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

Emily K Calton[1]
Mario J Soares[1, 3]
Anthony P James[1]
Richard J Woodman[2]

[1] Directorate of Nutrition, Dietetics & Food Technology
School of Public Health, Curtin Health Innovation Research Institute-Metabolic Health,
Faculty of Health Sciences,
Curtin University, Perth, Western Australia 6845.

[2]Flinders Centre for Epidemiology and Biostatistics
School of Medicine, Faculty of Medicine, Nursing and Health Sciences
Flinders University of South Australia, Adelaide, GPO Box 2100, SA 5001.

[3]Correspondence & reprints:
Associate Professor Mario J Soares
Directorate of Nutrition, Dietetics & Food Technology, School of Public Health,
Curtin Health Innovation Research Institute-Metabolic Health,
Curtin University, Perth, Western Australia 6845.
Email: m.soares@curtin.edu.au
Tel: +61-892663220
Fax: + 61-8-92662958

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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/m^2. 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^{o}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^{o}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) ≥ 20 kg/m^2, weight stable ($< \pm 2$ 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 $m^{3,}$ 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

159 Marken Lichtenbelt et al., 2006). Peripheral vasoconstriction was measured as the forearm
160 to fingertip temperature gradient (FFG) (Rubinstein and Sessler, 1990). Core temperature
161 is generally defined as the temperature measured within the pulmonary artery. IET
162 measurements have been known as accurate, valid and reproducible measurements of core
163 body temperature, reflecting temperature changes within the pulmonary artery (Benzinger,
164 1969; Erickson and Kirklin, 1993; Hasper et al., 2011). Thus, IET measurements were made
165 after 50 minutes in the chamber using a standard in the ear thermometer (Omron Model
166 MC-510). Three measurements of IET were made with the average of the two closest
167 measurements recorded.
168
169 *Resting metabolic rate*
170 Participants rested in the supine position for 30 minutes to equilibrate with the temperature
171 of the environmental chamber. RMR was measured via indirect calorimetry (Deltatrac II,
172 Datex Instrumentarium, Finland), according to a standardized protocol that emphasized 30
173 minute rest (Piers et al., 2002). A trial RMR measurement was made for 20 minutes for
174 participants to become accustomed to the canopy. After a 20 minute rest period, RMR was
175 again measured as described above. The RMRs reported represent the value after a 90 min
176 exposure to that temperature. RMR in kJ/d was derived from CO_2 production and O_2
177 consumption according to the Weir's formula, neglecting protein oxidation in the
178 fasting state (Weir, 1990). Substrate oxidation was calculated as volume CO_2/volume O_2.
179 The Deltatrac II apparatus was calibrated with gas mixtures of known composition (95%
180 O_2, 5% CO_2) before each measurement session and regularly checked by 30 minute ethanol
181 burn tests (mean and SD for six tests during the study was RQ = 0.66 ± 0.02).
182 *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

208 Primary study outcomes were RMR, irisin and FFG.

209

210 *Statistical analysis*

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

222

223 **Results**

224 *Baseline demographics, body composition and key MetS variables*

225 Twenty two Australians of European origin (9 males, 13 females) were recruited into the
226 study and there were no dropouts. Participants had a mean ± SD (range) age of 57.73 ± 10.07
227 years (29-68 years), weight 79.80 ± 17.82 kg (49.65-109.1 kg), BMI of 27.80 ± 4.52 kg/m^2
228 (19.89-33.73 kg/m^2), fat mass of 60.54 ± 14.06 kg (41.7-87.3 kg) and fat-free mass of 20.88
229 ± 7.93 kg (7.9-36.5 kg) (Table 1).

230

231 [Table 1 here]

232 *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 20ºC compared to 25ºC (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 ºC greater vasoconstriction and males showing a greater thermogenic response by 80 kJ (Table 3).

[Table 2 here]

[Table 3 here]

Correlations - Change in IET, RMR, FFG and irisin

Gender was not associated with fold change in IET (r=100, 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

283 males significantly increased their SBP 3 times more than the females (Table 3) and
284 decreased their HR 4-fold compared to the females (Table 3). Overall, male subjects
285 responded by increased SBP 12 ± 16 mmHg ($p = 0.02$) and DBP 6 ± 7 mmHg ($p = 0.02$),
286 while female subjects responded mainly through vasoconstriction (Table 3; 4.16 ± 2.98 °C
287 , $p < 0.001$).
288

289 **Discussion**

290 The current study examined the effect of mild cold exposure on thermoregulatory variables
291 and investigated whether irisin played a role in thermoregulation. Our RMR measures at
292 25ºC are lower than predicted from FFM, based on a published equation (Cunningham,
293 1991). This is possibly due to a significant overestimation of FFM by the BIA device used
294 in this study rather than errors in RMR. Unpublished observations in our laboratory suggest
295 a ~10% (~4.7 kg) overestimation of FFM compared to DEXA, despite an excellent
296 correlation coefficient of 0.97 between the two techniques. Other authors have also noted
297 similar outcomes for a comparable BIA model (Bolanowski and Nilsson, 2001). In this
298 paired design, the ability to detect changes in RMR due to temperature was critical. We
299 obtained an intra-individual CV in RMR of 4%, which if separated from technical error (1.5-
300 3%), is well within the expected range for variations in RMR (Adriaens et al., 2003; Adzika
301 Nsatimba et al., 2015; Soares and Shetty, 1986).
302

