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Concurrent validity of supraclavicular skin temperature measured with iButtons and infrared thermography as a surrogate marker of brown adipose tissue

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ABSTRACT

Brown adipose tissue (BAT) thermogenic activity is commonly assessed with a positron emission tomography with computed tomography scan (PET/CT). This technique has several limitations and alternative techniques are needed. Supraclavicular skin temperature measured with iButtons and infrared thermography (IRT) has been proposed as an indirect marker of BAT activity. We studied the concurrent validity of skin temperature measured with iButtons vs. IRT and the association of supraclavicular skin temperature measured with iButtons and IRT with BAT. We measured skin temperature upon a shivering threshold test with iButtons and IRT in 6 different regions in 12 participants (n = 2 men). On a separate day, we determined supraclavicular skin temperature with an iButton and IRT after 2 h of a personalized cooling protocol. Thereafter, we quantified BAT volume and activity by PET/CT. We observed that the absolute differences between the devices were statistically different from 0 (all $P < 0.05$) after the shivering threshold test. Moreover, we did not find any association between supraclavicular skin temperature measured with iButtons or IRT and BAT ^{18}F-FDG activity ($r = -0.213$; $P = 0.530$ and $r = -0.079$; $P = 0.817$). However, we observed a negative association of supraclavicular skin temperature measured by IRT with BAT ^{18}F-FDG volume ($r = -0.764$; $P = 0.006$), but not with supraclavicular skin temperature measured with iButtons ($r = -0.546$; $P = 0.082$). In light of these results, we concluded that the measurement of skin temperature obtained by iButtons and IRT are not comparable. Furthermore, it seems that supraclavicular skin temperature is not associated with BAT ^{18}F-FDG activity, but it appears to be negatively associated with BAT ^{18}F-FDG volume in the case of IRT.

1. Introduction

Brown adipose tissue (BAT) is a thermogenic tissue able to release heat through the action of the uncoupling protein 1, an inner mitochondrial protein which uncouples oxidation from ATP synthesis (Cannon and Nedergaard, 2004). In 2009, a set of studies confirmed the presence of metabolically active BAT in adults (Aaron M Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). Nowadays, human BAT is being studied as a possible tool to combat obesity and metabolic-related diseases (Ruiz et al., 2018). The main benefits of activating BAT is that it could play an important role in adaptive thermogenesis (Acosta et al., 2018; Ravussin and Galgani,

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Abbreviations

^{18}F-FDG	^{18}F-Flurodeoxyglucose
ANOVA	one-was analyses of variance
BAT	Brown adipose tissue
BMI	Body Mass Index
DXA	Dual-energy X-ray
HU	Hounsfield unit
IRT	Infrared thermography
PET/CT	Positron Emission Tomography- Computer Tomography
Personal-ET	Personal environmental temperature
ROI	region of interest
SUV	Standardized uptake value

2011), as an endocrine organ (Villarroya et al., 2017) or in the thermoregulatory system (Tan and Knight, 2018).

The most used technique to activate human BAT consists in exposing participants to cold temperatures for approximately 2 h (Brychta and Chen, 2016). Normally, after the first hour, an 18F-Fluorodeoxyglucose (^{18}F-FDG) dose is injected, and, after the second hour, a positron emission tomography with computed tomography scan is performed (PET/CT) to assess BAT volume and activity (Chen et al., 2016; Chondronikola et al., 2017). However, this technique presents several limitations which hamper the understanding of this tissue in humans (Chondronikola et al., 2017). Consequently, there is a need to develop alternative and valid techniques which could solve these limitations (Chondronikola et al., 2017).

Supraclavicular skin temperature has been previously proposed as a surrogate marker of human BAT (Boon et al., 2014; Law et al., 2018b; Symonds et al., 2012; van der Lans et al., 2016). A recent study showed that the temperature of the BAT depots located at the supraclavicular site can be measured by magnetic resonance spectroscopy (MRS) (Koskensalo et al., 2017), whereas the supraclavicular skin temperature is normally measured with iButtons (small devices attached to the skin) (Boon et al., 2014) or by infrared thermography images (IRT) (Law et al., 2018a). Two studies reported that supraclavicular skin temperature measured with iButtons is an indirect marker of BAT activity (Boon et al., 2014; van der Lans et al., 2016). Both studies presented controversial data despite having used a similar sample and study design. On the other hand, the validity of supraclavicular skin temperature measured by IRT was examined by Law et al. (2018b), who showed that it could be used as a surrogate marker of BAT activity. Nonetheless, this study presents several limitations: (i) the small sample size (n = 8) and (ii) the fact that the ^{18}F-FDG-PET/CT scan and the IRT pictures

