An optimal condition for the evaluation of human brown adipose tissue by infrared thermography

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Abstract

Brown adipose tissue (BAT) is responsible for non-shivering thermogenesis and is an attractive therapeutic target for combating obesity and related diseases. Human BAT activity has been evaluated by 18F-fluorodeoxyglucose-positron emission tomography/computed tomography (18FDG-PET/CT) under acute cold exposure, but the method has some serious limitations, including radiation exposure. Infrared thermography (IRT) may be a simple and less-invasive alternative to evaluate BAT activity. In the present study, to establish an optimal condition for IRT, using a thermal imaging camera, skin temperature was measured in the supraclavicular region close to BAT depots (T_{scv}) and the control chest region (T_{c}) in 24 young healthy volunteers. Their BAT activity was assessed as the maximal standardized uptake value (SUV_{max}) by 18FDG-PET/CT. Under a warm condition at 24–27˚C, no significant correlation was found between the IRT parameters (T_{scv}, T_{c}, and the difference between T_{scv} and T_{c}, Δtemp) and SUV_{max}, but 30–120 min after cold exposure at 19˚C, T_{scv} and Δtemp were significantly correlated with SUV_{max} (r = 0.40–0.48 and r = 0.68–0.76). Δtemp after cold exposure was not affected by mean body temperature, body fatness, and skin blood flow. A lower correlation (r = 0.43) of Δtemp with SUV_{max} was also obtained when the participant’s hands were immersed in water at 18˚C for 5 min. Receiver operating characteristic analysis revealed that Δtemp after 30–60 min cold exposure can be used as an index for BAT evaluation with 74% sensitivity, 92% specificity, and 79% diagnostic accuracy. Thus, IRT may be useful as a simple and less-invasive method for evaluating BAT, particularly for large-scale screening and longitudinal repeat studies.

Introduction

Brown adipose tissue (BAT) is responsible for non-shivering thermogenesis (NST) and is therefore involved in the regulation of whole-body energy expenditure and body fatness [1].
humans, the current gold standard method to assess BAT is $^{18}$F-fluorodeoxyglucose (FDG)-
positron emission tomography (PET) in combination with computed tomography (CT) and
cold exposure, which uses cold-activated glucose uptake as an index of BAT activity [2]. How-
ever, this $^{18}$FDG-PET/CT method has some serious drawbacks such as radiation exposure, the
need for cold exposure, and the high cost of the device, which have limited its frequent use in
both experimental and clinical studies. Although several alternative methods to overcome
these limitations have been developed, including magnetic resonance imaging [3], near-infra-
red time-resolved spectroscopy [4], and contrast-enhanced ultrasound [5], they are also rela-
tively expensive and not yet soundly confirmed for their validity and reliability [6].

There have been reports to assess the thermogenic activity of BAT by monitoring the temper-
ature of the skin ($T_{sk}$) overlying BAT depots. A few studies using a wire-less thermistor probe
revealed that cold-induced changes in $T_{sk}$ of the supraclavicular region ($T_{scv}$) close to BAT
depots positively correlated with the activity and volume of BAT estimated by $^{18}$FDG-PET/CT
[7,8]. Infrared thermography (IRT) can also be used to evaluate $T_{sk}$ with visualization by measur-
ing the infrared radiation emitted from the body surface. Jang et al. showed by IRT that differ-
ences between $T_{sk}$ in a control chest region and $T_{scv}$ ($\Delta temp$) were greater in subjects having
higher BAT activities after a 2-h cold exposure [9]. Furthermore, a significant relationship
between the IRT method and $^{18}$FDG-PET/CT also has been confirmed [9,10]. However, during
the cold exposure experiments of these studies, $T_{sk}$ was measured before and after a 2-h cold
exposure protocol, where the subjects with light-clothing were kept in a room at 19˚C (cold
exposure) or on mattresses perfused with cooled water at ~17˚C; these protocols may not induce
muscle shivering, but are apparently uncomfortable, stressful, and intolerable for most individu-
als, particularly those with cardiovascular diseases. Therefore, less invasive and easier protocols
are needed for frequent assessment of BAT, in both experimental and clinical studies.

