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Subcutaneous and Visceral Fat: Relation with Brown Adipose Tissue Activation in Women

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ABSTRACT

Brown adipose tissue (BAT) helps control body weight and is inversely correlated with body fat, but it is unclear whether it is subcutaneous adipose tissue (SAT) or visceral adipose tissue (VAT) that is related to BAT activation. The presented study aimed to verify the relation of SAT and VAT on BAT activation through infrared thermography (IRT) and cold stimulation in adult women. Forty women were evaluated in body composition and skin temperature (Tskin) acquisition by IRT. Student's independent t-test, Pearson's correlation, and two-way repeated measures ANOVA with Tukey post-hoc were applied. Women with low amounts of SAT and VAT had a significant increase in supraclavicular Tskin (SCVT). Medium negative degrees of linear variation were found before and after cold stimulation between SCVT, SAT and VAT. A significant effect of the moment factor and the group factor on the SCVT between subjects divided into the groups were pointed out. No difference was found in the relation between SAT, VAT, and BAT in adult women, pointing out that both types of fat are equally related. These results can help clinical practice understand clearly, through IRT, that the high accumulation of SAT and VAT can impair the activation of BAT and hinder the loss of weight in women.

Introducion

The scientific literature is quite robust regarding the importance of adipose tissue acting as an essential endocrine organ in the regulation of energy metabolism [1]. Adipose tissue can be classified according to its morphological and functional characteristics into white adipose tissue (WAT), beige adipose tissue (BeAT) and brown adipose tissue (BAT) [2, 3]. It is understood that most body adipose tissue is formed by WAT whose main function is to store energy in the form of triglycerides [1]. WAT may also be called subcutaneous adipose tissue (SAT) when deposited under the skin and visceral adipose tissue (VAT) when it is deposited around the viscera [1]. BeAT originating from WAT precursor cells can be defined as a cell with mixed characteristics of WAT and BAT and localized in small amounts between WAT [3]. BAT, whose main function is the production of energy in the form of heat, is deposited, in adults, mostly in the supraclavicular region (SCV) [2].

In the fight against the health problems caused by excess weight and possible non-transmissible chronic diseases, BAT appears as an important element [4–6]. Through its thermogenic activity, BAT can help control body weight and reduces risk factors for metabolic diseases [4, 7]. The oxidation of lipids to produce heat stimulated by cold or diet in BAT cells is caused by the high intensity of mitochondrial and consequently uncoupling protein-1 (UCP-1) [8]. The uncoupling between oxygen consumption and ATP synthesis induces the energy dissipation in the form of heat, thus helping to reduce glucose and lipid levels and, consequently, weight loss [7–9]. It is important to note that, like WAT, BAT is found in greater amounts in women than in men [10, 11]. Herz et al. [11] pointed out that BAT activation by cold stimulation and PET-CT was greater, but not significant, in premenopausal women than in men.

Analyzing BAT activation did not seem like such an easy task, because it used to require examination of data obtained with positron emission tomography (PET-CT) using 18F-fluordeoxyglucose (FDG), which made it difficult. After all, it is an expensive and time-consuming test, with a high rate of exposure to radiation [12]. On the other hand, with the evolution of infrared thermography (IRT), evaluating the BAT became simpler, because by measuring the skin temperature (Tskin) in the SCV region, it is possible to estimate the activation of the BAT in less time, with low risk and good accuracy [12, 13].

BAT activation is inversely correlated with body mass index (BMI), percentage of total body fat (%BF) and VAT, proving that somehow WAT is negatively associated with non-shivering thermogenesis [14, 15]. A recent survey analyzed 4852 patient scans performed with PE-CT-FDG and found that VAT and SAT were independent factors related to BAT [15]. However, this same study concluded that VAT, age and sex were more important in BAT activity under thermoneutral conditions [15].

