Non-invasive assessment of human brown adipose tissue: development of robust imaging methods to facilitate clinical translation

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Chapter 2

Association of shivering threshold time with body composition and brown adipose tissue in young adults

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ABSTRACT

Purpose: Brown adipose tissue (BAT) increases metabolic heat production in response to cold exposure. Body size and composition are involved in the human cold response, yet the influence of BAT herein have not fully been explored. Here, we aimed to study the association of the cold-induced shivering threshold time with body composition, BAT, the perception of shivering and skin temperature in young adults.

Methods: 110 young healthy adults (81 females; age=21.7±2.1 years, BMI=24.2±4.3 kg/m^2) underwent 2 hours of individualized cooling, followed by the quantification of BAT using a ^{18}F-fluorodeoxyglucose ([^{18}F]FDG) positron emission tomography-computed tomography (PET-CT) scan. Body mass index (BMI), lean mass, fat mass and body surface area (BSA) were also measured. Shivering threshold time was defined as the time until shivering occurred using an individualized cooling protocol.

Results: The shivering threshold time was on average 116.1 minutes for males and 125.8 minutes for females, and was positively associated to BMI (β=3.106; R2=0.141; p=0.001), lean mass (β=2.295; R2=0.128; p=0.001) and fat mass (β=1.492; R2=0.121; p=0.001) in females, but not in males (all p≥0.409). The shivering threshold time was positively associated with BSA in males (p=0.047) and females (p=0.001), but it was not associated with BAT volume or [18F]FDG uptake nor with the perception of shivering and skin temperature perception in both sexes.

Conclusion: The shivering threshold time is positively associated with whole-body adiposity and lean mass in females, but not in males. The shivering threshold time was positively associated with BSA, but no association was observed with BAT nor with the perception of shivering or skin temperature. Future research should consider the influence of body composition when applying cooling protocols among individuals with different phenotypical features.

2.1 INTRODUCTION

Since the discovery of metabolically activated brown adipose tissue (BAT) in human adults[1–3], many studies have addressed the physiological implication of this tissue in energy metabolism and cardiovascular health[4]. BAT has been identified as a thermogenic tissue due to its ability to dissipate energy as heat via uncoupling protein 1 (UCP-1), a process that mainly utilizes intracellular triglyceride-derived fatty acids as an energy source. A well-established physiological BAT activator is cold exposure[5,6]. To maintain core body temperature during cooling, heat production will increase, a process that is known as cold-induced thermogenesis (CIT). CIT is divided into shivering and non-shivering thermogenesis (NST)[7,8], shivering thermogenesis is defined as the increase in heat production due to skeletal muscle contraction, whereas NST is defined as heat production that does not involve contractions of skeletal muscles, such as BAT activation[9]. However, recent studies[10–12] demonstrate that skeletal muscles may contribute to NST via futile cycles, which is a mechanism that generates heat independently of skeletal muscle contractions.

The most common technique to assess human BAT activity is by quantifying the uptake of a radioactively-labeled glucose tracer, ^{18}F-fluorodeoxyglucose ([^{18}F]FDG), after intravenous injection, by using a static positron emission tomography/computed tomography (PET-CT)[13] scan. To ensure BAT activation, a variety of cooling strategies have been described[14]. These strategies differ in terms of duration, intensity, type of the cold stimulus (e.g., air-conditioned room, ice-blocks, water-perfused vests) or cooling strategy (i.e., standardized or individualized)[13]. In standardized cooling protocols, participants are exposed to a predetermined temperature for a certain duration, whereas in individualized cooling protocols the temperature is tailored to certain participant responses, such as heat production, skin temperature, perception of skin temperature, or the estimated onset of shivering[15]. Individualized cooling protocols based on the shivering threshold were developed to maximally stimulate NST in order to avoid an underestimation of BAT activity[16].

Body composition refers to the amount of relative fat to muscle that is present in the body, which seems to be involved in the individualization of a cooling protocol. For instance, large individuals that are commonly characterized by a small body surface area to volume ratio can preserve heat better compared to smaller individuals[17]. Differences in body size may, therefore alter the cold tolerance, defined as the ability to withstand low temperatures[18]. Additionally, the cold tolerance may increase with the abundance of subcutaneous white adipose tissue (WAT)[19,20] and lean mass[21]. Thus, larger individuals may have a higher cold tolerance capacity, although this response is quite variable between individuals[6]. Moreover, it has been shown that 10-days of cold acclimation in overweight individuals[22,23] increases BAT volume and BAT [^{18}F]FDG uptake. Curiously, the authors also

found that cold perception of skin temperature decreases after 10-days of cold acclimation, although subcutaneous WAT and lean mass were not altered. These findings suggest that human BAT may be involved in the cold tolerance capacity of an individual. Therefore, we hypothesize that individuals with a higher cold tolerance capacity have different body composition characteristics (i.e., a higher lean mass and/or fat mass), and higher BAT volume and activity compared to individuals with a lower cold tolerance capacity.