Symonds et al. [11] and Ang et al. [12] tested the feasibility of IRT as a non-invasive method
by monitoring the changes in $T_{scv}$ 5 min after placing the hand and/or feet of the participant in
water at 20˚C. This hand immersion protocol is apparently much less invasive and is easily
applicable in various experimental and clinical settings; however, they did not validate the cor-
relation of their data with the BAT activity assessed by $^{18}$FDG-PET/CT. In the present study,
to establish optimal conditions for IRT assessment of human BAT, we monitored the response
of $T_{sk}$ to cold exposure for 10–120 min, in healthy subjects with a wide range of BAT activity.
We also examined $T_{sk}$ response after 5-min hand immersion in the same subjects, and com-
pared the two protocols. Our results revealed that $\Delta temp$, only after 30-min exposure to cold
at 19˚C, correlated well with the BAT activity assessed by $^{18}$FDG-PET/CT, indicating that this
protocol can be used for BAT evaluation with an accuracy of approximately 80%.

Methods

Twenty-four healthy male volunteers (age: 23.5 ± 3.6 years; body mass index [BMI]: 21.6 ± 2.5
kg/m²) participated in this study in winter from December 2017 to March 2018. This study
was carried out in accordance with the principles of the Declaration of Helsinki (Fortaleza
2013). The protocol was approved by the institutional review boards of Kyoto Medical Center
(no. 15–092) and was registered at the University Hospital Medical Information Network
(UMIN) center (UMIN000029206). Written informed consent was obtained from all
participants.

$^{18}$FDG-PET/CT

After overnight fasting for ~12 h, subjects were exposed to cold by being kept in an air-condi-
tioned room at 19˚C with standardized light clothing (a patient gown), with intermittent
placement of their feet on an ice block wrapped in cloth for ~4 min at 5-min intervals to avoid cooling-associated pain [13]. After 1 h under these cold conditions, each subject was intravenously injected with $^{18}$F-FDG (1.66–5.18 mega [106] Becquerel (MBq)/kg body weight) and kept under the same cold conditions. At 1 h after the $^{18}$F-FDG injection, $^{18}$FDG-PET/CT scans were obtained with a PET/CT system (Aquiduo; Toshiba Medical Systems, Otawara, Japan). BAT activity in both the right and left supraclavicular regions was quantified based on the maximum standardized uptake value (SUV$_{max}$), defined as the radioactivity per ml within the region of interest divided by the injected dose in mBq/g body weight. BAT was defined as tissue with Hounsfield units $-300$ to $-10$ on CT with an SUV $\geq 1.5$. PET and CT images were co-registered and analyzed using VOXBASE workstation (J-MAC System, Sapporo, Japan).

**IRT**

IRT was carried out using a thermal imaging camera (DE-TC1000T; D-eyes Inc., Osaka, Japan) fastened to a tripod. The thermal resolution was $160 \times 120$. The T$_{sec}$ of both the right and left sides was measured from each image. The T$_{sk}$ of the chest region (T$_{c}$) immediately lateral to the sternum approximating the second intercostal space, which is apart from the underlying BAT depots, was simultaneously measured as a control [13]. The subjects fasted for ~12 h, wore a light patient gown (about 0.2 clo), and underwent IRT successively for the following two tests: 5-min hand immersion into 18˚C water and 120-min cold exposure at 19˚C, as described below. IRT images were analyzed using a modified (D-eyes Inc.) version of Thermal-Cam v.1.1.0.9 software (Laon People Inc., Seoul, Korea).

**Cold exposure test**

Cold exposure was performed using two adjacently located rooms controlled at 27˚C and 19˚C, respectively, with 40% relative humidity. The coefficient of variance (CV) was 2.5% in the 27˚C room and 1.1% in the 19˚C room. Subjects were seated in an upright position looking straight ahead for ~30 min in the 27˚C room and underwent IRT and other measurements including skin blood flow (SkBF), then moved to the 19˚C room and underwent IRT at 10–30 min intervals for 120 min.

**Hand immersion test**

For the hand immersion test, the ambient room temperature and water temperature were 24˚C and 18˚C, respectively, with 40% relative humidity. The water temperature in a tank was maintained using a thermostatic water circulator (LV-200; Toyo Roshi Kaisha, Tokyo, Japan), and the CV of water temperature was 5.4%. After more than 30 min of rest, the subjects immersed both hands into the water tank for 5 min [11,12].

