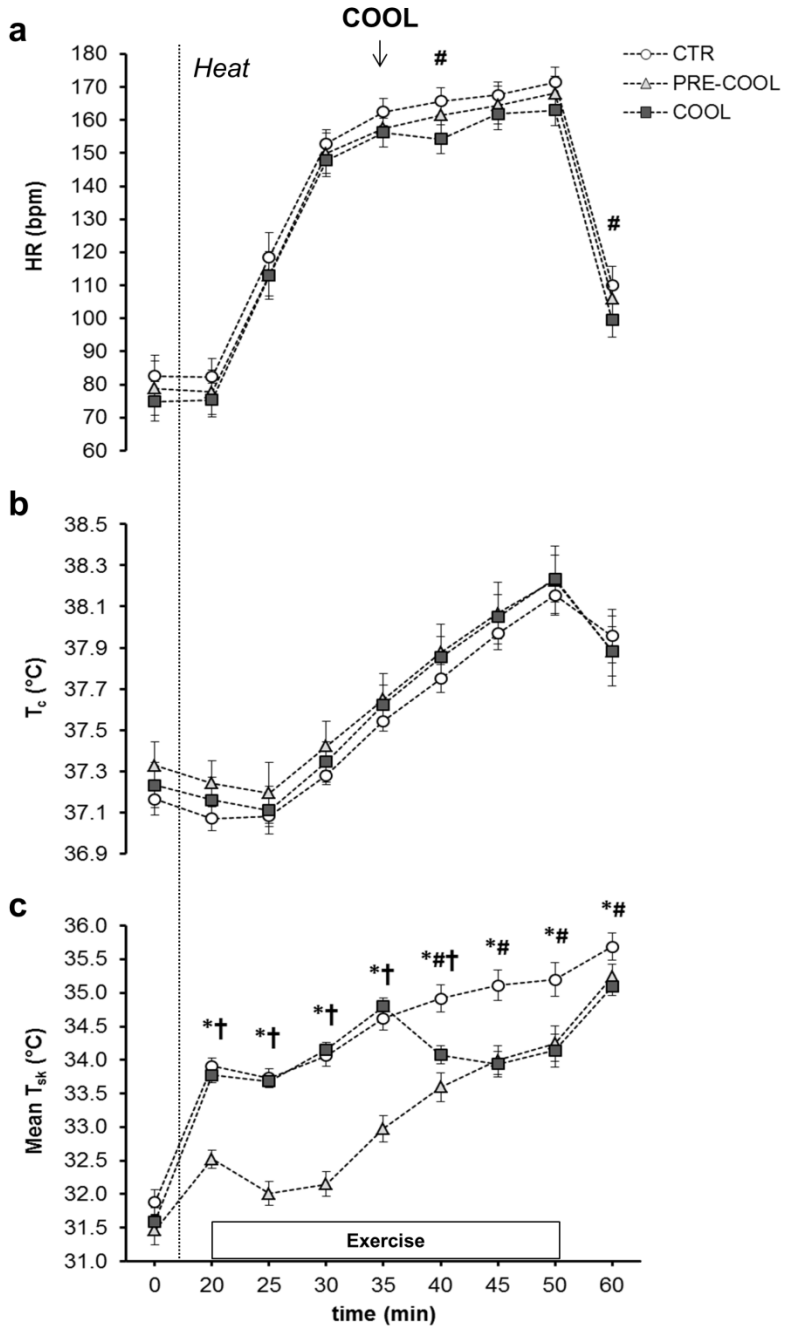

Figure 2: Mean (± standard error) HR (a), T_c (b) and T_{sk} (c) values as recorded during the CTR, PRE-COOL and COOL trials. The application of cooling during exercise (15 min after the exercise was started, COOL trial) is marked by an arrow. * (CTR ≠ PRE-COOL), # (CTR ≠ COOL), † (PRE-COOL ≠ COOL) refer to significant differences between trials as computed using $p<0.05$.

3.5.2 **Core temperature**

Mean T_c values for each trial are shown in figure 2b. No significant main effect of condition was found on the T_c values recorded during the exercise protocol ($F= 0.6_{(2, 16)}$, $p= 0.53$; observed power= 0.14). Only a significant main effect of time was observed ($F= 176.5_{(5, 40)}$, $p<0.001$; observed power= 1), with an average increase in T_c of 1.0 ± 0.1 °C from a baseline value of 37.2 ± 0.1 °C. No interaction between time and condition was found ($F= 0.84_{(10, 80)}$, $p =0.58$; observed power= 0.4). Post-exercise T_c values did not differ amongst conditions ($F= 0.15_{(2, 16)}$, $p =0.85$; observed power= 0.4).

3.5.3 **Mean skin temperature**

Mean T_{sk} values for each trial are shown in figure 2c. A significant main effect of condition ($F= 74.2_{(2, 16)}$, $p<0.001$; observed power= 1), time ($F= 41.67_{(1.33, 10.65)}$, $p<0.001$; observed power= 1) and a significant interaction between these two ($F= 67_{(10, 80)}$, $p<0.001$; observed power= 1) was found on mean T_{sk}. At the end of the familiarisation with the hot environment-period, and as a result of the application of the cooling garment, the mean T_{sk} was significantly lower in the PRE-COOL than in the CTR (-1.4 ± 0.1°C, $p<0.001$) and COOL trials (-1.2 ± 0.1°C, $p<0.001$). No differences were found between CTR and COOL trials (0.13 ± 0.10 °C, $p= 0.22$). During the exercise protocol, the mean T_{sk} was significantly lower in the PRE-COOL than in the CTR trial (from a minimum of -0.9 ± 0.1 to a maximum of -2.0 ± 0.1 °C). When compared to the COOL trial, PRE-COOL mean T_{sk} was significantly lower up until 20 min from when the exercise was started. From this point, and until the end of the exercise protocol, COOL and PRE-COOL mean T_{sk} values did not differ significantly. These results indicated that the application of the cooling during the exercise (COOL trial) reduced mean T_{sk} to values similar to the ones observed when the cooling was applied prior to start exercising (PRE-COOL trial). Post-exercise mean T_{sk} was found to differ significantly between conditions, with the values recorded during the PRE-COOL and COOL trials being respectively 0.4 ± 0.2 ($p= 0.045$) and 0.6 ± 0.2 °C ($p= 0.018$) significantly lower than in the CTR trial. No differences were found between PRE-COOL and COOL trials ($p= 0.28$).

3.5.4 **Local skin temperature**

Local T_{sk} values for each body region (i.e. chest, abdomen, upper and lower back) are shown in figure 3. Local T_{sk} showed similar trends amongst all the regions investigated. These patterns were similar to the one observed for the mean T_{sk}. Before starting the exercise protocol, local T_{sk} was significantly lower in the PRE-COOL trial than in the CTR and COOL trials for all the regions investigated. These differences varied in a range of -1.9 ± 0.2 °C (i.e. abdomen) to -5.3 ± 0.3 °C (i.e. chest). During the exercise protocol, local T_{sk} was significantly lower in the PRE-COOL trial than in the CTR and COOL trials up until 15 min from when the exercise was started. Notably, chest local T_{sk} recorded in the PRE-COOL trial showed the greatest regional difference amongst the regions investigated. Specifically, ten minutes after the exercise was started, chest local T_{sk} was 5.3 ± 0.3 °C lower than during the CTR and COOL trials. From 20 min onwards and until the end of the exercise protocol, COOL and PRE-COOL local T_{sk} values did not differ significantly. This was observed for all the regions but the chest. Chest local T_{sk} was significantly lower in the COOL than in the PRE-COOL from 25 min after the exercise was started until the end of the test. Post-exercise PRE-COOL and COOL local T_{sk} values were found to be significantly lower than CTR only for the chest and abdomen regions.

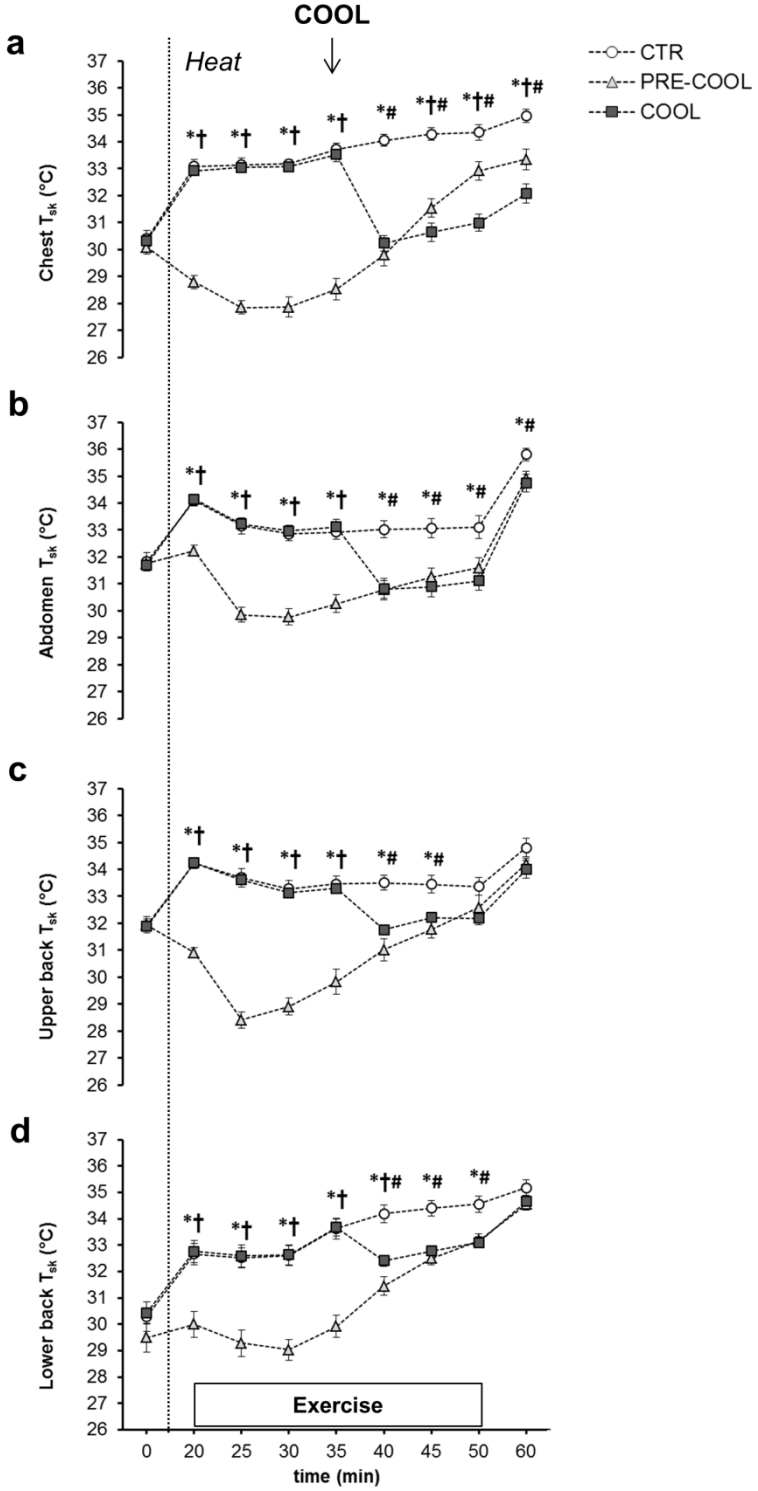

Figure 3: Mean (± standard error) local T_{sk} values for chest (a), abdomen (b), upper (c) and lower back (d) as recorded during the CTR, PRE-COOL and COOL trials. The application of cooling during exercise (15 min after the exercise was started, COOL trial) is marked by an arrow. * (CTR ≠ PRE-COOL), # (CTR ≠ COOL), †

(PRE-COOL ≠ COOL) refer to significant differences between trials as computed using $p<0.05$.

3.5.5 **Body sweat losses**

Water ingestion during the exercise protocol did not differ between CTR (226 ± 30 g), PRE-COOL (213 ± 42 g) and COOL trial (181 ± 32 g) ($F= 0.9_{(2, 16)}$, $p= 0.4$; observed power= 0.2). A significant main effect of condition was found on body sweat loss ($F= 2.5_{(2, 16)}$, $p= 0.025$; observed power= 0.7). Post-Hoc analysis indicated that the body sweat loss was significantly lower ($p= 0.018$) in the PRE-COOL (630 ± 100 g) than in the CTR condition (775 ± 90 g). No significant differences ($p= 0.4$) were found between PRE-COOL and COOL trials (768 ± 85 g).

3.5.6 **Thermal sensation**

Mean thermal sensation scores for each trial are shown in figure 4a. A significant main effect of condition ($F= 9.8_{(2, 16)}$, $p= 0.002$; observed power= 0.9) and time ($F= 26.92_{(5, 40)}$, $p<0.001$; observed power= 1) was found on the thermal sensations. Overall, the average thermal sensations recorded during the PRE-COOL, COOL and CTR trials were respectively 2.2 ± 0.4 (i.e. “slightly warm”), 2.0 ± 0.5 (i.e. “slightly warm”) and 3.8 ± 0.3 (i.e. “hot”). Also, a significant interaction between condition and time was found ($F= 18.1_{(10, 80)}$, $p<0.001$; observed power= 1). Post-hoc analyses indicated that thermal sensations were significantly lower in the PRE-COOL than in the CTR and COOL trial up until 15 min from when the exercise was started. From 20 min onwards and until the end of the exercise protocol, COOL thermal sensations were significantly lower than in the PRE-COOL and CTR trials. Post-exercise thermal sensations were found to differ significantly between conditions, with scores recorded during the COOL trial being significantly lower than in the PRE-COOL ($p= 0.048$) and CTR ($p= 0.013$) trials. No differences were found between PRE-COOL and CTR trials ($p= 0.24$). Expressed in terms of semantic labels, COOL thermal sensations corresponded to “neutral” to “slightly warm”, PRE-COOL thermal sensations corresponded to “slightly warm” to “hot”, and CTR thermal sensations corresponded to “hot”.

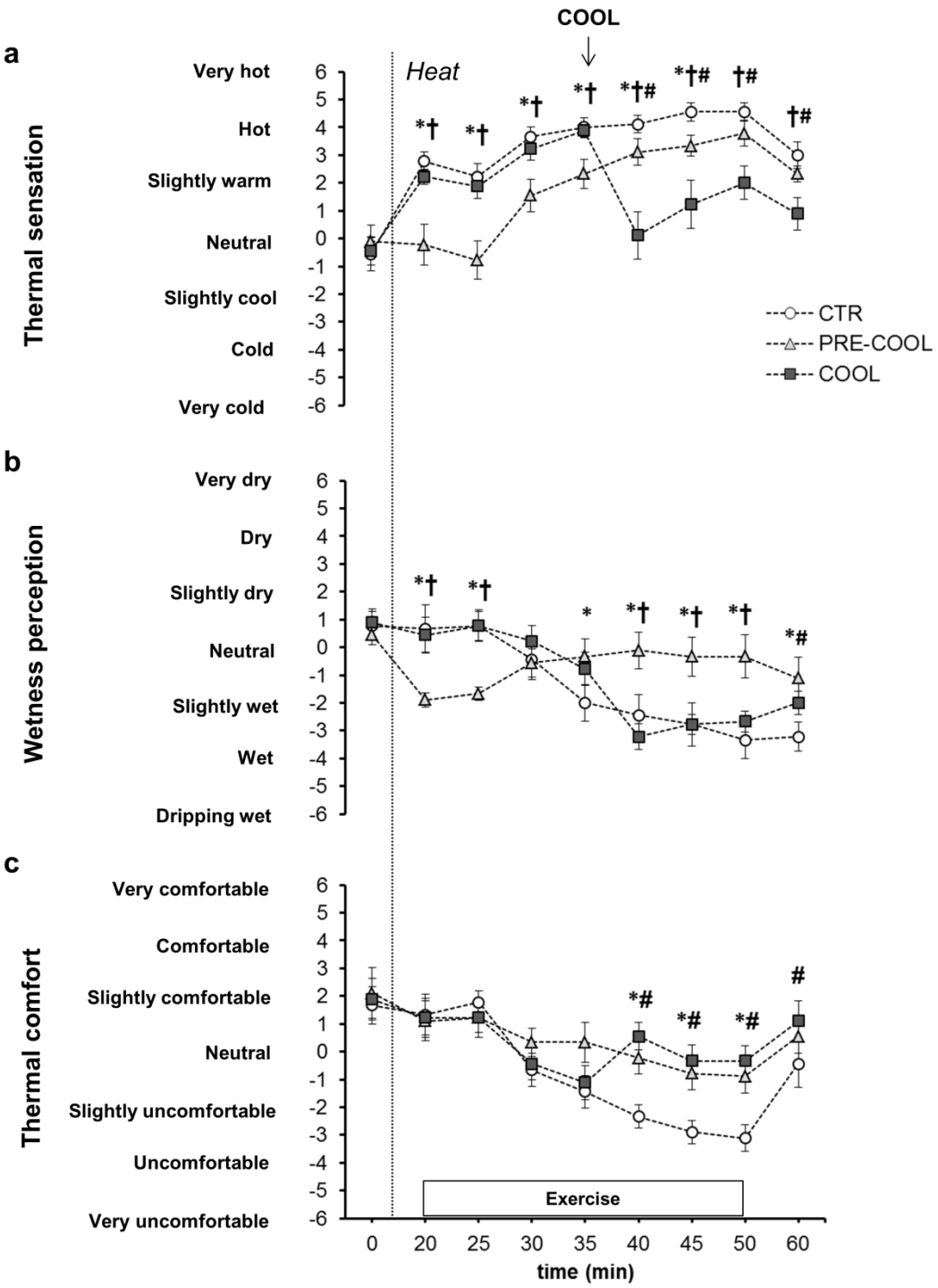

Figure 4: Mean (± standard error) thermal sensation (a), wetness perception (b) and thermal comfort (c) values as recorded during the CTR, PRE-COOL and COOL trials. The application of cooling during exercise (15 min after the exercise was started, COOL trial) is marked by an arrow. * (CTR ≠ PRE-COOL), # (CTR ≠ COOL), † (PRE-COOL ≠ COOL) refer to significant differences between trials as computed using $p<0.05$.

3.5.7 **Wetness perception**

Mean wetness perception scores are shown in figure 4b. No significant main effect of condition ($F= 3.15_{(2, 16)}$, $p= 0.07$; observed power= 0.5), a significant effect of time ($F= 13.07_{(2.19, 17.5)}$, $p<0.001$; observed power= 1) and a significant interaction between condition and time ($F= 12.35_{(10, 80)}$, $p<0.001$; observed power= 1) was found on the wetness perceptions. The perceptual scores indicated that during the PRE-COOL trial, wetness perception was initially "slightly wet" when the cooling garment was worn and then "neutral" during the rest of the trial. On the contrary, during the CTR and COOL trials, an opposite trend was observed, with a significant increase in the level of wetness perceived from the beginning till the end of the trials (i.e. from "neutral" to "slightly wet"). Post-exercise wetness perceptions were found to differ significantly between conditions (p=0.025). Expressed in terms of semantic labels, PRE-COOL and COOL wetness perceptions corresponded to "neutral" to "slightly wet" whereas CTR wetness perceptions corresponded to "slightly wet" to "wet".

3.5.8 **Thermal comfort**

Mean thermal comfort scores for each trial are shown in figure 4c. A significant main effect of condition ($F= 11.2_{(2, 16)}$, $p= 0.001$; observed power= 1) and time ($F= 12.05_{(1.9, 15.5)}$, $p<0.001$; observed power= 1) was found on the thermal comfort. Overall, the average thermal comfort recorded during the PRE-COOL, COOL and CTR trials was respectively 0.0 ± 0.4 (i.e. "neutral"), -0.7 ± 0.5 (i.e. "neutral" to "slightly uncomfortable") and -1.4 ± 0.4 (i.e. "neutral" to "slightly uncomfortable"). Also, a significant interaction between condition and time was found ($F= 6.4_{(10, 80)}$, $p<0.001$; observed power= 1). Post-hoc analyses indicated that, as compared to the CTR trial, thermal comfort was significantly improved in the PRE-COOL and COOL trials between 20 and 30 min from when the exercise was started. Post-exercise thermal comfort was found to differ significantly between conditions, with participants being more comfortable at the end of the COOL than the CTR trial (p= 0.01). Expressed in terms of semantic labels, COOL thermal comfort corresponded to "neutral" to "slightly comfortable", whereas CTR thermal comfort corresponded to "neutral" to "slightly uncomfortable".

3.5.9 **Rate of perceived exertion**

Mean rating of perceived exertion for each trial are shown in figure 5a. No significant main effect of condition ($F= 2.09_{(2, 16)}$, $p= 0.15$; observed power= 0.4), a significant effect of time ($F= 79.32_{(2, 16)}$, $p<0.001$; observed power= 1) and a significant interaction between condition and time ($F= 3.4_{(10, 80)}$, $p= 0.001$; observed power= 1) was found on ratings of perceived exertion. Post-hoc analyses indicated that, during the COOL trial, RPE scores were significantly lower than during the CTR trial, both after 25 ($p= 0.024$) and 30 min ($p= 0.001$) from when the exercise was started. The analysis of session RPE data indicated a significant effect of condition ($F= 18.2_{(1.2, 9.9)}$, $p<0.001$; observed power= 1). Post-hoc analyses indicated that session RPE recorded after the COOL trial was significantly lower than after the PRE-COOL ($p= 0.002$) and CTR ($p= 0.001$) trails (fig. 5b).

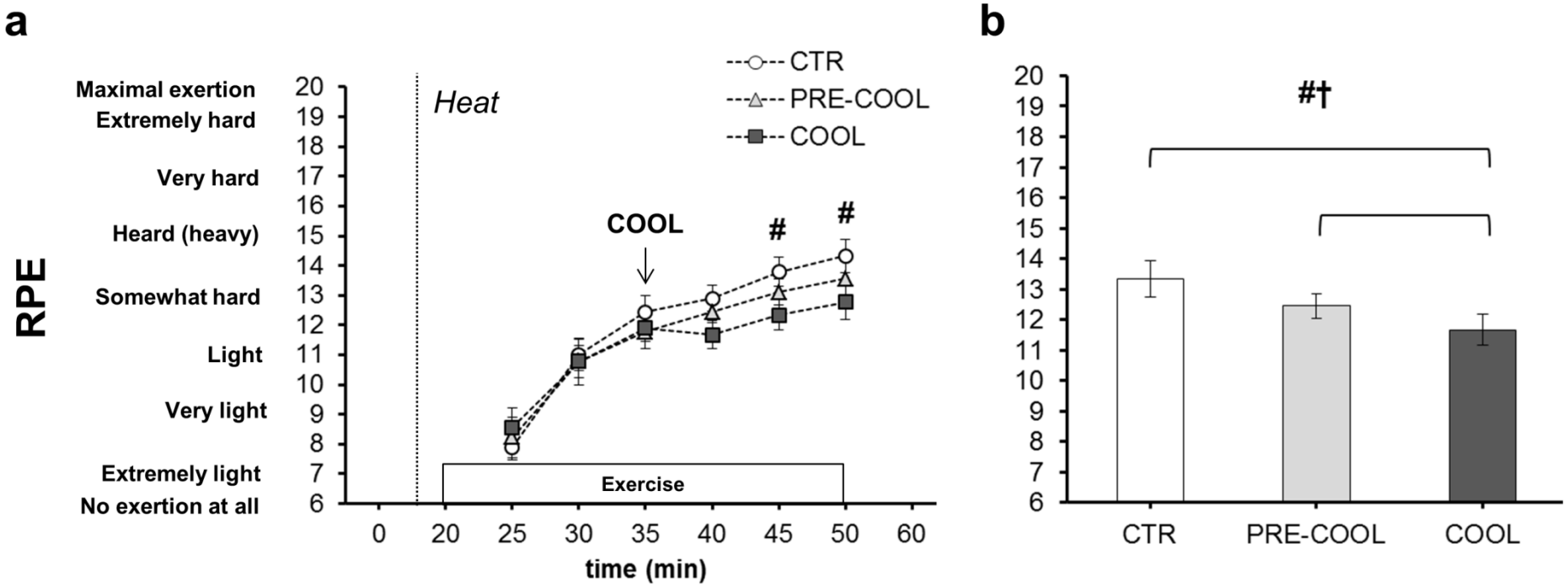

Figure 5: Mean (± standard error) RPE (a) and session RPE values (b) as recorded respectively during the exercise phase and 10 min after the exercise was terminated for the CTR, PRE-COOL and COOL trials. In panel a, the application of cooling during exercise (15 min after the exercise was started, COOL trial) is marked by an arrow. * (CTR ≠ PRE-COOL), # (CTR ≠ COOL), † (PRE-COOL ≠ COOL) refer to significant differences between trials as computed using $p<0.05$.

3.6 Discussion

The aim of this study was to investigate the physiological and perceptual effects of applying mild evaporative cooling to the torso during moderate exercise in the heat, and to investigate whether varying the timing of cooling (i.e. before or during exercise) had an impact on the same physiological and perceptual parameters.

The outcomes of this study indicated that, when applied before exercise (PRE-COOL trial) and when compared to the CTR trial, the evaporation-induced mild cooling of the torso significantly lowered both local (from -1.9 ± 0.2 up to -5.3 ± 0.3 °C) and mean T_{sk} (from -0.9 ± 0.1 up to -2.0 ± 0.1 °C). This was accompanied by significantly lower body sweat loss. Also, significantly "less hot" thermal sensations and a significant reduction in thermal discomfort were reported during the PRE-COOL trial. Furthermore, this study has shown that when applied during exercise (COOL trial), the evaporation-induced mild cooling significantly decreased local and mean T_{sk} to an extent similar to the one observed during the PRE-COOL trial from the time of application. No effects were observed on body sweat loss in this case. However, when compared to the CTR trial, applying the cooling when participants were already exercising significantly lowered the HR. Although its impact on HR was limited to the initial 10 min of application, on the perceptual side, cooling applied during exercise lowered thermal heat sensations, decreased the thermal discomfort, and also reduced the perceived exertion for up to 25 min from when cooling was initially applied. This resulted in the COOL trial being perceived as the overall least demanding experimental trial.

In summary, the outcomes of this study indicated that, despite its low cooling power (compared to e.g. ice vests), due to its main effect on mean and local T_{sk}, the mild evaporative cooling method tested in this study provided significant thermoregulatory benefits during exercise in the heat. Furthermore, these findings indicated that varying the timing of cooling had a major impact on the magnitude of the physiological (e.g. HR) as well as perceptual (e.g. RPE) responses. These outcomes have both a fundamental as well an applied significance.

As no differences in T_c were observed amongst the experimental conditions, the outcomes of this study provide novel insights on the role played by T_{sk} in influencing

the physiological and perceptual responses occurring during exercise in the heat. With regards to pre-cooling, this was observed to significantly improve thermal sensation and comfort during exercise in the heat, regardless of the rate of increase in T_c. T_{sk} seems therefore to have a larger contribution than core, in characterizing thermal sensation and comfort during exercise in the heat. This is in line with classic studies suggesting skin, more than core temperature, to drive thermal comfort in resting individuals (Gagge et al., 1967). Furthermore, this is aligned to more recent studies, which have shown that sensory feedback arising from changes in T_{sk} can significantly contribute to behavioural adjustments (e.g. pacing strategies) in temperature regulation while exercising (Schlader et al. 2011a; 2011b; 2011c). Thermal sensations arising from variations in T_{sk} seem indeed to initiate behavioural thermoregulation prior to any change in T_c. This is suggested as an anticipatory response to prevent the activation of autonomic thermoregulatory responses (i.e. sweating or shivering) and to maintain heat balance (Schlader et al. 2011a; 2011b; 2011c).

In the present study it was also found that pre-cooling significantly reduced the body sweat loss (-143 ± 40 g), regardless of the rate of increase in T_c. This result seems aligned to the ones reported by Webster et al. (2005) and Hasegawa et al. (2005) who have respectively shown that wearing a cooling vest (for 35 to 50 min) can significantly reduce the total sweat losses (i.e. -100 g when compared to no cooling) during exercise in the heat. A possible explanation to the lower sweat loss could be that the lower rate of increase in body heat content resulting from the pre-cooling intervention translated into lower evaporative requirements for heat balance, thus inducing a significantly lower sweat production (Gagnon et al., 2013). Alongside this change in the central thermoregulatory drive, significant changes in local T_{sk} (such as the ones observed in this study on the chest, abdomen, upper and lower back), could have also contributed to a significant reduction in local sweat rates. In this respect, evaporation-based local skin cooling has been previously shown to affect local sweating response in the early stages of exercise (Kondo et al., 1997), due to possible changes in the amount of transmitter substance being released at the neuroglandular junction for each nerve impulse (MacIntyre, 1968). As the torso is amongst the most active body regions in terms of sweat production (Smith & Havenith, 2012), a reduction in the torso sweat production could have contributed significantly to the

reduction in the total body sweat loss recorded during the PRE-COOL trial. This hypothesis was perceptually matched by the significantly "less wet" wetness perceptions reported by participants during the exercising phase of the PRE-COOL trial.

From an applied point of view, the fact that pre-cooling significantly reduced body sweat loss is of interest for its potential implications in the context of limiting the amount of fluid loss and thus the rate of dehydration when exercising in the heat. Indeed, although sweating represents the main avenue for heat loss to the environment under heat stress, and should be therefore encouraged to limit core overheating under conditions that permit full evaporation (Bain et al., 2012), when the skin reach sweat saturation (i.e. maximal skin wetness) (Gagge, 1937) and sweat starts to drip off the skin, any further sweating does not contribute to any further heat loss (i.e. reduced evaporative efficiency of sweating) (Candas et al., 1979), but only to an increase in fluid loss and thus dehydration (Bain et al., 2012). As elevated skin temperature and dehydration have been shown to have a detrimental and interactive effect on aerobic performance (Kenefick et al., 2010), it is therefore clear how a reduction in both T_{sk} and sweat loss (i.e. when this is no longer efficient) is of fundamental importance in order to preserve homeostasis and thus support performance in the heat.

With regards to the application of cooling during exercise in the heat, we observed that suddenly cooling the skin (mean T_{sk} change of ~1 °C) of exercising individuals resulted in significantly lowering the HR, perceived exertion and the resulting session RPE, regardless of the rate of increase in T_c. The changes in the HR could be explained by the effect of skin cooling in reducing the skin-muscle competition for the available cardiac output (Shaffrath & Adams, 1984). It was indeed hypothesised that by suddenly lowering T_{sk}, the skin blood flow requirements for temperature regulation would be also suddenly lowered (Sawka et al., 2012), thus resulting in a reduced cardiovascular strain. In this respect, by lowering T_{sk}, the resulting increase in the core to skin heat gradient facilitated the heat losses and therefore contributed to further decrease the cardiovascular strain and ultimately the HR,. Although the effect on HR was short-lived (i.e. only 10 min from cooling was applied) this outcome confirms the role of T_{sk} as being critical in the modulation of the

cardiovascular response during exercise in the heat (Nybo, 2008). Also, this outcome indicates that the manipulation of T_{sk} can be used as a potential method to modulate the cardiovascular challenge of exercising under heat stress, thus helping in preserving maximal aerobic power during exercise under heat stress (Kenefick et al., 2010), at least within short-duration exercise conditions.

It is of interest that the physiological impact of suddenly cooling the skin translated into a significant decrease in the perceived exertion during the exercise, regardless of changes in T_c. This fact was also confirmed by the session perceived effort, which was the lowest when cooling was applied during exercise. This finding highlights the complex nature of RPE, and indicates that this conscious perception results from the integration of sensory inputs arising not only from systems such as the cardiovascular and musculoskeletal (Crewe et al., 2008), but also from the skin.

When compared to each other, the PRE-COOL and COOL interventions resulted in different physiological and perceptual responses. This could be due to the overall magnitude and different duration of the two experimental interventions. The PRE-COOL intervention was beneficial in terms of lowering mean T_{sk} (and related thermal sensations and comfort) and the body sweat loss but it did not have any significant effect on the HR and RPE. The absence of an effect on the HR during the PRE-COOL trial could be due to the mild nature of the cooling used for this study. Previous studies have shown that greater cooling (i.e. able to significantly lower T_c) is indeed needed to result in a significant and prolonged attenuation of the cardiovascular strain resulting from the heat exposure (Marino, 2002; Hasegawa & Takatori, 2005). Based on the linear relationship between RPE and HR (Borg, 1982), the mild effect of this type of cooling on the cardiovascular response could also explain why during the PRE-COOL trial the RPE was not significantly lowered. The COOL intervention was beneficial in terms of lowering mean T_{sk} (and related thermal sensations and comfort), HR and RPE, but it did not have any effect on the body sweat loss. As during the COOL trial the cooling was applied only for the last 25 min of the trial (as opposed to the 55 min application of the PRE-COOL trial), this might have therefore not been sufficient to significantly reduce the sweat production.

In summary, these results indicate that the magnitude, as well as the timing (PRE-COOL vs. COOL) and duration of the cooling intervention (i.e. 55 vs. 25 min) could

have played a role in the different thermoregulatory responses observed during the different experimental trials. Furthermore, these outcomes highlighted the important role of cutaneous thermoreceptors and T_{sk} in body temperature regulation, thermal sensation and comfort during exercise under heat stress.

We conclude that the mild evaporative cooling method used in this study can provide significant thermoregulatory benefits during exercise in the heat, by significantly lowering mean and local T_{sk} as well as thermal discomfort. We have also shown that the timing of application of mild cooling (prior vs. during exercise) can significantly change the magnitude of the physiological as well as of the perceptual benefits occurring during exercise in the heat. Cooling the skin prior to exercise resulted in significantly reducing the sweat production and the overall thermal discomfort experienced. Suddenly cooling the skin during the exercise resulted in significantly lowering the heart rate, as well as the overall thermal discomfort and perceived exertion. These findings provide fundamental insights on the role of skin temperature and thermal comfort in the thermoregulatory processes occurring when exercising under heat stress. Also, these open to potential applications of a newly developed and practical mild evaporative cooling method within elite and recreational sport contexts.

In this respect, it has to be highlighted that the practical applications and potential benefits of such mild evaporative cooling garments should be carefully considered in light of the exercise modality performed, the environmental conditions and the resulting thermal load. As previously reported (see Wegmann et al. 2012), the beneficial effects of external cooling when exercise is performed in the heat can vary largely, particularly across different exercise modalities. For example, due to the greater thermal stress posed by repeated sprint performance (e.g. intermittent exercise) as opposed to single sprint activities performed in hot conditions, the possibility of investigating the effects of such cooling garment in sports such as e.g. Football could be of interest, as cooling has been already shown to be beneficial for intermittent exercise performed under high thermal loads (Wegmann et al. 2012). From an applied point of view, this seems very relevant in light of the recent 2014 FIFA Football World Cup and of the introduction of cooling breaks, used to reduce the risk of heat-related injuries during those matches performed in high ambient temperatures and humidity (see e.g. BBC SPORT 2014 FIFA WORLD CUP.

http://www.bbc.co.uk/sport/0/football/28075216). In this context, the practicality of the light weight cooling method presented in this study, as well as the possibility to change the timing of wetting as well as of re-wetting the garment (e.g. during "cooling breaks") could represent an advantage in maximizing the performance benefits of such novel method during sport competitions (e.g. Football matches) performed under heat stress. Hence, further studies investigating specific performance benefits of this novel method are warranted and recommended.

3.7 Conclusions

The mild evaporative cooling method we tested has important practical implications. This method could be integrated with more traditional pre-cooling strategies which aim to lower T_c prior to exercise (e.g. ice slurry ingestion). Also, this method could be used alone to manipulate T_{sk} prior as well as during exercise (e.g. by changing the timing of wetting and of re-wetting the garment), the latter possible given the low weight of the garment. This could be considered for those sports in which the pacing strategy is essential to maximise performance (e.g. cycling and running). Also, this could be useful for intermittent-exercise-based sports (e.g. Football) when the environmental conditions pose a greater thermal load than normal. Finally, reducing the sweat loss by cooling the skin (as observed in this study) could attenuate the rate of dehydration during prolonged performance in the heat. This, as well as the possibility to lower thermal discomfort during exercise in the heat could contribute to performance benefits in competitive sport, as well as could to increase the activity levels of healthy individuals performing in the heat.

4 CHAPTER FOUR – Laboratory study 2: The role of decreasing contact temperatures and skin cooling in the perception of skin wetness

Publication(s) based on this chapter:

Filingeri, D., Redortier, B., Hodder, S., Havenith, G. (2013) The role of decreasing contact temperatures and skin cooling in the perception of skin wetness. *Neuroscience Letters*, 551:65-69 (Appendix D).

4.1 Abstract

Cold sensations are suggested as the primary inducer of the perception of skin wetness. However, limited data are available on the effects of skin cooling. Hence, we investigated the role of peripheral cold afferents in the perception of wetness. Six cold-dry stimuli (producing skin cooling rates in a range of 0.02 to 0.41 $°C \cdot s^{-1}$) were applied on the forearm of 9 female participants. Skin temperature and conductance, thermal and wetness perception were recorded. Five out of 9 participants perceived wetness as a result of cold-dry stimuli with cooling rates in a range of 0.14 to 0.41 $°C \cdot s^{-1}$, while 4 did not perceive skin wetness at all. Although skin cooling and cold sensations play a role in evoking the perception of wetness, these are not always of a primary importance and other sensory modalities (i.e. touch and vision), as well as the inter-individual variability in thermal sensitivity, might be equally determinant in characterising this perception.

4.2 Introduction

Humans interact with their immediate environment through the medium of sensory experiences. However, the way we perceive the world differs qualitatively from the way we sense it (Parsons and Shimojo, 1987). This difference between perception and sensation relies on the fact that our nervous system extracts only certain information from each stimulus and these are then interpreted according to the current situation and previous experiences (Kandel et al., 2000). Furthermore, perception often results from multisensory experiences as our sensory systems operate within interconnecting, intermodal and cross modal networks (McGlone and Reilly, 2010).

The ability of the central nervous system to combine and process different sensory information into particular perceptions provides the basis for understanding why some of the perceptions we experience are not directly linked to just one specific sensory system. For instance, we experience the perception of "wetness" on the skin though we are not provided with specific receptors for this sensation (Clark and Edholm, 1985; Lee et al., 2011). This somatosensory experience is considered a result of the integration of the somatosensory sub-modalities of touch and temperature (Bentley, 1900; Bolanowski et al., 2001; Ackerley et al., 2012; Bergmann Tiest et al., 2012a). However, the way in which touch and temperature senses interact to generate the perception of wetness is still unclear (Storaas and Bakkevig, 1996; Li, 2005; Lee et al., 2011). It has been hypothesised that the activity of thermoreceptors responding to specific drops in skin temperature, such as the ones occurring during the evaporation of sweat from the skin, represents the primary inducer of this perception (Li, 2005; Daanen, 2009; Lee et al., 2011). Nevertheless, the role played by cold thermoreceptors (i.e. small myelinated Aδ and unmyelinated C fibers) (Campero and Bostock, 2010) is still unclear and might vary according to the location of these cold sensitive free nerve endings. Indeed, Belmonte and Gallar (Belmonte and Gallar, 2011) suggest that the augmented activation of cold thermoreceptors (i.e. corneal trigeminal neurons) located on the human cornea recorded during evaporation-induced ocular surface cooling, seems to be responsible for the perception of ocular dryness. The same physical process (cooling) encoded by the same type of thermoreceptors (cold sensitive) might be therefore primarily

responsible for two completely opposite perceptions: dryness and wetness. Furthermore, it could be reasonable hypothesising the interaction of other sensory systems such as vision or touch (in terms of pressure and distribution of pressure) in characterising the perception of wetness (Wang et al., 2002). For these reasons, it is still unclear which sensory modality plays the primary input, to what extent, and how it relates with the potentially secondary sensory inputs which overall contribute to characterize wetness as a synthetic perception (Bentley, 1900; Li, 2005). Increasing the knowledge about the neurophysiological bases of the perception of wetness can be useful both for clinical and industrial applications. On the clinical side, it might be used for diagnostic purposes in patients with sensory disorders e.g. diabetic neuropathy (Mano et al., 2006; Bergmann Tiest and Kappers, 2009; Gin et al., 2011). On the industrial side, it might support the development of new strategies in clothing design, as this perception has been shown to play a significant role in the onset of thermal discomfort (Fukazawa and Havenith, 2009).

The present study focuses on the sensation of skin temperature and perception of wetness using a single-blinded psychophysical approach. The aim of the study was to investigate the role of peripheral cold afferents in evoking the perception of skin wetness. Although it has been suggested that this perception can be evoked by the application onto dry skin of a cold-dry stimulus producing a cooling rate of 0.05 to 0.2 $°C \cdot s^{-1}$ (Daanen, 2009), no experimental data are currently available involving human participants exposed to different levels of skin cooling. Therefore we investigated a wide range of temperatures, where cold stimuli were applied to the forearm.

4.3 Material and methods

4.3.1 Participants

Nine healthy female university students (27 ± 8 years) with no history of sensory-related diseases volunteered to participate in this study. Female participants were preferred to male as they are generally less hairy on the ventral side of the forearm. All participants gave their informed consent for participation. The test procedure and the conditions were explained to each participant. The study design had been

approved by the Loughborough University Ethics Committee and testing procedures were in accordance with the tenets of the Declaration of Helsinki.

4.3.2 **Experimental design**

The experimental design was based on the application of six cold-dry stimuli of different strength in a balanced order on the bare, left forearm of each participant, while they were resting in an environmental chamber (set at 20 °C and 50 % relative humidity). Ten minutes were allowed for acclimation and preparation for the test. An s-shaped wooden panel (width: 81 cm; length: 74 cm; height: 60 cm) was placed on a table. A hole (width: 12 cm; height: 13 cm) in the panel allowed participants to enter their left forearm and lay it down with the palm facing upward. This setup did not allow the participants to see the stimuli that were applied on their forearm. Participants were informed only about the body region subjected to the stimulation. No information was provided on the type and magnitude of the stimulation to limit any expectation effects. To avoid an effect of surprise on the transient cold sensation and wetness perception, a verbal warning was given prior to stimulation during the test. The exact temperatures of cold-dry stimuli were calculated on an individual basis and consisted of a short contact (30 s) with a cold surface set at -2, -5, -7, -10, -15 or -20 °C than the individual's forearm resting skin temperature [which was recorded using an infrared thermometer (Fluke Corporation, USA)]. The cold-dry stimuli were delivered by a thermal probe (Physitemp Instruments Inc., USA) with a contact surface of 25 cm^2 and a weight of 269 g.

During the test, participants were asked to maintain their forearm in the required position while the thermal probe was applied to a point corresponding to the mid distance between the elbow and the wrist, on the ventral side. Skin conductance was recorded from the beginning and throughout the whole test using the MP35 system (Biopac Systems Inc., USA) which was connected to two electrodes placed on the participant's forearm at a set distance (7cm), allowing the thermal probe to be applied in between them. The skin conductance was monitored to estimate sudo-motor activity (Vetrugno et al., 2003; Tronstad et al., 2008) and in the present study was used as a control to establish that no sudo-motor activity occurred i.e. the participant was not sweating due to stress.

4.3.3 **Experimental Protocol**

Participants were asked to rate their thermal sensation and wetness perception using psychological rating scales during each of four experimental phases: A) rest; B) cold-dry stimulus; C) bare skin; D) re-warming. In phase A, participants were asked to rate their local thermal sensation and wetness perception at rest without stimulation while forearm skin temperature was recorded with the infrared thermometer. In phase B, the thermal probe (set to the required temperature) was applied to the forearm and left in full contact with the skin site for 30 s, while participants were asked to rate their local thermal sensation and wetness perception 10 s after the application. The probe was then removed and the skin temperature was immediately recorded. The skin site was left bare for 30 s (phase C). At the end of this phase participants were asked to rate their local thermal sensation and wetness perception, and skin temperature was again recorded. Finally (phase D – re-warming), the thermal probe was set at a temperature corresponding to the one recorded at the beginning of the test (the individual's baseline) and then applied for 30 s to re-warm the skin. Participants were then asked to rate their thermal sensation and wetness perception for the last time and skin temperature was recorded immediately after the thermal probe was removed. This sequence was repeated for each stimulus allowing at least one minute in between. Each participant had only one presentation of each stimulus. The order of the stimuli was balanced within and between the tests to avoid any order effect.

4.3.3.1 Psychological rating scales

We designed three psychological rating scales to record individual thermal sensation and wetness perception (Olesen and Brager, 2004). An 11 point thermal sensation scale (-5 extremely cold; -4 very cold; -3 cold; -2 cool; -1 slightly cool; 0 neutral; +1 slightly warm; +2 warm; +3 hot; +4 very hot; +5 extremely hot) was used at rest and during the re-warming; a seven points thermal sensation scale [from 0 to 6, where 0 was labelled as not cold at all and 6 as extremely cold (with no labels in between them)] was used during both cold stimulus and bare skin phases. Finally, a seven point wetness perception scale [from 0 to 6, where 0 was labelled as dry and 6 as extremely wet (with no labels in between them)] was used during all the phases of each test. We defined the value "1" of the scale as our set threshold to identify a clearly perceived wetness. Participants familiarised with the scales during the

acclimation period. During the experimental protocol, participants rated verbally their sensations, which were immediately recorded by the investigator.

4.4 Statistical Analysis

In the present study, the independent variable was the temperature of the thermal probe (the relative cold stimulus based on the individual baseline skin temperature) and the dependent variables were the forearm skin temperature, skin conductance, thermal sensation and wetness perception. Data were tested for normality of distribution using Shapiro-Wilk test. Skin temperature data were analysed by a one way repeated measures analysis of variance. (ANOVA) Post-hoc analyses using a Tukey's test were performed to account for multiple comparisons and sample size effect.

Thermal and wetness ratings were analysed using a Friedman test (non-parametric randomized block ANOVA) and post-hoc analyses were performed using a Wilcoxon signed rank tests. Huynh–Feldt, Geisser–Greenhouse, and lower bound corrections were undertaken to adjust the degrees of freedom for the averaged tests of significance. A linear regression analysis was performed to assess the relationship between the variation in skin temperature from baseline and the relative cold stimuli. Ordinal regression analyses were performed between the thermal and wetness ratings and the relative cold stimuli. Finally, a Spearman's rank correlation coefficient was calculated to investigate the degree of association between thermal sensation and wetness perception. All data were analysed using SPSS Statistics 19 (IBM, Armonk, NY) and were reported as means ± standard deviation (SD). In all analyses, $p<0.05$ was used to establish significant differences.

4.5 Results

4.5.1 Skin temperature

Skin temperature data were normally distributed and were thus analysed by a repeated measure ANOVA and Tukey's test. The resting skin temperature before stimulation (29.7 ± 1.4 °C) did not significantly differ between each of the six conditions ($p>0.05$) confirming the effectiveness of the balanced order of the stimuli

in avoiding any order effect. Furthermore, no differences were recorded in the post re-warming skin temperature (29.5 ± 1.2 °C) between conditions (p>0.05), confirming that the skin was effectively re-warmed to the resting value.

During the stimulation, each cold-dry stimulus produced significantly different decreases in the skin temperature ($F = 71.61_{(2.32, 18.57)}$, p<0.001) varying in a range between -0.8 ± 0.8 to -12.3 ± 2.7 °C from the baseline skin temperature, corresponding to a cooling rate range of 0.02 ± 0.02 to 0.41 ± 0.09 $°C \cdot s^{-1}$ (Fig. 1a).

