Metabolic effects of cold exposure

The potential role of irisin in the thermoregulatory responses to mild cold exposure in adults.

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Abstract

Objectives: To determine the acute effect of a mild cold exposure on thermoregulatory thermogenesis and the role of circulating irisin in the process.

Methods: We studied twenty two adults (9 males, 13 females) aged 57.7 ± 10.07 years and body mass index 27.8 ± 4.52 kg/m². Participants experienced a 90 min exposure to 20°C and 25°C In a randomized cross-over design. Resting metabolic rate (RMR), forearm to finger-tip gradient (FFG), blood pressure (BP), in-the-ear temperature (IET), and fasting bloods were measured on each occasion.

Results: There were significant increases in FFG [mean ± SD: +3.8 ± 3.0°C, p < 0.001], SBP [+8 ± 13 mmHg, p = 0.015] and DBP [+4 ± 6 mmHg, p=0.005] and decreases in IET [-0.24 ± 0.29°C, p=0.001]. Overall, RMR [+190 ± 570 kJ/d, p=0.135], irisin, glucose or insulin did not differ between temperatures. There were no significant between-gender differences, but males significantly increased SBP (+12 ± 16, p= 0.02) and DBP (+6 ± 7, p= 0.02) with decreases in heart rate (-4 ± 3, p= 0.002), while females did not. Moreover men had ~50% higher thermogenic response while women had a ~25% greater vasoconstrictor response. Adjusted for age, gender, insulin sensitivity and body composition, fold changes in irisin was inversely related to RQ (r= -0.54, P=0.048), while IET was related to FFG (r= -0.55, p=0.043).

Conclusions: Mild cold exposure increased vasoconstriction with a drop in IET and these were related. Greater irisin was related to a greater fasting fat oxidation in the absence of shivering. A potential gender bias in thermoregulation was noted.

Word count: 250

Key words: resting metabolic rate; cold-induced thermogenesis; vasoconstriction; irisin
Introduction

There is a pandemic of obesity and its associated co-morbidities. At the population level, current recommendations to prevent weight gain through dietary modification and exercise are not having their desired effect, as overweight and obesity is still on the rise (Ng et al., 2014). There is an urgent need for novel prevention and treatment strategies to help reverse these detrimental trends. On exposure to a cold environment, humans respond by decreasing heat loss via increased vasoconstriction, increasing RMR or both (Milan, 1980; Young et al., 1996). Resting metabolic rate (RMR) makes the greatest contribution to total energy expenditure in man. The demonstration that mild cold exposure is able to activate brown adipose tissue and thereby increase RMR in adults, suggests cold exposure may be a potential novel strategy in obesity prevention and treatment (Chen et al., 2013; van Marken Lichtenbelt et al., 2009; Yoneshiro et al., 2013). Even small increases in cold-induced thermogenesis have the potential to significantly impact on long-term energy expenditure if sustained during both day and night (van Marken Lichtenbelt and Schrauwen, 2011).

Cold-induced thermogenesis is one form of adaptive thermogenesis and represents the increase in heat production in response to ambient temperatures below the thermo-neutral range. The spectrum of cold-induced thermogenesis includes non-shivering thermogenesis and shivering thermogenesis. In defense of a drop in core body temperature, the body increases non-shivering thermogenesis. This stimulation of energy expenditure is mostly at the site of skeletal muscle and brown adipose tissue, whereby heat production occurs via mitochondrial uncoupling of oxygen consumption and adenosine triphosphate production (Wijers et al., 2008). In an acute setting, when the required demand for heat production cannot be met, ST is activated to dramatically increase heat production through vigorous muscle contractions. Irisin, is a newly discovered myokine that was first described as being
stimulated by exercise. Very recently it has been demonstrated that irisin secretion is also increased on exposure to low ambient temperatures and closely follows the degree of shivering; hence contributing to ST (Lee et al., 2014). Hence, irisin may represent a cold-activated thermogenic factor that is potentially exploitable toward obesity prevention and treatment. Various factors such as age (Charkoudian, 2010; Kingma et al., 2011), gender (Graham, 1988), and adiposity (Ooijen et al., 2006) have been shown to influence the physiological response to cold stress. The current study was designed to determine the effect of a mild cold exposure on thermoregulatory variables, RMR and vasoconstriction, in adults who span a spectrum of age, gender and body composition. To the best of our knowledge the response of irisin to mild cold induced thermoregulation has not be studied before.

