Use of Infrared Thermography to Estimate Brown Fat Activation After a Cooling Protocol in Patients with Severe Obesity That Underwent Bariatric Surgery

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Abstract

Background In contrast to the energy-storing role of white adipose tissue (WAT), brown adipose tissue (BAT) acts as the main site of non-shivering thermogenesis in mammals and has been reported to play a role in protection against obesity and associated metabolic alterations in rodents. Infrared thermography (IRT) has been proposed as a novel non-invasive, safe, and quick method to estimate BAT thermogenic activation in humans. The aim of this study is to determine whether the IRT could be a potential new tool to estimate BAT thermogenic activation in patients with severe obesity in response to bariatric surgery.

Methods SuprACLAVicular BAT thermogenic activation was evaluated using IRT in a cohort of 31 patients (50 ± 10 years old, BMI = 44.5 ± 7.8; 15 undergoing laparoscopy sleeve gastrectomy and 16 Roux-en-Y gastric bypass) at baseline and 6 months after a bariatric surgery. Clinical parameters were determined at these same time points.

Results SuprACLAVicular BAT-related activity was detected in our patients by IRT after a cooling stimulus. The BAT thermogenic activation was higher at 6 months after laparoscopy sleeve gastrectomy (0.06 ± 0.1 vs 0.32 ± 0.1), while patients undergoing to a roux-en-Y gastric bypass did not change their thermogenic response using the same cooling stimulus (0.09 ± 0.1 vs 0.08 ± 0.1).

Conclusions Our study postulates the IRT as a potential tool to evaluate BAT thermogenic activation in patients with obesity before and after a bariatric surgery. Further studies are needed to evaluate differences between LSG technique and RYGB on BAT activation.

Keywords Infrared thermography · Brown adipose tissue · Obesity · Metabolic surgery
Introduction/Purpose

White adipose tissue (WAT) is involved in the regulation of energy balance and glucose homeostasis, owing to its function as a lipid-storing and endocrine organ. Numerous studies have demonstrated that abnormal WAT function is linked to obesity, whole body insulin resistance, and T2D [1]. However, few studies have comprehensively addressed the effect of obesity and weight loss on the thermogenic capacity of BAT, to the healthy effects of active BAT on systemic metabolism [2].

In contrast to the energy-storing role of WAT, brown adipose tissue (BAT) acts as the main site of non-shivering thermogenesis in mammals due to the presence of uncoupling protein-1 in mitochondria of brown adipocytes, which uncouples mitochondrial oxidative processes and generates heat [2]. BAT plays a major role in protection against obesity and associated metabolic alterations in rodents due to its draining of glucose and lipids from circulation to sustain thermogenesis [3, 4]. Moreover, sustained thermogenic activation leads also to the so-called “browning” of adipose tissue: the appearance of beige (brown adipocyte-like) adipocytes in anatomical sites corresponding to WAT depots [5, 6]. Chronic exercise, cold exposure, and chronic β3-adrenergic stimulation have been shown to promote browning of WAT in experimental models [7]. A higher capacity of browning has been directly associated with protection against experimental obesity and improved glucose tolerance in mice [8]. Moreover, BAT has been also reported to secrete endocrine regulatory factors which contribute, in addition to the intrinsic energy expending properties of BAT, to the healthy effects of active BAT on systemic metabolism [9, 10].

Increased body mass is associated with numerous metabolic diseases, including type 2 diabetes (T2D). Nowadays, it has been estimated that approximately 350 million people have diabetes, representing almost 6% of the world population [11]. Thus, it is not surprising to find out that, concomitantly with the dramatic increase in obesity, T2D has become the most common metabolic disorder, being recognized as one of the deadliest non-communicable diseases worldwide.

Laparoscopy sleeve gastrectomy (LSG) and Roux-en-Y gastric bypass (RYGB) are two types of bariatric surgery, the most effective therapy to avert obesity and T2D currently available [12–15]. One of the consequences of bariatric surgery is activation of BAT and browning of WAT, which is hypothesized to contribute to the increased energy expenditure, weight loss, and overall improvement in systemic glucose and lipid metabolism after surgery [16, 17]. In 2009, several studies confirmed that active BAT is present in adult humans, and that, accordingly, BAT activity is reduced in patients with obesity [18–21]. One of the techniques currently used to quantify BAT is positron emission tomography with 2-deoxy-2-[fluorine-18] fluoro-d-glucose integrated with computed tomography (18F-FDG-PET-CT). This technique is considered the “gold standard” to measure BAT/beige activity and metabolism [22]; it is an expensive, time-consuming, and relatively invasive technique [23]. For the moment, the lack of non-invasive and low-cost methods to measure BAT activation before and after surgery in patients makes it difficult to implement this parameter in the clinical practice for a better evaluation of the patients.

