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Thermosensory mapping of skin wetness sensitivity across the body of young males and females at rest and following maximal incremental runningAlessandro Valenza^{1,2}, Anntonino Bianco², Davide Filingeri¹*¹THERMOSENSELAB, Environmental Ergonomics Research Centre, Loughborough University, Loughborough, UK**²Sport and Exercise Sciences Research Unit, SPPF Department, University of Palermo, Palermo, Italy***Running head:** Sex- and exercise-induced regional differences in wetness perception**Key words:** Wetness, Thermoreceptors, Sensation, Sex, Skin, Body Temperature Regulation**Corresponding author's address:**

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Key points summary

- Humans lack skin receptors for wetness, i.e. hygroreceptors, yet we present a remarkable wetness sensitivity;
- Afferent inputs from skin cold-sensitive thermoreceptors are key for sensing wetness; yet it is unknown whether males and females differ in their wetness sensitivity across their body and whether high intensity exercise modulates this sensitivity;
- We mapped sensitivity to cold, neutral, and warm wetness across five body regions and show that females are more sensitive to skin wetness than males, and that this difference is greater for cold than warm wetness sensitivity;
- We also show that a single bout of maximal exercise reduced both sexes' sensitivity to skin wetness, i.e. *hygro-hypoesthesia*, as a result of concurrent decreases in thermal sensitivity;
- These novel findings clarify the physiological mechanisms underpinning this fundamental human sensory experience, they elucidate sex differences in thermoregulatory responses, and will inform the design of more effective sport and protective clothing, and thermoregulatory models;

Abstract

Humans lack skin hygroreceptors and we rely on integrating cold and tactile inputs from A-type skin nerve fibres to sense wetness. Yet, it is unknown whether sex and exercise independently modulate skin wetness sensitivity across the body. We mapped local sensitivity to cold, neutral and warm wetness of the forehead, neck, underarm, lower back, and dorsal foot in 10 males (27.8 ± 2.7 y; 1.92 ± 0.1 m² BSA) and 10 females (25.4 ± 3.9 y; 1.68 ± 0.1 m² BSA), at rest and post maximal incremental running. Participants underwent our quantitative sensory test where they reported the magnitude of thermal and wetness perceptions (Visual Analogue Scales) resulting from the application of a cold (5°C below skin temperature) wet (0.8ml water), neutral wet, and warm wet (5°C above skin temperature) thermal probe (1.32cm^2) to 5 skin sites.

We found that: 1) females were ~ 14 to $\sim 17\%$ more sensitive to cold-wetness than males, yet both sexes were as sensitive to neutral- and warm-wetness; 2) regional differences were present for cold-wetness only, and these followed a cranio-caudal increase that was more pronounced in males (i.e. the foot was $\sim 31\%$ more sensitive than the forehead); 3) maximal exercise reduced cold-wetness sensitivity over specific regions in males (i.e. $\sim 40\%$ decrease in foot sensitivity), and it also induced a generalised reduction in warm-wetness sensitivity in both sexes (i.e. ~ 4 to $\sim 6\%$). For the first time, we show that females are more sensitive to cold wetness than males, and that maximal exercise induce *hygro-hypoesthesia*. These novel findings expand our knowledge on sex differences in thermoregulatory physiology.



Alessandro Valenza recently completed an MSc in sciences and techniques of preventive and adapted sports activities at the University of Palermo (Thesis: Monitoring of the Young Soccer Player via GPS: Comparison of Exercises). In 2015 he qualified as "Professional Athletic Trainer" (Thesis: Aerobic power: the intermittent race as a method of development and evaluation methods), and in 2018 as "Basic Football Technician: UEFA B". Alessandro joined the THERMOSENSELAB as a visiting researcher in April 2018, and his research now focuses on the impact of maximal exercise on thermoregulation and local thermosensitivity in males and females.

Introduction

The perception of skin wetness is a fundamental sensory experience for humans (Filingeri & Havenith, 2015) and a key contributor to our thermoregulatory behaviours (Vargas *et al.*, 2018). Sensing *when* and *where* we get wet on the skin due to sweating or contact with a wet surface (e.g. a wet t-shirt), i.e. *hygro-sensation*, contribute to the awareness of our own thermal state (Filingeri *et al.*, 2015a) and of that of our surrounding environments (Filingeri, 2015). For example, the experience of exercise- and sweat-induced skin wetness is a well-known trigger of thermal discomfort (Gagge *et al.*, 1967) and it provides a drive for thermal behaviours (e.g. active body cooling) (Vargas *et al.*, 2019a).

As humans, we present a very well developed skin wetness sensitivity (i.e. we can discriminate wetness levels differing by as little as of 0.04 ml) (Ackerley *et al.*, 2012); yet our skin is not provided with a specific hygroreceptor (Clark & Edholm, 1985). In the absence of a skin hygroreceptor, humans have developed alternative sensory integration mechanisms to sense skin wetness (Filingeri *et al.*, 2014a), which appear to be shared by other hygroreceptor-lacking species (e.g. nematodes) (Russell *et al.*, 2014; Filingeri, 2015).

In the past 6 years, we have repeatedly shown that humans infer about the presence of physical wetness on their skin by using thermal and skin cooling-related sensory cues triggered by conductive and evaporative heat transfer in the presence of moisture on the skin (Filingeri *et al.*, 2013, 2014a, 2014b, 2014e, 2015c), in combination with mechanical and skin deformation-related cues arising from the movement of moisture across the skin (Filingeri *et al.*, 2014a, 2014e, 2015a). The role of thermal cues in sensing wetness is so pronounced that an illusion of skin wetness can be induced in blindfolded participants by cooling their skin with a dry-cold stimulus inducing skin cooling at a rate (i.e. $0.14\text{--}0.41^{\circ}\text{C}\cdot\text{s}^{-1}$) equivalent to that resulting from actual moisture evaporation (Filingeri *et al.*, 2013; Filingeri, 2014). Furthermore, regional differences in cold sensitivity across the torso positively correlate with regional differences in wetness sensitivity (Filingeri *et al.*, 2014b). Similarly, sweat-induced skin wetness perceptions can be significantly reduced independently of the level of physical skin wetness, by limiting the extent of intermittent, sweat-induced mechanical stimulation of the skin arising from wearing wet clothes (Filingeri *et al.*, 2015a). Our findings have contributed to the empirical demonstration that afferent inputs from peripheral A-type afferent nerve fibers innervating the skin and sub serving cold and touch sensing play a fundamental role in the conscious experience of skin wetness (Filingeri *et al.*, 2014a; Filingeri & Havenith, 2018). However, while our understanding of the physiology of human skin wetness sensing has undoubtedly expanded (Filingeri & Havenith, 2018), our

knowledge on the mechanisms and modulators of skin wetness perception remain somewhat fragmentary.

First, there is a lack of empirical data on whether sex independently modifies wetness sensitivity in humans. Females are generally more thermally sensitive than males (Gerrett *et al.*, 2014; Filingeri *et al.*, 2018) and present more sensitive thermal behaviours during exercise (Vargas *et al.*, 2019b); yet male and female skin wetness sensitivity has never been formally compared. Given the critical role that thermal (cold) sensitivity plays in sensing wetness (Filingeri *et al.*, 2013), and the importance of thermal afferents for the regulation of thermal behaviour (Schlader *et al.*, 2011), it would be reasonable to expect that females show greater wetness sensitivity than males.

Second, there is limited evidence regarding the presence of regional differences in wetness sensitivity over body regions (e.g. forehead, neck, underarm, lower back, foot) that experience high-levels of sweat-induced wetness following high intensity exercise (Smith & Havenith, 2011, 2012). Our previous data (Filingeri *et al.*, 2014b, 2015a) and that of others (Ackerley *et al.*, 2012) indicate that regional differences in wetness sensing exist and that these are highly dependent on regional patterns of cold sensitivity. Given that regional patterns of perceptual sensitivity often correlate with regional thermoeffector sensitivity (e.g. decreases in local skin temperature of the forehead produce more intense cold sensations and greater decreases in local sweating than similar changes over the abdomen) (Crawshaw *et al.*, 1975), it might be expected that regions with high local sweat rates such as the forehead, neck, underarm, lower back, foot, present high wetness sensitivity (Smith & Havenith, 2012). Third, there is a paucity of data regarding the independent effect of maximal exercise on local skin wetness sensitivity. Acute bouts of sub-maximal exercise are known to induce transient reductions in thermal sensitivity, i.e. exercise-induced *thermo-hypoesthesia* (Ouzzahra *et al.*, 2014; Gerrett *et al.*, 2014), via potential changes in circulating stress hormones (Koltyn, 2000). Furthermore, exercise-induced hypoalgesia is more consistently observed following high-intensity exercise (Koltyn, 2002). Hence, it might be expected that maximal exercise is likely to reduce wetness sensitivity via large changes in local sensitivity to thermal stimuli. Increasing our fundamental understanding on the independent and interactive effects of sex, regional differences, and maximal exercise, on human skin wetness sensitivity has important implications for better clarifying the drivers of sex differences in human thermoregulatory behaviour at rest and during exercise (Vargas *et al.*, 2019b), for optimizing the design of sport and protective clothing (Filingeri *et al.*, 2014b), and to further develop individualised thermoregulatory models (Havenith, 2001).