Methods

Study design and participants

Participants were recruited via flier advertisement, radio advertisement, community newspapers and social media websites. Interested participants were assessed for eligibility by means of a short screening questionnaire. Inclusion criteria were as follows: Australians of European origin; aged between 20–70 years; body mass index (BMI) \( \geq 20 \text{ kg/m}^2 \), weight stable (\(< \pm 2 \text{ kg}\)) in the previous 3 months with no intention for weight loss within the next 3 months and absence of the following by self-report: fever, thyroid disease, polycystic ovarian syndrome, type 2 diabetes, previous heart attack; current pregnancy, lactation, peri-menopausal status, smoking, use of thermogenic agents, hormonal contraception and hormone replacement therapy. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Curtin University Ethics Committee [HREC number
20/2012] and registered at www.ANZCTR.org.au (ACTRN12612000509864). Written informed consent was obtained from all participants.

**Intervention**

The study took place in the environmental chamber housed at the School of Public Health, Curtin University, Western Australia, between September and November 2012. The chamber is a purpose built structure within a large room of the building. It has a volume of 57.75 m³, insulated walls and roof and is independently controlled for temperature range from 4°C to 50°C. The study used a randomized crossover design, and participants were studied at two temperatures, mean (within day SD) 20°C (0.37)°C (the intervention) and 25°C (0.4)°C (the control temperature). Exposure to each temperature was for 90 mins. Randomization to the first temperature condition was determined using block randomization with random block sizes and randomly generated codes for each block (Random Allocation Software, version 1.0, Isfahan University of Medical Sciences, Isfahan, Iran). The two measurement sessions were separated by no more than 2 weeks. Participants arrived at the laboratory by car or public transportation and were instructed not to perform any strenuous activity the day before the experiment to avoid any effects of activity on RMR. Participants presented in the fasting state, 10-12 hours after a standardized evening meal (2010 kJ; 15.73% protein, 31.85% total fat, 14.73% saturated fat, 52.43% carbohydrate), which we provided in advance, to avoid any effects of previous evening meal on the next day’s metabolic measurements.

**Physical characteristics and body composition**

Participants voided their bladder and changed into a provided gown with only their under garments, for weight, height and waist circumference measurements. Weight was measured
using an electronic platform balance (CW-11, Precision Balances Pty Ltd). Height was measured using a stadiometer fixed to a wall (Seca, Hamburg, Germany). Waist circumference was measured midway between the lowest rib of the ribcage and the iliac crest (Ashwell and Browning, 2011). Body composition was assessed by bioelectrical impedance analysis with the InBody 3.0 Body Composition Analyzer and software (Biospace, Korea).

Clothing, shivering response and comfort levels

Clothing worn by participants in the chamber was standardized to within 0.5 clo. All participants were provided with a gown (0.46 clo), females wore their own bra and underwear (0.04 clo) and males wore their own briefs (0.04 clo). Participants were asked to verbally notify the investigators if they started to shiver. At 30 minutes in the chamber, a 10mm visual analogue scale (VAS) questionnaire was administered to determine comfort levels at 20°C and 25°C, similar to the VAS used by others (Westerterp-Plantenga et al., 2002b). Question 1 (VASQ1) asked the participant “How agreeable do you find the room temperature right now?” (anchored; very agreeable/not agreeable at all). Question 2 (VASQ2) asked the participant “Do you feel comfortable?” (anchored; very comfortable/not comfortable at all).

Body temperature

To assess peripheral vasoconstriction, iButtons (iButton type DS1921H-F#, Maxim Integrated Products, Inc., USA) were placed on the left middle fingertip on the ventral side and the dorsal left forearm, midway between the elbow and wrist using fixomull tape (BSN, Hamburg, Germany). The iButtons measured skin temperatures continuously during the experimental days and have been recently validated for this use (van
Peripheral vasoconstriction was measured as the forearm to fingertip temperature gradient (FFG) (Rubinstein and Sessler, 1990). Core temperature is generally defined as the temperature measured within the pulmonary artery. IET measurements have been known as accurate, valid and reproducible measurements of core body temperature, reflecting temperature changes within the pulmonary artery (Benzinger, 1969; Erickson and Kirklin, 1993; Hasper et al., 2011). Thus, IET measurements were made after 50 minutes in the chamber using a standard in the ear thermometer (Omron Model MC-510). Three measurements of IET were made with the average of the two closest measurements recorded.