Despite these findings, to our knowledge there are no studies that provide joint information on the possible effects of SAT and VAT, using IRT, on the physiological functioning of BAT cells when exposed to cold in humans. It is still important to emphasize that research involving BAT and IRT in only groups of women, who notably have more adipose tissue than men, is scarce [16–18]. It is hypothesized in this study that VAT is more closely related to BAT activation, since it eliminates more inflammatory cytokines when compared to SAT, which may cause some alteration in the non-shivering thermogenesis process.

Thus, the presented study aimed to verify the relation of SAT and VAT on BAT activation through IRT and cold stimulation in adult women. This study is justified by the need for further clarification as to which type of adipose tissue can be more harmful to the BAT activation process, negatively influencing body weight loss.

Methods

Study population

The present research included a total of 40 healthy female members of the military divided into two groups using the median of variables: %SAT (Over vs. Under) and %VAT (Over vs. Under). Female active-duty military personnel aged between 27 and 56 years were included. Every subject who underwent surgery in the entire trunk region and in menopause was excluded. This study was designed according to Ethics Commission approved by Doc103-CE-202 and CAAE:40495120.9.0000.9433, in agreement with the Declaration of Helsinki [19].

Data collection

On the day before the beginning of the data collection, the subjects were instructed to: 1) remain fasting for 12 hours, 2) not perform physical exercises 24 hours before the collection, 3) not shave the clavicular and sternum regions, 4) not consume alcohol 24 h beforehand, 5) avoid direct sun or UV, and 6) avoid any medication with a thermogenic substance. On the day of collection, the subjects could not wear accessories next to the body, not ingest caffeinated substances, not use cosmetic products and not smoke [20]. All data were collected in the morning starting from 7:30 am to 11:30 am, between the months of February (summer) and June (autumn) of 2022.

Body composition by anthropometry

Applying the protocol standardized by the International Society for the Advancement of Kinanthropometry (ISAK), total body mass (BM), height, and body mass index (BMI) was estimated by a physical education professional [21]. For this purpose, a digital scale model P150M (Lider, São Paulo, Brazil) and a stadiometer model SN ES2030 (Sanny, São Bernardo do Campo, São Paulo, Brazil) were used.

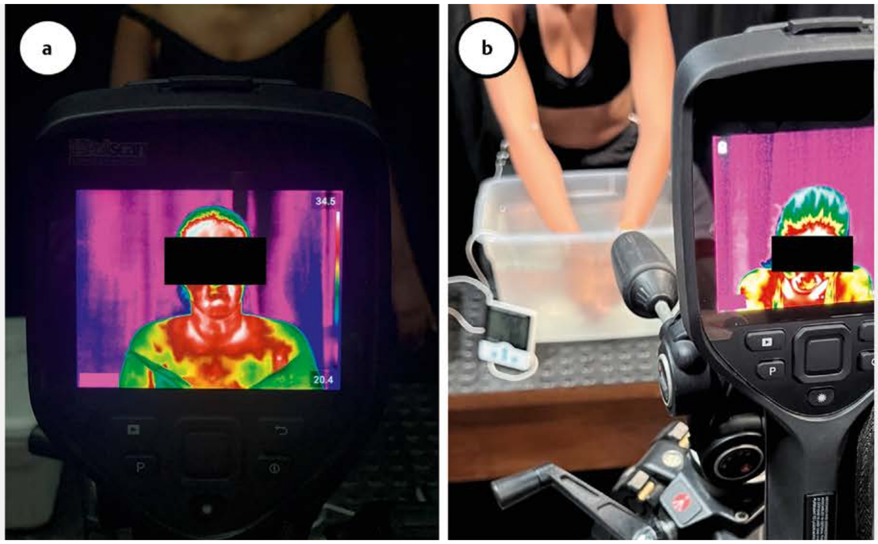
Body composition by dual X-ray absorptiometry (DXA)

This exam was performed by a radiology technician following the protocols defined by the manufacturer, using a DXA, iLunar model, from GE Healthcare (GE Healthcare, Madison, WI, USA), with the enCore 2015 software (version 14.10.022). After the scanning process, the variables of total fat mass (FM), percentage of total body fat (%BF), VAT (g), and fat mass index (FMI) produced by the equipment were used for analysis [22–24]. Using the FM and VAT values, the percentage of VAT (% VAT) was estimated. The percentage of SAT (% SAT) was also calculated, decreasing %BF by %VAT. The equipment was calibrated before starting the daily assessments.