The aim of this study was threefold. We ought to determine: 1) whether healthy males and females differ in their ability to sense wetness on their skin; 2) whether the forehead, neck, underarm, lower back, and foot present different levels of wetness sensitivity; 3) whether wetness sensitivity decreases following maximal exercise. We hypothesized that females present greater wetness sensitivity than males, that regional differences in sensitivity are present for both sexes, and that maximal exercise similarly reduces wetness sensitivity in both sexes.

Methods

Ethical approval

The testing procedure and the conditions were explained to each participant and they all gave written informed consent for participation. The study was approved by the Loughborough University Ethics Sub-Committee for Human Participants (#R18-P083), and testing procedures were in accordance with the tenets of the Declaration of Helsinki (note: the study was not registered in a database). All testing took place at Loughborough (UK) between June and September 2018.

Participants

We performed an a priori sample size calculation using an effect size corresponding to 15% mean difference ($\pm 8\%$ standard deviation) in wetness perception between sexes. This value derived from pilot data and from the experimental assumption that this mean difference (equivalent to 1.5cm on the VAS scale) would be the minimum required to infer the presence of meaningful differences in wetness perception between sexes. The resulting effect size $f=0.93$, combined with an $\alpha=0.05$ and a β (power)=0.8, determined a minimum sample of 8 participants per group. We recruited 10 participants per group.

Twenty non-smoking, recreationally active (i.e. ≥ 3 exercise sessions per week) participants (i.e. 10 males and 10 females), with no history of cardiovascular, neurological and skin-related conditions (e.g. eczema), and familiar with treadmill running, were recruited from the student population of Loughborough University to take part in this study. Participants characteristics are presented in Table 1. Males and female participants were matched for age. Male participants presented a greater body surface area than females, which resulted in a smaller proportion of their body being stimulated by thermal probe (surface area: 1.32cm^2) that we used to deliver the wet stimuli (see *Experimental design*). Female participants were spread across a typical 28-day menstrual cycle (day of cycle: 16.3 ± 8.1), and only two of

them were taking oral contraceptives at the time of this study. Participants were instructed to refrain from: 1) performing strenuous exercise in the 48h preceding testing; 2) consuming caffeine or alcohol in the 24 h preceding testing; 3) consuming food in the 3 h preceding testing.

Experimental design

We used a single-blind psychophysical approach based on a well-established quantitative sensory test of skin wetness sensing that we have developed (Filingeri *et al.*, 2014a), to map sex differences in regional wetness sensitivity at rest and following a maximal incremental running test performed in a thermoneutral environment (ambient temperature: 25°C; relative humidity: 45%).

All participants took part in one experimental session, during which we performed the same quantitative sensory test prior to and following a maximal running test. We opted for a maximal exercise protocol in order to induce the greatest systemic perturbation achievable within a single bout of acute exercise [e.g. large changes in heart rate (HR), core temperature (T_{core}), mean and local skin temperatures (T_{sk})]. Furthermore, evidence indicates that exercise-induced hypoalgesia is consistently observed following high-intensity exercise (Koltyn, 2002). Previous investigations on exercise-induced thermo-hypoesthesia have utilised sub-maximal exercise intensities (Ouzzahra *et al.*, 2012; Gerrett *et al.*, 2015), and so no study has determined the impact of maximal exercise on local non-noxious thermo- and wetness sensitivity.

The quantitative sensory test that we used was based on our established protocol (Filingeri *et al.*, 2014a) and consisted in participants having to report the perceived magnitude of local thermal and wetness perceptions arising from the short-duration (i.e. 5s) static application of a cold-wet (i.e. 5°C below local T_{sk}), neutral-wet (i.e. equal temperature as local T_{sk}), and warm-wet (i.e. 5°C above local T_{sk}) hand-held temperature-controllable probe (surface area: 1.32cm², water content: 0.8ml). Participants reported the magnitude of their local perceptions on two digital visual analogue scales for thermal sensation (length 200 mm; anchor points: 0 very cold, 100 neutral, 200 very hot) and wetness perception (length: 100 mm; anchor points: 0 dry, 100 completely wet). We used stimuli whose temperatures were relative to the local T_{sk} pre-stimulation (i.e. $\pm 5^\circ\text{C}$ or equal to local T_{sk}) to account for the expected exercise-induced changes in local T_{sk} . In this way we ensured that the same relative thermal stimulus would be applied pre and post exercise, as the difference between the temperature of a stimulus and

that of the skin, is an important determinant of the magnitude of a resulting thermal sensation (i.e. the greater the difference, the more intense the sensation) (Darian-Smith, 1984).

We mapped thermal and wetness sensitivity at five different locations over the body, i.e. the centre of the forehead (i.e. 5 cm above the pupillary line), the posterior neck (i.e. over the process spinous of cervical 4), the centre of the underarm (i.e. over the midaxillary line, 10 cm above the nipple line), the lower lateral back (i.e. over the posterior superior iliac crest) and the dorsal foot (i.e. midpoint between the 2nd and 3rd metatarsal joints). We chose those body regions because: 1) they present high exercise-induced local sweat rates (e.g. forehead and lower back) (Smith & Havenith, 2012); 2) they are generally reported to trigger wet-induced thermal discomfort (e.g. underarm and lower back) (Fukazawa & Havenith, 2009); 3) there is limited evidence of their intrinsic wetness sensitivity in males and females. As per our previous studies (Filingeri *et al.*, 2014a, 2014b, 2018), all participants were blinded to the nature and application of the stimuli to limit expectation biases, and they were only informed about the location of the stimulation. Furthermore, participants underwent a systematic familiarization and calibration to the testing procedures and perceptual scales prior to testing (Filingeri *et al.*, 2014a, 2018). The same investigator performed all testing, to limit any inter-individual variability arising from the procedures carried out.

Experimental protocol

Participants arrived at the laboratory on testing days and underwent preliminary measurements and preparation. They changed into running shorts (and sport-bra) before we assessed their semi-nude body mass on a precision scale (SECA 874, Germany), and their height on a wall stadiometer. Six skin thermistors (Grant, Cambridge, UK) were taped to six location on the left side of the body (i.e. cheek, upper chest, outer mid lower arm, hand dorsum, anterior thigh and lower lateral back) to record local T_{sk} for the estimation of mean T_{sk} according to the following equation (Lund & Gisolfi, 1974):

$$\begin{aligned} \text{mean } T_{sk} = & (\text{cheek } T_{sk} \times 0.14) + (\text{upper chest } T_{sk} \times 0.19) \\ & + (\text{outer mid lower arm } T_{sk} \times 0.11) + (\text{hand dorsum } T_{sk} \times 0.05) \\ & + (\text{anterior thigh } T_{sk} \times 0.32) + (\text{lower lateral back } T_{sk} \times 0.19) \end{aligned}$$

Local T_{sk} was recorded at 2Hz via a dedicated data acquisition system (USB-Temp, MCCdaq, USA) and custom-written software (DASYLab, MCCdaq, USA). Participants then wore a

HR monitor and chest strap (Ambit 3 sport, Suunto, Finland). We used a washable marker to mark the skin sites to be stimulated, and we gently shaved each site to limit any insulative effect of hairiness on heat transfer during the application of the stimuli.

Following on this preparation, participants underwent 20 minutes of resting on a chair to adjust to the environmental conditions. During this time, participants were familiarised with the experimental procedures, and calibrated to the visual analogue scales. Calibration procedures consisted of the following. Six stimuli varying in temperature and wetness (i.e. 0.8 ml wet, or dry) were applied to the volar surface of both forearms (i.e. midpoint between wrist and antecubital fossa) in a randomized order, and participants were instructed to associate each stimulus to a specific descriptor on the thermal scale. The stimuli and related descriptors were: 1) wet stimulus, 10°C above local skin temperature - scale descriptor: Very hot; 2) wet stimulus, 5°C above local skin temperature - scale descriptor: midpoint between Neutral and Very hot; 3) wet stimulus, equal temperature as local skin temperature - scale descriptor: Neutral; 4) dry stimulus, equal temperature as local skin temperature - scale descriptor: Neutral; 5) wet stimulus, 5°C below local skin temperature - scale descriptor: midpoint between Neutral and Very cold; 6) wet stimulus, 10°C below local skin temperature - scale descriptor: Very cold. During each of the six stimuli applications, participants were instructed to freely determine the level of wetness experienced on the wetness visual analogue scale. This procedure ensured that all participants had comparable experiences of the different stimuli and related perceptual anchor points to be used during testing. The forearm was chosen as a “neutral” calibration site to avoid any priming, given that this region was not going to be tested during the mapping protocol.

Upon termination of calibration, recordings of local T_{sk} and HR were started and continued throughout the testing session. Furthermore, spot measurements of tympanic temperature (ThermoScan IRT 6520, Braun, Germany) were taken at this stage and every 3 min thereafter and until completion of the testing session and used as an indicator of T_{core} .