Resting metabolic rate

Participants rested in the supine position for 30 minutes to equilibrate with the temperature of the environmental chamber. RMR was measured via indirect calorimetry (Deltatrac II, Datex Instrumentarium, Finland), according to a standardized protocol that emphasized 30 minute rest (Piers et al., 2002). A trial RMR measurement was made for 20 minutes for participants to become accustomed to the canopy. After a 20 minute rest period, RMR was again measured as described above. The RMRs reported represent the value after a 90 min exposure to that temperature. RMR in kJ/d was derived from CO₂ production and O₂ consumption according to the Weir’s formula, neglecting protein oxidation in the fasting state (Weir, 1990). Substrate oxidation was calculated as volume CO₂/volume O₂. The Deltatrac II apparatus was calibrated with gas mixtures of known composition (95% O₂, 5% CO₂) before each measurement session and regularly checked by 30 minute ethanol burn tests (mean and SD for six tests during the study was RQ = 0.66 ± 0.02).

Blood pressure and heart rate
Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured after 50 minutes in the chamber using an automatic blood pressure monitor (IA2, Omron Healthcare Co., Ltd Japan). The average of two consecutive measurements was recorded. Heart rate was recorded in the same fashion (Pulse Trace, Viasys, Pa, USA).

**Blood collection and analysis**

A fasting venous sample was obtained after 90 mins exposure to the set temperature by experienced phlebotomists in EDTA-containing tubes to prevent clotting, centrifuged for 10 minutes and stored at -80°C for analysis 2 months later. Briefly, triglyceride, total cholesterol, high-density lipoprotein cholesterol and fasting blood glucose were determined using routine automated procedures on an Architect c16000 analyzer that used specific enzyme-based colorimetric reagents (Abbott Diagnostics; CV < 2%). Low-density lipoprotein cholesterol was estimated using the Friedewald equation (Friedewald et al., 1972). Fasting insulin was determined by Pathwest Laboratories Perth Australia using an Architect i2000SR Analyser (Abbott Diagnostics; CV < 3%). McAuley’s index for insulin resistance was calculated from triglyceride and insulin concentrations (Hettihewa et al., 2006). Plasma irisin, was measured using an ELISA kit (AdipoGen, AG-45A-0046PP-KI01; inter-assay CV<7%). Due to technical reasons, we could not obtain complete blood samples from three participants.

**Repeatability Trial**

To examine repeatability of measurements, 6 subjects returned for a second measure of the protocol at 20°C.

**Primary study outcomes**
Primary study outcomes were RMR, irisin and FFG.

Statistical analysis

Our sample size of 22 provided more than 80% power at a two-tailed alpha level = 0.05, to detect a moderate effect size of 0.53 in RMR. Our sample size was similar to previous studies examining physiological responses to cold exposure (Celi et al., 2010; Westerterp-Plantenga et al., 2002a; Wijers et al., 2010). Normally distributed data are presented as mean (SD). Paired t tests were used to assess the effect of temperature on clinical characteristics and thermoregulatory variables. We used IET, as the proxy variable for core temperature since previous research had established its validity (Benzinger, 1969; Erickson and Kirklin, 1993; Hasper et al., 2011). Univariate analysis was performed using a Spearman’s rho correlation matrix to examine factors associated with fold changes in IET, RMR FFG, BP and irisin. All statistics were analyzed using SPSS for Windows version 22.0 (SPSS, Chicago, IL). Statistical significance was accepted at p < 0.05.

Results

Baseline demographics, body composition and key MetS variables

Twenty two Australians of European origin (9 males, 13 females) were recruited into the study and there were no dropouts. Participants had a mean ± SD (range) age of 57.73 ± 10.07 years (29-68 years), weight 79.80 ± 17.82 kg (49.65-109.1 kg), BMI of 27.80 ± 4.52 kg/m² (19.89-33.73 kg/m²), fat mass of 60.54 ± 14.06 kg (41.7-87.3 kg) and fat-free mass of 20.88 ± 7.93 kg (7.9-36.5 kg) (Table 1).