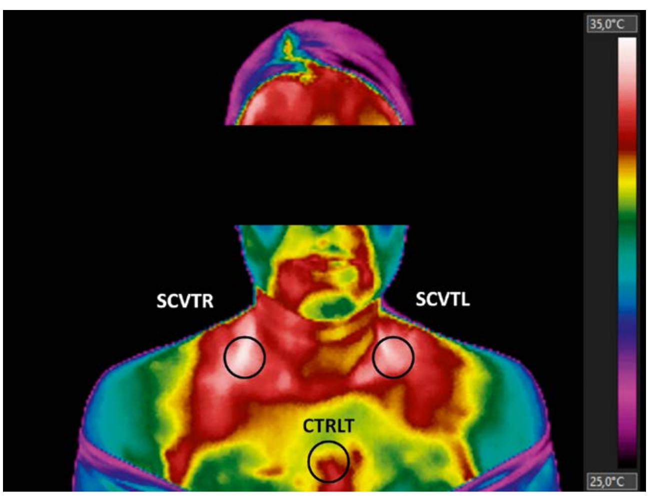
IRT image acquisition

For the analysis of thermal images, a 3.0 × 2.5-m room with control of air temperature (22.0 ± 0.5 °C) and relative air humidity (55.0 ± 1.2 %) was used. Also, a FLIR E75 camera (FLIR Systems, Inc., Wilsonville, OR, USA) (▶ **Fig. 1a**), with a thermal resolution of 320 × 240 pixels and a 4-inch LCD screen, spectral range of 7.5–14.0 μm, a 180-pixel rotating lens platform, and 0.98 % emissivity and fixed into a tripod was operated. All preparation and collection follow the recommended protocol for thermographic imaging in sports and exercise medicine (TISEM) [20]. After 15 minutes of acclimatization, the IRT images were captured in two moments: 1) right after the end of acclimatization (T0); and 2) right after the cold stimulation (T5) protocol **(**▶ **Fig. 1b**), which consisted of keeping both hands in a bowl of water at 13.0 ± 0.6 ºC for 5 min [25].

In this research, IRT images of some regions of interest (ROIs) were acquired: 1) mean Tskin in the right SCV (SCVTR) and mean Tskin in the left SCV (SCVTL) for BAT activation analysis, and 2) mean Tskin in the sternum as the control region (CTRLT). The same



▶ **Fig. 1** (**a**) Camera FLIR E75 positioned for acquisition before cold protocol; (**b**) immersion of both hands in a container of cold water for 5 min.



▶ **Fig. 2** Regions of Interest (ROIs) evaluated. SCVTR: right mean supraclavicular skin temperature, SCVTL: left mean supraclavicular skin temperature, CTRLT: mean control skin temperature.

ROI identification protocol created by da Rosa et al. [25] was applied (▶ **Fig. 2**).

The women were positioned sitting on a chair perpendicular to the thermographic camera, one meter away, keeping their heads in the Frankfurt plane, wearing a top t-shirt without a shoulder strap. Water temperature were monitored by a hygrometer (HTC-1, Rio de Janeiro, RJ, Brazil). The images were captured using the FLIR ResearchIR professional Analyzing software (version 1.5, FLIR Systems).

