Journal of Thermal Biology 77 (2018) 137–144

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Sex difference in cold perception and shivering onset upon gradual cold exposure

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ARTICLE INFO

Keywords:
Cold temperature
Sex characteristics
Shivering
Skin temperature
Thermogenesis
Thermosensing

ABSTRACT

To maintain a thermal balance when experiencing cold, humans reduce heat loss and enhance heat production. A potent and rapid mechanism for heat generation is shivering. Research has shown that women prefer a warmer environment and feel less comfortable than men in the same thermal condition. Using the Blanketrol® III, a temperature management device commonly used to study brown adipose tissue activity, we tested whether the experimental temperature (T_E) at which men and women start to shiver differs. Twenty male and 23 female volunteers underwent a cooling protocol, starting at 24 °C and gradually decreasing by 1–2 °C every 5 min until an electromyogram detected the shivering or the temperature reached 9 °C. Women started shivering at a higher T_E than men (11.3 ± 1.8 °C for women *vs* 9.6 ± 1.8 °C for men, $P = 0.003$). In addition, women felt cool, scored by a visual analogue scale, at a higher T_E than men (18.3 ± 3.0 °C for women *vs* 14.6 ± 2.6 °C for men, $P < 0.001$). This study demonstrates a sex difference in response to cold exposure: women require shivering as a source of heat production earlier than men. This difference could be important and sex should be considered when using cooling protocols in physiological studies.

1. Introduction

Humans tightly control their core body temperature (T_c) by balancing heat loss and heat production (Costanzo, 2017). When exposed to mild cold, humans first reduce heat loss by energy-inexpensive mechanisms such as the constriction of blood vessels supplying the peripheral tissues. This peripheral vasoconstriction not only reduces the heat transfer from the isothermal core to the non-isothermal shell, but also increases the insulating capacity of the skin and subcutaneous tissues. The shift of blood from superficial layers to deeper vessels results in an increased total body insulation since the bloodless layer, where the convective heat loss substantially diminishes, becomes thicker (Anderson, 1999). However, if the thermal balance cannot be accomplished by a reduction in heat loss, heat production is required (Tansey and Johnson, 2015).

Heat production can be achieved by many mechanisms (Castellani and Young, 2016; Hall, 2015). Exposure to cold activates the sympathetic nervous system (SNS), immediately increasing the metabolic rates of all cells in the body that consequently generate heat as a by-product of metabolism. The direct stimulation of β-adrenergic receptors by the SNS also activates brown adipose tissue (BAT), a specialized metabolic tissue that can convert energy into heat (Lee et al., 2013). When the metabolic heat production (non-shivering thermogenesis) together with the cutaneous vasoconstriction is not sufficient to maintain the optimal T_c, shivering begins.

Shivering, which is the involuntary rhythmic contraction of skeletal muscles, is the most potent and rapid mechanism to generate heat in response to cold stress. When the skin senses cold via the transient receptor potential cation channel subfamily M member 8 (TRPM8) on the sensory nerves (Voets et al., 2004), it signals to the temperature center in the hypothalamus. The primary motor center for shivering in the posterior hypothalamus is then activated and transmits signals to the skeletal muscles to initiate shivering throughout the body (Hall, 2015). At the maximum intensity of shivering, metabolic heat production can rise to five times of the resting levels (Eyolfson et al., 2001).

Various cooling techniques have been used to study the physiological responses to cold environment, especially after the rediscovery of BAT in adult humans in the last decade (Nedergaard et al., 2007) because cold is a well-known stimulant for the thermogenic function of BAT. The cooling methods include cold-water immersion, cold-air

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https://doi.org/10.1016/j.jtherbio.2018.08.016
Received 10 May 2018; Received in revised form 21 August 2018; Accepted 22 August 2018

exposure, cold-air exposure combined with localized cooling e.g. feet cooling in ice water, and water-filled cooling blankets or suits (Castellani and Young, 2016; van der Lans et al., 2014). The cooling blanket with temperature of the filling water set at 1–2 °C above the shivering point (also known as a personalized cooling protocol) is likely the method that maximally activates BAT (Bahler et al., 2017). Using this cooling method, conduction will be the mode of heat transfer at those body areas that are covered and in direct contact with the cooling blankets whereas convection will occur at areas without direct contact with the blankets. One of the frequently used cooling blankets is the Blanketrol® III, a temperature management device that can control the temperature of the circulating water in a range from 4 °C to 42 °C.

Research has shown that sex is one of the important factors that influence thermal perception and physiological responses to cold. Karjalainen (2007) demonstrated that women prefer a higher ambient temperature and feel less comfortable than men in the same thermal environment, especially during the winter season. Furthermore, Kingma and van Marken Lichtenbelt (2015) showed that women require more heat production than men in the standard indoor climate setting that was mainly based on male metabolic rates. Castellani and Young (2016) revealed that the primary source of the variable capability to maintain a normal T_c between men and women during whole-body cold exposure is body anthropometric and body composition characteristics. At the same body mass and surface area, women generally have a higher subcutaneous fat content than men that enhances insulation (Anderson, 1999; Castellani and Young, 2016; Kuk et al., 2005). On the other hand, when the subcutaneous fat thickness is equal between a man and a woman, the latter in general will have a larger body surface area (BSA) and a smaller body mass contributing to a greater total heat loss and a lower heat-production capacity during resting cold exposure (Castellani and Young, 2016; Graham, 1988).

Understanding sex differences in thermal regulation and cold-induced physiological responses is beneficial in many aspects. For instance, Iyoho et al. (2017) proposed a sex-specific modification of the thermoregulation model for predicting thermal response in a wide range of the operational conditions for military relevant tasks, especially in the cold-stress responses. Chaudhuri et al. (2018) showed that different physiological parameters from male and female occupants were needed to accurately predict the thermal comfort status in a range of the general indoor climate setting. Graham (1988) demonstrated that men and women respond differently in many physiological parameters when exercising or resting in either a cold room or cold water, which frequently could not be explained solely by sex-specific morphological differences. A review of chamber experiments and field studies to identify the factors influencing individual differences on thermal comfort by Wang et al. (2018) revealed that women are more critical about indoor thermal settings and more sensitive to deviations of thermal environment than men, but a consistent conclusion on sex differences in thermal comfort could not be drawn. Moreover, it has not been addressed whether men and women differ in the shivering onset after a gradually cold exposure using the Blanketrol® III, a common method for studying BAT activation. To test this, we exposed healthy volunteers to cold progressively and determined the experimental temperature (T_E) at which the volunteers started to shiver. This study demonstrates sex differences in the physiological responses to a gradual cold exposure.