At this point, the pre-exercise quantitative sensory test commenced. This lasted 20 min and was executed as following. Depending on the body region to be tested, we first recorded local T_{sk} of the testing site with an infrared thermometer (Spot IR Thermometer TG54, FLIR, USA). We then determined the temperature of the first wet stimulus (e.g. cold wet, 5°C below local skin temperature) and applied a 100% cotton fabric on the hand-held, round thermal probe (surface area: 1.32 cm²; NTE-2A, Physitemp, USA), that was then wetted with a pipettor with 0.8ml of water to ensure its full saturation. Following a verbal warning, the wet stimulus was applied statically on the participant's skin for 5s, during which the

participant was encouraged to rate their very first thermal and wetness perception.

Application pressure was not measured but was controlled to be sufficient to ensure full contact, while not resulting in pronounced skin indentation. Upon acquisition of the perceptual rating, we removed the stimulus, gently dried the skin, and then repeated the same procedure for the other stimuli (e.g. neutral and warm wet) on the same skin site, before proceeding to the next skin region. The order of testing region was counter-balanced between participants and the order of stimuli (e.g. warm vs. neutral vs. cold wet) was counter-balanced between and within participants. Immediately after completion of the quantitative sensory test for all 5 regions, participants moved to a motorised treadmill (Jet 200, Reebok, UK) to start the maximal incremental running testing.

The incremental test comprised 7 steps, consisting of a combination of increases in speed and inclination at 3-min intervals (i.e. step 1: $6.5\text{km}\cdot\text{h}^{-1}$, 0%; step 2: $8.5\text{km}\cdot\text{h}^{-1}$, 0%; step 3: $8.5\text{km}\cdot\text{h}^{-1}$, 5%; step 4: $8.5\text{km}\cdot\text{h}^{-1}$, 10%; step 5: $8.5\text{km}\cdot\text{h}^{-1}$, 15%; step 6: $10.5\text{km}\cdot\text{h}^{-1}$, 15%; step 7: $12\text{km}\cdot\text{h}^{-1}$, 15%). This was carried out until participants reached their age-predicted maximum HR (i.e. calculated as $220 - \text{age}$), or until they verbally signalled the obtainment of volitional fatigue.

Upon termination of the running test, participants returned to their seated position where any sweating was dried off with a towel, and the same quantitative sensory test, as described above, was immediately performed (note: we continued to dry off any sweat before any stimulus application as the test continued).

Statistical analysis

We analysed HR, mean T_{sk} , and T_{core} for the independent and interactive effect of sex (2 levels: male vs. female) and exercise (2 levels: pre- vs. post maximal tests), by means of 2-way mixed ANOVAs. We assessed the independent and interactive effect of sex (2 levels: male vs. female), body region (5 levels) and exercise (2 levels: pre- vs. post maximal tests) on baseline local T_{sk} (i.e. prior to application of wet stimuli) by means of a 3-way mixed ANOVA.

We evaluated the independent and interactive effect of sex (2 levels: male vs. female), body region (5 levels) and exercise (2 levels: pre- vs. post maximal tests) separately for thermal and wetness perceptions and for each stimulus (i.e. cold-wet, neutral-wet, and warm-wet), by means of 3-way mixed ANOVAs.

Also, we evaluated the independent effect of the temperature of the stimuli (3 levels: cold-wet, neutral-wet, and warm-wet) on wetness perceptions collapsed over body region (i.e.

cumulative mean perception of the 5 regions tested for each participant) separately for males and females and for rest and post exercise, by means of a 1-way repeated measure ANOVA. In the event of statistically significant main effects or interactions, post-hoc analyses were conducted with Tukey's tests.

Finally, we assessed the relationship between cold-wet-, neutral-wet-, and warm-wet induced wetness perceptions and thermal sensations separately for males and females, and for rest and post exercise, by means of regression analyses. First, we assessed the relationship between thermal and wetness sensations for each individual participant, and separately for Males vs. Females, and for Rest vs. Post exercise. Individual data sets were plotted, visually inspected, and then analysed. We first compared which one between a linear model (simpler) and a quadratic polynomial (more complex) would best fit the data by means of an extra-sum-of-squares F test. Depending on the test results, a linear or quadratic model would be fitted, and we calculated related R^2 values. Individual R^2 values arising from best fitting model (i.e. linear vs. quadratic) were analysed by means of a 2-way mixed ANOVA for the independent effects of sex and maximal exercise. Following on the individual analyses we went on developing group models that could provide a generalisable relationship between thermal and wetness perceptions which accounted for the inter-individual variability observed in the individual models. Mean and standard deviation data, along with sample size ($N=10$) for thermal and wetness perceptions in males and females at rest and post exercise entered 4 separate regression models. Accounting for mean and standard deviation, along with sample size, ensured our group models provided a better representation of the relationship between thermal and wetness perception for our entire sample.

Normality testing using Shapiro-Wilk test was performed for all datasets. Data are reported as means, standard deviations (SD), and 95% Confidence Intervals (CI). Observed power was computed using $\alpha=0.05$. Statistical analysis was performed using GraphPad Prism (version 8.0; GraphPad Software, La Jolla, CA, USA).

Results

Physiological responses at rest and post maximal exercise

The maximal incremental running test lasted 16.7 ± 1.4 min for males and 14.2 ± 1.7 min for females ($p=0.002$). Exercise elevated participants' HR (main effect of exercise: $F_{(1, 18)}=1706$; $p<0.001$) and similarly (main effect of sex: $F_{(1, 18)}=0.925$; $p=0.348$) in males (pre-exercise= 58 ± 9 bpm; post-exercise= 197 ± 11 bpm) and females (pre-exercise= 63 ± 8 bpm; post-exercise= 195 ± 7 bpm) (Tab. 2). When expressed as a percentage of the age-predicted

maximal HR, participants' post-exercise HR corresponded to $102 \pm 6\%$ in males and to $100 \pm 4\%$ in females.

Participants tympanic T_{core} was significantly elevated following the maximal test (main effect of exercise: $F_{(1, 18)}=79.9$; $p<0.001$) and similarly (main effect of sex: $F_{(1, 18)}=0.043$; $p=0.837$) in males (pre-exercise= $36.9 \pm 0.3^\circ\text{C}$; post-exercise= $37.7 \pm 0.3^\circ\text{C}$) and females (pre-exercise= $37.1 \pm 0.3^\circ\text{C}$; post-exercise= $37.6 \pm 0.3^\circ\text{C}$) (Tab. 2). On the contrary, the maximal test reduced participants' mean T_{sk} (main effect of exercise: $F_{(1, 18)}=52.3$; $p<0.001$), which tended to be lower in females than in males (main effect of sex: $F_{(1, 18)}=7.06$; $p=0.016$), prior to (males: $32.95 \pm 0.76^\circ\text{C}$; females: $32.22 \pm 0.53^\circ\text{C}$) and following exercise (males: $32.13 \pm 0.90^\circ\text{C}$; females: $31.31 \pm 0.63^\circ\text{C}$) (Tab. 2).

Participants' baseline local T_{sk} (i.e. prior to the wet stimuli application) varied significantly across body regions (main effect of body region: $F_{(4, 72)}=67.1$; $p<0.001$) and similarly for males and females (main effect of sex: $F_{(1, 18)}=3.51$; $p=0.077$). Specifically, we observed a clear cranio-caudal pattern of decrease in local skin temperature from the forehead to the foot in both sexes (Fig. 1A). Exercise resulted in a decrease in local T_{sk} in all skin regions but the dorsal foot (interaction body region with exercise: $F_{(4, 72)}=62.5$; $p<0.001$), which on the contrary showed a significant increase in local T_{sk} in both males (mean change in foot T_{sk} : $+3.49^\circ\text{C}$ [95%CI 2.65, 4.32]; $p<0.001$) and females (mean change in foot T_{sk} : $+3.47^\circ\text{C}$ [95%CI 2.63, 4.30]; $p<0.001$) (Fig. 1B).

Pre and post exercise thermal and wetness perception: cold wet stimulus

Thermal sensations resulting from the application of the cold wet stimulus varied significantly as a function of sex (main effect: $F_{(1, 18)}=12.1$; $p=0.009$) and of body region (main effect: $F_{(2.9, 52.9)}=4.3$; $p=0.003$) (Fig. 2A & D). Irrespective of body region, females generally perceived the same cold wet stimulus as colder when compared to males (compare males and females in fig. 2A & D), both at rest (female mean thermal sensation collapsed over body region: 38.2 ± 18.1 mm; male mean thermal sensation collapsed over body region: 53.5 ± 15.2 mm), and following exercise (female mean thermal sensation collapsed over body region: 33.6 ± 10.6 mm; male mean thermal sensation collapsed over body region: 61.4 ± 10.8 mm). When expressed as percentage of the thermal VAS scale used, those sex differences corresponded to females being $\sim 8\%$ and $\sim 14\%$ more cold sensitive than males at rest and post exercise, respectively.

