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Title

Multiorgan contribution to non-shivering and shivering thermogenesis and vascular responses during gradual cold exposure in humans

Authors

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Abstract

Purpose: Human brown adipose tissue (BAT) is known to be a significant thermoeffector in non-shivering thermogenesis (NST), albeit with individual variations in the BAT activity. We hypothesized that humans with less BAT would have more contribution from the skeletal muscle (SM) to NST or earlier shivering onset and greater vasoconstriction to compensate for less BAT-mediated thermogenesis.

Methods: Eighteen males participated in this study. Their BAT activity and detectable volume were investigated. A gradual cold exposure was conducted for inducing NST at 18.6 °C and initiating shivering at 11.6 °C. The energy expenditure, electromyograph of the pectoralis major, skin blood flow, and rectal (T_{re}) and skin temperatures were evaluated.

Results: BAT volume significantly correlated with the change in metabolic heat production during mild cold phase relative to baseline (*NST*; r=0.562, P<0.05), but not with shivering initiation phase (*NST+ST*). SM mass correlated with baseline metabolic heat production (M_{base}; r=0.839, P<0.01) but not with *NST* or *NST+ST*. A positive correlation was noted between BAT volume and T_{re} at the end of the 18.6 °C exposure period (r=0.586, P<0.05), which positively correlated with shivering onset time (r=0.553, P<0.05). The skin blood flow, mean skin temperature, and forearm and finger skin temperature difference at the end of the 18.6 °C exposure period did not correlate with *NST* or BAT volume.

Conclusion: BAT volume positively correlated with NST. Notably, lower T_{re} in individuals with less BAT volume induced earlier shivering onset for offsetting the less NST. Whereas, no correlation between metabolic and vasomotor responses was observed.

Keywords

brown adipose tissue, skeletal muscle, cold-induced thermogenesis, whole-body contribution

Abbreviations

BAT	Brown adipose tissue
CIT	Cold-induced thermogenesis
EMG	Electromyography
I_{tissue}	Tissue insulation
M	Metabolic heat production
MVC	Maximal voluntary contraction
NST	Non-shivering thermogenesis
SkBF	Skin blood flow
SM	Skeletal muscle
ST	Shivering thermogenesis
SUV_{mean}	Mean standard uptake value
$T_{f\text{-}f}$	Forearm and finger skin temperature difference
T_{re}	Rectal temperature
$\bar{T}_{sk}$	Mean skin temperature
$\dot{V}O_2$	Oxygen uptake

Introduction

Studies have shown the cold-induced activation of human brown adipose tissue (BAT) by assessing glucose uptake in BAT during mild cold exposure using ^{18}F-fluorodeoxyglucose (^{18}FDG) positron emission tomography (PET;(Saito et al. 2009; van Marken Lichtenbelt et al. 2009; Virtanen et al. 2009). Since then, researchers have focused on BAT as a potential tissue for increasing energy expenditure and improving metabolic health. However, considering the limited amount of BAT in particular body regions, several studies indicated the contribution of other organs to cold-induced thermogenesis (CIT) (Blondin et al. 2015b; Din et al. 2016). An evaluation of tissue activity using [^{15}O]O_2 and ^{18}F-fluoro-6-thia-heptadecanoic acid PET technique suggested that a major contribution to CIT without visible shivering came from the skeletal muscle (SM), whereas BAT had a minor contribution (Din et al. 2016). Moreover, another report indicated that patients with type 2 diabetes, who have less BAT volume, exhibited shivering and greater net ^{18}FDG uptake into SM during mild cold exposure (Blondin et al. 2015a). Based on this evidence, one can envision that even in healthy individuals who have less BAT activity there could be more contribution from the SM toward CIT to compensate for lesser BAT-mediated thermogenesis.

Regarding individual variations in BAT activity, a report revealed less volume of active BAT in South Asians than in Caucasians during mild cold exposure (Bakker et al. 2014). A significant correlation was revealed between the living latitude and frequency of an uncoupling protein 1 haplotype, which showed highest non-shivering thermogenesis (NST) (Nishimura et al. 2017). Moreover, summarizing previous investigations from several countries, the Japanese had less cold-induced activation of BAT compared with Europeans (Saito 2013).

Based on these observations, we conducted this study with Japanese participants who were supposed to have less BAT activity than other populations (Saito 2013; Bakker et al. 2014; Nishimura et al. 2017). It was hypothesized that especially in some individuals possessing less BAT activation, there could be more contribution from the SM to the NST or earlier onset of shivering to compensate for the less BAT-mediated

thermogenesis. Moreover, whole-body contribution of vasomotor and metabolic response to CIT should be considered when investigating thermoregulation in the cold. Possibly, greater vasoconstriction might be observed in the individuals with less BAT volume for balancing heat loss and thermogenesis. Hence, this study investigated the hypothesis that humans with less BAT would have more contribution from the SM to CIT and/or more significant vasoconstriction for offsetting the less BAT-mediated thermogenesis.

Methods

Participants

Eighteen healthy Japanese males living in Sapporo ([mean±standard deviation] age: 21.8±1.3 years; height: 173.1±4.3 cm; body weight: 61.5±6.7 kg; percentage of body fat: 14.5%±3.6%; surface area: 1.73±0.10 m^2) participated in a series of experiments during winter from the end of December to February. Their body fat percentages were estimated using a body composition analyzer (RD-800; TANITA, Japan) based on the bioelectrical impedance method. The body composition analysis was conducted in a laboratory controlled at 28°C within a few hours' individual variation. Participants were asked to keep fasting, except drinking water, for 3 hours before the measurement. The SM mass was estimated from the lean body mass, assuming that 40% of lean body mass was composed of SM (Abe et al. 2003). Body surface area (SA, m^2) was estimated from height (H, m) and bodyweight (BW, kg) as follows: $SA=0.1644\times H^{0.4225}\times BW^{0.5146}$ (Gehan and George 1970). Participants were equally separated into high and low BAT groups based on a cluster analysis using the detectable BAT volume (describe later). All experimental protocols in this study were designed as per the principles of the Helsinki Declaration. The FDG-PET/CT protocol for assessing BAT activity was approved by the Institutional Review Board (IRB) of the Tenshi College. In addition, the IRB of the Hokkaido University approved the gradual cold exposure protocol. All participants were informed of all the experimental protocols and gave their written informed consent before participation.