2. Methods

2.1. Participants

We recruited 43 participants (20 men and 23 women) who met the inclusion criteria: age 16–35 years; being physically healthy; Caucasian ethnicity; body mass index (BMI) 18.5–29.9 kg/m^2 for participants aged more than 20 years old, or BMI standard deviation score (BMI-SDS) between −2 and + 2 for participants aged 16–19 years old. The following exclusion criteria were used: diabetes mellitus, thyroid disorders, substance use disorders, pregnancy, breastfeeding, and using β-adrenergic blocking medication. Participants were requested to eat, drink, and sleep as their usual routines, and requested not to smoke, eat, or drink any caffeinated or alcohol beverage within one hour before an appointment.

Since female sex hormones fluctuate during the reproductive cycle and could potentially influence the thermal balance (Charkoudian and Stachenfeld, 2014), female participants were included as follows. When a female participant was using contraceptive pills, she could only participate on a day she was taking a hormone-containing pill. When the female participant was not using contraceptive pills, she could not participate in the early follicular phase of her menstrual cycle, i.e. her menstruation period. In addition, we asked the female participants about their menstrual history to identify the phase of reproductive cycle at the day of experiment.

The experiment was performed after the participants had signed the written informed consent. The study was conducted according to the principles of the Declaration of Helsinki (version 19 October 2013). The procedures had been approved by the IRB of Erasmus MC, University Medical Center Rotterdam, the Netherlands.

2.2. Study design

To limit the influence of the environmental temperature on thermal perception (Makinen et al., 2004), the experiment was performed during the summer (July–September 2017). Daily mean temperatures of Rotterdam, the city where the experiment was performed, were obtained from the Royal Dutch Meteorological Institute (KNMI) via a publicly accessible database (KNMI, 2017). For further analysis, the outside temperature for each individual was calculated from three-day daily-mean-temperatures (2 days before and the day of the experiment).

The experiment was performed in the same laboratory with a standard heating, ventilation and air-conditioning system at a spot without direct air flow. The overall experimental design is illustrated in Fig. 1. Room temperature was recorded to verify the thermal condition. After arriving at the laboratory, participants acclimatized to the thermal setting of the room for at least 30 min. During the acclimatization period, participants changed their clothing to shorts and a T-shirt, filled in a questionnaire (Fig. 2A), rated their thermal perception,

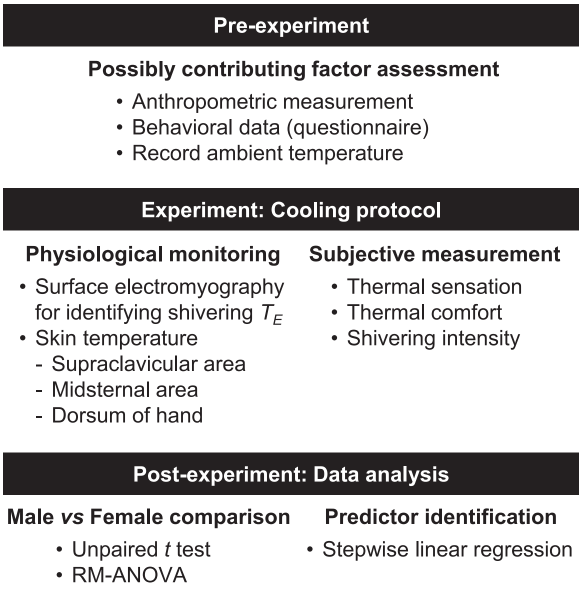


Fig. 1. Overall experimental procedure, The primary outcome is to identify a shivering T_E: the experimental temperature at which shivering started.

A

1. Do you smoke? If yes, how many cigarettes per day?
   ☐ Never
   ☐ Rarely / Sometimes
   ☐ 1–10 cigarettes per day
   ☐ > 10 cigarettes per day

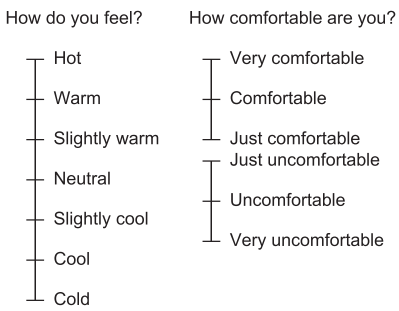
2. Do you drink alcoholic beverages? If yes, how many drinks per day?
   ☐ Never
   ☐ Rarely / Sometimes
   ☐ 1 drink per day
   ☐ 2–3 drinks per day
   ☐ ≥ 4 drinks per day

3. Do you drink caffeinated beverages (e.g. coffee, cola, energy drink)? If yes, how many cups/cans per day?
   ☐ Never
   ☐ Rarely / Sometimes
   ☐ 1 cup/can per day
   ☐ 2–3 cups/cans per day
   ☐ ≥ 4 cups/cans per day

4. How often do you do physical exercises / sports?
   ☐ Never
   ☐ Rarely / Sometimes
   ☐ Once per week
   ☐ 2–3 days per week
   ☐ ≥ 4 days per week

5. Do you have any problems or feel uncomfortable with thermal conditions at home or workplace?

B



C

Time (minute)	T_E (°C)
0	24
5	22
10	20
15	18
20	16
25	14
30	12
35	11
40	10
45	9
50 or at shivering	30

Fig. 2. Tools for evaluating thermal perception, (A) A questionnaire for evaluating the possible factors that may influence thermal perception. **(B)** Visual analogue scales (VAS) for assessing thermal perception: $VAS_{sensation}$ and $VAS_{comfort}$. **(C)** A cooling protocol showing the experimental temperature (T_E) setup of the Blanketrol at each indicated time.

and were measured for anthropometric characteristics.