Irrespective of sex, we observed a cranio-caudal increase in the magnitude of cold sensations resulting from the application of the same cold wet stimulus at rest (Fig. 2A), with the

forehead presenting some of the less intense cold sensations while the foot some of the most intense, in both males (mean difference forehead vs. foot: 26.5 mm [95%CI 8.0, 45.0]; $p=0.010$; corresponds to $\sim 13\%$ difference) and females (mean difference forehead vs. foot: 39.5 mm [95%CI 17.6, 61.4]; $p=0.003$; corresponds to $\sim 20\%$ difference). The only exception to this trend concerned the underarm, which presented similar responses as those of the forehead, in both males (underarm at rest: 73.3 ± 27.7 mm; forehead at rest: 57.5 ± 18.3 mm) and females (underarm at rest: 56.7 ± 30.2 mm; forehead at rest: 52.6 ± 8.6 mm) (Fig. 2A). Exercise modulated thermal sensations to the cold wet stimulus, yet this only occurred for some specific regions (interaction body region with exercise: $F_{(3, 62)}=5.4$; $p=0.001$) (Fig. 2D). The most pronounced of such exercise-induced changes occurred for the dorsal foot in males, where a large reduction in cold sensation arising from stimulation of this region took place following exercise (mean difference: 47.5 mm [95%CI 15.8, 79.1]; $p=0.008$) (compare fig. 2A and 2D). When expressed as percentage of the thermal VAS scale used, this region-specific difference corresponded to the foot being $\sim 24\%$ less cold sensitive post exercise. Wetness perceptions resulting from the application of the cold wet stimulus varied significantly as a function of sex (main effect: $F_{(1, 18)}=5.6$; $p=0.029$), with females generally reporting greater wetness sensations than males (Fig. 3A & D), both at rest (female mean wetness perception collapsed over body region: 69.0 ± 7.6 mm; male mean thermal sensation collapsed over body region: 51.7 ± 18.6 mm) and following exercise (female mean wetness perception collapsed over body region: 64.4 ± 8.3 mm; male mean thermal sensation collapsed over body region: 50.6 ± 13.1 mm). When expressed as percentage of the wetness VAS scale used, those sex differences corresponded to females being $\sim 17\%$ and $\sim 14\%$ more wetness sensitive than males at rest and post exercise, respectively.

We observed a significant interaction for sex, body region and exercise (interaction: $F_{(4, 72)}=4.6$; $p=0.002$), indicating that certain regional differences in wetness perception were present in one sex, and that these regional patterns changed as a result of exercise. For example, similarly to what observed for thermal sensations, males showed a clear cranio-caudal increase in wetness perception at rest, with the forehead presenting lower sensitivity than the foot (mean difference: 30.6 mm [95%CI 8.6, 52.6]; $p=0.012$; corresponds to $\sim 31\%$ difference), and with the only exception to this trend being the underarm, which presented the lowest wetness sensitivity (Fig. 3A). Contrary to what seen for thermal sensation, the cranio-caudal trend was not as pronounced in females (mean difference forehead vs. foot: 15.2 mm [95%CI -2.8, 33.2]; $p=0.089$) (Fig. 3A). It is of note that exercise induced a clear inversion in the cranio-caudal trend observed in males at rest, with the male forehead showing an increase

in wetness sensitivity to the extent that this became the most sensitive region (mean difference pre- vs. post-exercise: 25.9 mm [95%CI 9.2, 42.6]; $p=0.007$; corresponds to ~26% difference), and with the male foot showing a decrease in wetness sensitivity to the extent that this became the least sensitive region (mean difference pre- vs. post-exercise: 40.2 mm [95%CI 18.1, 62.3]; $p=0.003$; corresponds to ~40% difference), following the maximal incremental running test (compare fig. 3A and 3D). We did not observe any clear change in wetness sensitivity over any region in females following exercise (Fig. 3D).

All in all, these findings indicated that females were generally more sensitive to coldness (i.e. ~8% rest; ~14% post exercise) and cold wetness (i.e. ~17% rest; ~14% post exercise) than males; that a cranio-caudal increase (i.e. 31%) in cold wetness sensitivity was present in males only (despite both sexes showed a cranio-caudal increase in cold sensitivity, i.e. ~13% males; ~20% females); and that exercise contributed to reductions in local cold sensitivity (i.e. ~24%) and in cold wetness sensitivity (i.e. ~40%) over the male dorsal foot only.

Pre and post exercise thermal and wetness perception: neutral wet stimulus

Thermal sensations resulting from the application of the neutral wet stimulus did not vary neither as a function of sex (main effect: $F_{(1, 18)}=4.3$; $p=0.052$), nor as a function of body region (main effect: $F_{(3.6, 65.3)}=2$; $p=0.109$) (Fig. 2B & E). While there was a trend for women to present slightly lower thermal sensations than males, it is important to note that average thermal sensations (collapsed over body region) in both sexes generally aligned to the “Neutral” descriptor located at the 100th mm of the 200-mm visual analogue scale, both at rest (female: 102.5 ± 8.7 mm; male: 107.6 ± 6.3 mm) and following exercise (female: 92.0 ± 12.3 mm; male: 107.8 ± 15.9 mm). This confirmed that the neutral wet stimulus triggered minimal thermosensory cues, and that the stimulus was generally perceived as neither warm nor cold (Fig. 2B & E). It is of note that following exercise, there was a greater heterogeneity in the thermal sensations reported across body regions (interaction body region with exercise: $F_{(3.3, 59.1)}=2.9$; $p=0.036$) (compare fig. 2B and 2E). For example, the male underarm presented a lower thermal sensation (i.e. more on the cold side of the scale, mean: 84.9 ± 28.0 mm) than the forehead (i.e. more on the warm side, mean: 119.9 ± 28.8 mm) as a result of the neutral wet stimulus following exercise (Fig. 2E).

Wetness perceptions resulting from the application of the neutral wet stimulus did not vary neither as a function of sex (main effect: $F_{(1, 18)}=8.9$; $p=0.015$), nor body region (main effect: $F_{(3.4, 60.8)}=4.3$; $p=0.615$), nor exercise (main effect: $F_{(1, 18)}<0.001$; $p=0.983$) (Fig. 3B & E). Average wetness perceptions (collapsed over body region) corresponded to 19.6 ± 6.2 mm

and 28.14 ± 3.6 mm in males and females at rest, respectively; and to 16.1 ± 6.1 mm and 31.6 ± 8.1 mm in males and females following exercise, respectively.

All in all, these findings indicated that the neutral wet stimulus did not trigger neither cold nor warm sensations, and that this induced minimal wetness sensations (e.g. when compared to the cold wet stimulus) in males and females that did not differ neither as a function of the region stimulated nor following exercise.

Pre and post exercise thermal and wetness perception: warm wet stimulus

Thermal sensations resulting from the application of the warm wet stimulus varied significantly as a function of body region (main effect: $F_{(3,3, 59.7)}=10.2$; $p<0.001$), but not of sex (main effect: $F_{(1, 18)}=3.5$; $p=0.079$) (Fig. 2C & F). Irrespective of sex, we observed a cranio-caudal decrease in the magnitude of warm sensations experienced as a result of the same warm wet stimulus at rest (Fig. 2C), with the neck presenting the most intense warm sensations while the foot the least intense, in both males (mean difference neck vs. foot: 68.3 mm [95%CI 46.6, 90.3]; $p<0.001$; corresponds to ~34% difference) and females (mean difference forehead vs. foot: 46.6 mm [95%CI 35.9, 57.3]; $p<0.001$; corresponds to ~23% difference). Exercise induced decreases in warm sensations to the same warm wet stimulus (main effect: $F_{(1, 18)}=5.4$; $p=0.032$), with this effect being more pronounced for certain regions (interaction body region and exercise: $F_{(2,4, 43.5)}=3.5$; $p=0.030$) such as the male neck (mean difference pre- vs. post-exercise: 23.1 mm [95%CI 9.2, 37.0]; $p=0.004$; corresponds to ~11% difference) (compare fig. 2C and 2F).

Wetness perceptions resulting from the application of the warm wet stimulus did not vary neither as a function of sex (main effect: $F_{(1, 18)}=2.6$; $p=0.123$), nor body region (main effect: $F_{(2,7, 48.8)}=2.2$; $p=0.107$) (Fig. 3C & F). Irrespective of sex and body region, exercise induced a general reduction in wetness sensations arising from the warm wet stimulus (main effect: $F_{(1, 18)}=7.3$; $p=0.015$) in both males (pre-exercise mean wetness perception collapsed over body region: 31.9 ± 15.8 mm; post exercise: 20.3 ± 4.6 mm) and females (pre-exercise mean wetness perception collapsed over body region: 46.0 ± 5.4 mm; post exercise: 38.7 ± 1.6 mm) (compare fig. 3C and 3F). When expressed as percentage of the wetness VAS scale used, those exercise-induced differences corresponded to males and females being ~6% and ~4% less warm sensitive than males at rest and post exercise, respectively.

All in all, the findings indicated that the warm wet stimulus induced similar warm sensations in both males and females, with both sexes showing a similar pattern of cranio-caudal decrease in warm sensitivity (i.e. ~34% males; ~23% females); they also indicated that the

warm wet stimulus induced wetness sensations that did not differ between sexes nor across different body regions, and that these wet sensations generally decreased in intensity following exercise (i.e. ~6% males; ~4% females).