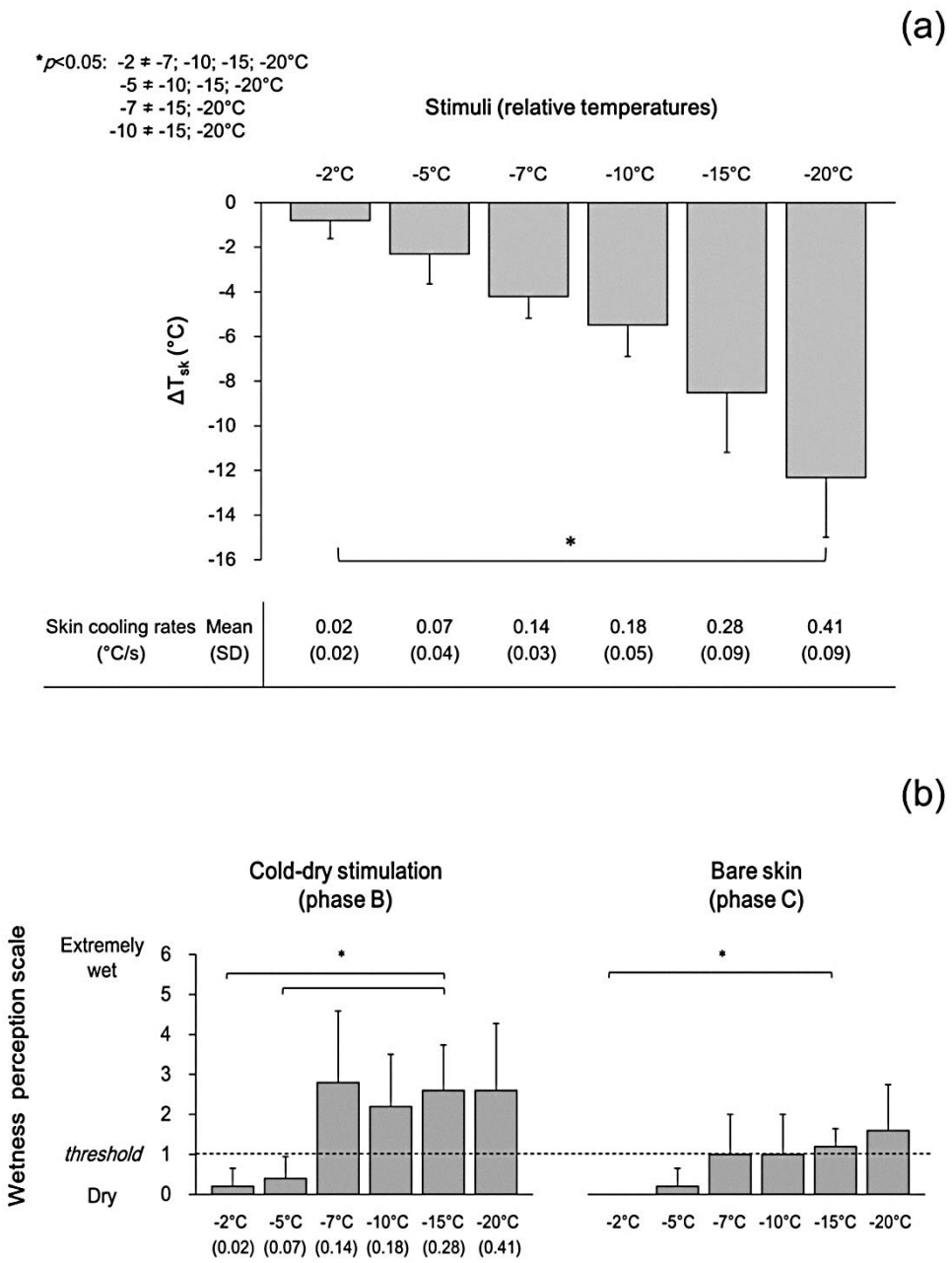

Figure 1: (a) Relative variations in skin temperature drop from baseline (ΔT_{sk}) and corresponding cooling rates as a result of each of the six cold-dry stimuli. (b) Wetness perception scores recorded in the responders sub-group as a result of each of the six cold-dry stimuli (phase B) and during the following bare skin phase (C) (*p<0.05). Skin cooling rates corresponding to each stimulus are reported between brackets. The point "1" of the wetness perception scale corresponds to the threshold set to identify perceived skin wetness.

4.5.2 **Thermal sensation and wetness perception**

Thermal sensation and wetness perception data were analysed by a Friedman test and Wilcoxon signed rank tests. Resting thermal sensation and wetness perception did not significantly differ between the six conditions (p>0.05) with an average score of -0.2 ± 0.2 and 0.2 ± 0.1 respectively. Furthermore, no differences were found during the re-warming phase of each condition (p>0.05), as shown by a recorded average thermal sensation of +1.4 ± 0.2 and an average wetness perception of 0.2 ± 0.2.

Stimuli produced statistically significant differences (χ^2= $34.7_{(5,\ 9)}$, p<0.001) in thermal sensation both during stimulation (varying in a range between 0.7 ± 1 to 4.1 ± 1.8) as well as during the bare skin phase (varying in a range between 0.8 ± 1.1 to 2.3 ± 1.1). Data related to wetness perception showed that overall, in 19 out of 54 scores (35 %) recorded during phase B (cold-dry stimulation), a cold-dry stimulus was perceived as cold-wet. We then proceeded with the analysis of individual data which showed the existence of two sub-groups within the whole sample tested in this experiment. Indeed, five out of nine participants reported wetness perceptions varying significantly according to the rate of skin cooling, either during the cold-dry stimulation and the following bare skin phase, whereas four out of nine participants did not perceive wetness at all. At this point we decided to identify the two groups as "responders" and "non-responders" (Carter and Ray, 2009) to the cold-dry stimuli we used in this study and thus performing a separate analysis in terms of wetness perception.

Data related to the responders group showed statistically significant differences (χ^2= $16.2_{(5,\ 5)}$, p<0.01) in the wetness perception scored during both the cold-dry stimulation and the bare skin phase (Fig. 1b), with the threshold we set (point "1" of the scale) to identify a clearly perceived wetness reached during four out of the six conditions (-7, -10, -15 and -20 °C respectively).

4.5.3 **Regression and correlation analysis**

The relationship between the variation in skin temperature from baseline and the relative cold stimuli (assessed by a linear regression analysis which included data from the whole sample) was found to be statistically significant (p<0.001; r^2=0.83; regression coefficient b_0=0.605; regression coefficient b_1= 0.632). Similarly, the relationship between the thermal ratings and the relative cold stimuli (assessed by an ordinal regression analysis which included data from the whole sample) was found to

be statistically significant [$p<0.001$; Chi-square analysis (Pearson; Deviance): $p>0.05$; Nagelkerke (pseudo r^2) = 0.58; Test of parallel lines: $p>0.05$]. The relationship between the wetness ratings and the relative cold stimuli (assessed by an ordinal regression analysis which included only the data from the responders sub-group) was also found to be statistically significant [$p<0.001$; Chi-square analysis (Pearson; Deviance): $p>0.05$; Nagelkerke (pseudo r^2) = 0.57; Test of parallel lines: $p>0.05$]. Finally, the degree of association between thermal sensation and wetness perception (assessed by a Spearman's rank correlation test which included only the data from the responders sub-group) was found to be statistically significant ($p<0.001$; Spearman's rho= 0.78).

4.5.4 **Skin conductance**

Average values did not significantly change during testing procedures and were observed to remain constantly at a level below 0.5 µS. These results confirm that no variations in sudo-motor activity occurred during the experiment.

4.6 **Discussion**

The aim of this study was to investigate the mechanisms responsible for the perception of skin wetness with regard to cold temperature sensing. The experimental protocol was designed to ensure that a dry skin site would be exposed for a relatively short time to a wide range of local cold-dry stimuli. This approach resulted in evoking artificial wetness perceptions, with 35 % of the cold-dry stimuli applied on the participants' forearms being perceived as cold-wet.

This first outcome showed that the wetness perception did relate to the activation of the thermal afferents responding to skin cooling. However, this was true only for a sub-group of five participants. Data from this sub-group seem aligned to the findings of Daanen (Daanen, 2009) who measured the temperature course of the skin (i.e. temperature' s drop of 1 to 5 °C with a 0.05 to 0.2 $°C \cdot s^{-1}$ cooling rate) when this was wetted with drops of water with volumes in a range of 10 to 100 µl. The author suggested that the cold sensations experienced when such skin cooling occurs can contribute to the perception of skin wetness. Therefore, exposing the skin to a cold-dry stimulus producing such skin cooling was hypothesised to evoke an illusory

perception of skin wetness. In our study, this hypothesis was confirmed, as when the application of cold-dry stimuli produced a drop in skin temperature ranging between 1.4 and 4.1 °C with a cooling rate of 0.14 to 0.41 $°C \cdot s^{-1}$, a clear wetness perception was evoked, whereas when the cold-dry stimulation produced a drop in skin temperature of 0.2 to 0.7 °C with a cooling rate of 0.02 to 0.07 $°C \cdot s^{-1}$, wetness was little evoked and decreasing thermal sensations prevailed.

Therefore we suggest that, the rate of heat transfer from the skin to a colder surface seems to play a significant role not only in thermal and touch discrimination of different materials (Bergmann Tiest and Kappers, 2009) but also in characterising the perception of a cold stimulus as simply cold or as also wet. During our experimental conditions a skin cooling rate threshold for the perception of "cold-dryness" and "cold-wetness" was identified (i.e. between 0.07 and 0.14 $°C \cdot s^{-1}$) and further evidence has been added to the work of Daanen (Daanen, 2009), as we observed that greater skin cooling rates (up to 0.41 $°C \cdot s^{-1}$) than the one proposed by the author (0.05 to 0.2 $°C \cdot s^{-1}$), can also contribute to evoke a wetness perception.

However, although at this point it might be proposed that skin cooling and thus temperature sensations alone might be sufficient to generate the perception of skin wetness, {as suggested by Bergmann Tiest et al. (Bergmann Tiest et al., 2012b) in their recent work in which phase-change materials inducing cool sensations were perceived as wet}, the presence of a non-responders sub-group within the whole sample, who did not perceive wetness during any of the experimental conditions, contrasts with this conclusion. A possible explanation of the incongruent sensory perceptions recorded in the two sub-groups might be related the properties of the stimulus, which were voluntarily limited to focus on the effects produced by skin cooling. The lack of intra- and inter-sensory interaction, particularly in terms of touch and vision (the probe was applied but not moved and participants could not see the stimulation area), might be primary responsible for the heterogeneity of the responses. Indeed, it has been shown that the co-activity of highly specialised receptors with different individual properties is essential in generating the variety of cutaneous sensations we encounter in everyday life, particularly in complex perceptions such as skin wetness (McGlone and Reilly, 2010; Ackerley et al., 2012) . Thus, the role of the other somatosensory sub-modalities might be equally as important as the skin cooling itself (Ackerley et al., 2012), which can therefore not always be sufficient in evoking the perception of wetness. In the work of Bergmann

Tiest et al. (Bergmann Tiest et al., 2012b), no non-responders group was identified, a fact which might be the reason why the author concluded that touch-related sensations seem unnecessary and thermal sensations can be sufficient in evoking the perception of skin wetness. However, it has to be observed that in the mentioned work, participant where asked to choose which one felt wetter between a treated (with phase-change materials) and an untreated fabric. In our view, this experimental approach affected the participants' responses as no option of reporting the absence of wetness was given to them. In principle, if both samples had been experienced as dry, the lower score observable in the group would have been a 50 %, which means that neither in that case a non-responders subgroup would have been identified.

Therefore, although decreases in skin temperature may sometimes be sufficient, a more complex sensory-blending hypothesis should be considered to explain the psycho-physiological process responsible of the perception of skin wetness (Jousmäki and Hari, 1998; Guest et al., 2002). Studies by Gerrett (2012) and everyday experience suggest that we are able to perceive the wetness even when the skin temperature does not decrease (e.g. during exposure to hot environmental conditions or when in contact with hot water). Thus, defining some particular activations of the cold afferents as sufficient to generate this perception (regardless of other sensory interactions) might be limiting in the light of the complex interconnecting, intermodal and cross modal networks our sensory systems operate within (McGlone and Reilly, 2010).

The way we perceive "feelings" from our body results from complex integrations between the activity of the exteroceptive and interoceptive systems (Craig, 2003). Furthermore, converging evidence suggests a phylogenetically new system (which integrates information about the overall homeostatic condition of the body) as one of the principal neuroanatomical structures that differentiate humans from non-human primates (Craig, 2002). This hypothesis confirms the multimodal as one of the most appropriate approaches when investigating the mechanisms of sensory integration. As the perception of skin wetness represents one of the numerous somatosensory experiences that allow us to sense and perceive our immediate environment (and eventually interact with it) (McGlone and Reilly, 2010), it is reasonable to hypothesise that other sensory inputs than just temperature (i.e. touch, vision) can significantly influence the way we experience this complex perception.

Finally, although the neurological and molecular basis of thermal sensations have been largely investigated and described (Tominaga and Caterina, 2004; Schepers and Ringkamp, 2010; McKemy, 2013), individual thermal sensations are much more difficult to predict due to other parameters relating to wider and more complex relationships between physiological and psychological responses (McKemy, 2005; Lee et al., 2010a). For instance, the inter-individual variability is a critical factor in determining the psychological responses resulting from somatic stimulation, as shown in the role played by individual characteristics such as gender, age, ethnicity and physical fitness in influencing the cutaneous thermal thresholds and thus the variability of thermal sensations (Havenith, 1990; Lee et al., 2010a).

4.7 Conclusion

In this study we found that skin cooling and thermal sensations can contribute significantly to the perception of skin wetness. We have shown that a cooling rate threshold for a cold stimulus to be perceived as wet is identifiable based on the rate of heat transfer from the skin. Also, greater cooling rates than the ones currently proposed, were shown to evoke wetness perceptions. However, the activity of peripheral cold afferents as a result of skin cooling has been shown to not always be sufficient in evoking the perception of wetness. This suggests that the intra- and inter-sensory interaction with other modalities (i.e. touch, vision), as well as the inter-individual variability, might have a role as equally determinant as the one played by the temperature sense in affecting individual thresholds for the perception of complex somatosensory experiences such as skin wetness. Little is known about the temperature sensing system across the body and even less is known on how this specifically interacts with the other sensory systems to produce the variety of somatosensory perceptions we experience every day.

5 CHAPTER FIVE – Laboratory study 3: Thermal and tactile interactions in the perception of local skin wetness at rest and during exercise in thermo-neutral and warm environments

Publication(s) based on this chapter:

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5.1 Abstract

The central integration of thermal (i.e. cold) and mechanical (i.e. pressure) sensory afferents is suggested as to underpin the perception of skin wetness. However, the role of temperature and mechanical inputs, and their interaction, is still unclear. Also, it is unknown whether this intra-sensory interaction changes according to the activity performed or the environmental conditions. Hence, we investigated the role of peripheral cold afferents, and their interaction with tactile afferents, in the perception of local skin wetness during rest and exercise in thermo-neutral and warm environments. Six cold-dry stimuli, characterised by decreasing temperatures [i.e. -4, -8 and -15 °C below the local skin temperature (T_{sk})] and by different mechanical pressures [i.e. low pressure (LP): 7 kPa; high pressure (HP): 10 kPa], were applied on the back of 8 female participants (age 21 ± 1 years), while they were resting or cycling in 22 or 33 °C ambient temperature. Mean and local Tsk, thermal and wetness perceptions were recorded during the tests. Cold-dry stimuli produced drops in Tsk with cooling rates in a range of 0.06 to 0.4 $°C \cdot s^{-1}$. Colder stimuli resulted in increasing coldness and in stimuli being significantly more often perceived as wet, particularly when producing skin cooling rates of 0.18 and 0.35 $°C \cdot s^{-1}$. However, when stimuli were applied with HP, local wetness perceptions were significantly attenuated. Wetter perceptions were recorded during exercise in the warm environment. We conclude that thermal inputs from peripheral cutaneous afferents are critical in characterizing the perception of local skin wetness. However, the role of these inputs might be modulated by an intra-sensory interaction with the tactile

afferents. These findings indicate that human sensory integration is remarkably multimodal.

5.2 Introduction

The perception of skin wetness is a complex somatosensory experience which seems to result from the intra-sensory integration of temperature and mechanical inputs (Ackerley et al. 2012; Bergmann Tiest et al. 2012; Bentley, 1900). Although humidity-receptors have been previously described in some insects (Yokohari and Tateda, 1976), these receptors have not been identified in human skin (Clark and Edholm, 1985). It is currently suggested that as human beings, we "learn" to perceive the wetness experienced when our skin is in contact with a wet surface, when a liquid is touched, or when sweat is produced (Bergmann Tiest et al., 2012a) through a complex multisensory integration (Driver and Spence, 2000; Gescheider and Wright, 2012). The physical processes which occur when the skin is in contact with moisture (i.e. heat transfer and mechanical interactions between the skin and the environment) generate thermal and mechanical inputs which could be integrated and combined at different anatomical levels through specific multisensory pathways (Cappe et al., 2009). Hence, it is not the contact of the skin with moisture *per se*, but rather the integration of particular sensory inputs which seems driving the perception of local skin wetness during the contact with a wet surface (Bentley, 1900). It could therefore be suggested that the perception of local skin wetness is a "perceptual illusion" shaped by sensory experience.

The thermal sense, and specifically the cold sensations (as resulting from the afferent activity of the cold sensitive skin's thermo-receptors, i.e. small myelinated Aδ and unmyelinated C-fibers) (Campero and Bostock, 2010), could play a critical role in the ability to perceive local skin wetness. For example, we seem to interpret the coldness experienced during the evaporation of water from the skin as a signal of the presence of water (and thus wetness) on the skin' surface (Bergmann Tiest et al. 2012; Daanen, 2009). The importance of sensing coldness in order to experience local skin wetness has been highlighted by our previous findings. We have demonstrated that an illusion of local skin wetness can be evoked during the skin's contact with a cold-dry surface producing a range of skin cooling rates of 0.14 to

0.41 °C·s^{-1} (Filingeri et al., 2013) (see Chapter Four). Nevertheless, the mechanical sense could play a role as determinant as the one played by the thermal sense in characterising this perception. Everyday experience indicates that we perceive skin wetness even in the absence of coldness, e.g. when in contact with warm liquids. Bergmann Tiest et al. (2012) have shown that, when thermal cues (e.g. thermal conductance of a wet material) provide insufficient sensory inputs, individuals seem to use mechanical cues (e.g. stickiness resulting from the adhesion of a wet material to the skin) to aid them in the perception of wetness. Thus, in particular conditions, the mechanical and pressure related sensations, as resulting from the afferent activity of the cutaneous mechano-receptors(for review, see Abraira and Ginty, 2013), might contribute significantly to the perception of wetness (Wang et al., 2002; Ackerley et al., 2012). However, although thermal and mechanical inputs seem to be acknowledged as the principal inducers of the perception of local skin wetness (Bentley, 1900; Ackerley et al. 2012; Bergmann Tiest et al. 2012), to date it is unclear how and to what extent these sensory inputs interact in characterising this complex perception. Furthermore, to our knowledge, whether and how this intra-sensory interaction is influenced by factors such as the activity performed (i.e. rest vs. exercise) and the ambient temperature (i.e. thermo-neutral vs. warm) has never been investigated.

Thermal sensitivity to cold has been previously shown to be reduced during exercise, possibly due to hormonal and neurological factors (Ouzzahra et al., 2012). Also, local thermal sensations resulting from the same thermal stimulation have been shown to change according to the whole-body thermal state (e.g. greater cold sensitivity can be observed during heat exposure) (Cabanac et al., 1972; Attia and Engel, 1982; Arens and Zhang, 2006). Thus, as we believe that sensing coldness is the primary inducer of the “perceptual illusion” of skin wetness (Filingeri et al., 2013) (see Chapter Four), it would be reasonable to hypothesise that the perception of local skin wetness is reduced during exercise (due to a reduced sensitivity to cold), as well as increased during warm environmental conditions (e.g. due to an increased sensitivity to cold).

The aim of this study was therefore to investigate the role of thermal and mechanical inputs, as well as their interaction, in the perception of local skin wetness, using a single-blinded psychophysical approach. Also, we investigated whether and how this

intra-sensory interaction is influenced by factors such as the activity performed (i.e. rest vs. exercise) and the ambient temperature (i.e. thermo-neutral vs. warm).

We hypothesised that, due to its synthetic nature, an illusion of skin wetness can be evoked through the application of particular cold-dry stimuli, resulting in specific rates of skin cooling (i.e. range of 0.14 to 0.41 $°C \cdot s^{-1}$) (Filingeri et al., 2013) (see Chapter Four). Also, we hypothesised that, as the mechanical inputs generated by experiencing skin wetness (e.g. when sweating or immerging a body part into a liquid) usually refers to modest levels of pressure, and due to the complex interconnecting, intermodal and cross modal networks our sensory systems operate within (McGlone and Reilly, 2010), the interaction of different mechanical inputs (in the form of higher pressures) might attenuate the way this illusion is evoked.

5.3 Materials and methods

5.3.1 Participants

Eight healthy university female students (age 21 ± 1 years; height 166 ± 6 cm; body mass 60.5 ± 8 Kg; body composition by skinfold analysis 16.8 ± 3.4 % body fat) with no history of sensory-related disorders volunteered to participate in this study. Female participants were preferred to male as they are less hairy. All participants gave their informed consent for participation. The test procedure and the conditions were explained to each participant. The study design had been approved by the Loughborough University Ethics Committee and testing procedures were in accordance with the tenets of the Declaration of Helsinki.

5.3.2 Experimental design

The experimental design was based on the application (in a balanced order) of six cold-dry stimuli with different temperatures and mechanical pressures, on the bare upper and lower back of each participant. During the application of the stimuli participants were resting or cycling in an environmental chamber set at 22 °C (thermo-neutral exposure) or at 33 °C (warm exposure) and 50 % relative humidity. Each participant took part in four experimental tests: i) thermo-neutral rest; ii) warm rest; iii) thermo-neutral exercise; iv) warm exercise. These were performed in a balanced order, on separate days with at least 48 hours in between of them. The data

collection took place during May and June. A single-blind psychophysical approach was used for this study. Participants were informed only about the body region objected to the stimulation. No information was provided on the type and magnitude of the stimulation to limit any expectation effects.

5.3.3 **Stimuli**

Six cold-dry stimuli, resulting from combining three relative temperatures [-4, -8 and -15 °C below the local skin temperature (T_{sk})] and two mechanical pressure [low pressure (LP): 7 kPa; high pressure (HP): 10 kPa] were used in this study: -4 °C LP; -4 °C HP; -8 °C LP; -8 °C HP; -15 °C LP; -15 °C HP. The stimuli were delivered by a square thermal probe (Physitemp Instruments Inc., USA) with a contact surface of 25 cm^2. The exact temperatures of the stimuli were calculated on an individual basis, by measuring the local T_{sk} with an infrared thermometer (Fluke Corporation, USA).

To manipulate and control the mechanical pressures applied by the thermal probe, we designed and developed a pressure control system (Fig. 1). The system consisted of an air bladder, inserted into a frame attached to the thermal probe, which was connected to a manometer (containing water) throughout a silicon tube. The frame consisted of two wooden discs laid one upon the other and coupled by three springs which allowed the top disc to scroll down freely. A handle was attached to the top disc so that the probe could be applied to the skin. When this happened, the air bladder deformed, producing a pressure change in the system which resulted in displacing the water in the manometer from its set “null” point (no pressure applied). The point reached by the water in the tube as a result of the pressure change was used as an indicator to control the mechanical pressure. To calibrate and standardize this last one, a digital scale (Mettler Toledo Inc., USA) was used to measure the force resulting from the application of the probe. The range between the lowest and the highest pressure applicable and measurable by the system resulted in 7 to 55 kPa. For the purposes of this study, two levels of mechanical pressure were chosen. The LP represented the pressure applied by the probe when this was just in contact with the skin surface (i.e. light touch). This pressure (i.e. 7 kPa) was considered as a reference pressure, as it was the lowest applicable and measurable by the pressure control system. The HP (i.e. 10 kPa) was then chosen to be just slightly greater than the reference pressure. We wanted our participants to perceive a difference between the two stimuli, without however applying an excessive mechanical stimulation.

Preliminary data indicated that individuals were able to perceive differences between the two levels of pressure chosen for this study.

Tests were performed prior to the main experiment to check the accuracy and repeatability of the nominal pressures applied with the pressure control system. 100 trials (i.e. 50 for the LP and 50 for HP) were conducted. These consisted of measuring the force resulting from the application of the probe on a digital scale (Mettler Toledo Inc., USA) while controlling that the water displacement on the manometer was the one required for the pressures selected. 95% confidence interval values were calculated for the two nominal pressures and resulted as follow: LP (i.e. 7 kPa) = 7.1 kPa (lower bound) – 7.2 kPa (upper bound); HP (i.e. 10 kPa) = 10.4 kPa (lower bound) – 10.6 kPa (upper bound). To ensure precision in the application of the stimuli and repeatability of the data, the same investigator conducted all trails.

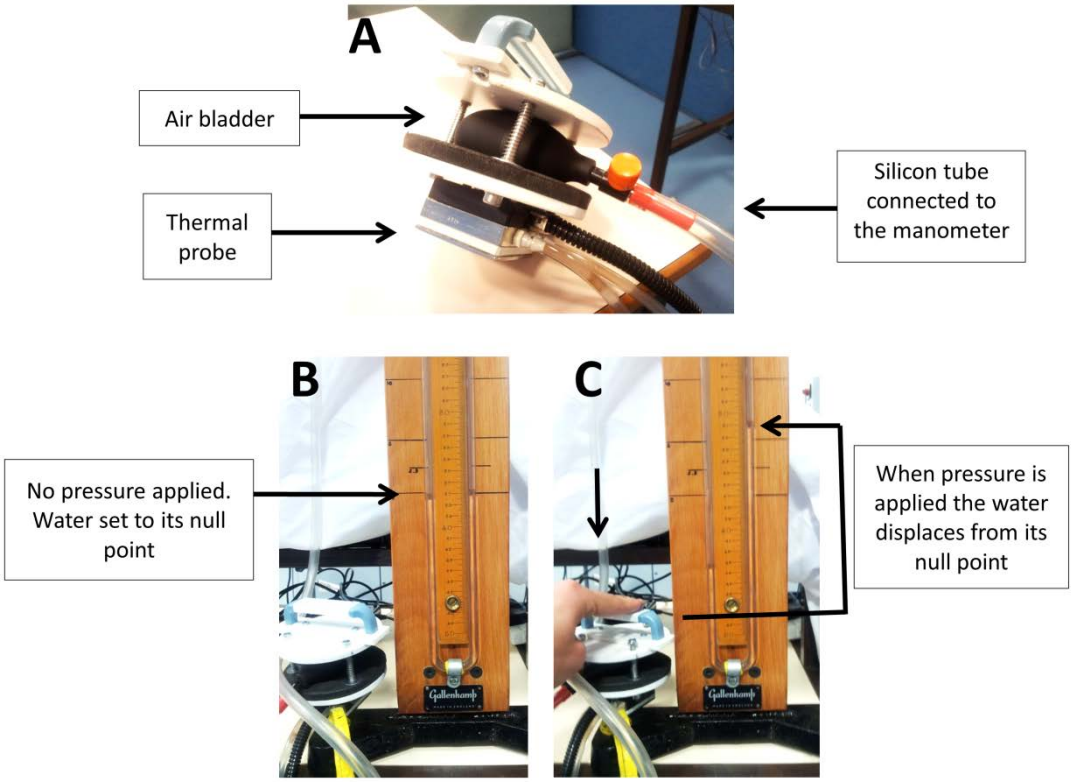


Figure 1: The thermal probe and pressure control system used in this study. The system consists of an air bladder, inserted into a frame attached to the thermal probe. The air bladder is connected to a manometer (containing water) throughout a silicon tube (A). When no pressure is applied to the system, the water in the manometer sets to its "null" point (B). When pressure is applied, the air bladder deforms, producing a

pressure change in the system which displaces the water in the manometer from its set "null" point (C). The point reached by the water in the tube, as a result of the pressure change, was used as an indicator to control the mechanical pressure applied to the skin.

5.3.4 **Experimental protocol**

Participants arrived to the laboratory 30 min before the time scheduled for the test to allow preparation procedures. During the first visit, semi-nude body mass, height and skinfolds thickness (seven sites) were recorded. For body composition calculations ACSM's guidelines for exercise testing and prescription were used (Thompson et al. 2010).

Participants then changed into sport bra, shorts, socks and trainers. Five iButtons (Maxim, USA) were taped to five left skin sites (cheek, abdomen, upper arm, lower back and back lower thigh) to record T_{sk} (1-min intervals). The five temperature measurements were recorded at 1 min intervals throughout the tests, averaged every 5 min, and then weighted according to the work of Houdas, to give an estimate of mean T_{sk} for the entire body (Choi et al. 1997; Houdas and Ring, 1982). The skin sites targeted for stimulation were marked with a washable marker to assure consistency in the location of stimulation. These corresponded to: 5 cm upwards the inferior angle of the right scapula (i.e. upper back skin site); 5 cm upwards the right posterior superior iliac spine (i.e. lower back skin site). The back was chosen as targeted area for stimulation in order to eliminate any visual feedback which could have affected the way participants perceived the stimuli.

After preparation, participants entered the environmental chamber and 10 min were allowed for acclimation. During this period, participants familiarised with the rating scales designed to record individual thermal sensations and wetness perceptions: an 11 point thermal scale (-6 very cold; -4 cold; -2 slightly cool; 0 neutral; +2 slightly warm; +4 warm); an 11 point wetness scale (-6 dripping wet; -4 wet; -2 slightly wet; 0 neutral; +2 slightly dry; +4 dry) (Olesen and Brager, 2004). No descriptors were applied to intermediate scores (i.e. -5; -3; -1; +1; +3). We defined the value -2 (labelled: "slightly wet") of the wetness scale as our set threshold to identify a clearly perceived local wetness. After the acclimation period, participants were asked to

maintain a seated position, or to move to an electromagnetically braked cycle ergometer (Lode Excalibur, The Netherlands) and start cycling at 40 rpm, with a workload of 60 W. During the experimental test, participants were first asked to rate their thermal sensations and wetness perceptions just before the application of the stimulus (i.e. baseline whole-body sensation), while the local T_{sk} of the skin site targeted for stimulation was measured with the infrared thermometer. Then the thermal probe was set to the required relative temperature and applied by hand to the skin site with the set pressure. To avoid an effect of surprise on the transient sensations, a verbal warning was given prior to stimulation. The application of the probe consisted of a short contact lasting 10 s. During the stimulation, the probe was not moved and participants could not see the stimulated area. At the end of the 10 s stimulation, participants were instructed and encouraged to verbally report their local sensation and perception, using whatever number in the scales seemed appropriate (integers only). Immediately after this the probe was removed and T_{sk} of the stimulated area was recorded with the infra-red thermometer. This method allowed rating to be made consistently close to the time when post-stimulation T_{sk} was recorded. This sequence was repeated for each stimulus allowing at least one minute in between them. This time interval, as well as the short duration of the stimulation and the balanced order of application (e.g. upper vs. lower back) allowed the local T_{sk} to return to baseline values before a new stimulus was applied. Each participant had only one presentation of each stimulus for each body region. All participants completed all conditions.

5.4 Statistical Analysis

In the present study, the independent variables were the probe temperature (the relative cold stimulus based on the individual baseline Tsk) and pressure, the body region stimulated, the activity performed and the environmental condition. The dependent variables were mean, local Tsk, average variations in local Tsk (ΔTsk) (from pre- to post-stimulation), thermal sensation and wetness perception.

All data were first tested for normality of distribution and homogeneity of variance using Shapiro-Wilk and Levene's tests respectively. Mean Tsk data were analysed by a 2-way repeated measure analysis of variance, with activity performed (2 levels: rest

and exercise) and ambient temperature (2 levels: thermo-neutral and warm) as repeated measures variables. Local ΔTsk data were analysed by a 5-way repeated measure analysis of variance, with temperature of the stimuli (3 levels: -4, -8 and -15°C), pressure (2 levels: 7 and 10 kPa), body region (2 levels: upper and lower back), activity (2 levels: rest and exercise) and ambient temperature (2 levels: thermo-neutral and warm) as repeated measures variables. Data were tested for sphericity and if the assumption of sphericity was violated, Huynh–Feldt or Greenhouse-Geisser corrections were undertaken to adjust the degrees of freedom for the averaged tests of significance. Estimated marginal means and 95% confidence intervals were used to investigate the main effects and interactions of the variables. When a significant main effect was found, Tukey's post-hoc analyses were performed. Observed power was computed using $\alpha=0.05$ and reported when a significant effect was observed.

Thermal sensation and wetness perception scores were analysed by Friedman's analysis of variance (X^2) and Wilcoxon signed rank tests (Z). First, the main effect of each independent variable was tested by collapsing the data over probe temperature (3 levels of comparison), pressure, body regions, activity and ambient temperature (2 levels of comparison) respectively. A Friedman's analysis of variance was performed for the 3 levels comparisons whereas a series of Wilcoxon Signed-ranks tests were performed for each of the 2 levels comparisons. Then, interactions between variables were investigated, using Friedman's analysis of variance (main effect) and Wilcoxon Signed-ranks test (post-hoc comparisons). It was decided to focus on specific interactions (i.e. probe temperature with pressure, 6 levels of comparison; activity with ambient temperature, 4 levels of comparison) in order to restrict the number of comparisons and thus reducing the risk of Type II errors. Effect size was calculated and reported as r. This analysis was considered advantageous for its "planned comparison-approach" to interactions, drawing on clear conceptualization (Acock, 2010). Although the authors acknowledge that non-parametric statistics tend to have less power for well distributed dependent variables, they can be more sensitive to effects when variables are not normally distributed, as in the case of this study (Acock, 2010). Statistical analysis was performed using IBM SPSS Statistics 19 (IBM, USA). In all analyses, $p<0.05$ was used to establish significant differences. Data are reported as mean ± standard error of the mean.

5.4.1 **Frequency distribution analysis of wetness scores**

To further investigate the effect of temperature and pressure of the stimuli on wetness perception scores, a frequency distribution analysis was performed. Wetness perception scores were averaged by temperature and pressure of the stimuli and collapsed over condition (i.e. activity and ambient temperature) and body region. Then, as the value -2 of the wetness scale (labelled: "slightly wet") was defined as our set threshold to identify a clearly perceived local wetness, wetness scores from -2 (i.e. "slightly wet") to -6 (i.e. "dripping wet") were grouped and considered as referring to a clear perception of wetness ("wet"), whereas any score in between -1 and +4 (i.e. "dry") was considered as representing no perception of wetness ("dry"). At this point, the frequency of times the same cold-dry stimulus was perceived as "dry" or as "wet" was calculated and analysed by a Chi-square test.

A similar frequency distribution analysis of thermal ratings has been previously reported in the literature (see Gan et al., 2012). In line with Gan et al. 2012, we believe that because of the variable nature of subjective responses, reorganizing the collected data in this format would make the potential thermal-tactile interaction in the perception of wetness easier to identify.

5.5 <u>Results</u>

5.5.1 **Parametric data**

5.5.1.1 Mean T_{sk}

Mean Tsk values were calculated for each condition and found to be normally distributed (p>0.05). A significant main effect of activity performed ($F= 18.89_{(1, 7)}$, p<0.01, observed power= 0.96), ambient temperature ($F= 300.23_{(1, 7)}$, p<0.01, observed power= 1) and a significant interaction between these two ($F= 6.54_{(1, 7)}$, p <0.05, observed power= 0.6) was found on the mean T_{sk}, whose values (as recorded and averaged for each test) were respectively: 31 ± 0.2 °C (thermo-neutral rest); 33.5 ± 0.2 °C (warm rest); 31.2 ± 0.3 °C (thermo-neutral exercise); 34.5 ± 0.2 °C (warm exercise). Post-hoc analysis indicated that conditions of exercise and warm ambient temperature resulted in a significantly higher mean T_{sk} than conditions of rest and thermo-neutral ambient temperatures (p<0.01).

5.5.1.2 Local T_{sk}

Baseline local Tsk values (pre-stimulation) varied in a range between 29.6 ± 0.2 °C (thermo-neutral exercise) and 33.6 ± 0.2 °C (Warm rest) for the upper back, and between 27 ± 0.2 °C (thermo-neutral exercise) and 32.1 ± 0.2 °C (Warm rest) for the lower back. Average ΔT_{sk} from pre- to post-stimulation (as a result of each of the six stimuli, applied to each skin site, during each of the four experimental conditions), were calculated and found to be normally distributed (p>0.05). These varied in a range of -0.6 ± 0.08 to -4 ± 0.2 °C (depending on probe condition), corresponding to a range of skin cooling rates of 0.06 ± 0.01 to 0.4 ± 0.02 $°C \cdot s^{-1}$. These values were calculated as the ratio between the ΔT_{sk} from pre- to post-stimulation and the contact time (i.e. 10 s). The data analysis indicated that only the temperature of the stimuli had a significant main effect on the local ΔT_{sk} ($F= 123.36_{(1.17, 8.2)}$, p<0.01, observed power= 1). No significant effect of the pressure applied ($F= 3.66_{(1, 7)}$, p>0.05), the body region stimulated ($F= 0.2_{(1, 7)}$, p>0.05), the activity performed ($F= 0.3_{(1, 7)}$, p>0.05) and the ambient temperature ($F= 2.13_{(1, 7)}$, p>0.05) was found. Figure 2 shows ΔT_{sk} and corresponding cooling rates, as a result of each cold-dry stimulus applied with LP and HP. Data were collapsed over the conditions performed (i.e. resting or exercising in thermo-neutral and warm environment) and the skin sites where the stimuli were applied. Post-hoc analysis indicated that colder stimuli resulted in significantly greater decreases in local T_{sk} (p<0.01). No significant interactions between the temperature of the stimuli and any other repeated-measures variables were found (p>0.05).

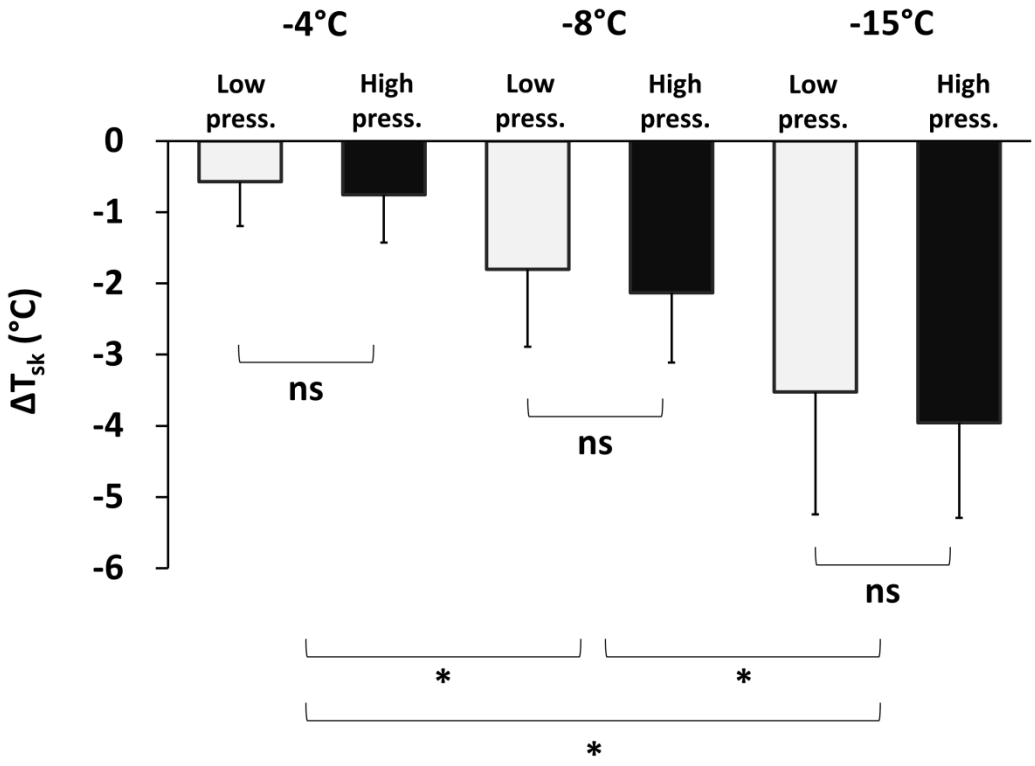


Figure 2: Relative variations in skin temperature drop from baseline (ΔT_{sk}), and corresponding cooling rates, as a result of each cold-dry stimulus applied with low (i.e. grey bars) and high pressure (i.e. black bars). Data were collapsed over the conditions performed (i.e. resting or exercising in thermo-neutral and warm environment) and the skin sites where the stimuli were applied. Differences are reported as statistically (*p<0.05) or as not statistically significant (i.e. ns).

5.5.2 **Non-parametric data**

5.5.2.1 Thermal sensation

Baseline thermal sensation scores (pre-stimulation) were respectively: -1.1 ± 0.1 (thermo-neutral rest); +0.9 ± 0.1 (Warm rest); +0.7 ± 0.1 (thermo-neutral exercise); +2.8 ± 0.1 (Warm exercise). Expressed in terms of semantic labels, these were in a range going from “slightly cold” to “warm”.

A first analysis was performed to investigate the main effects of temperature and pressure of the probe. A significant effect of temperature [X^2 (2, N = 128) = 187.69,

$p<0.01$] and a significant effect of pressure of the stimuli (Z= 4.26, $p<0.01$, r= 0.3) on local thermal sensations was found. At this point, the interaction between temperature and pressure of the probe was investigated. Figure 3 shows the local thermal sensation scores as a result of each cold-dry stimulus applied with LP and HP, with data collapsed over the conditions performed and the skin sites where the stimuli were applied. A significant interaction between the temperature and pressure of the stimuli was found [X^2 (5, N = 64) = 204.51, $p<0.01$] caused by the presence of a pressure effect at -8 °C (Z= -3.26, $p<0.01$, r= -0.4) and -15 °C (Z= -2.52, $p<0.01$, r= -0.32), but absence of this at -4 °C (p >0.05). The results confirmed that colder stimuli resulted in significantly colder sensations, and indicated that stimuli of same relative temperature (i.e. -8 °C and -15 °C) were perceived as significantly less cold when were applied with HP than when they were applied with LP.

A subsequent analysis was performed to investigate the main effect of ambient temperature and activity on thermal sensations.

A significant main effect of ambient temperature (Z= 2.91, $p<0.01$, r= 0.21) and activity (Z= 3.1, $p<0.01$, r= 0.22) was found on thermal sensations. At this point, the interaction between activity and ambient temperature was investigated and found to be statistically significant [X^2 (3, N = 96) = 20.18, $p<0.01$]. Significant differences were found only between conditions of rest in the thermo-neutral and warm environment (Z= -2.56, $p<0.01$, r= -0.26). These results indicated that stimuli were perceived as being less cold when participants were resting in a warm environment than when they were resting in a thermo-neutral one. No significant main effect of body region was found ($p>0.05$).

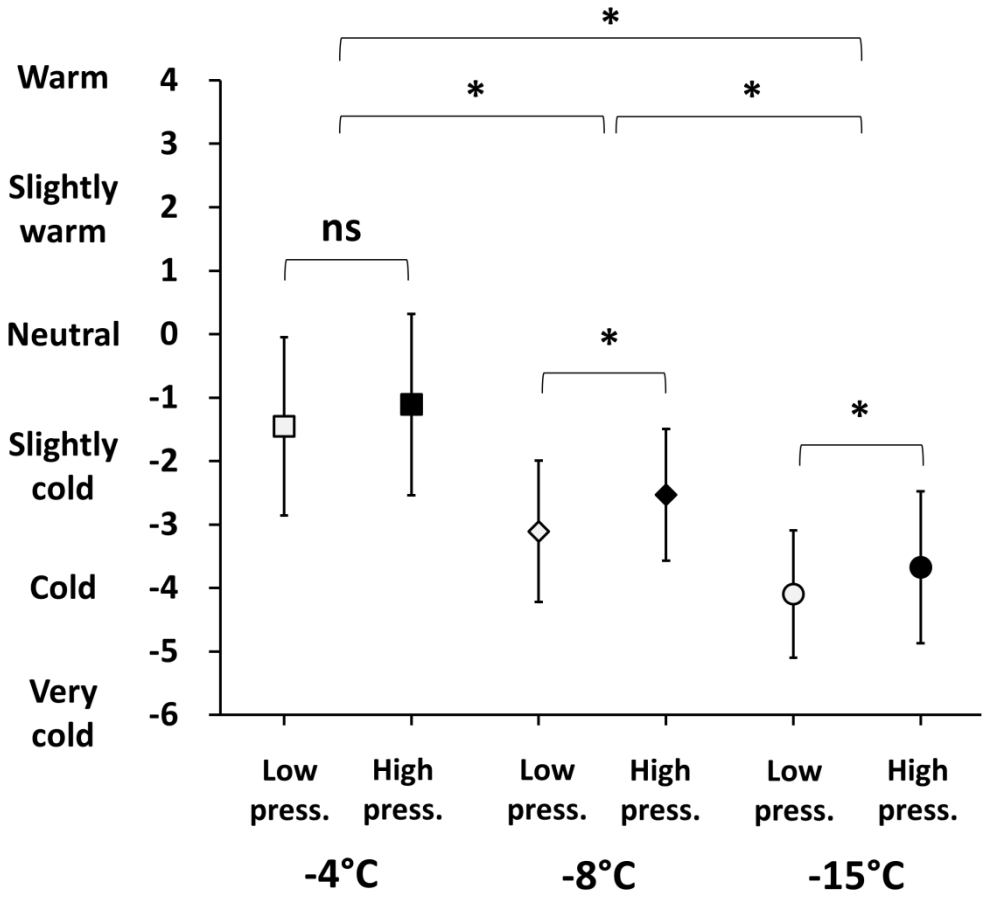


Figure 3: Local thermal sensation scores as a result of each cold-dry stimulus applied with low (i.e. grey dots) and high pressure (i.e. black dots). Data were collapsed over the conditions performed (i.e. resting or exercising in thermo-neutral and warm environment) and the skin sites where the stimuli were applied. Differences are reported as statistically (*p<0.05) or as not statistically significant (i.e. ns).

5.5.2.2 Wetness perception

Baseline wetness perception scores (pre-stimulation) were respectively: 0 ± 0.1 (thermo-neutral rest); 0 ± 0.1 (Warm rest); -0.5 ± 0.1 (thermo-neutral exercise); -2.2 ± 0.1 (Warm exercise). Expressed in terms of semantic labels, these were in a range going from “neutral” to “slightly wet”.

A first analysis was performed to investigate the main effect of temperature and pressure of the probe. A significant effect of temperature [X^2 (2, N = 128) = 75.36,

$p<0.01$], and a significant effect of pressure of the stimuli (Z= -3.27, $p<0.01$, r= -0.23) on local wetness perceptions was found. At this point, the interaction between temperature and pressure of the probe was investigated. Figure 4 shows the local wetness perception scores as a result of each cold-dry stimulus applied with LP and HP, with data collapsed over the conditions performed and the skin sites where the stimuli were applied. A significant interaction between temperature and pressure of the stimuli was found [X^2 (5, N = 64) = 87.31, p <0.01], caused by the presence of a pressure effect at -8 °C (Z= -2.98, $p<0.01$, r= -0.4) and -15 °C (Z= -2.3, $p<0.05$, r= -0.3), but absence of this at -4 °C ($p>0.05$).

These results indicated that colder stimuli resulted in significantly wetter sensations, and that stimuli of same relative temperature (i.e. -8 °C and -15 °C) were perceived as significantly less wet when were applied with HP than when they were applied with LP.

A subsequent analysis was performed to investigate the main effect of ambient temperature and activity on wetness perceptions. A significant effect of ambient temperature (Z= -3.65, $p<0.01$, r= -0.26), and a significant effect of activity (Z= -4.25, $p<0.01$, r= -0.32) on local wetness perceptions was found. At this point, the interaction between the activity and ambient temperature was investigated and found to be statistically significant [X^2 (3, N = 96) = 20.97, $p<0.01$]. Significant differences were found only between conditions of exercise in the thermo-neutral and warm environment, as well as between rest and exercise performed in the warm environment. These results indicated that stimuli were perceived as being wetter when participants were exercising in a warm environment than when they were resting in the same environment (Z= -3.75, $p<0.01$, r= -0.4), as well as when they were exercising in the thermo-neutral one (Z= -3.75, $p<0.01$, r= -0.38). No significant main effect of body region was found ($p>0.05$).

