Effect of BMI on the Thermogenic Response to Cold Exposure and Associated Changes in Metabolism and Browning Markers in Adult Humans

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Keywords

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Abstract

Introduction: Brown adipose tissue (BAT) serves to produce heat by nonshivering thermogenesis. Activation of BAT increases energy expenditure and is seen as a putative strategy to treat obesity. There are conflicting data on the capacity for cold-induced thermogenesis in individuals with higher BMI. **Methods:** To investigate the effect of BMI on cold-induced stimulation of energy expenditure, changes in the metabolic profile, and the expression of browning markers in subcutaneous white adipose tissue (scWAT), healthy adults ($N = 173$, 50.9% females) with a median age of 26.0 (interquartile range [IQR]: 23.0; 28.0) years and a median body mass index (BMI) of 23.6 [IQR: 21.9; 26.6] kg/m² were exposed to short-term mild cold exposure (CE). Resting energy expenditure (REE) was measured by indirect calorimetry and blood sampling was conducted at baseline and after CE. In a subgroup of participants with obesity, subcutaneous

abdominal fat biopsies were taken before and after CE. **Results:** The cold-induced median increase in REE was 74 (IQR: –28; 241) kcal/day ($p < 0.001$). This increase negatively correlated with BMI ($p < 0.001$). Participants with BMI 18.5–24.9 kg/m² displayed a significant median increase of 103 kcal/day ($p < 0.001$), participants with overweight or obesity were not able to increase REE (23, $p = 0.468$ or –30 kcal/day, $p = 0.917$, respectively). In participants with obesity, expression of cell death activator in scWAT after CE was upregulated in females ($p = 0.034$). **Conclusions:** Persons with overweight and obesity do not increase REE in response to CE, presumably reflecting lower BAT activity. Likewise, the metabolic response to cold is diminished in participants with elevated BMI.

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Introduction

The existence of brown adipose tissue (BAT) in humans was first described in the early 1900s in dissection studies [1, 2]. In contrast to rodents, the evidence is unclear in humans if BAT plays a role in the development of

obesity and its metabolic disturbances. Early attempts to develop sympathomimetic compounds that activate BAT turned out to either be ineffective to treat obesity or to cause adverse effects on the cardiovascular system [3]. Interest in BAT was renewed more than 10 years ago with studies using 18-fluoro-labeled 2-deoxy-glucose positron emission tomography combined with computed tomography (^{18}F -FDG-PET-CT) imaging [4–6]. These studies showed the presence and activation of BAT in adult humans particularly after cold exposure (CE). The presence of active BAT was lower in elderly persons as well as in people with higher body mass index (BMI) [7, 8]. In contrast to white adipose tissue (WAT), BAT does not serve for lipid storage, but facilitates nonshivering thermogenesis (NST) by burning lipids, thus releasing energy as heat. A unique feature of brown adipocytes is the expression of uncoupling protein 1 (UCP1) which is present in the membrane of the highly abundant mitochondria in these cells [9].

In studies using ^{18}F -FDG-PET-CT analysis, the gold standard for detecting active BAT, a negative correlation between BAT mass and BMI, was reported [5, 7, 10]. In other studies, BAT mass was found to be reduced or not detectable in participants with obesity [11, 12]. Conventional MRI analyses showed a positive correlation of BMI with the fat fraction of the supraclavicular area, a location where BAT is mostly found, suggesting that this depot contains less BAT in individuals with higher BMI [13]. Activation of BAT after CE using ^{18}F -FDG-PET-CT analysis was found to be associated with improved whole-body glucose homeostasis and insulin sensitivity in humans [14, 15]. Upon acute CE in lean, young adults, BAT was shown to be present and activated [16]. It was also reported that in subjects with a BMI of 28.1–36.8 kg/m², a substantial amount of BAT can be recruited after cold acclimation [17]. Large-scale retrospective studies suggested that individuals with active BAT have fewer cardiovascular symptoms and generally better blood lipid and blood glucose levels [18, 19].

Despite the growing number of studies, most were small in sample size (mostly between 10 and 30 participants), and there is still some inconsistency in the literature. In the present study, we were interested to assess changes in energy expenditure and metabolic parameters induced by an individualized CE in a large sample of 173 healthy adults across a wide BMI range. We analyzed whether the cold-induced change in resting energy expenditure (REE) is associated with BMI and changes in metabolic parameters. Furthermore, we took biopsies of subcutaneous WAT (scWAT) from 17 participants with

obesity before and after CE to examine changes in messenger RNA expression of selected browning marker genes.