Statistical analysis

First, the Shapiro–Wilk test was performed to check the data distribution. Thereby, a parametric analysis was applied. Descriptive statistics were applied to characterize the sample using mean and standard deviation. The Student's independent t-test was applied to compare descriptive data. Pearson's correlation between Tskin, %SAT, and %VAT was used to assess the relationship between variables. Two-way repeated measures ANOVA with Tukey's post-hoc comparisons (Over vs. Under) were conducted to assess group and moment factors effects of Tskin ROIs (T0 vs. T5). A *p*-value <0.05 was considered significant. Data analysis was conducted using the Jamovi statistical software, version 2.3.16.

Results

The descriptive data of the entire sample point out that the sample had an average age = 36.1 ± 6.6 years, BM = 66.1 ± 13.4, height = 164.6 ± 6.2 cm, BF = 23.3 ± 8.2 kg, %BF = 34.7 %, SAT = 22.9 ± 7.9 kg, %SAT = 33.3 ± 5.5, VAT = 0.36 kg, %VAT = 1.4 %, BMI = 24.4 ± 3.9 kg/m^2 and FMI = 8.6 ± 2.7 kg/m^2. ▶ **Table 1** shows the descriptive data from all samples extracted by type of fat (%SAT and %VAT) and points out that subjects classified as Over the amount of adipose tissue have worse health indicators than those classified as Under.

▶ **Table 2** makes the descriptive data of the average temperatures within each group quite clear. It is noted that, in both extracts (%SAT and %VAT), only the subjects classified with the lowest amount of fat had a significant increase in SCV skin temperatures after applying the cold stimulation protocol. Sternum ROI did not show a Tskin increase.

In the application of the Pearson's correlation between SCVT, %SAT, and %VAT, medium negative degrees of linear variation were found before and after cold stimulation (▶ **Table 3**). However, the same significance was not found when the values were compared with the delta (Δ) of SCVT (▶ **Table 3**).

The 2-way repeated measurements ANOVA identified a significant effect of the moment factor (BAT activation) and the group factor (Over vs. Under) on the SCVTR and SCVTL between subjects divided in the two extracts (%SAT and %VAT) (▶ **Table 4**). In intragroup analysis after the cold stimulation protocol was applied, we noticed that only the Under %SAT and Under %VAT groups had a

▶ **Table 1** Data of age, anthropometrics, body composition (n = 40 women) extracted Over and Under %SAT and Over and Under %VAT.

Parameters	Classification by SAT and VAT	%SAT (n = 20) Mean ± SD	*p* value*	%VAT (n = 20) Mean ± SD	*p* value*
Age	Over	36.1 ± 6.6	0.739	37.9 ± 6.7	0.007*
	Under	36.0 ± 6.8		34.2 ± 6.1	
Height (cm)	Over	164.2 ± 5.7	0.643	164.0 ± 7.2	0.513
	Under	165.2 ± 6.9		165.3 ± 5.2	
BM (kg)	Over	69.6 ± 14.7	0.153	71.4 ± 16.0	0.010*
	Under	62.6 ± 11.4		60.7 ± 7.2	
FM (kg)	Over	27.5 ± 8.5	0.001*	27.3 ± 9.2	0.001*
	Under	19.1 ± 5.2		19.2 ± 4.1	
%BM	Over	38.9 ± 4.0	< 0.001*	37.5 ± 5.1	< 0.001*
	Under	30.3 ± 4.1		31.7 ± 5.2	
SAT (kg)	Over	27.0 ± 8.2	0.001*	26.7 ± 9.0	0.001*
	Under	18.8 ± 5.0		19.1 ± 4.1	
%SAT	Over	37.3 ± 3.5	< 0.001*	35.4 ± 5.0	0.009*
	Under	29.1 ± 3.6		31.0 ± 5.1	
VAT (kg)	Over	472.9 ± 336.1	0.024*	589.6 ± 275.3	< 0.001*
	Under	254.6 ± 228.3		137.9 ± 83.6	
%VAT	Over	1.6 ± 1.0	0.131	2.1 ± 0.6	< 0.001*
	Under	1.2 ± 0.8		0.7 ± 0.3	
FMI (kg/m²)	Over	10.1 ± 2.7	0.032*	10.4 ± 2.8	< 0.001*
	Under	6.9 ± 1.5		7.1 ± 1.4	
BMI (kg/m²)	Over	25.9 ± 4.3	< 0.001*	26.5 ± 4.2	< 0.001*
	Under	22.9 ± 2.8		22.2 ± 1.9	