BAT activity evaluation (FDG-PET/CT test)

After overnight fasting for approximately 12 h, the participants wearing standardized light clothing (disposable gown) were exposed to a mild cold room controlled at 19 °C with the intermittent placement of their feet on an ice block wrapped with a towel to avoid cooling-associated pain (Saito et al. 2009). Participants were asked to remove their feet from the ice block when they subjectively felt or the experimenter observed initiation of shivering. This protocol enabled each participant to adjust the cold stimulus for maximizing BAT activity without shivering. In fact, in this protocol we did not detect any measurable change in the electromyograms at the pectoralis, suggesting negligible shivering (Yoneshiro et al. 2016). After the first hour of mild cold exposure, ^{18}F-FDG (4.0 MBq/kg body weight) was intravenously injected and the participants were exposed to cold for an additional hour. After mild cold exposure for 2 h, the radioactivity of ^{18}F-FDG was scanned for every 5 mm slice using the PET/CT system (Aquiduo, Toshiba Medical Systems, Otawara, Japan). BAT activity in the supraclavicular region was quantified based on the mean standardized uptake value (SUV_{mean}), defined as the average radioactivity per milliliter within the spherical region of interest (12 mm in diameter) divided by the injected dose of ^{18}F-FDG in mBq/g body weight. BAT was defined as tissue with Hounsfield units −300 to −10 on CT with an SUV>1.5. PET and CT images were co-registered and analyzed using the VOXBASE workstation (J-MAC System, Sapporo, Japan). Detectable BAT volume around the clavicular, cervical, and axillary regions with SUV>2.0 was analyzed using an image processing program (Image J v1.51; Wayne Rasband, NIH). To summarize, the area of BAT (SUV>2.0) was detected for every transverse 5-mm-thick slice of PET/CT image and the detectable BAT volume was then calculated as the sum of the product of the BAT area in each slice and the slice thickness (5 mm). In addition, the SUV_{mean} at the pectoralis major was assessed to confirm the absence of shivering during the protocol.

Gradual cold exposure test

On a separate day from the PET/CT test, a gradual cold exposure test was conducted in a climatic chamber. Participants wearing sports shorts were rested in a spinal position on a bed in a thermoneutral condition at 28 °C for at least 1 h before starting the experiment protocol. Following a 10-min baseline measurement at 28 °C and 40% relative humidity, the ambient temperature was gradually decreased during 20 min and maintained at 18.6 °C (mild cold condition) for 90 min (to induce NST). The temperature was almost matched with previous reports (Saito et al. 2009) and PET/CT protocol in this study. Based on the pilot tests, we set the temperature at 18.6 °C with intermittent foot cooling to adjust the cold stimulus for maximizing NST. An ice block wrapped with a towel was intermittently placed on their feet by an experimenter according to the subjective sensation of shivering. Subsequently, the ambient temperature was gradually lowered to 11.6 °C during 30 min to assess the initiation of minimal shivering thermogenesis (ST).

Continuous breath-by-breath measurement of respiratory gases was performed using an automated respirometer (AE-300S; Minato Medical Science, Japan). The oxygen uptake ($\dot{V}O_2$) and respiratory exchange ratio (RER) were averaged every minute for subsequent data analyses. Metabolic heat production was calculated from $\dot{V}O_2$ (L/min) and RER using the following formula (Tikuisis and Giesbrecht 1999):

$$M\ [\mathrm{W}] = (281.65 + 80.65\mathrm{RER}) \times \dot{V}O_2$$

The muscle activity of the pectoralis major was measured using surface electromyography (EMG). The skin cuticle on the right pectoralis major was removed by rubbing with a skin preparation abrasive paste (SkinPure; Nihon Kohden, Japan) and alcohol wipes. A pair of silver-silver-chloride surface EMG electrodes 5 mm in diameter (DL-941; S&ME, Japan) were placed on the right pectoralis major. A reference electrode was placed on the clavicle. The EMG signal amplified using an active electrode (DL-140; S&ME, Japan) and an analog output system (DL-720; S&ME, Japan) was transferred to digital data using an A/D converter (PowerLab16/35; AD Instruments, Australia), and recorded at a 1-kHz sampling rate and filtered using band-pass filters ranging from 20 to 500 Hz using a data acquisition and analysis software (LabChart

v8.1; AD Instruments). Before starting the cold exposure protocol, the participants underwent isometric maximal voluntary contraction (MVC) involving bilateral palm press for 5 sec with the shoulders horizontally flexed, elbows flexed at 90°, and wrists dorsally flexed at 90°. The root mean square (RMS) of the EMG signal was calculated for the 5-sec MVC (RMS_{mvc}), the 10-min thermoneutral baseline (RMS_{base}), and during the cold exposure every min. The shivering intensity (EMG_{shiv}) was evaluated in percentages of MVC (MVC%), adjusting the baseline to 0%, using the following formula (Haman et al. 2004):

$$EMG_{shiv}\ [MVC\%]=(RMS-RMS_{base})/(RMS_{MVC}-RMS_{base})\times 100$$

Onset time of vigorous shivering during the shivering initiation phase was manually detected by two examiners based on the EMG_{shiv} curve.

Rectal temperature (T_{re}) was measured using a thermistor probe inserted 13 cm beyond the anal sphincter. Skin temperature was measured using thermistor probes at eight body sites (forehead, chest, forearm, hand, thigh, calf, foot, and fingertip). The T_{re} and skin temperatures were monitored every second using data loggers (NR543R; Nikkiso-Therm Co. Ltd., Japan), and averaged every minute for subsequent data analyses. Mean skin temperature ($\bar{T}_{sk}$) was estimated using Hardy and DuBois' equation (Hardy and DuBois 1937). The difference between forearm and finger skin temperature (T_{f-f}) was calculated for assessing cutaneous vasoconstriction (House and Tipton 2002). The mean body temperature ($\bar{T}_b$) was estimated using the following formula:

$$\bar{T}_b=0.67T_{re}+0.33\bar{T}_{sk}$$

Whole-body tissue insulation (I_{tissue}) during the 90-min mild cold exposure was calculated from the thermal gradient between core and skin temperature and heat loss from the skin (H_s) using the following body heat balance equations (Rennie et al. 1962):

$$I_{tissue}\ [°C\cdot m^2/W]=(T_{re}-\bar{T}_{sk})/H_s$$

$$H_s\ [W/m^2]=M_s-S_s$$

M_s [W/m^2]=(M−0.08M)/SA

S_s [W/m^2]=C_b×$\Delta\bar{T}_b$×BW/SA

where H_s was calculated from the metabolic heat production per unit skin surface (M_s), excluding the respiratory heat loss assumed to be 8% of the total metabolic heat production (M) and body heat storage (S_s). S_s was estimated from $\Delta\bar{T}_b$ during the 90-min mild cold exposure, body weight (BW), and the human body specific heat capacity (C_b). I_{tissue} was only calculated at the end of 90-min mild cold exposure when body temperatures reached stable condition, but not for the shivering initiation phase during nonsteady state in skin temperature.