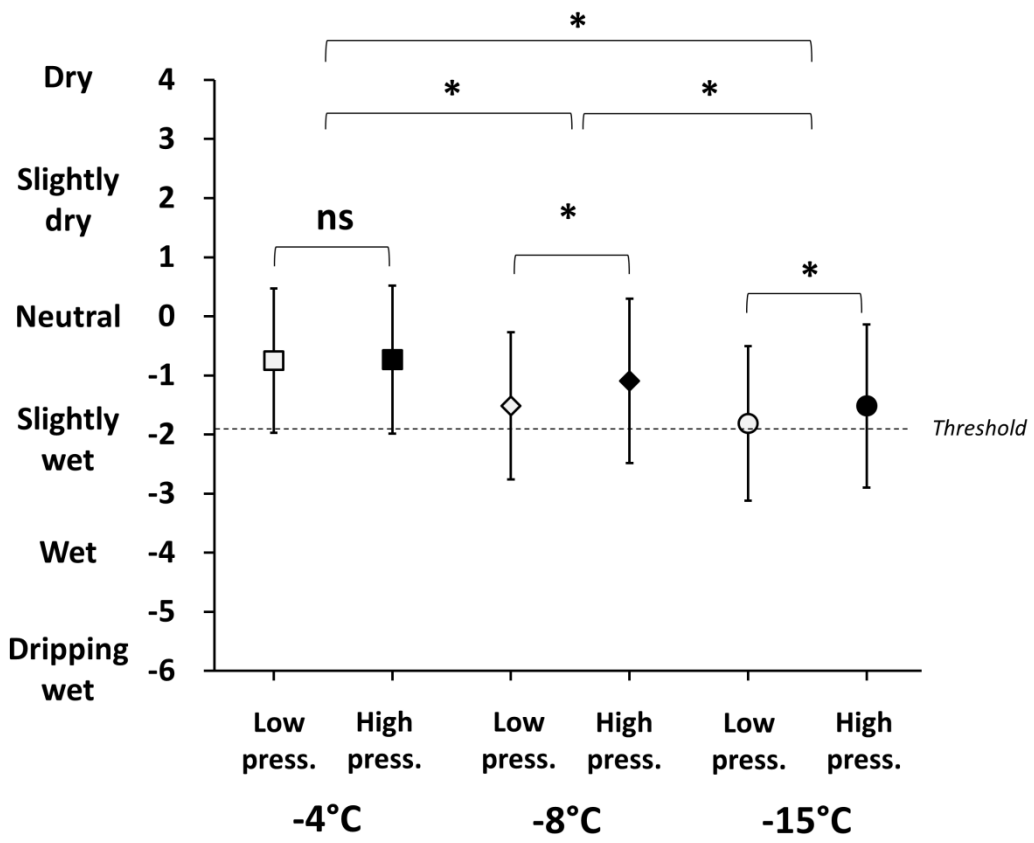


Figure 4: Local wetness perception scores as a result of each cold-dry stimulus applied with low (i.e. grey dots) and high pressure (i.e. black dots). Data were collapsed over the conditions performed (i.e. resting or exercising in thermo-neutral and warm environment) and the skin sites where the stimuli were applied. Differences are reported as statistically (*p<0.05) or as not statistically significant (i.e. ns).

5.5.2.2.1 Frequency distribution analysis of wetness scores

A frequency distribution analysis of wetness scores was performed and data for each of the six cold-dry stimuli are shown in figure 5. The results indicated a main effect, as well as a significant interaction, between temperature and pressure of the stimuli on the frequency of "wet" scores (Pearson Chi-square p<0.01). Colder stimuli were significantly more often perceived as wet (i.e. -4 °C LP= 21.9 %; -8 °C LP= 46.9 %; -15 °C LP= 60.9 %). However, when stimuli with the same relative temperature were applied with HP, local wetness perceptions were significantly attenuated (i.e. -4 °C HP= 20.3 %; -8 °C HP= 32.8 %; -15 °C HP= 45.3 %).

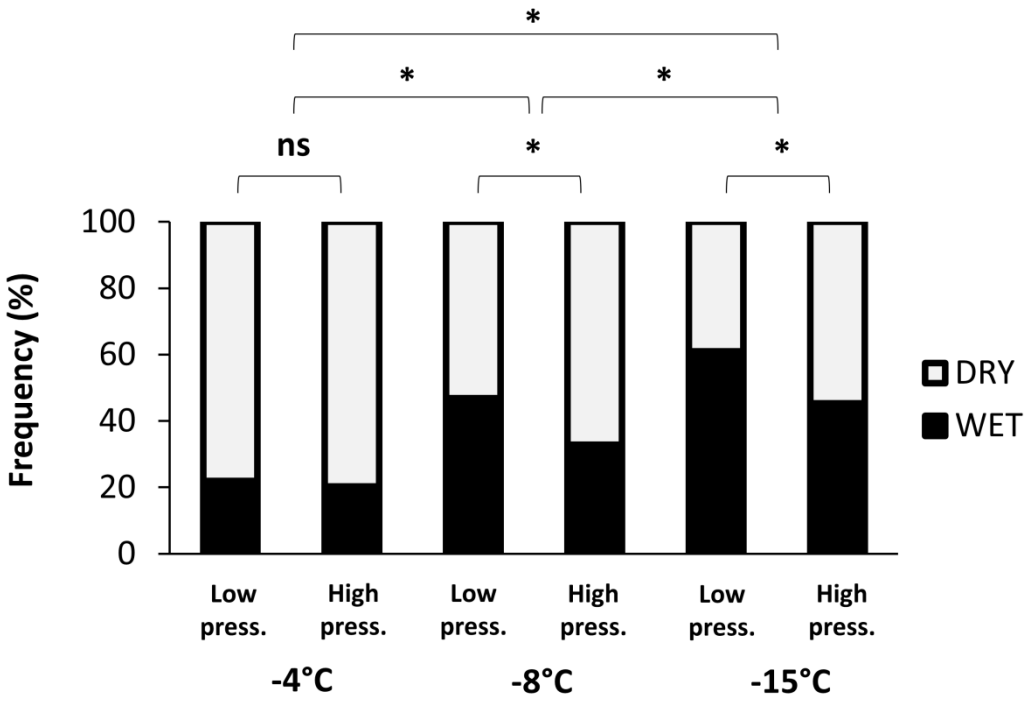


Figure 5: Frequency distribution of local wetness perception scores as a result of each cold-dry stimulus applied with low and high pressure. The frequency of times the same cold-dry stimulus was perceived as "dry" (i.e. wetness scores in between -1 and +4, labelled "dry"), or as "wet" (i.e. wetness score between -2, labelled "slightly wet", and -6, labelled "dripping wet"), is indicated as a fraction (%) of the total responses recorded for each stimulus. Data were collapsed over the conditions performed (i.e. resting or exercising in thermo-neutral and warm environment) and the skin sites where the stimuli were applied. Differences are indicated as statistically (*p<0.05) or as not statistically significant (i.e. ns).

5.6 Discussion

The aim of this study was to investigate the sensory integration responsible for the perception of local skin wetness, with regards to thermal (i.e. cold) and mechanical (i.e. pressure) afferents. The experimental protocol was designed to assure that two bare and dry skin sites would be exposed to the contact with a range of cold-dry

stimuli, applied with two different mechanical pressures, during experimental trials consisting of resting or exercising in a thermo-neutral or warm environment.

The results of this study indicated that cold-dry stimulations can evoke artificial wetness perception, with colder stimuli resulting in a higher frequency and magnitude of wet perceptions. Also, we observed that the application of stimuli with a higher mechanical pressure on the skin reduced the frequency of times artificial wetness perceptions were evoked. Finally, we found that cold-dry stimuli were perceived as being wetter during exercise performed in the warm environment than during rest in the same environment, as well as than during exercise in the thermo-neutral one.

5.6.1 **The role of thermal inputs in the perception of local skin wetness**

The first main outcome of this study is that the perception of local skin wetness did relate to the activation of the thermal afferents responding to skin cooling. When cold-dry stimuli, resulting in skin cooling rates in a range of 0.06 to 0.4 $°C \cdot s^{-1}$, were applied on participants' skin, these were frequently perceived as being not only cold, but as also wet. Cold-dry stimuli were more frequently perceived as cold-wet (i.e. 46.9 and 60.9 % of times they were applied) when these resulted in skin cooling rates of 0.18 $°C \cdot s^{-1}$ (i.e. -8 °C LP stimulus) and 0.35 $°C \cdot s^{-1}$ (i.e. -15 °C LP stimulus). This is aligned to our previous findings. We have recently shown that an illusion of local skin wetness can be evoked during the skin's contact with a cold-dry surface producing skin cooling rates in a range of 0.14 to 0.41 $°C \cdot s^{-1}$ (Filingeri et al., 2013) (see Chapter Four). This range of skin cooling rates is also aligned to the one which occurs during the evaporation of water from the skin' surface as suggested by Daanen (2009), who measured the temperature course of the skin (i.e. temperature's drop of 1 to 5°C with a 0.05 to 0.2 $°C \cdot s^{-1}$cooling rate) when this was wetted with drops of water with volumes in a range of 0.01 to 0.1ml. However, in the present study, and in line with our previous findings (Filingeri et al., 2013) (see Chapter Four), we observed that the cooling rates which more often evoked perceptions of wetness (i.e. 0.18 and 0.35 $°C \cdot s^{-1}$) were slightly faster than the ones proposed by Daanen (2009). A possible explanation to this difference might be related to the different types of cooling used in the two experiments, as in Daanen' s work, skin cooling resulted from evaporation whereas in our study cooling resulted from conduction (i.e. contact with a surface colder than the skin). Recent evidence has

indicated that the perception of skin wetness comprises a number of different cues, amongst which evaporation and thermal conductance, and that evaporation might require slower cooling rates than thermal conductance to evoke the perception of wetness (Bergmann Tiest et al., 2012a). This seems to be due to the fact that evaporation is only sensed with a thin layer of moisture on the skin, whereas increased thermal conductance is only a factor with a larger volume of liquid (Bergmann Tiest et al., 2012a). This could result in greater heat extraction from the skin and thus greater coldness experienced. In the light of this, the outcomes of this study provide evidence in support of the hypothesis that different thermal cues (i.e. evaporation or conductance) might require different rates of skin cooling to evoke the perception of local skin wetness.

The fact that an illusion of local skin wetness was experienced when the skin was in contact with a cold-dry surface resulting in particular rates of skin cooling (and thus cold sensations), unmasked the synthetic nature of this complex perception (Bentley, 1900). Furthermore, it highlighted the remarkable ability of the central nervous system to learn through sensory experiences (Gescheider and Wright, 2012). Perceptual learning, and specifically somatosensory-decision making, seems to be a critical neuronal process which underlines our ability to link sensation, memory and decision making (Pleger and Villringer, 2013). Studies in primates have shown how somatosensory stimuli might be represented in the brain, and how such representation relates to sensation, memory and decision making (Romo and Salinas, 1999). The somatosensory cortex seems to be involved in generating a neural representation of the sensory stimulus, which is used for further processing in downstream areas. These areas transform the neural representation into a simple firing rate code representing the stimulus frequency during presentation, working memory and decision components (Lemus et al., 2007). Thus, we hypothesise that a similar process might occur during the experience of skin wetness. As we are apparently not provided with specific hygro-receptors (Clark and Edholm, 1985), the somatosensory inputs which our brain encodes when the skin is wet (e.g. thermal cues due to skin cooling), might be coded into particular neural representations and then associated to the perception of skin wetness. This hypothesis could explain why in our study the exposure to thermal inputs similar to the ones occurring when the skin is physically wet, evoked a perceptual illusion of wetness, even if no contact with moisture occurred. However, this speculation needs further experimental

evidence, as somatosensory decision making is still an almost unexplored area in humans (Pleger and Villringer, 2013).

5.6.2 **The interaction between thermal and mechanical inputs**

The second main outcome of this study is that the illusion of local skin wetness was significantly attenuated by an increase in the mechanical pressure applied to the skin. Although thermal stimuli applied with HP and LP resulted in similar skin cooling rates, HP were perceived as significantly less cold and less wet. This finding is of high interest, as to our knowledge this is the first study to report an interaction between thermal and mechanical inputs, which attenuated the perceptual illusion of local skin wetness.

Interactions between thermal and mechanical inputs during dynamic contact cooling (i.e. skin cooling occurs when the thermal probe first contacts the skin) have been previously reported (see Green, 2004 for an extensive review). Based on the outcomes of these studies, cold sensations have been suggested to involve interactions between the pathways for cold, nociception and touch. These interactions seem to occur particularly at mild temperatures (Green and Pope, 2003; Green and Schoen, 2005; Green and Schoen, 2007), such as the ones resulting from the stimuli used in this study (i.e. skin temperature' s drop between 0.6 and 4 °C). Green et al. (2003, 2005, 2007) have reported an attenuation (i.e. -13 %) in cold sensation by dynamic contact cooling (as opposed to static contact, i.e. skin cooling occurs when the thermal probe is already in contact with the skin), during the application of stimuli with a mild temperature (i.e. 31 °C) to the volar surface of the forearm (when this had a baseline T_{sk} of 33 °C). In these studies, thermal sensations were unaffected by dynamic touch at lower temperatures (i.e. 27, 24 and 20 °C).

The outcomes of our study seems aligned to the ones reported by Green et al. (2003, 2005, 2007) as we observed attenuations in thermal sensation (and wetness perception) due to an increased mechanical stimulation to the skin. This attenuation was significantly accentuated by those stimuli which reduced T_{sk} by 1.8 to 4 °C (i.e. -8 and -15 °C stimuli respectively), from an average baseline value of 30.5 °C. Although Green et al. concluded that their results are a demonstration that tactile stimulation has only a relatively weak inhibitory effect on the cold pathway (which quickly becomes insignificant at colder levels of stimulations) (Green and Schoen, 2007), we believe that this "weak" inhibitory effect could have been sufficient

enough to alter the cold sensations, and thus the evoked skin wetness, experienced by our participants. As we have previously shown that local skin wetness is strongly related to the level of coldness experienced (Filingeri et al., 2013), we believe that even small changes in the cold sensations occurring during contact cooling might affect the way skin wetness is evoked. Furthermore, as stimulation of the rapidly-adapting skin mechanoreceptors during dynamic touch has been shown to be critical for other previously described intra- and inter-sensory interactions (e.g. touch-pain and thermal-pain, in which touch and thermal stimuli reduce the perception of pain) (Bolanowski et al., 2001; Green, 2009; Green and Pope, 2003; Green and Schoen, 2005), it is reasonable to hypothesise that changes in mechanical afferents might influence the way a complex perception such as skin wetness is experienced. It could be suggested that the LP stimuli used in this study (i.e. light touch) generated mechanical sensations which could have been closer to the mechanical inputs experienced when individuals are "physically wet" (e.g. when sweating or immerging a body part into a liquid). As these inputs usually refers to modest levels of pressure (Bergmann Tiest et al. 2012), it would be then reasonable to expect that LP stimuli, as opposed to HP ones, would increase the occurrence of wetness perceptions, as observed in this study. High static pressures during contact cooling of the skin, despite providing more cooling, might have generated "unfamiliar" sensations which are not commonly associated to the way we *learn* to perceive skin wetness.

Perception is well known to be a cognitive process which relies on the multisensory integration of information from different sensory systems, which are combined at different levels of the neuraxis (Cappe et al. 2009; Driver and Spence, 2000; Stein et al. 2009). The impact of multisensory integration on cognition and behaviour has been amply demonstrated by sensory phenomena such as the "skin parchment illusion", in which audio-tactile interactions change the perception of roughness (Jousmäki and Hari 1998). The outcomes of this study might therefore provide evidence in support of the hypothesis of a tactile-mediated attenuation of the perception of local skin wetness. Also, these findings indicate that cold sensation and wetness perception might not depend solely on the parameters of the thermal stimulus. However, one should note that any generalization of these findings should be carefully considered in the light of the regional differences (e.g. glabrous vs. hairy skin) in the thermal and spatial sensitivity (i.e. thermo- and mechano-receptors

innervation) across the body (Abraira and Ginty, 2013; Ackerley et al. 2012; Nakamura et al. 2008; Ouzzahra et al. 2012).

5.6.3 Effects of activity performed and ambient temperature on thermal and wetness perceptions

The third main outcome of this study is that cold-dry stimuli were perceived as wetter during exercise performed in the warm environment than during rest in the same environment, as well as than during exercise in the thermo-neutral one. This outcome might indicate that environmental factors, such as exercise and ambient temperature, could have a central effect on modulating the sensory pathway of complex perceptions such as skin wetness. However, we hypothesised that the changes observed in the local wetness perception during the condition of exercise in the warm environment are more likely to be related to an effect of the whole body level of wetness, than to a central sensory modulation. Indeed, by the end of this trial, participants' skin was wet due to sweat production. It is therefore reasonable to hypothesise that experiencing a whole body perception of wetness during the trial might have influenced the way cold-dry stimuli were perceived locally on the skin (Fukazawa & Havenith 2009). Our previous findings (Filingeri et al., 2013), as well as the results of this study, indicate that local wetness is strongly driven by local coldness. Hence, if local changes in the sensory pathway for this perception occurred due to a central effect of exercise or ambient temperature, we would have expected similar changes in local thermal sensations. However, local thermal sensations were significantly different only between the conditions of rest in thermo-neutral and warm ambient, with cold-dry stimuli being perceived as less cold during exposure to the warm than to the thermo-neutral ones. The different trends observed between thermal sensation and wetness perceptions amongst conditions might therefore highlight the possibility that other factors than ambient temperature and exercise (e.g. the level of moisture on the skin regions not targeted for stimulation, as well as the whole body perception of wetness) might have influenced the perception of local wetness. Nevertheless, the lack of studies investigating the central effects of factors such as exercise or ambient temperature on complex percepts makes any conclusion on this topic difficult to draw. Most of the studies looking into sensory perception have focused on exercise and/or ambient temperature-induced changes in thermal sensation (Burke and Mekjavic, 1991; Nakamura et al. 2008; Norrsell et al. 1999,

Ouzzahra et al. 2012). More studies are therefore needed in order to appraise how e.g. different levels of whole body wetness could affect the perception of local skin wetness.

5.7 Limitations

The absolute values for skin cooling reported in this study should be carefully considered. Indeed, the cooling rates presented should not be indented as the exact representation of the skin cooling profiles which occurred during the stimulations, but rather, as a close approximation. These values were calculated as the ratio between the ΔT_{sk} from pre- to post-stimulation and the contact time (10 s). Thus, the resulting skin cooling profile was in principle assumed to be linear. However, based on the skin's biological characteristics, it is more likely that the skin cooling had a an exponential profile, with a greater drop in temperature during the first seconds of contact, followed but a smaller one towards the end (Jay and Havenith, 2004a; Jay and Havenith, 2004b). Therefore, it is reasonable to hypothesise that the values we calculated represent an underestimation of the skin cooling rates which occurred during the first seconds of stimulation, though a high correlation of these rates with the presented ones can be assumed based on the nature of the cooling curve (Jay and Havenith, 2004a; Jay and Havenith, 2004b).

5.8 Conclusion

We conclude that thermal inputs from peripheral cutaneous afferents are critical in characterizing the perception of skin wetness. However, the role of these inputs might be modulated by an intra-sensory interaction with the tactile afferents. Taken together, these findings indicate that human sensory perception is remarkably multimodal. The outcomes of this study have a fundamental as well as an applied significance. On the fundamental side, these could contribute to a better understanding of how the peripheral and central nervous system interact to generate complex somatic perceptions. On the applied side, taking into account the neurophysiology of the perception of skin wetness might help to improve the design

of protective clothing and thus thermal comfort in strenuous work conditions (e.g. fire-fighting).

6 CHAPTER SIX – Pilot study: Biophysical effects of moisture evaporation on local skin temperature

6.1 Abstract

We have previously demonstrated that an illusion of local skin wetness can be evoked during the skin's contact with a cold-dry surface producing drops in skin temperature of 1.4 to 4.1 °C·s^{-1} (with a cooling rate of 0.14 to 0.41 °C·s^{-1}; Chapter Four) and of 1.8 to 3.5 °C (with a cooling rate of 0.18 to 0.35 °C·s^{-1}; Chapter Five). However, as limited data are available on the effects that the presence of different volumes of external moisture on the skin has on local skin temperature, the possibility of a direct comparison between the cooling rates we observed during dry contact cooling and the ones occurring when actual moisture evaporates from the skin is limited. Hence, we tested the biophysical effects of applying water drops of different volumes on local skin temperature. Also, we tested the effect of evaporative cooling resulting from dipping the hand in water at ~30 °C and then exposing it to the ambient for 30 s (with or without artificially-generated additional convection). We found that, when water drops with a volume of 20, 60 and 120 µl and a temperature of ~30 °C were applied (total duration: 30 s) on the skin, these resulted in an immediate drop in skin temperature (i.e. first second of contact) of -2.2, -3.7 and -4.6 °C respectively, from a baseline value of ~30 °C. After 10 s, skin temperature was -1.2, -1.9 and -2.1 °C lower than baseline. Overall, the average change in skin temperature during the first 10 s of application was -1.5, -2.7 and -2.7 °C for the 20, 60 and 120 µl water drops respectively, corresponding to average cooling rates of 0.15, 0.27 and 0.27 °C·s^{-1}. Also, we found that evaporative cooling occurring post water immersion resulted in an immediate drop in skin temperature (i.e. first second of exposure) of -0.4 °C (i.e. no convection) and -1.5 °C (i.e. additional convection), from a baseline value of ~30 °C. After 10 s, skin temperature was -0.5 °C (i.e. no convection) and -2.0 °C (i.e. additional convection) lower than baseline. Overall, the average change in skin temperature during the first 10 s of exposure to the ambient was -0.7 °C (i.e. no convection) and -2.7 °C (i.e. additional convection), corresponding to average cooling rates of 0.07 and 0.27 °C·s^{-1}. We conclude that the immediate changes in skin temperature recorded as a result of the

evaporation of different volumes of moisture from the skin (i.e. 2.2 to 4.6 °C) appeared to be remarkably similar to the changes in skin temperature resulting from dry contact cooling (i.e. 1.4 to 4.1 °C) which we have previously reported to induce an illusion of local skin wetness.

6.2 Introduction

We have previously demonstrated that an illusion of local skin wetness can be evoked during the skin's contact with a cold-dry surface producing drops in skin temperature of 1.4 to 4.1 $°C \cdot s^{-1}$ (with a cooling rate of 0.14 to 0.41 $°C \cdot s^{-1}$; Chapter Four) and of 1.8 to 3.5 °C (with a cooling rate of 0.18 to 0.35 $°C \cdot s^{-1}$; Chapter Five).
These results seemed aligned to the findings of Daanen (2009) who measured the temperature course of the skin (i.e. temperature' s drop of 1 to 5 °C with a 0.05 to 0.2 $°C \cdot s^{-1}$ cooling rate) when this was wetted with drops of water with volumes in a range of 10 to 100 µl. The author suggested that the cold sensations experienced when such skin cooling occurs can contribute to the perception of skin wetness. Therefore, exposing the skin to a cold-dry stimulus producing such skin cooling was hypothesised to evoke an illusory perception of skin wetness.
In our previous studies this hypothesis was confirmed, as for example, when the application of cold-dry stimuli produced a drop in skin temperature ranging between 1.4 and 4.1°C with a cooling rate of 0.14 to 0.41 $°C \cdot s^{-1}$, a clear wetness perception was evoked, whereas when the cold-dry stimulation produced a drop in skin temperature of 0.2 to 0.7 °C with a cooling rate of 0.02 to 0.07 $°C \cdot s^{-1}$, wetness was little evoked and decreasing thermal sensations prevailed (see Chapter Four).
However, as the data available on the effects that the presence of different volumes of external moisture on the skin has on local skin temperature is limited only to the study of Daanen (2009), the possibility of a direct comparison between the cooling rates we observed during dry contact cooling and the ones occurring when external moisture is applied on the skin is therefore limited.
Hence, in the present pilot study it was aimed to investigate the biophysical effects of applying water drops of different volumes (i.e. 20, 60 and 120 µl) on local skin temperature. The effect of evaporative cooling resulting from dipping a skin site in water and then exposing it to the ambient (with or without artificially-generated

additional convection) was also tested. This, in order to verify whether the changes in skin temperature which we have previously shown to induce an illusion of skin wetness during dry contact cooling, are similar to the ones occurring when actual moisture evaporates from the skin.

6.3 Materials and methods

To investigate the biophysical effects of applying water drops of different volumes on the skin on local skin temperature, water drops with a volume of 20, 60 and 120 µl and a temperature of ~30 °C were applied (total duration: 60 s) with a variable volume pipettor (SciQuip LTD, Newtown, UK) on the ventral forearm of a male participant (28 years old), while this was resting in a seated position in a thermo-neutral environment (air temperature: ~23 °C; relative humidity: ~50 %) (Fig. 1).

Local skin temperature (T_{sk}) at the site of application was monitored continuously through the application of a thin thermocouple (0.08 mm wire diameter, 40 Gauge; 5SRTC-TT-TI-40-2M, Omega, Manchester, UK) on the ventral side of the forearm using transpore tape (3M, Loughborough, UK), with the sensor tip touching the skin, but not covered by tape. T_{sk} was monitored using a Grant Squirrel SQ2010 data logger (Grant Instruments Ltd., Cambridge, UK). Water temperature was monitored with a thermistor (Grant Instruments, Cambridge, UK), which was immersed in the water container used to sample the water drops used in this pilot, and which was connected to the same data logger as for the skin thermocouple.

In order to avoid the conductive cooling effect that water drops with a temperature lower than the skin might have generated on skin temperature, and in order to focus on the potential evaporative cooling generated by the presence of moisture on the skin surface, water drops were applied with a temperature (~30 °C) which was similar to the forearm skin temperature recorded for the participant (~30.2 °C). Also, to assure that the pipettor tip presented the same temperature as water temperature, this was immersed in the water container for at least 30 s before sampling the drops.

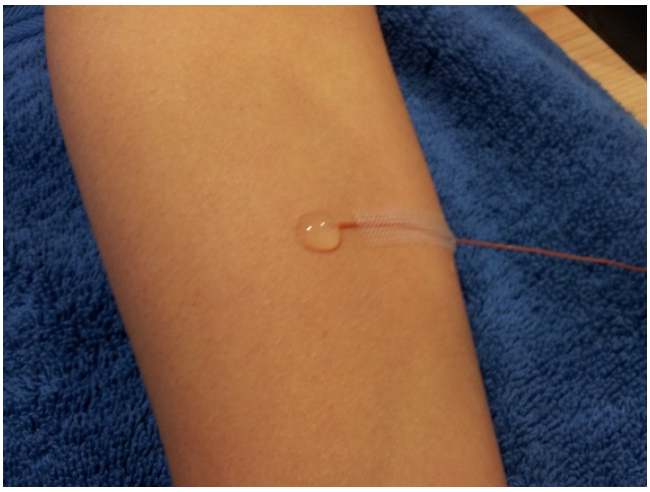


Figure 1: Water drop applied on the participant's ventral forearm at the skin site where the thermocouple used to monitor skin temperature was taped.

To investigate the effect of evaporative cooling resulting from post-water immersion on skin temperature, the same male participant was asked to fully immerse his right hand (up to the wrist) in a water container (water temperature: ~30 °C), until skin temperature reached water temperature (total duration: ~2 min). Local skin temperature (T_{sk}) was monitored continuously through the application of a thin thermocouple (0.08 mm wire diameter, 40 Gauge; 5SRTC-TT-TI-40-2M, Omega, Manchester, UK) on the dorsum of the hand using transpore tape (3M, Loughborough, UK), with the sensor tip touching the skin, but not covered by tape. T_{sk} was monitored using a Grant Squirrel SQ2010 data logger (Grant Instruments Ltd., Cambridge, UK). Water temperature was monitored with a thermistor (Grant Instruments, Cambridge, UK), which was immersed in the water container, and which was connected to the same data logger as for the skin thermocouple.

As soon as skin temperature was observed to reach water temperature (~30 °C), the participant was asked to remove the hand from the water container, leaving the skin exposed to the environment (air temperature: ~23 °C; relative humidity: ~50 %) for 30 s. This procedure allowed recoding of the effect of evaporative cooling (caused by the residual water on the skin after the immersion) on skin temperature. Following a break (~10 min), the same procedure (i.e. immersion and post-water immersion) was repeated, however with the addition of artificially-generated extra convection (i.e. the investigator blew air on the participant's hand) during the 30-s post water immersion exposure.

6.4 Results

Figure 2A shows the changes in skin temperature (ΔT_{sk}) during the initial 10 s of application of water drops (20, 60 and 120 µl) at skin temperature on the ventral forearm of the male participant. As soon as the 20, 60 and 120 µl water drops were applied, these resulted in an immediate drop in skin temperature (i.e. first second of contact) of -2.2, -3.7 and -4.6 °C respectively, from a baseline value of ~30 °C. After 10 s, skin temperature was -1.2, -1.9 and -2.1 °C lower than baseline. Overall, the average change in skin temperature during the first 10 s of application was -1.5, -2.7 and -2.7 °C for the 20, 60 and 120 µl water drops respectively, corresponding to average cooling rates of 0.15, 0.27 and 0.27 $°C \cdot s^{-1}$.

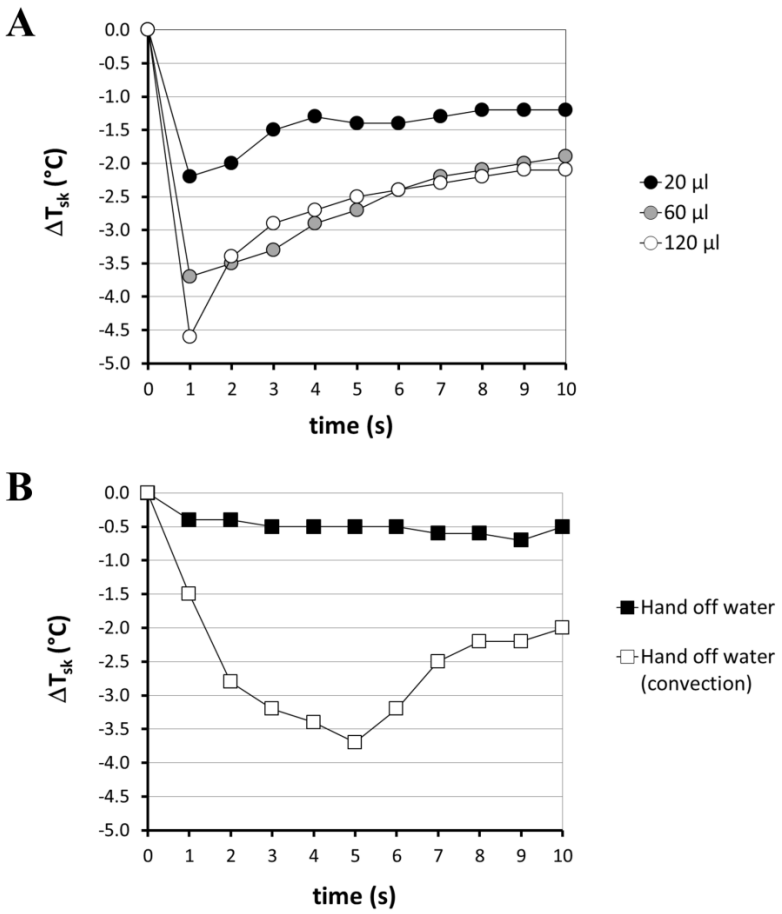


Figure 2: (A) Relative changes in skin temperature (ΔT_{sk}) during the first 10 s of application of the 20, 60 and 120 µl water drops. (B) Post water immersion ΔT_{sk} as recorded during the first 10 s of exposure to the environment, with (with squares) or without (black squares) additional convection.

Figure 2B shows the changes in skin temperature (ΔT_{sk}) during the initial 10 s post water immersion. As soon as the hand was removed from the water container, an immediate drop in skin temperature (i.e. first second of exposure) of -0.4 °C (i.e. no convection) and -1.5 °C (i.e. additional convection), from a baseline value of ~30 °C, was recorded. After 10 s, skin temperature was -0.5 °C (i.e. no convection) and -2.0 °C (i.e. additional convection) lower than baseline. Overall, the average change in skin temperature during the first 10 s of exposure to the ambient was -0.7 °C (i.e. no convection) and -2.7 °C (i.e. additional convection), corresponding to average cooling rates of 0.07 and 0.27 $°C \cdot s^{-1}$.

6.5 Discussion

In this pilot, it was observed that the immediate changes in skin temperature resulting from the application of water drops with volumes of 20, 60 and 120 µl (range: -2.2 to -4.6 °C) were remarkably similar to those changes in skin temperature resulting from dry contact cooling (range: -1.4 to -4.1 °C), which have previously been reported to induce an illusion of local skin wetness (Filingeri et al., 2013; 2014c; see Chapter Four and Five).

We also observed that the immediate changes in skin temperature resulting from post water immersion evaporative cooling (range: -0.4 to -1.5 °C) were above the lower threshold value (i.e. -0.7 °C) for wetness perception resulting from contact dry cooling (Filingeri et al., 2013; see Chapter Four). This finding indicates that the dry contact cooling previously shown to induce a perception of skin wetness is also similar to the evaporative cooling resulting from post water immersion. However, the immediate changes in skin temperature resulting from post water immersion evaporative cooling (range: -0.4 to -1.5 °C) appeared smaller than the ones observed as a result of the application of external drops (range: -2.2 to -4.6 °C). This difference could be due to the fact that, contrary to the application of water drops on the skin with a pipettor, removing the hand from the water container to allow evaporation left a very small amount of residual moisture on the skin site were temperature was recorded (due to drippage of water off the skin). Hence, this limited amount of residual moisture could have resulted in a limited amount of evaporative

cooling, as compared to the one generated when external drops were applied and remained on the skin.

6.6 Conclusion

In conclusion, the results of this pilot confirms what previously reported on the biophysical effects that the application of moisture has on skin temperature (i.e. skin cooling). Also, these findings support the hypothesis that the reason why specific cold-dry stimuli induced an illusion of skin wetness in blindfolded participants (see Chapter Four and Five) is because these stimuli resulted in similar skin cooling as the one occurring when actual moisture evaporates from the skin.

7 CHAPTER SEVEN – Laboratory study 4: Body mapping of cutaneous wetness perception across the human torso during thermo-neutral and warm environmental exposures

Publication(s) based on this chapter:

Filingeri, D., Fournet, D., Hodder, S., Havenith, G. (2014) Body mapping of cutaneous wetness perception across the human torso during thermo-neutral and warm environmental exposures. *Journal of Applied Physiology*. 117:887-897 (Appendix F)

7.1 Abstract

Sensing skin wetness is linked to inputs arising from cutaneous cold-sensitive afferents. As thermosensitivity to cold varies significantly across the torso, we investigated whether similar regional differences in wetness perception exist. Also, we investigated the regional differences in thermal pleasantness and whether these sensory patterns are influenced by ambient temperature. Sixteen males (20 ± 2yr) underwent a quantitative sensory test under thermo-neutral (T_{air}= 22 °C; RH= 50 %) and warm conditions (T_{air}= 33 °C; RH=50 %). Twelve regions of the torso were stimulated with a dry thermal probe (25 cm^2) with a temperature of 15 °C below local skin temperature (T_{sk}). Variations in T_{sk}, thermal, wetness and pleasantness sensations were recorded. As a result of the same cold-dry stimulus, the skin cooling response varied significantly by location (p=0.003). The lateral chest showed the greatest cooling (-5 ± 0.4 °C) while the lower back the smallest (-1.9 ± 0.4 °C). Thermal sensations varied significantly by location and independently from regional variations in skin cooling with colder sensations reported on the lateral abdomen and lower back. Similarly, the frequency of perceived skin wetness was significantly greater on the lateral and lower back as opposed to the medial chest. Overall wetness perception was slightly higher under warm conditions. Significantly more unpleasant sensations were recorded when the lateral abdomen and lateral and lower back were stimulated. We conclude that humans present regional differences in skin wetness perception across the torso, with a pattern similar to the regional differences in

thermosensitivity to cold. These findings indicate the presence of an inhomogeneous distribution of cold-sensitive thermo-afferent information.

7.2 Introduction

Thermosensitivity (*i.e.* the ability to perceive thermal changes in the surrounding environment) represents an important drive of thermoregulatory responses in humans and in other mammalian and non-mammalian species (Spray, 1986; Gallio et al., 2011). In humans, cutaneous thermosensitivity is peripherally sub-served by cold-sensitive, myelinated Aδ-nerve fibers (conduction velocities ranging from 5-30 $m \cdot s^{-1}$) and by cold- and warm-sensitive, unmyelinated C-nerve fibers (conduction velocities ranging from 0.2-2 $m \cdot s^{-1}$) (Campero et al., 2001; Schepers and Ringkamp, 2010) and centrally integrated by the primary and secondary somatosensory cortices as well as the insular cortex (a cortical region involved in cold and warm temperatures sensation, as well as pain and touch) (Craig et al., 2000) through the spino-thalamic tract and the the dorsal-column medial lemniscal pathway (McGlone and Reilly, 2010). Fluctuations in skin temperature (T_{sk}) due to environmental stimuli [e.g. changes in ambient temperature (T_{air}) and humidity (RH)] and the related thermal sensations have been shown to trigger autonomic (e.g. vasomotor tone and sweating/shivering response) (Kondo et al., 1997; Sendowski et al., 2000) and behavioral responses (e.g. adding or removing clothing) (Schlader et al., 2012). These responses aim to maintain thermal homeostasis and comfort (Cabanac et al., 1972; Schlader et al., 2010).

Despite the critical role of thermosensitivity, sensing temperature is not the only factor amongst the cutaneous sensory afferent to contribute to thermoregulatory responses in humans. Sensing cutaneous wetness is also critical both for behavioral and autonomic responses. Perceiving changes in both ambient humidity and skin wetness have been shown to impact thermal comfort (Fukazawa and Havenith, 2009) and thus the thermoregulatory behavior (Schlader et al., 2010), both in healthy and clinical populations (e.g. individuals suffering from rheumatic pain) (Strusberg et al., 2002). From an autonomic perspective, the degree of skin wetness influences sweat gland function through a progressive suppression of the sweat output (*i.e.* hidromeiosis) in the presence of wetted skin (Nadel and Stolwijk, 1973). This results

in a reduced ability to lose heat to the environment via evaporative cooling, potentially affecting the thermal balance of the body (Candas et al., 1979). However, although the ability to sense skin wetness plays an important role in several behavioral and thermophysiological functions, little it is known on how skin wetness is sensed in humans (Montell, 2008).

As opposed to insects, in which humidity receptors sub-serving hygrosensation have been identified and widely described (Tichy and Kallina, 2010), humans seem not to be provided with specific receptors for the sensation of wetness (Clark and Edholm, 1985). Thus, we seem to "learn" to perceive the wetness experienced when the skin is in contact with a wet surface or when sweat is produced (Bergmann Tiest et al., 2012a) through a complex multisensory integration (Driver and Spence, 2000) of thermal (*i.e.* heat transfer) and tactile (*i.e.* mechanical pressure and skin friction) inputs generated by the interaction between skin, moisture and (if donned) clothing (Fukazawa and Havenith, 2009). This hypothesis has been supported by our previous findings. We have recently demonstrated that an illusion of local skin wetness can be evoked during the skin's contact with a cold-dry surface producing skin cooling rates in a range of 0.14 to 0.41 $°C \cdot s^{-1}$ (Filingeri et al., 2013; 2014c) (see Chapter Four and Five), a temperature course which is similar to the one suggested to occur when the skin is physically wet (Daanen, 2009). This could be due to the fact that we seem to interpret the coldness experienced during the evaporation of moisture from the skin as a signal of the presence of moisture (and thus wetness) on the skin' surface. All in all, these recent findings have highlighted the critical role of thermosensitivity to cold in the ability to perceive skin wetness (Filingeri et al., 2013; 2014c).

Appraising the importance of cold afferents in the ability to sense cutaneous wetness has led us to hypothesize that regional differences in wetness perception might exist across the body and might depend upon the regional differences in thermosensitivity to cold. The distribution of cutaneous sensitivity to cold has been indeed repeatedly shown to vary significantly across different regions of the body (Keatinge and Nadel, 1965; Burke and Mekjavic, 1991; Nakamura et al., 2008) as well as within the same body region (Ouzzahra et al., 2012). For example, the torso is suggested as amongst the most sensitive regions to cold (Keatinge and Nadel, 1965; Burke and Mekjavic, 1991; Nakamura et al., 2008). In this regard, the recent work of Ouzzahra et al. (2012) has provided evidence for the presence of an uneven distribution of cold sensitivity across the front and back torso. If we accept the hypothesis that sensing skin wetness

is primarily driven by the level of coldness experienced, it is reasonable to hypothesize that wetness perception varies significantly across the torso, with a pattern which could be similar to the one of thermosensitivity to cold. To our knowledge, only few studies have investigated whether humans present regional differences in cutaneous wetness perception (Fukazawa and Havenith, 2009; Lee et al., 2011; Ackerley et al., 2012).

In a study in which thermal comfort sensitivity was investigated in relation to locally manipulated skin wetness (as resulting from sweat production), Fukazawa and Havenith (2009) (Fukazawa and Havenith, 2009) found that the torso seems to have a lower sensitivity to wetness than the limbs, while in a non-manipulated condition (natural wetness distribution across the torso) Gerrett et al. (2013) showed that the torso seemed to dominate wetness perception. Similarly, Lee et al. (2011) showed that when asked, individuals reported the torso (*i.e.* chest and back) to be the region more often perceived as wet during rest and moderate exercise in 25 and 32 °C T_{air} and 50 % RH. In line with Lee et al. (2011), Ackerley et al. (2012) have recently shown that when wet stimuli with different moisture contents (range: 20-160 µl over a 0.0024 m^2 surface) were applied to different body regions, individuals were able to differentiate between moisture levels, with a tendency of the back as being amongst the most sensitive region to wetness. The outcomes of these studies have provided initial insights about the regions on which skin wetness might be perceived to a larger extent (e.g. the torso). However, by only measuring the physical wetness (whether due to sweat production or to contact with a wet surface) these studies have failed to provide a link between the thermal changes occurring locally at the skin's surface when this is wet [variation in local T_{sk} (ΔT_{sk})], and how these are perceived in terms of thermal sensations and perception of skin wetness.

The aim of the present study was to investigate the regional distribution of skin wetness perception across the torso, in relation to the distribution of thermosensitivity to cold. Also, as local thermal sensations resulting from the same thermal stimulation have been shown to change according to the body's thermal state (e.g. greater cold sensitivity can be observed during heat exposure) (Cabanac et al., 1972; Attia and Engel, 1982; Filingeri et al., 2014c), we investigated whether the regional distribution of skin wetness perception is influenced by the environmental conditions (thermo-neutral vs. warm). Finally, as it has been previously suggested that the hedonic attribute (*i.e.* pleasure) of a thermal stimulus is dependent on the

perception of the actual thermal state of the body (e.g. if the direction of the thermal stimulus is oriented towards a shift in the thermal state of the body from its natural homeostasis, then this will result in thermally unpleasant sensations) (Cabanac, 1971; Attia and Engel, 1982), we investigated whether regional differences in thermal pleasantness in response to local skin cooling exist across the torso.

We tested the hypothesis that during the short contact with the same cold-dry stimulus (*i.e.* 15°C lower than local T_{sk}) which we have previously shown to induce an illusion of skin wetness (Filingeri et al., 2014c) (see Chapter Five), local T_{sk}, thermal and wetness sensations will vary significantly by location of stimulation. Regions with a high thermosensitivity to cold were expected to present a higher perception of skin wetness. Also, we hypothesized that, as local thermal sensations resulting from the same thermal stimulation have been shown to change according to the body's thermal state (Cabanac et al., 1972; Attia and Engel, 1982), thermal and wetness perceptions will be higher during a warm as opposed to a thermo-neutral environmental exposure. This was also hypothesized to impact the hedonic component of thermal stimulation (*i.e.* greater displeasure will be recorded during thermo-neutral as opposed to warm exposure), with regional differences in thermal pleasure/displeasure expected to follow a pattern similar to the one for thermosensitivity to cold.

7.3 Materials and methods

7.3.1 Participants

Sixteen healthy Caucasian male students (age 20 ± 2 yr; height 1.78 ± 0.10 m; body mass 77.4 ± 10 Kg; body composition by skinfold analysis 8.0 ± 3 % body fat) with no history of sensory-related disorders volunteered to participate in this study.

To account for the inter-individual variability in the hairiness of the torso, participants' hair growth was visually graded using a modified Garn (1951) scoring system (for an extensive review see Yildiz et al. (2010). Photos of the front and back torso of each participant were taken. A score of 0–4 was assigned to chest, abdomen and upper and lower back, based on the visual density of terminal hairs. A score of 0 represented the absence of terminal hairs, a score of 1 minimally evident hair growth, and a score of 4 extensive hair growth (Yildiz et al., 2010). Thirteen out of 16

participants presented minimal hairs on the chest (score= 0.2 ± 0.1) and abdomen (score= 0.3 ± 0.1) and the absence of terminal hairs on the upper and lower back. Three out of 16 participants presented a higher level of hairiness on the chest (score= 3 ± 0.6) and abdomen (score= 2.3 ± 0.3) and the absence of hairs on the upper and lower back.

All participants gave their informed consent for participation. The test procedure and the conditions were explained to each participant. The study design had been approved by the Loughborough University Ethics Committee and testing procedures were in accordance with the tenets of the Declaration of Helsinki.

7.3.2 **Experimental design**

All participants underwent the same quantitative sensory test under thermo-neutral (T_{air}= 22 °C; RH= 50 %) and warm environmental conditions (T_{air}= 33 °C; RH= 50 %). The quantitative sensory test was based on the application of a cold-dry stimulus on 12 different skin sites distributed across the front and back torso of each participant. The exact anatomical locations of the areas targeted for stimulation are described in figure 1 and are in line with the work of Ouzzahra *et al.* (2012). All tested sites were medial or on the left side of the body, assuming symmetry (Claus and Hilz, 1987). During the contact with the stimulus, participants reported their local thermal, wetness and pleasantness sensations on Likert scales. Local T_{sk} at the contact site was measured before and immediately after the contact with the stimulus using a single spot infrared thermometer (FLUKE 566, Fluke Corporation, USA) with a temperature range of -40 to 800 °C and an accuracy of ± 1 °C. In order to maximize the accuracy of the temperature readings, during each test the infrared thermometer was calibrated against a black plate whose temperature was monitored with a thermistor (Grant Instruments, Cambridge, UK). This method has been previously used (Filingeri et al., 2014c) (see Chapter Five) and shown to be effective in allowing recording of post-stimulation T_{sk} to be made consistently close to the when subjective sensations were rated. The cold-dry stimulus was delivered by a square thermal probe (Physitemp Instruments Inc., USA) with a contact surface of 0.0025 m^2. The relative temperature of the stimulus was 15 °C lower than the local T_{sk} which was measured with the infrared thermometer. We chose a relative temperature of -15 °C as we have previously shown this to evoke the highest levels

of perceived wetness during a 10-s contact with the upper and lower back of resting and exercising individuals (Filingeri et al., 2014c) (see Chapter Five).

A single-blind psychophysical approach was used for this study. Participants were informed only about the body region objected to the stimulation, and no information was provided on the type and magnitude of the stimulation to limit any expectation effects. To assure that the participants could not see the stimulus applied on their torso, the following set up was designed. When the front torso was stimulated, participants were asked to lie on a bench on their back, with their arms alongside the body and a rectangular-shaped textile screen (length: 0.8 m; height: 0.7 m) was placed above participants' neck. The screen was adjusted until each participant confirmed that they could not see either their front torso or the investigator. When the back torso was stimulated, participants were asked to lie on their front, with their arms alongside the body, and to face towards the left, while the investigator was standing on their right hand side. Each participant confirmed that they could not see either their back torso or the investigator. The 12 skin sites were stimulated on a balanced order to prevent any order effect. The data collection took place in December (mean monthly temperature: 5.1 °C; min-max temperature range: 2.0 to 8.2 °C).

1. Medial chest
(270 mm above the umbilicus)

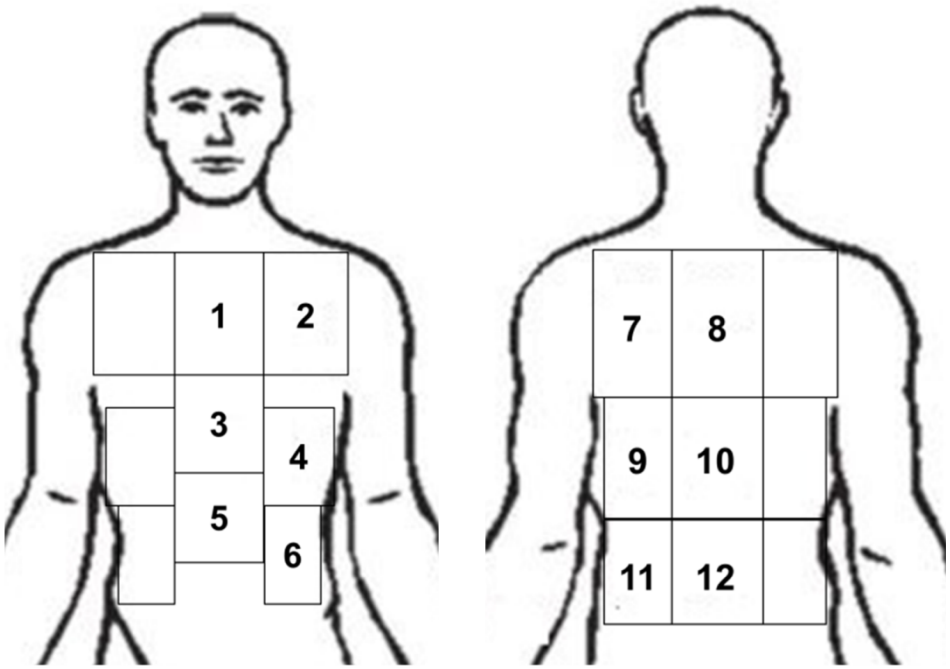
2. Lateral chest
(70 mm above the nipple)

3. Medial upper abdomen
(130 mm above the umbilicus)

4. Lateral upper abdomen
(30 mm below the nipple)

5. Medial abdomen
(30 mm above the umbilicus)

6. Lateral lower abdomen
(45 mm above the anterior superior iliac crest)



7. Lateral upper back
(50 mm above the inferior angle of the scapula)

8. Medial upper back
(30 mm medial to 7)

9. Lateral mid-back
(10 mm below the inferior angle of the scapula)

10. Medial mid-back
(30 mm medial to 9)

11. Lateral lower back
(165 mm below the inferior angle of the scapula)

12. Medial lower back
(30 mm medial to 11)

Figure 1: Name and exact anatomical locations of the 12 skin sites targeted for stimulation.