The aim of the present study was to investigate whether the shivering threshold time, as a proxy of the cold tolerance capacity, is associated with body composition (*i.e.,* body mass index, body surface area, lean mass and fat mass), BAT volume, [^{18}F]FDG uptake, mean radiodensity (i.e., as a proxy of BAT fat content)[24], and with the perception of shivering and skin temperature during cold exposure, in young adults.

2.2 MATERIAL AND METHODS

2.2.1 Study subjects, experimental procedures, and ethics statement

Participants that were initially included in the present study (n=147) were enrolled in the ACTIBATE study[25] (ClinicalTrials.gov, ID: NCT02365129). The study was conducted in accordance with the Declaration of Helsinki (revised in 2013), and approved by the Human Research Ethics Committee of the University of Granada (n° 924) and of the "Servicio Andaluz de Salud" (Centro de Granada, CEI-Granada). All participants were healthy, non-smokers, non-vigorous exercisers, and did not use any medication that could affect their energetic or neuromuscular responses to cold exposure. Before the start of the study visits, participants were instructed to refrain from vigorous exercise (during 24 hours before the visit), and from alcohol and stimulant beverages (during 6 hours before the visit).

Participants were asked to come to the research center after a 8 hours fasting period. During the first visit, the shivering threshold test was conducted to determine the water temperature of the cooling vest at the onset of shivering (shivering threshold temperature). During the second visit, a 2-hour individualized cooling protocol was conducted using the pre-determined shivering threshold temperature. After 2-hours of cooling, a static [^{18}F]FDG-PET-CT scan was acquired. The time between the study visits was 48-72 hours. In a third visit, body composition was measured. The procedures per visit are outlined below and are described in detail elsewhere[26]. The measurements were conducted during four time periods of approximately 12 participants in each period, from October 15th to November 28th, 2015 and 2016 in Granada (Spain).

2.2.2 Body composition and anthropometrics

Body composition (i.e., lean and fat mass) was assessed on a separate day by Dual Energy X-ray Absorptiometry (DEXA; HOLOGIC, Discovery Wi). Body weight and height were measured using a SECA scale and stadiometer (model 799, Electronic Column Scale, Hamburg, Germany), during which participants were wearing a T-shirt and shorts without shoes on. Lean mass (LM) and fat mass (FM) values were obtained from the DEXA software. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2). The body surface area (BSA) was determined according to the du Bois formula[27], in which the height is expressed in centimeters and the weight in kilograms:

$$BSA = 0.007184 * Height^{0.725} * Weight^{0.452} \quad [3]$$

2.2.3 Determination of the shivering threshold

At first, participants changed into standardized clothes [sandals, T-shirt and shorts, clo-value: 0.20[28]), and entered a warm room (22.1±1.6°C) for approximately 30 minutes. Subsequently, they entered an air-conditioned room (19.8±0.5°C) and sat down in a chair in the same position. After 15 minutes, participants were asked to wear a temperature-controlled water perfused cooling vest (Polar Products Inc., Ohio, USA). The water temperature was initially set at 16.6°C, and decreased every 10 minutes until the temperature reached 5.5°C. Participants were asked to self-report the occurrence of shivering, and one researcher visually monitored whether shivering occurred throughout the experiment. If participants did not report shivering and the researcher did not observe it either, the water temperature was decreased by 0.6°C every 15 minutes until 3.8°C. If shivering had not occurred, participants remained exposed to 3.8°C for another 45 minutes, after which the test was finished. The shivering threshold time was determined as the total time period, during which participants were exposed to cold until shivering occurred.

2.2.4 Perception of shivering and skin temperature

Perception of shivering was measured by a numeric rate scale of 10 points, where 0 represented “I am not shivering” and 10 represented “I am shivering a lot”. Additionally, the perception of skin temperature was measured using a seven-point scale from the American Society of Heating, Refrigerating and Air Conditioning Engineers (ASHRAE)[29]. This scale ranges from cold (-3), cool (-2), slightly cool (-1), neutral (0), slightly warm (1), warm (2) to hot (3). Participants were asked to score their perception of skin temperature for several anatomical areas, including the clavicular area, abdomen, arms, hands, legs, feet and whole-body areas. All scales were measured throughout the entire study, but only the measurements in the warm period and at end of the shivering threshold test were used in the analyses[30].