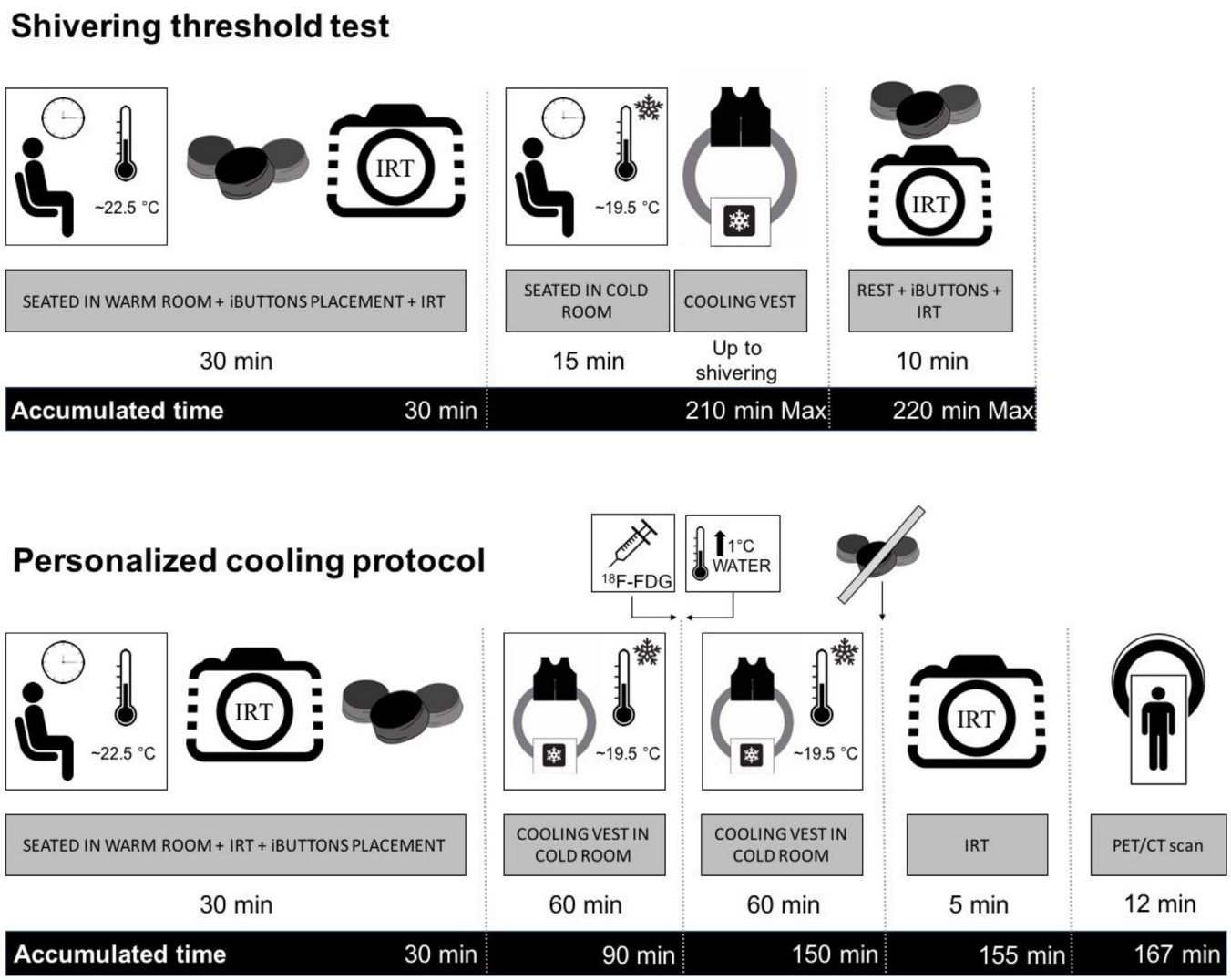

Fig. 1. Design of studies of both study days.

were performed on different days and with different cooling protocol durations. Moreover, Sarasniemi et al. (2018) showed that supraclavicular skin temperature measured by IRT positively correlates with the temperature of the BAT depots measured by MRS in lean adults, but negatively in obese adults. They observed that the fat layer (thickness) of the supraclavicular zone could play an important role, which concurs with other studies (Gatidis et al., 2016). It is important to note that these studies were performed in thermoneutral conditions, hence we do not know whether the supraclavicular fat layer could also play a role during cold exposures. Sarasniemi et al. (2018) highlighted the need to validate IRT against a method which truly measures the skin temperature. The supraclavicular fossa includes (Kellman et al., 1987) a set of vessels, lymph nodes, and fat and skeletal muscles, which have their own temperature, and therefore it may be involved in the supraclavicular skin temperature. In addition, skeletal muscle seems to be the main tissue involved in the cold-induced thermogenesis (U Din et al., 2016).

The present work has two aims: (i) to study the concurrent validity of skin temperature measured with iButtons and IRT and (ii) to study the association of supraclavicular skin temperature measured with iButtons and IRT with BAT volume and activity quantified by ^{18}F-FDG-PET/CT scan following the current recommendations (Chen et al., 2016).

2. Material & methods

2.1. Participants

A total of 12 young adults (2 men) aged 18–25 years old participated in the present study. The measurements were conducted in May 2017 after the exercise intervention of the ACTIBATE study in a subsample of participants in Granada (Spain)(Sanchez-Delgado et al., 2015). The inclusion criteria were healthy, non-smokers, with no family history of type 2 diabetes and sedentary behavior. The exclusion criteria were to did not take any medication that could affect thermoregulation and were not pregnant. They signed a written informed consent before their enrolment. The study protocols were approved by the Human Research Ethics Committee of the University of Granada (nº 924) and Servicio Andaluz de Salud (Centro de Granada, CEI-Granada) and were performed according to the Declaration of Helsinki (Fortaleza, Brazil, October 2013).

2.2. Design

The participants were assessed on two study days separated by 48–72 h (Fig. 1). They were asked to attend the research center by bus or car, with a minimum fasting time of 6 h on both days. Participants were given instructions for the study days: they were requested to sleep as usual, to avoid moderate or vigorous physical activities for 24 and 48 h, respectively, and to avoid any alcoholic or stimulant drink (at least 6 h), or the use of body lotions or drugs (at least 24 h) affecting the peripheral circulation. They were encouraged to be hydrated by drinking at least 1 L before starting the measurements.

2.3. Procedures

2.3.1. Shivering threshold test

An extended description of the shivering threshold test can be found elsewhere (Acosta et al., 2018; Martinez-Tellez et al., 2017b). On the first visit, the participants were required to wear standardized clothes (sandals, T-shirt, and shorts; clothing insulation value: 0.20), and they entered the warm room (~22.5 °C), where they remained seated for 30 min. They were informed about the protocol and were asked to stay as still and calm as possible, avoiding getting up, rubbing, or covering their bodies. Furthermore, women were asked to tie their hair up. After that, they entered a cold room (~19.5 °C) and remained seated for 15 min. At this point, a temperature-controlled water perfused cooling vest (Polar Products Inc., Ohio, USA) was placed and correctly adjusted on the participants' torsos, covering the anterior and posterior part of their trunk. Initially, water vest temperature was set at 16.6 °C and reductions of 0.6–2.2 °C were made every 10 min until a water temperature of 5.5 °C was reached. If the participants or the researchers reported or perceived no shivering, additional 0.6 °C reductions were performed every 15 min until either shivering or reaching a water temperature of 3.8 °C. Those participants who did not shiver by this time remained in the cold room for 45 extra minutes.

2.3.2. Personalized cooling protocol

After 48–72 h, we performed a personalized cooling protocol before the BAT quantification (Martinez-Tellez et al., 2017b). Upon arrival, the participants confirmed that the previous considerations were fulfilled. They were asked to urinate and to wear the same standardized clothes that they wore at the shivering threshold test. Women were asked to tie their hair up. Then, participants entered the warm room (~22.5 °C) and remained seated for 30 min. Subsequently, they entered the cold room (~19.5 °C) where participants put on the same cooling vests with an initial water temperature of ~ 4 °C above the personalized shivering threshold temperature (3.8 °C for those participants who did not shiver in the shivering threshold test). They were asked to stay in a sitting position during 120 min. If shivering was reported or detected, the water temperature was immediately increased by 1 °C and a bathrobe was worn for 2 min. After 60 min of cold exposure, 185 MBq of ^{18}F-FDG (~2.78 MBq/kg) were intravenously injected and the water temperature of the vest was increased by 1 °C for the second 60-min period. At this point, the PET/CT scan was conducted. A peak kilovoltage of 120 was applied for the CT acquisition, and for PET obtainment 2 bed positions (from atlas vertebra to mid-chest) were scanned, with an exposure time of 6 min per bed position (a total of 12 min).