BM: total body mass, FM: total fat mass, %BF: percentage total body fat, SAT: subcutaneous adipose tissue, %SAT: percentage subcutaneous adipose tissue, VAT: visceral adipose tissue, %VAT: percentage visceral adipose tissue, FMI: fat mass index, BMI: body mass index, *p value < 0.05 obtained by Student's independent t-test.

▶ **Table 2** Skin temperature before and after cold stimulation (n = 40 women)

%SAT (n = 20)			
	Tskin	**Before (T0)**	**After (T5)**
Over	SCVTR	33.4 ± 0.9	33.7 ± 0.8
	SCVTL	33.3 ± 0.7	33.6 ± 0.8
	CTRLT	31.1 ± 1.0	31.1 ± 0.8
Under	SCVTR	33.8 ± 0.6	**34.5 ± 1.1***
	SCVTL	33.9 ± 0.7	**34.6 ± 0.9***
	CTRLT	31.6 ± 0.6	32.1 ± 1.5
%VAT (n = 20)			
	Tskin	**Before (T0)**	**After (T5)**
Over	SCVTR	33.4 ± 0.8	33.6 ± 0.7
	SCVTL	33.5 ± 0.8	33.7 ± 0.8
	CTRLT	31.2 ± 0.9	31.2 ± 0.2
Under	SCVTR	33.7 ± 0.7	**34.5 ± 1.1***
	SCVTL	33.8 ± 0.7	**34.5 ± 1.0***
	CTRLT	31.5 ± 0.1	32.0 ± 1.6

%SAT: percentage subcutaneous adipose tissue, %VAT: percentage visceral adipose tissue, Tskin: skin temperature, T0: before cold stimulation, T5: after cold stimulation, SCVTR: right mean supraclavicular skin temperature, SCVTL: left mean supraclavicular skin temperature, CTRLT: men control skin temperature. *p value obtained by Tukey

significant increase in SCVTR and SCVTL. The same trend was observed in the intergroup analysis where the SCV Tskin averages of the group with Under %SAT and Under %VAT were significantly higher than subjects classified with Over %SAT and Over %VAT. Unexpectedly, no significant effect of the interaction of the factors was found (▶ **Table 4**). Otherwise, there was only a group factor effect on CTRLT (▶ **Table 4**). When we analyzed the size of the Tskin effects after cold stimulation for moment factor and group factor with a significant increase, four parameters were classified with a "medium" and four with a "large" effect size (▶ **Table 4**).

The presented results can help those in clinical practice understand more clearly, through IRT, that the high accumulation of SAT and VAT can impair the activation of BAT and, consequently, hinder the loss of body weight in women.

Discussion

This research aim of the present study was to verify the relation of SAT and VAT on BAT activation through IRT and cold stimulation in adult women. The main findings showed that Tskin SCV, right and left, despite small differences follow the same trend of relation with SAT and VAT levels. This indicates that the higher the level of body adipose tissue, the lower the increase in Tskin SCV, inferring the inverse relation between activation of BAT, SAT, and VAT.