Skin blood flow (*SkBF*) in the chest was measured using laser Doppler flowmetry (ALF21; ADVANCE, Japan) and sampled using an A/D converter (Powerlab16/35; AD Instruments) and recorded at every 1-sec interval. The voltage output of the laser Doppler measurement was normalized (*SkBF%*) relative to the thermoneutral baseline (100%) before starting the cold exposure. Because of the artifacts caused by the movement of the laser Doppler probe, data during the shivering initiation phase was not included in the analyses.

Statistical analysis

Ward's hierarchical cluster analysis was conducted to classify the participants into two groups according to their BAT volume data. Comparisons of every 10 min time-course datasets were performed using two-way (time×low or high BAT group) analysis of variance (ANOVA). If Mauchly's sphericity test was not satisfied, the degrees of freedom were adjusted using Greenhouse–Geisser's ε. Post-hoc test was conducted using an unpaired Student's *t*-test with Holm's multiple comparisons adjustment (Holm 1979) at various time points between the low and high BAT groups. Dunnet's multiple comparison was conducted for M and EMG_{shiv} data in each group during the shivering initiation phase (90–120 min) to assess the time when M and EMG_{shiv} were significantly greater compared with the end of the mild cold phase (90 min). I_{tissue} during

the 90-min mild cold exposure was compared using unpaired Student's *t*-tests between BAT groups. Pearson's correlation coefficients were calculated to examine the relationships between parameters. Cohen's *d* and partial η^2 (η_p^2) were calculated for assessing effect size for unpaired Student's *t*-test and ANOVA, respectively. Statistical significances were set at $P<0.05$. All data were presented as mean values and standard deviation.

Results

Physical characteristics of the participants

Based on the cluster analysis using detectable BAT volume, participants were equally separated into high and low BAT groups (n = 9 in each group). The physical characteristics of both groups are summarized in Table 1. No significant intergroup differences were noted related to age, height, body weight, percentage of body fat, and SM mass, whereas SUV_{mean} and BAT volume were higher in the high BAT group ($P<0.001$).

Time course of metabolic and vasomotor response

The time courses of metabolic heat production (*M*) and shivering intensity (EMG_{shiv}) during gradual cold exposure are presented in Figure 1. The EMG data of one participant in the low BAT group was excluded from the analysis due to excessive noise. A significant main effect of time was detected using a two-way ANOVA on *M* ($F_{1.5,\ 23.6}=23.688$, $\eta_p^2=0.597$, $P<0.01$) and EMG_{shiv} ($F_{1.2,\ 17.8}=9.578$, $\eta_p^2=0.390$, $P<0.01$). Compared with the EMG_{shiv} at the end of the mild cold phase (90 min), significantly greater EMG_{shiv} was observed at 110 and 120 min in the low BAT group (both $P<0.01$), whereas at 120 min in high BAT group ($P<0.01$). *M* at 110 and 120 min was significantly higher than that at 90 min in each group ($P<0.01$).

The time courses of T_{re}, $\bar{T}_{sk}$, and $T_{f\text{-}f}$ are illustrated in Figure 2. A significant main effect of time ($F_{1.4,\ 22.5}=7.154$, $\eta_p^2=0.309$, $P<0.01$) and BAT group ($F_{1.4,\ 22.5}=8.423$, $\eta_p^2=0.345$, $P<0.05$) was observed in T_{re}. A

significant main effect of time was detected in $\bar{T}_{sk}$ ($F_{2.6,\ 41.3}$=2262.15, η_p^2=0.993, P<0.01) and $T_{f\text{-}f}$ ($F_{1.9,\ 25.9}$=213.987, η_p^2=0.939, P<0.01).

Metabolic and vasomotor response in each phase

The baseline M (M_{base}), change in the last 60 min averaged M during mild cold phase relative to baseline (*NST*), and the final 10 min averaged M for the shivering initiation phase (*NST+ST*) was picked up as a phase representative value and summarized in Table 2. No intergroup differences were noted in M_{base} and *NST+ST*, whereas *NST* was significantly greater in the high BAT group than in the low BAT group (d=1.186, P<0.05).

T_{re}, $\bar{T}_{sk}$, and $T_{f\text{-}f}$ at the baseline and at the end of each mild cold exposure and shivering initiation phase are presented in Table 2. T_{re} was significantly higher in the high BAT group at the end of mild cold phase (d=1.395, P<0.01) and shivering initiation phase (d=1.586, P<0.01). No intergroup difference was observed in $\bar{T}_{sk}$ and $T_{f\text{-}f}$ at any time point of the experiment. No intergroup differences were noted during the final 10 min related to averaged *SkBF%* and I_{tissue} during mild cold exposure (Table 2).

Relationship between metabolic response and thermoeffectors

The metabolic heat production during cold exposure as a function of BAT volume and SM mass is summarized in Figure 3. BAT volume significantly correlated with *NST* (r=0.562, P<0.05), but not with M_{base} or *NST+ST*. The SM mass correlated with M_{base} (r=0.839, P<0.01) but not with *NST* or *NST+ST*. No significant correlation was observed between BAT volume and SM mass.

BAT volume positively correlated with T_{re} at the end of the mild cold exposure (r=0.586, P<0.05; Fig. 4a) and at the shivering initiation phase (r=0.626, P<0.01; Fig. 4b). In contrast, SM mass did not correlate with T_{re} at any time point. Shivering onset was clearly detected in 13 participants (7 in the high and 6 in the low BAT group), and the shivering onset time positively correlated with T_{re} at the end of the mild cold

exposure (r=0.553, P<0.05; Fig. 4c), but not with BAT volume (Fig. 4d).