2.2.5 Individualized cooling protocol prior to the [^{18}F]FDG-PET-CT acquisition

Upon arrival, all participants confirmed that they followed all pre-study instructions. Similar to the first visit, participants changed into standardized clothes [sandals, T-shirt and shorts, clo-value: 0.20[28]), and entered a warm room for 30 min (22.2±0.5°C) before they entered an air-conditioned room (20.2±0.3°C). Participants were then asked to wear the temperature-controlled water perfused cooling vest (Polar Products Inc., Ohio, USA) for 60 minutes. The water temperature was set at ~4°C above the water temperature that caused the onset of shivering during the first visit. The water temperature was set at 3.8°C for those participants who did not report shivering during the shivering threshold test [14,31]. During 60 minutes of individualized cooling, participants were asked to report whether they experienced shivering. In that case, the water temperature was increased by 1°C and participants wore a bathrobe until shivering subsided. After 60 minutes of cold exposure, [^{18}F]FDG was administered intravenously (185 MBq; ~2.78 MBq/kg), and the water temperature was increased by 1°C. This temperature was kept stable for the remaining 60 minutes of cooling. After two hours of individualized cooling, a [^{18}F]FDG PET-CT scan (Siemens Biograph 16 PET/CT, Siemens, Germany) was acquired. A CT scan was used for anatomical localization with an attenuation correction at a peak voltage of 120, and for the PET acquisition, a scan time of six minutes per bed position was implemented. Two bed positions were scanned ranging from the *cerebellum* to thoracic vertebrae 4 to 6, depending on the size of the person. The date of the PET/CT scan was annotated, as January 1st, day 1, and December 31st, day 365/366. The date (as a discrete number) of the PET/CT acquisition was used as a proxy of the outdoor temperature.

2.2.6 PET-CT data analysis

PET-CT scans were analyzed using Beth Israel plugin for FIJI[1] software (http://sourceforge.net/projects/bifijiplugins/)[32]. The standard uptake value (SUV) and the SUV threshold (SUV_{thres}) were calculated as follows[13]:

$$SUV = \frac{[18_F]\text{FDG uptake (kBq/mL)}}{\text{injected dose [kBq]/patient weight [g]}} \quad [1]$$

$$SUV_{thres}{:}SUV \geq \frac{1.2}{\text{lean body mass/body mass}} \quad [2]$$

A fixed segmentation range of [-190, -10] Hounsfield units was applied[13]. According to the pre-determined SUV and HU thresholds, regions of interest (ROIs) were outlined from the *atlas vertebrae* (Cervical 1) to the *thoracic vertebrae* 4. BAT volume and [^{18}F]FDG uptake outcomes (SUVmean and SUVpeak) were determined based on the BARCIST 1.0 recommendations[13,33]. As a measure for BAT metabolic activity, we multiplied BAT SUVmean by BAT volume. BAT mean radiodensity was calculated as the average radiodensity of those voxels meeting the aforementioned criteria in a single ROI from the atlas to thoracic ver-

tebrae 4, excluding the mouth. Twenty-five individuals presented several voxels classified as BAT which were outside these anatomical areas, and these participants were therefore excluded from the BAT mean radiodensity analyses. The [^{18}F]FDG uptake in skeletal muscle was retrieved from a single slice ROI placed in several skeletal muscles i.e., *cervical, scalene, longus colli, paravertebral, subscapular, sternocleidomastoid, supraspinous, trapezius, deltoid, pectoralis major, and triceps braquii*), which were averaged[8].

2.2.7 Statistical analysis

Data normality was confirmed using the Shapiro-Wilk test, visual histograms, and Q-Q plots. Unpaired t-tests were performed to compare participant characteristics between males and females, and between participants who reported shivering *vs.* who did not report shivering. For the main analysis, linear regression analyses were performed to study the association of the shivering threshold time with i) body composition (i.e. BMI, BSA, lean mass and fat mass) and ii) BAT volume, [^{18}F]FDG uptake (SUVmean and SUVpeak), and BAT mean radiodensity. These linear regression analyses were repeated with the date of the PET/CT acquisition as a confounder. Data were analyzed for males and females separately. In addition, the cohort was divided in tertiles based on the shivering threshold time for males and females [males: low (from 52 to 114 min)], medium (from 118 to 151 min) and high tertiles (from 153 to 175 min); and females: low (from 53 to 92 min), medium (from 93 to 120 min) and high tertiles (from 121 to 205 min)]. Based on these tertiles, one-way analyses of variance (ANOVA) were performed, where the perception of shivering and skin temperature were compared across the different groups. We repeated these ANOVAs adjusting for potential confounders, including BMI, BSA or the date of the PET/CT acquisition. All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences; version 22) and the level of significance was set to <0.05. All figures were made using GraphPad Prism (version 8.0.0).

2.3 RESULTS

2.3.1 Participant characteristics

A total of 37 participants were not included in the main analysis: five participants because the [^{18}F]FDG tracer was not correctly injected, seven participants due to technical problems with the cooling device, and 25 individuals were included in a secondary analysis because no shivering was visually detected by the researcher. The 25 individuals that did not shiver showed a higher whole-body adiposity and BAT volume[34], and reported a lower perception of shivering and skin temperature than individuals who shivered (n=110; all $p \leq 0.034$, **Table S1**). **Table 1** shows the characteristics of participants who reached the shivering threshold and were included in the main analyses. Males had a higher whole-body adiposity than females (all $p \leq 0.004$), but the shivering threshold time and BAT were

similar between sexes (all p≥0.05, **Table 1**). However, significant sex interaction effects were detected in many of the associations, and thus the results are separately presented for males (n=29) and females (n=81).