[Table 1 here]

Repeatability
Test-retest values at 20°C were non-significant for all parameters (paired t test) and intra-individual CV% were as follows: IET 0.6% [35.68 ± 0.42 °C vs 35.4 ± 0.34 °C], RMR 4.0% [5457 ± 2104 kJ/d vs 5274 ± 1933 kJ/d], substrate oxidation 2.7% [0.84 ± 0.049 vs 0.84 ± 0.034], FFG 15.2% [5.31 ± 1.43 °C vs 5.09 ± 1.13 °C], fat mass 1.1% [15.62 ± 6 kg vs 15.92 ± 6 kg], fat-free mass 4.5% [54.15 ± 17.49 kg vs 53.40 ± 17.10 kg], SBP 2.6% [129 ± 19 mmHg vs 128 ± 21 mmHg], and DBP 2.8% [70 ± 8 mmHg vs 61 ± 10 mmHg].

*IET, RMR, and FFG*

IET at 20°C was significantly lower than 25°C (Table 2; p = 0.001, Fig. 1A). We detected a 3.1% increase in RMR at 20°C compared to 25°C (range 10.01% decrease to a 20.62% increase), although this was non-significant (Table 2, Fig. 1B). Removal of the 6 participants in whom RMR dipped below the paired value at 25°C, resulted in a significant increase in RMR by 444 ± 442 kJ/d (p=0.001). The FFG, reflecting vasoconstriction, was significantly higher at 20°C than at 25°C (Table 2; change=3.75 ± 3.02 °C, p < 0.001, Fig. 1C). There was no significant gender difference in IET, RMR or FFG, however there was a sizeable difference in FFG response with females showing a 0.96 °C greater vasoconstriction and males showing a greater thermogenic response by 80 kJ (Table 3).

*Correlations - Change in IET, RMR, FFG and irisin*
Gender was not associated with fold change in IET (r=0.10, p=0.658), RMR (r=0.053, p=0.815), FFG (r=-0.250, p=0.263) or irisin (r=-0.248, p=0.306). Fold change in IET showed a trend towards an association with the change in RMR (r=0.41, p=0.058), but this was firmly non-significant after adjustment for age, FM(%) and FFM(kg) (r=0.429, p=0.126). Unadjusted fold change in irisin showed a trend towards an association with fold change in RMR (r=0.396, p=0.094). On adjustment for age, gender, McA and body composition, fold change in irisin was inversely correlated with RQ (r = -0.54, p=0.048), while fold change in IET was significantly related to changes in FFG ( r = -0.55, P=0.043).

No other significant associations were observed.

**Bloods, heart rate, blood pressure and thermal comfort**

Both SBP and DBP were higher at 20°C than at 25°C (Table 2, p < 0.015 and p < 0.005, respectively) while heart rate tended to be lower (p = 0.053). There were no differences in plasma glucose, insulin, triglyceride and irisin concentrations between the different temperatures (data not shown). VAS questions addressing thermal comfort, showed a significant preference for 25°C compared to 20°C (VASQ1: [1.4 ± 1.8 cm at 25°C vs 3.4 ± 3.1 cm at 20°C, p = 0.009]; VASQ2: [1.1 ± 1.3 cm at 25°C vs 3.3 ± 2.5 cm at 20°C, p = 0.001). None of the participants reported shivering during the protocol, nor was any physical signs of shivering observed.

**Gender bias**

There were no significant differences in responses between genders, however there was a trend for a gender bias in RMR response with males showing ~50% higher change related to females (Table 3; males 233 ± 773 kJ/d vs females 160 ± 418 kJ/d, p = 0.087). In contrast females had a ~25% greater vasoconstrictor response relative to males (Table 3). Moreover
Males significantly increased their SBP 3 times more than the females (Table 3) and decreased their HR 4-fold compared to the females (Table 3). Overall, male subjects responded by increased SBP $12 \pm 16$ mmHg ($p = 0.02$) and DBP $6 \pm 7$ mmHg ($p = 0.02$), while female subjects responded mainly through vasoconstriction (Table 3; $4.16 \pm 2.98 \, ^\circ C$, $p < 0.001$).