**Anthropometric parameters and others**

BMI was calculated as body weight in kilograms divided by the square of the height in meters, and body fat mass was estimated by the multifrequency bioelectric impedance method (Karada Scan HBF-701; Omron, Kyoto, Japan). Visceral and subcutaneous fat areas at the abdominal level of L4–L5 were estimated from the CT images. Total abdominal fat area was calculated as the sum of visceral and subcutaneous fat areas.

Tymppanic and sublingual temperature was measured using an earphone type infrared tympanic thermometer (CET-101; Nipro, Osaka, Japan) and an electronic thermometer before and after 2-h cold exposure (MC-172L; Omron Healthcare Co., Kyoto, Japan), respectively. A small disc-type temperature data logger (Thermochron SL; KN Laboratories, Osaka, Japan)
was used to monitor $T_{sk}$ on the forehead, left upper chest, non-dominant ventral forearm, non-dominant ventral middle finger, left shin, and left instep as reported previously [8]. The mean $T_{sk}$ was calculated according to a modified Hardy and DuBois’s equation [14].

SkBF in the supraclavicular region and back (left scapula) was measured using a laser tissue blood flowmeter (FLO-N1; Omegawave, Inc., Tokyo, Japan). Data were sampled using an A/D converter and recorded at 1-s intervals using a personal computer. In the subsequent analysis, artifacts observed in the raw data were eliminated using a 10-s median filter [15]. Before and after 2-h cold exposure, subjects were asked to rate shivering according to a modified version of a previously used scale [16] consisting of four levels: 1 = no shivering, 2 = slight shivering, 3 = moderate shivering, and 4 = heavy shivering. Cold sensation [17] and discomfort [18] were also assessed before and after 2-h cold exposure.

Statistical analyses

Data are expressed as mean ± standard deviation. Two-way analysis of variance with repeated measures was used to test interactions (group × time) and main effects (group, time). If there was a significant interaction or main effect, time or group differences in variables between baseline and after the test, were analyzed with the paired and unpaired t tests, respectively. The relationship between the data of IRT and $^{18}$FDG-PET/CT was analyzed by Pearson’s correlation analysis, where $SUV_{max}$ was log-transformed because of the non-normal distribution determined with the Shapiro-Wilk test. Values were considered statistically significant at $P < 0.05$. Receiver operating characteristic (ROC) analysis was performed to evaluate the area under the ROC curve (AUC), sensitivity, specificity, and the accuracy of IRT parameters. Then the AUC of after cold exposure was compared to that of 27˚C. The statistical analyses were performed using SPSS v.19 (IBM, Armonk, NY, USA) and Easy R software (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [19].

Results

$^{18}$FDG-PET/CT revealed that 5 of 24 subjects showed undetectably low BAT activity ($SUV_{max}$ < 1.5), and thus, were defined as BAT-negative, whereas the remaining 19 subjects showed a detectable activity ($SUV_{max}$ = 1.8~26.8), and thus, were defined as BAT-positive. There was no significant difference in the anthropometric parameters between the two subject groups (Table 1).

Fig 1 shows typical images of IRT and $^{18}$FDG-PET/CT at 27˚C and 2 h after cold exposure. $T_{sk}$ was considerably different between the two subjects, both under warm (27˚C) and cold (19˚C) conditions. Despite the individual differences, as summarized in Fig 2A and 2B, under the warm condition at 27˚C, $T_{scv}$ was insignificantly higher than $T_c$ in both BAT-negative and positive groups. After cold exposure, $T_{scv}$ and $T_c$ seemed slightly higher and lower, respectively, in the BAT-positive group. Although neither $T_c$ nor $T_{scv}$ was significantly different between the two groups at any time point during the 2-h cold exposure, the cold-induced drop in $T_{scv}$ was significantly smaller ($P < 0.05$) in the BAT-positive group (0.7 ± 0.6˚C) than in the BAT-negative group (1.7 ± 1.1˚C), while the drop in $T_c$ was comparable in the two groups (1.4 ± 0.7˚C vs. 1.8 ± 1.0˚C; $P = 0.16$).