Table 1. Characteristics of participants.

	All (n=110)		**Male (n=29)**		**Female (n=81)**		**P**
Age (years)	21.7	(2.1)	22.0	(2.1)	21.7	(2.1)	0.521
Weight (kg)	68.1	(14.7)	80.5	(15.6)	63.7	(11.7)	**<0.001**
Height (m)	1.67	(8.2)	174.8	(6.7)	164.0	(6.6)	**<0.001**
Body mass index (kg/m^2)	24.4	(4.3)	26.3	(5.0)	23.6	(3.9)	**0.004**
Lean mass (kg)	40.1	(8.7)	51.5	(6.5)	35.8	(5.0)	**<0.001**
Fat mass (kg)	24.2	(8.1)	24.3	(9.9)	24.2	(7.5)	0.945
Body surface area (m^2)	1.7	(0.2)	1.9	(0.2)	1.7	(0.2)	**<0.001**
Visceral adipose tissue mass (kg)	0.31	(0.17)	0.41	(0.17)	0.28	(0.16)	**<0.001**
Shivering threshold time (min)	116	(34)	126	(36)	113	(32)	0.072
BAT volume (ml)	64	(54)	69	(61)	62	(51)	0.542
BAT metabolic activity (g)	316	(327)	289	(329)	326	(327)	0.775
BAT SUVmean	3.7	(1.9)	3.2	(1.4)	3.9	(2.1)	0.064
BAT SUVpeak	10.8	(7.8)	9.1	(7.3)	11.4	(8.0)	0.171
BAT mean radiodensity (HU)*	-58.5	(13.3)	-55.7	(9.9)	-59.5	(14.3)	0.200

All data are presented as mean and standard deviation (SD). P values obtained from student t-test. *109 participants had valid data on this outcome (28 males and 65 females). BAT: brown adipose tissue; HU: Hounsfield units. SUV: standardized uptake value.

2.3.2 Association between the shivering threshold time with body composition

There were positive and significant associations between the shivering threshold time and BMI (β=3.106; R^2=0.141; p=0.001; **Fig. 1B**), lean mass (β=2.295; R^2=0.128; p=0.001; **Fig. 1D**) and fat mass (β=1.492; R^2=0.121; P=0.001; **Fig. 1F**) in females, but not in males (all p≥0.409, **Fig. 1A-C**). The shivering threshold time was positively associated with BSA in both sexes (males: β=73.056; R^2=0.138; p=0.047, **Fig. 1G**; and females: β=70.519; R^2=0.122; p=0.001, **Fig. 1H**).

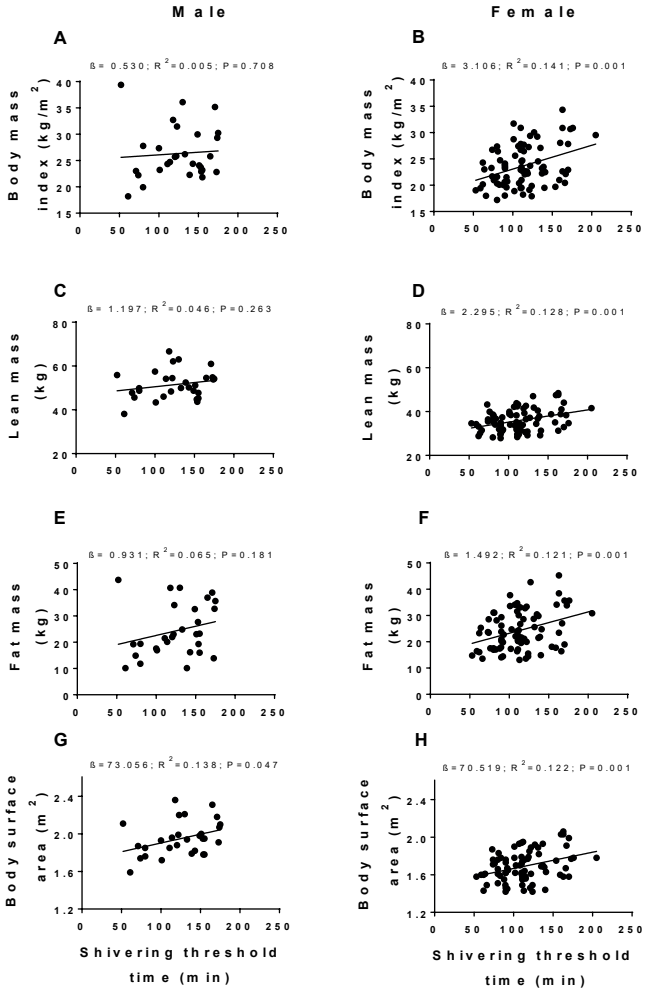

Figure 1. Associations between the shivering threshold time (minutes) and body composition: body mass index (**A** and **B**), lean mass (**C** and **D**), fat mass (**E** and **F**), and body surface area (**G** and **H**) by sex. Beta values, adjusted R squared (R^2) and P-values are shown.