In the last years, infrared thermography (IRT) has been proposed as a novel non-invasive, safe, cheap and quick method to estimate BAT thermogenic activation in humans [24]. IRT uses the heat-emitting properties of BAT and the relatively superficial position of the supraclavicular BAT depot. A rise in supraclavicular temperatures after cold exposure has been demonstrated using this methodological approach, and several studies correlated IRT data obtained in supraclavicular skin surface with BAT activity as determined using PET scans [25–29].

In this study, we determined for the first time the thermogenic activation of BAT after a hand-cold protocol using IRT in patients with severe obesity undergoing to two different types of bariatric surgery.

Materials and Methods

Subject Cohort and Surgical Interventions

A cohort of 31 patients with severe obesity was included in this study and stratified into two groups according to the type of surgery they underwent: (1) LSG (n = 15), in which the stomach is transected vertically from 5 cm proximal to the pylorus up to the His’ angle, using a 36Fr bougie as a calibrator. Hence, more than 70% of patient’s stomach (mainly fundus and body) volume is removed without modifying the rest of gastrointestinal tract [30]; (2) RYGB (n = 16), where the surgeons tailor a 40–60-mL gastric pouch that is anastomosed to the previously divided jejunum distal to the ligament of Treitz. Reconstruction of the gastrointestinal tract was completed in a “Y” configuration with a distal jejuno-jejunostomy [31]. All patients were evaluated by the same endocrinology specialist according to criteria formulated in Spanish Position Statement between Obesity, Endocrinology, Diabetes and Surgery Societies [32]. IRT, demographic, and clinical data, including age, diabetes, and hypertension, were recorded for all participants at baseline and 6 months after surgery.

The Institutional Ethics Committee, in accordance with the Declaration of Helsinki, approved the study (PI16–025). All participants gave their written informed consent before the IRT and the collection of clinical data and samples.

Study Visits and Protocols

For IRT acquisition, participants went to a specific room where the body area object of evaluation was uncovered and
sat-down during 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, and any long-haired participants were asked to tie their hair up to expose the supraclavicular area when necessary.

For the cold protocol, first of all, a pilot study was performed in order to select the correct time of cold stimulus, and thermal images were taken before and at 1, 2, 3, 4, and 5 min since the patients put the hand in the cold water (17 °C, according to Symonds and collaborators [26]). According to this pilot study, three thermal images were taken for each patient using a FLIR T420 infrared camera (FLIR T420 Systems AB, Sweden), with a thermal sensitivity of 0.05 °C and 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; in this moment, ambient temperature and relative humidity were registered for each set as well. 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, patients were asked to put their left hand in cold water (17 °C) during 5 min to stimulate BAT activation, after which the thermographic picture was taken. Temperature was controlled with a thermometer, and cold blocks were used to keep 17 °C when it was necessary.

**Analysis of IRT**

Thermal data were extracted from IRT pictures by using a region of interest (ROI)-based approach. The ROIs were manually drawn in the images on the supraclavicular and sternum region using the FLIR ResearchIR Max software version 4.40.6.24 for windows (FLIR Systems Inc., North Billerica, MA, USA). All analyses were adjusted by atmospheric temperature and distance to the participant and relative humidity, which, as previously stated, were recorded at the beginning of each IRT session. Moreover, 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 thermographic images, emissivity was set at 0.98 (human skin). Minimum, maximum, and mean values of each ROI were retained as variables. The temperature in the supraclavicular region was normalized by sternum region temperature for each participant at all time points before and after surgery.

We calculated the delta Supraclavicular T-Sternum T in thermal image number 2 (0 min) and in thermal image number 3 (5 min after cold stimulation), obtaining a value “ΔT0” for the thermal image 1 and a value “ΔT5” for the thermal image 2:

\[
\Delta T_0^{\text{scr-str}} = T_\text{supraclavicular area}^0 - T_\text{sternum area}^0
\]

\[
\Delta T_5^{\text{scr-str}} = T_\text{supraclavicular area}^5 - T_\text{sternum area}^5
\]

After that, we calculated the delta “ΔT0-5min,” which gave us the value of BAT thermogenic activation capacity elicited by cold stimulus. This is key readout of our study:

\[
\Delta T_0-5\text{min} = \Delta T_0^{\text{scr-str}} - \Delta T_5^{\text{scr-str}}
\]

We repeated this same sequence 6 months after a bariatric surgery, and we compared the values of the capacity of BAT thermogenic activation by cold stimulus 6 months after surgery, taking into account the different type of surgery.