Material and Methods

Study Design

This intervention study was conducted at two study sites of the Technical University of Munich (Munich and Freising) using identical equipment and with the same personnel. The study protocol was reviewed and approved by the Ethics Commission of the School of Medicine of the Technical University of Munich (#236/16S). Recruitment started in October 2016 and ended in April 2019. Participants were recruited by advertising via bulletins, university mailing lists, and a newspaper article. Each volunteer gave their written informed consent. The study was registered at the German Clinical Trials Register DRKS (accession number: DRKS00010489). A subgroup of this cohort was already described previously [20].

Individuals were enrolled for the study when they met pre-defined eligibility criteria as described recently in detail [20]. Briefly, participants were 18–60 years old, nonsmoking, and without severe diseases. The presence of diabetes was another exclusion criterion. In case of acute infection, such as common cold, appointments were postponed. Attrition of participants is displayed in the online supplementary Figure S1 (for all online suppl. material, see www.karger.com/doi/10.1159/000522218).

Anthropometric Measurements

Measurements started in the morning between 07:30 a.m. and 08:00 a.m. Participants were asked to refrain from caffeine and exercise for at least 24 h and from caloric beverages or food for at least 10 h. Body height (cm) was measured to the nearest 0.1 cm using a stadiometer (Seca, Hamburg, Germany). Body composition and weight were measured using the TANITA Body Composition Analyzer Type BC-418 MA (Tanita Europe GmbH, Sindelfingen, Germany). Systolic and diastolic blood pressure and heart rate were assessed in triplicate in a sitting position before each blood sampling using Omron M8 comfort (Omron Medizintechnik Handelsgesellschaft mbH, Mannheim, Germany).

Measurement of Temperatures

For assessing skin temperature, participants were equipped with eight wireless thermosensors (iButtons DS-1921 H, Thermochron; Maxim, Dallas, TX, USA) at positions of the body defined by ISO9886 [21]. One additional sensor was attached to the supraclavicular fossae. Outside temperature data were obtained from the website of the German Meteorological Service (Deutscher Wetterdienst [DWD], www.dwd.de) for the study site in Munich. For Freising, data were obtained from the meteorological station in Freising (<https://www.wetter-weihenstephan.de/>) which is run by DWD and LfL (Bayerische Landesanstalt für Landtechnik). Room temperature was kept stable and was monitored during the experiment.

Measurement of REE

REE was assessed at two time points: at thermoneutrality (TN) and after 2-h CE, for 30 min each. This was done by indirect calo-

rimetry using a ventilated canopy hood system (COSMED Quark RMR, Fridolfing, Germany). On each morning before the participant arrived, a volume calibration was done, and before each indirect calorimetry measurement, a gas calibration of the system was conducted. By applying the shortened Weir equation, conversion of the measurements of VO_2 (consumption of oxygen [mL/min]) and VCO_2 (production of CO_2 [mL/min]) to REE (kcal/day) was assessed [22]. The respiratory exchange ratio was calculated by dividing VCO_2 by VO_2 . To ensure data quality, REE measurements had to achieve at least 4 continuous minutes in which the variances of VCO_2 and VO_2 were less than 10% each [23]. This steady-state was not met by 10 participants and they were excluded from REE analysis.

Individualized Cooling Protocol

After anthropometric and cardiovascular measurements, participants were bedded between two water perfused blankets (Maxi-Therm Lite Blankets; Cincinnati Sub-Zero, Cincinnati, OH, USA) that were connected to a cooling device (WiseCircu type WCR-P8; Witeg Labortechnik, Wertheim, Germany). At TN, the water inlet temperature was set to 32°C, or lower if this temperature was sensed as too warm. After the first indirect calorimetry and blood sampling, the cooling protocol started. The water temperature was decreased stepwise, as described earlier [20]. As soon as participants reported shivering or showed any signs thereof, the shivering temperature was noted and the water inlet temperature was increased by 2°C. Then, CE was maintained for 120 min at this defined temperature (Texp). If shivering occurred during that time, the temperature was increased further until shivering stopped. Participants with shivering during the second REE measurement ($n = 2$) were excluded from the analysis.

Blood Analyses

Each indirect calorimetry was followed by blood sampling. Fasting plasma glucose was determined using a point-of-care glucometer system (HemoCue Glucose 201+ System; Radiometer GmbH, Willich, Germany). Enzyme-linked immunosorbent assays were used to measure plasma leptin, adiponectin (Duoset ELISA human; R&D, Wiesbaden, Germany), and insulin (DRG Insulin ELISA; DRG Instruments, Marburg, Germany). Plasma non-esterified fatty acids (NEFAs) were assessed by using an enzymatic colorimetric method (Wako Chemicals, Neuss, Germany). Serum triglycerides (TGs), C-reactive protein (CRP), and free tri-iodothyronine were measured using established commercial test kits by a certified laboratory service (SynLab Labordienstleistungen, Munich, Germany). One participant was excluded from analysis as her thyroid levels were out of the accepted range and for two participants blood sampling after CE was not successful for technical reasons. For one further participant, CE values for TG, CRP, and free triiodothyronine were not available. Insulin sensitivity was calculated by homeostasis model assessment (HOMA-IR): $\text{HOMA-IR} = \text{plasma glucose (mmol/L)} \times \text{plasma insulin} (\mu\text{U/mL}) / 22.5$ [24].