2.3.3. Skin temperature measurements: iButtons

A total of 5 iButtons (DS-1922 L, Thermochron; resolution: 0.0625 °C; Maxim, Dallas, USA) (Martinez-Tellez et al., 2018b) were used to measure skin temperature in both protocols (i.e. shivering threshold test and the personalized cooling protocol). The iButton placement was performed at the beginning of both study days, when the participants were in the warm room (Martinez-Tellez et al., 2018b, 2017a). For the current study, we analyzed data from the iButtons placed on the supraclavicular and sternal regions, forearm, index fingertip, and posterior part of the neck. One-minute intervals were selected as the recording frequency. The iButton data was analyzed using the Temperatus software (http://profith.ugr.es/temperatus?lang=en) (Martinez-Tellez et al., 2019). The validity and reliability of iButtons in the assessment of skin temperature in humans have been previously reported (Smith et al., 2010; Wouter D. van Marken Lichtenbelt et al., 2006). The supraclavicular, sternal, and forearm's temperatures were used in these analyses in order to include central, reference, and peripheral body regions.

2.3.4. Skin temperature measurements: IRT

For IRT acquisition, the participants went to a separate room where they took their T-shirts off and sat down for 5 min in a thermoneutral ambient for acclimatization (24.3 ± 1.6 °C). Women were asked to move the straps of their sports bra aside, as well as to tie their hair up to make the supraclavicular area visible. IRT acquisition was measured before and after the shivering threshold test and before and after the personalized cooling protocol, just before the ^{18}F-FDG-PET/CT scan. For each measurement, we took 4 thermal images using a FLIR E60 thermal imaging camera (FLIR Systems Inc., Wilsonville, USA) with thermal resolution set at 320 × 240 pixels. The first image was taken to an aluminum foil phantom (1 m away) to obtain a measurement of the reflected temperature for each set of images, and the real time ambient temperature and relative humidity were registered for each picture. For

the second thermal image, the participants remained seated in an upright position, with their arms relaxed on both sides of their legs. After a calculation of optimal distances, the camera was placed 1 m from the midpoint of the chair for these images. For the third image, we removed the chair and we placed the camera 3 m from the participant, who remained in an anatomical position facing the camera. For the last image, the participants turned 180° maintaining the same position and with their backs facing the camera, which was placed 3 m away. Two thermal images were taken and the clearest one was retained for analyses. All the images were taken perpendicularly with the 5 body regions selected, which were the same regions where we had placed the iButtons, in the contralateral side of the body. The pictures of the IRT were taken with the iButtons attached to the body, only in the shivering threshold test. We manually drew a region of interest (ROI) for each body region (Fig. S1). We tried to draw these ROIs as close as possible to the iButtons' locations but in the opposite side of the body to prevent the iButton temperature from contaminating the skin temperature, except for the sternal and upper back regions, where we drew the ROI just below the iButtons. All ROIs were performed using the FLIR ResearchIR Max software version 4.40.6.24 for Windows (FLIR Systems Inc., North Billerica, MA, USA). The supraclavicular and sternal ROIs were derived from the second image, the index fingertip ROI from the third image, and the forearm and upper back ROIs from the fourth image. All analyses were adjusted by atmospheric temperature and relative humidity, which were measured using a FLIR MR77 moisture meter (FLIR System Inc., Wilsonville, USA) at the beginning of each set of thermal images. Furthermore, the reflected temperature was obtained by placing a rounded ROI on the aluminum foil phantom of the first thermal image and retaining the mean value (°C) for adjustments. For all thermal images, emissivity was set at 0.98. Minimum, maximum, and mean values of each ROI were retained as variables.

2.4. PET/CT analyses

The Beth Israel plugin for FIJI software (Aaron M. Cypess et al., 2009) was used to analyze the PET/CT images. ROIs were semi-automatically drawn from the atlas vertebra to the thoracic vertebra 4 using a 3D-Axial technique (Leitner et al., 2017). Our protocol has recently shown high inter-observer reliability (Martinez-Tellez et al., 2018a). The standardized uptake value (SUV) was calculated as follows: ^{18}F-FDG uptake (kBq/mL)/(injected dose [kBq]/patient weight [g]). BAT volume, SUVmean, and SUVpeak were defined according to BARCIST 1.0 criteria [i.e. SUV threshold individualized to lean body mass in combination of Hounsfield Units (HU) range from −10 to −190] (Chen et al., 2016). BAT volume (ml) was calculated as the sum of the volumes defined as BAT in each ROI. SUVmean was obtained from the weighted average of the SUVmean of each ROI. Finally, SUVpeak was the highest average SUV in a 1 ml spherical volume. We drew a single ROI from 1 slice in supraspinatus, paracervical, sternocleidomastoid, longus colli, trapezius, parathoracic, subscapular, deltoid, pectoralis major, scalene, and triceps brachii muscles from both left and right side of the body (Blondin et al., 2015; Hanssen et al., 2016). An average of both sides including all skeletal muscles was performed in order to obtain a single representative value of the skeletal muscle ^{18}F-FDG uptake of the upper part of the body. Moreover, we performed different skeletal muscle groupings (Blondin et al., 2015).

2.5. Body composition

We measured the participants' weight and height barefoot and wearing the standardized clothes using a SECA scale and stadiometer (model 799, Electronic Column Scale, Hamburg, Germany). Body mass index (BMI) was calculated as weight/height squared (kg/m^2). Additional body composition measurements (i.e. lean mass and fat mass) were taken by Dual Energy X-ray Absorptiometry scan (Discovery Wi, Hologic, Inc., Bedford, MA, USA). Lean mass index and fat mass index were calculated as lean mass/height squared and fat mass/height squared (kg/m^2), respectively.

