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Supraclavicular skin temperature measured by iButtons and ^{18}F-fluorodeoxyglucose uptake by brown adipose tissue in adults

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ABSTRACT

Currently, 18 [F]-Fluorodeoxyglucose (^{18}F-FDG) in combination with a positron emission tomography/computed tomography (PET/CT) scan analysis is the most commonly used method to quantify human BAT volume and activity. However, this technique presents several drawbacks which negatively affect participant's health. The aim of the present work is to determine whether supraclavicular skin temperature can be used as an indirect marker of cold-induced BAT and skeletal muscle ^{18}F-FDG uptake in adults, while taking into account body composition. We performed a personalized cooling protocol just before an ^{18}F-FDG-PET/CT scan, and we measured supraclavicular skin temperature before (in warm conditions) and after the cooling protocol in 88 adults (n = 57 women, mean age: 21.9 ± 2.1 years old, body mass index: 24.5 ± 4.3 km/m^2). We found that supraclavicular skin temperature at the warm and cold periods was weakly and positively associated with BAT activity (SUV_{mean} and SUV_{peak}: β = 3.000; R^2 = 0.072; P = 0.022 and β = 2.448; R^2 = 0.060; P = 0.021), but not with skeletal muscle ^{18}F-FDG uptake, after controlling for body composition. We performed further analyses and the positive associations persisted only in the group of women. In conclusion, supraclavicular skin temperature in warm and cold conditions seems to be related with cold-induced ^{18}F-FDG uptake by BAT only in women, although the low explained variance of these associations means that there are other factors involved in the supraclavicular skin temperature.

1. Introduction

In 2009, several independent studies showed unequivocally that brown adipose tissue (BAT) is metabolically active in adults upon cold stimulation (Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). Currently, 18 [F]-Fluorodeoxyglucose (^{18}F-FDG) in combination with a positron emission tomography/computed tomography (PET/CT) scan analysis is the most commonly used method to quantify human BAT volume and activity (Chondronikola et al., 2017; Nedergaard et al., 2007). However, this scanning technique exposes participants to ionizing radiation, and therefore the number of scans that can be performed is limited. Moreover, the tracer used is a derivative of glucose, while BAT mainly combusts fatty acids for thermogenesis (Schilperoort et al., 2016), urging the need for alternative techniques to quantify BAT (Hadi et al., 2017; Paulus et al., 2017). Future research is thus warranted to accurately, reproducibly, and practically assess human BAT and its metabolic function (Chondronikola et al., 2017).

Brown adipocytes have a large number of mitochondria containing the uncoupling protein 1 (UCP1), the main function of which is to generate heat (Cannon and Nedergaard, 2004). Since ^{18}F-FDG is mainly absorbed by BAT depots that are localized in the supraclavicular fossa

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Abbreviations

^{18}F-FDG	^{18}F-Flurodeoxyglucose
BAT	Brown adipose tissue
BMI	Body Mass Index
CP	Cold period
DXA	Dual-energy X-ray
PET-CT	Positron Emission Tomography-Computer Tomography
SUV	Standardized uptake value
WP	Warm period

(Leitner et al., 2017), supraclavicular skin temperature has been postulated as an indirect marker of BAT volume and activity (Boon et al., 2014; Lee et al., 2016a; Symonds et al., 2012; van der Lans et al., 2016). Indeed, whereas the temperature of most other skin surface areas decreases upon cold exposure, the supraclavicular skin temperature does not decrease or even slightly increases (Boon et al., 2014; Martinez-Tellez et al., 2017a; van der Lans et al., 2016). Therefore, it is biologically plausible that local skin temperature actually reflects BAT activity.

Supraclavicular skin temperature is commonly measured by iButtons, small temperature sensors that are attached to the skin (Hasselberg et al., 2013; van Marken Lichtenbelt et al., 2006), or by infrared thermography (El Hadi et al., 2016; Jang et al., 2014; Symonds et al., 2012). Boon et al. (2014) were the first to show that supraclavicular skin temperature measured by iButtons at the end of 2 h of cold exposure was positively correlated with BAT volume ($R^2 = 0.28$) and activity ($R^2 = 0.32$) as measured by ^{18}F-FDG-PET/CT scans in young healthy lean men. Similar results were reported by Lee et al. (2016b) and Yoneshiro et al. (2016) in young healthy lean men. Van der Lans et al. (van der Lans et al., 2016) showed that the difference in supraclavicular skin temperature between the end of the cold exposure and the warm period was positively correlated with BAT activity ($R^2 = 0.23$) measured by ^{18}F-FDG-PET/CT scan in young healthy lean men.