The associations persisted when the date of the PET/CT acquisition was included as a confounder (data not shown). No associations were found between the shivering threshold time and BAT volume (**Fig. 2A** and **B**), BAT SUVmean (**Fig. 2C** and **D**), BAT SUVpeak (**Fig. 2E** and **F**), BAT mean radiodensity (**Fig. 2G** and **H**) or BAT metabolic activity (**Fig. S1A** and **B**) in both sexes (all p>0.05). This lack of significant associations between the shivering threshold time and BAT-related outcomes persisted when the date of the PET/CT acquisition was included as a confounder (data not shown). We repeated all the analyses including the participants who did not report shivering (n=25) and the linear regressions remained unaltered (data not shown).

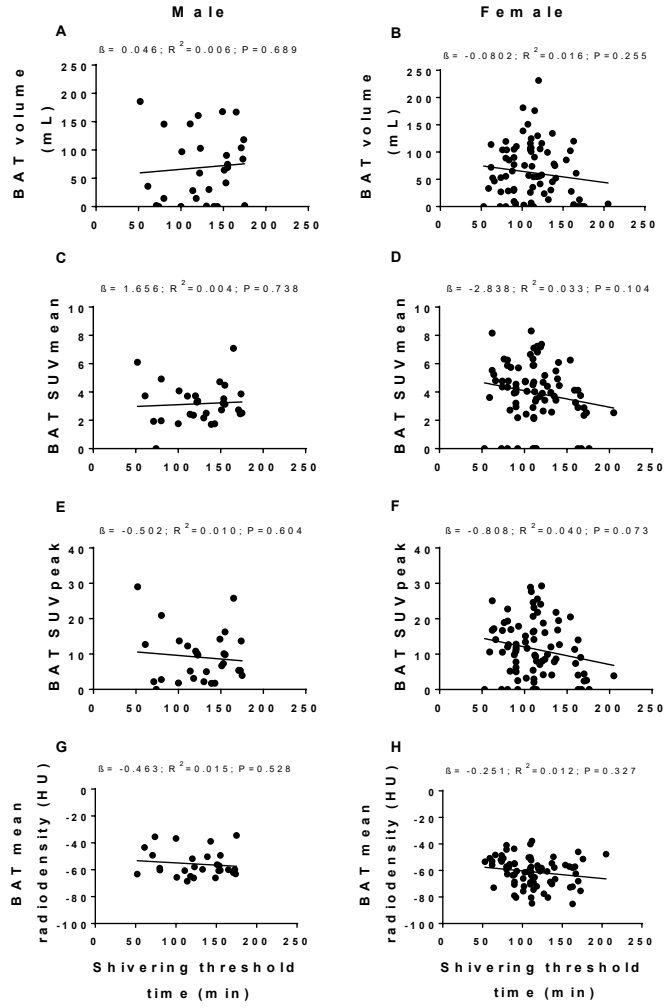

Figure 2. Associations between the shivering threshold time (minutes) and brown adipose tissue (BAT) related outcomes. BAT volume [ml] (**A** and **B**), BAT standardized uptake value (SUV) mean (**C** and **D**), BAT SUVpeak **(E** and **F**), and BAT mean radiodensity (**G** and **H**) by sex. 28 male and 65 female had valid data on BAT mean radiodensity. Beta values, adjusted R squared (R^2) and P-values are shown. HU: Hounsfield units.

2.3.3 Association between the shivering threshold time with the perception of shivering and skin temperature

Subsequently, we determined the tertiles in terms of the shivering threshold time (i.e., low, medium and high) for males and females separately. Similar perceptions of shivering at the end of cold exposure were reported by males and females (all p>0.05, **Fig. 3A** and **B**) across tertiles of the shivering threshold time. Males also reported a similar perception of skin temperature of the clavicular area (**Fig. 3C**); however, females in the highest tertile (i.e., longest shivering threshold time) reported a colder perception on skin temperature

of the clavicular area than females in the lowest tertile (average perception on skin temperature of clavicular area ± standard deviation: -2.4±0.7 points *vs.* -1.8±1.2 points; p=0.035, **Fig. 3D**). For other anatomical areas, no significant differences were observed across tertiles for both sexes (**Table S2**). We repeated all these analyses adjusting for BMI, BSA, and date of the PET/CT acquisition, and the results were unaltered (all p>0.05; data not shown).