To confirm the effect specific to the supraclavicular region, the difference between $T_{scv}$ and $T_c$ was calculated and expressed as $\Delta$temp. As shown in Fig 2C, $\Delta$temp in the BAT-positive group was 0.5 ± 0.3˚C at 27˚C, rose remarkably and significantly 10 min after cold exposure, reached a steady level of 1.3 ± 0.5˚C at 30 min, and was maintained at high levels of 1.2~1.3˚C thereafter. In contrast, $\Delta$temp in the BAT-negative group showed no significant change after cold exposure, being 0.5~0.6˚C, which was significantly lower ($P < 0.05$) than that of the BAT-
positive group. Thus, cold-induced change in Δtemp was observed only in the BAT-positive group. As the supraclavicular region, but not the control chest region, is close to the underlying BAT depots, Δtemp after cold exposure is likely to reflect the thermogenic activity of BAT and would serve as a BAT-specific index. Consistent with this idea, a fairly positive correlation ($r = 0.74$) was observed between the Δtemp at 2-h cold exposure and the BAT activity expressed as log SUV$_{\text{max}}$ (Fig 3). Significant correlations with SUV$_{\text{max}}$ were also found in $T_{scv}$ itself and the cold-induced $T_{scv}$ change ($T_{scv}$-time), but with lower correlation coefficients ($r = 0.48$ and $r = 0.59$, Table 2). In contrast, neither $T_{scv}$ nor Δtemp at 27˚C correlated with SUV$_{\text{max}}$. Comparative positive correlations between IRT parameters and log SUV$_{\text{max}}$ were also observed even at 30 min after cold exposure, including those for $T_{scv}$ ($r = 0.40$), Δtemp ($r = 0.68$) and $T_{scv}$-time ($r = 0.57$).

We also examined the effects of 2 h-cold exposure on tympanic and sublingual temperatures and skin temperature in various regions including the forehead, forearm, hand, finger, calf, and foot. Similar to the supraclavicular and chest regions, skin temperature in these regions dropped, showing the mean $T_{sk}$ from 33.1˚C ± 0.4˚C to 29.7˚C ± 0.3˚C ($P < 0.01$), but no notable difference was found between the BAT-positive and -negative groups (data not shown). The effects of cold-exposure on SkBF were also examined. After 2 h-cold exposure, SkBF decreased by 15.6% ($P < 0.05$) in the back, whereas it did not change in the supraclavicular region ($P = 0.51$), and no difference was found between the BAT-positive and -negative groups.

### Table 1. Characteristics of study subjects.

| Measurement                      | All         | BAT-positive | BAT-negative | $P$-value |
|----------------------------------|-------------|--------------|--------------|-----------|
| Number                           | 24          | 19           | 5            | -         |
| Age, years                       | 23.5 ± 3.6  | 23.8 ± 3.8   | 22.4 ± 2.2   | 0.44      |
| Height, cm                       | 172.1 ± 4.6 | 172.6 ± 5.0  | 170.2 ± 1.7  | 0.53      |
| Weight, kg                       | 64.0 ± 8.6  | 65.4 ± 8.9   | 58.9 ± 5.6   | 0.24      |
| BMI, kg/m$^2$                    | 21.6 ± 2.5  | 21.9 ± 2.6   | 20.3 ± 1.9   | 0.29      |
| Body fat, %                      | 16.6 ± 4.4  | 17.4 ± 4.4   | 13.7 ± 3.4   | 0.22      |
| Skeletal muscle, kg              | 35.6 ± 1.8  | 35.3 ± 1.8   | 36.6 ± 1.5   | 0.29      |
| Subcutaneous fat area, cm$^2$    | 42.8 ± 31.6 | 45.0 ± 33.9  | 34.6 ± 21.3  | 0.47      |
| Total abdominal fat area, cm$^2$ | 131.5 ± 72.7| 139.3 ± 74.5 | 102.0 ± 63.5 | 0.31      |
| SUV$_{\text{max}}$               | 7.0 ± 6.9   | 8.1 ± 6.1    | 0.6 ± 0.2    | < 0.01    |

Values represent mean ± standard deviation. BAT, brown adipose tissue; BMI, body mass index; SUV$_{\text{max}}$, maximal standardized uptake value.

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Fig 1. Typical images of $^{18}$FDG-PET/CT and IRT. Typical images of $^{18}$FDG-PET/CT in BAT-negative (A) and positive subjects (B). Typical images of IRT method in BAT-negative (C) and positive subjects (D) before (left) and after 2-h cold exposure (right).