2.6. Statistical analysis

The descriptive characteristics of the study sample are shown as mean ± standard deviation and as percentage when stated in Table 1. To study the concurrent validity between the iButtons and IRT, we performed paired T-tests comparing both devices' outputs measured in the shivering threshold test. We studied which outcome of skin temperature measured by IRT (i.e. minimum, maximum, or mean) in different body regions and in different conditions (i.e. before and after cold exposure) showed a more similar measurement to that obtained from the iButtons. The iButton output used for these comparisons was obtained at the same time as the thermal image was taken. In order to study the grade of agreement between iButtons and the mean IRT measurement we applied the Bland-Altman method (Martin Bland and Altman, 1986). Later, we performed one-sample t-tests to analyze whether the differences between both devices were statistically significantly different from 0. Additional one-sample t-tests were performed using the absolute difference between the methods to avoid potential balances between positive and negative values, which could lead to wrong conclusions.

To study the association between supraclavicular skin temperature measured with iButtons and IRT at the end of the personalized cooling protocol and human BAT ^{18}F-FDG volume and activity, we conducted simple linear regression analyses. We used additional simple linear regression analyses to observe whether sternal or supraclavicular skin temperature or the difference between supraclavicular minus sternal at the end of the cold exposure were able to predict human BAT ^{18}F-FDG as previously suggested (Law et al., 2018b). These analyses were repeated introducing the ^{18}F-FDG uptake by skeletal muscles in the model instead of BAT.

We performed one-way analysis of variance (ANOVA) with repeated measures to study the effect of cold on skin temperature parameters for both devices.

Table 1
Participants' characteristics (n = 11).

	Shivering threshold test			Personalized cooling protocol		
	Mean ± SD	Min	Max	Mean ± SD	Min	Max
Sex (% male)	(n = 2) 18.2%			(n = 2) 18.2%		
Age (years)	21.9 ± 2.2	18.3	25.8	22.2 ± 2.3	18.3	25.8
Body mass index (kg/m^2)	23.5 ± 4.8	18.4	34.8	23.9 ± 4.7	18.4	34.8
Lean mass index (kg/m^2)	14.6 ± 2.6	11.5	21.3	14.7 ± 2.5	11.5	21.3
Fat mass (%)	32.7 ± 6.9	16.6	40.7	32.7 ± 7.3	16.6	40.7
Fat mass index (kg/m^2)	7.7 ± 2.7	3.0	11.8	7.9 ± 2.7	3.0	11.8

Data are presented as mean ± standard deviation (SD) or percentage when stated.

3. Results

Table 1 shows the characteristics of the participants of this study. All of them participated on both study days. However, we could not obtain the iButtons and IRT data of one participant during the personalized cooling protocol, and the same occurred with another participant during the shivering threshold test. This is the main reason why we have the same sample size but different descriptive data. Of note is that there was a physiological outlier (BMI = 35 kg/m^2). We performed sensitivity analyses excluding this participant from the analyses and the results persisted (data not shown). Moreover, we observed that supraclavicular skin temperature in the same cohort measured by IRT from the right and left side was not different after the personalized cooling protocol (Fig. S2).

3.1. Concurrent validity

Table 2 shows the differences between the average of the iButton measurement of skin temperature in the different body regions and the minimum, maximum, and mean measurement of skin temperature by IRT. We observed that there were significant differences between the iButtons and the minimum measurement of IRT in 4 out 5 body regions (supraclavicular, sternal, forearm, and upper back) before and after the shivering threshold test (all $P < 0.05$). We found similar differences between the iButtons and the maximum measurement of IRT in the same body regions (all $P < 0.05$). However, regarding the differences between the iButtons and the mean measurement of IRT, the only difference found was in the sternal region ($P < 0.05$), while the supraclavicular, forearm, index fingertip, and upper back regions showed no differences (all $P > 0.05$). These results were similar before and after the shivering threshold test.

Based on the results displayed in Table 2, we selected the measurement of iButtons and the mean measurement of the IRT to perform the Bland-Altman plots (Fig. 2). The systematic error between the iButtons and the mean measurement of IRT was not significantly different from 0 for the supraclavicular region before and after the shivering threshold test (mean differences ranged from −0.15 to 0.27 °C, all $P > 0.05$, Fig. 2). For the sternal region, the systematic error was statistically significantly different from 0 ($P < 0.05$) before and after cold (mean differences ranged from −0.67 to −0.97 °C). Regarding the forearm, the systematic errors for the two different conditions did not differ from 0 (mean differences ranged from −0.11 to 0.74 °C, All $P > 0.05$). We transformed the value of the differences between the iButton and IRT to absolute values, and we studied whether these outcomes were statistically different from 0 (Fig. 3). We observed that the differences between instruments in all the regions and conditions (before and after cold exposure) were different from 0.

3.2. Association between supraclavicular skin temperature with BAT ^{18}F-FDG

Supraclavicular skin temperature measured with the iButtons and the mean of measurement of IRT were not associated with any BAT ^{18}F-FDG-related outcome (all $P \geq 0.165$, Fig. 4A and B for iButton and IRT). However, we found a negative association of supraclavicular skin temperature measured by IRT with BAT ^{18}F-FDG volume ($r = -0.764$; $P = 0.006$), but not with supraclavicular skin temperature measured with the iButtons ($r = -0.546$; $P = 0.082$). These results persisted when we included fat mass percentage as a covariate (data not shown). We selected the sternal region as a reference location because this region did not change upon the cold exposure whereas other regions did (Fig. S3). In addition, we did not observe any association between sternal skin temperature and BAT ^{18}F-FDG-related outcomes (Fig. 4C and D, for the iButton and IRT, respectively) (Fig. 4). All the analyses were performed with the data of skin temperature after the personalized cooling protocol. When we analyzed the association of the difference between supraclavicular minus the sternal skin temperatures and BAT ^{18}F-FDG-related outcomes, we did not find any significant association (All $P \geq 0.061$, Fig. 5A and B). When we studied these associations before cooling, the results persisted (data not shown). Moreover, we did not find any association between the differences in supraclavicular skin temperature (after-cold minus the measurement before-cold conditions) and BAT ^{18}F-FDG-related outcomes with both devices (data not shown). Furthermore, we repeated the associations with BAT ^{18}F-FDG-related outcomes obtained only in the supraclavicular fossa and the results persisted (data not shown). We repeated the associations introducing the minimum and the maximum supraclavicular skin temperature measured by IRT at the end of personalized cooling protocol with BAT ^{18}F-FDG and the results persisted. However, we observed that maximum supraclavicular skin temperature before the personalized cooling protocol was positively associated with BAT ^{18}F-FDG activity (i.e. SUVmean and SUV peak, data not shown). Finally, we repeated all the analyses between supraclavicular skin temperature with both devices and ^{18}F-FDG uptake by skeletal muscles and we did not find any association (data not shown).