To assess the thermal perception, we used the visual analogue scales (VAS; Fig. 2B) for thermal sensation ($VAS_{sensation}$) and thermal comfort ($VAS_{comfort}$), adapted from Zhang and Zhao (2008). $VAS_{sensation}$ is reported on ASHRAE 7-point scales: hot (+3), warm (+2), slightly warm (+1), neutral (0), slightly cool (−1), cool (−2), and cold (−3). $VAS_{comfort}$ includes 6-point scales: very comfortable (+3), comfortable (+2), just comfortable (+1), just uncomfortable (−1), uncomfortable (−2), and very uncomfortable (−3). Of note, the $VAS_{comfort}$ score does not contain 'neutral', to make a clear determination to either the 'comfortable' or 'uncomfortable' category.

We followed the American College of Sports Medicine (ACSM)'s Guidelines For Exercise Testing and Prescription (ACSM, 2013) for anthropometric measurement. In brief, body mass was measured with a 0.5-kg based scale, and body height was measured with a 0.5-cm based stadiometer. Waist circumference was measured at the height of the iliac crest, and hip circumference was measured at the maximal circumference of buttocks with the subject standing upright. BSA was calculated by the formula (Reading and Freeman, 2005):

$$BSA = 1/6\cdot(W\cdot H)^{0.5}$$

In this formula, BSA is in m^2, W is weight in kg, and H is height in m. Skinfold thickness was measured in mm at three sites, depending on the sex of the participant (Nieman, 2011). For men, skinfolds were measured at the chest (diagonal fold, halfway between the anterior axillary line and the nipple), the abdomen (vertical fold, 2 cm to the right side of the umbilicus), and the thigh (vertical fold, on the anterior midline, halfway between the proximal border of the patella and the inguinal crest). For women, skinfolds were measured at the triceps (vertical fold, halfway between the acromion and olecranon processes, with the arm held freely to the side of the body), the suprailiac site (diagonal fold, at the anterior axillary line immediately superior to the iliac crest), and the abdomen (as described above). Body density (BD) and body fat percentage (%BF) were calculated by the following formulas (ACSM, 2013):

$$BD_{men} = 1.10938–0.0008267\cdot(\text{sum of skinfolds}) + 0.0000016\cdot(\text{sum of skinfolds})^2–0.0002574\cdot\text{age}$$

$$BD_{women} = 1.089733–0.0009245\cdot(\text{sum of skinfolds}) + 0.0000025\cdot(\text{sum of skinfolds})^2–0.0000979\cdot\text{age}$$

$$\%BF = (457/BD)–414.2$$

For the mentioned formulas, sum of skinfolds is in mm, and age is in years.

After the acclimatization period, two surface electromyography (EMG) electrodes (Nutrode Mini-P10MO, O&R Medical) for monitoring the electrical activity of the rectus femoris muscle were placed at anterior mid-thigh with an inter-electrode center-to-center distance of 4 cm. The rectus femoris muscle was chosen for monitoring since it is one of the muscle groups recruited at onset of the shivering (Blondin et al., 2011; Tikuisis et al., 1991) and the movement artefact of the leg is easily detected by an investigator. The other electrode was placed on an ankle as a ground. EMG signals were transmitted using the Bio Amp Cable (model MLA2540, ADInstruments) to the PowerLab 26 T (model ML856, ADInstruments). The signals were monitored on LabChart software (version 7, ADInstruments) with a sampling rate of 1000 Hz, band-pass filters of 10 Hz and 500 Hz, a notch filter of 50 Hz, and an amplification range between ± 1 mV to ± 5 mV depending on the resting activity of each individual [modified from Blondin et al. (2011)]. Next, three Thermochron iButton® digital thermometers (model DS1921H, Maxim Integrated) were applied at the supraclavicular area, the midsternal area, and the dorsum of the hand with medical adhesive tapes (Micropore, 3 M) for continuous measuring of skin temperature (T_{sk}) with a one-minute interval. Then, the cooling protocol started.

2.3. *Cooling protocol*

The Blanketrol® III Hyper-Hypothermia System (model 233, Cincinnati Sub-Zero), the Maxi-Therm® Lite Patient Vest (model 800, Cincinnati Sub-Zero), and the Maxi-Therm® Lite Blanket (model 876, Cincinnati Sub-Zero) were used as a temperature management system. The Blanketrol regulates the temperature of the water that circulates through the Vest and the Blanket. The Vest covered shoulders, chest, abdomen, and back of the participant who was lying supine on a bed. The Blanket covered hip, groin, buttock, and anterior and posterior of thighs. Calculated with the Lund and Browder chart that is normally used to estimate the burn areas in patients (Hettiaratchy and Papini, 2004), the Vest and Blanket covered more than 50% of total BSA.

The cooling protocol started with the Blanketrol set at 24 °C after which the T_E was reduced by 1–2 °C every 5 min (a detailed setting is shown in Fig. 2C). During the cooling protocol, a participant was requested to lie still without any leg movement. When the EMG detected an onset of shivering burst (EMG amplitudes exceeded the resting values with a duration of > 0.2 s and an interburst interval of > 0.75 s; without any active movement of the leg observed by an investigator), the cooling protocol was terminated and the temperature of the Blanketrol was recorded as the shivering T_E. However, if the participant did not shiver after 50 min of the cooling protocol (at 9 °C), the experiment was also stopped and the shivering T_E of that participant was assumed to be 8 °C. Subsequently, the Blanketrol was set at 30 °C to warm up a participant for at least 10 min or until the participant was satisfied with the thermal comfort.

During the cooling protocol, a participant rated the $VAS_{sensation}$ score every 5 min before the next-step T_E setting and rated the $VAS_{comfort}$ score every 15 min at the 5th, 20th, 35th, and 50th minutes. At the end of the cooling protocol, we asked a participant to rate his or her shivering intensity as either no shivering, minimal shivering, moderate shivering, or profound shivering.

2.4. Data analysis and statistics

The statistical tests were performed using IBM SPSS Statistics for Windows (version 24, IBM Corp.) and GraphPad Prism for Windows (version 6, GraphPad Software Inc.). A difference between groups was analyzed by an unpaired *t*-test or a Mann–Whitney *U* test when the data were not normally distributed. For categorical data, a difference between groups was analyzed with a Fisher's exact test. The effects of sex and T_E on $VAS_{sensation}$ score, $VAS_{comfort}$ score, and T_{sk} were analyzed using 2-way repeated measures analysis of variance (RM-ANOVA) with a Bonferroni's multiple comparisons test when appropriate. Stepwise linear regression was used to identify factors influencing the shivering T_E. Statistical significance is considered when $P < 0.05$. Unless otherwise indicated, data are presented as mean ± SD.