7.3.3 **Experimental protocol**

Participants arrived at the laboratory 30 min before the time scheduled for the test to allow preparation procedures. First, semi-nude body mass, height and skinfolds thickness (seven sites) were measured and recorded. For body composition calculations ACSM's guidelines for exercise testing and prescription were used (Gordon, 2009). Body density was calculated using the following seven sites (chest, midaxillary, triceps, subscapular, abdomen, suprailiac and thigh) equation:

$$Body\ density = 1.112 - 0.00043499(sum\ of\ seven\ skinfolds) + 0.00000055(sum\ of\ seven\ skinfolds)^2 - 0.00028826(age)$$

Participants then changed into shorts, socks and running shoes. Five iButtons (Maxim, USA) were taped to five skin sites on the right side of the body (*i.e.* cheek, abdomen, upper arm, lower back and back lower thigh) to record local T_{sk}. The five temperature measurements were recorded at 1 min intervals throughout the tests, averaged every 5 min, and then weighted according to the work of Houdas and Ring (1982) to give an estimate of mean T_{sk} for the entire body. The 12 skin sites targeted for stimulation were marked with a washable marker to assure consistency in the location of stimulation.

After preparation, participants entered a first environmental chamber set for the thermo-neutral exposure (22 °C T_{air}, 50 % RH). Participants sat on a chair and waited 10 min to allow acclimation to the environmental conditions. During this period, participants were familiarized with the rating scales designed to record individual thermal, wetness and pleasantness sensations: an 11 point thermal scale (-6 very cold; -4 cold; -2 slightly cool; 0 neutral; +2 slightly warm; +4 warm); an 11 point wetness scale (-6 dripping wet; -4 wet; -2 slightly wet; 0 neutral; +2 slightly dry; +4 dry); an 11 point pleasantness scale (-6 very unpleasant; -4 unpleasant; -2 slightly unpleasant; 0 neutral; +2 slightly pleasant; +4 pleasant) (Olesen and Brager, 2004; Filingeri et al., 2014c). No descriptors were applied to intermediate scores (i.e. -5; -3; -1; +1; +3). We defined the value -2 (labelled: "slightly wet") of the wetness scale as our set threshold to identify a clearly perceived local skin wetness.

After the acclimation period and according to the order of stimulation, participants were asked to lie either on their front or back and the quantitative sensory test was initiated. Participants were first asked to rate their thermal and wetness sensations

only, just before the application of the stimulus (*i.e.* baseline whole-body sensation), while the local T_{sk} of the skin site targeted for stimulation was measured with the infrared thermometer. Then the thermal probe was set to the required relative temperature (*i.e.* 15 °C below the recorded local T_{sk}) and applied by hand to the skin site. To avoid an effect of surprise on the transient sensations, a verbal warning was given prior to stimulation. The application of the probe consisted of a short contact lasting 10s. During the stimulation, the probe was not moved and participants could not see the stimulated area. At the end of the 10 s stimulation, participants were instructed and encouraged to verbally report their local thermal, wetness and also pleasantness sensations, using whatever number in the scales seemed appropriate (integers only). Immediately after this the probe was removed and T_{sk} of the stimulated area was recorded with the infra-red thermometer. The same protocol was repeated for each of the 12 skin sites allowing at least one minute in between them. Each participant had only one presentation of each stimulus for each skin site. The quantitative sensory test lasted for 15 min.

After completion of the test, 10min were allowed before participants moved from the first to the second environmental chamber set for the warm exposure (33 °C T_{air}, 50 % RH). Once in the second chamber, 10min were allowed for acclimation before the same quantitative sensory test, as explained above, was performed.

7.4 Statistical Analysis

In the present study, the independent variables were the skin site stimulated and the environmental condition. The dependent variables were mean, local T_{sk}, ΔT_{sk} (*i.e.* variation from pre- to post-stimulation) and thermal, wetness and pleasantness sensation. All data were first tested for normality of distribution and homogeneity of variance using Shapiro-Wilk and Levene's tests, respectively.

Mean T_{sk} data for the thermo-neutral and warm exposure were compared using a paired t-test. Local ΔT_{sk} data were analysed by a 2-way repeated measures analysis of variance, with skin site stimulated (12 levels) and environmental condition (2 levels: thermo-neutral and warm) as repeated measures variables. Data were tested for sphericity and if the assumption of sphericity was violated, Huynh–Feldt or Greenhouse-Geisser corrections were undertaken to adjust the degrees of freedom for

the averaged tests of significance. Estimated marginal means and 95 % confidence intervals were used to investigate the main effects and interactions of the variables. Observed power was computed using $\alpha = 0.05$. When a significant main effect was found, Tukey's post-hoc analyses were performed.

As absolute thermal, wetness and pleasantness sensations were obtained in the form of ordinal ratings, these were analysed by means of non-parametric statistics. The main effect of the environmental condition (2 levels of comparison) was tested by a Wilcoxon signed rank test (Z) whereas the main effect of the skin site stimulated (12 levels of comparison) was tested by a Friedman's analysis of variance (X^2). Post-hoc analyses for the effect of skin site stimulated were performed by a Wilcoxon signed rank test (Z) and adjusted for multiple comparisons. Effect size was calculated and reported as *r*. Although the authors acknowledge that non-parametric statistics tend to have less power for well distributed dependent variables, they can be more sensitive to effects when variables are not normally distributed, as in the case of this study (Acock, 2010).

To further investigate the regional distribution of cutaneous wetness perception, a frequency distribution analysis of skin wetness was performed. Wetness perception scores as recorded during both environmental conditions were collapsed over the skin site stimulated. Then, as the value -2 of the wetness scale (labelled: "slightly wet") was defined as our set threshold to identify a clearly perceived local wetness, wetness scores from -2 (*i.e.* "slightly wet") to -6 (i.e. "dripping wet") were grouped and considered as referring to a clear perception of wetness ("wet"), whereas any score in between -1 and +4 (*i.e.* "dry") was considered as representing no perception of wetness ("dry"). At this point, the frequency of times (%) the cold-dry stimulus was perceived as "dry" or as "wet" was calculated and analysed by a Chi-square test. This analysis was performed for each of the 12 skin sites. Also, frequency data were grouped and compared between the front and back torso. The same frequency distribution analysis of wetness ratings has been performed in one of our recent studies (Filingeri et al., 2014c). Also, a similar frequency distribution analysis of thermal ratings has been previously reported in the literature (see Gan et al., 2012). In line with Gan et al. (2012) and with our previous findings, we believe that, because of the variable nature of subjective responses, reorganizing the collected data in this format would make the potential differences in the regional distribution of wetness perception across the torso easier to identify.

Finally, a Spearman's rank correlation coefficient was calculated to investigate the degree of association between: 1. thermal sensation and frequency of wetness perception; 2. pleasantness sensation and frequency of wetness perception; 3. thermal sensation and pleasantness sensation. Statistical analysis was performed using IBM SPSS Statistics 19 (IBM, USA). In all analyses, $p<0.05$ was used to establish significant differences. Parametric and non-parametric (perceptual scores) data are reported as mean ± standard error of the mean. Furthermore, median and inter-quartile ranges [median; percentile] are reported for non-parametric data.

7.5 Results

7.5.1 Mean and local T_{sk}

Mean T_{sk} was calculated for each exposure and found to be normally distributed ($p>0.05$). Mean T_{sk} values for thermo-neutral and warm exposures were respectively 32.4 ± 0.1 °C and 34.8 ± 0.1 °C. These values were found to be significantly different (mean difference= 2.4 °C; 95 % CI= 2.2, 2.5 °C; t= 36.8; *two tailed* $p<0.001$). This result confirms the effectiveness of the environmental conditions we designed in inducing a significant change in the skin's thermal state.

Baseline local T_{sk} values (pre-stimulation) varied in a range between 31.8 ± 0.1 °C (*i.e.* lateral chest) and 33.4 ± 0.2 °C (*i.e.* medial upper back) for the thermo-neutral exposure, and between 34.9 ± 0.2 °C (*i.e.* lateral chest) and 36.1 ± 0.1 °C (*i.e.* medial upper back) for the warm exposure. Local ΔT_{sk} (as a result of the relative cold-dry stimulus applied to each skin site during the thermo-neutral and warm exposures), was calculated and found to be normally distributed ($p>0.05$). The data analysis indicated that only the skin site stimulated had a significant main effect on the local ΔT_{sk} ($F= 4.4_{(4.6, 50.6)}$, $p=0.003$). No significant effect of the environmental condition ($F= 2.2_{(1, 11)}$, $p=0.17$) nor significant interaction between the skin site stimulated and the environmental condition was found ($F= 0.4_{(11, 121)}$, $p=0.4$). The regional distribution of ΔT_{sk} is shown in figure 2*A*. Post-hoc analyses indicated that, depending on skin site, local ΔT_{sk} varied significantly in a range of -1.9 ± 0.4 °C (i.e. medial lower back) to -5.0 ± 0.4 °C (i.e. lateral chest), corresponding to a range of skin cooling rates of 0.19 ± 0.04 to 0.5 ± 0.04 $°C \cdot s^{-1}$. These values were calculated as the ratio between the ΔT_{sk} from post- to pre-stimulation and the contact time (i.e.

10s). The significance levels are presented separately for sites of the front and back torso (Tab.1).

Overall, these outcomes indicated that, as a result of the same relative cold-dry stimulus, the skin cooling response varied significantly by location across the torso, with a pattern which did not change between the thermo-neutral and warm environmental exposure.

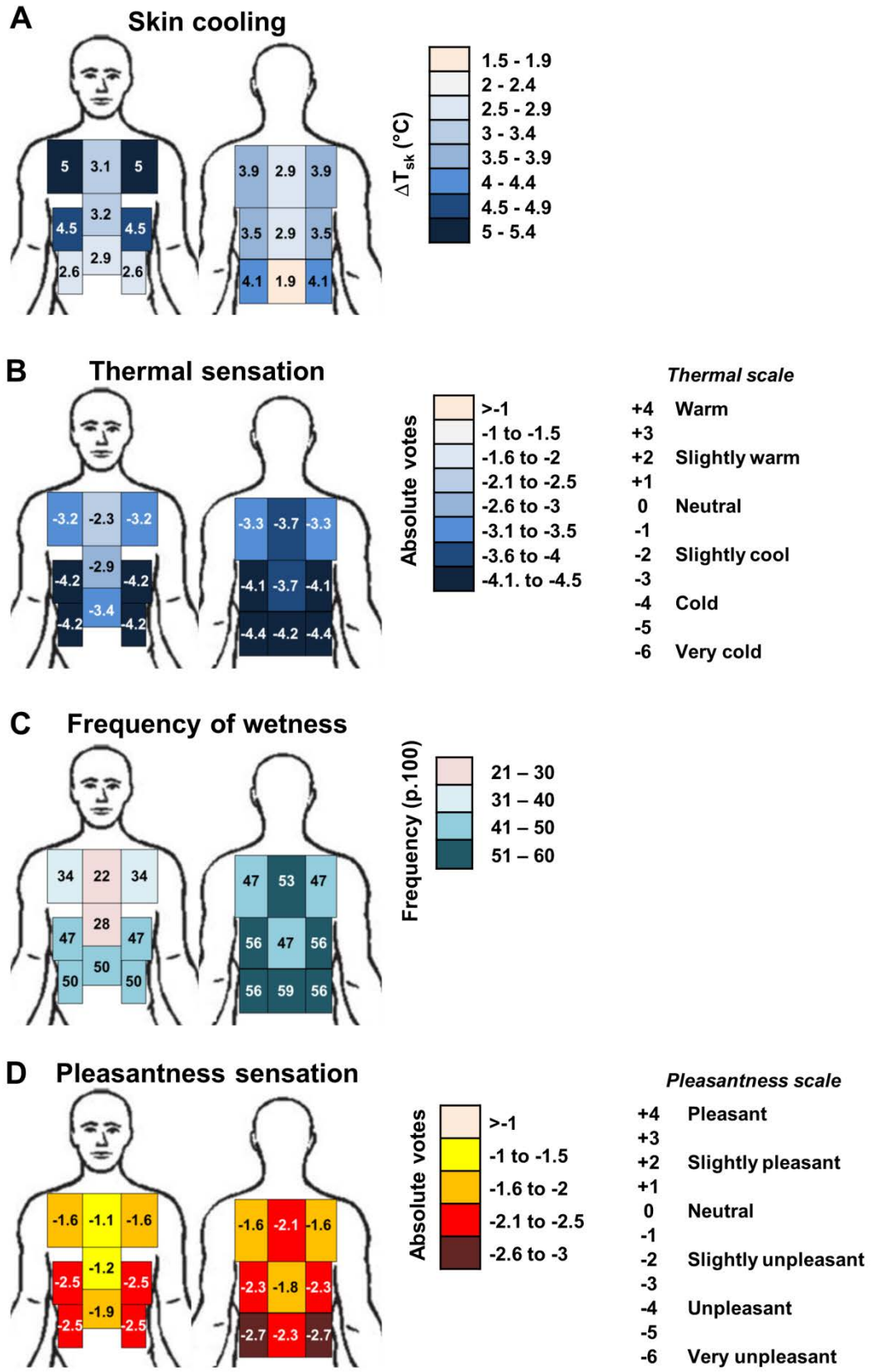


Figure 2: Body maps showing the regional distribution of (A) skin cooling (°C), (B) absolute mean votes for thermal sensation, (C) frequency of wetness perception and (D) absolute mean votes for pleasantness sensation, as a result of the 10 s application

of the relative cold-dry stimulus (15 °C lower than local T_{sk}) to each skin site, collapsed over all conditions. Data were collected on the left side of the body and the body maps presented were developed assuming left-right symmetry (see Ouzzahra et al., 2012). Regions showing greater skin cooling, colder sensations, more frequent wetness perceptions and more unpleasant sensations are represented in darker colors. The rating scales used by the participants to score their absolute thermal and pleasantness sensations are reported next to the respective body maps. Two main tendencies are shown. First, the regional differences in thermal, wetness and pleasantness sensation present a similar pattern across the torso (e.g. as opposed to the chest, the lateral and lower back appears more sensitive to cold, wetness and thermal displeasure). Second, these sensory patterns seem independent from the regional variations in skin cooling (*i.e.* regions which show greater skin cooling, such as the lateral chest, are not necessarily the ones in which the stimulus was perceived as colder, more often wet or more unpleasant).

7.5.2 **Thermal sensation**

Baseline thermal sensation scores (pre-stimulation) varied in a range of 0.1 ± 0.1 [median= 0; 0.0, 1.0] to 0.6 ± 0.2 [median= 1; 1.0, 1.0] for the thermo-neutral exposure and of 1.4 ± 0.3 [median= 1; 0.2, 2.7] to 1.7 ± 0.2 [median= 2; 1.0, 2.0] for the warm exposure. Expressed in terms of semantic labels, these were in the range of "neutral" for the thermo-neutral exposure and in a range going from "neutral" to "slightly warm" for the warm exposure.

In response to the stimuli, thermal sensation scores were overall "less cold" during the warm (-3.5 ± 0.1) [median= -4; -4.0, -3.0] than during the thermo-neutral exposure (-3.7 ± 0.1) [median= -4; -5.0, -3.0] (Z= -3.5, p=0.001, r= -0.25). Expressed in terms of semantic labels, these were in a range going from "slightly cool" to "cold" for the warm exposure and in a range going from "slightly cool" to "very cold" for the thermo-neutral exposure. Thermal sensations differed significantly according to the skin site stimulated [X^2 (11, N = 32) = 143.2, p<0.001], with scores varying in a range of -2.3 ± 0.2 [median= 2; -3.0, -1.2] (i.e. medial chest) to -4.4 ± 0.2 [median= 4; -5.0, -4.0] (i.e. lateral lower back) between sites. Expressed in terms of semantic labels, these were in a range going from "slightly cool" to "very cold". Mean thermal sensations, averaged over both environmental conditions, are shown in figure 2*B*.

The significance levels are presented separately for sites of the front and back torso (Tab.1).

Overall, these outcomes indicated that the same relative cold-dry stimulus evoked thermal sensations which were significantly "colder" when the stimulus was applied on specific regions (such as the lateral abdomen and the lateral and lower back) as opposed to other regions (such as the lateral and medial chest), in which the same stimulus evoked "less cold" thermal sensations. Also, the same relative cold-dry stimulus was overall perceived as slightly less cold during the warm than during the thermo-neutral exposure.

7.5.3 **Wetness perception**

Baseline wetness perception scores (pre-stimulation) varied in a range of 0.6 ± 0.3 [median= 0; 0.0, 2.0] to 1 ± 0.3 [median= 0; 0.0, 2.0] for the thermo-neutral exposure and 0.6 ± 0.4 [median= 0; 0.0, 1.7] to 0.8 ± 0.4 [median= 1; 1.0, 2.0] for the warm exposure. Expressed in terms of semantic labels, these were in a range going from "neutral" to "slightly dry".

In response to the stimuli, local wetness perception scores were overall slightly "wetter" during the warm (-1.7 ± 0.1) [median= -2; -2.0, -1.0] than during the thermo-neutral exposure (-1.4 ± 0.1) [median= -1; -2.0, -1.0] (Z= -2.9, p=0.004, r= -0.2). Expressed in terms of semantic labels, these were in a range going from "neutral" to "slightly wet" for both warm and thermo-neutral exposure. Wetness perceptions differed significantly according to the skin site stimulated [X^2 (11, N = 32) = 58.4, p<0.001], with scores varying in a range of -1.1 ± 0.1 [median= -1; -1.0, -1.0] (i.e. medial chest) to -2.1 ± 0.2 [median= -2; -3.0, -1.0] (i.e. medial lower back) between sites. Expressed in terms of semantic labels, these were in a range going from "neutral" to "slightly wet". The significance levels are presented separately for sites of the front and back torso (tab.1). To further investigate the regional distribution of wetness perception, a frequency distribution analysis of wetness scores was performed. The data analysis indicated a main effect of skin site stimulated on the frequency of "wet" scores (Pearson Chi-square p<0.001). Data for each of the 12 skin sites stimulated are shown in figure 2*C*. The results indicated that the relative cold-dry stimulus was significantly more often perceived as wet when applied to the lower back (lateral= 56 %; medial= 59 %) and the medial upper back (53 %). The same stimulus was significantly less often perceived as wet when

applied to the medial chest (22 %) and medial upper abdomen (28 %). Overall, the back presented a significantly greater frequency of wetness perception (53 %) than the front torso (39 %) (Pearson Chi-square $p = 0.047$).

Overall, these outcomes indicated that, the same relative cold-dry stimulus evoked wetness perceptions which were significantly "wetter", and more often perceived as wet, when the stimulus was applied on specific regions (such as the medial and lateral lower back) as opposed to other regions (such as the medial and lateral chest), in which the same stimulus evoked "less wet" and less frequent wetness perceptions.

7.5.4 **Pleasantness sensation**

Pleasantness sensations were recorded only during the stimulation as we were primarily interested in the affective and discriminative sensations aroused by the application of the thermal stimulus with regards to the whole body's thermal state.

Pleasantness sensation scores were overall "less unpleasant" during the warm (-1.8 ± 0.1) [median= -2; -3.0, -1.0] than during the thermo-neutral exposure (-2.2 ± 0.1) [median= -2; -3.0, -1.0] ($Z = -3.8$, $p<0.001$, $r = -0.3$). Expressed in terms of semantic labels, these were in a range going from "neutral" to "unpleasant" for both the thermo-neutral and warm exposure. Pleasantness sensation scores differed significantly according to the skin site stimulated [X^2 (11, N = 32) = 108.1, $p<0.001$], with scores varying in a range of -1.1 ± 0.2 [median= -1; -1.0, -0.2] (i.e. medial chest) to -2.7 ± 0.2 [median= -2; -4.0, -2.0] (i.e. lateral lower back). Expressed in terms of semantic labels, these were in a range going from "neutral" to "unpleasant". Mean pleasantness sensations averaged over conditions, as reported during the application of the relative cold-dry stimulus to each skin site, are shown in figure 2*D*.

Overall, these outcomes indicated that, the same relative cold-dry evoked sensations which were significantly "more unpleasant" when the stimulus was applied on specific regions (such as the lateral abdomen and lateral lower back) as opposed to other regions (such as the medial chest and medial upper abdomen), in which the same stimulus evoked "less unpleasant" sensations. Interestingly, the regional variation in displeasure showed a pattern similar to the regional distribution in thermosensitivity to cold. Finally, the same relative cold-dry stimulus was overall perceived as slightly less unpleasant during the warm than during the thermo-neutral exposure.

7.5.5 Correlation analysis between thermal sensation, frequency of perceived wetness and pleasantness sensation

The degree of association between the level of coldness experienced and the frequency of perceived wetness (assessed by a Spearman's rank correlation test) was found to be statistically significant ($p<0.01$; Spearman's rho= 0.79), indicating a significant correlation between increasing coldness and increasing frequency of perceived wetness (fig. 3*A*). Similarly, the degree of association between the level of pleasantness experienced and the frequency of perceived wetness was found to be statistically significant ($p<0.01$; Spearman's rho= 0.76), indicating a significant correlation between decreasing pleasantness and increasing frequency of perceived wetness (fig. 3*B*). Finally, the degree of association between the level of coldness and the level of pleasantness experienced was also found to be statistically significant ($p<0.01$; Spearman's rho= 0.97), indicating a significant correlation between increasing coldness and decreasing pleasantness (fig. 3*C*).

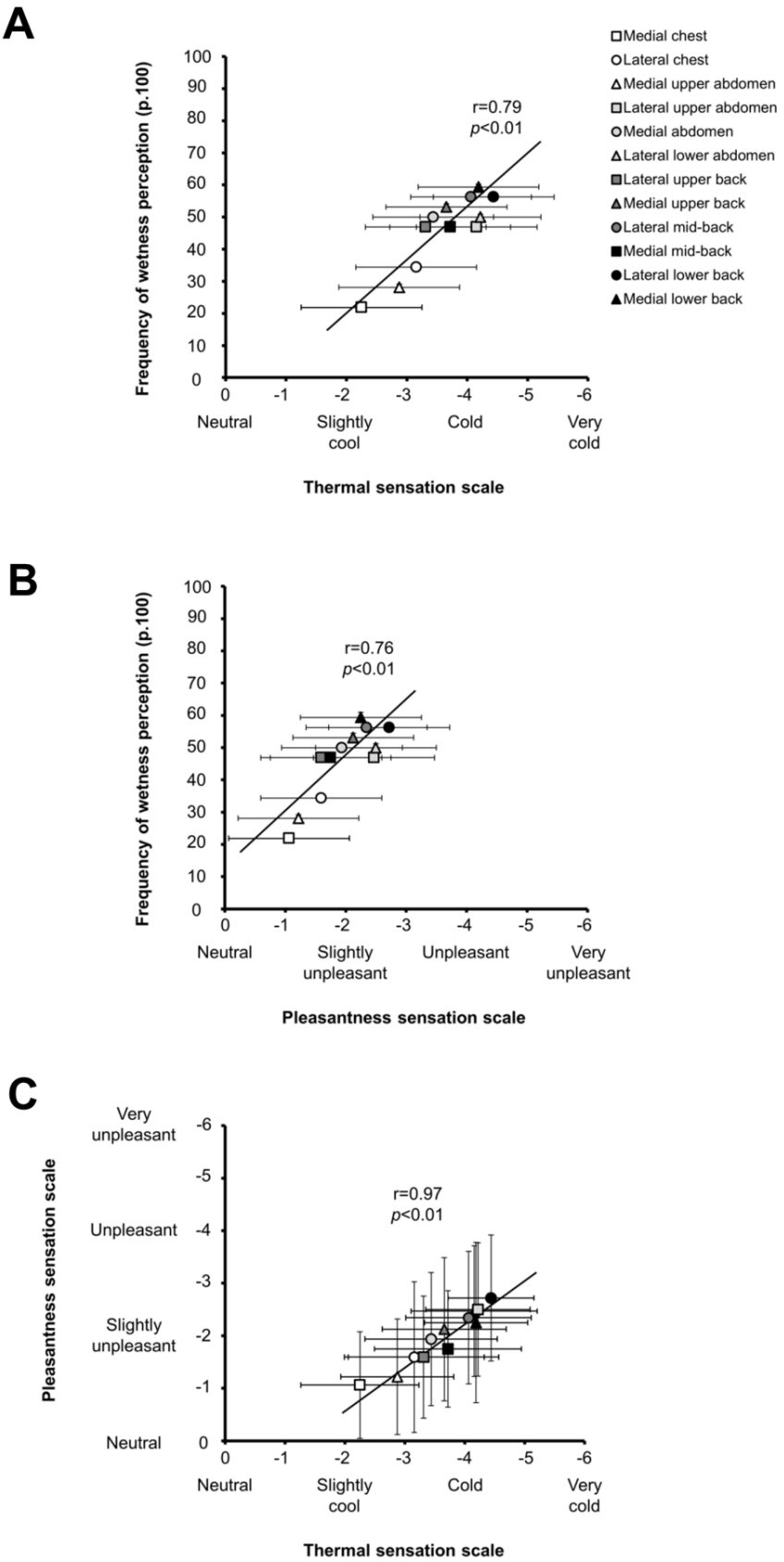


Figure 3: Relationship between: (A) thermal (cold) sensation and the frequency of perceived wetness; (B) pleasantness sensation and the frequency of perceived

wetness; (C) thermal (cold) sensation and pleasantness sensation. Data are reported as mean for each skin site, collapsed over all conditions, and standard deviation (horizontal and vertical lines). There is a highly significant correlation between the level of coldness experienced and the frequency of perceived wetness (*i.e.* increasing coldness and increasing wetness), the level of pleasure experienced and the frequency of perceived wetness (*i.e.* decreasing pleasantness and increasing wetness), and the level of coldness and pleasure experienced (*i.e.* increasing coldness and decreasing pleasantness).

			Front Torso						Back Torso					
			Medial chest (1)	Lateral chest (2)	Medial upper abdomen (3)	Lateral upper abdomen (4)	Medial abdomen (5)	Lateral lower abdomen (6)	Lateral upper back (7)	Medial upper back (8)	Lateral mid-back (9)	Medial mid-back (10)	Lateral lower back (11)	Medial lower back (12)
Front Torso	Medial chest (1)	ΔT_{sk}		†										*
		TS				‡		†		†	‡	‡	‡	‡
		WP								†			†	*
		PS				‡	*	‡		†	†			‡
	Lateral chest (2)	ΔT_{sk}	†		†		‡	†	*	*	*	*	*	†
		TS				†		*			*		†	‡
		WP												
		PS				*		*						†
	Medial upper abdomen (3)	ΔT_{sk}		†										*
		TS				‡		†			‡		‡	‡
		WP								†			†	*
		PS				‡		‡		*	†			‡
	Lateral upper abdomen (4)	ΔT_{sk}					*	‡			*			†
		TS	‡	†	‡				*					
		WP												
		PS	‡	*	‡				*					
	Medial abdomen (5)	ΔT_{sk}		‡		*								*
		TS												†
		WP												
		PS	*											
	Lateral lower abdomen (6)	ΔT_{sk}		†		‡								
		TS	†	*	†				†					
		WP												
		PS	‡	*	‡				*					
Back Torso	Lateral upper back (7)	ΔT_{sk}		*										†
		TS				*		†					*	†
		WP												
		PS				*		*						‡
	Medial upper back (8)	ΔT_{sk}		*		*								†
		TS	†											
		WP	†		*									
		PS	†		*									
	Lateral mid-back (9)	ΔT_{sk}		*										*
		TS	‡	*	‡									
		WP												
		PS	†		†									
	Medial mid-back (10)	ΔT_{sk}		*										
		TS	‡											
		WP												
		PS												†
	Lateral lower back (11)	ΔT_{sk}		*			*							†
		TS	‡	†	‡				*					
		WP	†		†									
		PS												
	Medial lower back (12)	ΔT_{sk}	*	†	*	†			†	*	†		†	
		TS	‡	‡	‡		†		†					
		WP	*		*									
		PS	‡	†	‡				‡			†		

Table 1: Significance levels of the multiple comparisons for the 12 skin sites are reported for the ΔT_{sk}, thermal (TS), wetness (WP) and pleasantness (PS) sensation. Table footnote: *p<0.05; †p<0.01; ‡p<0.001.

7.6 Discussion

The present study investigated the regional distribution of cutaneous wetness perception across the torso, in relation to the distribution of thermosensitivity to cold. Furthermore, we investigated whether these regional sensory patterns are influenced by different ambient temperatures as well as whether regional differences in thermal pleasantness in response to local skin cooling exist. During a thermo-neutral and warm environmental exposure, by exposing 12 skin sites of the torso to the static contact with the same relative cold-dry stimulus we demonstrated that: 1. cutaneous wetness perception varies significantly across the torso (see fig. 2*C*), with regions showing high thermosensitivity to cold (e.g. the lower and lateral abdomen and back, see fig. 2*B*) presenting wetness perception in larger magnitude and frequency (compare fig. 2*B* vs. 2*C*); 2. cutaneous wetness perception is slightly higher under warm than under thermo-neutral environmental conditions, despite thermosensitivity to cold appears to be slightly lower; 3. regional variations in thermal pleasure/displeasure exist across the torso, and show a pattern similar to the regional distribution in thermosensitivity to cold (*i.e.* greater coldness induced greater displeasure) (compare fig. 2*B* vs. 2*D*).

In summary, our results indicate that the existence of regional differences in cutaneous thermosensitivity to cold translates into significant regional differences in cutaneous wetness perception across the human torso. Interestingly, these regional sensory patterns were observed to be independent from the magnitude of local skin cooling. In other words, the regions in which the stimulus resulted in greater skin cooling (*i.e.* lateral chest) were not necessarily the ones in which the stimulus was perceived as colder, wetter and more unpleasant (compare fig. 2*A* with 2*B*, 2C and 2*D*). To our knowledge the present study is the first to take into account the regional variation in skin temperature occurring during contact cooling and to link this to the regional distribution of thermosensitivity to cold, skin wetness and thermal pleasure/displeasure across the human torso. The novelty of these findings is in providing the first detailed body maps of thermal, wetness and pleasantness sensation across the human torso.

7.6.1 **The role of thermosensitivity to cold in the ability to sense skin wetness**

With regards to the role of thermosensitivity to cold in characterizing the ability to sense cutaneous wetness, the outcomes of this study are in line with our previous findings, in which we have demonstrated that the contact with a cold-dry stimulus producing skin cooling rates in a range of 0.14 to 0.41 $°C \cdot s^{-1}$ can evoke an illusion of skin wetness (Filingeri et al., 2013; 2014c). In the present study, the relative temperature stimulus we used resulted in skin cooling rates ranging from 0.19 to 0.5 $°C \cdot s^{-1}$. Although generated by a dry stimulus, these fluctuations in T_{sk} evoked thermal sensations which were associated to the perception of skin wetness, particularly on the back torso. Hence, this finding supports the hypothesis that the central integration of coldness, as primarily sub-served by peripheral myelinated Aδ-nerve fibers, is critically involved in the neural processes underpinning humans' ability to sense wetness (Filingeri et al., 2013; 2014c). As the skin seems not to be provided with hygroreceptors (Clark and Edholm, 1985), it is indeed hypothesized that the somatosensory cortex could be involved in generating a neural representation of a *"typical wet stimulus"*. This could be based on the multimodal transformation (*i.e.* information from one sensory sub-modality can be transformed into a map or reference frame defined by another sub-modality) of the somatosensory inputs generated when the skin is physically wet (Haggard et al., 2013). As the sensory inputs associated to the physical experience of cutaneous wetness are often generated by heat transfer in the form of evaporative cooling (Ackerley et al., 2012), the typical neural representation of a wet stimulus might therefore rely on experiencing a certain degree of coldness. This neural representation could be transformed into a firing rate code and then associated to the perception of wetness (Pleger and Villringer, 2013). Hence, when the memorized stimulus (i.e. coldness), as coded by the specific afferents (*i.e.* Aδ-nerve fibers) is presented, wetness will be sensed.

The outcomes of this study, in which a cold-dry stimulus evoked an illusion of skin wetness in blindfolded individuals, are in agreement with this sensory model for wetness. However, although the relative temperature stimulus used in this study resulted in skin cooling rates which were within the range suggested to evoke wetness perceptions for all the regions investigated (*i.e.* 0.19 to 0.5 $°C \cdot s^{-1}$) (Daanen, 2009; Filingeri et al., 2013; 2014c), significant regional variations in wetness perception were observed across the torso. Hence, this indicates that other factors than the degree of local skin cooling (e.g. regional differences in thermal sensitivity

and habituation components) might play a significant role in characterizing the cutaneous distribution of wetness perception, at least across the human torso.

7.6.2 Physiological significance of regional differences in cutaneous skin wetness perception

Within the experimental conditions of this study, the lower back, lateral mid-back and medial upper back, as well as the lateral abdomen presented wetness perception in larger magnitude and frequency than the lateral and medial chest and medial upper abdomen (see fig. 2*C*). These outcomes are in line with the work of Lee et al. (2011) who have shown the upper and lower back to be most frequently perceived as wet during conditions of sweat-induced physical wetness. Although not statistically significant, a similar trend was observed by Ackerley et al. (2012) who reported the back to present higher wetness perception than other body regions. However, in the mentioned works, no data are reported on any physiological change (e.g. regional differences in ΔT_{sk}) which could have triggered the sensory inputs used by the participants to discriminate the level of wetness experienced regionally. In the present study, this issue was overcome by quantifing the local ΔT_{sk}, recording thermal sensations, and eventually comparing these with the regional distribution of wetness perception. Thus, for the first time we provide evidence in support of the physiological and behavioral significance of the regional differences in cutaneous wetness perception across the torso.

In the current study, the local thermal sensations in response to the cold stimulus were observed to be independent from the local ΔT_{sk}. A comparison of the body maps of ΔT_{sk} (fig. 2*A*) and thermal sensation (fig. 2*B*) shows that the cold-dry stimulus was perceived as colder when applied to the lower back than to the lateral chest, despite when stimulated, the lower back presented a significantly smaller drop in T_{sk} than the lateral chest. Interestingly, a similar trend was observed for the perception of wetness (see fig. 2*C*). Hence, it could be proposed that, as well as for the thermosensitivity to cold, the regional differences in wetness perception could depend upon an uneven weighting and integration of thermoafferent information, which seems independent from the regional variations in T_{sk} and, potentially, from the density of thermoreceptors (Burke and Mekjavic, 1991; Nakamura et al., 2008; Auliciems, 2013). As shown in figures 2*B* and 2*C*, the regions with high wetness

frequency presented a high sensitvity to cold, with the association between the level of experienced coldness and the frequency of perceived wetness being linear (*i.e.* greater coldness induces more frequent wetness) and statistically significant. Thus, it could be suggested that the sensitvity to coldness (*i.e.* a neurophysiological variable) rather then local ΔT_{sk} (*i.e.* a physical variable) might be more critical in characterizing the regional distribution of cutaneous wetness perception. From a neurophysiological point of view, this is in line with what has previously been proposed on the critical role of thermosensitivity to cold in sensing cutaneous wetness (Ackerley et al., 2012; Filingeri et al., 2014c). The higher sensitivity to cold of some regions of the torso could indeed result in these regions being more sensitive to perceive skin wetness. The possibility that colder sensations are more likely to translate in wetter perceptions, is also aligned to the work of Ackerley et al. (2012) (Ackerley et al., 2012). In their work, the authors have shown that individuals readily discriminated between very small amount of moisture on the skin (in the range of 40 µL over a surface of 0.0024 m^2). Altough in the mentioned study no recordings of local ΔT_{sk} and thermal sensations were performed, in line with the authors, we believe that participants distinguished the greater from the smaller levels of moisture due to the resulting greater evaporative cooling which induced colder thermal sensations.

The fact that humans seem to associate “feeling colder” with “feeling wetter” is not entirely surprising, and could be due to learning factors. For example, the contact with a wet surface or the exposure to a cold-humid environment often result in colder sensations than the ones resulting from the contact with a dry surface or the exposure to a cold-dry environment. In this regard, the skin’s contact with a wet fabric has been suggested to be perceived as wet, as the presence of moisture leads to higher heat losses from the skin (and thus colder sensations), due to a higher thermal conductivity of a wet as opposed to a dry fabric (Niedermann and Rossi, 2012). As for the same physical process (i.e. higher rate of heat losses), a cold-humid environment is perceived to be colder than a cold-dry one (Plante et al., 1995).

Habituation factors could also explain the observed regional pattern in wetness perception. As we are not provided with hygroreceptors (Clark and Edholm, 1985), if we assume that, based on the concept of perceptual learning (Pleger and Villringer, 2013), we learn to perceive cutaneous wetness, it would be reasonable to hypothesize that the body regions more sensitive to skin wetness are the ones in which we are

more used to experience high levels of physical wetness, e.g. due to sweating. The outcomes of this study could support this behavioral hypothesis. In the present study the back torso, and particularly the lower back, a region which has been repeatedly shown to present some of the highest levels of sweat production (Smith and Havenith, 2011, 2012), was indeed observed to be the most sensitive region to wetness across torso.

7.6.3 Role of the thermal state of the body and the affective component of thermal stimulation

The cutaneous wetness perception was observed to be slightly higher under warm than under thermo-neutral environmental conditions. As the thermosensitivity to cold was on the contrary found to be slightly lower during the warm environmental condition, the increase in overall wetness perception in the warm environment is more likely to be related to an expectation effect (*i.e.* participants might have expected to sweat under the warm exposure) than to a central sensory modulation of this perception. It could be argued that a higher level of whole-body wetness, which might have influenced the way the cold-dry stimulus was perceived locally on the skin (Fukazawa and Havenith, 2009), occurred during the warm exposure. However, as the baseline wetness perceptions recorded pre-stimulation did not differ between the thermo-neutral and the warm environmental exposures, and due to the resting condition of the participants, it is unlikely that a higher level of whole-body wetness occurred or was perceived by the participants. Nevertheless, the possibility to measure the skin's local hydration status should be considered in future studies, in order to investigate whether a swelling state of the skin (due to sweat production) can affect the regional perception of skin wetness (Gerrett et al., 2013).

With regards to the affective component of thermal stimulation, it deserves mention that the local cold-dry stimulation of the torso was overall perceived as being unpleasant and that the level of displeasure experienced varied significantly by location of stimulation. Interestingly, the topographical distribution of the displeasure resulting from local thermal stimulation corresponded to the regional distribution of cutaneous thermal and wetness perception (compare fig. 2*D* with 2*B* and 2*C*). In this respect, it was observed that regions with a higher thermosensitivity to cold and a higher frequency of wetness (e.g. the lower back, lateral mid-back and medial upper back, as well as the lateral abdomen) were the ones in which the application of the

stimulus resulted as the most unpleasant (see fig. 3*B* and 3*C*). These outcomes confirm the physiological bases of pleasure (Cabanac, 1971, 1992), particularly in the context of thermal sensation and comfort (Cabanac et al., 1972).

It has been previously suggested that the hedonic attribute of a thermal stimulus is dependent on the perception of the actual thermal state of the body: if the direction of the thermal stimulus is oriented towards a shift in the thermal state of the body from its natural homeostasis, then this will result in thermally unpleasant sensations; on the contrary, if the direction of thermal stimulus is towards a re-establishment of the thermal state to its set point, then this will result in thermally pleasant sensations (Attia and Engel, 1982). This concept, known as alliesthesia (Cabanac, 1971), underpins the reason why a cold stimulus applied on normothermic individuals might be perceived as more unpleasant than if the same was applied on hyperthermic individuals. As during our experimental conditions participants were not expected to become hyperthermic (due to resting conditions and short exposure duration), it is therefore clear why the application of the cold stimulus was overall perceived as unpleasant. However, the novelty of this study is to provide a detailed topographical distribution of the regions of the torso in which the exposure to cold stimuli might have a greater influence on the overall thermal displeasure and discomfort. The fact that the back as well as the lateral abdomen presented a higher sensitivity to thermal displeasure further our understanding of the role of the torso's thermal comfort in the whole-body thermal comfort. Nakamura et al. (2008, 2013) have repeatedly shown that humans prefer a warm trunk and that abdominal cooling is often perceived as more unpleasant than other regions' cooling. This is in line with the findings of the present study, in which e.g. we observed the lateral abdomen to be amongst the regions in which the application of the cold-dry stimulus was perceived as the most uncomfortable. As local cooling of the abdomen has been shown to induce vasoconstriction of the corresponding gastrointestinal tract, which in turn could affect the organ's function (Kuntz and Haselwood, 1940), it is therefore reasonable to hypothesize that the higher sensitivity to thermal displeasure of this region might represent a form of thermal protection aiming to maintain homeostasis (Nakamura et al., 2013).

It has to be acknowledged that, with regards to linking the changes in the internal state of the body with the affective component of local thermal stimulation of the torso, the absence of a direct measurement of core temperature represents a limitation

of the current study. It could be indeed speculated that, despite an increase in core temperature is unlikely to have occurred within the experimental conditions of this study, a potential (although slight) fall in this value could have occurred during the thermo-neutral exposure (due to the resting and semi-nude conditions of the participants). Therefore, the contribution of even small changes in core temperature to the overall hedonic component of thermal stimulation cannot be ruled out conclusively. Nevertheless, the outcomes of this study further our understanding of the role of cutaneous thermal afferents (as opposed to deep body) in influencing the hedonic attribute of tactile stimulations. Recent evidence on the neurophysiology of affective touch have indeed indicated that, apart from the role of core temperature, the presence of a particular class of cutaneous nerve fibers *i.e.* C-tactile afferents, which are specifically tuned to affective as opposed to discriminative touch, could also play a significant role in influencing the affective component of local thermal stimulation (Ackerley et al., 2014a). In a recent study in which stroking-like stimuli at 3 different temperatures [i.e. warm, neutral (same as skin temperature) and cold)] were applied on participants' skin, Ackerley et al. (2014a) have shown that stimuli with temperatures which deviated from neutrality (i.e. warm and cold) were perceived as less pleasant than thermo-neutral stimuli. The authors concluded that the activity and role of C-Tactile fibers in contributing to the hedonic component of tactile stimuli seems therefore to be specifically tuned to the neutral temperature of a skin-stroking caress (Ackerley et al., 2014a). These observations seem supporting the results of the present study, in which we have demonstrated that the further the stimuli deviated from thermo-neutrality (i.e. colder sensations), the greater the displeasure experienced by the participants (see fig. 3*C*). Therefore, our findings indicate that, despite the importance of monitoring core temperature, taking into account the potential contributions of cutaneous C-Tactile afferents should also be considered in future investigations as these could play a role in the hedonic component of local thermal stimulation.

7.7 Conclusion

In conclusion, the present study found that cutaneous wetness perception varies significantly across the human torso. We found that the existence of regional

differences in cutaneous thermosensitivity to cold translates into significant regional differences in cutaneous wetness perception: regions with a high thermosensitivity to cold (e.g. the lower and lateral abdomen and back) present skin wetness perceptions in greater magnitude and frequency. Also, it was found that the regional distribution of cutaneous thermal and wetness perception was matched by regional differences in the level of displeasure resulting from local thermal stimulation: regions with a higher thermal and wetness perception (e.g. the lower and lateral abdomen and back) present higher sensitivity to thermal displeasure. The outcomes of this study have a fundamental, clinical as well as an applied significance. From a fundamental point of view, these indicate that cutaneous thermal, wetness and pleasantness sensations do not depend solely on regional variations in T_{sk} but also on an uneven weighting and integration of peripheral thermoafferent information which could be influenced by behavioral and habituation factors. From a clinical point of view, due to a recent interest in mapping bodily sensations such as pain (Mancini et al., 2014), the body maps of torso thermal, wetness and pleasantness sensation developed in this study could be used as a frame of reference for normal and altered somatosensory function in the context of multiple sclerosis or polyneuropathies, diseases which are usually accompanied by alteration of normal somatosensory function (Rae-Grant et al., 1999; Susser et al., 1999; Nolano et al., 2008; Hulse et al., 2010). Finally, from an applied point of view, these body maps could be useful in improving the design of protective clothing in order to optimize thermal protection and maximize thermal comfort under extreme environmental conditions (e.g. cold air/water exposures).

8 CHAPTER EIGHT - Laboratory study 5: Warm temperature stimulus suppresses the perception of skin wetness during initial contact with a wet surface

Publication(s) based on this chapter:

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8.1 Abstract

In the absence of humidity receptors in human skin, the perception of skin wetness is considered a somatosensory experience resulting from the integration of temperature (particularly cold) and mechanical inputs. However, limited data are available on the role of the temperature sense. Wet and dry stimuli at 4 and 8 °C above local skin temperature were applied on the back of 7 participants (age 21 ± 2 years) while skin temperature and conductance, thermal and wetness perceptions were recorded. Resting local skin temperature always increased by the application of the stimuli (+0.5 to +1.4 °C). No effect of stimulus wetness was found on wetness perceptions ($p>0.05$). The threshold (point "-2 slightly wet" on the wetness scale) to identify a clearly perceived wetness was never reached during any stimulations and participants did not perceive that some of the stimuli were wet. Overall, warm temperature stimuli suppressed the perception of skin wetness. We conclude that it is not the contact of the skin with moisture per se, but rather the integration of particular sensory inputs (amongst which coldness seems dominant) which drives the perception of skin wetness during the initial contact with a wet surface.

8.2 Introduction

The perception of skin wetness is a complex somato-sensory experience which seems to result from the integration of temperature and mechanical (i.e. pressure) inputs (Bentley, 1900; Ackerley et al., 2012; Bergmann Tiest et al., 2012a). To date, a hygro-receptor has never been identified on the human skin (Clark and Edholm, 1985). Therefore, it has been suggested that human beings learn to perceive the wetness experienced when their skin is in contact with a wet surface, when a liquid is touched, or when sweat is produced (Bergmann Tiest et al., 2012a).This learning process seems to be based on a complex multisensory integration (Driver and Spence, 2000; Gescheider and Wright, 2012). The thermal and mechanical inputs which result from the physical processes occurring when the skin is in contact with moisture (i.e. heat transfer and mechanical interactions between the skin and the environment) could be integrated and combined at different anatomical levels through specific multisensory pathways (Cappe et al., 2009). However, although the interaction between thermal and mechanical inputs seems to be the principal inducer of the perception of skin wetness (Bentley, 1900; Ackerley et al., 2012; Bergmann Tiest et al., 2012a), to date it is unclear which sensory modality is predominant in driving this perception.

The thermal sense might play a significant role in this perception. We have recently shown that exposing the skin to cold-dry stimuli (resulting in cooling rates similar to the ones occurring during the evaporation of water from the skin) can evoke an illusion of local skin wetness (Filingeri et al., 2013; 2014a; 2014c) (see Chapter Four, Five and Seven). This indicated that in particular situations, individuals seem to associate local coldness with local skin wetness.

These recent findings have opened an interesting question: if skin wetness might be primarily driven by coldness, would individuals be able to perceive local skin wetness if exposed to a local warm-wet stimulus during which no coldness is experienced? It might be hypothesised that in that case, the ability to perceive local skin wetness would depend upon the mechanical cues available. Every day experience indicates that we are able to perceive the wetness of a warm liquid. Inserting the hand into a bucket of warm water generates a particular sensation of pressure around the wrist (i.e. “ring”) which individuals associate to the perception

of liquidity (Bentley, 1900). In this case, as cooling cues are not available, individuals rely more on mechanical cues to aid the perception of wetness (Bergmann Tiest et al., 2012a). However, in particular situations of local warm-wetness, mechanical cues might also be limited. Wearing feminine sanitary products (as well as incontinence products such as diapers) represents one of the real-life situations in which individuals can be exposed to a warm-wet surface and mechanical as well as cooling cues can be limited (Farage et al., 2004a, 2004b). Therefore, in the light of this common real-life situation, the fundamental question we posed would be of practical relevance.