Relationship between metabolic and vasomotor response

The relationship between vasomotor responses during cold exposure ($\bar{T}_{sk}$, $T_{f\text{-}f}$, *SkBF%*, and I_{tissue}), metabolic heat production (*NST* and *NST+ST*), and thermoeffectors (BAT volume and SM mass) is presented in Table 3. $\bar{T}_{sk}$, $T_{f\text{-}f}$, *SkBF%*, and I_{tissue} at the end of the mild cold phase did not correlate with *NST*. $\bar{T}_{sk}$ and $T_{f\text{-}f}$ at the end of the shivering initiation phase did not correlate with *NST+ST*. BAT volume significantly correlated with $\bar{T}_{sk}$ at the end of the shivering initiation phase (r=0.528, P<0.05), but not with $T_{f\text{-}f}$, *SkBF%*, or I_{tissue}. The SM mass did not correlate with any parameters of the vasomotor response at any time point.

Discussion

BAT and SM contribution to NST

The primary focus of this study was to evaluate the contribution of BAT and SM to NST during mild cold exposure. Only the BAT volume positively correlated with *NST*, but the SM mass did not (Fig. 3). This result at least suggested that BAT volume contributed to NST. It was previously reported that total ^{18}FDG uptake during mild cold exposure (with minimal shivering) was 42 times greater in SM compared with that in BAT (Blondin et al. 2015b). However, because this evaluation did not distinguish between the metabolic components of basal metabolism and CIT, the contribution of SM toward the NST was not investigated. In our data, the SM mass exhibited a strong correlation with M_{base} (Fig. 3), and was also related to the net heat production (M_{base}+*NST*) in mild cold (r=0.683, P<0.01), but not with the NST component (*NST*). Furthermore, because the cold exposure protocol induced 1.8 times the resting whole-body energy expenditure with minimal shivering (Blondin et al. 2015b), the contribution of the SM could have been overestimated.

Another study investigated tissue-specific energy expenditure (EE) in BAT and SM in thermoneutral and mild cold environment without any shivering using the $[^{15}O]O_2$ PET technique, which has an advantage in measuring local tissue oxygen consumption regardless of utilized substrates (Din et al. 2016). The NST amounted to 20% (on average) of thermoneutral metabolism (Din et al. 2016), which is comparable with that in the mild cold phase in the present study (13.5% on average). The authors reported a positive correlation between the whole-body EE and BAT mass, as observed in our study. On the other hand, when multiplying tissue oxygen uptake and mass (tissue-specific EE), cold-induced whole-body EE did not correlate with the change in BAT-specific EE, but significantly correlated with that in the SM. Based on these results, the authors suggested that the contribution of BAT to the NST was minor and that SM was the major thermoeffector for NST (Din et al. 2016). However, in our study, SM mass did not correlate with *NST*. Therefore, assessment of the thermoeffectors' contribution based on the mass of the tissue could have probably been limited. In addition, the whole-body SM mass estimation using bioelectrical impedance analysis has limitation in the accuracy, and regional variation in the muscle metabolism could not be evaluated. It was reported that tissue-specific metabolism in deep and centrally located muscles made a major contribution to the NST without shivering (Din et al. 2016) and CIT with minimal shivering (Blondin et al. 2015b). In this study, the limitation in the accuracy and whole-body assessment of the SM mass without considering the tissue metabolism might fail to find a relationship between SM and NST. Hence, further examination measuring tissue-specific EE, which takes into account the tissue metabolism, volume of the tissue and the regional variation, would determine their contribution to CIT.

Correlation between NST and shivering initiation

Contribution of different thermoeffectors (BAT and SM) for NST and ST was the original focus of this study. Compared with the EMG_{shiv} at the end of the mild cold phase, a significant increase in the EMG_{shiv} was observed later among those with more BAT than those with less BAT (Fig. 1b). Moreover, participants

with more BAT volume revealed greater NST and maintained higher T_{re} at the end of the mild cold exposure (Table 2, Fig. 4a), and the higher T_{re} delayed the onset of shivering (Fig. 4b). These results might indicate an indirect correlation between thermoeffectors because BAT-mediated NST during mild cold exposure maintained a higher core body temperature, resulting in delayed shivering onset mediated by the SM. However, it should be noted that earlier studies have suggested major contribution of deeper skeletal muscle to NST (Din et al. 2016) and minor contribution of BAT oxidative metabolism (Muzik et al. 2013; Din et al. 2016). One might expect the contribution of fat mass to core body temperature and shivering intensity, but no difference was observed in body fat percentage between high and low BAT groups.

A previous study indicated a tendency of higher shivering intensity during mild cold exposure in patients with type 2 diabetes mellitus with less BAT activity compared with healthy young controls, even though they had more fat mass as insulation (Blondin et al. 2015a). This finding might indicate that less NST in a BAT negative population might have more significant contribution of SM to offset the lack of BAT-mediated thermogenesis. However, the greater shivering intensity in type 2 diabetes patients was only observed in comparison with healthy young controls, who had higher EE from the state at thermoneutral control, and no difference was observed during CIT (Blondin et al. 2015a). Hence, the findings of the present study would add to the evidence regarding correlation between two components of CIT (NST and ST) with different thermoeffectors (BAT and SM) in healthy young individuals.

Several studies have reported habituation of shivering simultaneously with the enhancement of BAT activity after repeated exposure to cold (Blondin et al. 2017; Hanssen et al. 2015; Hanssen et al. 2016). Therefore, individuals with high BAT activity might exhibit lower shivering thermogenesis. Nevertheless, the contribution of different metabolic components remains uncertain because no significant correlation was observed between *NST* and the final 10 min averaged *M* during the shivering initiation phase (*NST*+*ST*). Moreover, no direct relationship was observed between BAT volume and shivering onset time, intensity, or *NST*+*ST*. This could be related to insufficient cold stimulus during the shivering initiation phase at 11.6 °C

air for 30 min and low metabolic heat production (around 1.5 times M_{base}), including four participants without shivering onset. In addition, metabolic rate and shivering intensity were still rising at the end of the phase. Therefore, further investigation in colder and longer exposure conditions that induce more shivering reaching a plateau would clarify the contribution between the amounts of CIT components. Furthermore, evaluation of tissue-specific oxidative metabolism, as described earlier, would further clarify the contribution of the thermoeffectors and components of thermogenesis.