3. Results

The characteristics and the anthropometric data of the recruited participants are shown in Table 1. The age was not different between the sexes ($P = 0.67$). Men were taller and heavier than women ($P < 0.001$ for both height and weight). When calculating BMI and categorizing to normal or overweight subgroups, there was no difference between the sexes ($P = 0.90$ for BMI and $P = 1.00$ for BMI category). Men had a larger BSA than women ($P < 0.001$) while women had a higher BSA-to-mass ratio than men ($P < 0.001$). Men had a larger waist circumference than women ($P < 0.001$) but they had equal hip circumferences ($P = 0.96$); hence, waist-to-hip ratio is higher for men than for women ($P < 0.001$). Women tended to have a greater sum of skinfold thickness ($P = 0.08$) and had a significantly higher body fat percentage than men ($P < 0.001$). Concerning the five behavioral data collected from the questionnaire (Fig. 2A), four of them revealed no significant difference between men and women, except that women reported more discomforts than men at their home or workplace thermal condition ($P = 0.01$). The outside temperature when the experiment was performed was slightly higher for women than for men ($P = 0.04$; Table 1). However, the room temperature at which all participants acclimatized before the cooling protocol was not different between men and women ($P = 0.09$).

Table 1
Characteristics of participants and ambient temperatures.

Parameters	**Men** (n = 20)	**Women** (n = 23)
Age (year)	23.5 (8.8)	22.0 (9.0)
Height (cm)*	184.4 (6.9)	170.9 (4.8)
Weight (kg)*	76.0 (9.8)	64.8 (9.0)
BMI (kg m^{-2})	22.4 (2.7)	22.2 (3.4)
Normal	16 (80%)	19 (83%)
Overweight	4 (20%)	4 (17%)
BSA (m^2)*	1.97 (0.14)	1.75 (0.12)
BSA-to-mass ratio (× 10^{-2} m^2/kg)*	2.61 (0.15)	2.72 (0.18)
Waist circumference (cm)*	83.7 (7.7)	74.7 (6.3)
Hip circumference (cm)	100.9 (4.5)	100.8 (7.0)
Waist-to-hip ratio*	0.83 (0.06)	0.74 (0.04)
Sum of skinfolds (cm)	46.0 (20.3)	56.9 (18.9)
Body fat percentage (%)*	13.1 (5.6)	23.6 (4.8)
Smoking history		
Never	16 (80%)	22 (96%)
Rarely/sometimes	3 (15%)	–
1–10 cigarettes per day	–	1 (4%)
> 10 cigarettes per day	1 (5%)	–
Alcohol consumption		
Never	4 (20%)	4 (17%)
Rarely/sometimes	13 (65%)	16 (70%)
One drink per day	3 (15%)	3 (13%)
Caffeine consumption		
Never	2 (10%)	–
Rarely/sometimes	6 (30%)	10 (43%)
One cup per day	3 (15%)	7 (30%)
2–3 cups per day	5 (25%)	6 (26%)
≥ 4 cups per day	4 (20%)	–
Exercise/sports frequency		
Never	2 (10%)	–
Rarely/sometimes	6 (30%)	8 (35%)
once per week	8 (40%)	11 (48%)
2–3 days per week	4 (20%)	4 (17%)
Thermal complaint/discomfort*		
No	20 (100%)	16 (70%)
Sometimes	–	5 (22%)
Often	–	2 (9%)
Outside temperature (°C)*	16.3 (4.7)	18.0 (1.8)
Room temperature (°C)	23.7 (0.7)	24.0 (0.7)

For BMI category, normal indicates BMI 18.5–24.9 kg/m^2 for adult (≥ 20 years) and BMI-SDS from –2 to 1 for adolescent (16–19 years); overweight indicates BMI 25.0–29.9 kg/m^2 for adult and BMI-SDS 1–2 for adolescent.
Data are shown as mean (SD), except age and outside temperature are shown as median (IQR), and BMI category and behavioral data are shown as n (%).
* indicates a statistically significant difference between male and female participants.

Experiencing the identical cooling protocol, women started shivering at a higher T_E than men (shivering T_E: 11.3 ± 1.8 °C for women *vs* 9.6 ± 1.8 °C for men, $P = 0.003$; Fig. 3A). At the average shivering T_E for women, 65% (15/23) of women shivered while only 25% (5/20) of men started shivering ($P = 0.014$). Throughout the cooling protocol, all participants felt colder when the T_E declined with women perceiving the T_E colder (lower $VAS_{sensation}$ score) than men ($P_{\text{Sex}\times\text{Temp}} < 0.001$, $P_{\text{Sex}} = 0.004$, $P_{\text{Temp}} < 0.001$; Fig. 3B). Remarkably, the difference in $VAS_{sensation}$ score between men and women was statistically significant when the T_E was between 14 °C and 20 °C. Furthermore, women felt more uncomfortable (lower $VAS_{comfort}$ score) than men during the experiment, and both groups felt more uncomfortable when the T_E declined ($P_{\text{Sex}\times\text{Temp}} = 0.006$, $P_{\text{Sex}} = 0.01$, $P_{\text{Temp}} < 0.001$; Fig. 3C).

Concerning thermal sensation and thermal comfort at room temperature before the cooling protocol started, female and male participants did not differ in both $VAS_{sensation}$ score ($P = 0.98$) and $VAS_{comfort}$ score ($P = 0.61$). Interestingly, women started to feel 'colder than neutral' ($VAS_{sensation}$ score < 0) at a higher T_E than men (22.1 ± 1.9 °C for women *vs* 20.3 ± 3.5 °C for men, $P = 0.04$; Fig. 3D). Women also started to feel 'cool' ($VAS_{sensation}$ score ≤ -2) at a higher T_E than men (18.3 ± 3.0 °C for women *vs* 14.6 ± 2.6 °C for men, $P < 0.001$; Fig. 3E). At the average 'cool' T_E for women, 65% (15/23) of women felt cool whereas only 20% (4/20) of men did ($P = 0.005$). At the end of the cooling protocol, when shivering was detected or T_E reached 9 °C, women felt colder than men ($VAS_{sensation}$ score –3 ± 0 for women *vs*