Comparison of cold-wetness, neutral-wetness, and warm-wetness perceptions

When comparing the overall level of wetness (i.e. collapsed over body region) experienced as a result of the cold-wet, neutral-wet, and warm-wet stimulus, we observed that the cold-wet stimulus induced consistently greater wetness perceptions than the neutral- and warm-wet stimuli (Fig. 4), despite all stimuli presenting the same level of wetness (i.e. 0.8 ml water). At rest, males perceived the cold-wet stimulus as wetter ($F_{(1.5, 13.3)}=19.7$; $p<0.001$) than both neutral-wet (mean difference: 32.1 mm [95%CI 20.9, 43.3]; $p<0.001$; corresponds to ~32% difference) and warm-wet (mean difference: 19.8 mm [95%CI 1.8, 37.9]; $p=0.033$; corresponds to ~20% difference), with no differences between neutral- and warm-wet (mean difference: -12.3 mm [95%CI -25.3, 0.7]; $p=0.064$) (Fig. 4A). Similarly, at rest females perceived the cold-wet stimulus as wetter ($F_{(1.6, 14.8)}=39.8$; $p<0.001$) than both neutral-wet (mean difference: 40.8 mm [95%CI 29.7, 51.9]; $p<0.001$; corresponds to ~41% difference) and warm-wet (mean difference: 23.0 mm [95%CI 7.5, 38.5]; $p=0.006$; corresponds to ~23% difference); they also perceived the warm-wet as wetter than the neutral-wet (mean difference: 17.9 mm [95%CI 6.6, 29.2]; $p=0.004$; corresponds to ~18% difference) (Fig. 4B). Post exercise, males perceived the cold-wet stimulus as wetter ($F_{(1.4, 12.7)}=31.5$; $p<0.001$) than both neutral-wet (mean difference: 34.5 mm [95%CI 25.1, 43.8]; $p<0.001$; corresponds to ~34% difference) and warm-wet (mean difference: 30.3 mm [95%CI 13.6, 47.1]; $p=0.002$; corresponds to ~30% difference), with no differences between neutral- and warm-wet (mean difference: -4.2 mm [95%CI -16.7, 8.4]; $p=0.636$) (Fig. 4C). Similarly, post exercise females perceived the cold-wet stimulus as wetter ($F_{(1.6, 14.8)}=19.9$; $p<0.001$) than both neutral-wet (mean difference: 32.8 mm [95%CI 20.6, 45.0]; $p<0.001$; corresponds to ~33% difference) and warm-wet (mean difference: 25.7 mm [95%CI 7.4, 43.9]; $p=0.009$; corresponds to ~26% difference), with no differences between neutral- and warm-wet (mean difference: -7.1 mm [95%CI -21.8, 7.6]; $p=0.403$) (Fig. 4D).

Relationship between wetness perception and thermal sensations

In males at rest, a quadratic model best fitted the data in 7 out 10 individual datasets (F-test $p<0.05$). In males post exercise and females at rest, a quadratic model best fitted the data (F-test $p<0.05$) in 5 out 10 individual datasets. In females post exercise, a quadratic model best

fitted) the data (F-test $p < 0.05$) in 4 out of 10 individual datasets. Individual R^2 values arising from best fitting individual model (i.e. linear vs. quadratic) are reported in Table 3. Analysis of individual R^2 values indicated that: 1) changes in the magnitude of thermal sensations explained an average 44% ($\pm 29\%$) and 42% ($\pm 40\%$) of changes in wetness perception in males at rest and post exercise, respectively; 2) changes in the magnitude of thermal sensation explained an average 33% ($\pm 32\%$) and 29% ($\pm 30\%$) of changes in wetness perception in females at rest and post exercise, respectively. Neither sex ($F_{(1, 18)} = 0.95$; $p = 0.342$) nor exercise ($F_{(1, 18)} = 0.09$; $p = 0.769$) had an independent effect on the variance in wetness perception explained by thermal sensations.

Following on the individual analyses we went on developing group models that could provide a generalisable relationship between thermal and wetness perceptions which accounted for the inter-individual variability observed in the individual models. Visual inspection and comparison between R^2 values resulting from linear vs. quadratic polynomial group model fitting indicated that second order (quadratic) polynomial regression models best fitted group data for the relationship between wetness perceptions and thermal sensations. This observation applied to both male and female data for both the rest and post exercise components of the test. Models parameters with 95% CIs and related R^2 values are summarised in figure 5. Based on the fitted group models, thermal and wetness perceptions presented a U-shaped relationship across the thermal sensation continuum (i.e. from very cold to very hot), with thermal sensations explaining 41% and 36% of the variability in wetness perceptions at rest in males (Fig. 5A) and females (Fig. 5B), respectively. Post exercise group models indicated a reduction in variance explained by thermal sensations in both males (i.e. 17%, Fig. 5C) and females (i.e. 20%, Fig. 5D), and they also showed a “downward” shift and a “shrinkage” over the horizontal axis in both sexes (see fig. 5C & D), likely owing to the exercise-induced reductions in thermal and wetness sensitivity as described above.

Discussion

The aim of this study was to determine the independent and interactive effect of sex, body region, and maximal incremental running on humans’ local sensitivity to cold, neutral, and warm skin wetness.

In relation to our initial hypotheses, our findings indicated that: 1) females were ~14 to ~17% more sensitive to cold-wetness than males, yet they were as sensitive to neutral- and warm-wetness as their male counterparts; 2) regional differences were present for cold-wetness only, and these followed a cranio-caudal pattern of increased sensitivity that was more

pronounced in males (i.e. the foot was ~31% more sensitive than the forehead); 3) maximal exercise reduced cold-wetness sensitivity over specific regions in males only (i.e. ~40% decrease in foot sensitivity), and it also induced a generalised reduction in warm-wetness sensitivity in both sexes (i.e. ~4 to ~6%). Additionally, we observed a clear U-shaped relationship between thermal and wetness perceptions (Fig. 5), where greater thermal sensations (and particularly cold sensations) induced greater wetness perceptions, and where exercise-induced reductions in thermal sensitivity translated in reduction in wetness sensitivity.

To our knowledge, this is the first study to provide empirical evidence for the fact that females are more sensitive to skin wetness than males, and that this difference is dependent on the thermal quality of the skin wetness experienced, i.e. there are greater sex differences for cold than warm wetness sensitivity. Importantly, our data provide clear evidence that the independent role of sex is rooted in sex-related differences in thermal sensing (i.e. females were ~8 to ~14% more cold sensitive than males), and that the relationship between thermal and wetness sensing is one that strongly determines the extent of wetness that a stimulus will induce based on its thermal qualities (consider fig. 5).

Finally, we showed for the first time that a single bout of maximal exercise can reduce both sexes' sensitivity to skin wetness to an extent that is dependent on the concurrent exercise-induced reduction in thermal sensation. Hence, our results provide novel evidence for the fact that the previously described exercise-induced thermo-hypoesthesia is accompanied by “*hygro-hypoesthesia*”, i.e. a reduction in skin wetness sensitivity, in healthy young males and females.

The role of biological sex in human wetness sensing

Our results indicate that females rely on similar integration mechanism for skin wetness sensing as the ones previously described (and also observed here) in males (Filingeri *et al.*, 2014a). This is confirmed by the fact that, despite all the wet stimuli used in the current study presented the same level of physical skin wetness (i.e. 0.8 ml of water), both male and female participants systematically perceived the cold-wet stimulus as largely wetter (i.e. ~20 to ~40%) than the neutral- and warm-wet stimuli, both at rest and post exercise (see fig. 4). This perceptual behaviour is well predicted by our neurophysiological model of skin wetness sensing, which has shown that irrespective of the physical presence of moisture on the skin, activations of cold-sensitive A-type skin thermoreceptors will trigger the neural representation of a typical wet stimulus (hence a perception of wetness), which is often

associated with the cooling sensations arising from evaporative skin cooling (Filingeri *et al.*, 2014a; Filingeri & Havenith, 2015, 2018). Humans are therefore more likely to perceive cold-wet (and cold-dry) (Filingeri *et al.*, 2013, 2014e) stimuli as wetter than equally wet warm (Filingeri *et al.*, 2015c) and neutral stimuli (Filingeri *et al.*, 2014a). The fact that we often struggle to determine whether the washing hanging on the line is wet or just cold, as well the common experience of not immediately realising to have a nose bleed (note: blood is often warmer than the skin), are good real-life examples of how much we rely on coldness to infer about skin wetness (Filingeri, 2016). Finally, the fact that both sexes presented a clear U-shaped relationship between their thermal and wetness perceptions (see fig. 5), which was asymmetrical between the cold and warm portions of the thermal sensation continuum (i.e. given the same magnitude of thermal sensation, cold stimuli induced greater wetness sensations than warm stimuli) provided further evidence for the presence of similar sensory integration mechanisms for wetness sensing in males and females.

While our male and female participants appeared to experience skin wetness according to similar thermosensory mechanisms, the extent of skin wetness experienced was different between sexes, with females being ~14 to ~17% more sensitive to cold wetness than males, despite both sexes were exposed to the same amount of physical moisture. Interestingly, the greater female sensitivity to cold-wetness correlated well with the fact that females were also ~8 to ~14% more cold sensitive than males. Once again, these findings fit well our neurophysiological model of skin wetness (Filingeri *et al.*, 2014a) and the fact that colder sensations are generally associated with wetter perceptions (Filingeri *et al.*, 2014b). It would therefore appear likely that the greater sensitivity to coldness of females is at the root of this sex's greater sensitivity to cold wetness. Importantly, this observation is confirmed by the fact that males and females presented similar sensitivity to warmth, and consequently they were equally sensitive to warm-wetness.