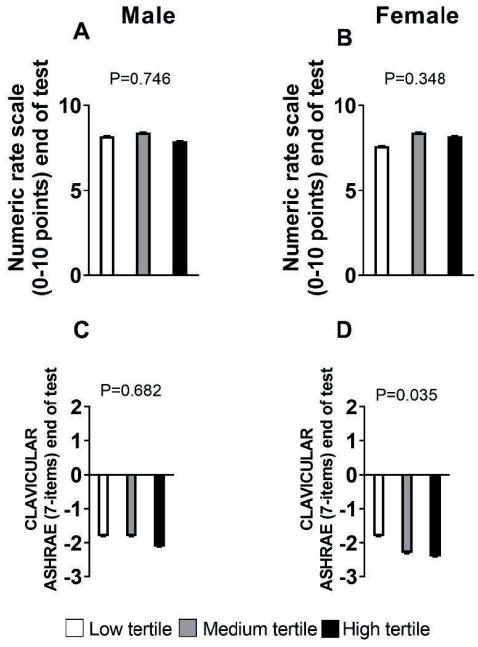

Figure 3. The perception of shivering and skin temperature in the clavicular area in males (A, C) and females (B, D). The perception of shivering was measured by a numeric rate scale, where 0 represented "I am not shivering" and 10 represented to "I am shivering a lot". Perception of skin temperature at clavicular area was measured by an ASHRAE scale that ranges from cold (-3), cool (-2), slightly cool (-1), neutral (0), slightly warm (1), warm (2) and hot (3). Data are divided by tertiles of shivering threshold time by males (i.e., 52-114, 118-151 and 153-175 minutes) and females (i.e., 53-92, 93-120 and 121-205 minutes) separately.

2.4 DISCUSSION

The present study shows that the shivering threshold time is positively related to whole-body adiposity and lean mass in females, but not in males. In both sexes, the shivering threshold time is positively associated with BSA, but it is not associated with BAT parameters nor with the perception of shivering and skin temperature in young adults. Thus, these findings suggest that the cold tolerance capacity might not be related to BAT activation in humans.

2.4.1 Role of body composition and brown adipose tissue in the shivering threshold time

Our data shows that the shivering threshold time is weakly related to both fat and lean mass in females (LM: β=2.295; R^2=0.128; p=0.001; FM: β=1.492; R^2=0.121; p=0.001), but not in males. This may be due to differences in body composition between both genders (**Table 1**). For instance, men had a higher average BMI than women (26.3 kg/m^2 vs. 23.6 kg/m^2; **Table 1**), with adipose tissue stored mainly in the trunk/abdominal area vs. hip/thigh area in females[35]. In the present study, we used a water-perfused cooling vest, which only covered the trunk and the abdomen. Thus, it might be possible that this specific protocol locally cooled several areas at which adipose tissue is more abundant in men than in women, which may have induced different physiological responses. Albeit not significant, females tended to shiver faster compared to males (p=0.072), which is in agreement with previous results[36]. This may be due to a better insulation in male participants considering that the BMI was higher in males than in females. Future studies using air-conditioning cooling protocols, or whole-body water-perfused cooling mattresses/suits, might be of interest to investigate the role of body composition in the regulation of the cold tolerance capacity in men versus women. Interestingly, the shivering threshold time was positively related to BSA, which is a well-known determinant of heat loss[37]. This finding concurs with studies showing that larger male individuals have an increased cold tolerance capacity[19,21]. In addition, it has been shown that total body weight and BSA were the main factors that explained sex differences in the thermogenic response during cold exposure[38].

Contrary to our hypothesis, we found no association between the shivering threshold time and BAT volume, [^{18}F]FDG uptake or BAT mean radiodensity in young adults, which suggests that BAT activity might not influence the cold tolerance. Interestingly, Van Der Lans et al. [39] reported that the shivering threshold time did not change after 10-days of cold exposure in young and lean individuals[39]. However, they observed that BAT volume and [^{18}F]FDG uptake substantially increased after the intervention, which was accompanied by a decrease in the perception of shivering. It is biologically plausible that the cold-induced recruitment of BAT may have contributed to the decrease in the perception of shivering, thereby improving cold tolerance capacity, although this finding was not replicated in another cohort of individuals with overweight[40]. In another study[15], it has been shown that shivering intensity was substantially reduced, while NST increased in healthy males after 7-days of cold exposure. It was suggested that these effects might be mediated by an enhancement in skeletal muscle function rather than an improvement in BAT function. In our study, we were not able to objectively quantify shivering. Therefore, we cannot distinguish between NST and shivering thermogenesis. Based on previous studies[10–12], we believe that skeletal muscles may be involved in the early stages of NST, but in this study, we cannot make any assumption on the contribution of thermogenic tissues during the

process of NST and shivering. Therefore, future studies are needed to gain further insight into the interplay between cold tolerance capacity, BAT and skeletal muscle activity.