Since all of these studies determined the association between supraclavicular skin temperature and BAT activity in lean men only (Carla et al., 2017), it is currently unknown whether these findings also apply to women or individuals with overweight or obesity. Gatidis et al. (2016) reported that body composition influences supraclavicular skin temperature, as measured by infrared thermography, which would hamper the detection of activated BAT using indirect thermic measurements. Moreover, skeletal muscles may also contribute to cold-induced thermogenesis (CIT) by increasing shivering and non-shivering thermogenesis (Brychta and Chen, 2016). Deep skeletal muscles of the neck area (Blondin et al., 2015b; U Din et al., 2016) seem to be involved in the non-shivering thermogenesis before the onset of shivering is visually detected or self-reported by participants (Blondin et al., 2015b; U Din et al., 2016). Based on that, it is plausible that skeletal muscles of the neck could also be involved in maintaining the supraclavicular skin temperature constant upon cold exposure. To our knowledge, the association of supraclavicular skin temperature with skeletal muscle ^{18}F-FDG uptake has not been examined before.

In the present study, we aimed to determine the association of supraclavicular skin temperature (in warm and cold conditions) with cold-induced BAT and skeletal muscle ^{18}F-FDG uptake in lean and men and women with overweight and obesity, taking into account body composition.

2. Material & methods

2.1. Participants

A total of 88 young adults ($n = 57$ women) aged 21.9 ± 2.1 years old took part in the present study (Table 1). The participants were enrolled in the ACTIBATE study (Sanchez-Delgado et al., 2015) (ClinicalTrials.gov ID: NCT02365129). All participants were healthy, non-smokers, who did not take any medication that could influence cardiovascular or thermoregulatory responses to cold exposure and who reported to be sedentary (< 20 min physical activity on < 3 days/week). The informed consent and study protocol were performed in accordance with the last version of the Declaration of Helsinki. The study was approved by the Ethics Committee on Human Research of the University of Granada (n°924) and of the Servicio Andaluz de Salud (Centro de Granada, CEI-Granada). All participants carefully read and signed the informed consent. The evaluations were performed in four waves of ~17 participants each, in Granada, Spain from October 15th to December 6th during two consecutive years (2015–2016).

2.2. Personalized cooling protocol

On both study days, the participants arrived in fasting condition (at

Table 1
Descriptive characteristics of the participants (n = 88).

	All (n = 88)					Women (n = 57)					Men (n = 31)				
	Mean		SD	Min	Max	Mean		SD	Min	Max	Mean		SD	Min	Max
Age (years)	21.9	±	2.1	18.2	26.6	21.8	±	2.1	18.2	26.6	22.2	±	2.2	18.7	25.9
Body mass index (kg/m^2)	24.5	±	4.3	17.9	38.4	23.5	±	3.5	17.9	30.9	26.3	±	5.1	18.2	38.4
Fat mass (%)	35.5	±	7.7	15.6	52.2	38.3	±	6.1	28.7	52.2	30.4	±	7.7	15.6	44.7
Lean mass index (kg/m^2)	24.5	±	4.6	18.1	40.6	21.8	±	2.4	18.1	28.3	29.4	±	3.6	22.6	40.6
Fat mass index (kg/m^2)	14.6	±	5.1	5.9	29.6	14.8	±	4.4	8.3	25.6	14.3	±	6.3	5.9	29.6
Water temperature (°C) cooling vest 2 h before BAT quantification	9.4	±	3.1	3.9	16.7	10.4	±	2.6	3.9	16.6	7.8	±	3.3	3.9	15.0
SUV individualized threshold	2.01	±	0.24	1.52	2.68	2.12	±	0.21	1.80	2.61	1.88	±	0.21	1.52	2.31
Brown adipose tissue															
Volume (ml)	79.7	±	60.4	0	232.2	76.7	±	54.4	0	231.4	85.3	±	70.8	0	232.2
SUV_{mean}	3.96	±	1.92	0	8.60	4.24	±	2.10	0	8.60	3.46	±	1.44	0	7.09
SUV_{peak}	12.27	±	8.31	0	43.66	13.23	±	8.78	0	43.66	10.51	±	7.16	0	28.22
Right muscular ROIs (SUV_{peak})															
Longus Colli	1.32	±	0.50	0	2.70	1.35	±	0.57	0	2.70	1.25	±	0.34	0.77	2.25
Sternocleidomastoid	0.73	±	0.19	0.32	1.73	0.75	±	0.20	0.39	1.73	0.69	±	0.18	0.32	1.22
Scalene	1.21	±	0.78	0	4.41	1.28	±	0.86	0	4.41	1.08	±	0.61	0.43	3.36
Pectoralis major	0.49	±	0.26	0	1.04	0.53	±	0.23	0	1.01	0.43	±	0.29	0.00	1.04
Reference tissue (SUV_{peak})															
Descending aorta	1.60	±	0.34	0.73	2.45	1.58	±	0.31	0.93	2.26	1.63	±	0.41	0.73	2.45

SD: Standard deviation; SUV: standardized uptake value; ROIs: Regions of interest.