The scientific literature points out that there is an inverse correlation between BAT activity and BF, and subjects classified with

▶ **Table 3** Correlation between skin temperature and %SAT and %VAT, before and after cold stimulation (n = 40 women)

	SCVTR(T0)	SCVTL(T0)	SCVTR(T5)	SCVTL (T5)	ΔTSCVR	ΔTSCVL
%SAT (n = 20)	r = −0.359	r = −0.501	r = −0.371	r = −0.407	r = −0.086	r = −0.006
	p = 0.023 *	**p < .001** *	**p = 0.018** *	**p = 0.009** *	p = 0.598	p = 0.969
%VAT (n = 20)	r = −0.376	r = −0.342	r = −0.442	r = −0.443	r = −0.034	r = −0.006
	p = 0.017 *	**p = 0.031** *	**p = 0.004** *	**p = 0.004** *	p = 0.834	p = 0.971

%SAT: percentage subcutaneous adipose tissue, %VAT: percentage visceral adipose tissue, T0: before cold stimulation, T5: after cold stimulation, SCVTR: right mean supraclavicular skin temperature, SCVTL: left mean supraclavicular skin temperature, CTRLT: men control skin temperature. ΔTSCVR = SCVTR(T0)−SCVT(T5); ΔTSCVL = SCVTL(T0)− SCVTL(T5); Pearson's correlation * $p < 0.005$ value significance.

▶ **Table 4** ANOVA two-way repeated sample (group × moment factor) for skin temperature and groups by %SAT (n = 20) and by %VAT (n = 20), before and after cold stimulation

%SAT vs. BAT							**%VAT vs. BAT**					
Tskin	**Source**	**df**	**MS**	**F**	**p**	**η²**		**df**	**MS**	**F**	**p**	**η²**
SCVTR	Moment Factor (BAT Activation)	1.00	5.25	8.34	**0.006** *	0.073	Moment Factor (BAT Activation)	1.00	5.23	8.63	**0.006** *	0.073
	Group Factor (%SAT)	1.00	7.50	8.38	**0.006** *	0.105	Group Factor (%VAT)	1.00	7.53	8.38	**0.006** *	0.105
	Interaction	1.00	0.90	1.43	0.239	0.036	Interaction	1.00	1.71	2.81	0.102	0.024
SCVTL	Moment Factor (BAT Activation)	1.00	4.73	0.04	**0.005** *	0.072	Moment Factor (BAT Activation)	1.00	4.73	9.44	**0.004** *	0.072
	Group Factor (%SAT)	1.00	12.40	16.8	**<0.001** *	0.189	Group Factor (%VAT)	1.00	5.46	5.92	**0.020** *	0.083
	Interaction	1.00	0.43	0.828	0.369	0.007	Interaction	1.00	1.27	2.53	0.120	0.019
CTRLT	Moment Factor (BAT Activation)	1.00	1.48	1.88	0.179	0.015	Moment Factor (BAT Activation)	1.00	1.48	1.85	0.182	0.015
	Group Factor (%SAT)	1.00	10.88	7.82	**0.008** *	0.112	Group Factor (%VAT)	1.00	6.44	4.27	0.066	0.101
	Interaction	1.00	1.71	2.16	0.149	0.054	Interaction	1.00	1.27	1.59	0.215	0.040

SCVTR: right mean supraclavicular skin temperature, SCVTL: left mean supraclavicular skin temperature, CTRLT: mean control skin temperature, %SAT: percentage subcutaneous adipose tissue, %VAT: percentage visceral adipose tissue,

positive BAT activity were female, younger, and with a normal/overweight BMI when compared to subjects with negative BAT [26].

This finding is in line with our results, which found that the group of women with a higher BMI and %BF were those with lower BAT activation. Matsushita et al. [27] confirmed that body fat mass, BMI, and abdominal fat area were lower in BAT-positive subjects than in BAT-negative subjects. Our study, despite using another BAT detection technique (IRT) but maintaining the cold stimulus, found the same trend in women with greater increases in SCVT. Mean Tskin in the SCV region in women, after cold stimulation in our study, was slightly higher than that found by da Rosa et al. [25] using the same protocol in men. However, in the study by Hartwig et al. [11] using IRT, after immersing the left hand in water at a temperature of 5 to 9 °C for 1 minute, overweight women had lower SCVTL increases than those with normal weight, corroborating our results where women with lower %SAT and %VAT also had greater increases.