4. Discussion

The present study shows that the skin temperature measured with iButtons and IRT is not comparable. Moreover, there is no association between supraclavicular skin temperature measured with iButtons or the mean measurement of IRT and BAT ^{18}F-FDG activity (SUVmean or

Table 2
Differences between skin temperatures of the selected body regions measured with iButtons and infrared thermography (IRT) before and after the shivering threshold test.

Before cold										
Body region	n iButtons	iButtons (°C)		n IRT	IRT minimum (°C)		IRT maximum (°C)		IRT mean (°C)	
		mean	SD		mean	SD	mean	SD	mean	SD
Supraclavicular	11	34.40	0.78	11	**32.40*****	1.38	**35.47*****	0.64	34.55	0.71
Sternal	11	33.38	1.08	10	**34.16***	0.89	**34.32****	0.84	**34.23***	0.88
Forearm	9	33.20	0.95	11	**30.44****	1.32	33.41	0.65	32.54	0.73
Index fingertip	11	32.16	1.74	11	31.21	1.79	31.21	1.79	31.21	1.79
Upper back	11	34.01	0.84	11	**31.12****	2.15	**34.77***	1.29	33.71	1.17
After cold										
Supraclavicular	10	34.56	0.55	10	**31.41*****	1.00	**35.50*****	0.56	34.29	0.83
Sternal	10	33.01	1.29	10	**33.61***	1.04	**33.76***	1.01	**33.68***	1.00
Forearm	8	29.87	1.23	10	**27.28****	1.32	**31.43****	1.01	29.98	1.08
Index fingertip	10	22.95	1.36	9	23.73	0.84	23.73	0.84	23.73	0.84
Upper back	10	32.91	1.44	10	**29.51*****	2.24	**34.60*****	1.08	32.98	1.31

$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ for differences between iButtons and IRT measurements. Significant differences are highlighted in bold in the IRT data.

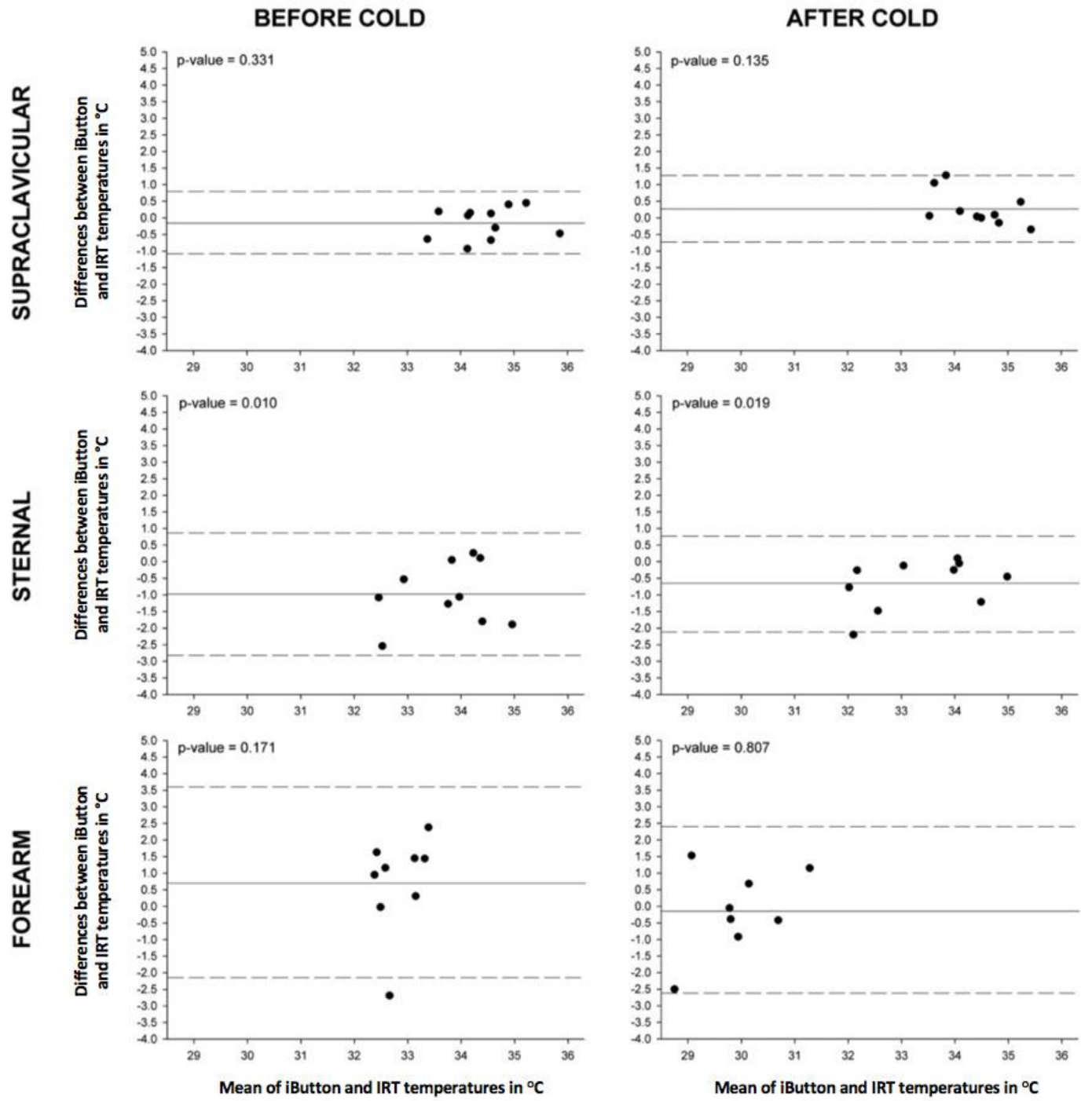

Fig. 2. Bland-Altman plots of the supraclavicular, sternal, and forearm mean skin temperatures measured with infrared thermography (IRT) [region of interest (ROI's) mean temperature] and iButtons during the shivering threshold test. The central lines represent the mean difference between the iButton and IRT measurements; the dashed lines represent the upper and lower 95% confidence intervals, respectively. The P values represent whether the mean differences were significantly different from 0.