least 6 h), after having slept as usual, having refrained from any moderate (for 24 h) or vigorous (for 48 h) physical activity, and not having consumed alcoholic or stimulant beverages (for 6 h) (Martinez-Tellez et al., 2017c; Sanchez-Delgado et al., 2017). During the measurements, the participants wore standardized clothes (shorts, standard T-shirt) and were barefoot (clo value = 0.20). A detailed description of the cooling protocol can be found elsewhere (Martinez-Tellez et al., 2017c). In brief, the participants waited 30 min in a warm room for acclimation. In order to determine their shivering threshold, we reduced the temperature of a water perfused cooling vest (Polar Products Inc., Ohio, USA) until shivering was self-reported and was visually observed by the researchers. After a period of 48–72 h, we subjected the participants to a personalized cold exposure (3.8 °C above their personal shivering threshold) during 2 h using the cooling vest and sitting in a mild cold-air room (19.5–20 °C). After the first hour of cold exposure, we administered ^{18}F-FDG intravenously (185 MBq: ~2.78 MBq/kg), and we increased the water temperature by 1 °C to avoid shivering. After the second hour of cold exposure, we removed all iButtons and performed a static PET/CT scan (Siemens Biograph 16 PET/CT, Siemens Germany). Two bed positions were scanned from approximately *atlas vertebrae* to *thoracic vertebrae* 4.

2.3. PET/CT analysis

The PET/CT images were analyzed using the Beth Israel plugin for FIJI (Cypess et al., 2009) software under the supervision of a nuclear medicine physician (Schindelin et al., 2012). The regions of interest (ROIs) were semi-automatically outlined from *atlas vertebrae* (Cervical 1) to *thoracic vertebrae* 4 using a 3D-Axial technique (Leitner et al., 2017). Regions such as mouth, nose, or thyroid were not included to avoid potential false positives within the ROIs. We calculated the standardized uptake value (SUV) as ^{18}F-FDG uptake (kBq/mL)/(injected dose [kBq]/patient weight [g])]. We defined BAT volume, SUV_{mean}, and SUV_{peak} following BARCIST 1.0 criteria [(SUV thresholds = (1.2/(lean body mass/body mass)); and Hounsfield Units (HU) between −10 and −190] (Chen et al., 2016). In short, BAT volume was calculated as the sum of the volumes identified as BAT in each ROI. SUV_{mean} was calculated by the weighted average of SUV_{mean} derived from each ROI(Martinez-Tellez et al., 2018a, 2018b). SUV_{peak} was the average SUV in a 1 ml spherical volume centered on the highest SUV_{max} over all ROIs. We also drew a ROI on the descending aorta as reference tissue (Chen et al., 2016). Since we only included the upper part of the body in our PET/CT scan, we quantified the FDG uptake (SUV_{peak}) of several skeletal muscles from atlas vertebrae to thoracic vertebra 4. We drew a single ROI of 1 slice in the paracervical, sternocleidomastoid, scalene, longus colli, trapezius, parathoracic, supraspinatus, subscapular, deltoid, pectoralis major, and triceps brachii muscles from both left and right side of the body (Blondin et al., 2015b; Hanssen et al., 2016). An average of left and right sides, and of all skeletal muscles was calculated in order to obtain a single representative value of the skeletal muscle ^{18}F-FDG uptake of the upper part of the body for every participant.

2.4. Skin temperature

Supraclavicular skin temperature was measured with one iButton (DS-1922 L, Thermochron; resolution 0.0625 °C; Maxim, Dallas, USA) placed on the right supraclavicular fossa (Boon et al., 2014; van der Lans et al., 2016). Moreover, we attached 16 additional iButtons on different parts of the body (Martinez-Tellez et al., 2018a, 2018b; Martinez-Tellez et al., 2017a) with adhesive tape (Fixomull, Beiersdorf AG, Hamburg, Germany). Skin temperature was recorded at 1 min intervals, and we estimated distal (Kräuchi et al., 1997) and mean (ISO-standard 9886:2004 Ergonomics – Evaluation of thermal strain by physio-logical measurements, International Standards Organization, Geneva, 2004) skin temperatures. We calculated the average of the last 5 min in the warm period (WP Supraclavicular skin temperature), the average of the last 5 min after the 2 h of personalized cooling protocol (CP Supraclavicular skin temperature), and the difference between CP Supraclavicular skin temperature and WP Supraclavicular skin temperature. We also calculated the average of every 5 min for distal and mean skin temperature parameters. All data recorded by the devices were processed and analyzed by the Temperatus® software (Version 1; Universidad de Granada, 2017; (Martinez-Tellez et al., 2019).

2.5. Body composition

The body composition was measured by DEXA (HOLOGIC, Discovery Wi). We measured the participants' weight and height without shoes while wearing standard clothes (see above) using a SECA scale and stadiometer (model 799, Electronic Column Scale, Hamburg, Germany), and we calculated their body mass index (BMI) (kg/m^2). The participants were categorized as normal-weight (BMI ≥18.5 and < 25 kg/m^2), overweight (BMI ≥25 and < 30 kg/m^2) and obese (BMI ≥30 kg/m^2) (Cole et al., 2007). Fat mass index (FMI) was calculated as kilograms (kg) of body fat divided by height in m^2, and lean mass index (LMI) was calculated as lean body mass in kg divided by height in m^2.