**Serum Samples**

Serum samples from the study participants were collected after a 12-h fasting period for solid food or energy-containing liquids, and 6 h for water at baseline and 6 months after surgery. All samples were stored at -80 °C in the Biobanc of the Health Sciences Research Institute Germans Trias i Pujol Foundation.

**Human Serological Analysis**

Fasted glucose and insulin levels, glycated hemoglobin, lipid profile (total cholesterol, HDL and LDL cholesterol, triglycerides), urea, creatinine, and C-reactive protein were measured in the certified core clinical laboratory at the hospital. Body mass index, waist circumference, and blood pressure were measured by the endocrinologists and dieticians in charge of the patients. The Homeostatic model Assessment-insulin resistance (HOMA-IR) was calculated through the following formula:

\[
\text{HOMA-IR} = \frac{\text{[Glucose (mg/dL)]} \times \text{[Insulin (m.u.int/dL)]}}{405}
\]

**Statistical Analysis**

Data are presented as mean ± sem. Statistical tests were performed with GraphPad Prism 6.0. Normality of datasets was assessed using the Kolmogorov-Smirnoff test. Wilcoxon matched pairs test was used to compare delta “ΔT0-5min” before and after a bariatric surgery. Grubbs test was used to remove outliers prior to statistical analyses. \( P < 0.05 \) was considered as the threshold of statistical significance in all analyses.
Results

BAT Thermogenic Capacity of Patients with Severe Obesity Increases After Surgery

Clinical data from 31 patients (19 females/12 males, aged = 50 ± 10 years old, BMI = 44.5 ± 7.8) are shown in Table 1. As expected, both LSG and RYGB led to weight loss and improved metabolic profile, including glucose and triglycerides levels. The group of individuals operated by LSG displayed significant lower levels of glucose and glycated hemoglobin before the surgery compared to the RYGB group.

Regarding the pilot study, a marked variability was detected at very short times between the different patients (Supplementary Figure S1), and considering our observations and the previous literature [26], we chose to perform 5 min of cold stimulus.

Figure 1 shows a representative example of IRT images of one participant from each type of surgery, which exhibit a lack of thermogenic activation by cold exposure before surgery in both patients. At 6 months after a bariatric surgery, cold exposure activated supraclavicular BAT in the patient from the LSG group but not in the patient from the RYGB group. The general data from the whole cohort of patients are shown in Fig. 2, where we observe a significant increase of the thermogenic activity 6 months after LSG but not RYGB. There were no significant differences between females and males before and after a bariatric surgery, and no correlation with weight loss was found (data not shown).

