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Note

Infrared thermography for indirect assessment of activation of brown adipose tissue in lean and obese male subjects

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Abstract

Brown adipose tissue (BAT) plays a key role in adaptive thermogenesis in mammals, and it has recently been considered as an attractive therapeutic target for tackling human obesity by increasing energy expenditure. Thermal imaging using infrared thermography (IRT) has emerged as a potential safe, rapid and inexpensive technique for detecting BAT in humans. However, little attention has been given to the reliability of this method in obese subjects. To this end, we evaluated the capacity of IRT to detect activated supraclavicular (SCV) BAT in 14 lean and 16 mildly obese young adults after acute cold exposure. Using IRT we measured the temperature of the skin overlying the SCV and sternal areas at baseline and after acute cold stimulation. Additionally, energy expenditure was measured by indirect calorimetry and body composition was estimated using bioelectrical impedance analysis. Energy expenditure and SCV skin temperature significantly increased in lean subjects upon cold exposure, while no significant changes were detected in the obese group. Furthermore, cold-induced variations in SCV skin temperature of obese subjects showed a negative correlation with body mass index.

This study suggests that in lean individuals BAT is a rapidly activated thermogenic tissue possibly involved in the regulation of energy balance, and can be indirectly assessed using IRT. In obese subjects, BAT seems less prone to be activated by cold exposure, with the degree of adiposity representing a limiting factor for the indirect detection of BAT activation by measuring the skin temperature overlying BAT.

Keywords: brown adipose tissue, energy expenditure, humans, obesity, infrared thermography, skin temperature, adaptive thermogenesis

(Some figures may appear in colour only in the online journal)

Abbreviations

BAT	Brown adipose tissue
BIA	Bioelectrical impedance analysis
BMI	Body mass index
IRT	Infrared thermography
PET/CT	Positron emission tomography/computed tomography
RQ	Respiratory quotient
SCV	Supraclavicular
18F-FDG	^{18}F-fluorodeoxyglucose

1. Introduction

It is well established that brown adipose tissue (BAT) is the major site for cold-induced non-shivering thermogenesis in human and rodent models (Van Der Lans *et al* 2013, Labbé *et al* 2015). In brown adipocytes, cold exposure via adrenergic stimulation activates a specialized mitochondrial protein called uncoupling protein-1 (UCP-1) that uncouples oxidative phosphorylation from adenosine triphosphate (ATP) synthesis and dissipating energy as heat. Different studies have estimated that in adult humans heat production by BAT, despite its low amount, contributes up to 15% of total energy expenditure (Van Marken Lichtenbelt and Schrauwen 2011).

Although the role of BAT in thermogenesis has been well known for many decades, the role of this tissue in the control of body weight has been considered only recently when BAT was detected in adults (Van Marken Lichtenbelt *et al* 2009, Virtanen *et al* 2009). Considering the effect of BAT on metabolic rate and nutrient consumption, interest in this tissue as a potential novel therapeutic target for obesity and associated metabolic diseases has progressively grown (Van Marken Lichtenbelt *et al* 2009, Geerling *et al* 2014). Currently, the 'gold standard' method for determination of the volume and activity of BAT in human subjects is cold-induced ^{18}F-fluorodeoxyglucose (18F-FDG) uptake assessed by positron emission tomography scan (PET-CT). The use of this imaging method has identified in the majority of human adults an adipose tissue with high metabolic activity in supraclavicular (SCV) regions, which on histological examination has been confirmed to be BAT (Saito *et al* 2009, Virtanen *et al* 2009).

However, the usefulness of PET-CT is limited by a number of factors as radiation exposure, duration of the procedure, susceptibility to variations in seasonal temperature and finally its high cost (Cohade *et al* 2003, Ouellet *et al* 2011). Therefore, given the promising role of BAT in the regulation of metabolic functions there is an increasing need for alternative methods to evaluate the pathophysiological characteristics of this tissue in humans.

Since BAT is a heat-producing tissue, thermal imaging using infrared thermography (IRT) has emerged as an alternative method for its detection (Jackson *et al* 2001, Sell *et al* 2004). PET-IRT correlative studies in mice reported a close correlation between the increase in 18F-FDG uptake within cold-stimulated BAT and the rise in depot temperature (Carter *et al* 2011). Furthermore, in adult humans, the temperature of skin overlying SCV BAT as measured by

IRT was higher than skin elsewhere during cold stimulation and meal challenge (Lee *et al* 2011). Although promising compared with PET/CT, the accuracy of IRT to determine activation of BAT in obese subjects needs further investigation since data in the literature are still scarce.