SUVpeak). There was a negative association between the supraclavicular skin temperatures measured by IRT and BAT ^{18}F-FDG volume. However, we observed that supraclavicular skin temperature was the only parameter that did not decrease after the cold exposure. Therefore, further studies are needed in order to elucidate which tissues are involved in this physiology fact.

4.1. Concurrent validity

The interest in the thermoregulatory response to different stimuli (exercise, meal intake, or cold) has increased given the facility and the relatively low cost of implementing these measurements (Chondronikola et al., 2017). iButtons are small and inexpensive thermometers used in different fields. However, their validity is questioned, since these devices are able to measure by both sides (Hasselberg et al., 2013; Wouter D van Marken Lichtenbelt et al., 2006), although this

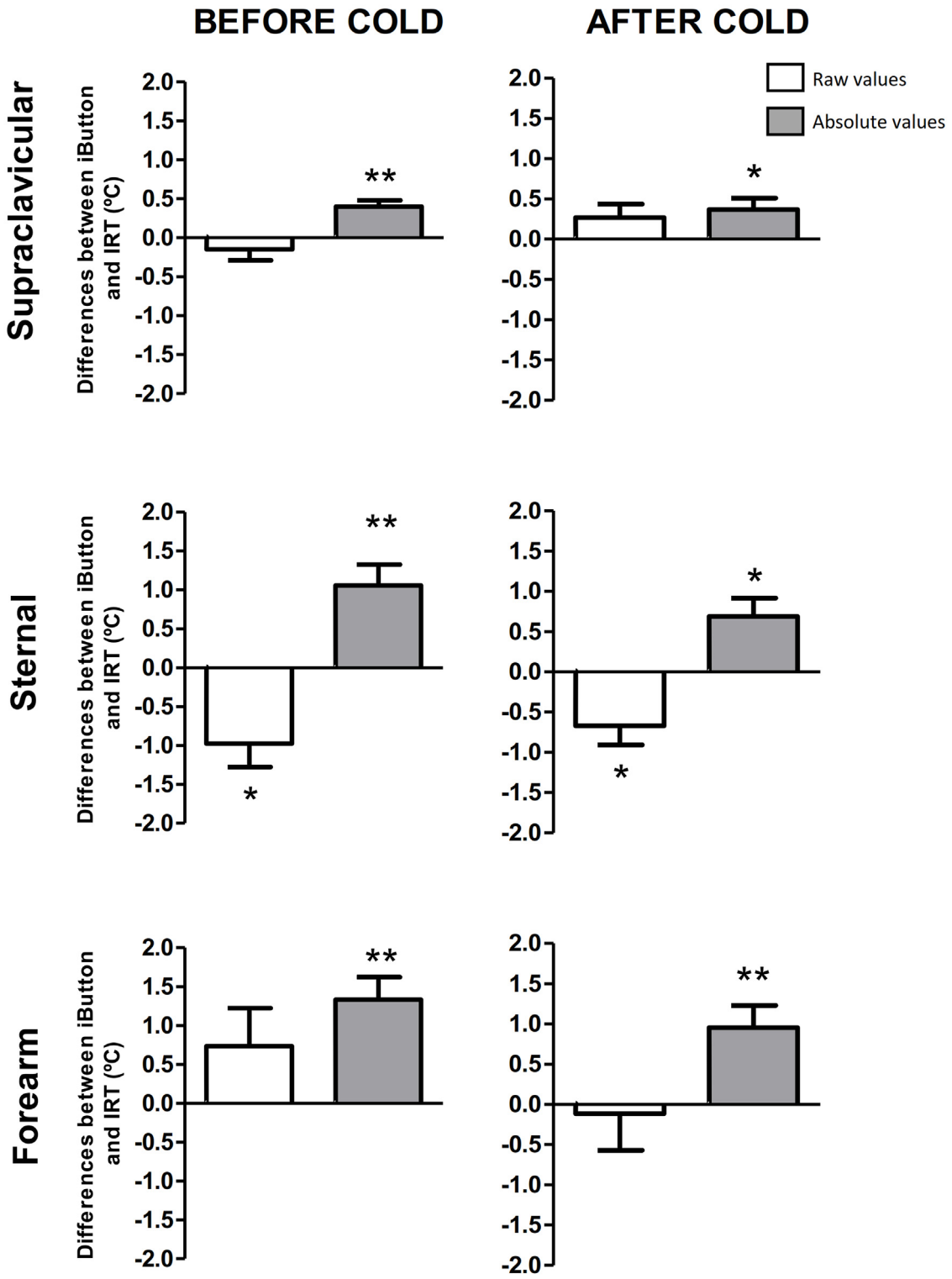

Fig. 3. Histograms show the values of skin temperature measured with iButtons minus the average of mean IRT in the supraclavicular, sternal, and forearm regions, before and after the cooling protocol. The white boxes depict raw values of the differences, whereas grey boxes show the absolute values. * Symbol means $P < 0.05$ in the one sample *t*-test from 0; ** means $P < 0.01$ in the one sample *t*-test from 0.