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groups. We also investigated the relationship between possible confounding factors and \( T_{sk} \) (Table 3). The % body fat was negatively correlated with \( T_{scv} \) and \( T_c \) at 27˚C. The mean \( T_{sk} \) was positively correlated with \( T_{scv} \) and \( T_c \) at 27˚C and \( T_{scv} \) at 19˚C. The SkBf was positively correlated with \( T_c \) at 27˚C and \( T_{scv} \) and \( T_c \) at 19˚C. However, \( \Delta temp \) did not correlate with any of the parameters or temperatures (Table 3). There was no perceived shivering either before (0.3 ± 0.5) or after (-0.3 ± 0.5) cold exposure, while cold sensation was -2.1 ± 1.0 (-2 = cool) and discomfort was -1.0 ± 0.8 (-1 = uncomfortable) at the end of cold exposure.

We also examined the effects of hand immersion in water for the same subjects participating in the above-described cold exposure test. As shown in Fig 2D and 2E, during 5-min hand immersion, \( T_c \) significantly decreased (\( P < 0.05 \)), while \( T_{scv} \) did not change. The calculated \( \Delta temp \) was increased after the 5-min hand immersion only in the BAT-positive group (\( P < 0.05 \)). A significant positive correlation between \( \Delta temp \) and log \( SUV_{max} \) was found, but the correlation coefficient (\( r = 0.43 \)) was lower than that after cold exposure (Fig 3, Table 4).

The ROC analysis between \( \Delta temp \) and log \( SUV_{max} \) revealed that AUCs were 0.80 and 0.85 after 30-min and 120-min cold exposure, respectively, but 0.59 at 27˚C and 0.77 after 5-min hand immersion. As summarized in Table 5, the cut-off value of \( \Delta temp \) and accuracy seemed to reach plateau levels 30 min after cold exposure. When the cut-off value for detecting BAT-positive subject was set as 1.01˚C for \( \Delta temp \) after 30-min cold exposure, the sensitivity, specificity, and diagnostic accuracy were 74.3%, 92.3%, and 79.2%, respectively, which were comparable with those after 120-min cold exposure.

![Fig 2](https://doi.org/10.1371/journal.pone.0220574.g002)

**Fig 2.** Skin temperature changes after cold exposure and hand immersion. \( T_{scv} \), skin temperature of the supraventricular region; \( T_c \), skin temperature of the chest region; \( \Delta temp \), differences between \( T_{scv} \) and \( T_c \) (A), \( T_c \) (B), and \( \Delta temp \) (C) during cold exposure. \( T_{scv} \) (D), \( T_c \) (E), and \( \Delta temp \) (F) during hand immersion. *vs 27˚C or 0 m.*

![Fig 3](https://doi.org/10.1371/journal.pone.0220574.g003)

**Fig 3.** Relationship between log \( SUV_{max} \) and \( \Delta temp \) in the cold exposure test (A) and the hand immersion test (B). \( \Delta temp \), difference between skin temperature on the supraventricular region (\( T_{scv} \)) and that on the chest region (\( T_c \)); \( SUV_{max} \), maximal standardized uptake value. The correlation coefficient in the cold exposure test (\( r = 0.74 \)) was significantly higher than that in the hand immersion test (\( r = 0.42 \) (\( P < 0.05 \)). Data were obtained from both the right and left sides in 24 subjects.
In this study, to investigate the optimal index for assessing BAT thermogenic activity using the IRT method, healthy volunteer subjects were exposed to the cold for 2 h, and the skin temperature of the supraclavicular region close to BAT depots (T_{scv}) was compared with the metabolic activity (SUV_{max}) assessed by the standard 18FDG-PET/CT method. Our results showed that the cold-induced response of Δtemp, reflecting the difference between T_{scv} and a control chest region apart from BAT depots (T_c), was the most relevant index of SUV_{max}.

Human BAT is mainly present in the supraclavicular region, which has been the focus of most studies measuring the temperature response by the IRT method or using wire-less thermistors. In a previous thermistor study, a correlation coefficient of r = 0.52 was found between T_{scv} after cold exposure and SUV_{max} [7], which is similar to our result (r = 0.48). However, the T_{sk} value itself may be affected by various factors such as the subcutaneous fat thickness [20,21], SkBF, and possibly other thermogenic tissues. Therefore, to minimize the influence of these factors, we calculated Δtemp as the difference between T_{scv} and T_c, and found a higher correlation coefficient (r = 0.74). In fact, T_{scv} and T_c correlated with body fatness (% body fat), mean body temperature, and SkBF, while Δtemp showed no significant correlation with these parameters.