**Discussion**

The current study examined the effect of mild cold exposure on thermoregulatory variables and investigated whether irisin played a role in thermoregulation. Our RMR measures at $25^\circ C$ are lower than predicted from FFM, based on a published equation (Cunningham, 1991). This is possibly due to a significant overestimation of FFM by the BIA device used in this study rather than errors in RMR. Unpublished observations in our laboratory suggest a $\sim 10\%$ ($\sim 4.7$ kg) overestimation of FFM compared to DEXA, despite an excellent correlation coefficient of 0.97 between the two techniques. Other authors have also noted similar outcomes for a comparable BIA model (Bolanowski and Nilsson, 2001). In this paired design, the ability to detect changes in RMR due to temperature was critical. We obtained an intra-individual CV in RMR of 4%, which if separated from technical error (1.5-3%), is well within the expected range for variations in RMR (Adriaens et al., 2003; Adzika Nsatimba et al., 2015; Soares and Shetty, 1986).

We detected a 3.1% non-significant increase in RMR upon exposure to $20^\circ C$ (Table 2), which was approximately half the increase reported by others (Celi et al., 2010; Westerterp-Plantenga et al., 2002a; Wijers et al., 2010). The stimulation of RMR in response to mild cold exposure has been reported (Celi et al., 2010; Maeda et al., 2007; Wijers et al., 2010) but it is not a consistent observation in every individual. Cold-induced thermogenesis is
highly variable between individuals (Ooijen et al., 2006). In fact a lower RMR in response
to cold is noted in several studies (Celi et al., 2010; van Marken Lichtenbelt et al., 2002) and
6 of 22 participants in the current trial showed no change or a lower RMR to cold exposure.
Some authors have explained this observation on the basis of a Q\textsubscript{10} effect, where a decrease
in energy expenditure accompanies a drop in tissue temperature (van Marken Lichtenbelt
and Schrauwen, 2011). Another likely explanation is that our participants were acclimatized
to mild cold, as the study was conducted towards the end of winter where an average
maximum of 18-20 °C in Perth was reached. Hence prior cold exposure could have blunted
thermogenic responses to mild cold (Armstrong and Thomas, 1991; Young, 1996). There is
a re-emergence of the role of BAT in human thermoregulation (van Marken Lichtenbelt et
al., 2009; Yoneshiro et al., 2013). It is hence possible that some participants had minimal
BAT, and therefore increases in energy expenditure were less than expected. Previous
studies have found an elevated metabolic rate only in those with high levels of BAT (Muzik
et al., 2013). Clearly future trials need to include measurements of BAT activity.

Similar to findings by previous investigators, we observed that core temperature
significantly decreased (van Marken Lichtenbelt et al., 2002), with an increase in peripheral
vasoconstriction (Table 2). Moreover, a decrease in heart rate (Celi et al., 2010; van Marken
Lichtenbelt and Schrauwen, 2011) and an increase in SBP and DBP (Celi et al., 2010) were
also noted (Table 2). Overall, such data are probably indicative of an increased sympathetic
nervous system activity that is seen with cold-induced thermogenesis and suggest that our
participants favored a vasoconstriction response to minimize heat loss. This study was not
specifically designed to examine gender differences in the physiological response to mild
cold stress. However some interesting but non-significant observations emerged. There were
greater decreases in heart rate, increases in blood pressure and a greater thermogenic
response in males relative to females (Table 3). Similar findings have been previously observed by others (Graham, 1988; Pettit et al., 1999; Stevens et al., 1987).

There were no differences between temperatures, in the circulating levels of the myokine irisin, and participants did not complain of shivering nor was it observed. Collectively, these observations would indicate that the adaptive increases in RMR observed in our participants, represent NST. Plasma irisin was not different between temperatures but was inversely related to adjusted RQ. These data suggest that circulating irisin may play a role in fat oxidation in the fasted state. The conditions for RMR measurement in a thermo-neutral range mandates the absence of small muscle activity and fidgeting, so that subjects are completely relaxed. However on exposure to cold, a pre-shivering increase in muscular tone is a physiological response (Meigal, 2002). Such changes can be expected to contribute to RMR, since forearm resting oxygen consumption makes a significant contribution to RMR even on adjusting for body composition (Zurlo et al., 1990). It remains to be determined whether irisin increases in response to heightened muscular tone as monitored by EMG recordings. The latter would indicate a more subtle role for irisin in human metabolism, other than its stated role in shivering thermogenesis.