2.6. Statistical analysis

The data are presented as mean ± standard deviation, unless otherwise stated. We calculated the difference between the last 5 min of the cold period minus the last 5 min of the warm period for mean, distal and supraclavicular skin temperature. To study whether these differences were statically different from 0, we performed one sample *t*-test. We calculated these differences in all the sample and then in women and men. To study the association of supraclavicular skin temperature (at the warm period, after the cold period, and the difference from cold

Table 2
Associations between the supraclavicular skin temperature in the warm period (WP), in the cold period (CP), and the difference in supraclavicular temperature from the warm to the cold period with brown adipose tissue (BAT)-related outcomes and the average of skeletal muscle 18F-Fluorodeoxyglucose uptake.

		WP Supraclavicular skin temp.				CP Supraclavicular skin temp.				ΔSupraclavicular skin temp.			
		n	β	R^2	P	n	β	R^2	P	n	β	R^2	P
BAT volume (ml)	Women	46	11.826	0.039	0.186	57	6.921	0.01	0.450	46	−4.039	0.004	0.664
	Men	27	−9.759	0.009	0.633	31	8.481	0.01	0.596	27	20.776	0.053	0.246
BAT SUVmean	Women	46	1.04	0.141	**0.010**	57	0.765	0.086	**0.027**	46	−0.327	0.013	0.448
	Men	27	0.218	0.009	0.631	31	0.291	0.028	0.369	27	0.193	0.009	0.632
BAT SUVpeak	Women	46	4.069	0.113	**0.013**	57	2.625	0.059	0.069	46	−1.705	0.022	0.327
	Men	27	0.237	< 0.001	0.916	31	1.38	0.025	0.392	27	1.435	0.021	0.469
SM SUVpeak	Women	46	−0.072	0.071	0.079	57	−0.035	0.019	0.313	46	0.033	0.014	0.437
	Men	27	−0.057	0.04	0.32	31	0.004	< 0.001	0.928	27	0.056	0.049	0.266

SM; Skeletal muscle; β, unstandardized beta coefficient; R^2, coefficient of determination. P-values in bold denote significant differences. Dashed lines show x-axes for 0.

to warm) with BAT-related outcomes (i.e. volume, SUV_{mean}, and SUV_{peak}) and skeletal muscle activity (i.e. SUV_{peak}), we conducted linear regression analyses with BAT-related outcomes and skeletal muscle activity as dependent variables and supraclavicular skin temperature as the independent variable. The analyses were conducted in separate regression models. The analyses were repeated including BMI or FMI or LMI as covariate in a single model each one. There was no interaction between sex, supraclavicular skin temperature, and the BAT- or skeletal muscle-related outcomes studied (all $P > 0.05$). Therefore, we conducted the main analyses in all the samples. Nevertheless, data are also reported in women and men separately (Table 2). All of the analyses were conducted using the Statistical Package for Social Sciences (SPSS, v. 22.0, IBM SPPS Statistics, IBM Corporation, Armonk, NY, USA), and the level of significance was set at < 0.05.

3. Results

3.1. Cold exposure and skin temperature

Two hours of personalized cold exposure decreased the mean (-2.7 ± 0.9 °C) and distal (-6.5 ± 1.6 °C) skin temperature (both $P \leq 0.001$) and these differences were statically different from 0 in the whole sample. Nevertheless, supraclavicular skin temperature was not statically different from 0 (-1.0 ± 0.7 °C, $P = 0.053$) (Fig. 1). We repeated the analyses separately by sex and we found similar results (Fig. 1).

3.2. Supraclavicular skin temperature and BAT volume and activity

No associations were observed between the supraclavicular skin temperature measured in the warm period (Fig. 2A), at the end of the cold period (Fig. 2D), and the difference (from cold to warm, Fig. 2G) with BAT volume. We repeated these associations by sex and we did not observe any significant association (see Table 2). The supraclavicular skin temperature measured in the warm and cold period was positively associated with BAT activity (SUV_{mean}) ($\beta = 0.822$; $R^2 = 0.097$; $P = 0.007$ and $\beta = 0.669$; $R^2 = 0.084$; $P = 0.006$, see Fig. 2B and E, respectively) and SUV_{peak} ($\beta = 3.000$; $R^2 = 0.072$; $P = 0.022$ and $\beta = 2.448$; $R^2 = 0.060$; $P = 0.021$, see Fig. 2C and F, respectively). There was no association between the difference in supraclavicular skin temperature and SUV_{mean} (Fig. 2H) or SUV_{peak} (Fig. 2I). The observed associations persisted after including BMI, LMI, or FMI as covariates (Table S1). Moreover, when we entered BMI in the model, the supraclavicular skin temperature in the cold period was positively associated with BAT volume ($\beta = 16.540$; $R^2 = 0.138$; $P = 0.039$). We repeated these associations by sex and we observed that supraclavicular skin temperature in the warm period were positively associated with BAT activity (SUV_{mean} and SUV_{peak}) only in women ($\beta = 1.040$ $R^2 = 0.141$; $P = 0.010$ and $\beta = 4.069$; $R^2 = 0.113$; $P = 0.013$), see Table 2. Moreover, in the cold period the positive results only persisted in women and with SUV_{mean} ($\beta = 0.765$; $R^2 = 0.086$; $P = 0.027$). We did not find any significant association between supraclavicular skin temperature and BAT-related outcomes in men (see Table 2).