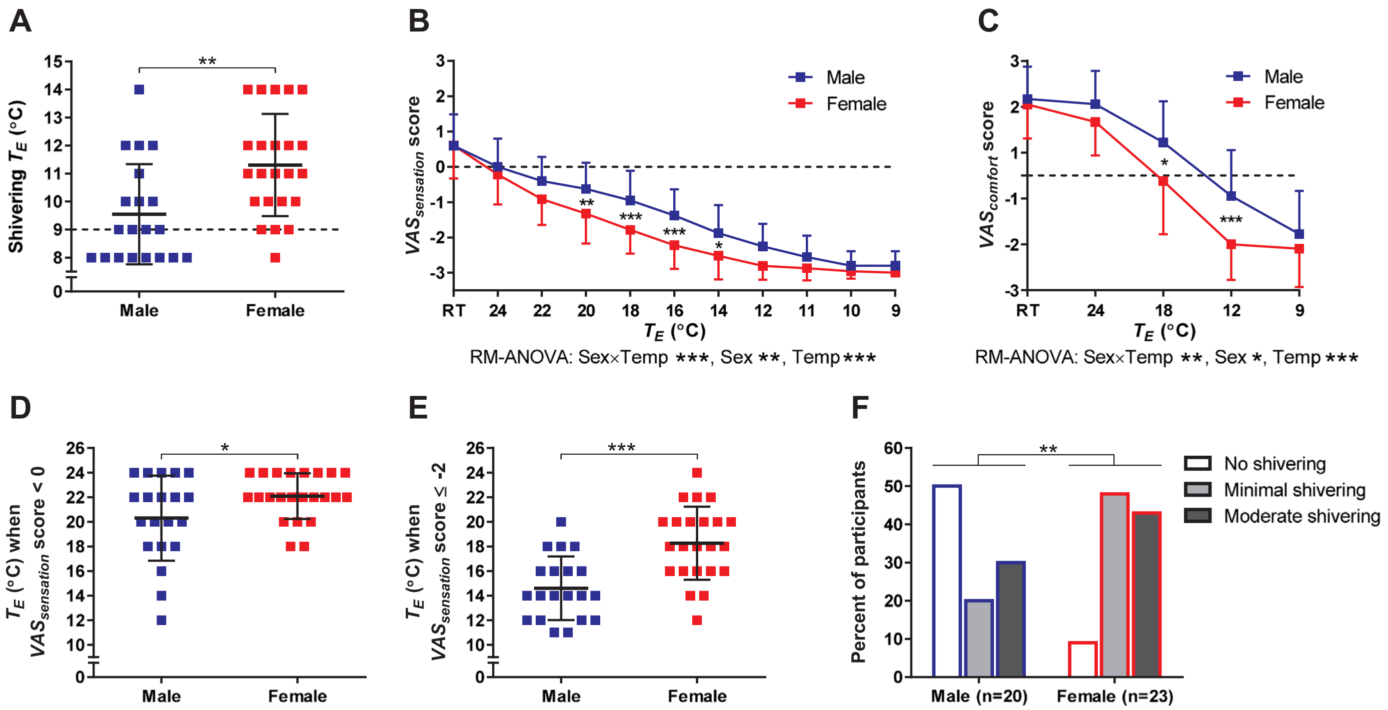


Fig. 3. Women shivered and perceived cold earlier than men. (A) Women started shivering at a higher experimental temperature (T_E) than men. The dotted line indicates the lowest experimental temperature. For those participants not shivering at 9 °C, the shivering temperature was set at 8 °C. **(B–C)** Women felt colder (lower $VAS_{sensation}$ score) and more uncomfortable (lower $VAS_{comfort}$ score) than men during the cooling protocol. Dotted lines indicate 'neutral' perception. **(D–E)** Women started to feel 'colder than neutral' ($VAS_{sensation}$ score < 0) and 'cool' ($VAS_{sensation}$ score ≤ -2) at higher temperatures than men. **(F)** Women perceived the shivering more intense than men. Statistical significance indicates by *P* values: $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***). Error bar indicates SD.

−3 ± 1 for men [median ± IQR], $P = 0.03$), but both sexes perceived the thermal discomfort equally ($VAS_{comfort}$ score −2 ± 1 for women *vs* −2 ± 1.25 for men [median ± IQR], $P = 0.29$). Women, moreover, perceived the shivering more intense than men at the end of cooling protocol ($P = 0.009$; Fig. 3F).

Factors that could possibly influence the shivering T_E are listed in Table 2. Only sex of the participants, $VAS_{comfort}$ score at room temperature, and $VAS_{sensation}$ score at room temperature were statistically significant predictors of the shivering T_E (stepwise linear regression: $F(3,39) = 9.352$, $P < 0.001$, $R^2 = 0.418$). Interestingly, when sex of the participants was removed from the analysis, the statistically significant predictors of the shivering T_E consisted of BSA-to-mass ratio, $VAS_{comfort}$ score at room temperature, and an amount of caffeine consumption (stepwise linear regression: $F(3,39) = 7.026$, $P = 0.001$, $R^2 = 0.351$).

Table 2
Factors that could influence the shivering T_E.

Factors	All factors			Excluding Sex		
	Beta	Sig.		Beta	Sig.	
Sex	0.444	0.001	**	–	–	–
Age (year)	0.049	0.71	ns	0.154	0.44	ns
BSA-to-mass ratio (m^2/kg)	0.148	0.31	ns	0.480	0.002	**
Body fat percentage (%)	−0.325	0.08	ns	0.231	0.08	ns
Sum of skinfolds (cm)	−0.239	0.07	ns	0.101	0.52	ns
Abdominal skinfold (cm)	−0.230	0.08	ns	−0.100	0.55	ns
Smoking	−0.072	0.57	ns	−0.212	0.15	ns
Alcohol consumption	0.156	0.21	ns	0.189	0.17	ns
Caffeine consumption	0.238	0.06	ns	0.299	0.04	*
Exercise frequency	0.056	0.66	ns	0.011	0.93	ns
Thermal complaint	0.240	0.07	ns	0.253	0.08	ns
$VAS_{sensation}$ score at room temperature	−0.282	0.03	*	−0.171	0.23	ns
$VAS_{comfort}$ score at room temperature	−0.339	0.009	**	−0.284	0.04	*
Baseline 3-site average T_{sk}	0.092	0.49	ns	−0.177	0.19	ns
Outside temperature (°C)	−0.087	0.53	ns	0.120	0.42	ns
Room temperature (°C)	−0.161	0.21	ns	−0.086	0.52	ns
Time of experiment	0.206	0.10	ns	0.239	0.08	ns

Stepwise linear regression was analyzed to predict the shivering T_E from indicated factors.
Time of experiment indicates the period of the day when the experiment was performed, dividing into 3 periods: 09:00–11:59, 12:00–14:59, and 15:00–17:59.
Sig. (statistical significance) indicates by *P* values: $P > 0.05$ (ns), $P < 0.05$ (*), and $P < 0.01$ (**).