Table 2. Correlation coefficients between IRT parameters and SUV_{max} in the cold exposure test.

|                  | 27˚C  | 19˚C (30 min) | 19˚C (120 min) |
|------------------|-------|---------------|---------------|
| T_{scv}          | -0.24 | 0.40*         | 0.48*         |
| T_c              | -0.25 | 0.08          | -0.002        |
| Δtemp            | 0.16  | 0.68*         | 0.74*         |
| T_{scv}-time     | -     | 0.57*         | 0.59*         |
| T_c-time         | -     | -0.14         | 0.22          |

SUV, standardized uptake value; T_{scv}, skin temperature on the supraclavicular region; T_c, skin temperature on the chest region; Δtemp, differences between T_{scv} and T_c; T_{scv}-time, difference in T_{scv} between 19˚C and 27˚C; T_c-time, difference in T_c between 19˚C and 27˚C.

* P < 0.05 (Pearson’s correlation analysis).

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Discussion

In this study, to investigate the optimal index for assessing BAT thermogenic activity using the IRT method, healthy volunteer subjects were exposed to the cold for 2 h, and the skin temperature of the supraclavicular region close to BAT depots (T_{scv}) was compared with the metabolic activity (SUV_{max}) assessed by the standard 18FDG-PET/CT method. Our results showed that the cold-induced response of Δtemp, reflecting the difference between T_{scv} and a control chest region apart from BAT depots (T_c), was the most relevant index of SUV_{max}.

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Table 3. The correlation coefficient between Δtemp and possible confounding factors.

|                  | 27˚C  | 19˚C 30 min | 19˚C 120 min |
|------------------|-------|------------|-------------|
| % Body fat       |       |            |             |
| T_{scv}          | -0.38*| -0.24      | -0.09       |
| T_c              | -0.39*| -0.35*     | -0.26       |
| Δtemp            | 0.14  | 0.15       | 0.20        |
| Mean T_{sk}      |       |            |             |
| T_{scv}          | 0.31* | 0.37*      | 0.40*       |
| T_c              | 0.36* | 0.39*      | 0.39*       |
| Δtemp            | -0.21 | -0.02      | 0.10        |
| SkBF             |       |            |             |
| T_{scv}          | 0.07  | -          | 0.56*       |
| T_c              | 0.34* | -          | 0.36*       |
| Δtemp            | -0.12 | -          | 0.22        |

T_{sk}, skin temperature; SkBF, skin blood flow; T_{scv}, T_{sk} on the supraclavicular region; T_c, T_{sk} on the chest region; Δtemp, changes in T_{scv}; ΔT_c, changes in T_c. The body fat was used as baseline data. The mean T_{sk} was calculated according to a modified Hardy and DuBois’s equation. The SkBF was evaluated in the supraclavicular and control regions at 27˚C and 19˚C.

* P < 0.05 (Pearson’s or Spearman’s correlation analysis).

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Previous reports have shown that BAT activity could be evaluated by the IRT method using thermoneutral conditioning [9,21] or the 5-min hand immersion test [11,12]. These methods are simple and less invasive, and thus would be useful in clinical settings. However, these methods have not been validated in relation to the BAT activity assessed by 18FDG-PET/CT.

In our study, under a thermoneutral condition without cold exposure, no correlation of $T_{scv}$ and $\Delta$temp with SUV$\text{max}$ was found. In the hand immersion test, however, $\Delta$temp correlated with SUV$\text{max}$, but with a lower correlation coefficient ($0.43$) than found in the cold exposure test ($r = 0.74$). Thus, the hand immersion test may be feasible only for subjects with relatively high BAT activity.

In most previous human studies, BAT was assessed after 2 h or longer cold exposure, regardless of the 18FDG-PET/CT or IRT method [1, 9]. In this study, we monitored $T_{sk}$ responses to cold exposure at 10~30-min intervals for 120 min, finding that the response in $\Delta$temp reached a steady level after 30 min and was maintained thereafter. In fact, comparative positive correlations between the IRT parameters and SUV$\text{max}$ were observed even at 30 min after cold exposure, including that for $\Delta$temp ($r = 0.68$). Accordingly, the ROC analysis for the data after 30-min cold exposure revealed that the sensitivity, specificity, and diagnostic accuracy were similar after a 120-min cold exposure. Thus, 120-min cold exposure, as applied in the previous studies, is not necessary. Our easier protocol of 30-min cold exposure is sufficient for BAT evaluation by the IRT method.