We repeated the analysis by BMI categories (Table S2) and found that the supraclavicular skin temperature at the end of the cold exposure was positively associated with BAT activity only in overweight individuals ($n = 20$; SUV_{mean}: $\beta = 0.900$; $R^2 = 0.243$; $P = 0.027$ and SUV_{peak}: $\beta = 5.284$; $R^2 = 0.258$; $P = 0.022$).

3.3. Supraclavicular skin temperature and skeletal muscle ^{18}F-FDG uptake

There was no association between the supraclavicular skin temperature in the warm period (Fig. 3A), in the cold period (Fig. 3B), and difference in supraclavicular skin temperature from the warm to the cold period (Fig. 3C) with skeletal muscle ^{18}F-FDG uptake (all $P > 0.05$). We found similar results when we conducted the analyses by sex (Table 2). Moreover, we observed that the supraclavicular skin temperature at the end of the cold exposure was positively associated with skeletal muscle uptake ($\beta = 0.274$; $R^2 = 0.423$; $P = 0.042$) in adults with obesity (Table S2).

3.4. Additional analysis

All associations persisted when we calculated the BAT volume and activity only from the right supraclavicular area. These results also remained unchanged when we quantified BAT for sensitivity analysis using other SUV and HU criteria (SUV: 2.0; HU: Not applied (Boon et al., 2014) and SUV: 1.5; HU: −10 to −180 (van der Lans et al., 2016)). Furthermore, the results persisted when BAT and skeletal muscle ^{18}F-FDG uptake were multiplied by LBM% (Leitner et al., 2017) (data not shown). These results also remained unaltered when we introduced as covariate the water temperature of the cooling vest (data not shown).

4. Discussion

The results of the present study show that supraclavicular skin temperature, measured before and after cold-exposure, is slightly associated with cold-induced BAT activity (SUV_{mean} and SUV_{peak}) in a group of men and women with a wide range of BMI, and that these associations are independent of body composition. Further analyses showed that the women group is driven this positive and significant associations. In contrast, supraclavicular skin temperature is not associated with BAT volume or with skeletal muscle ^{18}F-FDG uptake, either for women or men. Stratified analyses by BMI categories showed that supraclavicular skin temperature at the end of cold exposure is also associated with BAT volume, but only in overweight participants, and with skeletal muscle ^{18}F-FDG uptake in participants with obesity. Taken together, these findings suggest that supraclavicular skin temperature in both warm and cold conditions is slightly associated with cold-induced human BAT activity. These findings should, however, be taken with caution because the variance explained is relatively low and because we observed that this parameter of temperature in participants with obesity could be related to skeletal muscle ^{18}F-FDG uptake.

4.1. Supraclavicular skin temperature and BAT volume and activity

Our finding that supraclavicular skin temperature at the end of the cold period associates with ^{18}F-FDG uptake by BAT (all $R^2 \leq 0.1$)

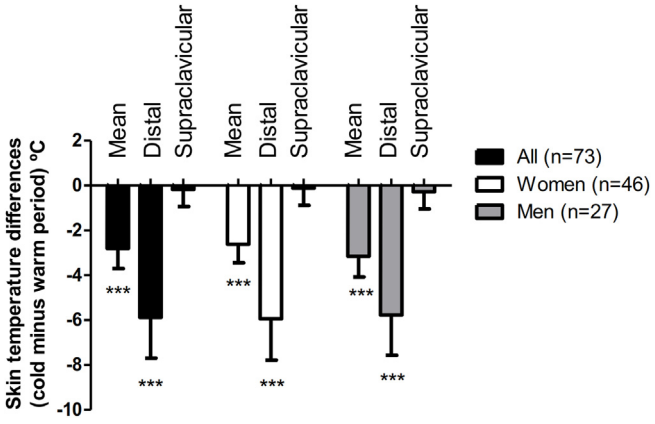

Fig. 1. Differences between mean, distal and supraclavicular skin temperature the last 5 min of the cold period minus the last 5 min of the warm period, in all (n = = 73), women (n = 46) and men (n = 27). *** means that the difference of skin temperature is significant different from 0 ($P \leq 0.001$). Data are presented as means and standard deviation.