Table 1

| Anthropometric and metabolic parameters from patients with morbid obesity before and after bariatric surgery |
|--------------------------------------------------|-----------------|-----------------|-----------------|
|                             | Basal | 6 months | \(\Delta 6-0\) months |
|                             | LSG (n = 15) | RYGB (n = 16) | LSG | RYGB | LSG | RYGB |
| Age (years)               | 46 ± 13 | 53 ± 8 | – | – | – | – |
| Sex (F/M)                 | 10/5 | 9/7 | – | – | – | – |
| Type 2 diabetes*          | 5     | 9     | 0 | 0 | – | – |
| Body mass index (kg/m²)   | 46 ± 9.7 | 42 ± 5.1 | 35 ± 9.0** | 31 ± 5.3** | −11.74 ± 2.82 | −10.91 ± 1.68 |
| Waist circumference (cm)  | 136 ± 14 | 130 ± 11 | 117 ± 16** | 105 ± 15** | −19.25 ± 11.23 | −25.96 ± 11.91 |
| Glucose (mg/dl)           | 94 ± 10 | 128 ± 44 | 86 ± 9 | 90 ± 16** | −7.60 ± 9.91 | −36.33 ± 30.68* |
| Glycated hemoglobin (%)   | 5.6 ± 0.38 | 6.2 ± 1.01* | 5.3 ± 0.32 | 5.3 ± 0.50** | −0.30 ± 0.27 | −0.89 ± 0.61* |
| Insulin (mu.int./l)       | 11.02 ± 8.48 | 12.07 ± 7.32 | 10.38 ± 8.34 | 7.14 ± 4.27** | −2.38 ± 9.32 | −10.72 ± 17.98 |
| Homeostatic model assessment (HOMA-IR) | 2.87 ± 2.21 | 4.50 ± 3.99 | 2.54 ± 2.11 | 1.63 ± 1.13** | −0.77 ± 2.3 | −4.48 ± 6.68 |
| Triacylglycerides (mg/dl) | 133 ± 46 | 130 ± 35 | 97 ± 39** | 100 ± 33** | −38.50 ± 54.37 | −29.48 ± 46.28 |
| Total cholesterol (mg/dl) | 153 ± 20 | 145 ± 33 | 181 ± 45 | 164 ± 34 | 30.00 ± 54.17 | 18.75 ± 51.36 |
| LDL-cholesterol (mg/dl)   | 85 ± 20 | 82 ± 34 | 113 ± 43 | 100 ± 30 | 30.50 ± 52.76 | 15.06 ± 46.65 |
| HDL-cholesterol (mg/dl)   | 41 ± 9 | 37 ± 4 | 48 ± 7 | 48 ± 22 | 8.41 ± 7.82 | 10.97 ± 22.97 |
| Creatinine (mg/dl)        | 0.91 ± 0.64 | 0.84 ± 0.26 | 0.86 ± 0.59 | 0.76 ± 0.18 | 0 ± 0.15 | −0.10 ± 0.14 |
| Urea (mg/dl)              | 39.13 ± 29.09 | 37.06 ± 13.74 | 34.80 ± 24.27 | 35.00 ± 7.65 | −4 ± 9.87 | −2.06 ± 11.62 |
| C-reactive protein (mg/l) | 8.89 ± 5.08 | 6.67 ± 5.17 | 7.12 ± 7.32 | 4.22 ± 3.59 | −3 ± 9.79 | 0.11 ± 10.92 |
| Systolic blood pressure (mmHg) | 136 ± 16 | 134 ± 13 | – | – | – | – |
| Diastolic blood pressure (mmHg) | 80 ± 7 | 80 ± 10 | – | – | – | – |
| Absolute temperature difference (°C)a | 0.59 ± 0.2 | 0.56 ± 0.2 | 0.44 ± 0.1 | 0.58 ± 0.3 | – | – |
| Relative temperature difference (°C)b | 0.06 ± 0.1 | 0.09 ± 0.1 | 0.32 ± 0.1* | 0.08 ± 0.1 | – | – |

Data in italics indicate statistically significant results

Data are shown as mean ± SD except when it is indicated. Two-tail Student’s t test was applied to compare two groups, and one-way ANOVA with Tukey’s post hoc test was used for comparisons between more than two groups.

LSG laparoscopic sleeve gastrectomy, RYGB roux-en-Y gastric bypass, LDL-Cho low-density lipoprotein cholesterol, HDL-Cho high-density lipoprotein cholesterol

*p < 0.05 between LSG and RYGB; **p < 0.05 between 0 and 6 post surgery

a This parameter is the difference between the supraclavicular skin temperature measured before the cold stimulation and the supraclavicular skin temperature measured after 5 min of cold exposure

b This parameter is the difference between the supraclavicular skin temperature minus the sternum temperature measured before the cold stimulation and the supraclavicular skin temperature minus the sternum temperature measured after 5 min of cold exposure

c Five T2D patients from LSG group: dapaglifozin 1 and metformin 4 and nine T2D patients from RPGY group: liraglutide 2, insulin 4, metformin 2, and linagliptin 1
In this study, we demonstrate for the first-time usage of IRT as a novel non-invasive technique to estimate BAT activation after hand-cold exposure in patients with severe obesity undergoing bariatric surgery. Due to IRT being a non-invasive method, a longitudinal approach could be used to estimate BAT activity at several time points after surgery in patients with obesity.

Patients undergoing bariatric surgery are currently evaluated before surgery in different aspects including endocrine-metabolic status and psychological traits. However, it is still challenging to predict the success of surgery because of the high variability in the extent of weight loss after surgery. Moreover, it is known that BAT thermogenic activation inversely correlates with BMI [18]. According to that, we have observed that at 6 months after surgery, BAT thermogenic capacity increases. Therefore, we propose that this parameter, obtained through a non-invasive method such as IRT, is worthy to be considered among the number of data that are taken into account to evaluate the physiological and metabolic status of patient candidates to bariatric surgery.