One of the limitations of this study is that all our participants were young and non-obese males. To confirm the overall feasibility of our IRT method, it should also be tested in other groups, particularly in female and/or obese individuals. They have more subcutaneous fat which is insulating, and may influence $\Delta$temp depending on the mass/thickness of the fat. Moreover, $\Delta$temp may not only be influenced by heat directly transmitted from underlying BAT, but also from blood flow in the carotid and subclavian arteries. Further studies are needed to examine the possible confounding effects of these factors.

| Table 4. Correlation coefficients between IRT parameters and SUV$\text{max}$ in the hand immersion test. |
|---------------------------------------------------------------|
| Parameter          | Before | After (5 min) |
|--------------------|--------|---------------|
| $T_{scv}$          | -0.01  | 0.14          |
| $T_c$              | -0.12  | -0.11         |
| $\Delta$temp       | 0.27   | 0.43*         |
| $T_{scv}$-time     | -      | 0.17          |
| $T_c$-time         | -      | 0.04          |

SUV, standardized uptake value; $T_{scv}$, skin temperature on the supraclavicular region; $T_c$, skin temperature on the chest region; $\Delta$temp, differences between $T_{scv}$ and $T_c$; $T_{scv}$-time, difference in $T_{scv}$ between before and after hand immersion; $T_c$-time, difference in $T_c$ between before and after hand immersion.

* $P < 0.05$ (Pearson’s correlation analysis).

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| Table 5. Accuracy for brown adipose tissue activity during cold exposure. |
|---------------------------------------------------------------|
| Cut-off value, °C | Sensitivity, % | Specificity, % | Accuracy, % | AUC |
|-------------------|----------------|----------------|-------------|-----|
| 27°C              | 0.78           | 28.6           | 100         | 47.9| 0.59 |
| 19°C 30 min       | 1.01           | 74.3           | 92.3        | 79.2| 0.80*|
| 19°C 60 min       | 1.03           | 85.7           | 84.6        | 85.4| 0.89*|
| 19°C 120 min      | 0.96           | 80.0           | 76.9        | 79.2| 0.85*|

* $P < 0.05$, vs. 27°C

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In conclusion, Δ\text{temp} calculated from IRT after 30-min cold exposure highly correlated with SUV_{\text{max}} assessed by^{18}\text{FDG-PET/CT}. Thus, the IRT method may be useful as a simple and less-invasive alternative for evaluating BAT, particularly for large-scale screening and longitudinal repeat studies.

Supporting information

S1 File. Data sheet Fig 2.

S2 File. Data sheet of Fig 3.

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References

1. Kajimura S, Saito M. A new era in brown adipose tissue biology: molecular control of brown fat development and energy homeostasis. Annu Rev Physiol. 2014; 76:225–249. https://doi.org/10.1146/annurev-physiol-021113-170252 PMID: 24188710

2. Chen KY, Cypess AM, Laughlin MR, Haft CR, Hu HH, Bredella MA, et al. Brown adipose reporting criteria in imaging studies (BARCIST 1.0): Recommendations for standardized FDG-PET/CT experiments in humans. Cell Metab, 2016; 24:210–222. https://doi.org/10.1016/j.cmet.2016.07.014 PMID: 27508870

3. Hu HH, Wu TW, Yin L, Kim MS, Chia JM, Perkins TG, et al. MRI detection of brown adipose tissue with low fat content in newborns with hypothermia. Magn Reson Imaging. 2014; 32:107–117. https://doi.org/10.1016/j.mri.2013.10.003 PMID: 24239336

4. Nirengi S, Homma T, Inoue N, Sato H, Yoneshio T, Matsushita M, et al. Assessment of human brown adipose tissue density during daily ingestion of thermogenic capsinoids using near-infrared time-resolved spectroscopy. J Biomed Opt. 2016; 21:91305.