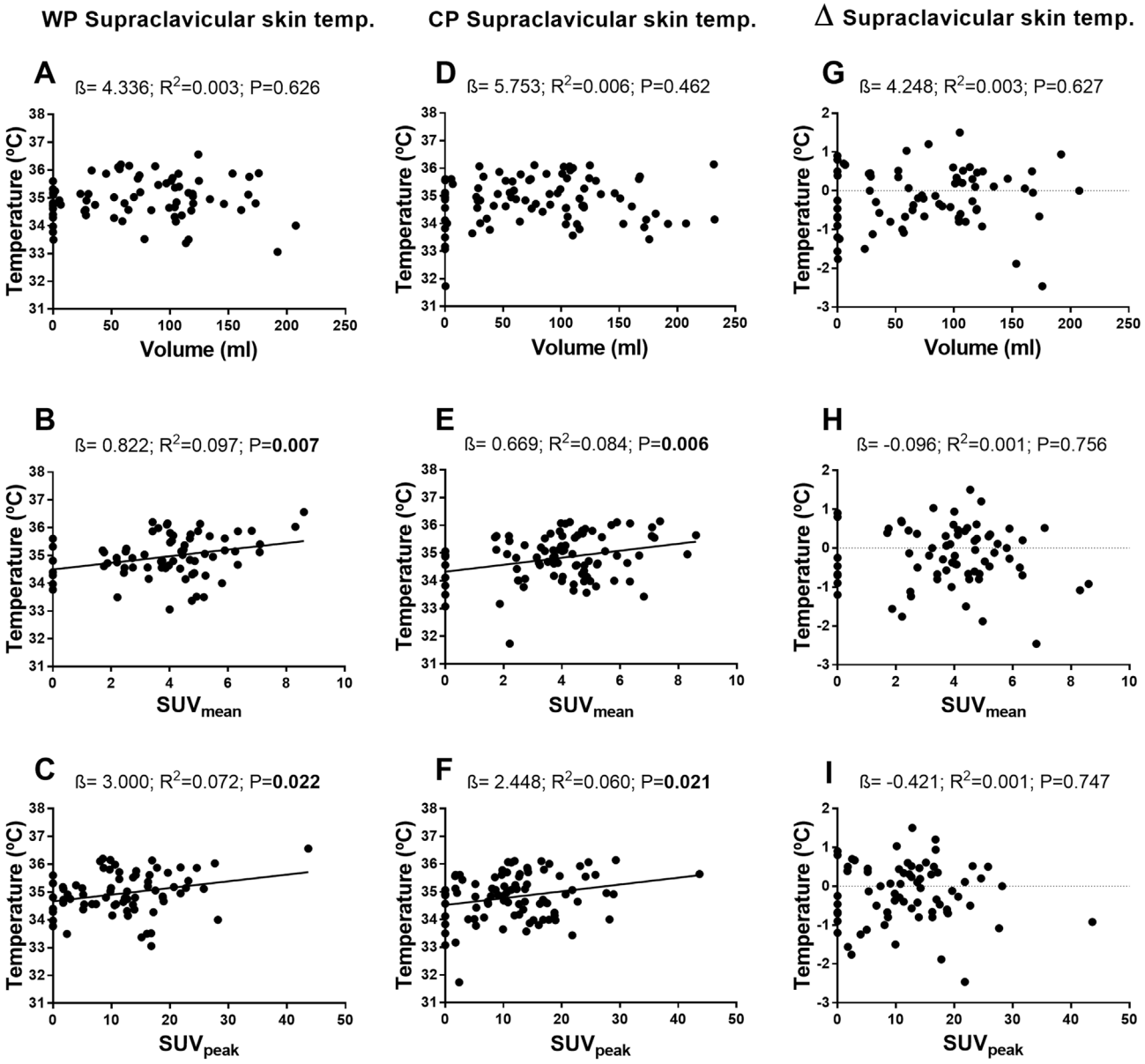

Fig. 2. Associations between the supraclavicular skin temperature in the warm period (WP) (A, B, C; n = 73), in the cold period (CP) (D, E, F; n = 88), and the difference in supraclavicular temperature from the warm to the cold period (G, H, I; n = 73) with brown adipose tissue (BAT) volume (A, D, G) and activity: SUV_{mean} (B, E, H), and SUV_{peak} (C, F, I). β, unstandardized beta coefficient; R^2, coefficient of determination. P-values in bold denote significant differences. Dashed lines show x-axes for 0.