Females have been previously reported to be more thermally sensitive than males (Gerrett *et al.*, 2014); yet sex-differences in thermal sensitivity are often ambiguous (Stevens & Choo, 1996), and so it remains to be fully elucidated whether sex has an independent physiological role in those difference (Filingeri *et al.*, 2018).

Body morphology is an important factor in driving sex-related thermoregulatory differences, and this also applies to thermosensation. Spatial summation in the thermal sense exists and it explains why, given the same thermal stimulus, stimulating a larger portion of skin induce more intense thermal sensations (Stevens *et al.*, 1974). In this respect, we have recently shown that body surface area-size matched males and females present limited differences in

warm and cold sensitivity across their hands and feet (Filingeri *et al.*, 2018). It could be speculated that the greater female cold (and wetness) sensitivity observed here could be driven by the fact that our female group had a smaller body surface area than males (see Tab. 1), and that this translated in a greater proportion of their skin being stimulated by the fixed size (1.32cm²) thermal probe we used (see tab. 1). However, it should be noted that, had body surface area driven sex-differences in thermal sensations, we would have expected our female group to be also more warm sensitive than males; yet this was not the case. Hence, it cannot be excluded that the sex differences in cold and cold-wetness sensitivity are dependent on either a greater density of cold sensitive afferents or in differently weighted central integration mechanism for thermal sensations in females (Filingeri, 2016). The greater female sensitivity could be driven by the greater thermoprotective needs that females have when exposed to the cold, given that they generally present smaller body masses and lower resting metabolic rates than males (Gagnon *et al.*, 2008).

Aside from their physiological purpose, it is worth noting that our observed sex differences in skin wetness sensing well complement some recent reports demonstrating that females present more sensitive thermal behaviours than males during exercise (Vargas *et al.*, 2019b). We feel that some of these recent behavioural observations could be explained by our observations that females are more cold and wetness sensitive and that this could underlie their greater behavioural sensitivity to thermal discomfort and to changes in body temperature (Vargas *et al.*, 2019b).

The role of body region in human wetness sensing

Our current study provides further evidence that skin wetness sensitivity does vary across the body, yet we show that these regional differences are dependent on the thermal quality of wetness, and that are indeed limited to cold-wet stimuli. Specifically, we observed a cranio-caudal increase in cold wetness sensitivity in males (and to a lesser extent in females) (see fig. 3A). This pattern was in line with the observed cranio-caudal increase in cold sensitivity in both sexes (see fig. 2A) and it therefore further supports the importance of cold sensing for discriminating wetness levels across the body (Filingeri *et al.*, 2014b). Interestingly, we did not observe any regional difference in neither neutral- nor warm-wetness sensitivity (see fig. 3B & C), despite warm thermal sensitivity presented a clear cranio-caudal decrease, with the foot being less sensitive than the forehead, in both males and females (see fig. 2C). We believe that these thermal-quality dependent patterns of regional wetness sensitivity are driven by changes in the relative importance of thermal cues for wetness sensing as one

moves from colder to warmer wet stimuli. As we previously described (Filingeri *et al.*, 2014a), when the key cold thermal cues that strongly underpin the neural representation of a typical wet stimulus are lacking (i.e. in the presence of neutral- and warm-wetness), humans increase their reliance on mechanosensory cues (i.e. movement of moisture across the skin, skin friction, stickiness and adhesion of wet skin with clothing), which are driven by the activation of A β skin mechanoreceptors (Bergmann Tiest *et al.*, 2012; Filingeri *et al.*, 2015a). Given that in the present study we only performed a static application of wet stimuli, it therefore appears likely that the lack of regional differences in neutral and warm wet sensitivity is due to the insufficient stimulation of those mechanosensory afferents that play a greater role in neutral and warm wetness sensing. Further support to the reduced role of thermal afferents in neutral and warm wetness sensing is provided by the observation that both sexes experienced ~20 to ~40% less wetness when the stimuli were neutral and warm than when they were cold (see fig. 4). Given that mechanosensory innervation varies greatly across the body (Johansson & Vallbo, 1979), that tactile sensitivity has been repeatedly shown to vary regionally (Ackerley *et al.*, 2014), and that humans discriminate regional wetness levels during exercise-induced sweating (i.e. likely inducing warm-wet sensations) (Lee *et al.*, 2011), it could be speculated that regional differences in warm wetness sensing could also exist in humans, yet these might become apparent only under conditions of dynamic skin interactions with warm wet stimuli.

The role of exercise in human wetness sensing: hygro-hypoesthesia

In showing that maximal incremental running induced a localised reduction in cold wetness sensitivity in males (i.e. foot, ~40%), as well as a generalised reduction in warm wetness sensitivity in both sexes (~4 to ~6%), our findings provide the first observation of exercise-induced *hygro-hypoesthesia*. It is noteworthy that the quality and extent of *hygro-hypoesthesia* observed here correlated well with a reduction in our participants' thermal sensitivity (e.g. ~24% reduction in male foot cold sensitivity; ~10% reduction in both sexes' warm sensitivity). Exercise-induced changes in thermosensing are therefore likely to trigger equivalent changes in hygrosensing.

The exact mechanisms for exercise-induced thermo-hypoesthesia and consequent hygro-hypoesthesia cannot be fully determined here and we can only speculate that an involvement of the endogenous opioid neural systems might have occurred as a result of high intensity running exercise, as it has previously been shown for pain (Janal *et al.*, 1984). Nevertheless, exercise-induced local T_{sk} changes could have also played a role in modulating some of the

perceptual changes observed. Our quantitative sensory test did account for exercise-induced changes in local T_{sk} (see fig. 1) as we used stimuli whose temperatures were relative to the local T_{sk} pre-stimulation. In this way we ensured that the same relative thermal stimulus would be applied pre and post exercise. Yet in doing so, we necessarily changed the absolute temperature of the stimuli applied pre and post exercise. For example, the absolute temperature of cold-wet stimulus applied to the foot of males was on average $\sim 25.1^{\circ}\text{C}$ pre exercise and $\sim 28.6^{\circ}\text{C}$ post exercise. While both stimuli were well within the range of activation of cold-sensitive thermoreceptors (Filingeri *et al.*, 2017), it could be argued that the “less cold” (in absolute terms) post exercise stimulus could have induced lower steady state discharge of cold sensitive thermoreceptors, which are known to have a peak frequency sensitivity at steady state temperatures of $\sim 27^{\circ}\text{C}$ (Hensel & Iggo, 1971). A similar scenario might have occurred with regards to the application of warm wet stimuli.

Finally, we have recently demonstrated that changes in whole-body thermal state can modulate local thermal sensitivity (Filingeri *et al.*, 2016), and so it cannot be excluded that exercise-induced changes in mean T_{sk} and T_{core} could have also shifted local thermal sensitivity (Cabanac *et al.*, 1972). The same considerations could apply to the differential changes in T_{core} occurring between males and females and their potential contribution to our observed sex-differences in wetness sensing.

Irrespective of whether exercise-induced neuroendocrine or biophysical changes are the primary trigger of hygro-hypoesthesia, our observation of a reduced skin wetness sensitivity is particularly relevant in the context of better understanding how thermoregulatory behaviours during and following exercise are modulated by changes in local sensitivity to temperature and skin wetness. Physical skin wetness has been recently shown to describe 52% of the variance in thermoregulatory behaviours during and following exercise, thereby proving to be the most significant drive to exercise-induced thermal behaviours (Vargas *et al.*, 2018). Yet it remains unclear whether *physical* as opposed to *perceived* skin wetness is a more important trigger of discomfort and related behaviours (Vargas *et al.*, 2018). Our results indicate that skin wetness sensitivity is likely to be reduced following exercise, and so it could be argued that if one were to observe a maintained behavioural response to the same level of physical wetness following exercise, then this is likely arising from physical skin wetness being a greater trigger of thermal behaviours than from its conscious experience. In support of the latter, we recently showed that despite we were able to modify skin wetness perception independently of physical skin wetness in exercising humans (Filingeri *et al.*, 2015a), this did not result in any meaningful change in thermal discomfort (i.e. a key trigger

of thermal behaviours), and the latter was better described by changes in physical than perceptual skin wetness (Gagge *et al.*, 1967). Nevertheless, future studies should combine perceptual and behavioural assessments to untangle the independent role of physical and perceptual skin wetness on human thermoregulatory responses.

Limitations and experimental considerations

There are two experimental considerations to be made when interpreting our findings. First, we did not control for the phase of menstrual cycle of our female participants. There is direct evidence that thermal sensations in females are not independently modified by menstruation (Matsuda-Nakamura *et al.*, 2015). Yet tactile sensitivity (which plays a role in dynamic skin wetness sensitivity), is influenced by the phase of the menstrual cycle (Robinson & Short, 1977). Accordingly, future studies should consider the independent role of menstruation on local skin wetness sensitivity, particularly under dynamic skin interactions with wet stimuli. Second, we recognize that infrared thermometry for measuring tympanic T_{core} and local T_{sk} carries an estimation error of up to 0.5°C and 1°C, respectively. This estimation error could have biased some perceptual responses based on local measurements of T_{sk} . To this end, we quantified the potential impact of this error in our findings, by determining the relationship between thermal sensations and absolute temperature of the stimuli in both males and females. The resulting regression model (i.e. stimuli temperature vs. thermal sensation; $y=11.152x-259.69$, $R^2=0.89$) allowed calculation of the perceptual change arising from a 1°C change in local stimulus temperature, which corresponded to the maximal error of our local T_{sk} measurement. When converted into a percentage of the 200-mm VAS scale, this gave a maximum perceptual change of 5.6%. This value is well below the range of effect sizes observed for the sex-, regional-, and exercise-induced differences in wetness perception reported here. Yet, the implications of those measurements' errors should be carefully considered when interpreting perceptual results similar methodologies to ours.