5. Flynn A, Li Q, Panagia M, Abdelbaky A, MacNabb M, Samir A, et al. Contrast-enhanced ultrasound: A novel noninvasive, nonionizing method for the detection of brown adipose tissue in humans. J Am Soc Echocardiogr. 2015; 28:1247–1254. https://doi.org/10.1016/j.echo.2015.06.014 PMID: 26255029

6. Chondronikola M, Beeman SC, Wahl RL. Non-invasive methods for the assessment of brown adipose tissue in humans. J Physiol. 2018; 596:363–378. https://doi.org/10.1113/JP274255 PMID: 29119565

7. Boon MR, Bakker LE, van der Linden RA, Pereira Arias-Bouda L, Smit F, Verberne HJ, et al. Supraclavicular skin temperature as a measure of 18F-FDG uptake by BAT in human subjects. PLoS One. 2014; 9:e98822. https://doi.org/10.1371/journal.pone.0098822 PMID: 24922545
8. Anouk AJJ, van der Lans AA, Vosselman MJ, Hanssen MJ, Brans B, van Marken Lichtenbelt WD. Supraclavicular skin temperature and BAT activity in lean healthy adults. J Physiol Sci. 2016; 66:77–83. https://doi.org/10.1007/s12576-015-0398-z PMID: 26420686
9. Jang C, Jalapu S, Thuzar M, Law PW, Jeavons S, Barclay JL, et al. Infrared thermography in the detection of brown adipose tissue in humans. Physiol Rep. 2014; 2:e12167. https://doi.org/10.14814/phy2.12167 PMID: 25413316
10. Law J, Morris DE, Izzi-Engbeaya C, Salem V, Coello C, Robinson L, et al. Thermal imaging is a noninvasive alternative to PET/CT for measurement of brown adipose tissue activity in humans. J Nucl Med. 2018; 59:516–522. https://doi.org/10.2967/jnumed.117.190546 PMID: 28912148
11. Symonds ME, Henderson K, Elvidge L, Bosman C, Sharkey D, Perkins AC, et al. Thermal imaging to assess age-related changes of skin temperature within the supraclavicular region co-locating with brown adipose tissue in healthy children. J Pediatr. 2012; 161:892–898. https://doi.org/10.1016/j.jpeds.2012.04.056 PMID: 22677567
12. Ang QY, Goh HJ, Cao Y, Li Y, Chan SP, Swain JL, et al. A new method of infrared thermography for quantification of brown adipose tissue activation in healthy adults (TACTICAL): A randomized trial. J Physiol Sci. 2017; 67:395–406. https://doi.org/10.1007/s12576-016-0472-1 PMID: 27443171
13. Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, et al. High incidence of metabolically active brown adipose tissue in healthy adult humans: Effects of cold exposure and adiposity. Diabetes. 2009; 58:1526–1531. https://doi.org/10.2337/db09-0530 PMID: 19401428
14. Hardy JD, Dubois EF. The technique of measuring radiation and convection. J Nutr. 1938; 15:461–475.
15. Wakabayashi H, Wijayanto T, Kuroki H, Lee JY, Tochihara Y. The effect of repeated mild cold water immersions on the adaptation of the vasomotor responses. Int J Biometeorol. 2012; 56:631–637. https://doi.org/10.1007/s00484-011-0462-1 PMID: 21695574
16. Nielsen R, Endrussick TL. Sensations of temperature and humidity during alternative work/rest and the influence of underwear knit structure. Ergonomics. 1990; 33:221–234. https://doi.org/10.1080/00140139008927112 PMID: 28080945
17. Gagge AP, Stolwijk JA, Hardy JD. Comfort and thermal sensations and associated physiological responses at various ambient temperatures. Environ Res. 1967; 1:1–20. PMID: 5614624
18. Wu Z, Li N, Cui H, Peng J, Chen H, Liu P. Using upper extremity skin temperatures to assess thermal comfort in office buildings in Changsha, China. Int J Environ Res Public Health. 2017; 14:E1092. https://doi.org/10.3390/ijerph14101092 PMID: 28934173
19. Kanda K. Investigation of the freely available easy-to-use software ‘EZR’ for medical statistics. Bone Marrow Transplant. 2013; 48:452–458. https://doi.org/10.1038/bmt.2012.244 PMID: 23208313
20. Sartorini JT, Koskensalo K, Raiko J, Nuutila P, Saunavaara J, Parkkola R, et al. Skin temperature may not yield human brown adipose tissue activity in diverse populations. Acta Physiol (Oxf). 2018; e13095.
21. Gatidis S, Schmidt H, Pfannenberg CA, Nikolau K, Schick F, Schwenzer NF. Is it possible to detect activated brown adipose tissue in humans using single-time-point infrared thermography under thermoneutral conditions? Impact of BMI and subcutaneous adipose tissue thickness. PLoS One. 2016; 11: e0151152. https://doi.org/10.1371/journal.pone.0151152 PMID: 26967519