2.4.2 The shivering threshold time does not relate to the perception of shivering and skin temperature for individuals who reported shivering

The shivering threshold time was not related to the perception of shivering and skin temperature. This might be explained by various factors: First, the range of the ASHRAE thermal perception scale may be too limited, as it only ranges from 0 to -3 points (for cooling), which could result in low sensitivity to detect whether participants perceived colder skin temperatures (below -3 points). Second, there could be a delay in processing of thermal perception and the onset of shivering as these physiological processes may be independently regulated. Shivering thermogenesis is regulated in the preoptic area within the hypothalamus, whereas afferent signals related to thermal perception are received by the thalamus and relayed onto the somatosensory cortex[41], and these might be independently regulated. Third, there was a subgroup of participants for whom shivering was not visually detected by the researcher (n=25; **Table S1**), and they self-reported a lower perception of shivering and skin temperature, even though they were exposed to cold for a substantially longer period (+1 h, **Table S1**) than individuals who reported shivering (n=110). Interestingly, the participants who did not shiver had a higher BSA, BMI, fat mass and lean mass, which suggests that they might have a higher cold tolerance capacity due to better insulation traits compared to the group that reported shivering. However, although this group had a higher BAT volume, they showed a similar BAT [^{18}F]FDG uptake compared to individuals who did report shivering, suggesting no differences in metabolic activity of BAT. Collectively, these hypotheses need to be further investigated to fully understand the possible mechanisms that are driving the two different phenotypes.

2.4.3 Limitations

A limitation of the present study is that no causality can be established, which however is inherent to all cross-sectional studies. The study population only included young and relatively healthy adults, which does not allow for extrapolation of results to populations of other age or health status. Additionally, there was a substantial sample size difference between male and female participants, and therefore, the results were not corrected for the False Discovery Rate. Another limitation is that we did not measure steroid hormones in this cohort, which hampers the possibility to perform further analyses investigating the role of sex in these associations. Moreover, a main limitation of the study is that the shivering threshold time was determined visually by the researcher and self-reported by participants; thus, no objective measurements were used. As such, visual inspection of shivering and self-reported shivering do not allow for the detection of invisible micro-shivering, which is defined as the thermoregulatory tonus prior to shivering[42]. Future studies should, therefore, use more objective measurements of shivering to confirm or

refute the present findings. Finally, although the static [^{18}F]FDG-PET/CT scan is commonly used for assessing BAT volume *in vivo*, it has some limitations. For example, it does not allow the observation of small brown fat deposits within white adipose tissue. Consequently, this study should be replicated if a new technology for BAT volume assessment *in vivo* becomes available. Alternatively, BAT detection can also be improved by using a multi-modality approach, for example by combining [^{18}F]FDG-PET/CT, magnetic resonance imaging and infrared thermography[43].

In conclusion, the shivering threshold time is positively, but weakly, related to whole-body adiposity and lean mass in females, but not in males. In addition, in both sexes, the shivering threshold time was positively related to BSA, but neither with BAT-related outcomes nor with perception of shivering and skin temperature. Future research could further explore the relation between the cold tolerance capacity and BAT by using objective study settings and a multi-modality approach to increase the sensitivity for BAT detection. Additionally, future studies should consider the potential influences of body composition more carefully when applying cooling protocols to heterogenous study populations.

SUPPLEMENTARY FIGURES

Table S1. Characteristics of participants who reported and did not report shivering

	Reporting shivering (n=110)		Not reporting shivering (n=25)		P
Age (years)	21.7	(2.1)	22.9	(2.1)	0.016
Sex (n; % male)	n=29;	26%	n=16;	64%	**<0.001**
Weight (kg)	68.1	(14.8)	84.7	(20.5)	**<0.001**
Height (m)	166.9	(8.2)	174.8	(7.7)	**<0.001**
Body mass index (kg/m^2)	24.4	(4.3)	27.5	(5.6)	**0.002**
Lean mass (kg)	40.1	(8.7)	49.9	(10.7)	**<0.001**
Fat mass (kg)	24.2	(8.1)	29.9	(11.7)	**0.005**
Body surface area (m^2)	1.7	(0.2)	2.0	(0.2)	**<0.001**
Visceral adipose tissue mass (g)	313.5	(169.8)	448.2	(206.6)	**0.001**
Shivering threshold time (min)	116.1	(33.6)	173.4	(20.2)	**<0.001**
BAT volume (ml)	64.0	(53.7)	99.0	(71.6)	**0.007**
BAT metabolic activity (g)	316	(327)	486	(363)	0.340
BAT SUVmean	3.7	(1.9)	4.1	(2.0)	0.389
BAT SUVpeak	10.8	(7.8)	14.1	(10.0)	0.078
BAT mean radiodensity (HU)*	-58.5	(13.3)	-56.5	(13.3)	0.489
Perception of shivering	8.1	(1.9)	5.8	(2.2)	**<0.001**
Cold perception on the clavicular	-2.1	(0.9)	-1.7	(0.9)	0.074
Cold perception on the abdomen	-2.5	(0.8)	-2.1	(0.9)	**0.008**
Cold perception on the arms	-2.7	(0.5)	-2.2	(0.7)	**<0.001**
Cold perception on the hands	-2.8	(0.5)	-2.2	(0.9)	**<0.001**
Cold perception on the legs	-2.6	(0.6)	-2.3	(0.8)	**0.034**
Cold perception on the feet	-2.8	(0.5)	-2.6	(0.6)	0.100
Cold perception on the whole body	-2.7	(0.5)	-2.3	(0.8)	**0.005**