Although the literature on the subjective perception of moisture in clothing is rather extensive within the textile engineering field (Sweeney and Branson, 1990a, 1990b; Li, 2005), the individual role of thermal and mechanical components in characterising this perception has been rarely investigated (Filingeri et al., 2013; 2014a; 2014c). Thus, there is a need to further the understanding of the psychophysical bases of this complex sensory experience. The aim of this study was to investigate the psychophysical bases of the perception of local skin wetness when the skin of blindfolded individuals was in initial contact with a wet surface with a temperature warmer than the skin. Our expectation is that, if cooling is the main driver for a static wetness perception, when a wet stimulus is applied to the skin with a temperature above the skin temperature, the resulting initial wetness perception will be lower than we observed in earlier experiments of skin cooling, despite the latter being dry stimuli (Filingeri et al., 2013; 2014a; 2014c) (see Chapter Four, Five and Seven).

8.3 Material and methods

8.3.1 Participants

Seven (5 females/2 males) healthy university students (age 21± 2 years) with no history of sensory-related diseases volunteered to participate in this study. All participants gave their informed consent for participation. The study design had been approved by the Loughborough University Ethics Committee and testing procedures were in accordance with the tenets of the Declaration of Helsinki.

8.3.2 **Experimental design**

The experimental design was based on the application in a balanced order of four different warm stimuli, varying in terms of temperature (i.e. +4 and +8 °C above local skin temperature) and wetness level (i.e. dry or wet). All stimuli were applied on both the bare right upper and lower back of each participant, while participants were resting on a chair in an environmental chamber (set at 22 °C and 50 % relative humidity). The stimuli were delivered by a thermal probe (Physitemp Instruments Inc., USA) with a contact surface of 25 cm^2. The stimulation consisted of a short contact (lasting no longer than 10s) with the probe' surface set at +4 °C or +8 °C above the individual's local skin temperature [determined using an infrared thermometer (Fluke Corporation, USA)]. To make the contact with the probe surface dry or wet, test fabrics (100 % cotton) with a surface of 100 cm^2 were placed either dry or wet on the probe' surface before the stimulation and fixed by an elastic band. Prior to testing, wet test specimens were soaked for few seconds in 22 °C water to ensure full saturation and then stored in sealed containers to avoid evaporation. Dry and soaked wet test specimens weight 1g and 3g respectively. Wet test specimens' water content was of 0.02 $g \cdot cm^{-2}$, which was considered acceptable for the purposes of this study as individuals have been previously shown to perceive wetness when in contact with wet surfaces containing an amount of water as little as of 0.0008 $g \cdot cm^{-2}$ (Ackerley et al., 2012)

To control that local skin hydration levels would not change significantly during testing procedures (i.e. participants were not sweating due to stress or environmental conditions), the sympathetic skin response was monitored from the beginning and throughout the whole test via galvanic skin conductance (Biopac Systems Inc., USA).

8.3.3 **Experimental Protocol**

Participants arrived to the laboratory 30 min before the time scheduled for the test to allow preparation procedures. Male participants wore shorts, socks and trainers whereas female participants wore sport bra, shorts, socks and trainers. Participants were informed only about the body region objected to the stimulation. No information was provided on the type and magnitude of the stimulation to limit any expectation effects. The exact anatomical locations of the areas targeted for stimulation were: 5cm upwards the inferior angle of the right scapula (upper back skin site); 5cm upwards the right posterior superior iliac spine (lower back skin site).

The back was chosen as targeted area for stimulation as it has been previously shown to be significantly sensitive to wetness perception (Lee et al., 2011; Filingeri et al., 2014c).

After preparation, participants entered the environmental chamber and 10 min were allowed for acclimation. During this period, participants were familiarised with the rating scales used to record thermal sensations and wetness perceptions: a modified 11 point thermal sensation scale (-6 very cold; -4 cold; -2 slightly cool; 0 neutral; +2 slightly warm; +4 warm) and a modified 11 point wetness perception scale (-6 dripping wet; -4 wet; -2 slightly wet; 0 neutral; +2 slightly dry; +4 dry) (Olesen and Brager, 2004). No descriptors were applied to intermediate scores (-5; -3; -1; +1; +3). We defined the value "-2" (Slightly wet) of the wetness scale as our set threshold to identify a clearly perceived local wetness.

During the test, participants were first asked to rate their thermal sensation and wetness perception before stimulation (i.e. baseline sensation). Then, the required fabric was applied on the thermal probe, which was set to the required relative temperature and then applied (and not moved) to the relevant skin site. As soon as the probe was applied, participants were instructed to report their local and very first sensation and perception, using whatever number in the scales seemed appropriate. The probe was then removed, the skin was gently wiped and its temperature immediately recorded. This sequence was repeated for each stimulus allowing at least one minute in between. Each participant had only one presentation of each stimulus for each body region.

8.4 Statistical Analysis

Data were tested for normality of distribution using Shapiro-Wilk test. Skin temperature data were analysed by a 3 way repeated measures analysis of variance (ANOVA),with temperature of the stimulus (+4 vs. +8 °C), type of stimulus (dry vs. wet), and body region (upper vs. lower back), as within subjects factors. Tukey's post-hoc analyses were performed accounting for multiple comparisons and sample size effect. Huynh–Feldt, Geisser–Greenhouse, and lower bound corrections were undertaken to adjust the degrees of freedom for the averaged tests of significance. Thermal and wetness ratings were analysed using a Friedman ANOVA test and post-

hoc analyses were performed using a Wilcoxon signed rank tests. All data were analysed using SPSS (IBM, Armonk, NY) and reported as means ± standard deviation. In all analyses, $p<0.05$ was used to establish significant differences.

8.5 Results

8.5.1 Skin temperature

Pre stimulation skin temperature was found to be on average 32.1 ± 1 °C for the upper back, and 30.7 ± 1 °C for the lower back. No effect of body region was observed on local skin temperature as a result of the stimulation (p=0.5). The +8 °C stimuli resulted in a greater increase in local skin temperature (+1.4 ± 0.8°C) than the +4 °C ones (+0.5 ± 0.4 °C) ($F=16.5_{(1, 6)}$, $p<0.01$). Dry and wet stimuli resulted in similar relative increases in local skin temperature (p=0.83). Overall, skin temperature always increased on application of the stimuli.

8.5.2 Thermal sensation

Pre stimulation thermal sensations ranged from neutral to slightly warm and were found to be not statistically different (p=0.8) between conditions. No effect of body region was found on the thermal sensations recorded during the stimulation (p=0.9). A significant effect of temperature was found, with warmer stimuli resulting in significantly warmer thermal sensations (Z= -2.04, $p<0.05$, r= -0.38). These varied in a range of +2 ± 1 (+4 °C stimuli) to +2.4 ± 1.5 (+8 °C stimuli), which corresponded to thermal sensations between slightly warm and warm. A significant effect of type of stimulus (dry vs. wet) was found, with wet stimuli resulting in significantly warmer thermal sensations (Z= -3.4, $p<0.01$, r= -0.64). These varied in a range of +1.7 ± 1 (dry stimuli) to +2.7 ± 1.3 (wet stimuli), which corresponded to thermal sensations between neutral and warm. A significant interaction between temperature and type of the stimuli was found (X^2= 19.64(3, 14), $p<0.01$).

8.5.3 Wetness perception

Pre stimulation wetness perceptions ranged from neutral to slightly dry and were found to be not statistically different (p=0.2) (fig. 1). No effect of body region (p=0.9), nor temperature (p=0.8) and type of the stimulus (p=0.1) was found on the

wetness perceptions recorded during the stimulation. These ranged from neutral to slightly dry. The threshold we set (point "-2 slightly wet" of the wetness perception scale) to identify a clearly perceived wetness was never reached during any of the four stimulations (fig. 1). To further elucidate the way warm-dry and warm-wet stimuli were perceived by the participants, with regards to their baseline wetness perception, the average change in the score from pre- to post-stimulation was calculated for each stimulus and then analysed. No effect of body region (p=0.8), nor temperature (p=1) was found on the average change in vote from pre to post stimulation, though type of the stimulus showed a trend of a bigger change in the wet stimulus (p=0.08). Changes in vote varied in a range of -0.6 ± 2.4 to +1 ± 1.2 votes (fig. 1).To further elucidate the way warm-dry and warm-wet stimuli were perceived by the participants, with regards to their baseline wetness perception, the average change in the score from pre- to post-stimulation was calculated for each stimulus and then analysed. No effect of body region (p=0.8), nor temperature (p=1) and type of the stimulus (p=0.08) was found on the average change in vote from pre to post stimulation. Changes in vote varied in a range of -0.6 ± 2.4 to +1 ± 1.2 votes (fig. 1).

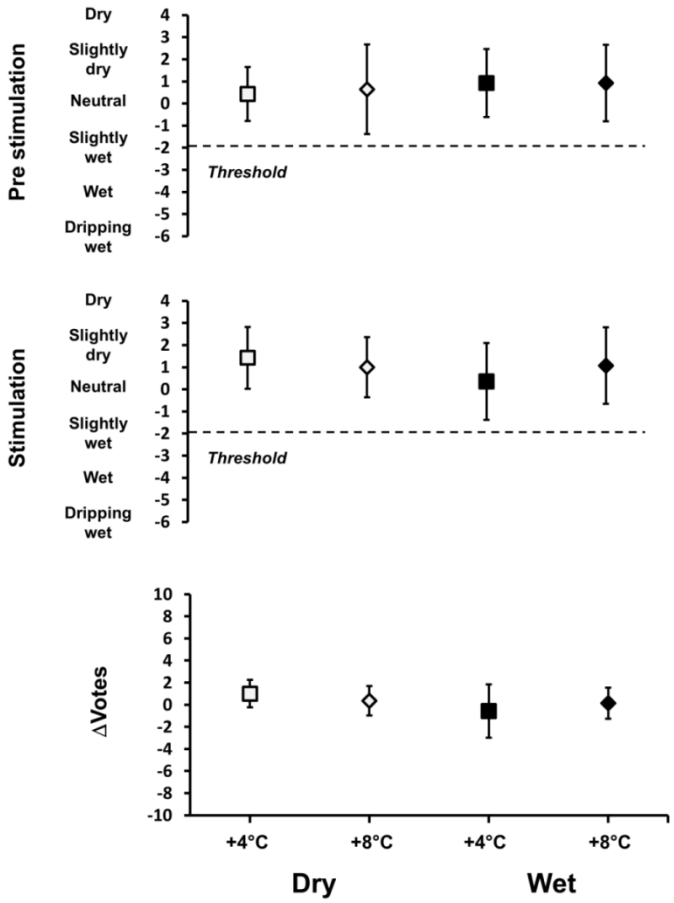

Figure 1: Wetness perception scores recorded before (Pre stimulation) and during (Stimulation) the application of the warm-dry and warm-wet stimuli. Average changes in vote (ΔVotes) from pre to post stimulation are also reported. Data were collapsed over the skin site where the stimulus was applied as no effect of body region (upper vs. lower back) was observed (p>0.05).

8.5.4 **Skin conductance**

Average skin conductance values did not significantly change during testing procedures and were observed to remain constantly at a level below 0.5 µS. These results confirm that no significant variations in the sudomotor activity occurred during the experiment.

8.6 <u>Discussion</u>

The aim of this study was to investigate the psychophysical bases of the perception of local skin wetness. Specifically, it was verified whether individuals would

perceive local wet stimuli as wet when these have a temperature warmer than the skin. The outcomes of this study indicated that participants did not perceive that some of the stimuli were wet and did not discriminate between warm-dry and warm-wet stimuli. This represents a novel and interesting finding, as to our knowledge no experimental data are currently available on the subjective thermal and wetness perceptions experienced during the initial contact of the skin with a warm-wet surface.

The possibility that warm sensations might suppress the perception of local wetness seems in line with the findings of our previous study, in which we have demonstrated the importance of experiencing coldness in order to perceive local skin wetness (Filingeri et al., 2013; 2014a; 2014c) (see Chapter Four, Five and Seven). We have recently shown that an illusion of local skin wetness can be evoked during the contact with a cold-dry surface inducing a skin cooling rate in a range of 0.14 to 0.41 $°C \cdot s^{-1}$ (Filingeri et al., 2013; 2014a; 2014c) (see Chapter Four, Five and Seven). This observation indicated that is not the contact of the skin with moisture per se, but rather the integration of specific sensory inputs which seems driving the perception of wetness during the contact with a wet surface (Bentley, 1900).

Amongst these sensory inputs, experiencing coldness seemed determinant in evoking the perception of local wetness. Although during the experimental test, participants' skin came in contact with a quantity of moisture (i.e. 0.02 $g \cdot cm^{-2}$) far greater than the threshold previously proposed for this perception (i.e. 0.0008 $g \cdot cm^{-2}$
) (Ackerley et al., 2012), as no skin cooling and thus cold sensations occurred, no perception of local wetness was reported at any time, and warm-wet stimuli were perceived as dry as warm-dry ones. The contact with a moist fabric has been suggested to be perceived as wet as the presence of moisture leads to higher heat losses from the skin (and thus colder sensations), due to the higher thermal conductivity of the wet fabric (Niedermann and Rossi, 2012). This phenomenon did not occur in the present study as the wet fabric was purposely in contact with a surface warmer than the skin, so that a heat gain, rather than a heat loss, would occur. This design resulted in our participants being unable to clearly perceive local wetness during the initial contact with a warm-wet surface. From a fundamental point of view, this furthers our understanding of the complex sensory integration underpinning the perception of skin wetness. The sensory integration of specific cooling cues seems to

critically determine the ability to perceive local skin wetness (Ackerley et al., 2012; Filingeri et al., 2013). This appears to be particularly true when intra- and inter-sensory interactions with other sensory modalities (e.g. mechanical sense and vision) are limited. However, one should note that the conclusions we propose cannot be generalised to any type of perception of wetness, and should be only limited to the ones resulting from the initial contact with a surface/object. Mechanical inputs could have a role as critical as thermal inputs in characterising this perception, particularly when cooling cues are not available (Bergmann Tiest et al., 2012a). If thermal cues are limited, individuals seem to rely more on mechanical sensations, such as "stickiness" (Guest et al., 2002), to characterise their perception of wetness when e.g. wearing wet clothes (Sukigara and Niwa, 1997) or manipulating wet surfaces (Essick et al., 2010).

The findings of the present study have an applied significance, as they could contribute to the design and optimization of sanitary products (e.g. diapers) for personal and patients care. As the occurrence of wetness could be a common event when wearing these products, the fact that warm-wetness might be sometimes difficult to perceive highlights the need to develop systems for alerting of the occurrence of wetness (Daanen, 2009). This could increase the awareness of local skin wetness, thus improving personal care (Akin and Lemmen, 1997; Farage et al., 2004a), particularly within clinical contexts. Skin wetness has been indeed shown to be a risk factor for pressure ulcers (Mayrovitz and Sims, 2001).

8.7 Conclusion

Warm temperature stimuli have been shown to suppress the perception of skin wetness during initial contact with a wet surface. Hence, we conclude that it is not the contact of the skin with moisture per se, but rather the integration of particular sensory inputs which drives the perception of skin wetness during the initial contact with a wet surface. When the contribution of other sensory inputs (i.e. dynamic pressure and vision) is limited, experiencing coldness could be the primary driver of the perception of wetness.

9 CHAPTER NINE - Laboratory study 6: Why wet feels wet? A neurophysiological model of human cutaneous wetness sensitivity

Publication(s) based on this chapter:

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9.1 Abstract

Although the ability to sense skin wetness and humidity is critical for behavioural and autonomic adaptations, humans are not provided with specific skin receptors for sensing wetness. It has been proposed that we "learn" to perceive the wetness experienced when the skin is in contact with a wet surface or when sweat is produced through a multisensory integration of thermal and tactile inputs generated by the interaction between skin and moisture. However, the individual role of thermal and tactile cues and how these are integrated peripherally and centrally by our nervous system is still poorly understood. Here we tested the hypothesis that the central integration of coldness and mechanosensation, as subserved by peripheral A-nerve afferents, might be the primary neural process underpinning human wetness sensitivity. During a quantitative sensory test, we found that individuals perceived warm-wet and neutral-wet stimuli as significantly less wet than cold-wet ones, although these were characterized by the same moisture content. Also, when cutaneous cold and tactile sensitivity was diminished by a selective reduction in the activity of A-nerve afferents, wetness perception was significantly reduced. Based on a concept of perceptual learning and Bayesian perceptual inference, we developed the first neurophysiological model of cutaneous wetness sensitivity centred on the multisensory integration of cold and mechano sensitive skin afferents. Our results provide evidence for the existence of a specific information processing model which underpins the neural representation of a typical wet stimulus. These findings contribute to explain how humans sense warm, neutral and cold skin wetness.

9.2 Introduction

The ability to sense humidity and wetness is an important attribute in the animal kingdom. For many insects, discriminating between dryness and wetness is vital for procreation and survival (Liu et al., 2007). Sensing wetness is also critical for humans, both for behavioural and autonomic adaptations. Perceiving changes in ambient humidity and skin wetness has been shown to impact thermal comfort (Fukazawa and Havenith, 2009) and thus the thermoregulatory behaviour (Schlader et al., 2010), both in healthy and clinical populations (e.g. individuals suffering from rheumatic pain) (Strusberg et al., 2002). From an autonomic perspective, decreases in ocular wetness seem to initiate the lacrimation reflex in order to maintain a tear film to protect the ocular surface (Hirata and Oshinsky, 2012). Also, tactile roughness and wetness discrimination is critical for precision grip (Augurelle et al., 2003) and object manipulation (André et al., 2010). However, although the ability to sense wetness plays an important role in many physiological and behavioural functions, the neurophysiological mechanisms underlying this complex sensory experience are still poorly understood (Montell, 2008).

In contrast with insects, in which humidity receptors sub-serving *hygrosensation* have been identified and widely described (Tichy and Kallina, 2010), humans' largest sensory organ i.e. the skin seems not to be provided with specific receptors for the sensation of wetness (Clark and Edholm, 1985). Thus, as human beings, we seem to *"learn"* to perceive the wetness experienced when the skin is in contact with a wet surface or when sweat is produced (Bergmann Tiest et al., 2012a) through a complex multisensory integration (Driver and Spence, 2000) of thermal (i.e. heat transfer) and tactile (i.e. mechanical pressure and friction) inputs generated by the interaction between skin, moisture and (if donned) clothing (Fukazawa and Havenith, 2009). The hypothesis of wetness as a *"perceptual illusion"* shaped by sensory experience has been supported by our previous findings. We have recently shown that exposing the skin to cold-dry stimuli (resulting in cooling rates similar to the ones occurring during the evaporation of water from the skin) can evoke an illusion of local skin wetness (Filingeri et al., 2013; 2014a; 2014c) (see Chapter Four, Five and Seven). This could be due to the fact that we seem to interpret the coldness experienced during the evaporation of moisture from the skin as a signal of the

presence of moisture (and thus wetness) on the skin surface. In line with this hypothesis, we have also observed that during the static contact with a warm-wet surface (with a temperature warmer than the skin) no local skin wetness was perceived, as no skin cooling, and thus no cold sensations occurred (Filingeri et al., 2014d) (see Chapter Eight).

These preliminary findings appeared to be in line with the Bayesian concept of perceptual inference (Knill and Richards, 1996). According to this framework, sensory systems (such as the somatosensory one) incorporate implicit knowledge of the environment and use this knowledge (i.e. sensory experiences) to infer about the properties of specific stimuli (Geisler and Kersten, 2002). As the sensory feedback received from the surrounding environment is by nature multimodal (i.e. involving different sensory cues), as well as noisy and ambiguous, perceptual systems are thought to perform on-line tasks aiming to predict the underlying causes for a sensory observation in a fashion which is considered as near optimal (Lochmann and Deneve, 2011). In this context, humans have been shown to integrate the different sensory cues associated with an external stimulus and to infer the most probable multimodal estimate (i.e. perception) by taking into account the reliability of each sensory modality involved in the perceptual process (Ernst and Banks, 2002; Weiss et al., 2002).

The potential ability of our neural systems to solve the inherent uncertainty associated with sensory interpretation in a probabilistic and predictive manner (Lochmann and Deneve, 2011), explains why many apparently idiosyncratic perceptual illusions (see e.g. the effects of luminance contrast on the perception of motion velocity) (Weiss et al., 2002) are instead what one would expect from a rational perceptual system (Geisler and Kersten, 2002). Thus, sensory illusions, such as the perception of wetness, can be used as a powerful method to gain conceptual and functional understanding of the sensory processing operated by specific sensory systems such as the somatosensory one (Lochmann et al., 2012).

In this respect, our previous work has shown that the cold sensations resulting from the afferent activity of the cutaneous cold-sensitive, myelinated Aδ-nerve fibers (with conduction velocities ranging from 5-30 $m \cdot s^{-1}$) (Campero et al., 2001), play a critical role in the ability to perceive skin wetness (Filingeri et al., 2013; 2014a; 2014c) (see Chapter Four, Five and Seven). Furthermore, we have recently demonstrated that tactile inputs, which are likely to be encoded by cutaneous

mechanosensory Aß-nerve fibers (with conduction velocities ranging from 16-100 $m \cdot s^{-1}$) (Tsunozaki and Bautista, 2009), could have a role in modulating the perception of skin wetness (Filingeri et al., 2014c) (see Chapter Five). Thus, these observations have led us to hypothesize that the central integration of coldness and mechanosensation, as subserved by peripheral myelinated A-nerve fibers, might be the primary neural process underpinning humans' ability to sense wetness. However, what remains unclear is the individual role of thermal and tactile cues and how these are integrated peripherally as well as centrally. If the multimodal integration of coldness and mechanosensation was the main neural process for sensing wetness, it would be reasonable to hypothesize that during the contact with a wet surface, the absence of any coldness and mechanosensation, either if naturally (i.e. contact with a warm-wet or neutral-wet surface) or artificially induced (i.e. during a selective reduction in the activity of A-nerve fibers), would result in a reduced cutaneous sensitivity to wetness. Hence, in the present study, we used psychophysical methods to investigate the role of thermal and tactile afferents and their central integration in the perception of skin wetness under normal fiber function and under a selective reduction in the activity of A-nerve afferents.

We tested the hypothesis that under normal nerve fiber function, wetness perception is primarily driven by the integration of cold and tactile inputs as subserved by A-nerve fibers. Furthermore, we hypothesized that during a selective reduction in the activity of A-nerve fibers, the artificially induced reduction in cutaneous cold and mechano sensitivity would translate in a significant reduction in the extent of perceived wetness. Finally, given the anatomical and functional differences in cutaneous thermal and mechano sensitivity between hairy and glabrous skin (Abraira and Ginty, 2013; Haggard et al., 2013; Pleger and Villringer, 2013), here we investigated whether the proposed neurophysiological model of wetness sensitivity applies similarly to the forearm (i.e. hairy) as well as to index finger pad (i.e. glabrous). As hairy and glabrous skin sites have been shown to differ in terms of innervation and particularly in terms of density of thermo- and mechano-sensory afferents as well as in their biophysical properties (e.g. thickness and thermal conductance) (Abraira and Ginty, 2013), it was hypothesized that, due to the primary role of thermal cues in sensing wetness (Filingeri et al., 2013, 2014a, 2014b, 2014c), the higher thermal sensitivity of the hairy skin (due to its larger density of thermoreceptors and to its lower thermal conductance) (Norrsell et al., 1999) would

translate in wetness being perceived in larger magnitude on this skin site as opposed to the glabrous skin. This, despite the latter presents a larger density of slowly adapting type 1 mechano-sensory afferents, also known as Merkel cells (low threshold mechanoreceptors transmitting acute spatial images of tactile stimuli with remarkably high spatial resolution) (Abraira and Ginty, 2013), which could potentially contribute to an increase in the haptic perception of wetness on this type of skin.

9.3 Materials and methods

9.3.1 Participants

Thirteen healthy university male students (mean age 21 years, SD 2; mean height 185 cm, SD 9; mean body mass 86 Kg, SD 12) with no history of sensory-related disorders volunteered to participate in this study. All participants gave their informed consent for participation. The test procedure and the conditions were explained to each participant. The study design had been approved by the Loughborough University Ethics Committee and testing procedures were in accordance with the tenets of the Declaration of Helsinki.

A sample size calculation was performed in order to determine the minimum number of participants required to be able to detect a significant change in thermal and mechano sensitivity as a result of the selective block protocol. Pilot tests data indicated that the difference in the thermal sensations of matched pairs (block vs. no block trials) was normally distributed with standard deviation of ~10 arbitrary units (a.u.) As we set the true difference in the mean thermal sensation of matched pairs at a value of 15 a.u., it was calculated that a minimum number of 12 participants was needed to be able to reject the null hypothesis that this response difference is zero with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis (α) was 0.05. Sample size calculations were performed using Power and Sample Size Calculation version 3.0, 2009 (Vanderbilt University).

9.3.2 Experimental Design

Participants took part in 3 experimental trials, during which the same quantitative sensory test was administered. The hairy skin of the ventral side of the left forearm (i.e. mid-distance between elbow and wrist) and the glabrous skin of the left index

finger pad were exposed to the contact with a warm-wet (35 °C), neutral-wet (30 °C) and a cold-wet (25 °C) stimulus during 3 phases: static, dynamic and evaporation (i.e. post-contact). During the contact with the stimuli, participants reported their local thermal and wetness perceptions on a hand-scored 100 mm visual analog scale for thermal (anchor points: hot and cold) and wetness perception (anchor points: completely dry and completely wet), while skin temperature at the contact site was continuously monitored. The 3 experimental trials differed with regards to the presence or absence of a selective reduction in the activity of A-nerve fibers and to the skin site stimulated. All 13 participants performed: one trial during which no nerve block was performed (NO-BLOCK) and the skin of the forearm and finger pad were exposed to the wet stimuli; two separate trials during which a selective reduction in the activity of A-nerve fibers was performed through local compression-ischemia, and the skin of the forearm (FA-BLOCK) or finger pad (FI-BLOCK) was exposed to the contact with the wet stimuli. Trials were performed on a balanced order, on separate days, with at least 72 h in between.

The thermal stimuli were delivered by a thermal probe (Physitemp Instruments Inc., USA) with a contact surface of 25 cm^2 and a weight of 269 g. To make the contact with the probe' surface wet, test fabrics (100 % cotton) with a surface of 100 cm^2, were placed on the thermal probe and fixed by an elastic band. These were wetted with 2000 µl water at ambient temperature (~23 °C), using a variable volume pipettor (SciQuip LTD, Newtown, UK). To ensure that the wet fabric would reach the required temperature (i.e. 35, 30 or 25 °C), the contact temperature between the probe and the test fabric was monitored with a thin thermocouple (0.08 mm wire diameter, 40 Gauge; 5SRTC-TT-TI-40-2M, Omega, Manchester, UK) placed on the thermal probe' surface. Also, local skin temperature (T_{sk}) at the contact site of stimulation was measured continuously through the application of a thermocouple on the ventral side of the forearm or index finger pad using transpore tape (3M, Loughborough, UK), with the sensor tip touching the skin, but not covered by tape. Probe-fabric temperature as well as T_{sk} was monitored using a Grant Squirrel SQ2010 data logger (Grant Instruments Ltd., Cambridge, UK).

During all the trials, participants rested in a seated position in a thermo-neutral environment (air temperature: ~23 °C; relative humidity: ~50 %). Participants were informed only about the skin site subjected to the stimulation and the trial to be performed (block vs. no block). No information was made available on the type and

magnitude of the stimulation to limit any expectation effects. To make this possible, an s-shaped wooden panel (width: 81 cm; length: 74 cm; height: 60 cm) was placed on a table. A hole (width: 12 cm; height: 13cm) in the panel allowed participants to enter their left forearm through the panel. This experimental setup did not allow the participants to see the stimulated area.

9.3.3 **Experimental protocols**

9.3.3.1 NO-BLOCK trial

In the NO-BLOCK trial, no compression ischemia was performed and participants interacted actively with the warm-wet, neutral-wet and cold-wet stimuli. Forearm and index finger pad skin sites were tested separately within this trial, allowing a 5min interval between them.

The thermal probe was secured with surgical tape on the side of the table which was not visible to the participants, with the thermally controlled surface facing upward. Prior to interacting with each wet stimulus, and in order to set a baseline T_{sk} of 30 °C, participants were asked to insert their left arm through the hole in the panel and place the forearm or index finger pad for 30 s on the dry thermal probe, which was set at 30 °C. Participants then removed the arm from the thermal probe, placed it on the side of the table visible to them, and waited 1min for the first stimulus to be prepared. During this time, the probe was set to the required temperature (i.e. 35 °C, 30 °C or 25 °C), the test fabric was secured to the probe and then wetted with the pipettor. Pilot tests indicated 1 min as the time required for the wet test fabric to reach the selected temperature. Once the stimulus preparation was completed, the interaction with the wet stimulus was initiated. This consisted of 3 phases (each lasting 10 s): static, dynamic and evaporation (i.e. post contact). First, participants were instructed to insert their left arm through the hole in the panel and to lower it until the forearm or index finger pad was in full contact with the thermal probe. As soon as in static contact, they were encouraged to rate their local thermal and wetness perceptions by marking a point on the thermal and wetness scales they were provided with on the side of the table which was visible to them (response time ~5 s). Then participants were asked to move the forearm or index finger pad forward (~2.5 cm) and backward (~2.5 cm) twice while maintaining full contact with the thermal probe. At the end of

this dynamic interaction they were asked again to rate their local thermal and wetness perceptions (response time ~5 s). Finally, they were asked to lift the forearm or index finger pad up from the thermal probe, thus allowing evaporation of any residual moisture on the skin, and as soon as not in contact with the probe, to rate their local thermal and wetness perceptions for the last time (response time ~5 s). This sequence (i.e. setting the baseline skin temperature, preparing and then interacting with the wet stimulus) was repeated for each of the 3 wet stimuli in a balanced order, with at least 1 min in between them.

As no visual feedback was available during the stimulation, to assure consistency in the interaction with the stimuli (i.e. pressure applied to the probe and horizontal displacement during the dynamic phase), the investigator gently guided the participants' arm throughout the interaction with each stimulus and provided verbal instructions on when to change the interaction (e.g. from static to dynamic). All participants were familiarized with the experimental protocol prior to testing. Participants also familiarized with the rating scales prior to testing. When reporting thermal sensations, they were instructed to associate the anchor point "Hot" (on the left of the scale) to the idea of a burning hot pan, and the anchor point "Cold" (on the right of the scale) to the idea of an ice cube, and to mark a point on the scale which corresponded to the level of warmness or coldness experienced. The midpoint of the scale was suggested as a neutral point (to be marked if neither hot nor cold sensations were experienced). When reporting wetness perceptions, they were instructed to associate the anchor point "Completely dry" (on the left of the scale) to the absence of any wetness. Thus, any marked point which was not on the left edge of the scale was to be considered as to correspond to the perception of wetness, with the closer this would be to the anchor point "Completely wet" (on the right of the scale), the greater the level of wetness experienced. The visual analog scales used in this study were hand-scored on laminated paper. Washable markers were used by the participants to mark their sensation so that the same scale could be re-used within the same test after participants' ratings were recording and cleaned off with a wet cotton pad.

9.3.3.2 FA-BLOCK and FI-BLOCK trials

In the FA-BLOCK and FI-BLOCK trials, participants underwent an initial selective reduction in the activity of A-nerve fibers and then were passively exposed to the

warm-wet, neutral-wet and cold-wet stimuli. The aim of this procedure was to reduce cutaneous cold and mechano sensitivity and it was performed through a modified local compression-ischemia protocol. This method has been previously shown to induce a dissociated reduction in A-fibers afferent activity (Yarnitsky and Ochoa, 1990, Davis 1998) as the compression ischemia impacts transmission in myelinated A-fibers before C-fibers (i.e. primarily sub-serving conscious warmth and pain sensitivity) are affected (Torebjörk and Hallin, 1973). Compression-ischemia was induced by inflating a sphygmomanometer cuff on the upper arm to a suprasystolic pressure (i.e.140 mmHg) for a maximum duration of 25 min. During the compression ischemia protocol, thermal sensitivity to warm (i.e. 35 °C) and cold dry stimuli (i.e. 25 °C) as well as mechanical sensitivity to light brush were checked every 5 min. It deserves mention that, despite of changes in mechano and cold sensitivity, the maximal duration of the compression-ischemia was set to 25 min in order to limit the discomfort and pain the participants could experience underneath the cuff (note: this duration does not include the sub-sequent stimulation with the wet stimuli, whose approximate duration was ~8 min). Although the literature reports compression blocks lasting between 27 to 60 min and performed with pressures up to 100 mmHg above systolic pressure (see e.g. Yarnitsky and Ochoa, 1990 and Davis 1998), our pilot studies indicated the duration chosen as well as the pressure used as to be sufficient to induce a gradual reduction in cold and mechano sensitivity, while maintaining to a minimum participants' overall discomfort. Indeed, during our preliminary testing, participants could not bear the 140 mmHg cuff pressure for longer than 35 to 40 min due to the excessive discomfort experienced underneath the cuff.

Prior to the application of the compression ischemia protocol, instrumentation and baseline measurements were performed. Participants were asked to sit on a chair for 15 min, at the end of which resting blood pressure was measured from the left wrist with a digital wrist blood pressure monitor (Speidel and Keller, Jungingen, Germany), while the arm was supported at heart level. Participants then entered their left arm through the hole in the panel, laid it down with the palm facing upward, while a 13 cm wide sphygmomanometer cuff (Hokanson Inc., Bellevue, USA) was placed around the arm (i.e. mid-distance between shoulder and elbow). The sphygmomanometer cuff was connected to a custom made cuff inflator. According to the experimental trial, a thermocouple was then taped to the ventral side of the

forearm or to the index finger pad to record T_{sk} throughout the test. An 8mm optic probe was taped to the ventral side of the forearm (proximal to the elbow joint) and connected to a Laser Doppler monitor (Moor Instruments, Devon, UK) to record skin blood flow. Finally, to allow thermal stimulation of the skin, the thermal probe, set at 30 °C, was secured with tape on the forearm or index finger pad (with the thermally controlled surface in full contact with the skin), where it rested during the first part of the test.

After instrumentation, baseline T_{sk} and skin blood flow were recorded for 5min, while participants were asked to maintain a comfortable seated position, having their left arm lying on the left hand side of the table (which was not visible to them) and their right arm on the right hand side, where the rating scale and washable marker were positioned to allow ratings of sensation when required. This position was maintained throughout the whole test. At this point pre-compression ischemia cutaneous thermal and mechano sensitivity was tested as follow: the thermal probe's temperature was first set to 35 °C (i.e. warm-dry stimulus) and as soon this temperature was reached (response time <4 s) participants were immediately asked to rate their thermal sensation only, by marking a point on the thermal sensation scale. The thermal probe was then re-set to 30 °C. As soon as the T_{sk} returned to 30 °C (this was monitored on-line on the data logger recording from the thermocouple placed on the skin site stimulated), the thermal probe's temperature was changed to 25 °C (i.e. cold-dry stimulus) and as soon this temperature was reached participants were asked again to rate their thermal sensation only. The thermal probe was then re-set to 30°C. Finally, the skin near the stimulated site was gently touched with a cotton pad and participants were asked to report verbally whether they could sense the touch. As soon as the baseline measurements were completed, the custom made cuff inflator was started, the sphygmomanometer cuff was inflated with the required pressure (time to reach the pressure: ~5 s), and the compression ischemia protocol initiated. The cutaneous sensitivity test was then repeated as above every 5 min. When the inability to perceive the light brush was observed, along with a reduction in thermal sensitivity to the cold stimulus, the thermal probe was removed from the skin site, and the warm-wet, neutral-wet and cold-wet stimuli were prepared and then applied following a protocol identical to the one performed during the NO-BLOCK trial (i.e. static, dynamic and evaporation phases), with the only difference being in the

investigator applying the thermal probe instead of the participants placing their forearm or finger pad on it.

9.4 Statistical analysis

In the present study, the independent variables were the temperature of the stimuli (i.e. 35, 30 and 25 °C), the different phases of stimulation (i.e. static, dynamic and evaporation), the skin site stimulated (i.e. forearm and index finger pad) and the condition (i.e. the presence or not of a selective reduction in A-fibers' activity). The dependent variables were local T_{sk}, thermal sensation and wetness perception. All data were first tested for normality of distribution and homogeneity of variance using Shapiro-Wilk and Levene's tests respectively. To investigate the role of thermal and mechanical cues on cutaneous thermal and wetness sensitivity, and whether differences exist between hairy and glabrous skin, data from the NO-BLOCK trial were analysed by a 3-way repeated measure ANOVA, with temperature of the stimuli (3 levels), phases of stimulation (3 levels) and skin site (2 levels) as repeated measure variables. To investigate whether the compression ischemia protocol resulted effective in selectively reducing A-nerve fibers' function in both forearm and index finger pad skin sites, thermal ratings recorded prior and at the end of the protocol (i.e. just before the wet stimuli were applied) were compared for both warm and cold stimulations by using paired t-tests. To investigate whether a reduction in cutaneous cold and mechano sensitivity decreased the ability to perceive skin wetness, data from the NO-BLOCK and BLOCK trials were analysed separately for the forearm and index finger pad by a 3-way repeated measure ANOVA, with condition (2 levels), temperature of the stimuli (3 levels) and phases of stimulation (3 levels) as repeated measure variables. Data were tested for sphericity and if the assumption of sphericity was violated, Huynh–Feldt or Greenhouse-Geisser corrections were undertaken to adjust the degrees of freedom for the averaged tests of significance. Estimated marginal means and 95 % confidence intervals (CI) were used to investigate the main effects and interactions of the variables. When a significant main effect was found, Tukey's post-hoc analyses were performed. In order to quantify the power associated with the statistically non-significant results, observed power was computed using $\alpha=0.05$ and reported. In all analyses, $p<0.05$ was used to establish significant differences. Furthermore, according to Curran-

Everett and Benos (2007), precise *p* values were interpreted as follow: $p>0.1$ data are consistent with a true zero effect; $0.05<p<0.1$ data suggest there may be a true effect that differs from zero; $0.01<p<0.05$ data provide good evidence that the true effect differs from zero; $p<0.01$ data provide strong evidence that the true effect differs from zero. Data were analysed using SPSS Statistics 19 (IBM, Armonk, USA) and are reported as means and standard deviation (SD) and 95 % CI.

9.5 Results

9.5.1 Cutaneous sensitivity to wetness under normal A-nerve fibers function (NO-BLOCK trial)

During the initial static contact with the warm-wet, neutral-wet and cold-wet stimuli, forearm skin and index finger pad T_{sk} respectively increased, remained unchanged or decreased (Fig. 1*A*,*C*). These variations in T_{sk} remained stable during the following dynamic phase. During the evaporation phase, T_{sk} started to return to pre-stimulation values after the warm-wet and cold-wet stimulations, whereas it started to decrease after the neutral-wet stimulation.

As a result, participants reported thermal sensations which varied significantly according to the temperature ($F= 28.8_{(1.2,\ 12.9)}$, $p<0.0001$) and phases of interaction ($F= 6.3_{(2,\ 22)}$, $p= 0.007$) with the wet stimuli. A trend was observed with the forearm being more thermally sensitive than the finger pad ($F= 3.6_{(1,\ 11)}$, $p= 0.085$, observed power= 0.4). Overall, thermal sensations matched the variations observed in local T_{sk}, with the warm-wet stimulus resulting in warmer sensations, the neutral-wet stimulus in neutral sensations and the cold-wet stimulus in colder sensations (Fig.1*E*,*G*).

With regards to wetness sensitivity, although all the stimuli presented the same level of physical wetness (i.e. 20 $\mu l \cdot cm^{-2}$), participants reported wetness perceptions which increased significantly with decreasing contact temperatures ($F= 5.3_{(2,\ 24)}$, $p= 0.012$) (Fig. 2*A*). Also, wetness perception increased significantly during the dynamic as opposed to the static contact ($F= 11.5_{(2,\ 24)}$, $p<0.0001$) (Fig. 2*B*). Overall a trend was observed in the interaction between temperature and phases of stimulation ($F= 2.38_{(4,\ 48)}$, $p= 0.064$, observed power= 0.6). This indicated that during the static phase, the cold-wet stimulus was perceived as “wetter” than the warm-wet and neutral-wet stimuli and that during the dynamic and evaporation phases, wetness perceptions

increased for all the stimuli (Fig.1*I*,*K*). Finally, a trend of the effect of skin site on wetness perception was observed (F= $3.5_{(1, 12)}$, p=0.086, observed power= 0.4), with the forearm showing a tendency in having a higher sensitivity to wetness [mean= 30.4 a.u.; CI= 21.8, 39 a.u.] than the index finger pad [mean= 18.2 a.u.; CI= 8.3, 28.1 a.u.].

Overall these results indicate that the perception of skin wetness was driven by the coldness experienced, and that when no coldness was perceived (e.g. warm-wet and neutral-wet stimulations), participants' ability to sense wetness relied on the mechanical inputs generated during the dynamic interaction with the wet surface.

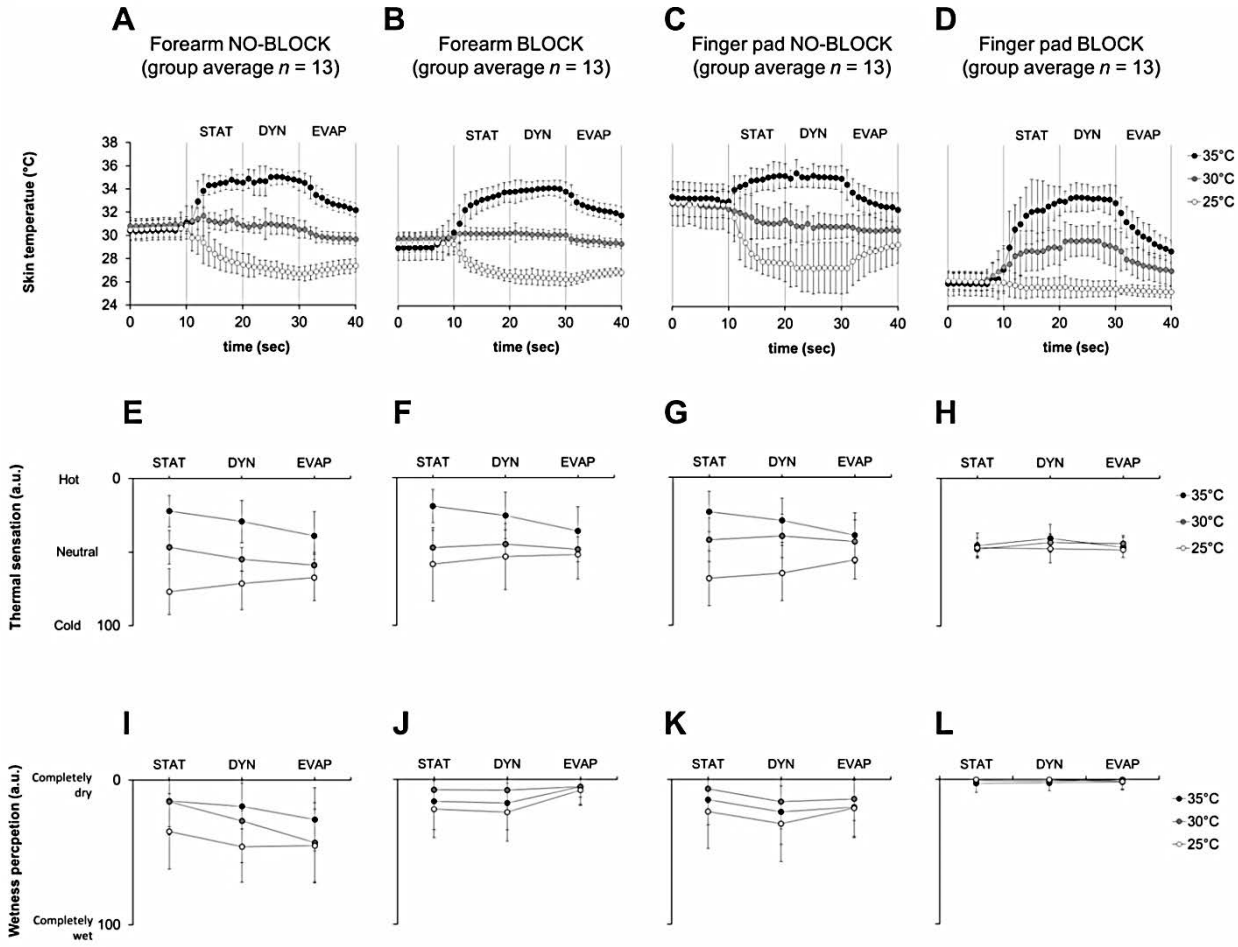


Figure 1: Forearm and finger pad skin temperature (°C) and corresponding ratings for thermal sensation and wetness perception (arbitrary units, a.u.) during the static (STAT), dynamic (DYN) and evaporation (EVAP) phases of contact with the warm-wet (35°C), neutral-wet (30°C) and cold-wet (25°C) stimuli. Panels *A* and *C*, panels *E* and *G* and panels *I* and *K* show skin temperature, thermal sensation and wetness perception data respectively as recorded during the NO-BLOCK trial for the forearm and finger pad. Panels *B* and *D*, panels *F* and *H* and panels *J* and *L* show skin temperature, thermal sensation and wetness perception data respectively as recorded during the BLOCK trial for the forearm and finger pad. Two tendencies are illustrated. In the NO-BLOCK trials, thermal sensations matched the variation in skin temperature and wetness perceptions increased with decreasing contact temperatures (static phase) and from static to dynamic to post contact (evaporation). In the BLOCK trials, cold sensitivity was reduced in the forearm, and both warmth and cold sensitivity were reduced on the finger pad. This resulted in a significant decrease in wetness perceptions during all temperature stimulations (and particularly during the cold one) and during all phases of interaction. Data are reported as mean (group average n= 13) and SD (vertical lines).

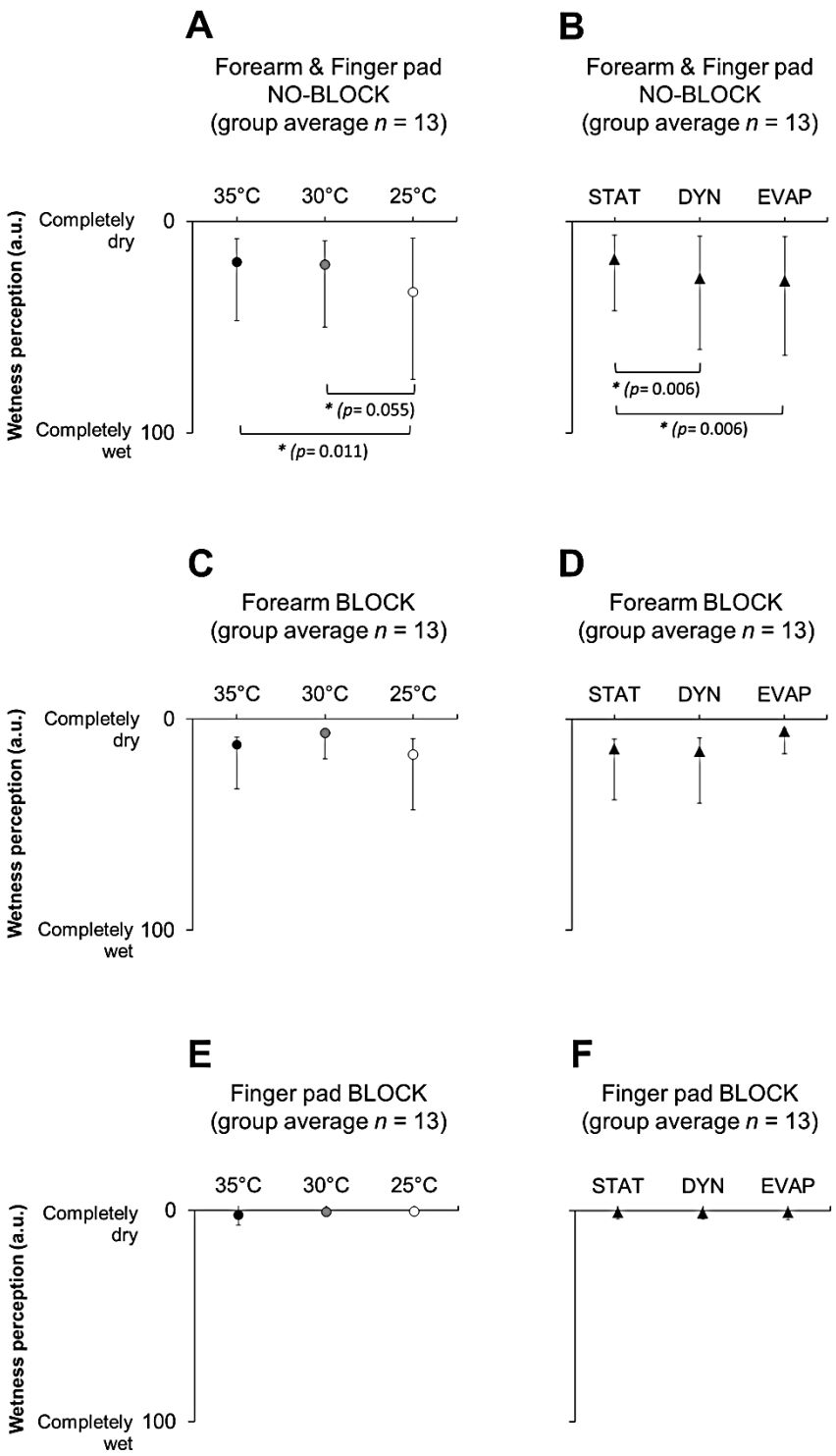


Figure 2: Ratings for wetness perception (arbitrary units, a.u.) grouped for forearm and finger pad and averaged over (*A*) temperature of the stimuli (35, 30 and 25 °C) and (*B*) phases of stimulation [static (STAT), dynamic (DYN) and evaporation (EVAP)] as recorded during the NO-BLOCK trial. Panels *C* and *D* show data as recorded for the forearm during the FA-BLOCK trial, whereas panels *E* and *F* show data as recorded for the finger pad during the FI-BLOCK trial. Two tendencies are

illustrated. During the NO-BLOCK trial, wetness perception increased with decreasing contact temperatures, and from static to dynamic and evaporation phases. During the BLOCK trials, wetness perception was reduced at any temperature for both forearm and finger pad, and no changes occurred from static to the dynamic phase. Data are reported as mean (group average n= 13) and 95 % CI (vertical lines).