Adipose Tissue Biopsy and Analysis

To obtain scWAT samples in a subgroup with overweight or obesity, needle aspiration biopsy at the region lateral from the umbilicus was conducted after each indirect calorimetry, subsequent to blood sampling, under sterile conditions providing enough scWAT (>1 g wet tissue per biopsy). This was done on a voluntary

basis and with written informed consent. For RNA processing, the fat tissue obtained was aliquoted into tubes containing sterilized zirconia glass beads (Carl Roth, Karlsruhe, Germany), RLT buffer (RNeasy Mini Kit; Qiagen, Hilden, Germany), and 1% (vol/vol) β -mercaptoethanol (Sigma-Aldrich, St. Louis, MO, USA).

Messenger RNA (mRNA) was isolated according to the protocol of the mirVana miRNA Isolation Kit (Thermo Fisher Scientific, Dreieich, Germany) for fat tissue. This was followed by transcribing complementary DNA according to the protocol of High-Capacity cDNA Reverse Transcription Kits (Thermo Fisher Scientific). Quantitative PCR was carried out with SYBRgreen MasterMix (QIAGEN GmbH, Düsseldorf, Germany) to measure the expression of being markers along with PPIA, GAPDH, and IPO8 as housekeeping genes. We performed gene expression analyses in scWAT samples of 17 individuals (12 females) before and after CE. The sequences of the primers used are listed in online supplementary Table S1.

Statistical Analysis

Data were analyzed using GraphPad Prism Version 5.2 (GraphPad Software, Inc., San Diego, CA, USA) and RStudio (RStudio Inc., Boston, MA, USA). The normality of data was formally tested by visual inspection of histograms. As most parameters were not normally distributed, we used nonparametric tests. Baseline characteristics and outcome variables, including REE, metabolic parameters, and skin temperatures, were compared between TN and CE using Wilcoxon signed-rank test and are presented as median including interquartile range [IQR]. For comparing the different BMI categories, Kruskal-Wallis analysis of variance was applied. For assessing differences between two groups, and between sexes, the Mann-Whitney U test was applied. Pearson and Spearman's rank correlation was used to identify correlations between variables. In simple linear regressions, Texp, sex, BMI, fat mass (FM), fat-free mass (FFM), age, study site, and ambient temperature were used as independent variables with ΔREE and $\Delta\text{REE} (\%)$ as dependent variables. Additionally, two multiple linear regression approaches were performed using BMI, ambient temperature, Texp, and FFM or FM, respectively, as the independent variables. A sensitivity analysis was done which excluded outliers that differed more than 3 SDs from the mean. Relative gene expression was calculated by the $\Delta\Delta\text{CT}$ method and normalized to the mean expression of the housekeeper genes. Bonferroni correction was applied. Results were considered statistically significant at $p < 0.05$. Due to the explorative nature of the study, no power calculation was done.

Results

Baseline characteristics of the study group are presented in Table 1. The median age of the participants was 26.0 [23.0–28.0] years, and the median BMI was 23.6 [21.9–26.6] kg/m^2 . The normal weight group (NW, BMI 18.5–24.9 kg/m^2) was significantly younger (25.0 [23.0–27.0] years) compared to the group with overweight (OW, BMI 25–29.9 kg/m^2 , 27.0 [25.0–28.5] years; $p = 0.012$) and the group with obesity (OB, BMI $\geq 30 \text{ kg/m}^2$, 28.0 [26.0–31.8] years; $p < 0.001$) (Table 2).

Table 1. Participant characteristics

	All (n = 170)	NW (n = 117)	OW (n = 27)	OB (n = 26)	p value
Sex (M/F)	83/87	58/59	14/13	11/15	
Age, years	26.0 [23.0; 28.0]	25.0 [23.0; 27.0]	27.0 [25.0; 28.5]	28.0 [26.0; 31.8]	<0.001
Weight, kg	74.8 [65.1; 86.2]	67.9 [61.1; 77.4]	84.8 [77.3; 88.8]	101.1 [96.5; 110.7]	<0.001
Height, cm	175.5 [169.5; 183.2]	176.0 [170.5; 185.1]	176.5 [167.8; 183.8]	174.3 [168.0; 177.7]	0.487
BMI, kg/m ²	23.6 [21.9; 26.6]	22.6 [21.3; 23.7]	26.8 [26.2; 27.8]	34.1 [31.7; 36.3]	<0.001
FM, n (%)	23.1 [14.4; 29.8]	19.0 [12.5; 25.2]	26.2 [19.1; 35.1]	38.2 [27.7; 43.1]	<0.001
FFM, kg	57.8 [47.4; 68.6]	53.2 [45.8; 81.5]	64.8 [51.4; 69.6]	63.7 [54.6; 77.2]	0.001