The present study evaluates the ability of IRT as an indirect technique to detect the thermogenic response of BAT in lean and obese male subjects after acute cold exposure.

2. Methods

2.1. Subjects

Fourteen healthy lean (BMI $< 25\,\mathrm{kg\,m^{-2}}$, age 24 ± 2.2 years) and 16 mildly obese male subjects (BMI 30–34.9 $\mathrm{kg\,m^{-2}}$, age 29 ± 6 years) were enrolled in the study. Subjects were studied in the morning, from approximately 9 a.m. to 12 a.m., after an overnight fast. Subjects were not allowed to exercise, take medications, drink coffee or tea or smoke in the 24 h prior to the study (Ring and Ammer 2000). The study was approved by the local university hospital ethics committee. All subjects were carefully instructed regarding the study and gave their informed consent to participation.

2.2. Study design

The examination procedure was illustrated for each subject by an expert operator. To achieve adequate stability of blood pressure and skin temperature, subjects rested for 1 h in a room at 24 °C before the examination began. After taking thermal images at baseline conditions within every region of interest, subjects lay on the office bed without moving, talking or sleeping, in a quiet room, for the measurement of resting energy expenditure. Oxygen and carbon dioxide exchange measurements were performed by computed open-circuit continuous indirect calorimetry (MMC HORIZON System, Sensor Medics, Anaheim, CA, USA). Each subject breathed through a face mask and expired air was collected by Beckman O_2 and CO_2 gas analyzers. O_2 consumption and CO_2 production were used to compute energy expenditure as expressed by resting metabolic rate using Weir's formula, $[(1.1 \times \mathrm{RQ}) + 3.9] \times \mathrm{VO_2} \times 4.18$) (Weir 1949), where RQ is the respiratory quotient. Resting energy expenditure was measured continuously under baseline conditions for 20 min, and once the steady state was obtained, two operators immersed simultaneously both hands of each subject in two basins of ice-cold water (5 °C) for a further 20 min, while continually monitoring the variations of caloric expenditure, heart rate and oxygen saturation. Energy expenditure data were expressed in kcal d^{-1}. The subject's hands were then removed from the water, and new thermal images were obtained.

Before the test, each subject underwent measurement of anthropometric parameters and body composition by bioelectrical impedance analysis (BIA; RSL system, mod. 103, AKERN, Florence, Italy).

2.3. Thermal imaging analysis

For acquisition of thermal images each subject was placed seating in upright position in a multi-position chair with his head in a neutral position and looking straight ahead. The room temperature was kept constant at 24 °C (Ring and Ammer 2000, Ammer 2008). The upper chest area and the neck region were exposed while subjects underwent thermographic analysis

using a FLIR® T450sc thermal imaging camera (FLIR Systems Inc., Wilsonville, OR, USA; sensor array size 320 × 240 pixels, NETD < 30 mK). The camera was positioned at the level of the neck 1 m from the subject's face. Bilaterally, two regions of the anterior thorax were chosen for analysis of surface temperature (figure 1): (a) the skin area overlying BAT in the SCV fossa and the lateral region of the neck and (b) the sternal area considered as a control (Van Der Lans *et al* 2014). As described in figure 1, triangular regions of interest (ROI) were placed in the left and right SCV areas, while a circular ROI was placed over the sternal region. The mean of these ROIs was then extracted for further analysis.

After acquisition, thermal images were analyzed using FLIR R&D software following the manufacturer's protocols.

2.4. Statistical analysis

Statistical analysis was performed with GraphPad prism version 6 for Windows (GraphPad software, CA). Two-sided t tests were used to test the statistical significance of differences between the findings at baseline and after cold exposure. Linear regression analysis was used to identify the relationship between the different considered variables. Data are reported as means ± SD. *P*-values < 0.05 were considered as statistically significant.

3. Results

3.1. Clinical characteristics

The clinical and anthropometric characteristics of lean and obese subjects are reported in table 1. All subjects were young adult men.