There are several strengths of the present study including its randomized cross-over design. We used temperatures that were mild, and reflected the seasonality in ambient temperatures encountered by participants living in Perth, Australia. We conducted repeatability studies for the cold exposure and obtained very good precision for all variables. We addressed the potential shivering response by inquiring about thermal comfort and monitoring plasma irisin. However the lack of EMG recordings meant that increased muscle tone or shivering per se could not be objectively confirmed in our participants. Further, we acknowledge that
participants are unlikely to detect the increased muscle tone which precedes shivering. However, given the mild temperature used in this study, and that VAS scores reflected only minor levels of discomfort, an increased pre-shivering muscle tone and shivering is unlikely. An unanticipated outcome was that a fairly large proportion of our participants (6 of 22) were non-responders in RMR. Whether this is due to a $Q_{10}$ effect or lack of BAT needs confirmation. Future studies examining thermoregulatory responses to mild cold must keep the possibility of no-responders in mind while determining the sample size of their study design.

In conclusion, the response to a mild cold exposure favored vasoconstriction to reduce heat loss and did not induce a statistically significant stimulation of RMR. Hence our data suggests that mild cold exposure may be an effective way to increase energy expenditure in some people, but may not be effective in others. The precise role of irisin in thermoregulatory thermogenesis needs confirmation. Our findings raise the possibility that subtle increases in irisin may increase fasting fat oxidation and hence play a role well before shivering has developed. As humans spends most of their time in a postprandial state, future studies could investigate whether food consumption is able to stimulate a greater thermogenic response to mild cold exposure through diet-induced thermogenesis.
378 Conflicts of interest

379 The authors have no conflict of interest to disclose based on the ICMJE guidelines.

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382 Author’s contributions

383 EKC, MJS, and APJ planned the study. EKC conducted all aspects of the data collection.

384 EKC, MJS and RW conducted the statistical analyses. EKS and MJS wrote the draft manuscript. TPJ and RW critically reviewed and co-wrote the manuscript.

387

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Literature cited


Table 1. Baseline characteristics of participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Whole group</th>
<th>Males</th>
<th>Females</th>
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<tbody>
<tr>
<td>Gender (M/F)</td>
<td>9/13</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.73 ± 10.07</td>
<td>58.11 ± 11.57</td>
<td>57.46 ± 9.38</td>
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<tr>
<td>Weight (kg)</td>
<td>79.80 ± 17.82</td>
<td>94.69 ± 12.76</td>
<td>69.50 ± 12.89</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>27.08 ± 4.52</td>
<td>29.82 ± 3.32</td>
<td>25.19 ± 4.35</td>
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<tr>
<td>WC (cm)</td>
<td>94.43 ± 12.87</td>
<td>104.72 ± 9.21</td>
<td>87.31 ± 9.94</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>20.88 ± 7.93</td>
<td>21.84 ± 8.03</td>
<td>20.21 ± 8.11</td>
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<tr>
<td>FFM (kg)</td>
<td>60.45 ± 14.06</td>
<td>72.84 ± 9.52</td>
<td>52.2 ± 9.84</td>
</tr>
<tr>
<td>HDL¹ (mmol/l)</td>
<td>1.18 ± 0.26</td>
<td>1.03 ± 0.21</td>
<td>1.30 ± 0.24</td>
</tr>
<tr>
<td>TG¹ (mmol/l)</td>
<td>1.22 ± 0.43</td>
<td>1.30 ± 0.34</td>
<td>1.17 ± 0.50</td>
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<tr>
<td>FBG¹ (mmol/l)</td>
<td>5.47 ± 0.31</td>
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<td>Insulin¹ (mmol/l)</td>
<td>6.30 ± 4.80</td>
<td>9.39 ± 4.66</td>
<td>5.47 ± 2.28</td>
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<tr>
<td>SBP (mmHg)</td>
<td>134 ± 24</td>
<td>143 ± 17</td>
<td>127 ± 26</td>
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<tr>
<td>DBP (mmHg)</td>
<td>74 ± 10</td>
<td>76 ± 10</td>
<td>73 ± 11</td>
</tr>
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</table>

Data are mean ± SD, n=22 except ¹ n=19 due to missing blood sample.

M, male; F, female; BMI, body mass index; WC, waist circumference; FM, fat mass; FFM, fat-free mass; HDL, high-density lipoprotein cholesterol; TG, triglycerides; FBG, fasting blood glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure.
Table 2. Thermoregulation and metabolic indices of participants exposed to two temperatures.