Since body composition was different between men and women, we performed a subgroup analysis of 19 men and 16 women by using the lowest BSA-to-mass ratio of the female participants as a minimal cut-off value and the highest BSA-to-mass ratio of the male participants as a maximal cut-off value (BSA-to-mass ratio 0.0263 ± 0.0014 m^2/kg for men *vs* 0.0264 ± 0.0016 m^2/kg for women, $P = 0.70$). The results showed the same pattern that women started shivering at a higher T_E than men (11.1 ± 1.9 °C for women *vs* 9.6 ± 1.8 °C for men, $P = 0.03$). Women also started to feel 'cool' ($VAS_{sensation}$ score ≤ -2) at a higher T_E than men (17.6 ± 2.7 °C for women *vs* 14.6 ± 2.7 °C for men, $P = 0.002$) whereas the T_E at which a participant started to feel 'colder than neutral' ($VAS_{sensation}$ score < 0) was not statistically significant (21.8 ± 1.9 °C for women *vs* 20.4 ± 3.5 °C for men, $P = 0.18$).

Throughout the cooling experiment, T_{sk} was monitored at 3 areas of the body. The T_{sk} at the supraclavicular area decreased with the declining T_E without a statistically significant difference between men and women ($P_{Sex \times Temp} = 1.00$, $P_{Sex} = 0.14$, $P_{Temp} < 0.001$; Fig. 4A). The T_{sk} at the midsternal area was not different between men and women; however, it was statistically significantly increased during the first few minutes of the cooling protocol, and remained stable until the end of the protocol ($P_{Sex \times Temp} = 0.64$, $P_{Sex} = 0.65$, $P_{Temp} < 0.001$; Fig. 4B). The T_{sk} at the dorsum of the hand decreased with the declining T_E and, interestingly, male participants had higher hand temperatures than female participants during the cooling protocol ($P_{Sex \times Temp} < 0.001$,

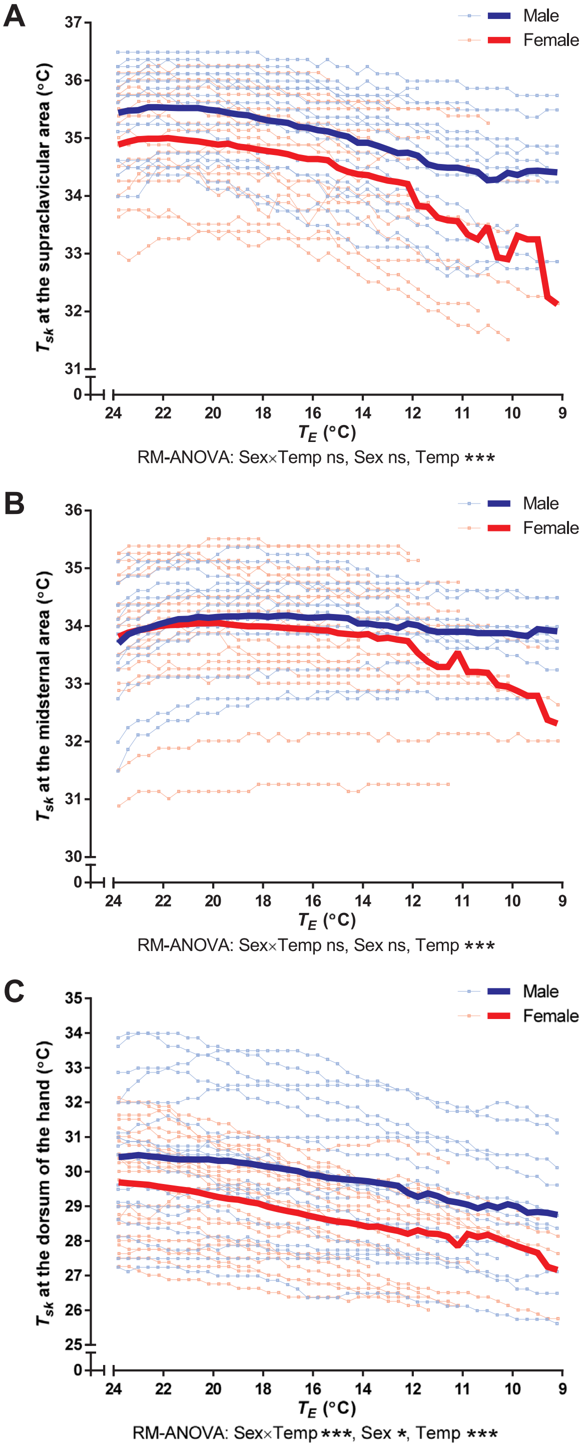


Fig. 4. Skin temperatures during the cooling protocol. Skin temperatures (T_{sk}) measured by the iButton digital thermometers at **(A)** the supraclavicular area, **(B)** the midsternal area, and **(C)** the dorsum of the hand. Statistical significance indicates by P values: $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***). Thin lines show the data of individual participants, and thick lines indicate the mean. The deviation in mean T_{sk} between men and women at T_E lower than 14 °C is mainly driven by the decreasing number of female participants that started shivering beyond this T_E.

$P_{\text{Sex}} = 0.02$, $P_{\text{Temp}} < 0.001$; Fig. 4C).