The monitoring of the heart rate showed that acute cold exposure stimulated a significant and rapid increase in heart rate in both lean (from 63 ± 7.4 to 76.3 ± 7.2, $p < 0.0001$) and obese subjects (from 64 ± 9.9 to 72.7 ± 10, $p < 0.0001$) confirming the activation of the adrenergic system. Shivering effects following cold exposure were not observed during the study, thus excluding any role of shivering-thermogenesis on metabolic rate.

3.2. Effect of cold exposure on skin temperature over the SCV fossa and sternal regions

After cooling we observed a significant increase in mean SCV skin temperature (34.3 ± 0.8 °C versus 35.2 ± 0.6 °C, $p < 0.001$) of lean subjects. On the other hand, cold exposure did not induce any significant variations in mean SCV skin temperature of obese subjects (33.6 ± 0.7 versus 33.6 ± 0.6, $p = 0.3$) (figure 1). The mean temperature of sternal skin regions in lean (33.1 ± 1.1 °C versus 33.3 ± 0.8 °C, $p = 0.4$) and obese (32.1 ± 1.3 °C versus 32 ± 1.4 °C, $p = 0.8$) subjects showed no significant variations compared with the baseline condition. The change in SCV skin temperature (ΔSCV skin temperature) of each individual in both groups is summarized in figures 2(A) and (B).

3.3. Effect of cold exposure on energy expenditure

In lean individuals, acute cold exposure by immersion of hands in iced water induced a rapid increase in energy expenditure from 1455.8 ± 192.2 kcal d^{-1} to 1635.9 ± 318.9 kcal d^{-1} (+11%, $p < 0.01$) (figure 2(C)). This phenomenon was consistently observed in all subjects

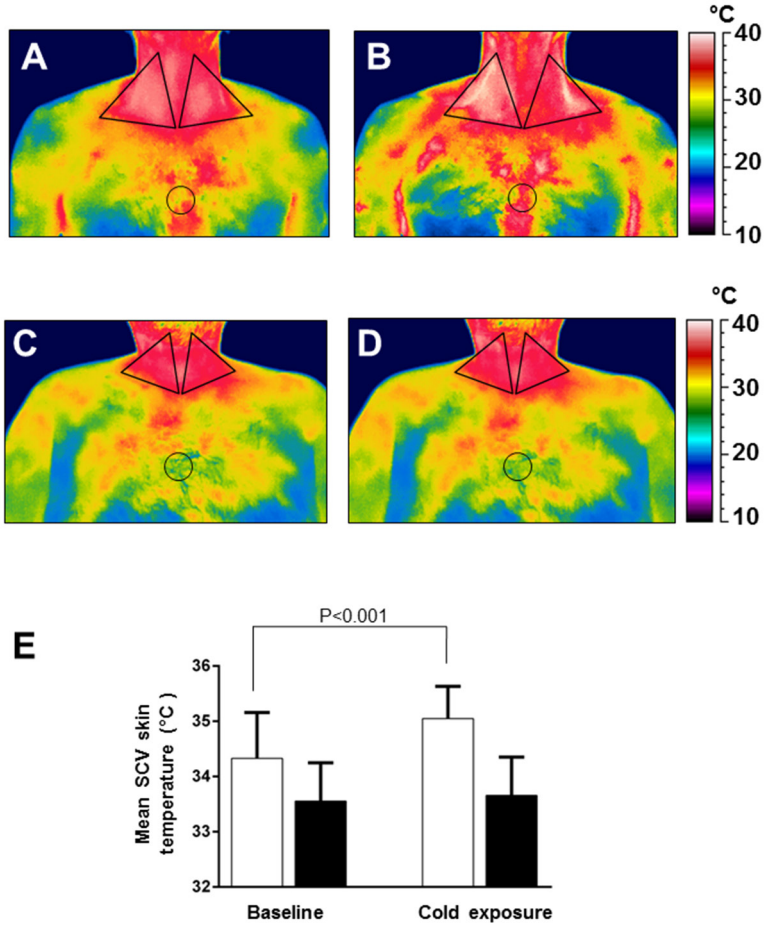

Figure 1. Representative example of thermal images of the skin overlying the ROI located at SCV and sternal (circular ROI) area in lean ((A) and (B)) and obese subjects ((C) and (D)) before ((A) and (C)) and after ((B) and (D)) 20 min of cold stimulation. (E) Mean values of SCV skin temperature in lean (white bars) and obese subjects (black bars) before (baseline) and after cold challenge.

although with different values. In parallel, the RQ showed a significant increase upon acute hand cooling, from 0.7 ± 0.1 to 0.8 ± 0.1 ($p < 0.05$) (data not shown).