Values are presented as median with [IQR]; differences between BMI groups were assessed by Kruskal-Wallis analysis with Bonferroni correction. BMI, body mass index; F, females; FM, fat mass; FFM, fat-free mass; IQR, interquartile range; M, males; NW, normal weight (BMI 18.5–24.9 kg/m²); OB, obese (BMI ≥30 kg/m²); OW, overweight (BMI 25.0–29.9 kg/m²).

Table 2. Multiple linear regression of REE TN, ΔREE, and ΔREE (%) as dependent variables

REE TN ~ BMI + sex + age + FFM								
	estimate	std error	t value	p value				
BMI, kg/m ²	8.22	3.43	2.40	0.018				
Sex (male)	−126.89	59.01	−2.15	0.033				
Age, years	−5.32	2.60	−2.05	0.042				
FFM	22.98	2.48	9.26	<0.001				
ΔREE ~ Texp + BMI + FFM + Tamb					ΔREE (%) ~ Texp + BMI + FFM + Tamb			
	estimate	std error	t value	p value	estimate	std error	t value	p value
Texp	−35.7	7.11	−5.02	<0.001	−2.18	0.43	−5.05	<0.001
BMI, kg/m ²	−12.07	3.83	−3.15	0.002	−0.73	0.23	−3.13	0.002
FFM	−3.38	1.56	−2.17	0.032	−0.29	0.09	−3.08	0.002
Tamb	−4.78	2.85	−1.68	0.096	−0.25	0.17	−1.47	0.142
ΔREE ~ Texp + BMI + FM + Tamb					ΔREE (%) ~ Texp + BMI + FM + Tamb			
	estimate	std error	t value	p value	estimate	std error	t value	p value
Texp	−36.89	7.08	−5.21	<0.001	−2.19	0.43	−5.08	<0.001
BMI, kg/m ²	−31.97	7.6	−4.21	<0.001	−2.18	0.46	−4.71	<0.001
FM	8.4	3.16	2.66	0.009	0.6	0.19	3.12	0.002
Tamb	−5.16	2.82	−1.83	0.069	−0.29	0.17	−1.69	0.093

Associations were assessed by a univariate analysis of variance approach. BMI, body mass index; CE, cold exposure; FM, fat mass; FFM, fat free mass; REE, resting energy expenditure; Std Error, standard error; Tamb, ambient temperature; Texp, exposure temperature; TN, thermoneutrality; ΔREE, change of REE after CE.

Changes in REE

Basal REE was significantly higher in the OB group compared to the NW group ($p < 0.001$). This difference remained statistically significant after adjustment for FFM and age ($p < 0.001$ data not shown). There was no statistical difference in REE at TN between the OW and

OB groups ($p = 0.079$, data not shown). In the whole cohort, REE increased from baseline 1,619 [IQR: 1,438–1,818] to 1,700 [1,503–1,934] kcal/day ($p < 0.001$) after CE with a high interindividual variance. While we observed an overall increase of 6.5% following CE ($p < 0.001$) in the NW group, there was no significant change in the

Fig. 1. a, b Changes in REE and RER after CE; for all participants and stratified for BMI group. Values are presented as median with IQR; Differences between BMI groups were assessed by the Kruskal-Wallis test; IQR, interquartile range; NW, normal weight (BMI 18.5–24.9 kg/m²); OW, overweight (BMI 25.0–29.9 kg/m²); OB, obese (BMI ≥30.0 kg/m²); ΔREE, differences in resting energy expenditure before and after CE; ΔRER, differences in respiratory exchange ratio before and after CE.

OW (1.6%, $p = 0.468$) or OB groups (−1.7%, $p = 0.917$, Fig. 1), respectively. The change in REE was significantly different between BMI groups ($p < 0.001$). As shown in Table 2, this finding remained significant after adjustment for age, Texp, sex, and FFM in a multiple regression analysis ($p = 0.019$) which demonstrates a negative association between BMI and ΔREE. Other putative predictors did not reveal an association with ΔREE (online suppl. Table S2). While there was no significant association between fat ratio (FM [%]) and ΔREE (%) ($p = 0.245$), we could observe an association after adjustment for age and gender ($p = 0.036$). There was a significant decrease in the respiratory exchange ratio after CE in all BMI groups with a tendency to decrease less depending on the BMI category, but there was no significant difference between groups (all $p > 0.094$; Fig. 1).