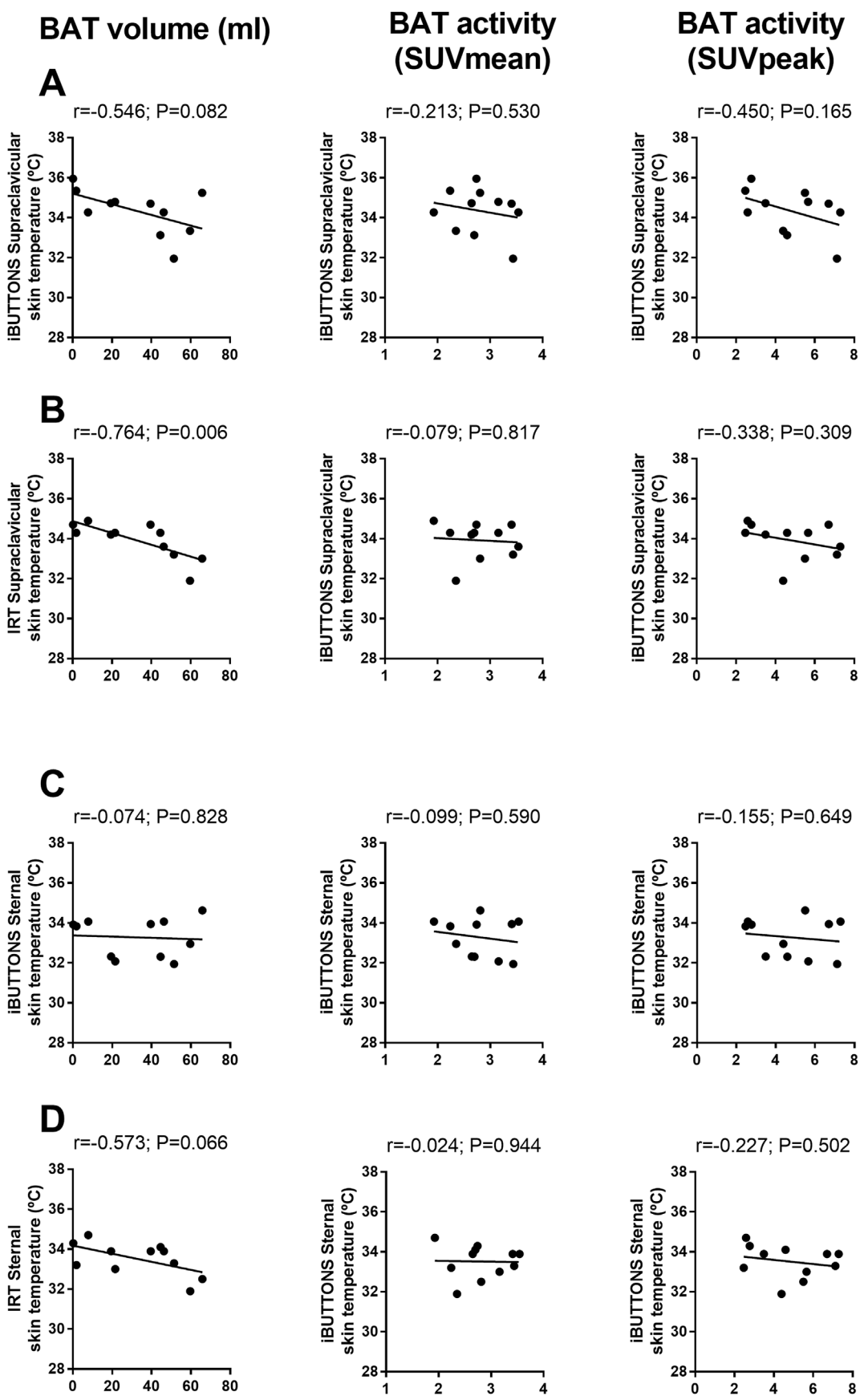

Fig. 4. Associations between supraclavicular skin temperature at the end of the personalized cooling protocol measured with iButtons (A) and infrared thermography (IRT) (B), and brown adipose tissue (BAT) volume and activity (SUV mean and SUVpeak). Panels C and D show the same association but with sternal instead of supraclavicular skin temperatures.

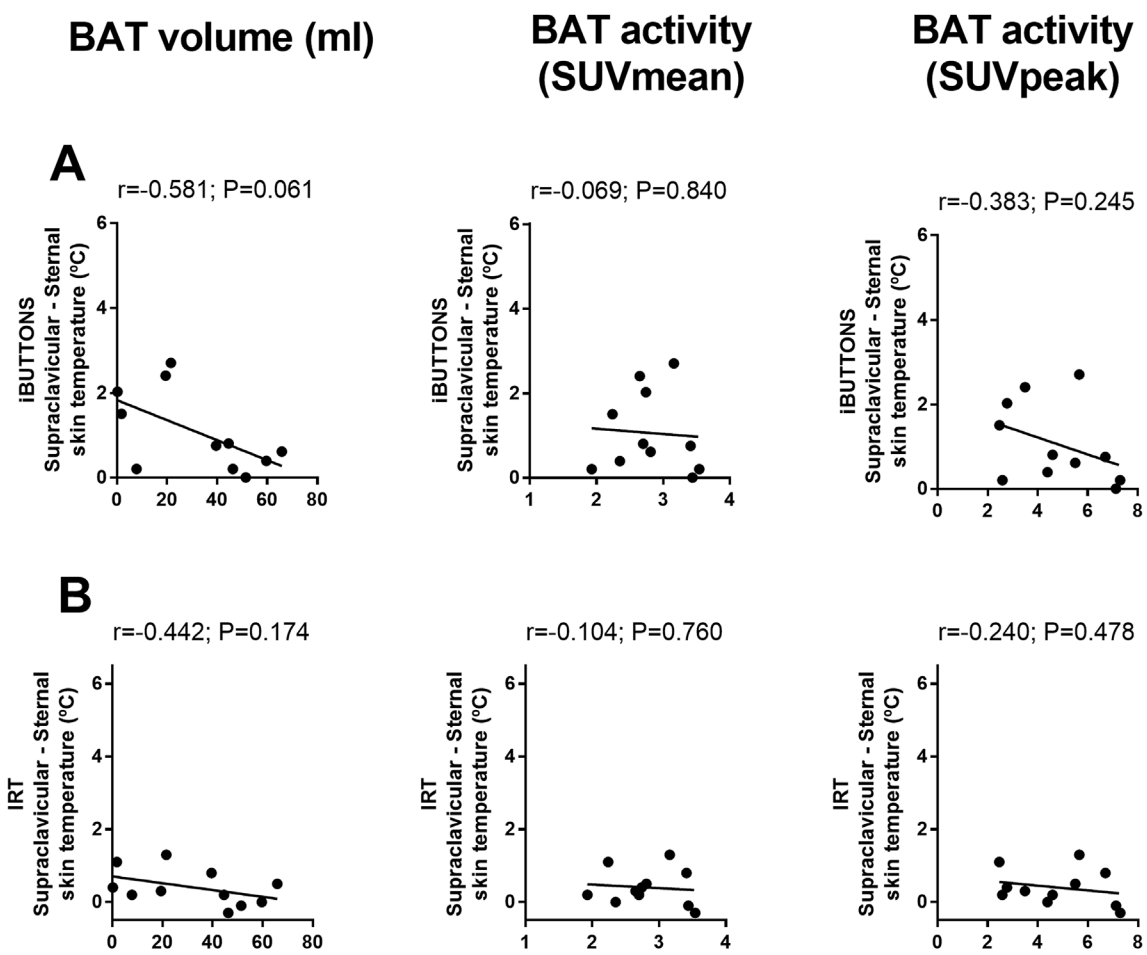

Fig. 5. Associations between supraclavicular skin temperature relative to sternal skin temperature at the end of the personalized cooling protocol and brown adipose tissue (BAT) volume and activity measured with iButtons (A) and by infrared thermography (IRT) (B).

issue has not been addressed yet. On the other hand, IRT creates an image by converting radiant heat energy into a signal (Chondronikola et al., 2017; Sarasniemi et al., 2018). In light of the present findings, we found that iButtons and IRT outcomes are not comparable. Currently, we do not know which device is better for the measurement of skin temperature, because these devices have not been compared with a device that truly measures skin temperature (for instance, mercury's thermometer or thermocouples), which is of pressing need in the field.