Concerning the 5-min average T_{sk} at each stage of the cooling protocol, women tended to have a lower baseline T_{sk} at the dorsum of the hand and supraclavicular area than men while the T_{sk} at the midsternal area was similar in both sexes (Table 3 and Fig. 4). The average T_{sk} from the 3 sites at baseline was significantly lower in women than in men. However, when the baseline 3-site average T_{sk} was added into the stepwise linear regression analysis, it was not a significant predictor for the shivering T_E (Table 2). When participants started to feel 'colder than neutral' ($VAS_{sensation}$ score < 0) or 'cool' ($VAS_{sensation}$ score ≤ -2), all of the measured T_{sk}'s and the declines of T_{sk}'s from the baseline values (ΔT_{sk}) were not different between the sexes (Table 3). At the end of the cooling protocol (shivering started or T_E reached 9 °C), women had a significantly lower hand T_{sk} than men while the supraclavicular and midsternal T_{sk}'s were not different between the sexes. The 3-site average T_{sk} at the end of cooling protocol was also significantly lower in women than in men; however, the decline in average T_{sk} (ΔT_{sk}) at the end of the cooling protocol was similar in both sexes. Interestingly, the midsternal T_{sk} did not differ between the sexes and remained unchanged during the cooling stages. As a result, the difference between the midsternal T_{sk} and the hand T_{sk} was greater after cold exposure and tended to be higher in women than in men (midsternal T_{sk} – hand T_{sk}: 4.5 ± 2.0 °C for women at baseline, 3.4 ± 2.6 °C for men at baseline, 6.0 ± 1.4 °C for women at shivering, and 4.6 ± 2.1 °C for men at shivering; $P_{Cold \times Sex} = 0.42$, $P_{Cold} < 0.001$, $P_{Sex} = 0.07$).

4. Discussion

This study revealed that women and men respond differently when experiencing cold. Female participants felt cold at a higher T_E and started shivering at a higher T_E than male participants upon the gradually cold exposure using the water-filled cooling blanket, a commonly used device to study the BAT activity in humans.

Shivering can be considered an indicator of cold stress since it is activated only if energy-inexpensive mechanisms are not sufficient to maintain a constant T_c (Daanen and van Marken Lichtenbelt, 2016). The stimulus for shivering is not only a drop in T_c, but also a cold stimulus that contacts the skin. The decrease in T_{sk} is transmitted as a cold signal by TRPM8, a temperature-sensitive receptor that can be activated when an ambient temperature is lower than 25 °C (Patapoutian et al., 2003). In mice, Caudle et al. (2017) found that TRPM8 receptors in females had a higher sensitivity to low temperatures than receptors in males. Our finding that women perceived the same cooling protocol colder than men might therefore partly be explained by a different sensitivity of the TRPM8 receptors.

Anderson et al. (1995) demonstrated that there was not a significant difference between men and women in the shivering threshold, which was defined by the deviation of the esophageal T_c from an individual baseline value when resting after exercise in 28 °C water. We could not address the shivering threshold in terms of the change of T_c because a lack of T_c monitoring is a limitation of our study. A study by Boon et al. (2014), which used the same cooling method as in this study to study the BAT activation, showed that T_c was unchanged after the 2-h cooling protocol. Moreover, Xu et al. (2013) showed that the T_{sk} measured from the sternum are best correlated with the T_c. Thus, it is likely that the T_c of the participants in this study was not remarkably affected since the midsternal T_{sk} remained unchanged during the cooling protocol. Interestingly, Boon et al. (2014) also found that the gradient between the proximal and the distal T_{sk} was greater after cold exposure, which was confirmed in our study. A decline in distal T_{sk} together with a greater gradient between the proximal and the distal T_{sk} reflects the vasoconstriction capacity of the peripheral tissues to conserve heat during an exposure to cold. Overall, our results suggest that women approach the maximal capacity of vasoconstriction earlier, and thus need shivering as a source of heat production sooner than men.

The BSA-to-mass ratio is an important factor explaining differences in the net heat transfer in cold conditions (Castellani and Young, 2016; Parsons, 2014). An increase in BSA results in a higher heat loss from the body to the environment. On the other hand, an increase in total body mass contributes to a higher heat production capacity (Arciero et al., 1993). A combination of high BSA and low body mass causes a low

Table 3
Skin temperatures measured at three sites during the cooling protocol.

Site and condition of measurement	T_{sk} (°C)			ΔT_{sk} (°C)		
	Men	**Women**	***P***	**Men**	**Women**	***P***
Supraclavicular area						
Baseline	35.4 ± 0.7	34.9 ± 1.1	0.08	–	–	–
At $VAS_{sensation}$ score < 0	35.3 ± 0.9	34.8 ± 1.1	0.16	−0.1 ± 0.3	−0.0 ± 0.1	0.44
At $VAS_{sensation}$ score ≤ -2	35.0 ± 1.0	34.7 ± 1.1	0.51	−0.4 ± 0.4	−0.2 ± 0.3	0.08
End of cooling protocol	34.5 ± 1.0	34.3 ± 1.2	0.48	−0.9 ± 0.5	−0.6 ± 0.9	0.25
Midsternal area						
Baseline	34.0 ± 0.8	34.0 ± 1.1	0.97	–	–	–
At $VAS_{sensation}$ score < 0	34.0 ± 0.7	33.9 ± 1.1	0.89	−0.1 ± 0.2	−0.0 ± 0.2	0.27
At $VAS_{sensation}$ score ≤ -2	33.8 ± 0.9	34.0 ± 1.0	0.56	−0.1 ± 0.7	0.0 ± 0.2	0.33
End of cooling protocol	34.0 ± 0.6	33.9 ± 1.0	0.62	0.0 ± 0.6	−0.1 ± 0.4	0.38
Dorsum of hand						
Baseline	30.5 ± 2.0	29.6 ± 1.5	0.12	–	–	–
At $VAS_{sensation}$ score < 0	30.3 ± 2.0	29.6 ± 1.5	0.19	−0.1 ± 0.3	−0.0 ± 0.2	0.16
At $VAS_{sensation}$ score ≤ -2	29.8 ± 1.8	29.0 ± 1.3	0.12	−0.7 ± 0.7	−0.6 ± 0.4	0.68
End of cooling protocol	29.0 ± 1.8	27.9 ± 1.2	**0.02**	−1.5 ± 1.1	−1.7 ± 1.0	0.47
Average 3-site T_{sk}						
Baseline	33.3 ± 0.6	32.8 ± 0.7	**0.02**	–	–	–
At $VAS_{sensation}$ score < 0	33.1 ± 0.6	32.8 ± 0.7	0.12	−0.1 ± 0.3	−0.0 ± 0.1	0.18
At $VAS_{sensation}$ score ≤ -2	32.8 ± 0.7	32.6 ± 0.8	0.24	−0.4 ± 0.5	−0.2 ± 0.2	0.17
End of cooling protocol	32.5 ± 0.6	32.0 ± 0.6	**0.02**	−0.8 ± 0.5	−0.8 ± 0.6	0.99