In contrast, obese subjects showed no significant variations in energy expenditure upon cooling (1871 ± 250.7 kcal d^{-1} versus 1880 ± 236.3 kcal d^{-1}, $p = 0.6$) (figure 2(C)).

3.4. Body composition, energy expenditure and SCV skin temperature

Following cold exposure, no significant correlations were found in either group between changes in energy expenditure (Δ energy expenditure) and the change in SCV skin temperature (ΔSCV skin temperature) (data not shown). In obese subjects, BMI was negatively correlated with SCV skin temperature after cold exposure ($r = -0.55$, $p = 0.02$) with no relation to the sternal skin temperature ($r = -0.24$, $p = 0.4$) (figure 3).

Table 1. Main clinical characteristics in lean and mildly obese subjects. Data are expressed as means ± SD.

	Lean		Obese	
	Baseline	Cold-induced	Baseline	Cold-induced
Number	14		16	
Age (years)	24 ± 2.2		29 ± 6	
Weight (kg)	73.1 ± 7.6		109 ± 14	
BMI (kg m^{-2})	22.9 ± 1.8		33.3 ± 1.7	
Fat mass (kg)	13.3 ± 4		37 ± 7.5	
Fat free mass (kg)	59.9 ± 3.7		72 ± 6.5	
Heart rate (bpm)	63 ± 7.4	76.3 ± 7.2[a]	64 ± 9.9	72.7 ± 10[a]
Energy expenditure (kcal d^{-1})	1455.8 ± 192.2	1635.9 ± 318.9[a]	1871 ± 250.7	1880 ± 236.3
Mean SCV skin temperature (°C)	34.3 ± 0.8	35.2 ± 0.6[a]	33.6 ± 0.7	33.6 ± 0.6
Mean sternal skin temperature (°C)	33.1 ± 1.1	33.3 ± 0.8	32.1 ± 1.3	32 ± 1.4

[a] $P < 0.05$ versus baseline condition.

4. Discussion

The recent demonstration of the presence of BAT in humans together with its activation by means of physical and pharmacological strategies (Van Marken Lichtenbelt *et al* 2009, Virtanen *et al* 2009, Van Der Lans *et al* 2014, Cypess *et al* 2015), has brought an increased interest in this tissue as a novel therapeutic target to counteract obesity and its associated comorbidities (Van Der Lans *et al* 2013, Lee *et al* 2014a).

Evaluation of the activity of BAT in humans has been widely investigated using PET-CT scan. This technique demonstrated the presence of BAT in discrete anatomical regions localized mainly in the SCV fossa and neck, followed by paravertebral, mediastinal, para-aortic and suprarenal foci (Sacks and Symonds 2013). In recent years research has focused on studying BAT activity using less invasive techniques with reliable and repeatable results, thus facilitating the evaluation of therapeutic strategies involved in the activation BAT or in browning of white fat to tackle obesity and its cardiovascular consequences (Lee *et al* 2014b, Cypess *et al* 2015).

Digital infrared thermal imaging is able to map the thermal pattern of different body regions. It has gained acceptance in the investigation of different inflammatory processes and neoangiogenetic phenomena such as those observed in cancer or in autoimmune disorders of the thyroid (Jiang *et al* 2005, Rossato *et al* 2015), although a strong clinical application has yet to be clearly realized.

Recently, IRT has started to provide new insights in assessment of active BAT by measuring the corresponding overlying skin temperature, given its thermogenic properties. Jang *et al* (2014) have clearly demonstrated that IRT, once compared with 18F-FDG PET/CT scan, can detect active BAT in humans. This technique has the advantage of being rapid and nontoxic and offers rapid real-time scanning that is easy to interpret (Usamentiaga *et al* 2014). Furthermore, it can be repeated frequently in the same subject with no side effects and it can also be performed also in more vulnerable subjects such as children and pregnant women.