4.2. Supraclavicular skin temperature does not seem to be a valid tool to quantify human BAT in young adults

To date, only 2 studies have validated the use of supraclavicular skin temperature (measured with iButtons) as a surrogate marker of BAT activity measured with the ^{18}F-FDG-PET/CT scan. Both studies were conducted in young lean and healthy adults, but their results were controversial. Boon et al. (2014) found that supraclavicular skin temperature was associated with BAT activity (i.e. SUVmean and SUVmax) at the end of the cold exposure, whereas van der Lans et al. (van der Lans et al., 2016) found that the difference between supraclavicular skin temperature after-cold minus before cooling was associated with BAT activity (i.e. SUVmean and SUVmax). We found, however, no association between supraclavicular skin temperature measured with iButtons or by IRT and BAT activity. Law et al. (2018b) studied the validity of supraclavicular skin temperature measured by IRT with BAT. They performed the IRT and dynamic ^{18}F-FDG-PET/CT quantification on separate days and, more importantly, the thermal images were performed after 10 min of cold exposure, whereas the metabolic rate of glucose [MR(gluc)] by BAT was obtained after 60 min. They reported that when the supraclavicular skin temperature was relativized to sternal skin temperature, it was positively correlated with MR(gluc) by BAT in 8 lean male individuals, yet the absolute values of supraclavicular skin temperature were not associated. They justified performing the IRT pictures after 10 min of cold exposure, because BAT shows its higher peak of activity at this point. However, this assumption has not been demonstrated with nuclear medicine techniques. In the present work, we quantified supraclavicular skin temperature before and after 2 h of a personalized cooling protocol, we performed the IRT measurements just before performing the BAT quantification, and we applied the latest recommendation for static ^{18}F-FDG-PET/CT scan (Chen et al., 2016). We also demonstrated that the sternal skin temperature, compared to chin and cheekbone skin temperature, is a real reference location upon a cold exposure (Fig. S3). However, we failed to replicate the results obtained by Law et al. (2018b). We included a larger sample size and both men and women in comparison to Law et al. (2018b) [12 individuals (men and women) vs. 8 men]. In addition, our sample was less homogeneous in terms of body composition in comparison to Law et al. (2018b). Moreover, the study of Law et al. (2018b) used a different cooling protocols in comparison to the present study and this could partially explain the differences observed between studies.

Sarasniemi et al. (2018) recently observed that the supraclavicular skin temperature measured by IRT was negatively and significantly associated with BAT-depot temperature located in the supraclavicular region ($r = -0.83$, $P = 0.042$) only in obese participants. Similarly, we observed that supraclavicular skin temperature measured by IRT was negatively associated with BAT volume. We thought that our outlier

according to BMI levels (35 kg/m^2) could be driving the association, but when we removed this participant the negative significant association remained. Saraniesmi et al. (Sarasniemi et al., 2018) discussed that the supraclavicular fat layer (which is positively related to BMI (Gatidis et al., 2016)) could be playing a cofounding role in the measurement of supraclavicular by IRT. Indeed, when they included the supraclavicular fat layer as a co-variate, the negative association disappeared. Of note is that, when we included the fat mass percentage or BMI the observed negative association persisted. A possible explanation could be that these outcomes are not representative of the supraclavicular fat layer. Moreover, they performed the complete experiment in thermoneutral conditions, whereas we performed the experiment after a personalized cold exposure, and we found the same direction of association only in BAT volume. We cannot discard that the supraclavicular fat layer played an important role in our study because our sample was comprised of mainly normal weight individuals. Therefore, future studies addressing this issue are warranted.

Blondin et al. (2015) and U Din et al. (U Din et al., 2016) showed that skeletal muscles of the neck are highly involved in the cold-induced thermogenesis, which are also presented in the supraclavicular fossa (Kellman et al., 1987). In line with this hypothesis, we did not find any association with ^{18}F-FDG uptake by skeletal muscles. In addition, supraclavicular depots are highly irrigated by vessels and lymph nodes placed around the neck, which may affect their temperature (Kellman et al., 1987). The instruments currently used in this field are not able to distinguish between tissues; therefore, supraclavicular skin temperature should be interpreted as a holistic measure of the reaction of all the tissues, which are located in the supraclavicular fossa to different environmental conditions.

We do not know whether these results would be replicated using a magnetic resonance imaging or a PET/CT scan with other tracers (Chondronikola et al., 2017). Moreover, these analyses should be replicated in older and obese individuals. For future studies, the measurement of the supraclavicular fat layer should be included as a covariate in order to account for its potential effect. Despite the fact that iButtons seem to be a valid and reliable tool for the assessment of skin temperature (Smith et al., 2010; Wouter D. van Marken Lichtenbelt et al., 2006), these devices are able to measure temperature by both sides (Hasselberg et al., 2013; Wouter D van Marken Lichtenbelt et al., 2006), which could be an important limitation in human physiology studies. Therefore, how this issue affects the measurement of the skin temperature should be addressed in the near future. As a result, more studies comparing the IRT measurements with alternative devices (i.e. mercury thermometers or thermocouples) are encouraged.

5. Conclusions

The present study shows that iButtons and the mean measurement of IRT are not comparable. Moreover, we did not observe any association between supraclavicular skin temperature measured with iButtons or by IRT and BAT ^{18}F-FDG activity, as well as with ^{18}F-FDG uptake by skeletal muscle. We confirm a negative association between supraclavicular skin temperature measured by IRT and BAT ^{18}F-FDG volume, which could be partially explained by the supraclavicular fat layer. In light of these findings, supraclavicular skin temperature (regardless of the instrument used) is not a valid instrument to quantify ^{18}F-FDG uptake by BAT in young adults.

6. Declaration of interest

The authors declare they have no actual or potential competing financial interests.

Author contribution

Conceptualization, B.M.T., A.P.B., G.S.D., and J.R.R.; Data curation, B.M.T., A.P.B., and J.C.P; Formal analysis, B.M.T. and A.P.B; Funding acquisition, J.R.R.; All authors drafted and reviewed the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2019.04.009.

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