9.5.2 **Selective reduction in the activity of A-nerve fibers**

To test the effectiveness of the selective reduction in the activity of A-nerve fibers, during the compression ischemia protocol, thermal sensitivity to warm (i.e. 35 °C) and cold dry stimuli (i.e. 25 °C) as well as mechanical sensitivity to light brush were checked every 5 min. As a result of the protocol, a statistically significant reduction in thermal sensitivity to cold was observed, both in the forearm (mean difference= -17.3 a.u.; CI= -2.9, -31.7 a.u.; t= -2.6; two-tailed *p*= 0.022; Fig. 3*A*) and index finger pad (mean difference= -16.8 a.u.; CI= -7.7, -25.9 a.u.; t= -1.5; two-tailed p= 0.002; Fig. 3*B*). No significant differences in thermal sensitivity to warmth were observed at the end of the selective block protocol, either in the forearm (mean difference= +5.1 a.u.; CI= -5.6, 15.9 a.u.; t= 1.04; two-tailed p= 0.32; Fig. 3*C*) or in the index finger pad (mean difference= -5.9 a.u.; CI= -14.4, 2.6 a.u.; t= -1.5; two-tailed p= 0.15; Fig. 3*D*). As the warm and cold-dry stimuli produced the same relative variations in local T_{sk} throughout the compression ischemia protocol (Fig. 4), these results indicate that this procedure was effective in selectively reducing cutaneous cold sensitivity of both forearm and finger pad, while maintaining warmth sensitivity intact. With regards to mechano sensitivity, at the end of compression ischemia protocol, 2 out of 13 participants were not able to sense the light brush on the forearm (FA-BLOCK trial), whereas during the FI-BLOCK trial 12 out of 13 participants were not able to sense the light brush on the finger pad.

Changes in cold and mechano sensitivity occurred earlier for the finger pad than for the forearm. For 11 out of 13 participants, the selective block lasted 20 min during the FI-BLOCK trial and 25 min during the FA-BLOCK trial. It deserves mention that the selective block resulted in paradoxical heat sensations during cold stimulation in 4 participants (i.e. FA-BLOCK trial) and 6 participants (i.e. FI-BLOCK trial). Before the application of the selective block, average values for resting systolic and diastolic pressure were 135 mmHg (SD 8) and 66 mmHg (SD 6) respectively.

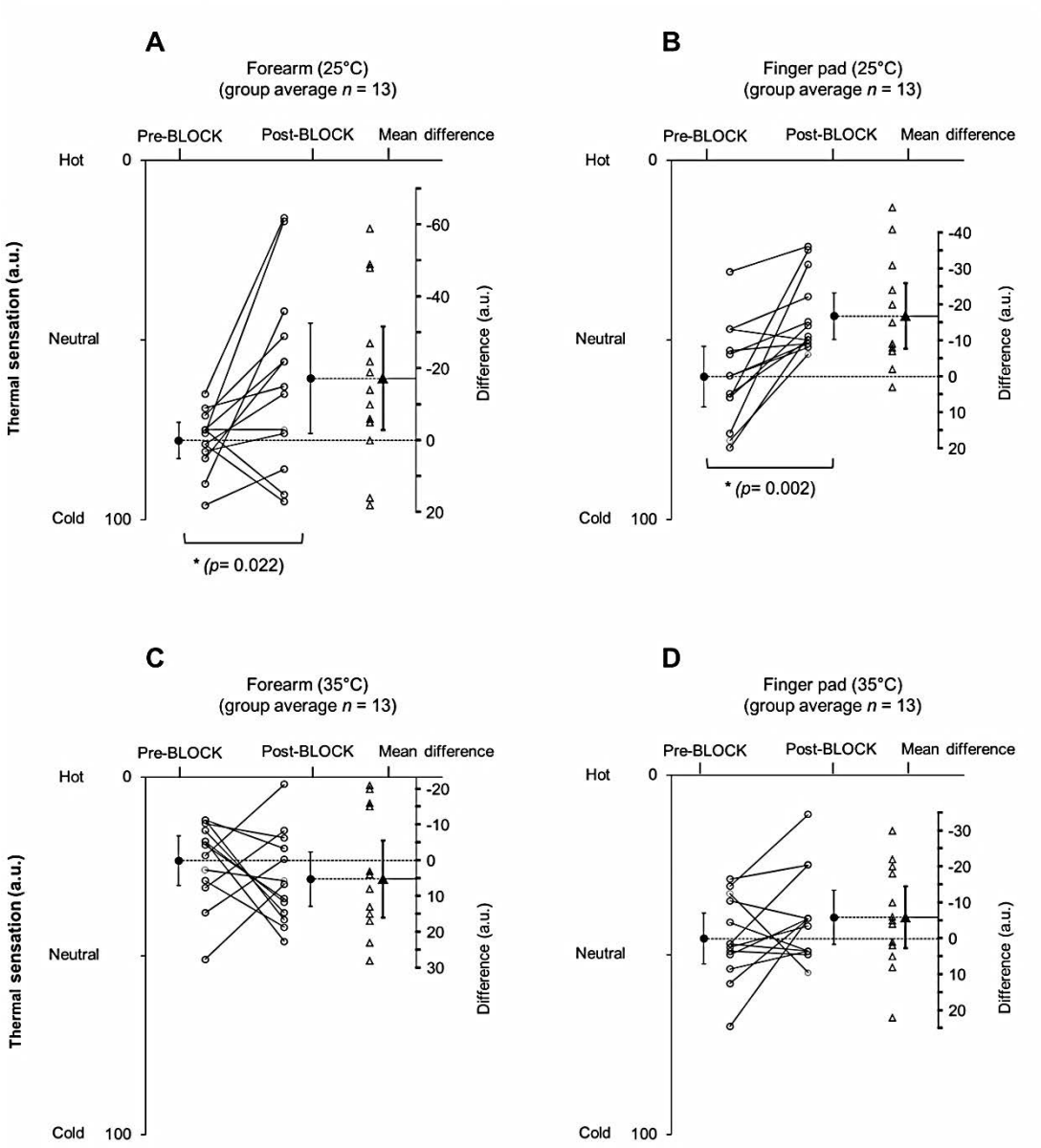


Figure 3: Ratings for thermal sensation (arbitrary units, a.u.) as a result of the cold (25°C) and warm (35°C) stimuli as recorded before (PRE-BLOCK) and at the end (i.e. just before application of wet stimuli, POST-BLOCK) of the compression ischemia protocol. Panels *A* and *C* show average and individual ratings for thermal sensation for the forearm. Panel *B* and *D* show average and individual ratings for thermal sensation for the finger pad. Mean difference (group average n= 13) and 95 % CI between pre and post-block are also shown. One main tendency is illustrated. At the end of the BLOCK trials, thermal sensitivity on the cold side was significantly reduced while no significant changes in sensitivity on the warm side occurred, both for forearm and finger pad. Data are reported as mean (group average n= 13) and 95 % CI (vertical lines).

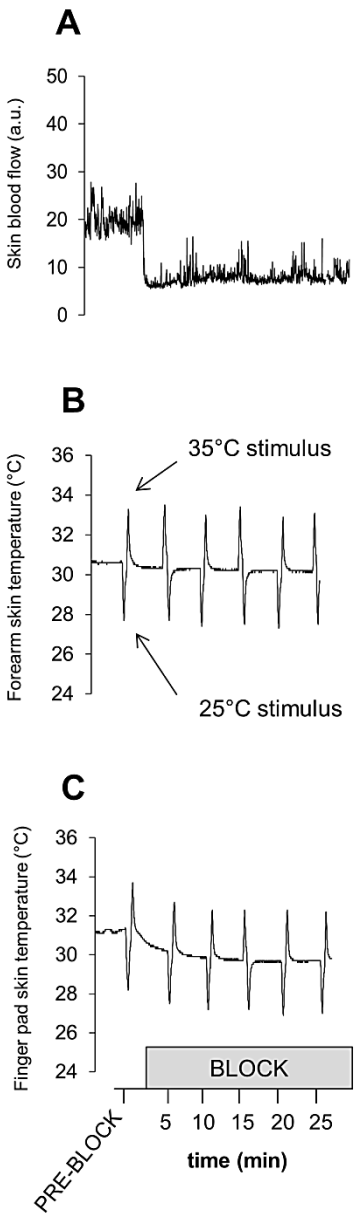


Figure 4: Representative skin blood flow (*A*) (arbitrary units, a.u.), forearm (*B*) and finger pad (*C*) skin temperature (°C) as recorded for participant 4 during the cutaneous thermal sensitivity test performed during the BLOCK trials. Cutaneous thermal sensitivity was tested as follow: the thermal probe's temperature was first set to 35 °C and as soon this temperature was reached (response time <4 s) participants were asked to rate their thermal sensation only. The thermal probe was then re-set to 30 °C. As soon as the skin temperature returned to 30 °C, the thermal probe's temperature was changed to 25 °C (i.e. cold stimulus) and participants were asked again to rate their thermal sensation only. The thermal probe was then re-set to 30 °C. Throughout the compression ischemia protocol, the cold and warm dry stimuli always resulted in the same variation in skin temperature.

9.5.3 Cutaneous sensitivity to wetness under selective reduction of A-nerve fibers' function

As soon as the compression ischemia protocol resulted effective, the quantitative sensory test was initiated. The results of the sensory test are presented individually for the forearm and then for the finger pad. Similar outcomes were observed for both forearm and finger pad during the contact with the wet stimuli, after cold and mechano sensitivity was reduced with the selective block protocol.

With regards to the forearm, during the initial static contact with the warm-wet, neutral-wet and cold-wet stimuli, forearm T_{sk} showed similar variations as the ones recorded during the NO-BLOCK trial (Fig. 1*B*). However, a significant effect of the compression protocol (F= $10.6_{(1,\ 11)}$, p= 0.008) was found on thermal sensation. During the contact with the warm-wet and neutral-wet stimuli, participants' thermal sensations did not differ significantly between NO-BLOCK and FA-BLOCK trials. However, as a result of the same cold-wet stimulus applied to the forearm, significantly "less cold" thermal sensations were reported during the FA-BLOCK trial [CI= 39.7, 65.5 a.u.] than during the NO-BLOCK trial [CI= 61.3, 82.5 a.u.] (Fig. 1*F*). These results confirmed that at the time of application of the wet stimuli, the forearm presented a reduced thermal sensitivity to cold.

This artificially induced reduction in cold sensitivity translated into a reduced perception of wetness of the forearm (Fig 1*J*). Overall, the magnitude of perceived wetness was significantly reduced during the FA-BLOCK [CI= 4.9, 18.8 a.u.] when compared to the NO-BLOCK trial [CI= 21.8, 39 a.u.] (F= $13.7_{(1,\ 12)}$, p= 0.003) (Fig. 5*A*). A trend in the interaction between the effect of the block and the temperature of the stimuli was observed (F=$3_{(2,\ 24)}$, p= 0.07, observed power= 0.5), with the greatest reduction in perceived wetness occurring during the cold-wet stimulation (see comparison between figures 1I and 1J). Finally, a significant interaction between condition and phases of stimulation was found (F= $11.7_{(2,\ 24)}$, p<0.0001). As opposed to the NO-BLOCK trial, during which wetness perception increased from static to dynamic and evaporation, during the FA-BLOCK trial, no changes in the forearm wetness perception from static to dynamic and a decrease from dynamic to evaporation occurred (Fig. 2*D*). Overall these results indicate that the significant reduction in the magnitude of perceived wetness observed during the FA-BLOCK

trial was mainly due to the reduced cutaneous cold and mechano sensitivity of the forearm.

Similar results were observed during the index finger pad contact with the wet stimuli (i.e. FI-BLOCK trial). During the initial static contact with the warm-wet, neutral-wet and cold-wet stimuli, finger pad T_{sk} respectively increased (i.e. warm and neutral-wet) or decreased (i.e. cold-wet) (Fig. 1*D*). As a result of the contact with the warm-wet and cold-wet stimuli, "less warm" and "less cold" thermal sensations were reported during the FI-BLOCK trial than during the NO-BLOCK trial (Fig. 1*H*). This interaction between condition (i.e. block vs. no block) and temperature of the stimuli was found to be statistically significant ($F= 13.1_{(1.5,\ 17.6)}$, $p= 0.001$). These results indicated that at the time of application of the wet stimuli, the index finger pad presented a reduced thermal sensitivity to warmth and cold. This translated into a reduced sensitivity to wetness (Fig. 1*L*). A significant effect of condition ($F= 13.9_{(1,\ 12)}$, $p= 0.003$), a trend in temperature of the stimuli ($F= 2.9_{(2,\ 24)}$, $p= 0.072$, observed power= 0.5) and a significant effect of phases of stimulation ($F= 5.9_{(2,\ 24)}$, $p= 0.008$) was found on wetness perception (Fig. 2*E*).

Overall wetness sensitivity was significantly reduced during the FI-BLOCK [CI= 0, 2.5 a.u.] as compared to the NO-BLOCK trial [CI= 8.3, 28.1 a.u.] (Fig. 5*B*). A significant interaction between condition and phases of stimulation was found ($F= 5.7_{(2,\ 24)}$, $p= 0.001$). As opposed to the NO-BLOCK trial, during which wetness perceptions increased from static to dynamic, during the FI-BLOCK trial no changes were observed from static to dynamic to evaporation (Fig. 2*F*). Overall these results reflect those observed with the forearm during the FA-BLOCK trial, and indicate that the significant reduction in wetness sensitivity observed on the finger pad during the FI-BLOCK trial was mainly due to the reduced cutaneous thermal and mechano sensitivity of this skin site.

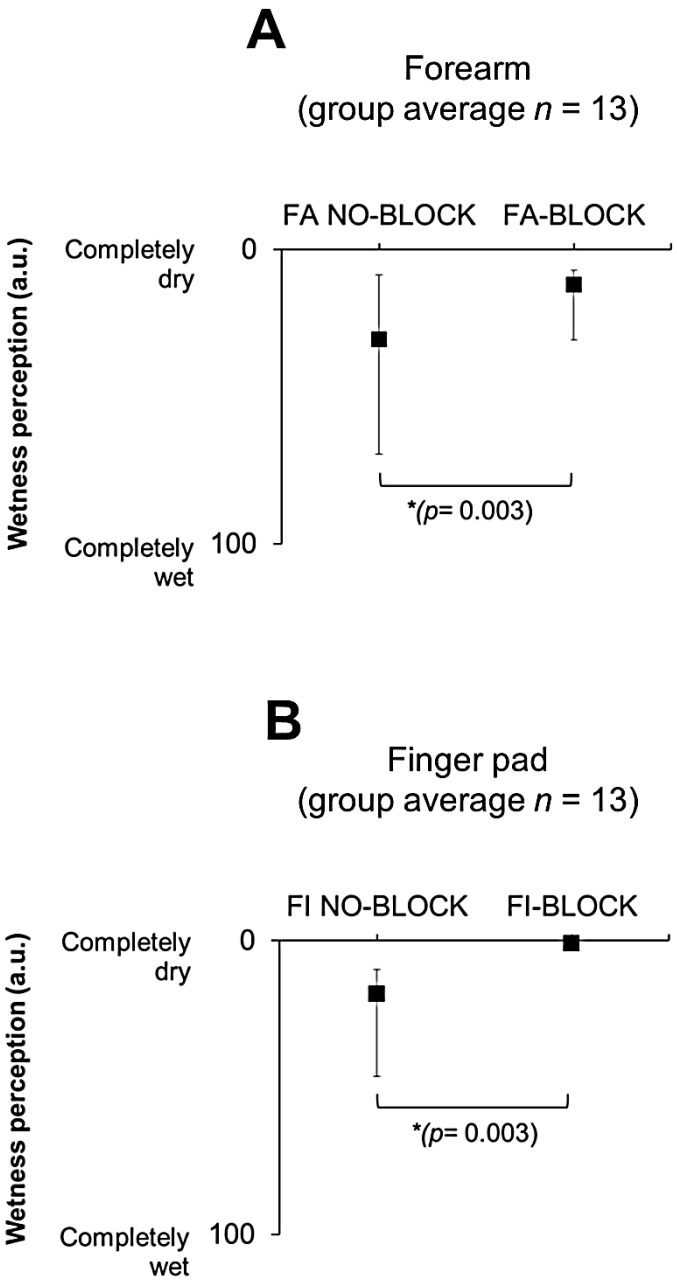


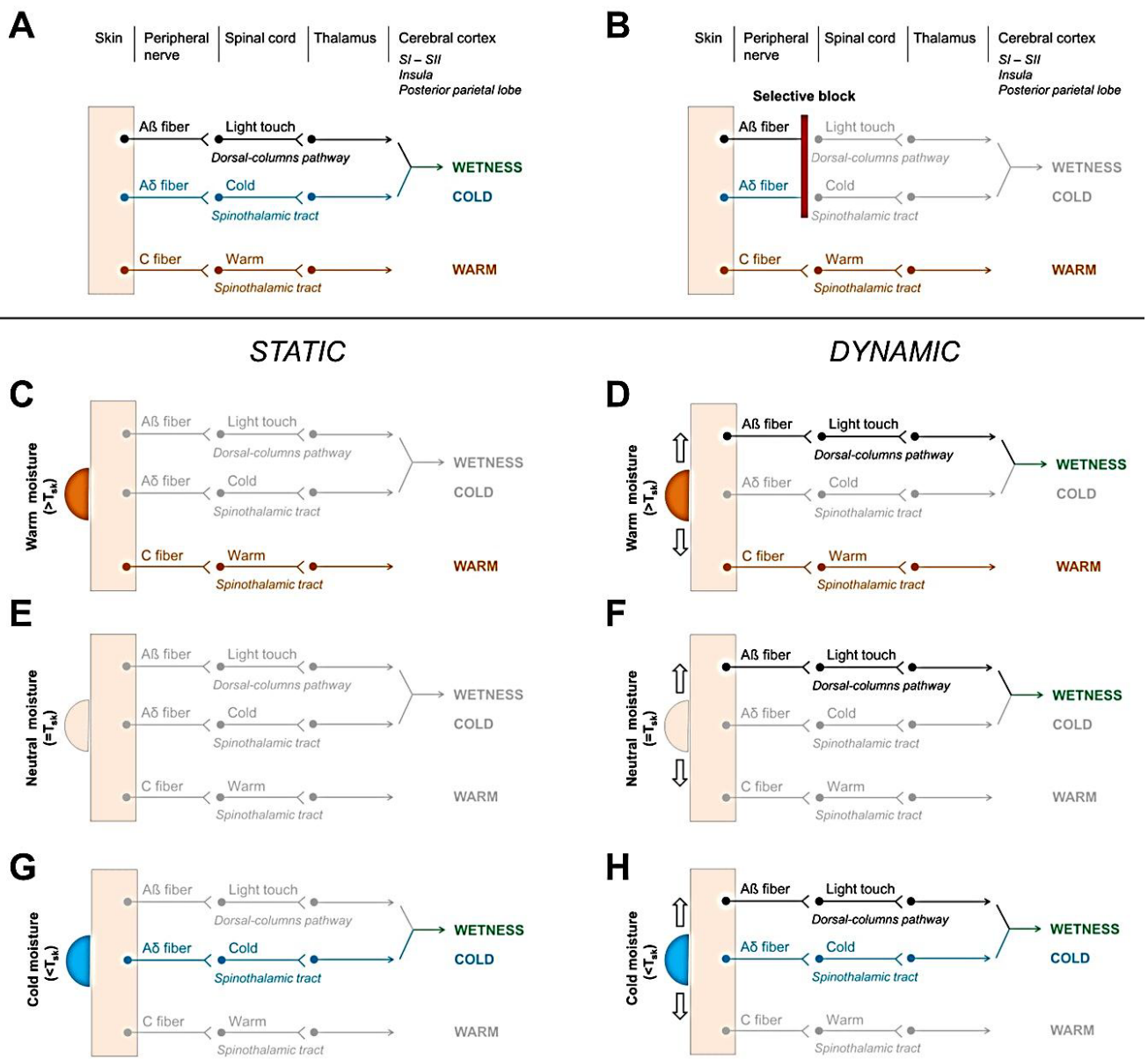
Figure 5: Ratings for wetness perception (arbitrary units, a.u.) averaged over condition (NO-BLOCK vs. BLOCK) for the forearm (*A*) and finger pad (*B*). A significant reduction in wetness sensitivity was recorded during the BLOCK trials as compared to the NO-BLOCK, both for the forearm and finger pad. Data are reported as mean (group average n= 13) and 95 % CI (vertical lines).

9.6 Discussion

The present study focused on the role of cutaneous thermal and tactile afferents and their central integration in the ability to sense wetness. By exposing hairy and glabrous skin sites to the static and dynamic contact with warm-wet, neutral-wet and cold-wet stimuli characterized by the same moisture content (i.e. 20µL/cm^2), we demonstrated that during a static contact, wetness perception increases with decreasing contact temperatures and that during a subsequent dynamic interaction, wetness perception increases regardless of the thermal inputs available. Also, we demonstrated that when cutaneous cold and mechano sensitivity was significantly diminished through a selective reduction in the activity of A-nerve afferents, the extent of perceived wetness was also significantly reduced, both on the forearm and index finger pad. Finally, a trend was observed with the extent of perceived wetness being higher on the hairy than on the glabrous skin.

In summary, our results indicate that the central integration of conscious coldness and mechanosensation, as sub-served by peripheral myelinated A-nerve fibers, could be the primary neural process underpinning humans' ability to sense wetness. To our knowledge the present study is the first to provide evidence in support of the hypothesis that a specific information processing model for cutaneous wetness sensitivity exists and that this is based on A-type somatosensory afferents. Based on these outcomes, we developed the first neurophysiological model of human cutaneous wetness sensitivity (Fig. 6).

Figure 6: Neurophysiological model of cutaneous wetness sensitivity. Mechano Aß, cold Aδ and warm C sensitive nerve fibers and their projections from the skin, through peripheral nerve, spinal cord (via the dorsal-column medial lemniscal pathway and the spinothalamic tract), thalamus and somatosensory cerebral cortex (including the primary and secondary somatosensory cortex cortices SI and SII, the insular cortex and the posterior parietal lobe) are shown. Panel A and B shows the neural model of wetness sensitivity (consisting of Aδ and Aß afferents) under normal and under selective reduction in the activity of A-nerve fibers respectively. Panel C, E and G show the pathways for wetness sensitivity during static contact with warm, neutral and cold moisture. Panel D, F and H shows the pathways for wetness sensitivity during dynamic contact with moisture



9.6.1 **A neurophysiological model of cutaneous wetness sensitivity**

The proposed neurophysiological model is based on the concept of Bayesian perceptual inference for which sensory processing is considered an inference problem (Knill and Richards, 1996). Given noisy and ambiguous sensory inputs (such can be thermal and mechanical stimuli on the skin), the brain is thought to estimate which events caused these inputs (e.g. the presence or not of physical wetness on the skin), based on prior knowledge which is acquired and shaped by sensory experience (Lochmann et al., 2012). In our proposed information processing model, two main neural pathways are suggested to subserve cutaneous wetness sensitivity: one referring to the afferent activity of cold sensitive Aδ-nerve fibers (projecting through the spinothalamic tract), and one referring to the afferent activity of mechano sensitive Aß fibers (projecting through the dorsal-column medial lemniscal pathway). The outcomes of this study have indeed indicated that in order to sense cutaneous wetness, a multimodal integration of thermal (i.e. cold) and mechanical sensory inputs had to take place (Fig. 6*A*). From a functional point of view, this was confirmed by the fact that when the activity of A-nerve fibers was selectively reduced, the extent of perceived wetness was also significantly reduced (Fig. 6*B*). From a central processing point of view, this was confirmed by the fact that, although all the stimuli had the same moisture levels, warm-wet and neutral-wet stimuli were sensed as significantly less wet than the cold-wet one.

Perceptual learning and somatosensory decision making could contribute to explain why the central nervous system processes sensory information about the perception of wetness in such fashion (Pleger and Villringer, 2013). As the skin seems not to be provided with hygroreceptors (Clark and Edholm, 1985), we hypothesized that the primary and secondary somatosensory cortices, the insular cortex (a cortical region involved in cold temperature sensation) (Craig et al., 2000) as well as the posterior parietal lobe (a cortical region concerned with integrating the different somatic sensory modalities necessary for perception) (McGlone and Reilly, 2010) could be involved in generating a neural representation of a *"typical wet stimulus"*. This could be based on the multimodal transformation (i.e. information from one sensory sub-modality can be transformed into a map or reference frame defined by another sub-modality) of the somatosensory inputs generated when the skin is physically wet (Haggard et al., 2013). As the sensory inputs associated to the physical experience of wetness are often generated by heat transfer in the form of evaporative cooling

(Ackerley et al., 2012), and mechanical pressure in the form of friction and stickiness (Adams, 2013), the typical neural representation of a wet stimulus might rely on perceiving coldness and stickiness. As for perceptual learning and somatosensory decision making (Pleger and Villringer, 2013), this neural representation could be transformed into a firing rate code, representing the wet stimulus, and then associated to the perception of wetness. Hence, only if the memorized combination of stimuli (i.e. coldness and stickiness), as coded by the specific afferents (i.e. A-nerve fibers) is presented, wetness will be sensed. In the occurrence of physical wetness on the skin, the bottom-up processes (i.e. combination of thermal and mechanical sensory afferents) as well as the top-down ones (i.e. inference of the potential perception based on the neural representation of a typical wet stimulus) might therefore interact in giving rise (or not) to the perception of wetness (Lochman and Deneve, 2011).

At this point however, although perceiving coldness and stickiness is likely to be determinant in the ability to process wetness at a central level, studies by Gerrett et al. (2013) and everyday experience suggest that we are able to sense wetness even in the absence of coldness (e.g. during exposure to warm-humid environments or when in contact with warm water). In these particular conditions, the mechanical and pressure related sensations resulting from the afferent information generated by cutaneous mechanosensitive fibers could therefore play a critical role in the ability to sense wetness. Based on the results of this study, as well as on the available literature, we hypothesized possible mechanisms through which wetness is sensed, according to the sensory inputs available when the skin is in contact with warm, neutral or cold moisture.

9.6.2 **Cutaneous sensitivity to warm, neutral and cold wetness**

Figure 6*C,D* shows the process through which warm moisture could be sensed. When the skin is in static contact with warm moisture (i.e. temperature above T_{sk}), no activation of cold sensitive Aδ-nerve fibers occurs, and only C-fibers, (subserving conscious warmth sensitivity), and Aß-nerve fibers (subserving light touch) are involved in the somato-sensation of moisture (Fig. 6*C*). In this scenario, as Aß are the only nerve fibers available within the processing model we suggest to subserve wetness, cutaneous wetness will be sensed only if a higher level of mechanosensory afferents i.e. a dynamic interaction between skin and warm moisture will occur (Fig. 6*D*). A similar mechanism applies if the skin is in contact with neutral moisture (i.e.

with a temperature equal to T_{sk}) (Fig. 6*E,F*). In support of this, Bergmann Tiest et al. (2012) have recently observed that, during the interaction with wet materials (i.e. cotton wool and viscose), Weber fractions for wetness discrimination thresholds decreased significantly when individuals were allowed dynamic as opposed to the static touching. This indicated that individuals' cutaneous sensitivity to wetness was increased by a higher availability of mechanosensory afferents, as occurring during the dynamic exploration of the wet materials. The authors concluded that, when thermal cues (e.g. thermal conductance of a wet material) provide insufficient sensory inputs, individuals seem to use mechanical cues (e.g. stickiness resulting from the adhesion of a wet material to the skin) to aid them in the perception of wetness.

In line with Bergmann Tiest et al. (2012), in this study we observed that the lack of thermal inputs (i.e. in the case of neutral wetness) translated in a reduced sensitivity to wetness. This, until a dynamic interaction with the wet stimuli was allowed, and a higher level of mechanosensory afferents was then made available for central integration (Fig. 6*E,F*). However, and in addition to the findings of Bergmann Tiest et al. (2012), in our proposed neural model we suggest that the extent of perceived wetness is reduced, and mechanosensory afferents are therefore more important, not only when thermal cues are insufficient, but also when these are the *"incorrect"* ones. This seems to happen when in contact with warm moisture (Fig. 6*C,D*). Although in this case thermal cues in the form of warm sensations are available, as these are generated by sensory afferents (i.e. C-nerve fibers) which are *"outside"* the proposed model for wetness (i.e. relying on A-nerve fibers) and which are not associated with the neural representation of a *"typical wet stimulus"*, wetness sensitivity to warm moisture is reduced unless more mechanosensory afferents are activated (i.e. stickiness due to the skin friction with the wet stimulus) (Gerhardt et al., 2008; Adams, 2013). In line with this, we have recently shown that during static contact with a wet surface, warm stimuli (i.e. temperature above T_{sk}) can suppress the perception of cutaneous wetness (Filingeri et al., 2014d) (see Chapter Eight).

Behavioural and learning components could contribute to the concept of *"incorrect"* thermal cues. Psychophysical studies have indeed shown that as humans we tend to associate the blend of warmth and light pressure more to the perception of oiliness (Cobbey and Sullivan, 1922) than to perception of wetness (Bentley, 1900). Everyday's life further provides evidence in support of why, in the absence of

stickiness, warm sensations only seem not to be associated to the perception of wetness. For example, a bleeding nose is an experience we usually become aware of only after this has been pointed out to us, and the "wet area" has been haptically explored by touch. This could be due to the fact that blood temperature (~37°C) is usually higher than T_{sk} (~30°C) (Mekjavic and Eiken, 2006).

A combination of anatomical, physiological and learning factors could also explain the trend observed with the forearm (i.e. hairy skin) being more sensitive to wetness than the finger pad (i.e. glabrous skin). Hairy and glabrous skin sites differ in terms of innervation and particularly in terms of density of thermo- and mechano-sensory afferents as well as in their biophysical properties. As observed in this study and as previously shown (Norrsell et al., 1999), the hairy skin seems indeed to be more sensitive to thermal stimuli than the glabrous skin, which on the contrary presents higher spatial acuity. From the receptors point of view, this could be due to the fact that, although both glabrous and hairy skin sites are innervated with slowly adapting type 1 mechano-sensory afferents, also known as Merkel cells (low threshold mechanoreceptors transmitting acute spatial images of tactile stimuli with remarkably high spatial resolution), glabrous skin presents a higher density of these specialized organs for tactile discrimination, a fact which could explain the higher spatial acuity to mechanical stimuli of this type of skin (Abraira and Ginty, 2013). From a biophysical point of view, the presence of a thicker stratum corneum (i.e. the outermost layer of the skin) on glabrous skin, resulting in a greater thermal insulation of this type of skin, contributes to the reduced thermal conductance of the finger pad (Rushmer et al., 1966) and therefore to the lower thermosensitivity of glabrous as opposed to hairy skin during short contact cooling and/or heating. This, as a result of the longer time that is needed for a given change in temperature of glabrous skin' superficial layers to penetrate to the underlying tissues (e.g. stratum granulosum) where the thermoreceptors lay (McGlone and Reilly, 2010). In this context, as thermal sensitivity seems to play the key role in sensing wetness, it is therefore reasonable to hypothesize that, despite a larger content in highly spatially sensitive mechanoreceptive afferents (Abraira and Ginty, 2013) which could potentially contribute to an increase in the haptic perception of wetness, the lower thermal sensitivity of the glabrous skin might translate in the palm of the hands being generally less sensitive to wetness than the rest of the body. From a thermoregulatory standpoint, this could be supported by the fact that, as opposed to regions covered by

hairy skin, human hands are indeed more of a specialized organ for heat exchange than a thermo-sensory organ (Romanowsky, 2014). Finally, from a behavioural point of view, the fact that the hairy skin presents a higher sweat production than the glabrous skin (due to thermoregulatory reasons) (Smith and Havenith, 2012) could result in individuals expecting to experience cutaneous wetness in larger magnitude on hairy than on glabrous skin sites.

Further support for the hypothesis of a possible neural representation of a *"typical wet stimulus"* being based primarily on cold and mechanosensory A-type afferent, could be found when looking at the perceptions evoked by the skin's contact with cold moisture (Fig. 6*G*,*H*). In case of skin's contact with cold moisture (i.e. temperature below T_{sk}), Aδ-nerve fibers (subserving cold sensitivity) and Aß-nerve fibers (subserving light touch) are involved in the somato-sensation of moisture. In this scenario, as both Aδ and Aß afferents are available within the processing pathway we suggest to subserve wetness, the extent of perceived wetness will be greater as compared to the wetness experienced when in contact with warm and neutral moisture. In this study we observed that, although all the stimuli had the same moisture levels, cold-wet stimuli were sensed as significantly wetter than the warm-wet and neutral-wet one, particularly during the static interaction, when only thermal cues were available (Fig. 6*G*). Also, the selective block trials indicated that the extent of perceived wetness was overall significantly decreased, mainly due to the reduced cutaneous cold and mechano sensitivity.

The critical role of experiencing coldness in the ability to sense wetness is in line with our previous findings. We have recently demonstrated that an illusion of local skin wetness can be evoked during the skin's contact with a cold-dry surface producing skin cooling rates in a range of 0.14 to 0.41 $°C \cdot s^{-1}$ (Filingeri et al., 2013; 2014a; 2014c) (see Chapter Four, Five And Seven), a temperature course which is similar to the one suggested to occur when the skin is physically wet (Daanen, 2009) (see Chapter Six). Evidence in support of the role played by thermal cold afferents in sensing wetness comes from studies investigating the role of cold-sensitive neurons in ocular dryness and wetness (Belmonte and Gallar, 2011; Hirata and Oshinsky, 2012). Hirata and Oshinsky (2012) have recently suggested that the sensation of "ocular wetness" could be based on the afferent activity of corneal cold-sensitive neurons, carrying a sensation of gentle cooling via a transient receptor potential (TRP) channel activation. The authors proposed this as a potential explanation to why tears

on the ocular surface could feel wet (Hirata and Oshinsky, 2012). The possibility that cold-sensitive neurons and TRP channels could be critical determinants of the human ability to sense wetness represents an intriguing possibility (Montell, 2008), particularly as TRP channels have been previously shown to be required for hygrosensation and detection of both dry and moist air in some insects, such as the fruitfly *Drosophila melanogaster* (Liu et al., 2007). However, the speculative nature of this hypothesis highlights the need for further experimental evidence in order to better understand the still little investigated neurophysiological mechanisms involved in such complex cognitive function such as wetness sensitivity. For example, it has to be highlighted that based on the present results, it cannot be concluded that coldness alone (without tactile component) is sufficient in generating a perception of wetness. Although we believe that a perception of wetness always results from the combination of thermal and tactile cues (and in this respect, our proposed processing model provides evidence in support of which cues the central nervous system relies more in its prediction of wetness) (Ernst and Banks, 2002) further research should deal with e.g. whether wetness could be evoked without any tactile component (e.g. through radiative cooling) or whether tactile stimuli only can evoke wetness, in order to further our understanding of somatosensation in the context of perceptual inference.

It deserves mention that C-nerve fibers (i.e. polymodal afferents responding to nociceptive, warm, cool and light mechanical stimulation with conduction velocities ranging from 0.2-2m/s) (McGlone et al. 2014) have been previously shown to respond to innocuous cold temperatures (Campero et al. 2001) as well as to touch (Lumpkin and Caterina, 2007). Therefore, it might be argued that these fibers could also contribute to the sensory processing of skin wetness. However, as their contribution to conscious cold sensations has not been proven conclusively (Schepers and Ringkamp, 2010) (therefore suggesting an alternative autonomic thermoregulatory function) and as their mechanical sensitivity seems to be specifically tuned to affective as opposed to discriminative touch (Loken et al., 2009; Olausson et al., 2010), the contribution of C-nerve fibers to the perception of wetness seemed not to be critical, at least not within the experimental conditions of the present study. Indeed, we observed that the reduction in A-nerve fibers' afferent activity, either when naturally (i.e. static contact with warm and neutral moisture) or artificially (i.e. during the compression ischemia protocol) induced, was sufficient to

significantly change the dynamic of the perception of wetness (i.e. significantly diminishing the extent of perceived skin wetness). Nevertheless, due to the polymodal nature of these nerve fibers (McGlone et al. 2014), and due to the absence of a direct measurement of peripheral neural activity in the present study (e.g. by microneurographic recording), the hypothesis of C-fibers significantly contributing to the sensory integration of skin wetness cannot be ruled out conclusively.

9.7 Conclusion

In summary, a neurophysiological model of cutaneous wetness sensitivity, based on the multimodal transformation of A-type somatosensory afferents, was developed, in order to explain how humans could sense warm, neutral and cold cutaneous wetness. This model supports the hypothesis that the brain infers about the perception of wetness in a rational fashion, taking into account the variance associated with thermal and mechano afferents evoked by the contact with wet stimuli, and comparing this with a potential neural representation of a *"typical wet stimulus"*, which is based on prior sensory experience. In this respect, our findings have both a fundamental, as well as a clinical significance. They provide insights on the integration and processing of somatosensory information occurring between peripheral and central nervous system. Also, they provide insights on the possible origin of symptoms such as spontaneous sensations of cold wetness experienced across the body by individual suffering from multiple sclerosis or polyneurophaties (Rae-Grant et al., 1999; Susser et al., 1999; Nolano et al., 2008; Hulse et al., 2010). As these disorders have been shown to affect peripheral A-nerve fibers functions and to alter somatic perception, the neurophysiological model of cutaneous wetness sensitivity developed in this study could be used as a frame of reference for normal and altered somatosensory function.

10 CHAPTER TEN - Laboratory study 7: Decreasing the tactile interaction between skin, sweat and clothing significantly reduces the perception of wetness independently of the level of physical skin wetness during moderate exercise

10.1 Abstract

We tested the hypothesis that the perception of skin wetness can be significantly manipulated independently from the level of physical skin wetness. Ten males repeated an incremental walking protocol (5 Km/h; gradient range: +2 to +16 %) during two trials designed to produce the same level of physical skin wetness, but to induce lower (i.e. TIGHT-FIT) and higher (i.e. LOOSE-FIT) perception of wetness. During the TIGHT-FIT trial, a tight fitting clothing ensemble was worn to limit the mechanical interaction and stickiness between skin, sweat and clothing. During the LOOSE-FIT trails, a loose fitting ensemble was used to augment this interaction. Heart rate, rectal temperature, mean skin temperature, whole body skin wetness (w_{body}) and galvanic skin conductance (GSC) as well as thermal, wetness and comfort sensation were recorded. Both sweat production (indicated by GSC) and physical skin wetness (indicated by w_{body}) increased significantly during the protocol (GSC range: 3.1 ± 0.3 to 18.8 ± 1.3 µS, $p<0.01$; w_{body} range: 0.26 ± 0.01 to 0.95 ± 0.2 n.d., $p<0.01$) with no differences between TIGHT-FIT and LOOSE-FIT ($p>0.05$). However, the reduced skin friction generated by the TIGHT-FIT ensemble lowered significantly the level of perceived skin wetness, both at a whole-body and at a regional level. Under conditions of sweat-induced whole-body wetness, the perception of skin wetness is primarily driven by the degree of tactile interaction between skin, sweat and clothing. By manipulating this interaction (e.g. changing the clothing fit), skin wetness perception can be significantly altered, independently of the level of physical wetness.

10.2 Introduction

As homeothermic mammals, humans need to maintain their core body temperature within a very narrow range (~37 °C) in order to ensure optimal cellular and molecular function (Nakamura and Morrison, 2007). Due to the variable nature of our surrounding environment, we constantly face the need of autonomically and behaviorally thermoregulate, as either core overheating or overcooling can pose a major challenge to our survival (Parsons, 2003). However, due to the asymmetry of our thermal physiology, which sees the normal core temperature being closer to its upper (≥40.5 °C) than its lower survival limit (≤18-20 °C) (Parsons, 2003), rises in core temperature are more dangerous than equivalent drops in this physiological parameter (Romanovsky, 2007).

Whether due to increases in metabolic heat production (e.g. as a result of exercise) or exposure to hot environments, core overheating is prevented, and heat balance maintained, by means of sweating (Candas et al., 1979). Evaporative heat loss through sweating plays a critical role in cooling the skin, thus maintaining a favourable core to skin gradient for heat losses from the core to the environment (Kondo et al., 1997). Therefore, within environmental conditions that allow full evaporation, the level of skin wetness represents an important parameter to ensure the evaporative efficiency of sweating (Candas et al., 1979).

As a physiological variable, skin wetness (*w*) was first introduced by Gagge (1937) who recognized its critical role in the heat balance of the body. Conceptually, *w* is defined as the fraction of the body covered by liquid at skin temperature (e.g. sweat), and it represents a physical measure of the degree of wetness involved in the process of evaporation (Gagge, 1937). Operationally, *w* can be determined as the ratio between a) the difference in water vapour pressure at the skin and in the air; and b) the difference between saturated water vapour pressure at the skin (calculated from skin temperature) and water vapour pressure in the air. *w* is usually expressed as a decimal fraction, with 1 representing the upper limit for a fully wet skin and 0.06 representing the minimal value due to insensible perspiration through the skin (Nishi and Gagge, 1977).

Since Gagge' seminal work, the measurement of *w* has received great attention, particularly in the context of predicting the body's heat balance during conditions of

increased metabolic heat production (e.g. resulting from exercising muscles), and decreased gradient for heat loss to the environment (e.g. resulting from high ambient temperatures) (Nadel and Stolwijk, 1973; Candas et al., 1979; Havenith, 2001a; Havenith et al., 2013). However, although much is known on the biophysical role of *w* in contributing to thermal homeostasis, surprisingly little has been done to elucidate how humans sense wetness on their skin and how the level of "physical" skin wetness relates to the level of "perceived" skin wetness. This is particularly relevant, as sensing skin wetness has been shown to be critical both for behavioural and autonomic responses. Perceiving changes in both ambient humidity and skin wetness have been shown to impact thermal comfort (Fukazawa and Havenith, 2009) and thus the thermoregulatory behaviour (Schlader et al., 2010), both in healthy and clinical populations (e.g. individuals suffering from rheumatic pain) (Strusberg et al., 2002). From an autonomic perspective, the degree of skin wetness influences sweat gland function through a progressive suppression of the sweat output (i.e. hidromeiosis) in the presence of wetted skin (Nadel and Stolwijk, 1973). This results in a reduced ability to lose heat to the environment via evaporative cooling, potentially affecting the thermal balance of the body (Candas et al., 1979). However, although the ability to sense skin wetness plays an important role in several behavioural and thermophysiological functions, little it is known on how skin wetness is sensed in humans (Montell, 2008).

As opposed to insects, in which humidity receptors sub-serving hygrosensation have been identified and widely described (Tichy and Kallina, 2010), humans seem not to be provided with specific receptors for the sensation of wetness (Clark and Edholm, 1985). Thus, we seem to "learn" to perceive the wetness experienced when the skin is in contact with a wet surface or when sweat is produced (Bergmann Tiest et al., 2012a) through a complex multisensory integration (Driver and Spence, 2000) of thermal (i.e. heat transfer) and tactile (i.e. mechanical pressure and skin friction) inputs generated by the interaction between skin, moisture and (if donned) clothing (Fukazawa and Havenith, 2009). This hypothesis has been supported by our previous findings. We have indeed repeatedly shown that the central integration of cold sensations (resulting from the afferent activity of cutaneous cold-sensitive, myelinated Aδ-nerve fibers) (Campero et al., 2001) and of tactile inputs (encoded by cutaneous mechanosensory Aß-nerve fibers) (Tsunozaki and Bautista, 2009), play a critical role in the ability to perceive skin wetness (Filingeri et al., 2013; 2014a;

2014c; 2014d) (see Chapter Four, Five Seven And Eight). This seems to be due to the fact that we interpret the coldness (i.e. thermal component) and stickiness (i.e. tactile component) experienced when the skin is wet as a signal of the presence of moisture (and thus wetness) on the skin' surface (Fig.1).

By appraising the central role of coldness and tactile sensory integration, our work has significantly contributed to elucidate the neural bases of the perception of skin wetness (Filingeri et al., 2014b) (see Chapter Nine). However, our investigations have so far focused on local skin wetness perceptions as evoked by the *passive* contact with an external wet stimulus. As a second way of experiencing this perception, skin wetness can also be evoked during the *active* production of sweat. In this respect, still little is known on the neurophysiological mechanisms by which skin wetness is sensed during conditions of sweat-induced whole-body wetness.

To our knowledge, only few studies have investigated how the level of physical skin wetness relates to the level of perceived skin wetness under conditions of sweat-induced whole-body skin wetness. In a study in which thermal comfort sensitivity was investigated in relation to locally manipulated skin wetness (as resulting from exercise-induced sweat production), Fukazawa and Havenith (2009) found that the torso seems to have a lower sensitivity to wetness than the limbs. Similar findings were also reported by Gerrett et al. (2013) in a non-manipulated condition (natural sweat distribution across the torso during exercise). On the contrary, Lee et al. (2011) showed that when asked, individuals reported the torso (i.e. chest and back) to be the region more often perceived as wet during rest and moderate exercise in 25 and 32°C ambient temperature and 50% humidity. Interestingly, in all these studies, skin temperature was always observed to significantly increase during the exercise protocols, suggesting that participants were able to both sense skin wetness as well as discriminate it regionally despite they did not experience any cold sensations. This is contrary to our earlier findings, in which we have observed that during the static contact with a warm-wet surface (with a temperature warmer than the skin) no local skin wetness was perceived as no skin cooling, and thus no cold sensations occurred (Filingeri et al., 2014d) (see Chapter Eight). It could be therefore suggested that in those conditions of sweat-induced skin wetness (Fukazawa and Havenith, 2009; Lee et al., 2011; Gerrett et al., 2013), participants relied more on tactile (i.e. stickiness of their clothing) than on thermal inputs (i.e. warm sensations) to characterize their wetness perception.

This hypothesis could be in line with what was previously shown on a local base (i.e. manual exploration of a wet material) by Bergmann Tiest et al. (2012), who reported that, when thermal cues (e.g. thermal conductance of a wet material) provide insufficient sensory inputs, individuals seem to use mechanical cues (e.g. stickiness resulting from the adhesion of a wet material to the skin) to aid them in the perception of wetness (Bergmann Tiest et al., 2012a). However, as in the above mentioned studies (Fukazawa and Havenith, 2009; Lee et al., 2011; Gerrett et al., 2013) the mechanical interaction at the skin was neither manipulated nor controlled, any hypothesis about the potential link between the thermal and mechanical changes occurring locally at the skin's surface when this was wet (due to sweating) and the resulting sensory inputs used by the participants to characterize their perception of skin wetness remains speculative.

To bridge this gap, the aim of this study was therefore to investigate the relationship between the level of physical wetness and the level of perceived wetness under conditions of sweat-induced whole-body skin wetness. Under conditions in which evaporation of moisture from the skin is limited (and therefore no skin cooling nor cold sensations are experienced), skin wetness perception is hypothesised to be primarily driven by the level of mechanical interaction (i.e. stickiness) between the skin and the wet surface (e.g. the clothing worn). The greater the mechanical interaction and skin friction, the higher the level of perceived skin wetness is expected to be (Gwosdow et al., 1986; Filingeri et al., 2014b). Therefore, we hypothesised that, during an incremental exercise protocol performed under conditions of restricted evaporation of sweat from the skin, at the same level of physical skin wetness, wearing a tight fitting clothing ensemble (which will limit the degree of mechanical interaction and stickiness at the skin) will result in a significant reduction in the level of perceived skin wetness when compared to wearing a loose fitting clothing ensemble (which on the contrary will increase the degree of mechanical interaction and stickiness at the skin). The overall aim of this investigation was to demonstrate that it is possible to manipulate significantly the level of perceived skin wetness, independently of the level of (sweat-induced) physical skin wetness, thus unveiling the synthetic nature of this complex sensory experience.