Correlation between metabolic and vasomotor response

Another original hypothesis in this study was whether stronger vasoconstriction was observed in the low BAT group for balancing heat loss and less thermogenesis. No intersystem correlation between metabolic heat production and any parameters of vasomotor responses was observed during mild cold exposure and shivering initiation phase (Table 3). Previous studies compared skin temperature between BAT positive and negative groups during mild cold exposure. No difference was observed in skin temperature at several body regions between BAT groups, whereas, skin temperature at the supraclavicular region, as a surrogate parameter for BAT activity, was higher in the BAT positive group (Yoneshiro et al. 2011; Yoneshiro et al. 2016; Nirengi et al. 2019). In addition to these reports, this study investigated $\bar{T}_{sk}$ and $T_{f\text{-}f}$ as parameters of peripheral vasoconstriction (House and Tipton 2002), as well as *SkBF%* and I_{tissue} calculated using the body heat balance equation. However, no intergroup differences or correlation between BAT activity and these vasomotor parameters was observed during mild cold exposure (Fig. 2; Tables 2 and 3). These observations indicate no correlation between BAT activity and vasoconstriction for balancing thermogenesis and heat loss in a mild cold environment. The lack of correlation between BAT and vasomotor response might be related to the relatively small contribution of BAT to CIT (Muzik et al. 2013; Din et al. 2016), as described earlier.

At the end of the shivering initiation phase, $\bar{T}_{sk}$ positively correlated with BAT volume (Table 3).

However, considering that no correlation between BAT volume and $T_{f\text{-}f}$ was observed, the lower $\bar{T}_{sk}$ observed in individuals with lower BAT volume would not reflect vasoconstriction. The high BAT group exhibited significantly higher T_{re} during cold exposure (Fig. 2, Table 2), whereas $\bar{T}_{sk}$ at the end of cold exposure tended to correlate with T_{re} at the end of mild cold exposure and shivering initiation phase (r=0.4365, P=0.07; r=0.4157, P=0.086). Hence, the higher $\bar{T}_{sk}$ observed in the high BAT group might be correlated to convective and conductive heat transfers from the core body region that was maintained warmer with greater NST.

Limitations

Because PET/CT measurement was conducted from the end of December to February, cold acclimatization differences between individuals might be included in our data. In addition, since the gradual cold exposure test was conducted from February to the first week of March, there might be some information bias due to a lengthy period between those two tests (26.3 days in average). However, we assumed that participants, who lived in Sapporo at the cold district, had mostly acclimatized to the cold season at the end of December and cold acclimatization difference would not be large between individuals and two tests. We also confirmed that there was no statistical difference in morphological characteristics of participants at two tests.

Additionally, it was suggested that there would be a methodological limitation in the repeatability for assessing the BAT volume using the simple cold exposure protocol with intermittent foot cooling on the ice block (Crandall et al. 2019). This is partly because the shivering might continue for a while after removing their feet from the ice. In our study, shivering was assessed based on the subjective sensation and experimenter's observation. Moreover, SUVmean at the pectoralis major was less than 0.6 in all participants (average, 0.48 ± 0.10), which would support the absence of continuous shivering during the protocol.

Conclusions

This study investigated the relationship between metabolic components, the contribution of thermoeffectors, and intersystem correlation between metabolic and vasomotor responses during gradual cold exposure. BAT volume significantly correlated with NST, regardless of the SM mass. The lower T_{re} in individuals with low BAT volume and less NST might induce an earlier shivering onset. Whereas, no precise intersystem correlation was observed between metabolic and vasomotor responses during gradual cold exposure.

Declarations

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Conflicts of interests None declared.

Ethics approval All experimental protocols in this study were approved by the IRB of the Tenshi College and Hokkaido University.

Author contributions HW, YK, KM, and TM conceived and designed research. TK, MM, and MS conducted the PET/CT experiments. YK, KM, and TE conducted the gradual cold exposure tests. TK, KM, YK, and HW analyzed data. KM and HW wrote the draft of manuscript. All authors read and approved the manuscript.

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Figure legend
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Figure legends

Figure 1 Time course of metabolic heat production (a) and shivering intensity (b) during gradual cold exposure in the high and low BAT volume groups.

The values are mean ± SD. *M*, metabolic heat production, EMG_{shiv}, shivering intensity. Time of 0, 10–90, and 100–120 min represent thermoneutral baseline, mild cold, and shivering initiation phase, respectively. † Significant difference compared with values at the end of mild cold exposure (90 min) in each group ($P<0.01$).

Figure 2 Time course of rectal (a) and mean skin temperature (b) and difference between finger and forearm skin temperature (c) during gradual cold exposure in high and low BAT volume groups.

The values are mean ± SD. T_{re}, rectal temperature; T_{sk}, mean skin temperature; $T_{f\text{-}f}$, forearm and finger skin temperature difference. Time of 0, 10–90, and 100–120 min represent thermoneutral baseline, mild cold, and shivering initiation phase, respectively.

Figure 3 Metabolic heat production during cold exposure as a function of BAT volume and skeletal muscle mass.

M_{base}, metabolic heat production at baseline; *NST* and *NST+ST* are change in metabolic heat production, relative to baseline, during mild cold phase and shivering initiation phase, respectively. R^2 for significant Pearson's correlation coefficients ($P<0.05$).

Figure 4 Relationship between BAT volume, rectal temperature, and shivering onset time.

T_{re} rectal temperature. R^2 for significant Pearson's correlation coefficients ($P<0.05$).