Temperature Monitoring

The median outdoor temperature on study days was 5.1°C [1.3–10.1], which was comparable between BMI groups ($p = 0.444$). Room temperature was 22.0°C [21.0–22.0] at TN and 23.0°C [21.0–24.0] at CE. Both temperatures were comparable between BMI groups ($p = 0.619$ and $p = 0.356$, respectively). Participants started shivering at 19.9°C [18.1–21.6] with no significant difference be-

tween BMI groups ($p = 0.398$). Likewise, Texp was similar in all BMI groups (22.8°C [21.0–24.3], $p = 0.106$). There were significant differences in the water inlet temperature at the beginning of the experiment due to the following reason: while 32°C was the official starting temperature, some participants perceived this level as too warm. To avoid sweating and thus elevated REE, we decreased the temperature to achieve the thermoneutral zone of these participants. This temperature was significantly lower in the OB group (31.0°C [30.1–32.0]) compared to the NW (32.0°C [32.0–32.0], $p < 0.001$) and OW (32.0°C [32.0–32.0], $p = 0.005$) groups.

CE led to a significant decrease in overall skin temperature by −3.5°C [−4.6 to −2.8] ($p < 0.001$). This decrease was not significantly different between BMI groups ($p = 0.891$; online suppl. Table S3). However, at TN and after CE, skin temperature was significantly higher in the NW group compared to the other two BMI groups ($p < 0.001$ for both conditions). Regarding skin temperature at the supraclavicular area (Tsupra), no changes in temperature were observed after CE (all $p > 0.136$). However, Tsupra was significantly lower in the OB group compared to the NW and OW groups at TN and after CE (all $p < 0.001$). In addition, there was a significant decrease in Tsupra in males from the NW and OB groups ($p = 0.034$ and $p = 0.002$, respectively). Linear regression showed that ΔTsupra was not associated with BMI ($p = 0.727$).

Cardiovascular Effects

Systolic blood pressure (BP) significantly increased in the NW and OB groups ($p < 0.001$ and $p = 0.024$, respectively) during CE, while the increase in systolic BP was not significant in the OW group ($p = 0.235$; Table 3). Diastolic BP significantly increased in all groups (all $p < 0.001$). However, the magnitude of these increases was comparable between the BMI groups. The heart rate significantly decreased in all groups (all $p < 0.002$) and there were no significant differences between the BMI groups ($p = 0.380$).

Circulating Metabolites

We observed that fasting glucose levels decreased during CE in all BMI groups (all $p < 0.002$). Neither basal values nor those after CE were significantly different between groups ($p = 0.172$ at TN and $p = 0.876$ after CE). Furthermore, the relative decrease was comparable across groups ($p = 0.120$, Fig. 2a). Plasma insulin levels were higher in the OB group compared to the NW and OW groups at both conditions (both $p < 0.001$). While CE had no significant effect on plasma insulin levels in the NW

Table 3. Changes in sysBP and diaBP and heart rate after CE, for the whole group and stratified for the BMI groups

	All (n = 170)			NW (n = 117)			OW (n = 27)			OB (n = 26)		
	TN	CE	p value TN versus CE	TN	CE	p value TN versus CE	TN	CE	p value TN versus CE	TN	CE	p value TN versus CE
SysBP, mm Hg	115.0 [108.0; 125.2]	119.0 [112.0; 129.2]		113.0 [107.0; 121.0]	117.5 [111.0; 127.8]		118.5 [108.0; 129.2]	120.5 [116.0; 127.0]		120.5 [115.5; 129.0]	125.5 [116.2; 135.0]	
ΔSysBP, mm Hg	4.0 [−1.0; 9.0]		<0.001	4.0 [−1.0; 9.0]		<0.001	0.5 [−4.0; 8.3]		0.235	4.5 [−1.8; 8.0]		0.024
ΔSysBP, n [%]	3.5 [−0.9; 7.5]			3.7 [−0.8; 7.8]			0.5 [−3.3; 6.9]			4.2 [−1.5; 7.1]		
DiaBP, mm Hg	76.0 [71.0; 81.0]	81.0 [77.0; 86.3]		74.0 [70.0; 79.0]	80.0 [75.0; 84.0]		77.0 [71.0; 81.5]	82.5 [77.0; 89.0]		82.5 [78.0; 87.8]	87.5 [85.0; 92.8]	
ΔDiaBP, mm Hg	5.0 [1.8; 8.0]		<0.001	6.0 [1.3; 8.0]		<0.001	5.5 [2.5; 7.0]		<0.001	4.5 [2.5; 8.0]		<0.001
ΔDiaBP, n [%]	6.9 [1.9; 11.5]			7.2 [1.5; 12.0]			6.9 [2.7; 9.5]			5.6 [2.8; 9.4]		
HR, bpm	69.0 [59.0; 77.0]	62.0 [55.0; 70.0]		69.0 [58.3; 78.0]	62.0 [54.0; 69.8]		67.5 [58.8; 71.0]	59.5 [55.0; 65.0]		72.0 [65.3; 82.5]	65.0 [60.0; 73.5]	
ΔHR, bpm	−6.0 [−12.0; −1.0]		<0.001	−6.0 [−12.0; −1.0]		<0.001	−4.0 [−7.3; −1.0]		0.002	−8.0 [−11.0; −2.5]		0.001
ΔHR, n [%]	−8.3 [−15.6; −1.5]			−9.2 [−16.6; −1.4]			−5.9 [−10.9; −1.9]			−11.1 [−15.2; −3.2]		