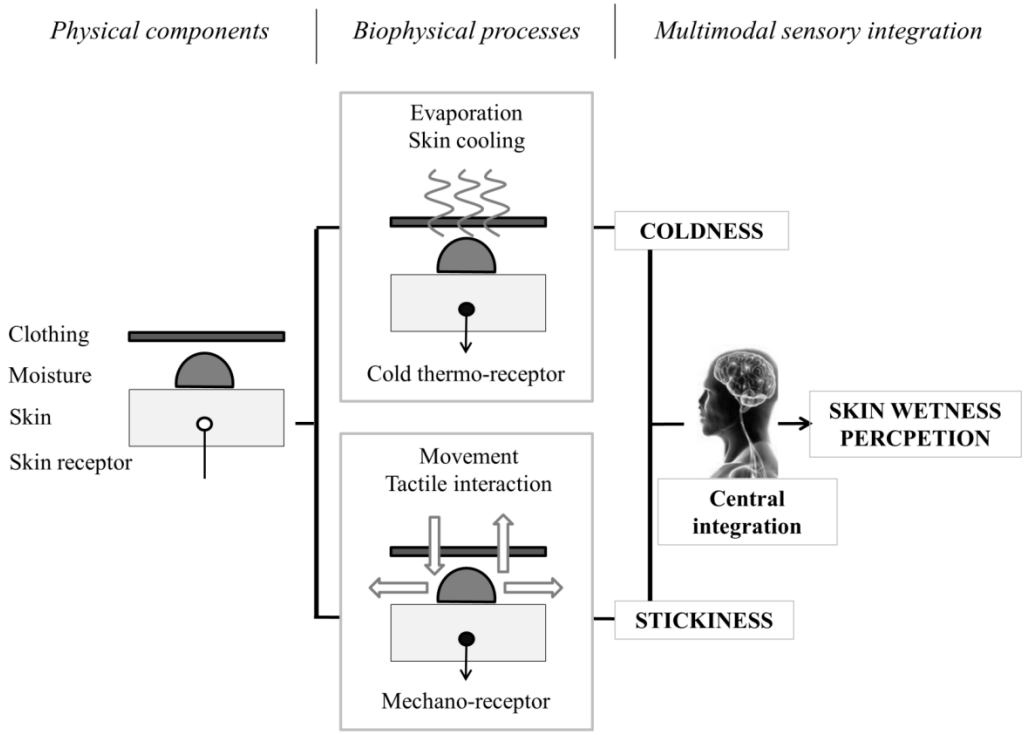


Figure 1: A schematic model of the psychophysical and neurophysiological processes underpinning the sensory experience of skin wetness. The physical components are: skin receptors, skin, moisture (sweat) and clothing. Altogether, these constitute the skin-sweat-clothing system. Within this system, two main biophysical processes occur: 1. evaporation of moisture, which generates skin cooling and thus activation of cold-sensitive skin receptors; 2. movement of moisture, which generates tactile inputs and thus activation of mechano-sensitive skin receptors. From a sensory point of view, the coldness and stickiness experienced due to the afferent inputs of the respective skin receptors are then integrated according to a multimodal sensory process, which, along with learning factors, contribute to give rise to the perception of skin wetness.

10.3 Materials and methods

10.3.1 Participants

Ten healthy male students [age 22 ± 2 years, height 180.3 ± 6 cm, body mass 79.6 ± 10 Kg, chest circumference 88.4 ± 6 cm, waist circumference 77.7 ± 8 cm, arm circumference 25.9 ± 4 cm, thigh circumference 49.4 ± 5 cm, maximum oxygen

consumption (VO_{2max}) 52.8 ± 7 $ml \cdot min^{-1} \cdot kg^{-1}$] volunteered to participate in this study. Inclusion criteria for this study were: 1. no history of cardio-vascular disease, sensory-related disorders and muscle-skeletal injuries in the previous 12 months; 2. being physically active (i.e. performing at least 4 to 6 h of regular exercise per week for at least the last 12 months). All participants gave their informed consent for participation. The test procedure and the conditions were explained to each participant. The study design had been approved by the Loughborough University Ethics Committee and testing procedures were in accordance with the tenets of the Declaration of Helsinki. For a period of 48 h before each trial, the participants were instructed to refrain from strenuous exercise. Furthermore, the participants were asked not to consume caffeine or alcohol 24 h before each trial, and to refrain from food 2 h before each trial.

10.3.2 **Experimental design**

The relationship between physical and perceived skin wetness was investigated during two different conditions specifically designed to produce the same level of physical skin wetness, but to induce a higher and a lower level of skin wetness perception. Each participant completed a pre-test session to assess fitness level and two experimental trials on separate days (with a minimum of 48 h separating tests) in a balanced order: tight fitting trial (TIGHT-FIT) and loose fitting trial (LOOSE-FIT). The experiment was treated as a repeated measures design.

As the aim of this investigation was to test the hypothesis that it is possible to manipulate the level of perceived skin wetness independently of the level of physical skin wetness, participants underwent an incremental exercise protocol performed under conditions of restricted evaporation of sweat from the skin, while wearing either a tight fitting clothing ensemble (associated with a lower level of mechanical interaction and skin friction) or a loose fitting clothing ensemble (associated with a higher level of mechanical interaction and skin friction). To limit the amount of moisture evaporation form the skin (and thus skin cooling), a vapour impermeable, loose fitting clothing ensemble was worn as a second layer on top of both the loose or the tight fitting garments. In this way, by reducing the chances of experiencing skin cooling and thus cold sensations during the exercise protocol, we aimed to isolate the contribution of tactile inputs (interaction skin-sweat-clothing) to the perception of sweat-induced whole-body skin wetness.

10.3.3 **Experimental protocol**

10.3.3.1 Preliminary session

Participants attended one preliminary session to determine their anthropometrical characteristics and aerobic capacity. Each participant's body mass and height, as well as chest, waist, arm and thigh circumferences were measured and recorded. A submaximal fitness test was then performed to estimate individuals' aerobic fitness level (expressed as VO_{2max}) using the Astrand-Rhyming method (Gordon, 2009). The test was completed on a treadmill (Woodway Pps Med, Woodway Incorporated, Waukesha, WI, USA) in a thermo-neutral environment (20 °C T_{air}, 50 % RH) to prevent any thermal strain.

10.3.3.2 Experimental trials

The preliminary session was then followed by the two experimental trials. The experimental trials differed in terms of the first layer of clothing the participants wore during the exercise protocol, being this composed of either a tight fitting, long sleeved top and trousers (Domyos, Oxylane, France; total clothing weight: 466 g) or a loose fitting, long sleeved top and trousers (Domyos, Oxylane, France; total clothing weight: 643 g). The tight and loose fitting test garments were made up of the same fabrics (85 % polyester and 15 % elastane) and had an intrinsic local thermal resistance of 0.112 and 0.140 $m^{2} \cdot KW^{-1}$ respectively. To ensure that the tight fitting clothing ensemble was in full and maximal contact with the skin over the whole body, a size "small" was used both for top and trousers. On the contrary, in order to increase the level of skin-clothing interaction over the whole body, a size "double extra-large" was used both for the top and trousers of the loose fitting clothing ensemble. A pressure sensor (PF2 n°37, +/- 0.1 mmHg, SIXAXES, Argenteuil, France) was used to measure the pressure applied by the tight fitting test garments on three different regions (i.e. thigh, chest and back) of a medium size manikin. The resulting clothing pressure for the tight fitting clothing ensemble was on average 2.5 ± 0.2 mmHg.

The second, vapour impermeable layer of clothing was the same for all conditions and was worn during the exercise protocol to limit evaporation of moisture. This

consisted of a vapour impermeable, loose fitting raglan jacket and trousers (total clothing weight of 427 g). The jacket and trousers were two-layered and 100 % polyester. In the front, the fastener was a zipper that closed to the top of the collar. A placket front was used to prevent air exchange through zipper. The sleeve and legs linings had also tight cuffs to prevent air exchange. When worn on top of the first layer of tight and loose clothing, this resulted in a total whole-body thermal insulation of 0.213 and 0.234 $m^{2}\cdot KW^{-1}$ respectively. Testing of clothing thermal properties were performed on a standing thermal manikin (Newton, Measurement Technology Northwest, USA) with a uniform skin temperature of 34 °C and environmental temperature of 20 °C and 51 % RH (Fig. 2).

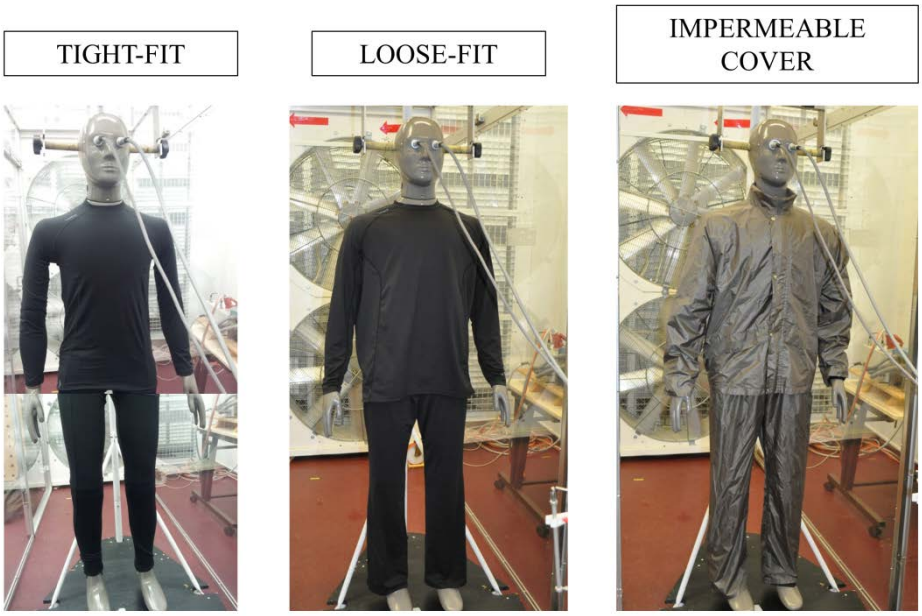


Figure 2: The tight and loose fitting test garments and the vapour impermeable layer of clothing used within the experimental conditions of this study.

Both TIGHT-FIT and LOOSE-FIT experimental trials consisted of 30min instrumentation and stabilization period, followed by a 45-min incremental walking protocol. This consisted of walking on a treadmill (Woodway Pps Med, Woodway Incorporated, Waukesha, WI, USA) at a fixed speed (5Km/h) while the treadmill's inclination was increased by 2 % every 5 min, until a maximum 16 % inclination was reached. This protocol was designed to slowly raise participants' sweat production

and physical skin wetness so that changes in skin wetness perception could be detected with sufficient sensitivity. This was confirmed during extensive piloting performed prior to testing, which indicated this exercise protocol to be effective in inducing a gradual and progressive increase in participants' sweat production, while maintaining the level of body movement to a minimum. All experimental trials were performed in a climatic chamber set for a thermo-neutral exposure (20 °C T_{air}, 50 % RH). These environmental conditions were chosen so that participants' thermal, wetness and comfort sensations would not be primarily influenced by the environment (being this neutral), but rather, by the way participants perceived their body under the vapour impermeable jacket.

On experimental days, participants arrived at the laboratory 30 min before the time scheduled for the experimental trial to allow preparation procedures and stabilization. Participants were first asked to void their bladder and semi-nude body mass was recorded on a digital scale (Sartorius Yacoila, Sartorius AG, Gottingen, Germany; precision 0.01 g). Then, they were instructed to self-insert a rectal thermometer (Grant Instruments Ltd., Cambridge, UK) 10cm beyond the anal sphincter for the measurement of core temperature (T_{rec}). Five iButtons (Maxim, San Jose, USA) were taped to five skin sites on the left side of the body (i.e. cheek, abdomen, upper arm, lower back and back lower thigh) to record local T_{sk} (1min intervals) to be used for the calculation of mean T_{sk}. Four humidity sensors (MSR electronics GmbH, Switzerland) were fixed to a holder and taped with surgical tape to the four skin sites on the right side of the body (i.e. chest, front arm, lateral lower back and front thigh) to record local relative humidity (1min intervals) in order to estimate local skin wetness. Sensors were located ~2 mm from the skin with the sensor tip not covered by tape. Four pairs of pre-gelled electrodes were attached to the same four skin sites as above for the measurement of local galvanic skin conductance (GSC) using the MP35 Biopac Systems (MP35 Biopac Systems, Goleta, California, USA), set to record at 35 Hz and 1-s intervals. The skin conductance was monitored in order to estimate local sudo-motor activity (Vetrugno et al., 2003). Gerrett et al. (2013) have recently proved this measurement to be a reliable indicator of sweat gland activity and intradermal sweat accumulation. Finally, each participant wore a Polar HR monitor (Polar Electro Oy, Kempele, Finland) to recorded heart rate at 10s intervals.

After preparation, and according to the trial, participants wore the first layer of tight or loose fitting long sleeved top and trousers and were asked to rate their thermal,

wetness and comfort sensations, while recording of the physiological parameters was started. Three modified rating scales were used to record individual thermal, wetness and thermal comfort sensations: a 7 points thermal sensation scale (i.e. -3 very cold; -2 cold; -1 cool; 0 neutral; +1 warm; +2 hot; +3 very hot); a 7 points wetness perception scale (i.e. -3 dripping wet; -2 wet; -1 slightly wet; 0 neutral; +1 slightly dry; +2 dry; +3 very dry); a 7 points thermal comfort scale (i.e. -3 very uncomfortable; -2 uncomfortable; -1 slightly uncomfortable; 0 neutral; +1 slightly comfortable; +2 comfortable; +3 very comfortable) (Olesen & Brager, 2004).

After scoring their baseline sensations, participants wore the second layer of clothing (i.e. vapour impermeable jacket and trousers), placed a head band over their forehead (to prevent sweat drippage over the face), and then moved to the treadmill where they started the 45min walking protocol. During the exercise protocol, participants were asked to rate their thermal, wetness and comfort sensations at 5min intervals. Furthermore, as soon as the votes "slightly wet" and "slightly uncomfortable" were reported on the respective wetness and comfort scales, participants were asked to indicate (in the following order): 1. which regions between chest, back, arms and thighs were perceived as wet; 2. which region was perceived as the wettest; 3. which region was perceive as the most uncomfortable. To make rating of regional distribution of wetness and discomfort sensations possible, participants were presented a whole body map (as modified from Lee et al. 2011) with the above mentioned four regions being highlighted by numbers (range: 1-4).

Upon completion of the 45min walking protocol, participants removed all clothing and sensors and semi-nude body mass was once again recorded.

10.3.4 **Measurements and calculations**

Body mass was measured at the beginning and at the end of each experimental trial to determine gross sweat loss in grams (g).

Mean T_{sk} was calculated according to the work of Houdas and Ring (1982) as follow:

$$Mean\ T_{sk} = (cheek\ \times 0.07) + (abdomen \times 0.175) + (upper\ arm \times 0.19) + (lower\ back \times 0.175) + (back\ lower\ thigh \times 0.39)$$

Skin wetness (*w*, dimensionless) is defined as the ratio between the evaporated heat flux from the body caused by regulatory sweating, and the maximal evaporative heat

flux from the body for a totally wet skin. In this study, local skin wetness was estimated for each of the four body regions (which were monitored with humidity sensors) according to Gagge (1937) as follow:

$$w_{local} = \frac{P_{sk} - P_a}{P_{sk,s} - P_a}$$

where P_{sk} is the water vapour pressure at the skin (measured using humidity sensors), Pa is the water vapour pressure in the air, and $P_{sk,s}$ is the saturated water vapour pressure at the skin calculated from skin temperature. P_a was calculated using the following equation:

$$P_a = \left(\frac{RH}{100}\right) \times P_{a,s}$$

where RH is ambient relative humidity and $P_{a,s}$ is saturated water vapour pressure in the air calculated from ambient temperature (T_{amb}) using the following equation (*exp* refers to an exponential function) according to Antoine (1888):

$$P_{a,s} = 0.1exp\left(18.956 - \frac{4030.18}{T_{amb} + 235}\right)$$

P_{sk} and $P_{sk,s}$ were calculated for each skin site using the above equations and by substituting ambient relative humidity with local relative humidity at the skin (measured using humidity sensors), and T_{amb} with local skin temperature.

Whole body wetness (w_{body}) was then calculated using the following equation based on four measurement sites (i.e. chest, front arm, lateral lower back and front thigh) (modified from Mitchell and Wyndham, 1969):

$$w_{body} = (chest \times 0.125) + (upper\ arm \times 0.07) + (lower\ back \times 0.125) + (front\ thigh \times 0.125)$$

Finally, mean GSC was averaged over the four sites (i.e. chest, front arm, lateral lower back and front thigh).

10.4 Statistical analysis

In the present study, the independent variables were condition (i.e. TIGHT-FIT vs. LOOSE-FIT) and time. The dependent variables were HR, T_{rec}, mean T_{sk}, w_{body}, mean GSC, gross sweat losses, thermal, wetness and comfort sensations.

Data were first tested for normality of distribution and homogeneity of variance using Shapiro-Wilk and Levine's tests respectively. With regards to parametric data such as HR, T_{rec}, mean T_{sk}, w_{body}, mean GSC, the main effect and interactions of each independent variable was analysed by a 2 way repeated measure ANOVA, with clothing fit and time as repeated measures variables. When a significant main effect was found, Tukey's post-hoc analyses were performed. Huynh–Feldt or Greenhouse-Geisser corrections were undertaken to adjust the degrees of freedom for the averaged tests of significance. With regards to the gross sweat loss data, these were compared between conditions by means of a paired t-test.

Non-parametric data such as thermal, wetness and comfort sensation scores were analysed by Wilcoxon signed ranks tests (Z) and by Friedman's analysis of variance (X^2). First, the main effect of each independent variable was tested by collapsing the data over condition (2 levels of comparison) and time (10 levels of comparison) respectively. A Wilcoxon signed ranks test was performed for the 2 levels comparison and a Friedman's analysis of variance was performed for the 10 levels comparison. Interactions between variables were investigated using Wilcoxon signed ranks test (post-hoc comparisons).

To investigate the differences in regional wetness perception and discomfort, a frequency distribution analysis was performed. Frequencies were calculated for the number of times each region was perceived as wet, as the wettest and as the most uncomfortable for each condition (i.e. TIGHT-FIT vs. LOOSE-FIT) and analysed by a Chi-square test.

Finally, regression analyses were performed to investigate the relationship between indicators of physical wetness (i.e. w_{body} and mean GSC) and perceived wetness, both for TIGHT-FIT and LOOSE-FIT conditions.

In all analyses, $p<0.05$ was used to establish significant differences. Estimated marginal means and 95 % confidence intervals were used to investigate the main

effects and interactions of the variables. Observed power was computed using α=0.05. Data are reported as mean ± standard deviation. Statistical analysis was performed using IBM SPSS Statistics 19 (IBM, USA).

10.5 Results

10.5.1 Physiological parameters

Figure 3 shows average values for HR, T_{rec}, mean T_{sk}, w_{body}, mean GSC as recorded during the TIGHT-FIT and LOOSE-FIT trials. No significant main effect of clothing fit (i.e. TIGHT-FIT vs. LOOSE-FIT) was found on HR (F= $0.16_{(1, 9)}$, p= 0.7), T_{rec} (F= $0.006_{(1, 9)}$, p= 0.94), mean T_{sk} (F= $0.8_{(1, 9)}$, p= 0.39), w_{body} (F= $0.43_{(1, 39)}$, p= 0.51) and mean GSC (F= $0.43_{(1, 39)}$, p= 0.83). Only a significant effect of time was found on the above mentioned physiological parameters. During the exercise protocol (and similarly between TIGHT-FIT and LOOSE-FIT) participants' HR was observed to increase significantly from an average baseline value of 81.8 ± 3.3 bpm to a maximum of 151.1 ± 5.1 bpm (F= $175.8_{(9, 81)}$, p<0.01); T_{rec} increased significantly from an average baseline value of 37.3 ± 0.1 °C to a maximum of 38 ± 0.1 °C (F= $106.9_{(9, 81)}$, p<0.01); mean T_{sk} increased significantly from an average baseline value of 30.2 ± 0.05 °C to a maximum of 33.5 ± 0.4 °C (F= $92_{(9, 81)}$, p<0.01); w_{body} increased significantly from an average baseline value of 0.26 ± 0.01 to a maximum of 0.95 ± 0.2 (n.d.) (F= $406.2_{(9, 351)}$, p<0.01); mean GSC increased significantly from an average baseline value of 3.1 ± 0.3 µS to a maximum of 18.8 ± 1.3 µS (F= $118.7_{(9, 351)}$, p<0.01). With regards to gross sweat loss, no significant differences were found between the recorded body mass changes for the TIGHT-FIT (721 ± 290 g) and LOOSE-FIT trials (758 ± 140 g) (mean difference= 37 g; 95% CI= -250, 176 g; t= -0.38; *two tailed* p= 0.7).

All in all, these results indicate that the protocol designed was effective in inducing a significant increase in participants' sweat production (as indicated by mean GSC) and physical skin wetness (as indicated by w_{body}), with no differences between TIGHT-FIT and LOOSE-FIT conditions.

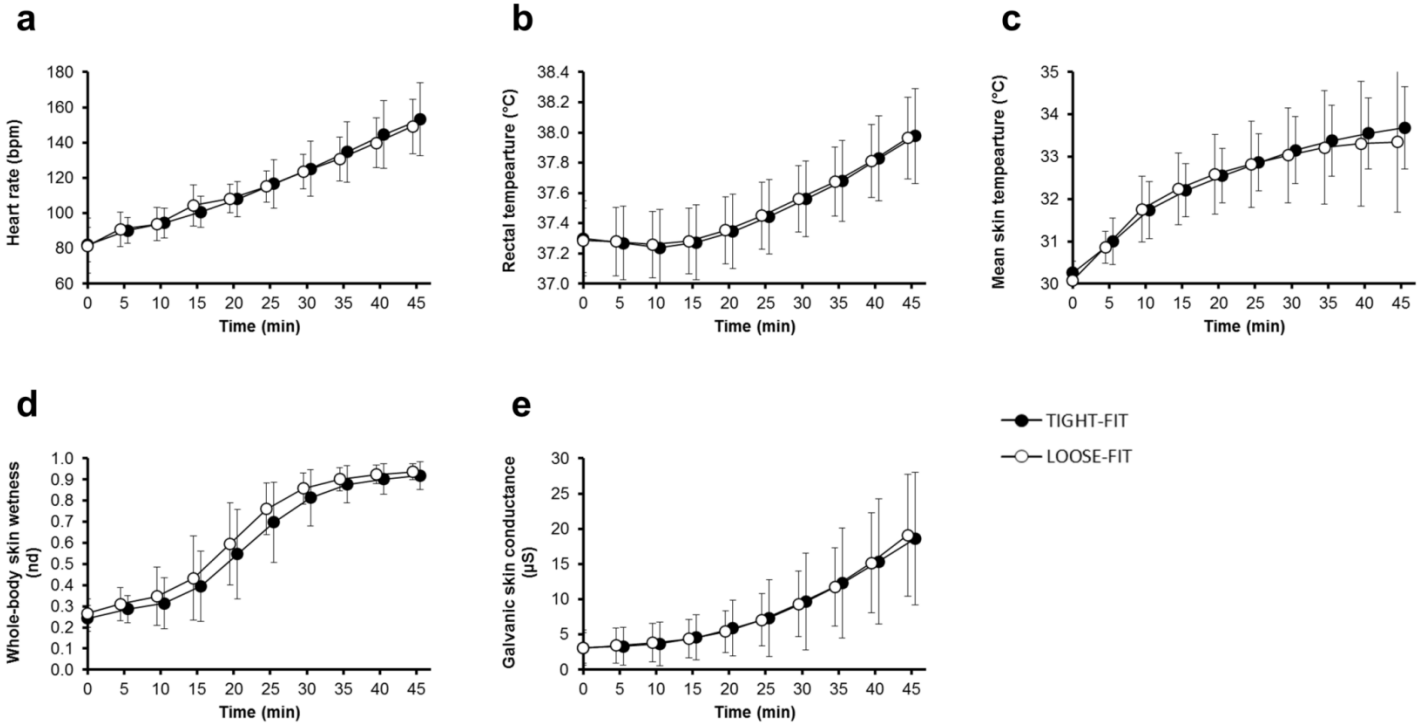


Figure 3: Average values (n=10) for heart rate (a), core (rectal) temperature (b), mean skin temperature (c), whole body skin wetness (d) and galvanic skin conductance (e), as recorded during the TIGHT-FIT and LOOSE-FIT trials. Data are reported as mean ± standard deviation. A main tendency is illustrated. A significant increase in participants' sweat production (as indicated by galvanic skin conductance) and physical skin wetness (as indicated by whole body skin wetness) can be observed. This was not different between TIGHT-FIT and LOOSE-FIT, thus confirming the effectiveness of the experimental protocol designed in inducing the same level of physical skin wetness during both trials.

10.5.2 **Perceptual parameters**

Figure 4 shows average values for thermal, wetness and comfort sensations as recorded during the TIGHT-FIT and LOOSE-FIT trials. No significant main effect of clothing fit (i.e. TIGHT-FIT vs. LOOSE-FIT) was found on thermal (Z=0.97, *p*=0.33) and comfort sensations (Z=-0.37, *p*=0.7). These varied significantly over the time (and similarly between TIGHT-FIT and LOOSE-FIT), with thermal sensations going from -0.4 ± 0.7 (label range: Neutral to Cool) to +2.5 ± 0.7 (label range: Hot to Very hot) [X^2(9, N=20)= 159.8, p<0.01], and thermal comfort going from +1 ± 1.5 (label range: Slightly comfortable) to -2.3 ± 0.8 (label range: Uncomfortable to Very uncomfortable) [X^2(9, N=20)= 159.5, p<0.01].

Contrary to what observed for thermal and comfort sensations, the clothing fit (i.e. TIGHT-FIT vs. LOOSE-FIT) had a significant effect on skin wetness perception (Z=-2.7, *p*<0.01), with the TIGHT-FIT trial resulting in overall significantly "less wet" perceptions (mean= -0.2 ± 1.8; 95% CI= -0.5, +0.1; label range: Slightly wet to Slightly dry) than the ones recorded during the LOOSE-FIT trial (-0.5 ± 1.7; 95% CI= -0.8, -0.1; label range: Slightly wet to Neutral). The effect of clothing fit on skin wetness perception showed a significant interaction with time. Indeed, although during both conditions skin wetness perception increased significantly over the time (from +1.4 ± 1.4 to -2.4 ± 0.5; label range: Dry to Dripping wet) [X^2(9, N=20)= 164.6, p<0.01], during the TIGHT-FIT trial skin wetness perception was significantly reduced 20min after the exercise was initiated when compared to the LOOSE-FIT trial (Z=-1.9, *p*=0.047) (see fig. 4c), despite no differences in indicators of physical wetness (i.e. w_{body} and mean GSC) were observed at any point in time between conditions (see fig. 3d and 3e).

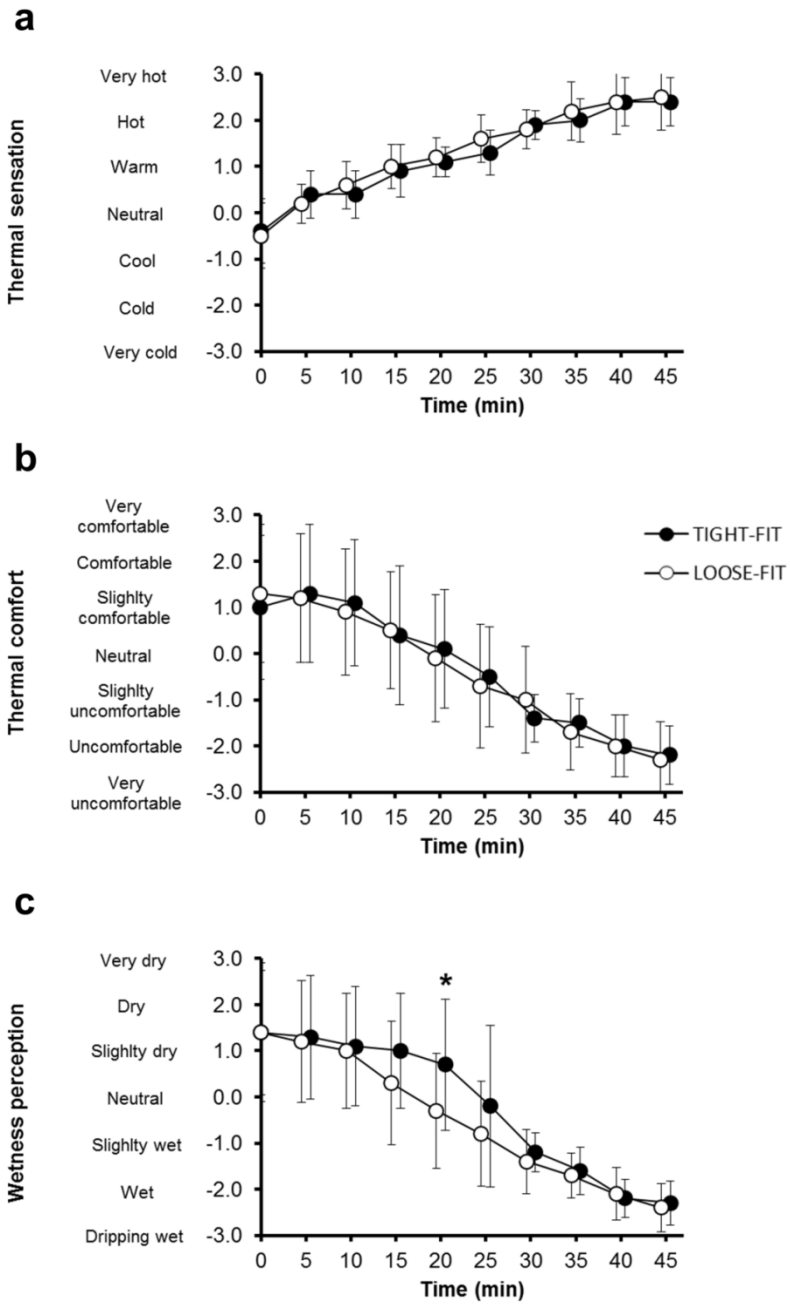


Figure 4: Average values (n=10) for thermal (a), comfort (b) and wetness perception (c) as recorded during the TIGHT-FIT and LOOSE-FIT trials. Data are reported as mean ± standard deviation. A main tendency is illustrated. Despite during both TIGHT-FIT and LOOSE-FIT trials, the level of physical skin wetness did not differ at any time point, with regards to the perception of skin wetness, this was overall significantly reduced during the TIGHT-FIT as opposed to the LOOSE-FIT trial. This main effect significantly interacted with time, 20 min after the exercise protocol was initiated (*: $p<0.05$). No differences in thermal and comfort sensations were recorded at any time between trials.

The regression analysis performed between indicators of physical wetness (i.e. w_{body} and mean GSC) and perceived skin wetness provided further support to the significant effect of clothing fit on skin wetness perception (Fig. 5). The relationship between w_{body} and perceived skin wetness was found to be statistically significant, both for the TIGHT-FIT (cubic curve estimation; $p<0.001$; $r^2= 0.98$) and LOOSE-FIT trial (linear curve estimation; $p<0.001$; $r^2= 0.94$). However, and as shown in figure 5a, during the TIGHT-FIT trial, this relationship was shifted to the right in the middle part of the curve. This indicated that, when w_{body} ranged from ~0.4 to ~0.8 (n.d.), skin wetness perception was significantly reduced when wearing tight as opposed to loose fitting garments.

The relationship between mean GSC and perceived skin wetness (fig. 5b) was also found to be statistically significant, both for the TIGHT-FIT (cubic curve estimation; $p<0.001$; $r^2= 0.98$) and LOOSE-FIT trial (cubic curve estimation; $p<0.001$; $r^2= 0.99$). However, and similarly to the w_{body}, during the TIGHT-FIT trial, this relationship was shifted to the right in the middle part of the curve. This indicated that, when the mean GSC ranged from ~4.5 to ~9.5 µS, skin wetness perception was significantly reduced when wearing tight as opposed to loose fitting garments.

All in all, these results indicate that, the level of perceived skin wetness was significantly reduced during the TIGHT-FIT when compared to the LOOSE-FIT trial, independently from the level of physical skin wetness.

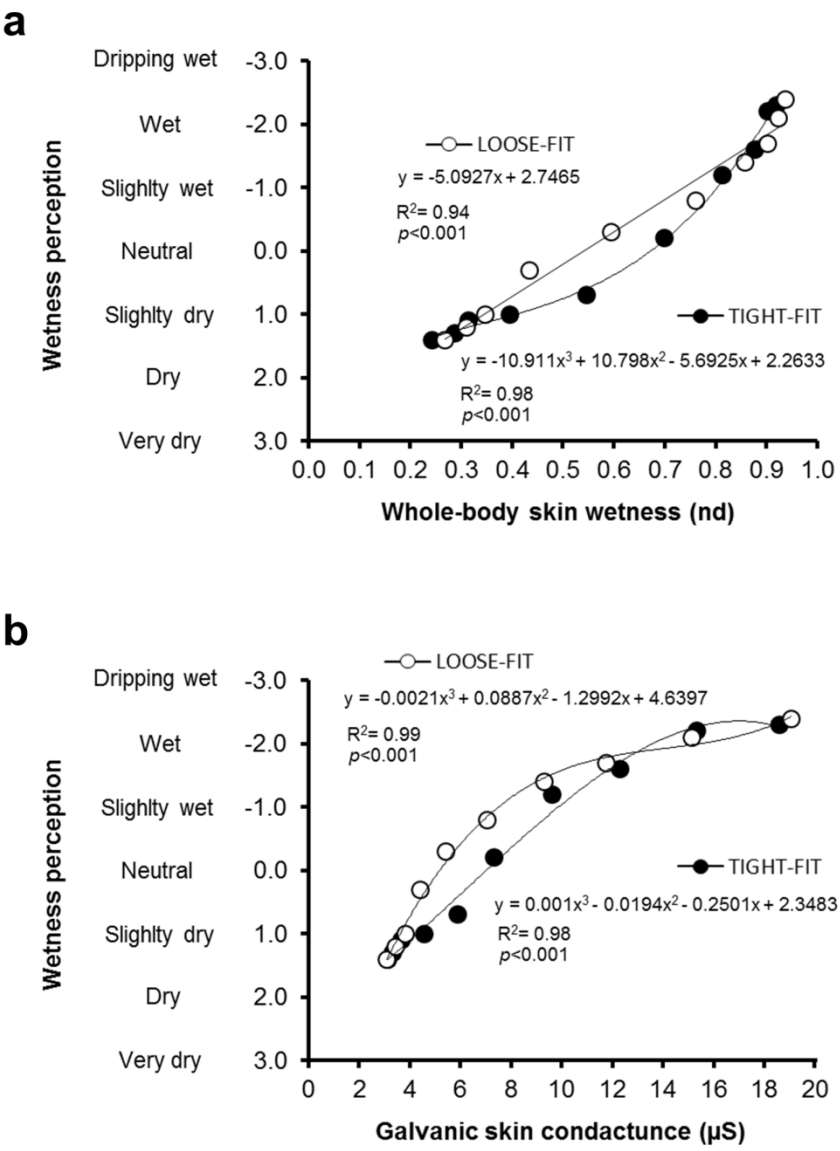


Figure 5: Regression analyses illustrating the relationship between whole body skin wetness and perceived skin wetness (a) as well as galvanic skin conductance and perceived skin wetness (b) for both TIGHT-FIT and LOOSE-FIT trials. Each data point represents group average (n=10) for a particular time point of the exercise protocol (each time point was calculated based on 5 min average). Two main tendencies are illustrated. Firstly, skin wetness perception showed a statically significant and positive relationship with both whole body skin wetness and galvanic skin conductance. Secondly, the slope of this relationship was significantly influenced by the clothing fit. This indicated that when wearing tight fitting clothing, a higher level of physical wetness was required to induce the same level of perceived wetness as observed when wearing loose fitting clothing.

10.5.2.1 Regional skin wetness perception, wettest and most uncomfortable body region

As well as for the perception of whole body skin wetness, the clothing fit had a significant effect on the local skin wetness perception. The Chi-square analysis indicated that the clothing fit had a main significant effect on all the regions investigated, with the overall frequency of local skin wetness perception being significantly reduced during the TIGHT-FIT as compared to the LOOSE-FIT trial, either for the chest (-11 %; Pearson Chi-square= 25.3; $p<0.001$), back (-7 %; Pearson Chi-square= 10.3; $p<0.01$), arm (-8 %; Pearson Chi-square= 13.8; $p<0.001$) and thigh (-9 %; Pearson Chi-square= 19.8; $p<0.01$). A significant interaction of clothing fit with time was observed, with the frequency of perceived skin wetness showing a significantly delayed onset during the TIGHT-FIT than during the LOOSE-FIT trial for all the regions investigated (fig. 6).

With regards to the region perceived as the wettest, the Chi-square analysis indicated that overall, during the TIGHT-FIT trail, the back was more frequently perceived as the wettest region (47 %), followed by the chest (29 %), arm (12 %) and thigh (12 %) (Pearson Chi-square= 44.7; $p<0.001$). During the LOOSE-FIT trial, the back was overall more frequently perceived as the wettest region (41 %), followed by the chest (25 %), arm (23 %) and thigh (11 %) (Pearson Chi-square= 24.3; $p<0.001$). The time-frequency distribution of how often each region was perceived as the wettest is shown in figure 7a and 7b. It should be noted that, although during both TIGHT-FIT and LOOSE-FIT trials the back and chest were amongst the regions which were more frequently perceived as the wettest, during the LOOSE-FIT trial the arms were also frequently perceived as the wettest region.

With regards to the region perceived as the most uncomfortable, the Chi-square analysis indicated that overall, during the TIGHT-FIT trail, the chest was more frequently perceived as the most uncomfortable region (40 %), followed by the back (31 %), arm (21 %) and thigh (8 %) (Pearson Chi-square= 30.2; $p<0.001$). During the LOOSE-FIT trial, the chest was overall more frequently perceived as the most uncomfortable region (41 %), followed by the back (24 %), arm (18 %) and thigh (16 %) (Pearson Chi-square= 20.7; $p<0.001$). The time-frequency distribution of how often each region was perceived as the wettest is shown in figure 6c and 6d. It should be noted that, although during both TIGHT-FIT and LOOSE-FIT trials the chest and back were amongst the regions which more frequently were perceived as the most

uncomfortable, during the LOOSE-FIT trial the thighs were also frequently perceived as the most uncomfortable region.

All in all, these results indicated that, not only did the TIGHT-FIT condition reduce the overall level of perceived skin wetness, independently from the level of physical skin wetness; but also, this had an effect on the regional sensitivity to wetness and comfort, with regions such as the arms and thighs being less frequently perceived as wet and uncomfortable during the TIGHT-FIT as opposed to the LOOSE-FIT trial. It is worth mentioning that during both conditions, the torso (i.e. chest and back) was more frequently reported as wetter and as more uncomfortable than the limbs (i.e. arms and thighs).

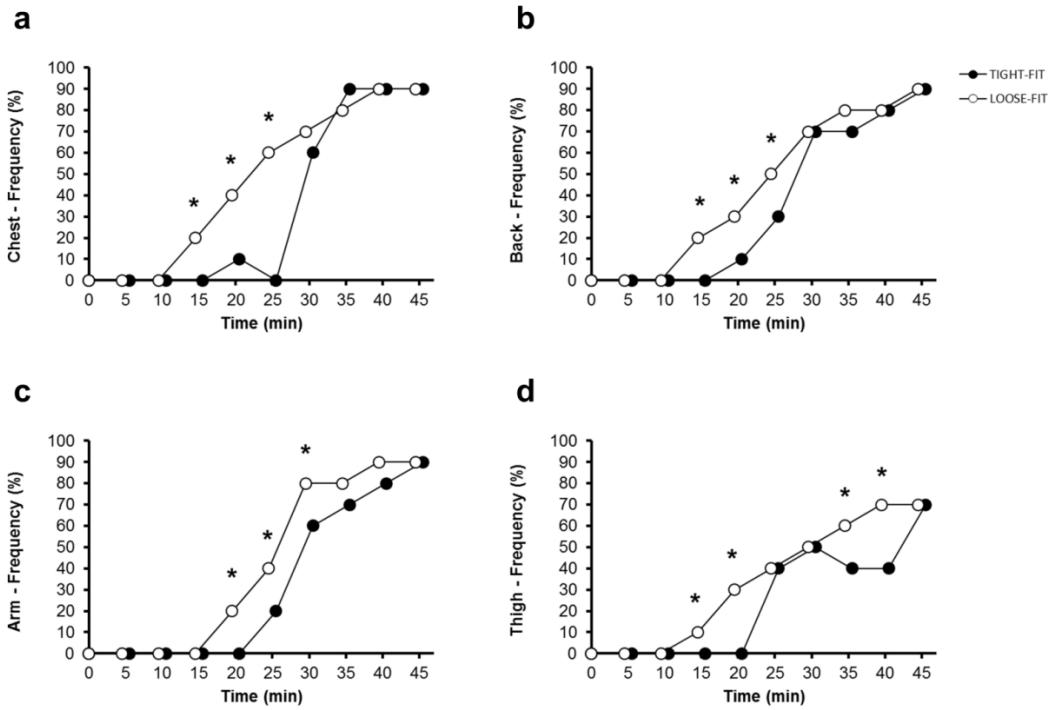


Figure 6: Time frequency distribution (%) of regional wetness perceptions based on the number of times the chest (a), back (b), arms (c) and thighs (d) were reported as being wet at each time point, during the TIGHT-FIT and LOOSE-FIT trials. A main tendency is illustrated. The frequency of perceived skin wetness show a significantly delayed onset during the TIGHT-FIT than during the LOOSE-FIT trial for all the regions investigated (*: $p<0.05$).

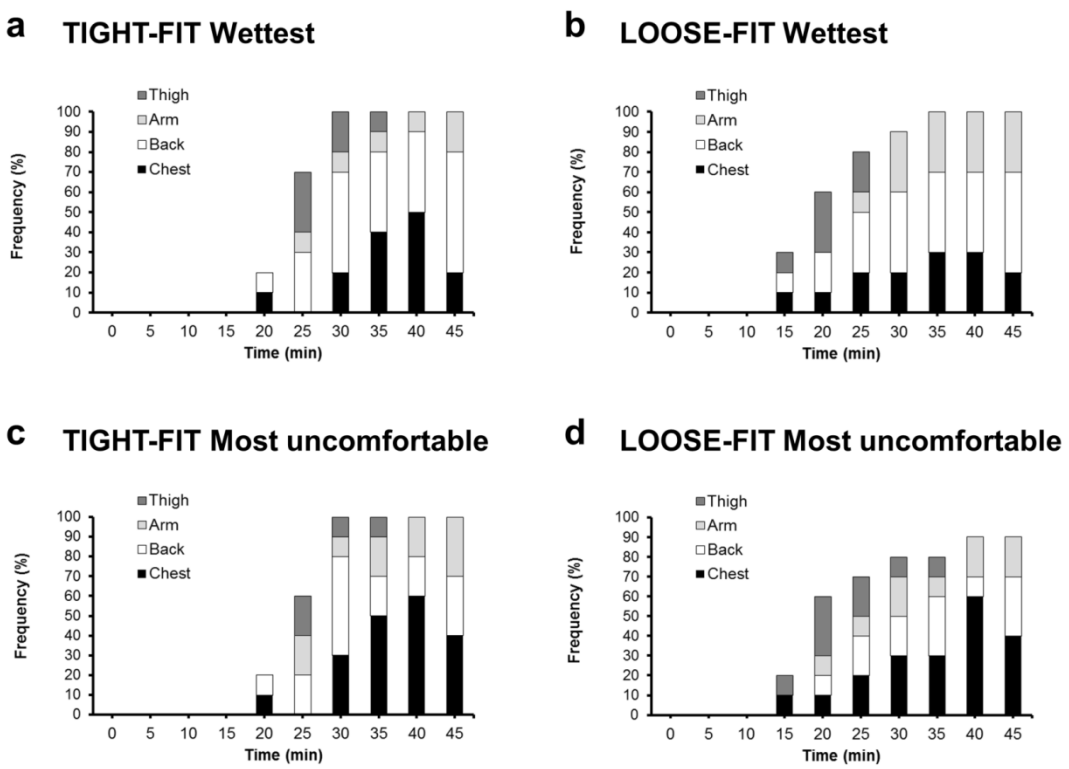


Figure 7: Time frequency distribution (%) based on the number of times each region amongst chest, back, arms and thighs was reported as being the wettest (panels a & b) and most uncomfortable region (panels c & d) at each time point, during the TIGHT-FIT and LOOSE-FIT trials. Two main tendencies are illustrated. Firstly, although during both TIGHT-FIT and LOOSE-FIT trials the back and chest were amongst the regions which were more frequently perceived as the wettest, during the LOOSE-FIT trial the arms were also frequently perceived as the wettest region (compare panels a & b). Secondly, although during both TIGHT-FIT and LOOSE-FIT trials the chest and back were amongst the regions which more frequently were perceived as the most uncomfortable, during the LOOSE-FIT trial the thighs were also frequently perceived as the most uncomfortable region (compare panels c & d).

10.6 Discussion

The aim of this study was to investigate the relationship between (sweat-induced) physical and perceived skin wetness and to test the hypothesis that the level of perceived skin wetness can be significantly manipulated, independently of the level of physical skin wetness, by changing the tactile interaction between skin and clothing. We hypothesised that, during an incremental exercise protocol performed under conditions of restricted evaporation of sweat from the skin, wearing a tight fitting clothing ensemble (which limited the degree of mechanical interaction and stickiness at the skin) would result in a significant reduction in the level of perceived skin wetness when compared to wearing a loose fitting clothing ensemble (which on the contrary increased the degree of mechanical interaction and stickiness at the skin). This, despite the exercise protocol being designed to induce the same level of physical skin wetness during both tight and loose fitting conditions.

The outcomes of this study have confirmed this hypothesis. Although during both TIGHT-FIT and LOOSE-FIT trials the level of physical wetness (w_{body}) was raised in the same pattern from a minimum of 0.24 ± 0.1 (n.d.) to a maximum of 0.92 ± 0.1 (n.d.) (TIGHT-FIT) and from a minimum of 0.26 ± 0.1 (n.d.) to a maximum of 0.94 ± 0.1 (n.d.) (LOOSE-FIT) (see fig. 3d), with average maximal values which correspond to an almost fully wet skin (Nishi and Gagge, 1977); and although the time-dependent increase in the sudomotor activity (as indicated by the mean GSC) was equal between conditions (see fig. 3e); the reduced mechanical interaction and skin friction generated by the TIGHT-FIT clothing ensemble resulted in significantly lowering the overall level of perceived wetness as well as delaying the onset of skin wetness perception, both at a whole-body (see fig. 4c) and at a regional level (see fig. 6).

In summary, and for the first time to our knowledge, these results contribute to provide evidence for the fact that: 1. under conditions of sweat-induced whole-body wetness, if no skin cooling occurs, the perception of skin wetness is primarily driven by the level of tactile interaction between skin, sweat and clothing; 2. by manipulating this interaction (e.g. by changing the clothing fit), skin wetness perception can be significantly changed, independently of the level of physical wetness on the skin.