**Fig. 1** Representative IRT images of two patients with severe obesity undergoing RYGB or LSG, respectively. IRT before RYGB at 0 min (a) and before RYGB at 5 min of cold exposure (b). IRT 6 months after RYGB at 0 min (c) and 6 months after RYGB at 5 min of cold exposure (d). IRT before LSG at 0 min (e) and before LSG at 5 min of cold exposure (f). IRT 6 months after LSG at 0 min (g) and 6 months after LSG at 5 min of cold exposure (h).

**Fig. 2** BAT thermogenic capacity of patients with severe obesity is increased after a bariatric surgery. Supraclavicular skin temperature corrected by sternum temperature before and 5 min after cold exposure. Thermogenic activation, measured as the difference between 5 and 0 min, is represented at basal time and 6 months after the RYGB (a) and SLG (b). Paired two-tailed t test was used. *p < 0.01
Interestingly, we found differences in the extent of BAT activation depending on the type of surgery, being patients undergoing LSG but not RYGB the ones who actually improved BAT activation capacity after surgery. In our study, differences in glucose levels and glycated hemoglobin were found between LSG and RYGB groups at baseline. Moreover, the LSG group showed normal glucose levels despite having the same degree of obesity in terms of BMI compared to RYGB group. Considering that BAT has demonstrated to exert a positive role in energy expenditure and insulin sensitivity, contributing to a better metabolic state [4], it is tempting to speculate that the BAT of patients with normoglycemia would maintain a better function than patients with pre-diabetes or diabetes in terms of endocrine activity. It could explain, at least in part, the improvement in insulin sensitivity and the increase in the capacity to activate BAT after cold exposure observed in LSG group compared to RYGB, which might be contributing to the success of surgery regarding weight loss.

Other researchers have described BAT activation measured by 18F-FDG-PET-CT after a RYGB, but interestingly, a lack of hypothalamic activity was reported in these patients with obesity before and after the RYGB [17]. Since hypothalamic signaling to BAT is the main neural circuit involved in thermogenic activation of this tissue [33], a potential explanation is that our protocol to expose patients to cold stress (5 min with their hand in cold water) might not be enough to activate BAT thermogenesis in the RYGB group compared to LSG group due to this impairment of sympathetic signaling in the former set of patients. Other mechanisms of action (e.g., specific changes in enterokine secretion, adipokine release or microbiota changes) could account for the systemic effects of RYGB without involving central nervous BAT activation. Further studies should be developed to decipher these potential differential effects of LSG and RYGB in the capacity to activate BAT thermogenesis.

Despite the advantages described above, IRT-based estimation of BAT activation is not exempt of limitations. Some researchers have pointed out some flaws in the quantification of BAT activity by IRT in subjects with obesity, suggesting that the changes in the layer of subcutaneous fat insulation could be a confounding factor to measure BAT temperature [34]. Although we assume this potential limitation in our study, it is important to remark that we did not use basal temperature at the supraclavicular area as index of BAT activity but the individual capacity to increase supraclavicular temperature before and after a single bout of cold stress in every individual. As the fat layer is the same before and after the 5-min cold-stimulus exposure in each individual patient, changes in fat layer width are not expected to significantly influence our estimation. PET-scan-based measurement of BAT activity may have less potential limitations in this regard. However, using PET-scan techniques rises as well a number of concerns (e.g., specific type of labeled metabolite used for the assay, stress associated with the scanning procedures, etc.) and are obviously not feasible for dynamic repeated measuring in the same individual as performed in our study, which can otherwise be assessed by IRT. Moreover, the lack of randomization in the type of surgery, the anti-diabetic medication, the differences in basal circulating parameters in patients subjected to RYGB and LSG, and no inclusion of normal weight individuals to compare at the same time their thermogenic activation by IRT are additional limitations of the study and might be a bias for assessment of a potential different response to RYGB-versus-LSG.

In conclusion, we report for the first time that IRT-based estimation of BAT thermogenic activity may be useful for a follow-up of patients after a bariatric surgery in a convenient, non-invasive manner. Thus, we propose that quantification of active BAT by IRT after a cold protocol may be useful in clinical practice as an additional measurement to evaluate the metabolic status and the potential success of surgery in patients with obesity. Further studies to check potential biological and clinical associations and to evaluate differences between LSG technique and RYGB on BAT activation would be necessary to establish its precise contribution to the clinical practice.

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Compliance with Ethical Standards

Conflict of Interest All authors declared that they have no conflict of interest.

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