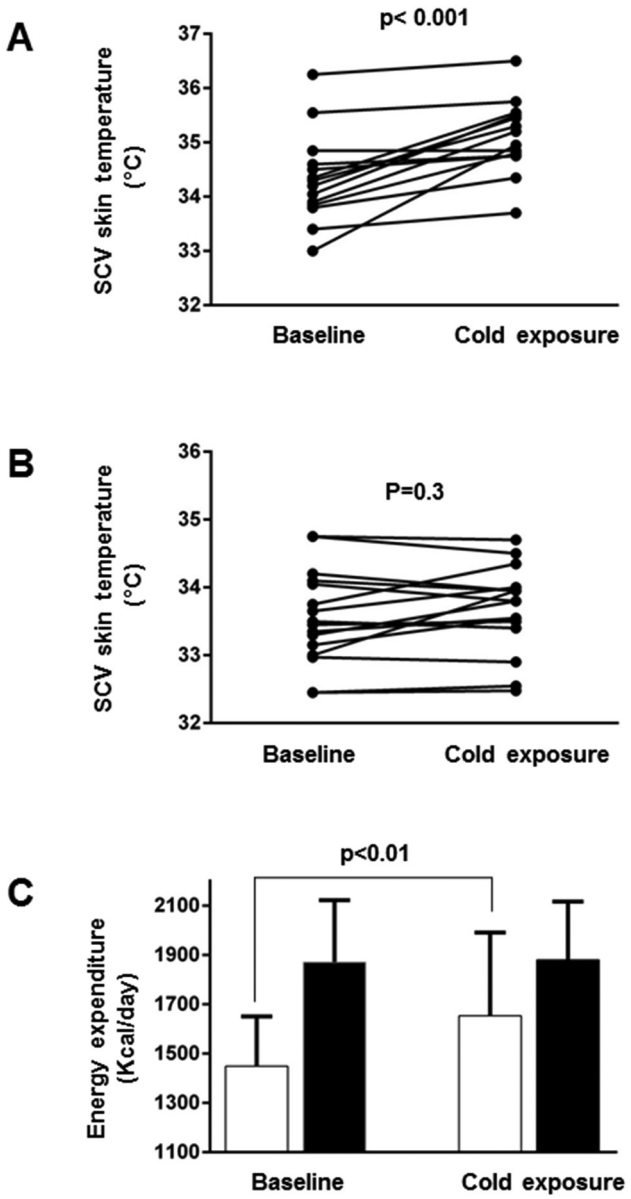

Figure 2. Effects of cold exposure on SCV skin temperature variation and on whole body energy expenditure. Panels (A) and (B) show the variations of SCV skin temperature of each subject in the lean and obese group, respectively, before (baseline) and after cold challenge. Panel (C) shows the mean value of energy expenditure in lean (white bars) and obese (black bars) subjects before (baseline) and after cold exposure.

The present study investigates the capacity of IRT to detect indirectly the thermogenic response of active BAT in young lean and obese male subjects after acute cold exposure. Both groups were comparable for age and race. All subjects showed a rapid increase in heart rate after cold challenge by the immersion of their hands in iced water, confirming activation of the adrenergic system (Ouellet *et al* 2012).

In the normal weight group, thermal changes in SCV and lateral neck regions following hand immersion in cold water occurred bilaterally in a rapid and localized manner, possibly excluding any interference of acute circulatory systemic effects related to increased blood flow/vasodilation (Deng and Liu 2004).

These observations are consistent with previous studies demonstrating that in humans BAT resides mainly in the SCV fossae and lateral neck skin regions as confirmed by PET-CT scan and overlying increases in skin temperature after its activation (Symonds *et al* 2012). The putative increase in BAT activity after cooling as shown by IRT in the present study was coupled with a significant increase in energy expenditure and respiratory quotient as evaluated by indirect calorimetry. These findings are in agreement with the previous observations of Yoneshiro *et al* (2011), which showed a positive correlation between energy expenditure and supraclavicular BAT metabolic activity detected using 18F-FDG-PET/CT after cold exposure in lean subjects. In that study, subjects with detectable BAT showed a significant increase in SCV skin temperature measured by means of small disk-type temperature data loggers. Since cold exposure induces activation of the sympathetic nervous system, these observations fit quite well with the well-known stimulatory effects of catecholamines on BAT activity and the inhibitory effects of beta-adrenergic receptor antagonists (Söderlund *et al* 2007, Yamaga *et al* 2008).