10.6.1 **Physical vs. perceived skin wetness: whole-body level**

The novelty and implications of the outcomes of this study is two-fold. Firstly, these findings confirm what is expected based on a neurophysiological model of wetness perception that we have recently developed (Filingeri et al., 2014b) (see Chapter Nine). That is, to characterize their perception of skin wetness, humans rely on specific sensory inputs which, if artificially manipulated (e.g. through a clothing intervention), can lead to a change in perception which is independent on its physical components (i.e. the level of physical wetness). Secondly, these findings expand our understanding of how humans sense skin wetness, not only when passively in contact with cold-dry and cold-wet surfaces (Filingeri et al., 2013; 2014a; 2014b; 2014c; 2014d) (see Chapter Four, Five, Seven, Eight and Nine), but also when actively producing sweat. This could provide mechanistic evidence for what was observed in those previous studies (Fukazawa and Havenith, 2009; Lee et al., 2011; Gerrett et al., 2013) which have reported that participants could perceive sweat-induced skin wetness even in the absence of any drops in skin temperature and any cold sensations. Our previous work on the neurophysiology of wetness perception has led to the development of a specific sensory processing model to help understanding how humans sense wetness on their skin (Filingeri et al., 2014b) (see Chapter Nine). Our results have demonstrated that, in order to sense skin wetness, humans rely on the cold and tactile sensations experienced when physically wet, and in this respect, we have observed that experiencing coldness seems to be a primary contributor (Filingeri et al., 2013; 2014a; 2014c). This, as one of the common features of skin wetness is to cool the skin down via evaporation, thus triggering cold sensations (Candas et al., 1979). In support of the above, we have recently shown that during the static contact with a warm-wet surface (with a temperature warmer than the skin) our participants did not perceive any local skin wetness, as no skin cooling, and thus no cold sensations, occurred (Filingeri et al., 2014d). However, the above referenced sensory framework for the perception of wetness was developed in the context of passive skin-contacts with wet surfaces, opening to the question of how skin wetness is sensed when sweat is actively produced by the body and clothing is worn. Indeed, and as apparently in contrast with our sensory model, previous studies investigating sweat-induced wetness perception have repeatedly shown that individuals seem to be able to sense skin wetness even in the absence of any skin cooling and cold

sensations (Fukazawa and Havenith, 2009; Lee et al., 2011; Gerrett et al., 2013), thus suggesting that the role of tactile inputs can potentially be more critical than coldness when skin wetness is due to sweating rather than to contact with wet surfaces.

To clarify this point, the experimental conditions of the present study were therefore designed to isolate the contribution of tactile components (see Fig. 1) to the perception of sweat-induced whole-body skin wetness. This was achieved by: 1. gradually raising the level of sweat-induced physical skin wetness (see Fig. 2d and 2e); 2. avoiding any drop in skin temperature (see Fig. 2c) (which could have triggered cold sensations; see Fig. 3a) 3. manipulating the level of mechanical interaction and friction (by changing the clothing fit) between skin, sweat and clothing. By doing this, we observed that a lower level of skin-sweat-clothing interaction (i.e. during the TIGHT-FIT trial) resulted in a significantly reduced perception of wetness. This finding indicates that the tactile stimuli occurring at the skin surface (i.e. mechanical interaction within the skin-sweat-clothing system) seem to be predominant in driving the perception of wetness under conditions of sweat-induced whole-body wetness and reduced evaporative cooling of sweat from the skin while wearing clothing. Therefore, when investigating the relationship between physical and perceived wetness, the clothing fit should be taken into account as a significant component in the skin-sweat-clothing system (see fig. 1). Indeed, changes in the fit could alter the tactile inputs arising from the contact of the skin with clothing, thus influencing the perception of skin wetness.

The relevance of the interaction skin-sweat-clothing in influencing the perception of wetness is not entirely surprising, and could be dependent upon the synthetic nature of this complex perception (Bentley, 1900). Being not provided with specific humidity receptors (Clark and Edholm, 1985), humans learn to perceive the wetness experienced when the skin is in contact with a wet surface or when sweat is produced (Bergmann Tiest et al., 2012a) through a complex multisensory integration (Driver and Spence, 2000) of thermal (i.e. heat transfer) and tactile (i.e. mechanical pressure and skin friction) inputs generated by the interaction between skin, moisture and (if donned) clothing (Fukazawa and Havenith, 2009). As previously shown by Bergmann Tiest et al. (2012), when thermal cues provide insufficient sensory inputs (e.g. absence of coldness), individuals seem to use mechanical cues (e.g. stickiness) to aid them in the perception of wetness (Bergmann Tiest et al., 2012a). Although referring to local skin wetness (i.e. participants haptically interacted with local wet

stimuli) the findings of Bergmann Tiest et al. (2012) support what was observed in the present study, that is, in conditions of sweat-induced whole-body wetness (and when wearing clothing) the mechanical and tactile interaction at the skin surface are predominant in driving the perception of skin wetness.

When the skin is exposed to external stimuli, surface' textures and properties are usually discriminated based on the amount of skin displacement as well as the rate of movement of the stimuli on the skin (Gwosdow et al., 1986). For example, when in contact with fabrics, the level of skin wetness has been shown to increase the amount of friction within the skin-clothing system, a fact which in turn may alter the sensations arising from the skin's mechanical contact with the fabric (Gwosdow et al., 1986). Gwosdow et al. (1986) have observed that increases in physical skin wetness result in increases in the frictional force required to pull a fabric across the skin, with this being positively correlated with the level of subjective displeasure experienced. Therefore, and in line with the above, the results of the present study have confirmed that, by reducing the level of skin friction (due to the lower chances for the garments to move across the skin) the tight fitting clothing ensemble resulted in significantly lowering the perception of skin wetness independently from the level of physical wetness.

The critical role of tactile stimuli occurring on the skin' surface in the perception of wetness, is in line with the neurophysiological model of skin wetness sensitivity that we have recently developed based on one of our most recent studies, in which we observed participants showing a higher discriminatory ability to perceive the local skin wetness of a wet test fabric when they were allowed a dynamic (i.e. resulting in increased tactile inputs) as opposed to a static interaction with the stimulus (Filingeri et al., 2014b) (see Chapter Nine). Therefore, the outcomes of this study expand our understanding of the neurophysiological and psychophysical mechanisms underlying humans' ability to sense wetness on their skin. Interestingly, these mechanisms (i.e. integration of thermal and tactile sensory cues) appear to be remarkably consistent (at least conceptually) regardless of the modality for which skin wetness is experienced, i.e. whether due to passive contact with a wet stimulus or due to active production of sweat. From a mechanistic standpoint, this could be explained by the fact that, independently from the modality (passive exposure vs. active sweat production), when the skin becomes wet (and clothing is worn), the components necessary for sensing wetness (i.e. skin, moisture/sweat, external stimulus) and the

resulting sensory inputs (i.e. thermal and tactile) will always be the same. In other words, skin wetness perception will always be the result of a central integration of thermal and tactile interaction between the skin and the wet stimulus (see Fig. 1). Therefore, by either assessing or manipulating these sensory cues, skin wetness perception can be confidently predicted also within conditions of sweat-induced whole-body wetness.

10.6.2 **Physical vs. perceived skin wetness: regional level**

The reduced skin friction resulting from the tight fitting clothing ensemble reduced the perception of skin wetness significantly, not only at a whole body level, but also regionally. Amongst the region investigated, the limbs (i.e. arm and thighs) were indeed less frequently perceived as wet during the TIGHT-FIT as opposed to the LOOSE-FIT trial. Interestingly, this finding highlights what seems to be the ability of humans to regionally discriminate their sensation of skin wetness, despite being not provided with specific humidity receptors on the skin.

Within the experimental conditions of this study, we observed that during both TIGHT-FIT and LOOSE-FIT trials, the back and chest (as opposed to arm and thighs) were overall more frequently perceived as the wettest regions. This is in line with what observed by Lee et al. (2011), who have showed that when asked, individuals reported the torso (i.e. chest and back) to be the region more often perceived as wet during rest and moderate exercise in 25 and 32 °C Tair and 50 % RH. Also, this outcome seems to be confirmed by the work of Ackerley et al. (2012), who have recently shown that when wet stimuli with different moisture contents (range: 20-160 µl over a 24cm^2 surface) were applied to different body regions, individuals were able to differentiate between moisture levels, with a tendency of the back as being amongst the most sensitive region to wetness. Finally, we have recently demonstrated that due to its higher thermosensitivity to cold, the (lower) back seems to be more sensitive to skin wetness (Filingeri et al., 2014a) (see Chapter Seven).

The fact that the torso was more frequently perceived as wet than the limbs is in apparent contrast with the findings of Fukazawa and Havenith (2009) who reported that this body region presented a lower sensitivity to wetness than the limbs. A potential explanation for these apparently contrasting results could be due the differences in the approaches used by these studies, being these either qualitative (i.e. sensation-oriented) or quantitative (i.e. sensitivity-oriented).

In the study by Lee et al. (2011) (as well as in the present study), participants' ability to regionally discriminate skin wetness was tested by analysing the frequency of wetness scores reported for each body region during conditions of natural sweat distribution (i.e. qualitative approach). This approach considered the subjective perception of wetness as the primary variable for comparing different regions. On the contrary, Fukazawa and Havenith (2009) calculated local *w* values for which comfort was no longer maintained (during conditions of artificially manipulated sweat distribution). This value was then used as a threshold to compare regional sensitivity (i.e. quantitative approach). This approach considered the sensitivity to wetness (i.e. changes in perception for a given change in the physical *w*) as the primary variable to be used for comparing different regions.

In light of the above, it is therefore clear that the two types of studies targeted different variables (sensation vs. sensitivity) which, although providing information on regional differences in wetness perception, in fact refer to different components of the relationship between stimulus (e.g. physical *w*) and resulting sensation (e.g. wetness perception). Indeed, the fact that the torso was perceived as wetter than the limbs [as observed in Lee et al. (2011) as well as in the present study] does not necessary imply that this region presented higher sensitivity to skin wetness [as shown by Fukazawa and Havenith (2009)]. The more frequent perception of wetness recorded for the torso could just indicate that in the whole, this region prevailed in terms of the absolute magnitude of the sensation generated by the presence of sweat on the skin. Indeed, in natural conditions (and under higher metabolic rates), owing to its higher sweat rate than the limbs (Smith and Havenith, 2011), the torso will prevail in the amount of sweat produced, and potentially, in the sensory inputs (i.e. thermal and tactile) generated as a result of the greater moisture levels produced on this large skin region. Hence, although the limbs could present an intrinsically greater sensitivity to wetness [as shown by Fukazawa and Havenith (2009)], the torso is likely to be overall and more frequently experienced as wetter due to: 1) a larger sweat production; 2) a resulting greater volume of moisture present the skin; 3) a resulting larger number of skin receptors which could be concurrently stimulated and which could ultimately contribute to a greater perception of wetness via spatial summation.

This potential explanation is supported by the findings of Gerrett et al. (2013) who have observed that, despite in conditions of natural sweat distribution the limbs

appear to be more sensitive to skin wetness, the overall magnitude of wetness perception and thermal discomfort was ultimately higher for the torso than for the arms and legs (Gerrett et al., 2013). The authors suggested that this area could present higher rates of discomfort and wetness perception as a combination of its intrinsic sensitivity to sweat as well as the amount of sweat effectively present on the skin (Gerrett et al., 2013). As the latter is directly related to local sweat rate, the fact that the torso has been repeatedly shown to have some of the highest sweat rates on the body (Smith and Havenith, 2011) could provide further support to the above. Therefore, although the need for mixed experimental approaches (combining qualitative and quantitative measurements) translates into the results of this study being not conclusive, in this context, the hypothesis of the torso being a region which ultimately prevails in regionally driving the perception of wetness, appears to be consistent with previous literature.

10.7 Conclusions

We conclude that, under conditions of sweat-induced whole-body wetness while wearing clothing, if no skin cooling occurs, skin wetness perception is primarily driven by the level of tactile interaction between skin, sweat and clothing. In this respect, by manipulating this interaction (e.g. changing the clothing fit), skin wetness perception can be significantly altered, independently from the level of physical wetness on the skin. These findings confirm the synthetic nature of the perception of skin wetness. Furthermore, these expand our understanding of the neurophysiological and psychophysical mechanisms underlying humans' ability to sense wetness on their skin. Interestingly, these mechanisms (i.e. integration of thermal and tactile sensory cues) appear to be remarkably consistent (at least conceptually) regardless of the modality for which skin wetness is experienced, i.e. whether due to passive contact with a wet stimulus or due to active production of sweat. From an applied point of view, due to the primary role of skin wetness on the development of thermal and clothing discomfort, the implications of our findings could be highly relevant for protective and sport clothing design.

11 CHAPTER ELEVEN – Summary and Conclusions

11.1 Summary

11.1.1 Conditions of skin wetness perception induced by the contact with an external stimulus

1. When the application of cold-dry stimuli on participants' hairy skin produced a drop in skin temperature ranging between 1.4 and 4.1 °C with a cooling rate of 0.14 to 0.41 $°C \cdot s^{-1}$, an illusion of skin wetness perception was evoked (note: 4.1 °C was the highest value tested and thus this is not necessarily the upper cooling limit for skin wetness perception); when cold-dry stimulations produced a drop in skin temperature of 0.2 to 0.7 °C with a cooling rate of 0.02 to 0.07 $°C \cdot s^{-1}$, skin wetness perception was little evoked and decreasing thermal sensations prevailed (Chapter Four).

2. Cold-dry stimulations inducing drops in skin temperature ranging between 0.6 and 4°C with skin cooling rates of 0.06 to 0.4 $°C \cdot s^{-1}$ were shown to evoke artificial skin wetness perceptions, with colder stimuli resulting in a higher frequency and magnitude of wetness perception (note: 4 °C was the highest value tested and thus this is not necessarily the upper cooling limit for skin wetness perception). However, it was observed that the application of stimuli with a higher mechanical pressure on the skin (10 vs. 7 kPa) reduced the frequency of times artificial wetness perceptions were evoked. Also, it was found that cold-dry stimuli with the same difference from actual skin temperature, were perceived as being wetter during exercise performed in the warm environment than during rest in the same environment, as well as than during exercise in the thermo-neutral one (Chapter Five).

3. The application of water drops with volumes of 20, 60 and 120 µl on the forearm skin resulted in changes in skin temperature (range: -2.2 to -4.6 °C) which were remarkably similar to the changes in skin temperature resulting from dry contact cooling (range: -1.4 to -4.1 °C), which were observed to induce an illusion of local skin wetness (Chapter Six).

4. The existence of regional differences in cutaneous thermosensitivity to cold has been shown to translate into significant and matching regional differences in cutaneous wetness perception across the human torso. Interestingly, these regional sensory patterns were observed to be independent from the magnitude of local skin cooling. In other words, the regions in which the stimulus resulted in greater skin cooling (i.e. lateral chest) were not necessarily the ones in which the stimulus was perceived as colder, wetter or more unpleasant (Chapter Seven).

5. Warm temperature stimuli have been shown to suppress the perception of skin wetness during initial static contact with a wet surface (Chapter Eight).

6. It was found that individuals perceived warm-wet and neutral-wet stimuli as significantly less wet than cold-wet ones, even when these were characterized by the same moisture content. Also, it was shown that when cutaneous cold and tactile sensitivity was diminished by a selective reduction in the activity of A-nerve afferents, wetness perception was significantly reduced. Finally, a trend was observed with the extent of perceived wetness being higher on the hairy than on the glabrous skin. This seems to be due to the structural (i.e. glabrous skin presents thicker stratum corneous and higher thermal insulation) and functional differences (i.e. glabrous skin presents higher density of mechano receptors while hairy skin has a higher density of thermoreceptors than for thermal sensation) between hairy (more of a thermo-sensory organ) and glabrous skin (more of an organ for heat exchange) (Chapter Nine).

7. Based on a concept of perceptual learning and Bayesian perceptual inference, the first neurophysiological model of cutaneous wetness sensitivity centred on the multisensory integration of cold and mechano sensitive skin afferents was developed in order to explain how humans sense warm, neutral and cold skin wetness (Chapter Nine).

11.1.2 **Conditions of skin wetness perception induced by sweating**

1. Under conditions of sweat-induced whole-body wetness, if no skin cooling occurs, skin wetness perception appeared to be primarily driven by the level of tactile interaction between skin, sweat and clothing. In this respect, by manipulating this interaction (e.g. changing the clothing fit), skin wetness perception was significantly altered, independently from the level of physical wetness on the skin (Chapter Ten).

11.2 Conclusions

1. The findings of this Thesis confirm the synthetic nature of the perception of skin wetness. It is concluded that it is not the contact of the skin with moisture per se, but rather the integration of particular sensory inputs which drives the perception of skin wetness during both the contact with an external (dry or wet) surface as well as during the active production of sweat.

2. The role of thermal (cold) afferents appears to be of a primary importance in driving the perception of skin wetness during the contact with an external stimulus.

3. The rate of heat transfer from the skin to a colder surface seems to play a significant role not only in thermal and touch discrimination of different materials but also in characterising the perception of a cold stimulus as simply cold or as also wet.

4. When thermal cues are limited, individuals seem to rely more on tactile cues (i.e. stickiness and skin friction) to characterize their skin wetness perception.

4. The central integration of conscious coldness and mechanosensation, as sub-served by peripheral myelinated A-nerve fibers, seems therefore the primary neural process underpinning humans' ability to sense wetness.

5. Interestingly, these mechanisms (i.e. integration of thermal and tactile sensory cues) appear to be remarkably consistent (at least conceptually) regardless of the

modality for which skin wetness is experienced, i.e. whether due to passive contact with a wet stimulus or due to active production of sweat.

6. From a mechanistic standpoint, this could be explained by the fact that, independently from the modality (passive exposure vs. active sweat production), when the skin becomes wet, the components necessary for sensing wetness (i.e. skin, moisture/sweat, external stimulus) and the resulting sensory inputs (i.e. thermal and tactile) will always be the same. In other words, skin wetness perception will always be the result of a central integration of thermal and tactile interaction between the skin and the wet stimulus.

7. Due to its synthetic nature (i.e. no humidity receptors are present on the skin), by either assessing or manipulating the thermal and tactile sensory cues which enter the neural processing of this complex sensory experience, skin wetness perception can be manipulated independently from the level of physical skin wetness (e.g. an illusory perception of skin wetness can be evoked with a dry stimulus or a reduction in the perceived skin wetness can be induced independently from the level of physical wetness).

8. The novelty of these findings is that, for the first time to our knowledge, this Thesis has provided mechanistic evidence for the neurophysiological and psychophysical processes which underpin humans' ability to sense wetness on their skin.

9. Based on these findings, the first neurophysiological sensory model for human skin wetness perception has been developed. This model helps explaining how humans sense warm, neutral and cold wetness on their skin. Finally, this model provides the first frame of reference for this complex somatic experience.

11.3 Application of the findings

The outcomes of this Thesis have a fundamental, clinical as well as an applied significance.

From a fundamental point of view, these findings further our knowledge on how human beings sense wetness on their skin, despite being not provided with specific humidity receptors. Furthermore, the comprehensive experimental analysis of the neurophysiology of skin wetness perception as performed in this Thesis expands our understanding on how peripheral and central nervous systems process complex somatosensory experiences.

From a clinical point of view, these findings provide insights on the possible origin of symptoms such as spontaneous sensations of cold wetness experienced across the body by individual suffering from multiple sclerosis or polyneurophaties. As these disorders have been shown to affect peripheral A-nerve fibers functions and to alter somatic perception, the neurophysiological model of cutaneous wetness sensitivity developed in this Thesis could be used as a frame of reference for normal and altered somatosensory function. Furthermore, due to a recent interest in mapping bodily sensations such as pain (see Mancini et al. 2014), the body maps of torso' thermo, wetness and pleasantness sensation developed in this Thesis could be used as a frame of reference for normal and altered somatosensory function in the context of multiple sclerosis or polyneuropathies, diseases which are usually accompanied by alteration of normal somatosensory function.

Finally, from an applied point of view, the knowledge produced on the sensory processing of skin wetness, as well as on the relationship between physical and perceived skin wetness, has practical implications for thermal modelling and clothing design. As the perception of skin wetness perception has been shown to have a critical role in the onset of thermal and clothing discomfort, taking into account the neurophysiological and psychophysical bases of this perception (as elucidated in this Thesis) could be useful to support the development of new strategies in sport and protective clothing design aiming to improve thermal comfort. Furthermore, the body maps developed in this Thesis provide practical guidance on which regions of the torso should be targeted when designing protective clothing aimed to optimize thermal protection and maximize thermal comfort under extreme environmental conditions (e.g. cold air/water exposures).

With regards to the above, Oxylane Research (the industry partner of this PhD) has implemented the findings of this Thesis in its product design and development.

12 CHAPTER TWELVE - Future research

The findings of the studies performed as part of this Thesis provide suggestions for future research. Three main areas have been identified which require further investigation in order to expand our understanding of the mechanisms underlying the perception of skin wetness:

1. The recent identification of molecular candidates (e.g. mechano- and temperature-sensitive TRP cation channels) for non-specific humidity sensation in an animal lacking of specific hygroreceptive organ (i.e. the free-living roundworm *Caenorhabditis elegans*) (Russell et al., 2014) has opened to the question of what potential molecular bases could underpin humidity sensation in humans. The remarkable similarities in the temperature- and mechano-dependent mechanisms for humidity detection used by species lacking hygroreceptors such as *Caenorhabditis elegans* and humans might indicate shared molecular mechanisms between species. However, the molecular mechanisms for hygrosensation in humans remain entirely unexplored. Indeed, our understanding of the molecular bases of peripheral temperature and mechano-transduction in humans has only recently started to be uncovered (for an extensive review see Vriens et al., 2014) and whether both temperature gated TRP channels and mechanically activated DEG/ENaC/ASIC channels (Tsunozaki and Bautista, 2009) could also be functionally essential for human hygrosensation, remains a matter of speculation. Future investigations should therefore deal with the question of whether pharmacological manipulation of these temperature and mechanical activated channels could disrupt/rescue human ability to sense humidity and wetness.

2. The experimental work presented in this Thesis has focused on a specific age group (18-30 years old) and has not directly focused on any gender comparison.
With regards to ageing, as age-related alterations of the peripheral nervous system has been shown to result in decreases in human thermal sensitivity, investigating whether age has an effect on the sensory mechanisms which drive the perception of skin wetness would be of interest. Elucidating how age-related changes in the neurophysiology of the thermosensitive nerve afferents impacts thermal and skin wetness sensitivity, and thus the thermal behaviour in the elderly, has important

applied implications. For example, if the ability to detect certain changes in skin temperature and wetness is impaired with ageing, medical devices could be designed to signal these changes and thus aid the elderly to adjust their thermal behaviour accordingly.

With regards to gender, as gender-differences in thermosensitive have been shown to exist (however only investigated for the warm side of thermal sensation spectrum), investigating whether gender has a direct effect on the sensory mechanisms which drive the perception of skin wetness would be of interest.

3. The experimental work presented in this Thesis has focused on the mechanisms for which skin wetness perception is sensed in healthy individuals.

As numerous and widely spread diseases such as Multiple Sclerosis and Diabetic neuropathies have been shown to affect peripheral A-nerve fibers functions and to alter somatic perception (i.e. thermal and tactie sensitivity), investigating whether and how the mechanisms for which skin wetness is sensed are altered in these clinical populations would be of interest. For example, this could support the development of specific diagnostic tests which could help the early identification of the development of a specific somatosensory-related pathology (e.g. diabetic neuropathy). Furthermore, this could be combined with the investigation of the molecular bases of human humidity and wetness perception. Increasing understanding of the molecular bases of human hygrosensation is indeed relevant for its clinical significance. For instance, undesired symptoms such as spontaneous sensations of cold wetness are often experienced across the body by individuals suffering from multiple sclerosis or polyneuropathies. Hence, understanding the molecular mechanisms of human hygrosensation and wetness perception could provide insights into the pathological mechanisms involved in the altered somatosensory function observed in these patients. This knowledge could then be used to develop specific treatment strategies targeting rescue and/or amelioration of sensory function in these pathological conditions. The fact that such an approach has already been used in other research areas (e.g. investigation of the role of temperature sensitive TRP channels in the development of acute and chronic pain and development of specific analgesic drugs targeting these channels) (Vriens et al., 2014) represents a promising avenue for future research aiming to elucidate the molecular mechanisms of human humidity and wetness perception.

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Appendix A

Loughborough University

INFORMED CONSENT FORM

(to be completed after Participant Information Sheet has been read)

The purpose and details of this study have been explained to me. I understand that this study is designed to further scientific knowledge and that all procedures have been approved by the Loughborough University Ethical Approvals (Human Participants) Sub-Committee.

I have read and understood the information sheet and this consent form.

I have had an opportunity to ask questions about my participation.

I understand that I am under no obligation to take part in the study.

I understand that I have the right to withdraw from this study at any stage for any reason, and that I will not be required to explain my reasons for withdrawing.

I understand that all the information I provide will be treated in strict confidence and will be kept anonymous and confidential to the researchers unless (under the statutory obligations of the agencies which the researchers are working with), it is judged that confidentiality will have to be breached for the safety of the participant or others. `

I agree to participate in this study.

Your name _____

Your signature _____

Signature of investigator _____

Date _____

Appendix B

Name/Number .....

Health Screen Questionnaire for Study Volunteers

Note to Investigators: This HSQ can be used in its entirety but you can also remove some of the questions if you know they are not relevant to your study.

As a volunteer participating in a research study, it is important that you are currently in good health and have had no significant medical problems in the past. This is (i) to ensure your own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

If you have a blood-borne virus, or think that you may have one, please do not take part in this research ***[only include for projects involving invasive procedures].***

Please complete this brief questionnaire to confirm your fitness to participate:

1. **At present,** do you have any health problem for which you are:

(a)	on medication, prescribed or otherwise	Yes	☐	No	☐
(b)	attending your general practitioner	Yes	☐	No	☐
(c)	on a hospital waiting list	Yes	☐	No	☐

2. **In the past two years,** have you had any illness which required you to:

(a)	consult your GP	Yes	☐	No	☐
(b)	attend a hospital outpatient department	Yes	☐	No	☐
(c)	be admitted to hospital	Yes	☐	No	☐

3. **Have you ever** had any of the following:

(a)	Convulsions/epilepsy	Yes	☐	No	☐
(b)	Asthma	Yes	☐	No	☐
(c)	Eczema	Yes	☐	No	☐
(d)	Diabetes	Yes	☐	No	☐
(e)	A blood disorder	Yes	☐	No	☐

(f)	Head injury	Yes	☐	No	☐
(g)	Digestive problems	Yes	☐	No	☐
(h)	Heart problems	Yes	☐	No	☐
(i)	Problems with bones or joints	Yes	☐	No	☐
(j)	Disturbance of balance/coordination	Yes	☐	No	☐
(k)	Numbness in hands or feet	Yes	☐	No	☐
(l)	Disturbance of vision	Yes	☐	No	☐
(m)	Ear / hearing problems	Yes	☐	No	☐
(n)	Thyroid problems	Yes	☐	No	☐
(o)	Kidney or liver problems	Yes	☐	No	☐
(p)	Allergy to nuts	Yes	☐	No	☐

4. **Has any,** otherwise healthy, member of your family under the age of 35 died suddenly during or soon after exercise? Yes ☐ No ☐

If YES to any question, please describe briefly if you wish (eg to confirm problem was/is short-lived, insignificant or well controlled.)

.....

5. **Allergy Information**

(a)	are you allergic to any food products?	Yes	☐	No	☐
(b)	are you allergic to any medicines?	Yes	☐	No	☐
(c)	are you allergic to plasters?	Yes	☐	No	☐

If YES to any of the above, please provide additional information on the allergy

……

5. **Additional questions for female participants**

(a)	are your periods normal/regular?	Yes	☐	No	☐
(b)	are you on “the pill”?	Yes	☐	No	☐
(c)	could you be pregnant?	Yes	☐	No	☐

(d) are you taking hormone replacement therapy (HRT)? Yes ☐ No ☐

Please provide contact details of a suitable person for us to contact in the event of any incident or emergency.

Name:
……

Telephone Number:
……

Work ☐ Home ☐ Mobile ☐

Relationship to Participant:……

Are you currently involved in any other research studies at the University or elsewhere?

Yes ☐ No ☐

If yes, please provide details of the study

……

Appendix C

Mild evaporative cooling applied to the torso provides thermoregulatory benefits during running in the heat

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Running head: EVAPORATIVE COOLING AND EXERCISE IN THE HEAT

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Abstract

We investigated the effects of mild evaporative cooling applied to the torso, before or during running in the heat. Nine males performed 3 trials: control-no cooling (CTR), pre-exercise cooling (PRE-COOL) and during-exercise cooling (COOL). Trials consisted of 10 min neutral exposure and 50 min heat exposure (30 °C; 44 % humidity), during which a 30 min running protocol (70% VO_{2max}) was performed. An evaporative cooling t-shirt was worn before the heat exposure (PRE-COOL) or 15 min after the exercise was started (COOL). PRE-COOL significantly lowered local skin temperature (T_{sk}) (up to -5.3 ± 0.3 °C) (p<0.001), mean T_{sk} (up to -2 ± 0.1 °C) (p<0.001), sweat losses (-143 ± 40 g) (p=0.002) and improved thermal comfort (p=0.001). COOL suddenly lowered local T_{sk} (up to -3.8 ± 0.2 °C) (p<0.001), mean T_{sk} (up to -1 ± 0.1 °C) (p<0.001), heart rate (up to -11 ± 2 bpm) (p=0.03), perceived exertion (p=0.001) and improved thermal comfort (p=0.001). We conclude that the mild evaporative cooling provided significant thermoregulatory benefits during exercise in the heat. However, the timing of application was critical in inducing different thermoregulatory responses. These findings provide novel insights on the thermoregulatory role of T_{sk} during exercise in the heat.

Keywords

Cooling garment, heat stress, thermoregulation, skin temperature, exercise

Appendix D

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The role of decreasing contact temperatures and skin cooling in the perception of skin wetness

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HIGHLIGHTS

- A skin cooling rate threshold for cold stimuli to evoke wetness was identified.
- The rate of heat transfer plays a role in characterising the perception of cold stimuli as cold-wet.
- Touch might be as determinant as temperature inputs in characterising the perception of wetness.

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ABSTRACT

Cold sensations are suggested as the primary inducer of the perception of skin wetness. However, limited data are available on the effects of skin cooling. Hence, we investigated the role of peripheral cold afferents in the perception of wetness. Six cold-dry stimuli (producing skin cooling rates in a range of 0.02–0.41 °C/s) were applied on the forearm of 9 female participants. Skin temperature and conductance, thermal and wetness perception were recorded. Five out of 9 participants perceived wetness as a result of cold-dry stimuli with cooling rates in a range of 0.14–0.41 °C/s, while 4 did not perceive skin wetness at all. Although skin cooling and cold sensations play a role in evoking the perception of wetness, these are not always of a primary importance and other sensory modalities (i.e. touch and vision), as well as the inter-individual variability in thermal sensitivity, might be equally determinant in characterising this perception.

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1. Introduction

Humans interact with their immediate environment through the medium of sensory experiences. However, the way we perceive the world differs qualitatively from the way we sense it [30]. This difference between perception and sensation relies on the fact that our nervous system extracts only certain information from each stimulus and these are then interpreted according to the current situation and previous experiences [21]. Furthermore, perception often results from multisensory experiences as our sensory systems operate within interconnecting, intermodal and cross modal networks [26].

The ability of the central nervous system to combine and process different sensory information into particular perceptions provides the basis for understanding why some of the perceptions we experience are not directly linked to just one specific sensory system. For instance, we experience the perception of "wetness" on the skin though we are not provided with specific receptors for this sensation [10,22]. This somatosensory experience is considered a result of the integration of the somatosensory sub-modalities of touch and temperature [1,3,5,7]. However, the way in which touch and temperature senses interact to generate the perception of wetness is still unclear [18,20]. It has been hypothesised that the activity of thermoreceptors responding to specific drops in skin temperature, such as the ones occurring during the evaporation of sweat from the skin, represents the primary inducer of this perception [11,18,32]. Nevertheless, the role played by cold thermoreceptors (i.e. small myelinated Aδ and unmyelinated C fibers) [8] is still unclear and might vary according to the location of these cold sensitive free nerve endings. Indeed, Belmonte and Gallar [2] suggest that the augmented activation of cold thermoreceptors (i.e. corneal trigeminal neurons) located on the human cornea recorded during evaporation-induced ocular surface cooling, seems to be responsible for the perception of ocular dryness. The same physical process (cooling) encoded by the same type of thermoreceptors (cold sensitive) might be therefore primarily responsible for two completely opposite perceptions: dryness and wetness. Furthermore, it could be reasonable hypothesising the interaction

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Appendix E

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THERMAL AND TACTILE INTERACTIONS IN THE PERCEPTION OF LOCAL SKIN WETNESS AT REST AND DURING EXERCISE IN THERMO-NEUTRAL AND WARM ENVIRONMENTS

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Abstract—The central integration of thermal (i.e. cold) and mechanical (i.e. pressure) sensory afferents is suggested as to underpin the perception of skin wetness. However, the role of temperature and mechanical inputs, and their interaction, is still unclear. Also, it is unknown whether this intra-sensory interaction changes according to the activity performed or the environmental conditions. Hence, we investigated the role of peripheral cold afferents, and their interaction with tactile afferents, in the perception of local skin wetness during rest and exercise in thermo-neutral and warm environments. Six cold-dry stimuli, characterized by decreasing temperatures [i.e. −4, −8 and −15 °C below the local skin temperature (T_{sk})] and by different mechanical pressures [i.e. low pressure (LP): 7 kPa; high pressure (HP): 10 kPa], were applied on the back of 8 female participants (age 21 ± 1 years), while they were resting or cycling in 22 or 33 °C ambient temperature. Mean and local T_{sk}, thermal and wetness perceptions were recorded during the tests. Cold-dry stimuli produced drops in T_{sk} with cooling rates in a range of 0.06–0.4 °C/s. Colder stimuli resulted in increasing coldness and in stimuli being significantly more often perceived as wet, particularly when producing skin cooling rates of 0.18 °C/s and 0.35 °C/s. However, when stimuli were applied with HP, local wetness perceptions were significantly attenuated. Wetter perceptions were recorded during exercise in the warm environment. We conclude that thermal inputs from peripheral cutaneous afferents are critical in characterizing the perception of local skin wetness. However, the role of these inputs might be modulated by an intra-sensory interaction with the tactile afferents. These findings indicate that human sensory integration is remarkably multimodal. © 2013 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: Skin wetness, Thermo-receptors, Mechano-receptors, Sensory integration, Perception.

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Abbreviations: T_{sk}, skin temperature; LP, low pressure; HP, high pressure; ΔT_{sk}, variation in local skin temperature.

INTRODUCTION

The perception of skin wetness is a complex somatosensory experience which seems to result from the intra-sensory integration of temperature and mechanical inputs (Bentley, 1900; Ackerley et al., 2012; Bergmann Tiest et al., 2012). Although humidity-receptors have been previously described in some insects (Yokohari and Tateda, 1976), these receptors have not been identified in human skin (Clark and Edholm, 1985). It is currently suggested that as human beings, we "learn" to perceive the wetness experienced when our skin is in contact with a wet surface, when a liquid is touched, or when sweat is produced (Bergmann Tiest et al., 2012) through a complex multisensory integration (Driver and Spence, 2000; Gescheider and Wright, 2012). The physical processes which occur when the skin is in contact with moisture (i.e. heat transfer and mechanical interactions between the skin and the environment) generate thermal and mechanical inputs which could be integrated and combined at different anatomical levels through specific multisensory pathways (Cappe et al., 2009). Hence, it is not the contact of the skin with moisture *per se*, but rather the integration of particular sensory inputs which seems driving the perception of local skin wetness during the contact with a wet surface (Bentley, 1900). It could therefore be suggested that the perception of local skin wetness is a "perceptual illusion" shaped by sensory experience.

The thermal sense, and specifically the cold sensations (as resulting from the afferent activity of the cold sensitive skin's thermo-receptors, i.e. small myelinated Aδ and unmyelinated C-fibers) (Campero and Bostock, 2010), could play a critical role in the ability to perceive local skin wetness. For example, we seem to interpret the coldness experienced during the evaporation of water from the skin as a signal of the presence of water (and thus wetness) on the skin' surface (Daanen, 2009; Bergmann Tiest et al., 2012). The importance of sensing coldness in order to experience local skin wetness has been highlighted by our previous findings. We have demonstrated that an illusion of local skin wetness can be evoked during the skin's contact with a cold-dry surface producing a range of skin cooling rates of 0.14–0.41 °C/s (Filingeri et al., 2013a). Also, we have observed that no local wetness was perceived during the contact with a warm-wet surface (with a temperature warmer than the skin),

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Appendix F

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Body mapping of cutaneous wetness perception across the human torso during thermo-neutral and warm environmental exposures

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Filingeri D, Fournet D, Hodder S, Havenith G. Body mapping of cutaneous wetness perception across the human torso during thermo-neutral and warm environmental exposures. *J Appl Physiol* 117: 887–897, 2014. First published August 7, 2014; doi:10.1152/japplphysiol.00535.2014.—Sensing skin wetness is linked to inputs arising from cutaneous cold-sensitive afferents. As thermosensitivity to cold varies significantly across the torso, we investigated whether similar regional differences in wetness perception exist. We also investigated the regional differences in thermal pleasantness and whether these sensory patterns are influenced by ambient temperature. Sixteen males (20 ± 2 yr) underwent a quantitative sensory test under thermo-neutral [air temperature (T_{air}) = 22°C; relative humidity (RH) = 50%] and warm conditions (T_{air} = 33°C; RH = 50%). Twelve regions of the torso were stimulated with a dry thermal probe (25 cm^2) with a temperature of 15°C below local skin temperature (T_{sk}). Variations in T_{sk}, thermal, wetness, and pleasantness sensations were recorded. As a result of the same cold-dry stimulus, the skin-cooling response varied significantly by location (P = 0.003). The lateral chest showed the greatest cooling (−5 ± 0.4°C), whereas the lower back showed the smallest (−1.9 ± 0.4°C). Thermal sensations varied significantly by location and independently from regional variations in skin cooling with colder sensations reported on the lateral abdomen and lower back. Similarly, the frequency of perceived skin wetness was significantly greater on the lateral and lower back as opposed to the medial chest. Overall wetness perception was slightly higher under warm conditions. Significantly more unpleasant sensations were recorded when the lateral abdomen and lateral and lower back were stimulated. We conclude that humans present regional differences in skin wetness perception across the torso, with a pattern similar to the regional differences in thermosensitivity to cold. These findings indicate the presence of a heterogeneous distribution of cold-sensitive thermo-afferent information.

skin wetness; temperature; body mapping; thermoreceptors; pleasure

THERMOSENSITIVITY (i.e., the ability to perceive thermal changes in the surrounding environment) represents an important drive of thermoregulatory responses in humans and in other mammalian and nonmammalian species (23, 54). In humans, cutaneous thermosensitivity is peripherally subserved by cold-sensitive, myelinated Aδ-nerve fibers (conduction velocities ranging from 5–30 m/s) and by cold- and warm-sensitive, unmyelinated C-nerve fibers (conduction velocities ranging from 0.2–2 m/s) (11, 48) and is centrally integrated by the primary and secondary somatosensory cortices, as well as the insular cortex (a cortical region involved in cold and warm temperature sensation, as well as pain and touch) (15), through the spino-thalamic tract and the dorsal-column medial lemniscal pathway (36). Fluctuations in skin temperature (T_{sk}) attributable to environmental stimuli [e.g., changes in ambient temperature (T_{air}) and relative humidity (RH)] and the related thermal sensations have been shown to trigger autonomic (e.g., vasomotor tone and sweating/shivering response) (32, 51) and behavioral responses (e.g., adding or removing clothing) (50). These responses aim to maintain thermal homeostasis and comfort (8, 49).

Despite the critical role of thermosensitivity, sensing temperature is not the only factor among the cutaneous sensory afferent to contribute to thermoregulatory responses in humans. Sensing cutaneous wetness is also critical both for behavioral and autonomic responses. Perceiving changes in both ambient humidity and skin wetness have been shown to impact thermal comfort (22) and thus the thermoregulatory behavior (49), both in healthy and clinical populations (e.g., individuals suffering from rheumatic pain) (55). From an autonomic perspective, the degree of skin wetness influences sweat gland function through a progressive suppression of the sweat output (i.e., hidromeiosis) in the presence of wetted skin (38). This results in a reduced ability to lose heat to the environment via evaporative cooling, potentially affecting the thermal balance of the body (12). However, although the ability to sense skin wetness plays an important role in several behavioral and thermophysiological functions, little it is known on how skin wetness is sensed in humans (37).

As opposed to insects, in which humidity receptors subserving hygrosensation have been identified and widely described (57), humans seem not to be provided with specific receptors for the sensation of wetness (13). Thus we seem to "learn" to perceive the wetness experienced when the skin is in contact with a wet surface or when sweat is produced (6) through a complex multisensory integration (17) of thermal (i.e., heat transfer) and tactile (i.e., mechanical pressure and skin friction) inputs generated by the interaction between skin, moisture, and (if donned) clothing (22). This hypothesis has been supported by our previous findings. We have recently demonstrated that an illusion of local skin wetness can be evoked during the skin's contact with a cold-dry surface producing skin-cooling rates in a range of 0.14 to 0.41°C/s (19, 21), a temperature course that is similar to the one suggested to occur when the skin is physically wet (16). This could be due to the fact that we seem to interpret the coldness experienced during the evaporation of moisture from the skin as a signal of the presence of moisture (and thus wetness) on the skin' surface. In line with this hypothesis, we have also observed that during the static contact with a warm-wet surface (with a temperature warmer than the skin), no local skin wetness was perceived, as no skin cooling and thus no cold sensations occurred (20). Finally, this concept has been further confirmed in our most

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Appendix G

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Skin Research and Technology

Warm temperature stimulus suppresses the perception of skin wetness during initial contact with a wet surface

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Background/purpose: In the absence of humidity receptors in human skin, the perception of skin wetness is considered a somatosensory experience resulting from the integration of temperature (particularly cold) and mechanical inputs. However, limited data are available on the role of the temperature sense.

Methods: Wet and dry stimuli at 4°C and 8°C above local skin temperature were applied on the back of seven participants (age 21 ± 2 years) while skin temperature and conductance, thermal and wetness perceptions were recorded.

Results: Resting local skin temperature was always increased by the application of the stimuli (+0.5–+1.4°C). No effect of stimulus wetness was found on wetness perceptions ($P > 0.05$). The threshold (point '–2 slightly wet' on the wetness scale) to identify a clearly perceived wetness was never reached during any stimulations and participants did not perceive that some of the stimuli were wet. Overall, warm temperature stimuli suppressed the perception of skin wetness.

Conclusion: We conclude that it is not the contact of the skin with moisture *per se*, but rather the integration of particular sensory inputs (amongst which coldness seems dominant) which drives the perception of skin wetness during the initial contact with a wet surface.

Key words: skin wetness – temperature – thermoreceptors – perception

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THE PERCEPTION of skin wetness is a complex somatosensory experience which seems to result from the integration of temperature and mechanical (i.e. pressure) inputs (1–3). To date, a hygroreceptor has never been identified on the human skin (4). Therefore, it has been suggested that human beings learn to perceive the wetness experienced when their skin is in contact with a wet surface, when a liquid is touched, or when sweat is produced (3). The thermal and mechanical inputs which result from the physical processes occurring when the skin is in contact with moisture (i.e. heat transfer and mechanical interactions between the skin and the environment) could be integrated and combined at different anatomical levels through specific multisensory pathways (5). However, although the interaction between thermal and mechanical inputs seems to be the principal inducer of the perception of skin wetness (1–3), to date it is unclear which sensory modality is dominant in driving this perception.

The thermal sense might play a significant role in this perception. We have recently shown that exposing the skin to cold-dry stimuli (resulting in cooling rates similar to the ones occurring during the evaporation of water from the skin) can evoke an illusion of local skin wetness (6, 7). This indicated that in particular situations, individuals seem to associate local coldness with local skin wetness. These recent findings have opened an interesting question: if skin wetness might be primarily driven by coldness, would individuals be able to perceive local skin wetness if exposed to a local warm-wet stimulus during which no coldness is experienced? It might be hypothesised that in that case, the ability to perceive local skin wetness would depend upon the mechanical cues available. Every day experience indicates that we are able to perceive the wetness of a warm liquid. Inserting the hand into a bucket of warm water generates a particular sensation of pressure around the wrist (i.e. 'ring') which individuals associate to the perception of liquidity (2). In this case, as cooling cues are not available, individuals rely more on mechanical cues to aid the perception of wetness (3).

Appendix H

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Why wet feels wet? A neurophysiological model of human cutaneous wetness sensitivity

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Filingeri D, Fournet D, Hodder S, Havenith G. Why wet feels wet? A neurophysiological model of human cutaneous wetness sensitivity. *J Neurophysiol* 112: 1457–1469, 2014. First published June 18, 2014; doi:10.1152/jn.00120.2014.—Although the ability to sense skin wetness and humidity is critical for behavioral and autonomic adaptations, humans are not provided with specific skin receptors for sensing wetness. It has been proposed that we "learn" to perceive the wetness experienced when the skin is in contact with a wet surface or when sweat is produced through a multisensory integration of thermal and tactile inputs generated by the interaction between skin and moisture. However, the individual roles of thermal and tactile cues and how these are integrated peripherally and centrally by our nervous system is still poorly understood. Here we tested the hypothesis that the central integration of coldness and mechanosensation, as subserved by peripheral A-nerve afferents, might be the primary neural process underpinning human wetness sensitivity. During a quantitative sensory test, we found that individuals perceived warm-wet and neutral-wet stimuli as significantly less wet than cold-wet stimuli, although these were characterized by the same moisture content. Also, when cutaneous cold and tactile sensitivity was diminished by a selective reduction in the activity of A-nerve afferents, wetness perception was significantly reduced. Based on a concept of perceptual learning and Bayesian perceptual inference, we developed the first neurophysiological model of cutaneous wetness sensitivity centered on the multisensory integration of cold-sensitive and mechanosensitive skin afferents. Our results provide evidence for the existence of a specific information processing model that underpins the neural representation of a typical wet stimulus. These findings contribute to explaining how humans sense warm, neutral, and cold skin wetness.

skin; wetness; thermoreceptors; mechanoreceptors; A-nerve fibers

THE ABILITY TO SENSE HUMIDITY and wetness is an important attribute in the animal kingdom. For many insects, discriminating between dryness and wetness is vital for procreation and survival (Liu et al. 2007). Sensing wetness is also critical for humans, for both behavioral and autonomic adaptations. Perceiving changes in ambient humidity and skin wetness has been shown to impact thermal comfort (Fukazawa and Havenith 2009), and thus thermoregulatory behavior (Schlader et al. 2010), in both healthy and clinical populations (e.g., individuals suffering from rheumatic pain) (Strusberg et al. 2002). From an autonomic perspective, decreases in ocular wetness seem to initiate the lacrimation reflex in order to maintain a tear film to protect the ocular surface (Hirata and Oshinsky 2012). Also, tactile roughness and wetness discrimination is critical for precision grip (Augurelle et al. 2003) and object manipulation (André et al. 2010). However, although the ability to sense wetness plays an important role in many physiological and behavioral functions, the neurophysiological mechanisms underlying this complex sensory experience are still poorly understood (Montell 2008).

In contrast with insects, in which humidity receptors subserving hygrosensation have been identified and widely described (Tichy and Kallina 2010), humans' largest sensory organ, i.e., the skin, seems not to be provided with specific receptors for the sensation of wetness (Clark and Edholm 1985). Thus, as human beings, we seem to "learn" to perceive the wetness experienced when the skin is in contact with a wet surface or when sweat is produced (Bergmann Tiest et al. 2012) through a complex multisensory integration (Driver and Spence 2000) of thermal (i.e., heat transfer) and tactile (i.e., mechanical pressure and friction) inputs generated by the interaction between skin, moisture, and (if donned) clothing (Fukazawa and Havenith 2009). The hypothesis of wetness as a "perceptual illusion" shaped by sensory experience has been supported by our previous findings. We have recently shown that exposing the skin to cold-dry stimuli (resulting in cooling rates similar to those occurring during the evaporation of water from the skin) can evoke an illusion of local skin wetness (Filingeri et al. 2013, 2014a, 2014b). This could be due to the fact that we seem to interpret the coldness experienced during the evaporation of moisture from the skin as a signal of the presence of moisture (and thus wetness) on the skin surface. In line with this hypothesis, we have also observed that during static contact with a warm-wet surface (with a temperature warmer than the skin) no local skin wetness was perceived, as no skin cooling, and thus no cold sensations, occurred (Filingeri et al. 2014c).

These preliminary findings appeared to be in line with the Bayesian concept of perceptual inference (Knill and Richards 1996). According to this framework, sensory systems (such as the somatosensory system) incorporate implicit knowledge of the environment and use this knowledge (i.e., sensory experiences) to infer about the properties of specific stimuli (Geisler and Kersten 2002). As the sensory feedback received from the surrounding environment is by nature multimodal (i.e., involving different sensory cues), as well as noisy and ambiguous, perceptual systems are thought to perform online tasks aiming to predict the underlying causes for a sensory observation in a fashion that is considered as near optimal (Lochmann and Deneve 2011). In this context, humans have been shown to integrate the different sensory cues associated with an external stimulus and to infer the most probable multimodal estimate (i.e., perception) by taking into account the reliability of each

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Appendix I

Humidity sensation, cockroaches, worms and humans: are common sensory mechanisms for hygrosensation shared across species?

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Appendix J

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