Conclusions

For the first time to our knowledge, we show that young healthy females are more sensitive to cold, but not neutral nor warm, skin wetness than healthy young males. We also show that regional differences to skin wetness exists, yet under static contact with moisture, these are greater for cold than warm wet stimuli. Finally, we demonstrate that maximal incremental running induces *hygro-hypoesthesia*, which is strongly driven by the quality and extent of exercise-induced thermo-hypoesthesia. Our findings confirm the importance that afferent

thermosensory inputs from cold-sensitive skin thermoreceptors play in human wetness sensing and demonstrate that the central integration mechanisms for wetness sensing are shared by males and females. The outcomes of this study carry fundamental physiological significance as they provide mechanistic evidence for sex differences in thermoregulatory behaviours. Also, they carry applied significance, as the body maps created, along with the wetness models developed, will inform the design of more effective sport and protective clothing, as well as they will feed into the optimization of individualised thermoregulatory models.

Competing interests

The authors report no conflict of interest.

Author contributions

AV, AB and DF conceived and designed the work. AV acquired the data. AV, AB, and DF analysed and interpreted the data, drafted the work and revised it critically for intellectual content. All authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Tables

Table 1. Participants' characteristics, including age, mass, height, body surface area (BSA), proportion of BSA stimulated by the fixed-size (i.e. 1.32cm²) thermal probe used, are reported for the male and female groups. Menstrual cycle and oral contraceptive information are also reported for the female group only. Statistical differences between groups for each characteristic were assessed by means of independent group t-tests, with cut-off probability value for significance set at p=0.05.

	Age (years)	Mass (Kg)	Height (m)	BSA (m ²)	Proportion of BSA stimulated (%)	Self-reported day of menstrual cycle	Oral contraceptive Y (N)
Males (N=10)	27.8 ± 2.7	76.4 ± 10.2	1.77 ± 0.1	1.92 ± 0.1	0.0069 ± 0.0005		
Females (N=10)	25.4 ± 3.9	62.7 ± 8.0	1.65 ± 0.1	1.68 ± 0.1	0.0079 ± 0.0006	16.3 ± 8.1	2 (8)
Probability	0.130	0.004	<0.001	0.001	0.001		

Table 2. Physiological responses to the maximal exercise test. Data are reported as means with [95% confidence intervals]. * denotes statistical difference between rest and exercise with cut-off probability value for significance set at $p=0.05$.

	Males (N=10)	Females (N=10)
Δ HR (bpm)	+113.6 [+104.7, +122.5]*	+100.6 [+91.6, +109.5]*
Δ Tympanic T_{core} ($^{\circ}$C)	+0.81 [+0.54, +1.07]*	+0.56 [+0.29, +0.82]*
Δ Mean T_{sk} ($^{\circ}$C)	-0.81 [-1.22, -0.39]*	-0.93 [-1.34, -0.51]*

Table 3. Summary data for individual model fitting for the relationship between thermal sensations and wetness perceptions for each participant, at rest and post exercise. A quadratic polynomial or linear model best fit was determined based on the outcome of an extra-sum-of squares F test (probability values are reported; * denotes statistical difference at $p < 0.05$). Variance in wetness perception explained by thermal sensation is reported as R^2 values.

Male Rest				Female Rest			
Participant	Model	R^2	Probability	Participant	Model	R^2	Probability
	(polynomial=1; linear=0)		(polynomial best fitting vs. linear)		(polynomial=1; linear=0)		(polynomial best fitting vs. linear)
1	1	0.57	0.011*	1	1	0.87	0.001*
2	1	0.37	0.010*	2	0	0.27	0.368
3	1	0.68	0.001*	3	1	0.50	0.003*
4	0	0.10	0.387	4	0	-0.09	0.154
5	1	0.86	0.001*	5	1	0.61	0.032*
6	1	0.67	0.001*	6	1	0.44	0.018*
7	1	0.30	0.028*	7	0	-0.07	0.669
8	0	0.18	0.276	8	1	0.50	0.045*
9	1	0.63	0.001*	9	0	-0.07	0.070
10	0	0.01	0.311	10	0	0.34	0.287
Mean \pm SD	0.70 \pm 0.48	0.44 \pm 0.29		Mean \pm SD	0.50 \pm 0.53	0.33 \pm 0.32	

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Male post exercise				Female post exercise			
Participant	Model	R ²	Probability	Participant	Model	R ²	Probability
	(polynomial=1; linear=0)		(polynomial best fitting vs. linear)		(polynomial=1; linear=0)		(polynomial best fitting vs. linear)
1	1	0.75	0.007*	1	1	0.91	0.001*
2	1	0.98	0.001*	2	1	0.42	0.004*
3	0	-0.06	0.119	3	1	0.24	0.025*
4	0	0.33	0.473	4	0	-0.03	0.728
5	1	0.81	0.004*	5	0	0.57	0.150
6	1	0.31	0.030*	6	0	0.23	0.060
7	0	0.08	0.257	7	1	0.33	0.027*
8	1	0.91	0.001*	8	0	0.33	0.987
9	0	-0.04	0.107	9	0	-0.03	0.502
10	0	0.15	0.059	10	0	-0.03	0.174
Mean ± SD	0.50 ± 0.53	0.42 ± 0.40		Mean ± SD	0.40 ± 0.52	0.29 ± 0.30	

Figures

Figure 1. Body maps of pre-stimulation local T_{sk} in males (N=10) and females (N=10) at rest (A) and following maximal incremental running (B). Numerical data represent group means. Symbols denote statistical differences at $p<0.05$, where α = different from forehead; β = different from neck; γ = different from underarm; δ = different from lower lateral back; ϵ = different from dorsal foot; $\#$ = interaction body region with exercise.

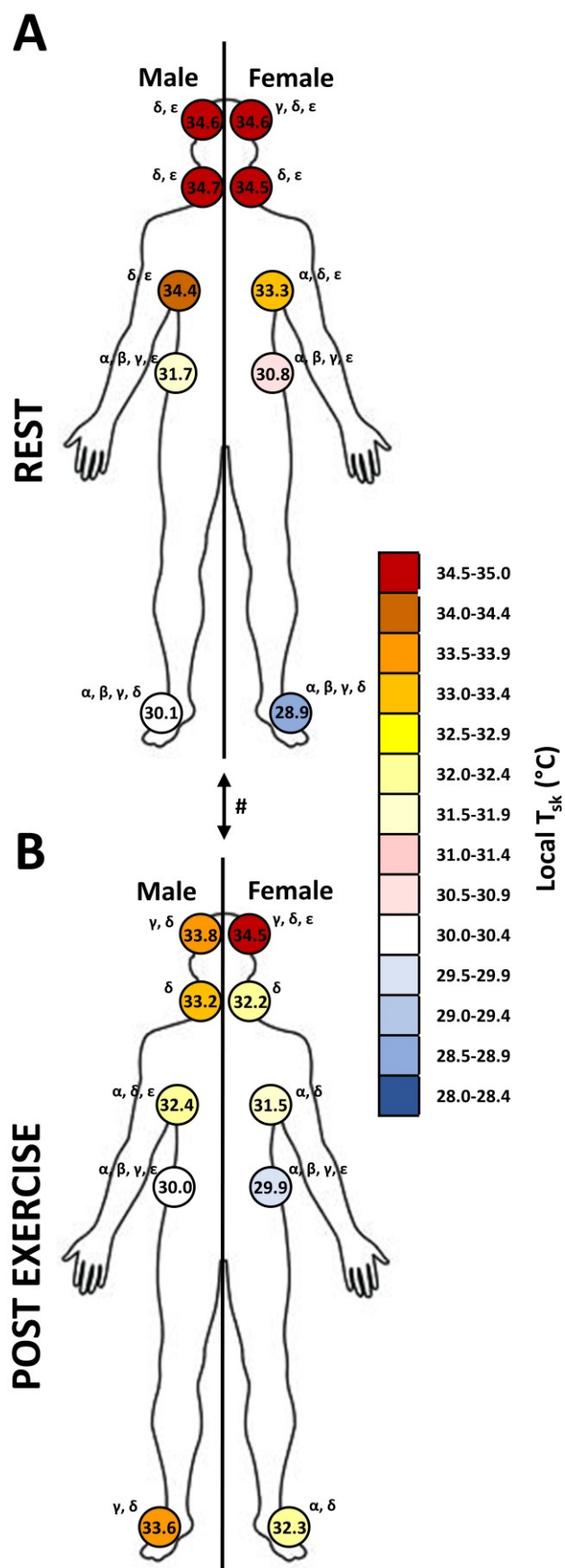


Figure 2. Body maps of thermal sensations in males (N=10) and females (N=10) resulting from the application of the cold wet (A, D), neutral wet (B, E), and warm wet stimulus (C, F), at rest and following maximal incremental running. Numerical data represent group means. Symbols denote statistical differences at $p < 0.05$, where α = different from forehead; β = different from neck; γ = different from underarm; δ = different from lower lateral back; ϵ = different from dorsal foot; *= main effect of sex; #= interaction body region with exercise.