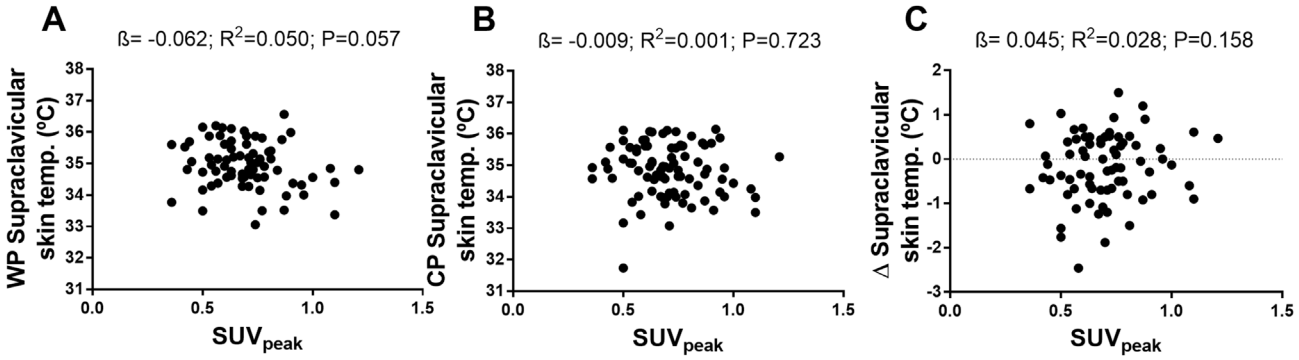

Fig. 3. Associations between the supraclavicular skin temperature in the warm period (A; n = 73), in the cold period (B; n = 88), and the difference in the supraclavicular temperature from the warm to the cold period (C; n = 73) and the average of the skeletal muscle 18 [F]-Fluorodeoxyglucose uptake after 2 h of a personalized cold exposure. Dashed lines show x-axes for 0.

concur with those reported by Boon et al. (2014), although they found a somewhat stronger association between supraclavicular skin temperature at the end of cold exposure and BAT ^{18}F-FDG uptake (SUV_{mean} and SUV_{max}, R^2 up to 0.20). Boon et al. (2014) also showed that supraclavicular skin temperature is associated with BAT volume, which is in agreement with our findings when BMI was included in the analysis. On the other hand, van der Lans et al. (van der Lans et al., 2016) showed that the difference in supraclavicular skin temperature from the warm to the cold period was positively associated with BAT activity (SUV_{mean}; $R^2 = 0.23$), yet no data were reported about supraclavicular skin temperature at the end of warm and cold exposures or its association with BAT volume, similar results were reported by Lee et al. (2016b) and Yoneshiro et al. (2016) in young healthy lean men. Therefore, the lower explained variance in our study could means that the temperature of the supraclavicular part of the body is not solely responsible to BAT activity, as others recently suggested (Sarasniemi et al., 2018).

Discrepancies across studies can be partially due to differences in (i) SUV and HU criteria to quantify BAT (Martinez-Tellez et al., 2017b), (ii) cooling protocols, (iii) characteristics of the participants (sex, age, body composition, and ethnicity), and (iv) sample size. We repeated the BAT analyses after applying the SUV and HU criteria used in the study by Boon et al. (2014) and van der Lans et al. (van der Lans et al., 2016), and found that our significant associations persisted. Of note is that we used a similar personalized cooling protocol as the aforementioned studies. The only difference was that they determined the shivering threshold just before the 2 h of the personalized cooling protocol, whereas we determined the shivering threshold on a previous day (Martinez-Tellez et al., 2017c). Since our cooling protocol has a shorter accumulative cold exposure during the day, our protocol possibly induces less fatigue on BAT (because with this protocol, BAT (Martinez-Tellez et al., 2017c) needs to be active less time), as occurs with other tissues (Haman and Blondin, 2017). Boon et al. (2014) studied lean young adults of two different ethnicities (white Caucasian and south Asian), and van der Lans et al. (van der Lans et al., 2016) studied lean young white Caucasians. Our participants were mostly white Caucasians (more than 98%) who were slightly younger and showed higher SUV_{peak} values compared to the participants in the study by Boon et al. (2014) and van der Lans et al. (van der Lans et al., 2016) (21.9 ± 2.1; 24.1 ± 0.6 vs. 23.4 ± 3.6 years old, respectively) and it is known that the prevalence and activity of BAT, at least based on ^{18}F-FDG uptake, is higher at young age (Ouellet et al., 2011). In addition, while Boon et al. (2014) and van der Lans et al. (van der Lans et al., 2016) only included men, we included both men and women with different ranges of BMI. We observed no sex interaction between supraclavicular skin temperature and BAT-related parameters, which suggests that the pattern of the association is similar in men and women. Nevertheless, we repeated the analyses by sex and the positive associations only persisted in women. This could be explained by the higher sample size in women than men, or the fact that our women group were leaner in comparison to men. Furthermore, the total sample size in our study is higher (n = 88) than that of Boon et al. (2014) (n = 24) and van der Lans et al. (van der Lans et al., 2016) (n = 36), which implies that we had more statistical power (Field, 2009) while reducing and potential spurious associations.