In the present study the increase in metabolic rate after cold exposure observed in lean subjects depended solely on the presence of metabolically active BAT, since cold challenge was confined only to the hands without observing diffuse shivering phenomena. By examining the relationship between changes in skin temperature in the SCV region and nonshivering thermogenesis expressed as the change in energy expenditure before and after cold exposure, no significant correlation between these parameters was found. This can be explained by the fact that nonshivering thermogenesis might not be solely related to the activation of BAT in the SCV region, but also to BAT stimulation in other areas. This observation is supported by the finding of Boon *et al* (2014), who reported a significant increase in thermogenic response and energy expenditure following cold exposure together with BAT activation. Interestingly the same authors did not observe any correlation between cold-induced changes in SCV skin temperature measured by iButtons and total BAT volume quantified by 18F-FDG PET/CT scan.

In contrast to what was observed in lean subjects, cooling in obese subjects did not induce any significant changes, either in SCV skin temperature or in energy expenditure. Many different studies have previously revealed an inverse relationship between BAT activity and obesity as evaluated by different methods including BMI, body fat percentage and body fat content (Cypess *et al* 2009, Saito *et al* 2009, Virtanen *et al* 2009). An investigation of morbidly obese patients before and after weight loss induced by bariatric surgery reported an increase in the number of subjects with active BAT 1 year after surgery, as measured by FDG-PET/CT. After weight loss, the 'BAT positive' subjects showed higher nonshivering thermogenesis, suggesting the recruitment of BAT (Vijgen *et al* 2012). The mechanism of impaired BAT activity in obesity is likely to be multi-factorial. Recently it was shown that obesity can trigger insulin resistance and inflammatory pathways in BAT (Roberts-Toler *et al* 2015). In addition, morbidly obese subjects are characterized by lower expression of UCP-1 in the intraperitoneal adipose tissue compared with lean controls (Oberkofler *et al* 1997). Furthermore, the negative relationship between SCV skin temperature after cold exposure and BMI can be also explained by the thick layer of subcutaneous adipose tissue in the SCV area. In this regard, adipose tissue is known to provide thermal energy insulation which

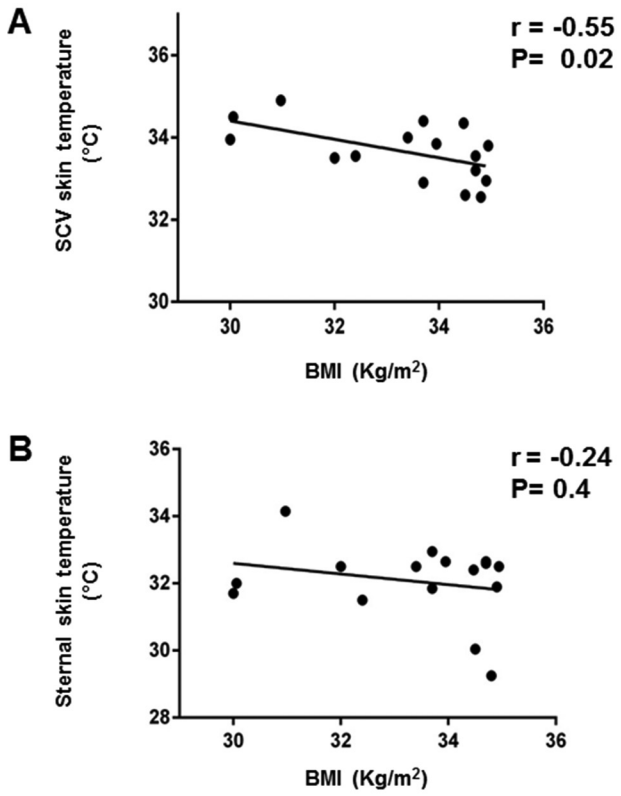

Figure 3. Relationship between cold-induced SCV (A) and sternal (B) skin temperature with BMI in obese subjects.

has been shown to be positively related to the degree of obesity (Cooper and Trezek 1971, Jéquier *et al* 1974).

5. Conclusion

The results of the present paper add evidence that BAT in lean subjects is a rapidly activated thermogenic tissue involved in heat production and it can be indirectly evaluated by means of simple techniques such as IRT and indirect calorimetry. Concerning obese subjects, at least in males, even though the activation of BAT cannot be excluded in certain subjects activation by cold exposure seems to be less effective than in lean subjects, with the degree of adiposity representing an important limiting factor that should be considered when using IRT in these subjects to monitor BAT activity.

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