303 We detected a 3.1% non-significant increase in RMR upon exposure to 20°C (Table 2),
304 which was approximately half the increase reported by others (Celi et al., 2010; Westerterp-
305 Plantenga et al., 2002a; Wijers et al., 2010). The stimulation of RMR in response to mild
306 cold exposure has been reported (Celi et al., 2010; Maeda et al., 2007; Wijers et al., 2010)
307 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_{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.

Conflicts of interest

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

Author's contributions

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

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Table 1. Baseline characteristics of participants

Variable	Whole group	Males	Females
Gender (M/F)	9/13	9	13
Age (years)	57.73 ± 10.07	58.11 ± 11.57	57.46 ± 9.38
Weight (kg)	79.80 ± 17.82	94.69 ± 12.76	69.50 ± 12.89
BMI (kg/m^2)	27.08 ± 4.52	29.82 ± 3.32	25.19 ± 4.35
WC (cm)	94.43 ± 12.87	104.72 ± 9.21	87.31 ± 9.94
FM (kg)	20.88 ± 7.93	21.84 ± 8.03	20.21 ± 8.11
FFM (kg)	60.45 ± 14.06	72.84 ± 9.52	52.2 ± 9.84
HDL[1] (mmol/l)	1.18 ± 0.26	1.03 ± 0.21	1.30 ± 0.24
TG[1] (mmol/l)	1.22 ± 0.43	1.30 ± 0.34	1.17 ± 0.50
FBG[1] (mmol/l)	5.47 ± 0.31	5.66 ± 0.22	5.33 ± 0.28
Insulin[1] (mmol/l)	6.30 ± 4.80	9.39 ± 4.66	5.47 ± 2.28
SBP (mmHg)	134 ± 24	143 ± 17	127 ± 26
DBP (mmHg)	74 ± 10	76 ± 10	73 ± 11

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.

Variable	25°C	20°C	Change (20°C -25°C)	Paired t te
	Mean ± SD	Mean ± SD	Mean ± SD	P value
RMR (kJ/d)	5938 ± 1415	6127 ± 1467	190 ± 573	0.135
RQ	0.84 ± 0.04	0.83 ± 0.04	-0.003 ± 0.05	0.745
IET (°C)	35.69 ± 0.38	35.49 ± 0.47	-0.24 ± 0.28	0.001
FFG (°C)	1.37 ± 2.96	5.24 ± 2.51	3.75 ± 3.02	<0.001
SBP (mmHg)	134 ± 23	142 ± 23	8 ± 13	0.015
DBP (mmHg)	75 ± 10	79 ± 10	4 ± 6	0.005
HR (bpm)	57 ± 7	56 ± 7	-2 ±5	0.053
McA[1]	7.96 ± 1.82	8.13 ± 1.79	0.17 ± 0.87	0.396
Irisin[1] (ug/ml)	1.15 ± 0.63	1.8 ± 0.54	-0.08 ± 0.51	0.527

Data are mean ± SD, n=22 except [1]n=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.

606 **Table 3.** Potential influence of gender on thermoregulation and metabolic indices.

Variable	Males change (20-25ºC)	Females change (20-25 ºC)	Between-gender difference†
	Mean ± SD	Mean ± SD	P value
RMR (kJ/d)	233 ± 773	160 ± 418	0.087
RQ	-0.011 ± 0.064	0.002± 0.043	0.938
IET (°C)	–0.21 ± 0.195*	-0.27 ± 0.342*	0.794
FFG (°C)	3.15 ± 3.15*	4.16 ± 2.975*	0.274
SBP (mmHg)	12.11 ± 16*	4 ± 11	0.920
DBP (mmHg)	6.22 ± 7*	2.58 ± 5	0.391
HR (bpm)	-3.79 ± 3.42*	-1.42 ± 6.30	0.941
McA[1]	-0.13 ± 0.46	-0.21. ± 1.10	0.906
Irisin[1] (ug/ml)	-0.3 ± 0.41	0.08 ± 0.54	0.380

607 Data are mean ± SD, n=22 except [1]n=19 due to missing blood sample.
608 *p<0.05, paired t test for within-gender effects.
609 † Multivariate ANOVA adjusted for age, and body composition
610 RMR, resting metabolic rate; RQ, respiratory quotient; IET, in the ear temperature; FFG,
611 forearm minus fingertip temperature gradient; SBP, systolic blood pressure; DBP, diastolic
612 blood pressure; HR, heart rate; McA, McAuley's index