<table>
<thead>
<tr>
<th>Variable</th>
<th>25°C Mean ± SD</th>
<th>20°C Mean ± SD</th>
<th>Change (20°C -25°C) Mean ± SD</th>
<th>Paired t test P value</th>
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</thead>
<tbody>
<tr>
<td>RMR (kJ/d)</td>
<td>5938 ± 1415</td>
<td>6127 ± 1467</td>
<td>190 ± 573</td>
<td>0.135</td>
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<td>RQ</td>
<td>0.84 ± 0.04</td>
<td>0.83 ± 0.04</td>
<td>-0.003 ± 0.05</td>
<td>0.745</td>
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<td>IET (°C)</td>
<td>35.69 ± 0.38</td>
<td>35.49 ± 0.47</td>
<td>-0.24 ± 0.28</td>
<td>0.001</td>
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<td>FFG (°C)</td>
<td>1.37 ± 2.96</td>
<td>5.24 ± 2.51</td>
<td>3.75 ± 3.02</td>
<td>&lt;0.001</td>
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<tr>
<td>SBP (mmHg)</td>
<td>134 ± 23</td>
<td>142 ± 23</td>
<td>8 ± 13</td>
<td>0.015</td>
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<tr>
<td>DBP (mmHg)</td>
<td>75 ± 10</td>
<td>79 ± 10</td>
<td>4 ± 6</td>
<td>0.005</td>
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<tr>
<td>HR (bpm)</td>
<td>57 ± 7</td>
<td>56 ± 7</td>
<td>-2 ± 5</td>
<td>0.053</td>
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<tr>
<td>McA1</td>
<td>7.96 ± 1.82</td>
<td>8.13 ± 1.79</td>
<td>0.17 ± 0.87</td>
<td>0.396</td>
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<tr>
<td>Irisin1 (ug/ml)</td>
<td>1.15 ± 0.63</td>
<td>1.8 ± 0.54</td>
<td>-0.08 ± 0.51</td>
<td>0.527</td>
</tr>
</tbody>
</table>

Data are mean ± SD, n=22 except 1n=19 due to missing blood sample.

RMR, resting metabolic rate; RQ, respiratory quotient; IET, in the ear temperature; FFG, forearm minus fingertip temperature gradient; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; McA, McAuley’s index.
Table 3. Potential influence of gender on thermoregulation and metabolic indices.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males change (20-25°C)</th>
<th>Females change (20-25°C)</th>
<th>Between-gender difference†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>P value</td>
</tr>
<tr>
<td>RMR (kJ/d)</td>
<td>233 ± 773</td>
<td>160 ± 418</td>
<td>0.087</td>
</tr>
<tr>
<td>RQ</td>
<td>-0.011 ± 0.064</td>
<td>0.002 ± 0.043</td>
<td>0.938</td>
</tr>
<tr>
<td>IET (°C)</td>
<td>-0.21 ± 0.195*</td>
<td>-0.27 ± 0.342*</td>
<td>0.794</td>
</tr>
<tr>
<td>FFG (°C)</td>
<td>3.15 ± 3.15*</td>
<td>4.16 ± 2.975*</td>
<td>0.274</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>12.11 ± 16*</td>
<td>4 ± 11</td>
<td>0.920</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>6.22 ± 7*</td>
<td>2.58 ± 5</td>
<td>0.391</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>-3.79 ± 3.42*</td>
<td>-1.42 ± 6.30</td>
<td>0.941</td>
</tr>
<tr>
<td>McA</td>
<td>-0.13 ± 0.46</td>
<td>-0.21 ± 1.10</td>
<td>0.906</td>
</tr>
<tr>
<td>Irisin (ug/ml)</td>
<td>-0.3 ± 0.41</td>
<td>0.08 ± 0.54</td>
<td>0.380</td>
</tr>
</tbody>
</table>

Data are mean ± SD, n=22 except †n=19 due to missing blood sample.

*p<0.05, paired t test for within-gender effects.
† Multivariate ANOVA adjusted for age, and body composition.
RMR, resting metabolic rate; RQ, respiratory quotient; IET, in the ear temperature; FFG, forearm minus fingertip temperature gradient; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; McA, McAuley’s index