All data are presented as mean and standard deviation (SD). P values from student t-test. P value from the sex outcome was obtained from chi-square test. *109 participants had valid data on this outcome (108 participants who reported and 25 who did not report shivering). The perception of shivering was measured by a numeric rate scale, where 0 represented "I am not shivering" and 10 represented to "I am shivering a lot". Perception of skin temperature areas were measured by ASHRAE scales that ranges from cold (-3), cool (-2), slightly cool (-1), neutral (0), slightly warm (1), warm (2) and hot (3). These scores were determined in the clavicular, abdomen, arms, hands, legs, feet and whole-body areas. BAT: brown adipose tissue; HU: Hounsfield Units; SUV: standardized uptake value.

Table S2. Perception of skin temperature (i.e., abdomen, arms, hands, legs, feet and whole-body areas) at the end of the shivering threshold test in males and females.

	Male (n=29)							Female (n=81)						
	Low (n=10)		Medium (n=10)		High (n=9)		p	Low (n=27)		Medium (n=27)		High (n=27)		p
Cold perception on the abdomen	-2.7	(0.7)	-2.7	(0.5)	-2.8	(0.4)	0.939	-2.4	(1.0)	-2.5	(0.6)	-2.6	(0.8)	0.687
Cold perception on the arms	-2.7	(0.5)	-2.7	(0.5)	-2.3	(0.5)	0.194	-2.8	(0.4)	-2.8	(0.4)	-2.7	(0.5)	0.580
Cold perception on the hands	-3.0	(0.0)	-2.6	(0.7)	-2.6	(0.5)	0.120	-2.9	(0.3)	-2.7	(0.6)	-2.7	(0.6)	0.263
Cold perception on the legs	-2.7	(0.5)	-2.2	(0.6)	-2.6	(0.5)	0.135	-2.6	(0.7)	-2.6	(0.6)	-2.6	(0.6)	0.967
Cold perception on the feet	-2.8	(0.4)	-2.7	(0.5)	-2.9	(0.3)	0.624	-2.8	(0.5)	-2.8	(0.5)	-2.8	(0.7)	0.962
Cold perception on the whole body	-2.9	(0.3)	-2.6	(0.5)	-2.7	(0.5)	0.315	-2.7	(0.6)	-2.7	(0.5)	-2.7	(0.5)	0.960

Data are divided by tertiles of shivering threshold time by male and female separately, and are presented as mean and standard deviation. P values obtained from univariate analyses of variance. Perception of skin temperature areas were measured by ASHRAE scales that ranges from cold (-3), cool (-2), slightly cool (-1), neutral (0), slightly warm (1), warm (2) and hot (3). These scores were determined in the abdomen, arms, hands, legs, feet and whole-body areas. These tertiles range from 52-114, 118-151 and 153-175 minutes of shivering threshold time in male, and from 53-92, 93-120 and 121-205 minutes in female. L: Low tertile; M: Medium tertile; H: High tertile.

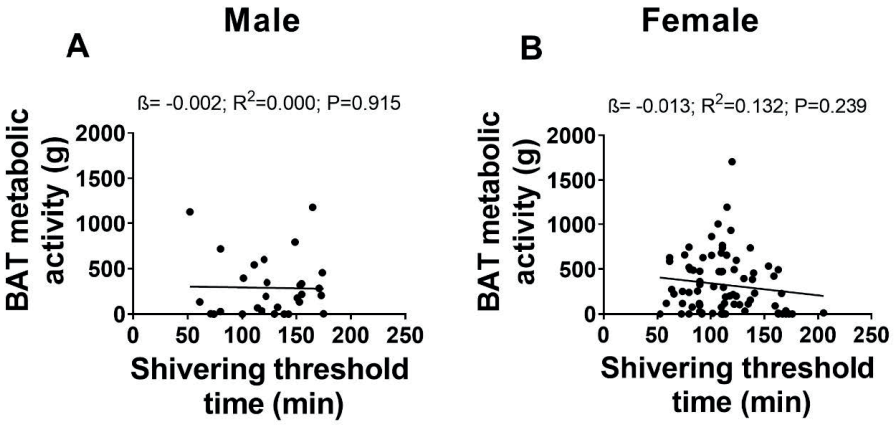

Figure S1. Associations between the shivering threshold time (minutes) and brown adipose tissue (BAT) metabolic activity, defined as BAT SUVmean * volume in males (A) and females (B). Unstandardized Beta values, adjusted R squared (R^2) and P-values are shown.

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