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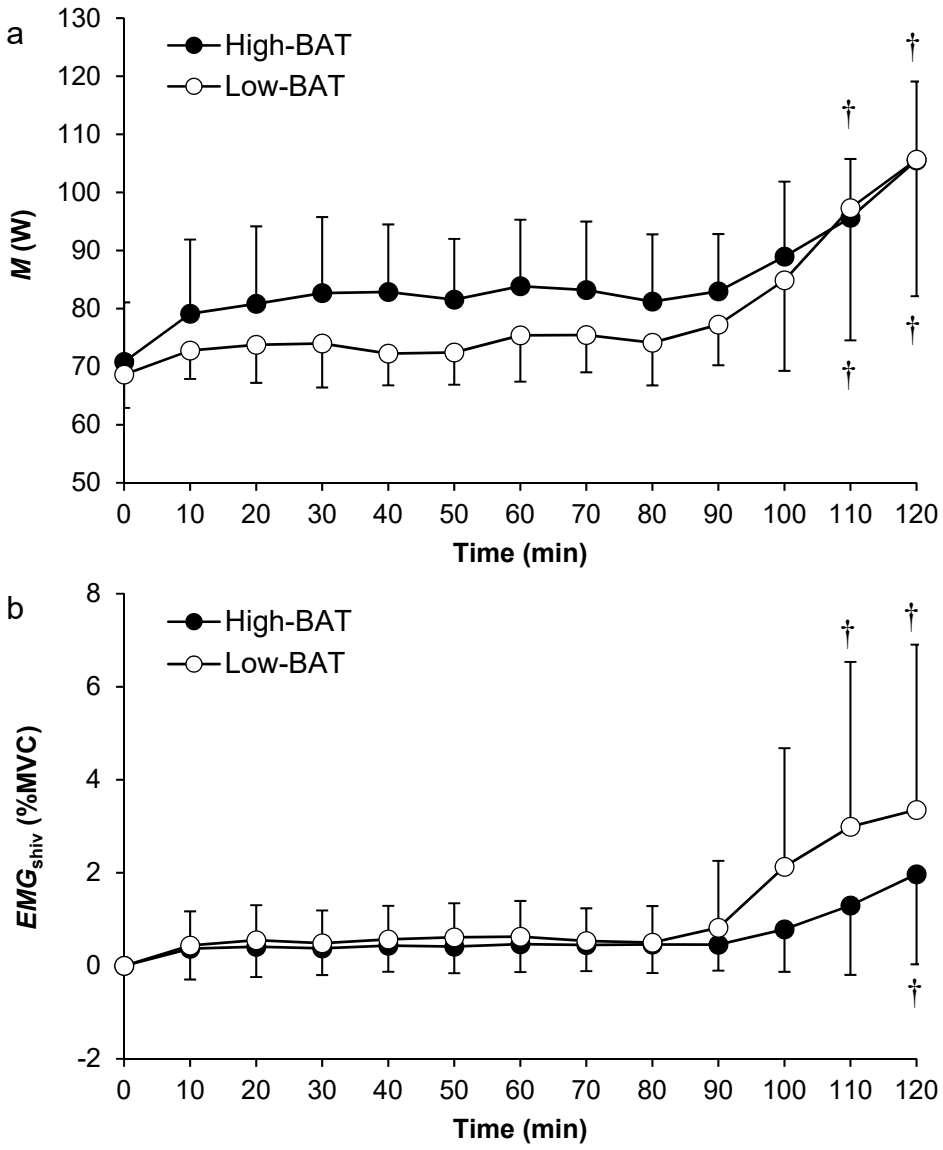

Figure 1

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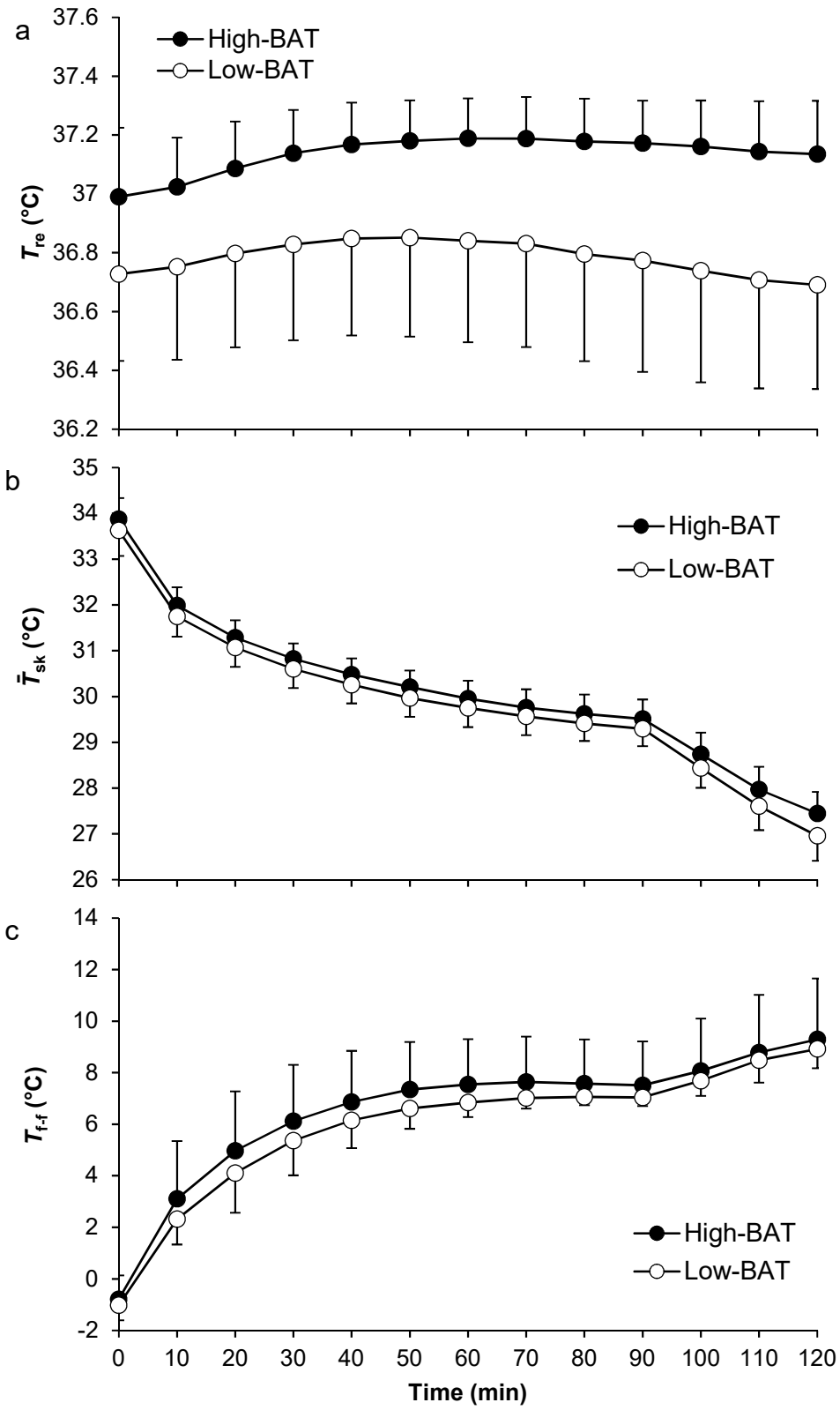