Values are presented as median and [IQR]; difference between TN and CE within one group assessed by Wilcoxon signed rank test; p values between groups were assessed by the Kruskal-Wallis test for the two time points (last column). CE, cold exposure; DiaBP, diastolic blood pressure; HR, heart rate; IQR, interquartile range; NW, normal weight (BMI 18.5–24.9 kg/m²); OW, overweight (BMI 25.0–29.9 kg/m²); OB, obese (BMI ≥30.0 kg/m²); SysBP, systolic blood pressure; TN, thermoneutrality.

Fig. 2. a–g Changes in circulating metabolic and hormonal parameters upon CE; Data are presented as median with IQR; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ as assessed by Kruskal-Wallis analysis of variance with Bonferroni correction. HOMA-IR, homeostasis model assessment of insulin resistance; NEFA, nonesterified fatty acids; NW, participants with BMI 18.5–24.9 kg/m² ($n = 115$); OW, participants with BMI 25–29.9 kg/m² ($n = 27$); OB, participants with BMI ≥ 30 kg/m² ($n = 26$); TGs, triglycerides; Δ = CE values – basal values.

Fig. 3. Expression of beiging markers in human scWAT: mRNA expression levels of the beiging markers UCP1, PGC1 α , PRDM16, and CIDEA, measured by qPCR in human scWAT of 17 participants with elevated BMI (5 males and 12 females, BMI 28.0–41.2 kg/m²). Importin 8, peptidyl-prolyl isomerase A, and glyceraldehyde 3-phosphate dehydrogenase mRNA levels served as HKGs for normalization of the data. Values are presented as fold change; * $p < 0.05$ as assessed by Wilcoxon signed rank test. HKGs, housekeeping genes.

and OW groups, insulin levels decreased in the OB group by 2.6 μ U/mL [–44.1 to –7.6%] ($p = 0.031$, Fig. 2b). At baseline, HOMA-IR as a crude measure of insulin resistance calculated from fasting glucose and insulin [23] was significantly higher in the OB group (3.40 [2.17–7.67]) compared to the OW (1.48 [0.66–2.12], $p < 0.001$) and NW groups (1.26 [0.71–1.96], each $p < 0.001$). After CE, HOMA-IR decreased significantly in the OB group by

0.87 [–1.27 to –0.17] ($p = 0.003$), whereas the levels in the NW and OW groups were unchanged ($p = 0.067$ and $p = 0.248$, respectively, Fig. 2e).

We further observed that serum TG significantly increased during CE in the NW ($p < 0.001$) and OW ($p = 0.001$) groups, but not in the OB group ($p = 0.084$, Fig. 2c). Although TG levels at TN and after CE were lower in the NW and OW groups than in the OB group (both $p <$

0.001), the increase in TG levels was significantly lower in the OB group ($p = 0.041$). Likewise, there was a significant increase in plasma NEFA in all groups (all $p < 0.001$). This increase was significantly higher in the NW and OB groups compared to the OW group ($p = 0.024$, Fig. 2d). However, in males there was also a significant difference between the NW and OB groups with men in the NW group having a higher increase compared to those with obesity ($p = 0.020$).

Serum levels of adiponectin significantly increased during CE in all BMI groups (NW $p < 0.001$; OW $p = 0.004$; OB $p = 0.010$), and the increase was comparable across BMI groups ($p = 0.856$, Fig. 2f). In contrast, there was a significant decrease in plasma leptin concentrations after CE in all groups (all $p < 0.001$). This decrease was highest in the OB group compared to the other groups ($p < 0.001$, Fig. 2g). However, considering the relative decrease in leptin levels, no difference was observed between the BMI groups ($p = 0.552$).