4.2. Supraclavicular skin temperature and skeletal muscle ^{18}F-FDG uptake

During cold exposure, energy expenditure increases to maintain a stable core body temperature in a process defined as cold-induced thermogenesis (CIT) (Brychta and Chen, 2016). For many years, it was thought that the main contributor to non-shivering thermogenesis (Brychta and Chen, 2016) was BAT by means of UCP1, yet growing evidence indicates that skeletal muscle might also play a key role in non-shivering thermogenesis by sarcoplasmic/endoplasmic reticulum calcium ATPases (SERCAs) (Betz and Enerbäck, 2017). Skeletal muscles of the neck (U Din et al., 2016) and deep muscles (Blondin et al., 2015b) seem to contribute to CIT. Anatomically, supraclavicular and cervical BAT depots are very close to neck and deep skeletal muscles. Based on the arguments that other studies have put forward, there is no reason to think that the increase or slight decrease of the supraclavicular skin temperature during a cold exposure could be due to the activity of both thermogenic tissues (BAT and skeletal muscles). Indeed, there are also several important blood vessels in the supraclavicular/neck zone (as carotid artery) that could also be involved in the supraclavicular skin temperature (Kellman et al., 1987). With the available instruments (iButtons or infrared thermography), we cannot distinguish the contribution of different tissues to supraclavicular skin temperature. Without doubt, further studies with more advanced instruments are needed to better understand what is actually involved in the supraclavicular skin temperature, especially the possible involvement of the skeletal muscles in this region.

4.3. Body composition

Participants with obesity have higher subcutaneous adipose tissue stores in the fossa supraclavicular (Gatidis et al., 2016). Therefore, supraclavicular BAT depots are less close to the skin surface in participants with obesity in comparison to some skeletal muscles (e.g. trapezious), whereas BAT and skeletal muscles are both closer to the skin surface in lean participants. This could partially explain why we found such a high R^2 (0.42) between supraclavicular skin temperature and ^{18}F-FDG uptake by skeletal muscles in adults with obesity. However, in normal-weight individuals we did not find any relationship between supraclavicular skin temperature and BAT-related outcomes or skeletal muscle ^{18}F-FDG uptake. In fact, the results remained unchanged when we entered BMI in the model, and we also found a positive and significant association of supraclavicular skin temperature at the end of the cold period with BAT volume, as reported previously (Boon et al., 2014). Thus, these results show that supraclavicular skin temperature is influenced by body composition. Moreover, Sarasniemi et al. (2018) recently showed that supraclavicular skin temperature measured by infrared thermography is highly influence by supraclavicular fat layer, especially in subjects with obesity, although they did not apply a cold exposure before performing the measurements.

The significant associations found between the supraclavicular skin temperature at the warm period and BAT activity suggest that this instrument could be used as an indirect marker of cold-induced BAT activity. However, the low explained variance showed that supraclavicular skin temperature depends on the factors, not only BAT activity. Likewise, Nirengi et al. (2015) found that near-infrared time-resolved spectroscopy (NIR_{TRS}) parameters in thermoneutral conditions correlated with cold-induced BAT measured by ^{18}F-FDG-PET/CT scan. In addition, Law et al. (2018) reported that the difference between sternal and supraclavicular skin temperature measured by IRT positively correlated with BAT activity in 8 lean adults. Currently, we do not know whether skin temperature measured by iButtons or IRT are measuring the same and therefore, further studies are needed to confirm these findings and to validate the possible use of supraclavicular skin temperature as an indirect marker of BAT activity in thermoneutral or cold conditions.

4.4. Limitations

We measured the supraclavicular skin temperature with iButtons (Martinez-Tellez et al., 2017a), which may not only reflect heat production by BAT, but also by surrounding tissues. We did not measure core body temperature. Furthermore, we measured ^{18}F-FDG uptake by skeletal muscles of the upper part of the body with a static PET/CT scan, neglecting potential contributions of other skeletal muscles including *psoas-iliac* and *rectus femoralis*. We have determined associations between supraclavicular temperature and uptake of ^{18}F-FDG by BAT only, hence the associations with other measures of BAT activity

(e.g. ^{18}F-FTHA uptake or ^{11}C-acetate uptake (Blondin et al., 2015a), magnetic resonance imaging (Gifford et al., 2016), or dynamic acquisition) are still unknown. Moreover, further studies are needed in older men and women with different ranges of BMI and ethnicity to translate our findings to the general population.

5. Conclusions

Supraclavicular skin temperature before and after cold exposure is slightly associated with cold-induced BAT activity (SUV_{mean} and SUV_{peak}). These positive associations seem to be driven by the women group. We observed that this positive association persisted independently of the body composition. However, the low explained variance of these associations means that there are other tissues involved in the supraclavicular skin temperature.

Competing financial interest

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

Author contributions

Conception and design of the study: B.M.T. and J.R.R.; B.M.T., G.S.D., Y.G.R., H.X., F.J.A.G., F.A.M., M.R.B., P.C.R., J.M.L., and J.R.R. performed the experiments; B.M.T., G.S.D., Y.G.R., H.X., F.J.A.G., F.A.M., M.R.B., P.C.R., J.M.L., and J.R.R. analyzed the data; B.M.T., G.S.D., Y.G.R., H.X., F.J.A.G., F.A.M., M.R.B., P.C.R., J.M.L., and J.R.R. interpreted the results; B.M.T. and J.R.R. prepared the figures and drafted the manuscript. All authors critically revised the manuscript and approved the final version.

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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.2019.04.006.

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