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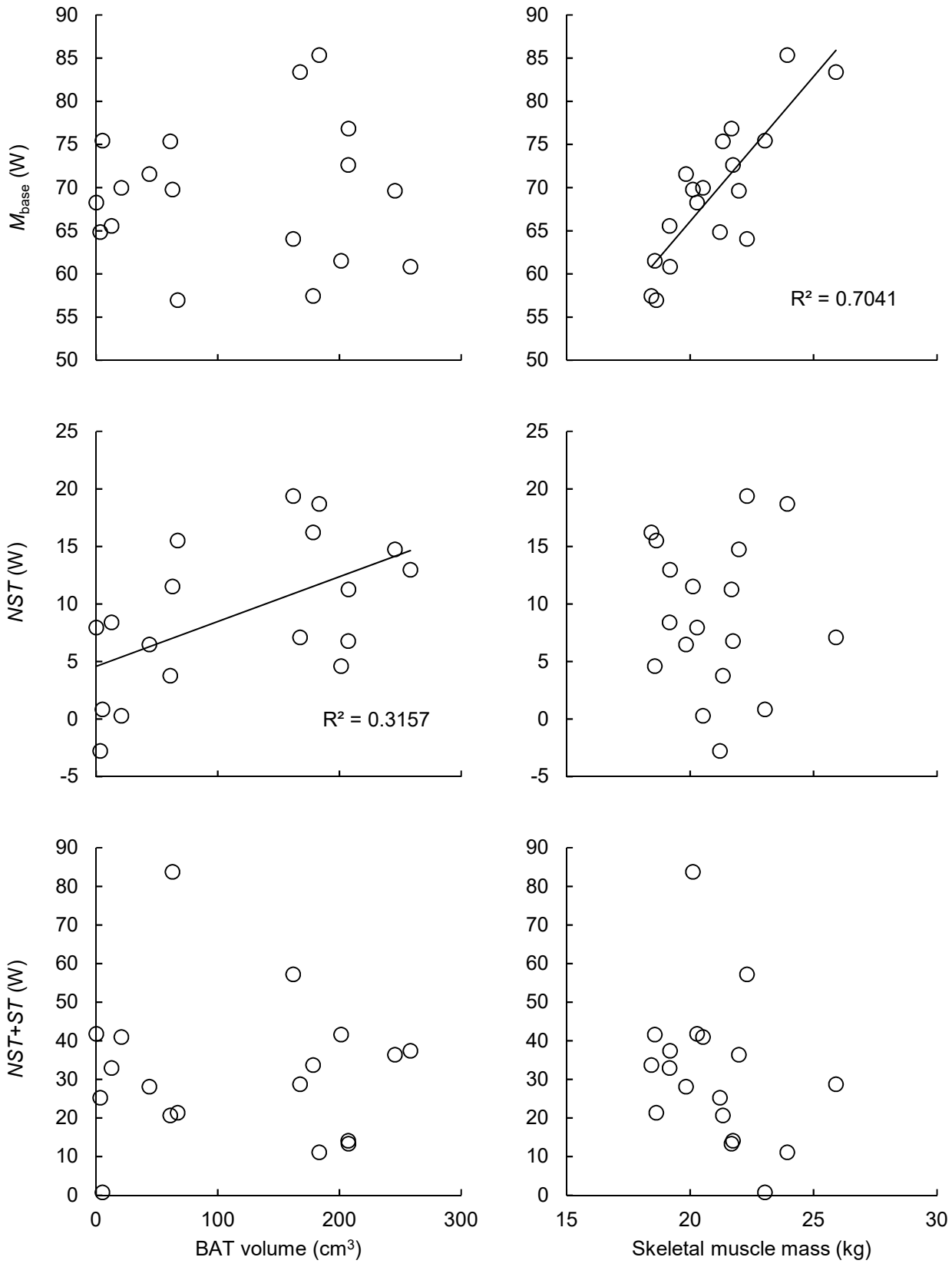

Figure 3

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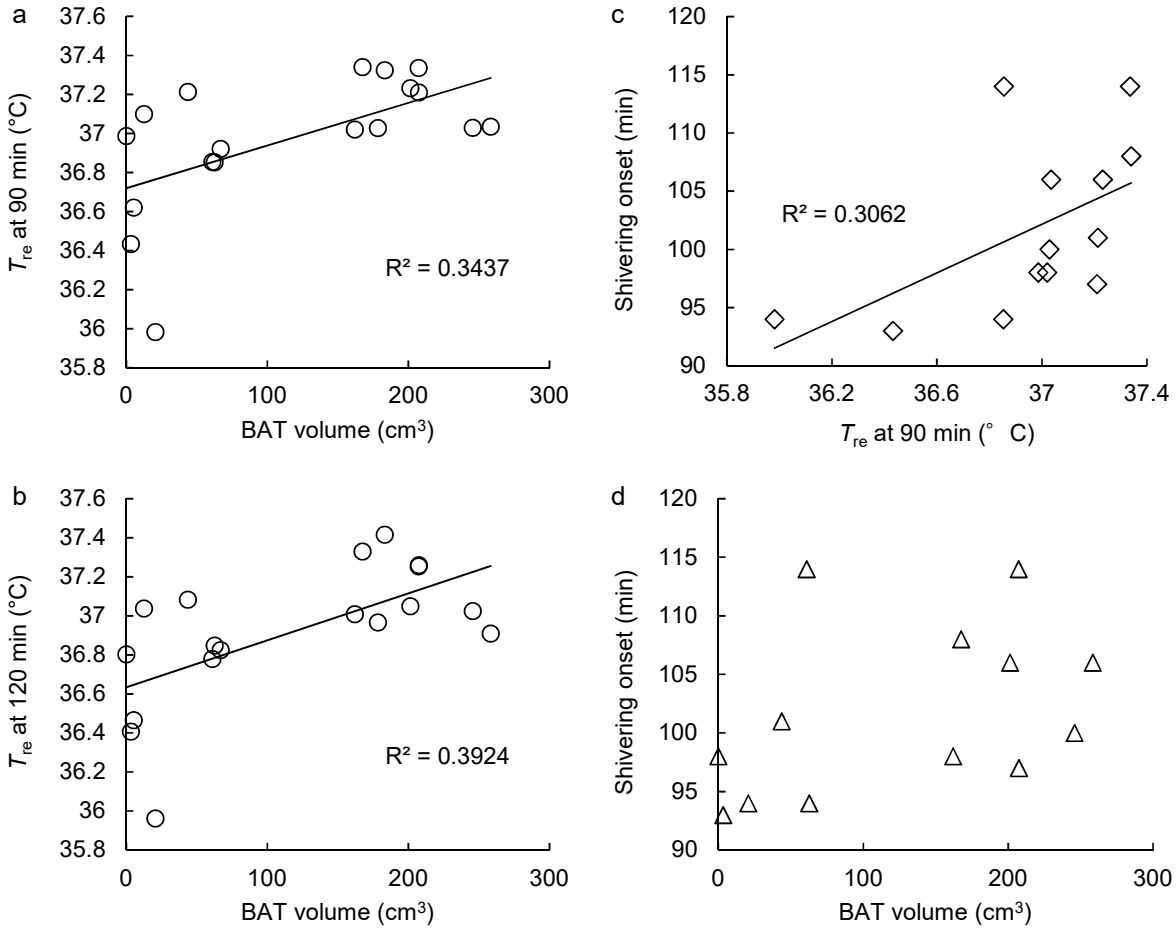