Furthermore, we observed a significant increase in serum CRP levels in all BMI groups (all $p < 0.014$), but this increase was comparable across BMI groups ($p = 0.280$). Basal levels and levels of CRP after CE were significantly higher in the OB group compared to the NW and OW groups ($p < 0.001$ each, data not shown).

Relationship between Dietary Intake and Cold-Induced Increase in REE

As dietary habits may influence the response to mild CE, we related total energy intake as well as macronutrient composition over 3 days prior to CE to cold-induced changes in REE. It turned out that there was no significant association of Δ REE and Δ REE (%) with calorie intake ($p = 0.511$, $p = 0.329$), nor percent fat intake ($p = 0.456$, $p = 0.323$), nor percent carbohydrate intake ($p = 0.914$, $p = 0.970$), nor percent protein intake ($p = 0.061$, $p = 0.051$).

mRNA Expression of scWAT

To evaluate the impact of CE on beiging in WAT, we performed a marker gene expression analysis by qPCR in an exploratory approach. Beiging marker expression levels in paired scWAT samples from 17 obese participants revealed no significant changes between baseline and after CE for UCP1, PRDM16, and PGC1 α (all $p > 0.962$), as demonstrated in Figure 3. However, there was a significant increase in cell death activator (CIDEA) mRNA in females ($p = 0.034$), whereas no significant change was seen in the much smaller subgroup of males ($p = 0.313$).

Discussion

This study addressed differences in the thermogenic and metabolic response to short-term mild CE across three BMI classes. We observed significant differences in changes of REE upon a 2-h CE between the BMI groups. While participants with normal weight showed a significant increase in REE by 6.5%, participants with overweight or obesity did not exhibit a significant increase in REE after CE. Linear regression analysis revealed an inverse association between BMI and Δ REE.

To date, reports on this topic from other research groups were conflicting [5, 17]. These studies were carried out under less strict conditions and with much smaller sample sizes. In contrast, our large cohort of 173 adult participants undergoing a highly standardized and individualized cooling protocol after an overnight fast has a high potential to elucidate the relationship between BMI and changes in REE upon CE. However, due to the cross-sectional design of the study, it remains unclear if the impaired thermogenic response to cold is a cause or consequence of obesity.

It is noteworthy that there was high interindividual variability in the thermogenic response to CE across the three BMI categories and it is likewise unclear which factors are driving this variability. Additionally, there was also a high variability in the metabolic changes among individuals of all BMI groups. This phenomenon is usually poorly recognized but may point to a general high interindividual variability in metabolic functions. Such variability is also observed after defined challenges as reported by our group in a previous study performed on healthy men [25].

It is interesting to note that TGs increased to a higher magnitude in participants with normal weight compared to the OW and OB groups. An overall increase in TGs and NEFAs upon CE was also described by others [26–28]. It is known that BAT not only preferentially oxidizes intracellular fatty acids but can also metabolize other substrates such as glucose [29]. This may explain why we saw a decrease in plasma glucose concentrations in all groups, in agreement with previous observations on the effect of CE on glucose uptake into BAT [30, 31].

Insulin levels decreased significantly in the OB group, but not in the NW and OW groups after CE. Likewise, HOMA-IR significantly improved supporting the concept that CE might be beneficial for insulin sensitivity and whole-body glucose homeostasis, particularly in insulin-resistant persons [14, 30]. Along this line, we recently showed that mild CE induces Tregs in WAT, suggesting

a local immunosuppressive and anti-inflammatory action [32].

Skin temperatures were slightly lower in participants with overweight and obesity at TN and after CE compared to participants with normal weight. Despite this difference, the decrease in skin temperature was comparable across groups which is in accordance with the published literature [5, 33]. A possible explanation is that a higher body FM may lead to better insulation, resulting in lower skin temperature of participants with obesity [34]. This improved insulation may be beneficial for maintaining a stable core temperature and may shift the thermoneutral zone to lower skin temperatures [35]. This phenomenon was also observed in our study. Thus, individuals with obesity may not need to activate their metabolism in order to defend core temperature as readily as persons with normal weight [36]. However, cold perception was similar across BMI categories in our study which resulted in comparable shivering and, thus, exposure temperatures. This indicates that individuals with normal weight increase their REE at mild cold mainly via NST and subjects with a higher BMI have impaired NST leading to a similar shivering threshold even though their thermoneutral zone is at lower temperatures. Tsupra, measured over the location with a residual BAT depot, was significantly lower in the OB group which may also be explained by lower supraclavicular BAT activity. Superficial skin temperature in the supraclavicular area was reported to be associated with BAT activity in women [37].