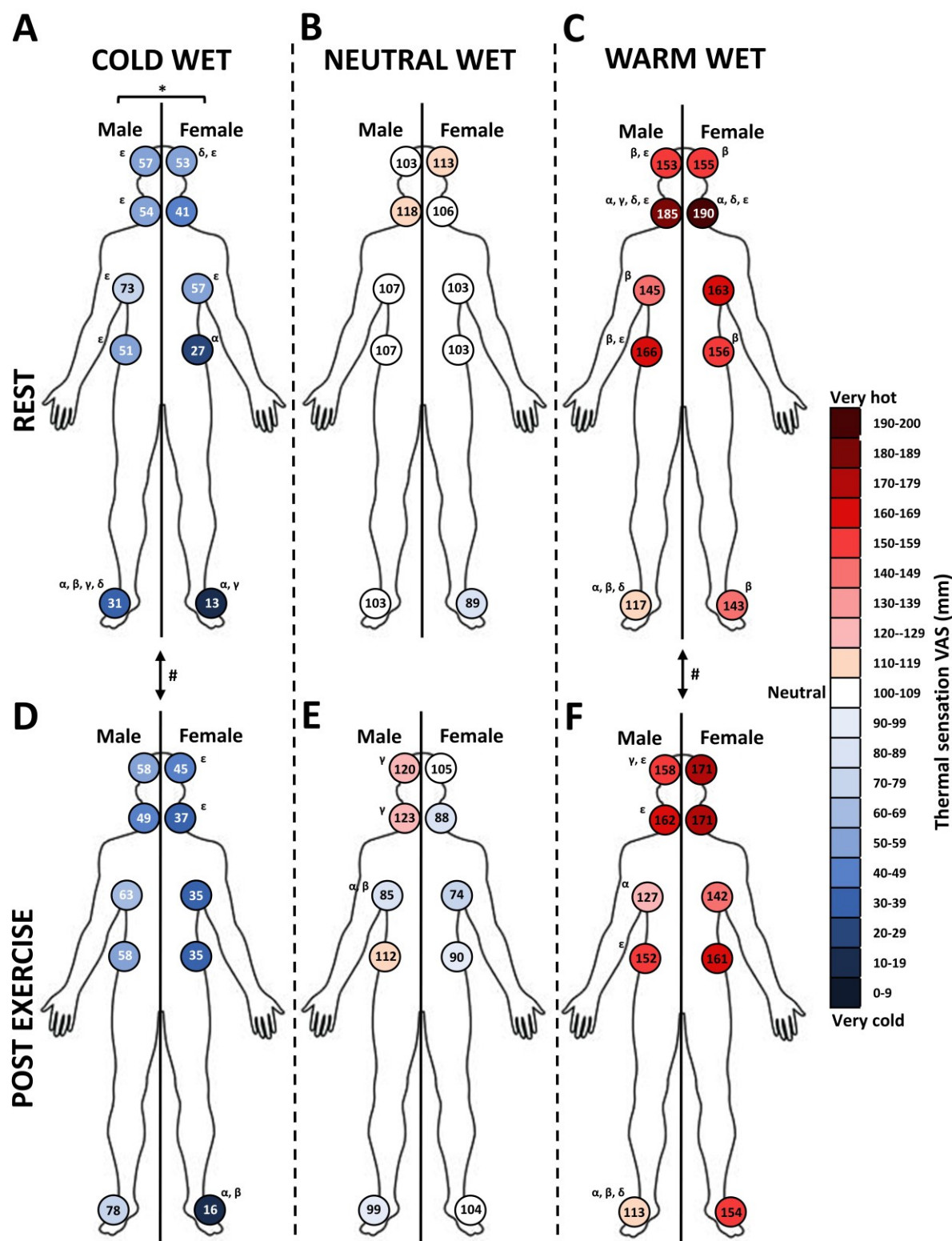


Figure 3. Body maps of wetness perceptions in males (N=10) and females (N=10) resulting from the application of the cold wet (A, D), neutral wet (B, E), and warm wet stimulus (C, F), at rest and following maximal incremental running. Numerical data represent group means. Symbols denote statistical differences at $p < 0.05$, where α = different from forehead; β = different from neck; γ = different from underarm; δ = different from lower lateral back; ϵ = different from dorsal foot; *= main effect of sex; #= interaction body region with exercise; \forall = main effect of exercise.

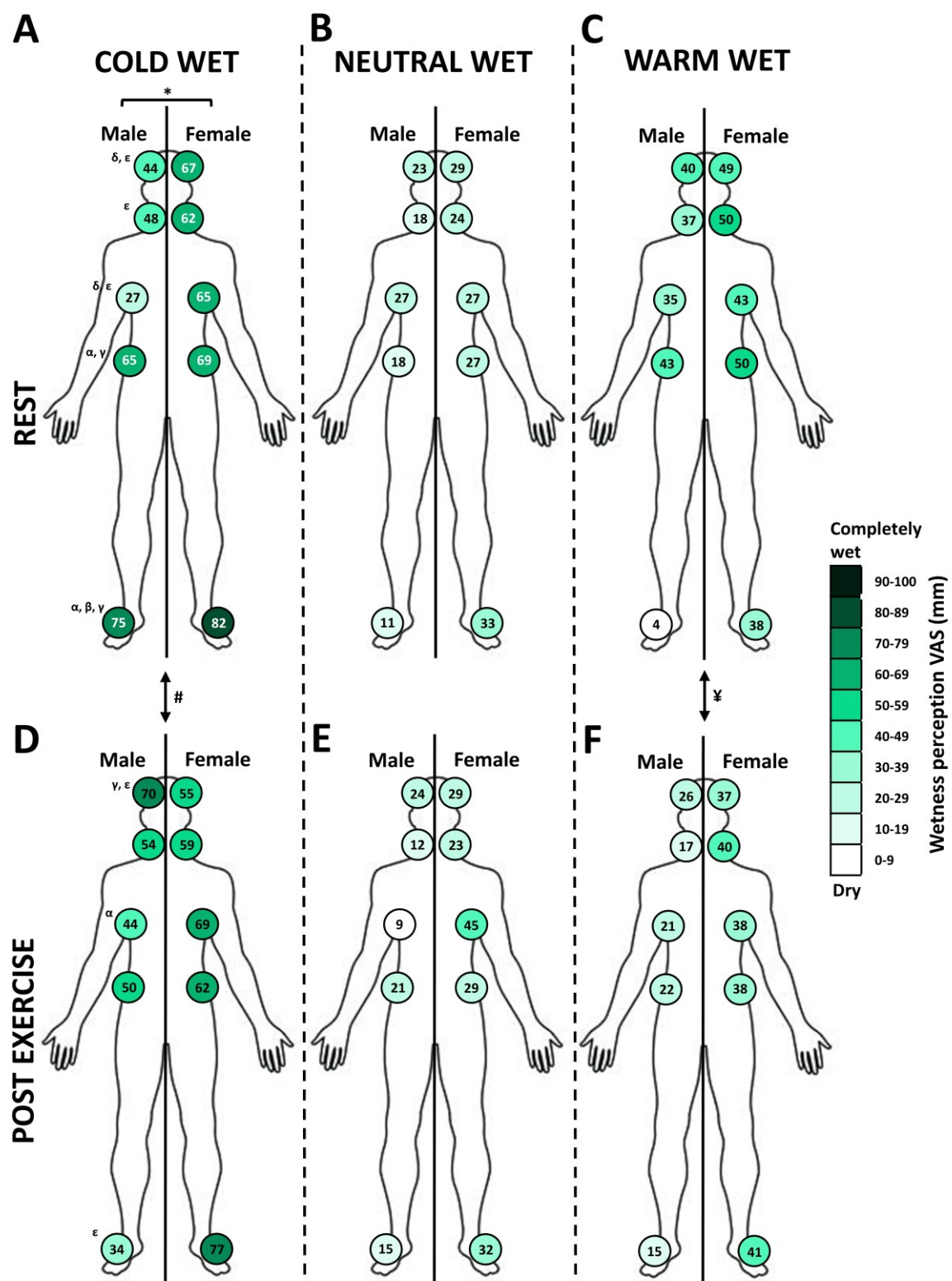


Figure 4. Box and whisker plots and individual data (N=10 per group) for wetness perceptions arising from the application of the cold wet, neutral wet, and warm wet stimulus, at rest and following maximal incremental running in males (A, C) and females (B, D).

Wetness perception data are collapsed over body region for each data point (i.e. participant).

*denotes statistical significance at $p < 0.05$.



Figure 5. Quadratic regression models of the relationship between mean (and SD) thermal sensations and wetness perceptions at rest and post exercise in males (A, C) and females (B, D). Dedicated tables present model parameters with 95%CI and R^2 values. Model fit lines are depicted in red with 95%CI grey bands.