Figure 4

Table

Table 1 Physical characteristics of high and low BAT groups

	High BAT		Low BAT	
	mean	(SD)	mean	(SD)
Age (years)	21.6	(1.2)	22.1	(1.4)
Height (cm)	172.8	(4.8)	173.5	(4.1)
Body weight (kg)	63.0	(8.8)	60.1	(3.8)
Percent body fat (%)	14.2	(4.3)	14.8	(3.0)
Skeletal muscle mass (kg)	21.5	(2.5)	20.5	(1.3)
Surface area (m^2)	1.74	(0.14)	1.71	(0.06)
SUV_{mean}	7.38	(1.37)	1.90	(1.18) *
BAT volume (cm^3)	201.3	(33.2)	30.7	(27.8) *

Values are mean (SD) for both the high and low BAT groups. SUV_{mean}, mean standard uptake value; BAT, brown adipose tissue, SD, standard deviation. * Significant difference between groups (P<0.05).

Table 2 Metabolic response, body temperature, skin blood flow, and tissue insulation during cold exposure

	High BAT		Low BAT	
	mean	(SD)	mean	(SD)
M_{base} (W)	70.2	(10.1)	68.6	(5.7)
NST (W)	12.4	(5.4)	5.8	(5.8) *
NST+ST (W)	30.4	(15.3)	32.8	(22.7)
T_{re} baseline (°C)	36.99	(0.23)	36.73	(0.29)
T_{re} at 90min (°C)	37.17	(0.14)	36.77	(0.38) *
T_{re} at 120min (°C)	37.14	(0.18)	36.69	(0.35) *
$\bar{T}_{sk}$ baseline (°C)	33.88	(0.46)	33.63	(0.56)
$\bar{T}_{sk}$ at 90min (°C)	29.51	(0.43)	29.29	(0.38)
$\bar{T}_{sk}$ at 120min (°C)	27.45	(0.47)	26.95	(0.54)
$T_{f\text{-}f}$ baseline (°C)	−0.79	(0.92)	−1.02	(0.59)
$T_{f\text{-}f}$ at 90min (°C)	7.52	(1.70)	7.03	(0.33)
$T_{f\text{-}f}$ at 120min (°C)	9.29	(2.36)	8.92	(0.75)
SkBF% at 90min (%)	61.8	(14.9)	59.3	(16.0)
I_{tissue} in mild cold ($℃\cdot m^2\cdot W^{-1}$)	0.105	(0.009)	0.105	(0.008)

Values are mean (SD) for each high and low BAT groups. *M*, metabolic heat production; *NST*, non-shivering thermogenesis; *NST+ST*, non-shivering and shivering thermogenesis (change in *M* during shivering initiation phase relative to baseline); T_{re}, rectal temperature; $\bar{T}_{sk}$, mean skin temperature; $T_{f\text{-}f}$, forearm and finger skin temperature difference; *SkBF%*, percentage of skin blood flow in the chest; I_{tissue}, tissue insulation. Values at 90 and 120 min represent the end of the mild cold phase and shivering initiation phase, respectively. * Significant difference between groups ($P<0.05$).

Table 3 Relationship between metabolic and vasomotor responses during the cold exposure

	NST	*NST+ST*	SM mass	BAT volume
$\bar{T}_{sk}$ at 90min	0.187	−0.002	0.041	0.320
$\bar{T}_{sk}$ at 120min	0.307	0.272	0.017	0.528 *
$T_{f\text{-}f}$ at 90min	−0.050	−0.211	0.205	0.237
$T_{f\text{-}f}$ at 120min	0.133	−0.093	0.280	0.105
SkBF% at 90min	0.077	-	0.035	0.183
I_{tissue} in mild cold	−0.183	−0.125	−0.413	0.061

Values are Pearson's correlation coefficients. *NST*, non-shivering thermogenesis; *NST+ST*, non-shivering and shivering thermogenesis (change in metabolic heat production during shivering initiation phase relative to baseline); SM, skeletal muscle; $\bar{T}_{sk}$, mean skin temperature; $T_{f\text{-}f}$, forearm and finger skin temperature difference; *SkBF%*, percentage of skin blood flow in the chest; I_{tissue}, tissue insulation. Values at 90 and 120 min represent data at the end of the mild cold phase and shivering initiation phase, respectively. * Significant correlation between parameters (P<0.05).

Author contributions

HW, YK, KM, and TM conceived and designed research. TK, MM, and MS conducted the PET/CT experiments. YK, KM, and TE conducted the gradual cold exposure tests. TK, KM, YK, and HW analyzed data. KM and HW wrote the draft of manuscript. All authors read and approved the manuscript.