The scarcity of studies involving analysis of thermal images and BAT activation with cold stimulation and associations between SAT and mainly VAT make the discussion somewhat unclear, as we should draw on studies that use PET-CT as a standard method for comparison [14, 15, 28]. However, analyzing the correlations found in our paper between %SAT, %VAT, and SCVT, right and left, they are consistent with previous research. For example, Gatidis et al. [14] evaluated BAT activation in 120 patients (44 female, 59 male) and found a significant inverse correlation between mean SCVT and %SAT ($r = -0.65$, $p < 0.001$). In a study with 86 prepubertal children (47 girls and 39 boys) between 6.5 and 10.2 years of age, with IRT and cold stimulation, Malpique et al. [17] investigated whether the BAT activation differs between prepubertal children born small-for-gestational-age (SGA) or appropriate-for-gestational age (AGA) and found significant negative associations between the change in the area of the thermally active supraclavicular region after the cold stimulus with SAT ($r = -0.243$, $p = 0.034$) and negative and non-significant associations with VAT ($r = -0.194$, $p = 0.077$). Another recent finding using PET-CT, IRT, and cold protocol proved the same significant inverse trend of standardized uptake values of 18-FDG and SCVT with the percentage of fat fraction ($r = -0.628$, $p = 0.012$) [12].

In a study analyzing 18 FDG PET-CT scans of 4,852 individuals, it was clear that, regardless of age groups and gender analyzed, VAT was more associated with BAT activation than with SAT [15]. However, men showed slightly higher correlation values between BAT, VAT ($r = -0.4945$, $p < 0.000$) and SAT ($r = -0.3902$, $p < 0.000$) activity, when compared to women's VAT ($r = -0.3433$, $p < 0.000$) and SAT ($r = -0.2131$, $p < 0.000$) [15].

These existing media correlations between BAT activation through analysis of SCVT, VAT, and SAT were confirmed in our research, but we did not find great differences between the correlations when we extract the groups with the highest and lowest amount of body fat, what points to a balance. To better understand this statement, we must verify that the effect of the moment and group factor on the SCVTR showed the same significance and the same large effect size when we extract the general group by %SAT and %VAT ($p = 0.006$, $\eta^2 = 0.073$) and ($p = 0.006$, $\eta^2 = 0.105$). On the SCVTL, the effect of the moment factor had the same medium effect size between variables and a small *p*-value difference. However, we observed that effect size of group factor in %SAT was classified as very large ($p < 0.001$, $\eta^2 = 0.189$) and in %VAT as large ($p = 0.020$, $\eta^2 = 0.089$). Our greatest contribution is to present an original study that, using a non-invasive, easy-to-apply, and effective technique, confirms that no matter where the fat is accumulated, it maintains the same degree of correlation with BAT activation. This minimum difference found in the Pearson's correlation and in the value p/size of the effect of the ANOVA are irrelevant to define which of the types of body fat percentage is more related to BAT activation.

Obviously, it is important to emphasize that within our limitations is the fact that we did not measure skin thickness in the SCV region. We must take this into account, as already raised by other authors, since Tskin has a direct influence on the amount of SAT due to its thermal insulation [29, 30]. However, our sample comprised women with normal BMI and low overall %BF.

Conclusion

The results of the present research infer that, using IRT, were not found difference in the relation between %SAT, %VAT, and BAT activation in adult women, pointing out that both types of fat are equally related to BAT thermogenesis quite, because this is the same thing right in last line. However, subjects with higher amounts of BF do not seem to achieve a significant increase in SCVT that characterizes non-thermogenic tremor when exposed to cold.

It is suggested that future studies include a sample of women with a larger age range, classified by age as well as nutritional status (BMI) and evaluating the skinfolds of the SCV region to estimate local SAT.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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