We were also interested to investigate whether CE may lead to changes in the expression of browning markers in biopsies from scWAT. However, this could only be done in a subgroup of volunteers with overweight and obesity. Among the selected markers, CIDEA mRNA levels increased after CE in both male and female participants. However, this upregulation reached statistical significance only in the female participants. CIDEA codes for a lipid droplet-associated protein, which has been reported to correlate with insulin sensitivity and lipid disorders in persons with obesity [38]. CIDEA protein has also been shown to regulate UCP1 expression in human adipocytes and as a result is known to coregulate browning, although there was no significant change in UCP1 expression upon CE. A possible explanation is that the short-term duration of CE may have not allowed to elicit significant changes in UCP1 mRNA expression.

In our study, samples of scWAT were taken immediately after each REE measurement. In a recent study, fat biopsies were collected 4 h after CE [39]. In the same study,

scWAT samples were taken in summer and winter, but there was an increase in UCP1 and PGC1 α expression after CE only in the samples collected during summer. The authors postulated that mRNA expression of these markers is already high in winter due to natural CE and that additional CE does not lead to a further increase. Additionally, they saw no effect of CE in participants with a BMI of over 30 kg/m², regardless of season [39]. As our biosamples were collected only during the winter months and only from individuals with obesity, this could explain why we were unable to see changes in the expression of those genes. In contrast to another group, we could not detect higher basal levels of UCP1 mRNA expression in females compared to males [40]. However, despite having comparable basal values of CIDEA mRNA expression in men and women, which is in line with another study [41], the observed increase in CIDEA mRNA expression in women upon CE may also indicate a sex-specific difference in browning of scWAT. This finding is consistent with previous reports suggesting that females have a higher browning capacity [42, 43]. However, our recent analysis of potential sex differences in thermogenic response to cold did not provide sufficient evidence for a sex difference [20].

Strengths and Limitations

The study population was rather young and healthy and was of Caucasian origin eliminating age, ethnicity, and disease as potential confounders. Therefore, the results might not be valid for the general population and specific population groups such as other ethnicities or elderly. Furthermore, the subgroup of participants with obesity consisted largely of individuals with grade 1 obesity, so we cannot draw conclusions about individuals with more severe obesity.

The increase in REE upon CE as described here may be largely due to an increase in NST, but we cannot exclude shivering of skeletal muscle contributing to this increase in energy expenditure. Therefore, an individualized cooling protocol was applied to assess the onset of shivering. Participants were also asked to report any signs of involuntary muscle contractions. By increasing the temperature at the slightest sign of shivering, we are convinced that muscle shivering was minimized. We did not apply surface electromyography, as only surface muscle shivering can be ruled out by this method, but not shivering of deep muscles. Participants who showed signs of shivering during the second REE measurement were excluded from the analysis. Furthermore, we did not directly measure BAT activity, therefore, our results might not reflect true cold-induced BAT activation.

The capacity for NST is subject to intraindividual variation [33]. Participants in our setting were only exposed to cold on a single study day. During the establishment of the protocol, we exposed 3 volunteers 3 times to the same CE and found a coefficient variation under 10% (data not shown). Together with the rigorous application of the protocol and the large number of study participants, we are rather confident that the decrease in NST upon CE with increasing BMI is rather robust. However, there is a possibility that we could not utilize the full capacity of cold-induced thermogenesis under fasting conditions and we cannot exclude a different picture under feeding.

Due to the limited amount of biopsy material we had to restrict our analysis of changes in gene expression. Further studies should address the expression of other genes that are important for non-UCP futile cycling or insulin signaling.

In conclusion, cold-induced thermogenesis, assessed by the increase in REE upon defined CE, was found to be impaired in individuals with overweight and obesity. If this impairment is caused by a higher FM or if a higher BMI is due to impaired thermogenesis cannot be answered due to the cross-sectional design of the study. The results also suggest that CE improves insulin sensitivity in participants with obesity underpinning the beneficial effects of CE on metabolism. Further research is warranted to explore the long-term effects of cold-induced thermogenesis on body weight and metabolism.

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Statement of Ethics

This study protocol was reviewed and approved the Ethics Commission of the School of Medicine of the Technical University of Munich (#236/16S).

Each volunteer gave their written informed consent.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Hans Hauner and Melina Claussnitzer conceived and designed the study. Laura Aline Mengel, Bahareh Nemati Moud, Hatti Seidl, Alberto Mesas-Fernández, and Claudine Seeliger performed the experiments. Laura Aline Mengel analyzed data and prepared the manuscript. Christina Holzapfel, Beate Brandl, and Thomas Skurk assisted with experimental design, recruitment of volunteers, and data interpretation. All authors were involved in the final revision of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary Files. Further enquiries can be directed to the corresponding author.

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