ΔT_{sk} demonstrates the change in T_{sk} at each condition relative to baseline value. T_{sk} at the end of the cooling protocol was measured when shivering started or T_E reached 9 °C. Data are shown as mean ± SD. Statistically significant difference between men and women is marked in bold.

capability to maintain a proper thermal balance for a constant T_c during cold stress. In general, the body composition of adult men and women shows a sexually dimorphic pattern (Kuk et al., 2005; Wells, 2007), which is the same pattern observed in this study cohort. Female participants in our study cohort had a slightly higher BSA-to-mass ratio than male participants and the BSA-to-mass ratio was a statistically significant determinant for the shivering T_E. Hence, the BSA-to-mass ratio is likely a principal factor determining this sex difference (Castellani and Young, 2016; Tikuisis et al., 2000). However, this anthropometric characteristic is not the only factor underlying the sex difference in our cohort since the subgroup analysis of an equivalent BSA-to-mass ratio between the sexes still showed the sex-dependent pattern. Further research is needed to identify why women need shivering as a source of heat production earlier than men to maintain their thermal balance when experiencing the same cold stress.

Concerning the thermal sensation and thermal comfort over the whole cooling period as a subjective method to evaluate cold perception, we found that women did feel colder and less comfortable than men. Intriguingly, the T_E's at which the participants started to feel ‘colder than neutral’ ($VAS_{sensation}$ score < 0) and ‘cool’ ($VAS_{sensation}$ score ≤ -2) were both higher for women than for men. Karjalainen (2012) and Wang et al. (2018) illustrated that women feel colder and are less comfortable than men in a standard indoor climate setting. Our results, however, did not find a difference in thermal sensation or thermal comfort between men and women at the acclimatization period before the cooling protocol started. We previously performed a behavioral mouse study to identify sex differences in thermal preference of adult mice (Kaikaew et al., 2017) and found that female mice preferred to reside at a higher ambient temperature than male mice. Overall, our data of both the previous mouse study and this current human study confirmed the sex difference in thermal perception.

Since our institutionally approved cooling protocol allowed us to study the effect of cold exposure in a healthy individual up to a minimum T_E of 9 °C only, we assumed a shivering T_E of 8 °C in those participants that failed to shiver at this minimum T_E. Using this assumption, we found that male participants started shivering at a statistically lower T_E than female participants. It is very unlikely that this assumption led to a false conclusion since 89% (8 out of 9) of the participants with undetectable shivering at 9 °C were male participants. Thus, this assumption may even underestimate the effect size in the difference in shivering T_E between the sexes. Although this conclusion is based on a rather small cohort (20 men and 23 women), our power analysis to determine a potential sex difference in shivering T_E indicated that this sample size is sufficient as a minimum of 16 participants per sex was needed.

Cold-induced activation of BAT, the thermogenic organ that utilizes energy to generate heat, can be detected in many regions of the body, including the supraclavicular area (van der Lans et al., 2014). The current ‘gold standard’ method to study the activity of human BAT is the ^{18}F-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography integrated with computed tomography (PET/CT) imaging (Blondin and Carpentier, 2016). ^{18}F-FDG uptake in BAT was detected in only ~6% of individuals when the PET/CT scan was performed in unstimulated conditions, with a significantly higher prevalence in women than in men (Cypess et al., 2009; Ouellet et al., 2011). Nevertheless, when participants were exposed to 19 °C for 2 h before the scan, ^{18}F-FDG uptake in BAT was observed in 52% of young participants (aged 23–35 years), without an apparent sex difference (Saito et al., 2009). Thus, an individualized cooling protocol could be beneficial for studying BAT activity.

^{18}F-FDG-PET/CT imaging has limitations such as underestimating a weakly activated BAT in normal physiological conditions, requiring expensive equipment, and exposing a subject to ionizing radiation (Cypess et al., 2014). A proposed non-invasive method to determine the activity of BAT is measurement of the T_{sk} at the supraclavicular area. Unlike previous reports that cold exposure enhanced the supraclavicular T_{sk} suggesting activation of BAT (Boon et al., 2014; van der Lans et al., 2016), our cooling protocol did not increase the supraclavicular T_{sk}. This conflicting result is likely explained by the shorter duration of cold exposure in our protocol compared to those published previously, which is possibly not potent enough to stimulate BAT to a detectable level.

5. Conclusions

This study demonstrates that women and men respond differently to low temperatures. Women not only started shivering at a higher T_E than men, they also felt colder and less comfortable than men throughout the same cooling protocol using the Blanketrol® III. These sex differences could be important for studying the physiological responses at temperatures lower than the thermoneutral zone, such as those using cooling protocols for radiologic diagnostic imaging in patients or those studying BAT activity in the general population.

Acknowledgements

The authors would like to thank Johan J.M. Pel and Marcel P.J.M. van Riel for the EMG instruments and advices; Jelmer Alsma and Niala den Braber for the iButtons; and Amir Abdelmoumen, Annemarie Mangnus, Anouk Franken, Anouk Versnel, Emma Arman, Indira Schouten, Margreet Vonk Noordegraaf, Miliaan Zeelenberg, and Suzanne van Woudenberg for their valuable assistance during the experiment.

Author contributions

JCvdB, SJCMMN, APNT, and AG conceived the ideas; KK, JCvdB, SJCMMN, JAV, and AG designed the experiment; KK performed the experiment and collect the data; KK, JAV, and AG analyzed and interpreted the data; KK drafted the manuscript. All authors provided intellectual feedbacks on the manuscript and approved the final version of the manuscript.

Declarations of interest

The authors declare no conflicts of interest.

Funding

This research was supported mainly by the Department of Internal Medicine, Erasmus MC, Rotterdam, the Netherlands, and partly by the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

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