**Objective:** Brown adipose tissue (BAT) is a thermogenic tissue with potential as a therapeutic target in the treatment of obesity and related metabolic disorders. The most used technique for quantifying human BAT activity is the measurement of $^{18}$F-fluorodeoxyglucose uptake via a positron emission tomography/computed tomography scan following exposure to cold. However, several studies have indicated the measurement of the supraclavicular skin temperature (SST) by infrared thermography (IRT) to be a less invasive alternative. This work reviews the state of the art of this latter method as a means of determining BAT activity in humans.

**Methods:** The data sources for this review were PubMed, Web of Science, and EBSCOhost (SPORTdiscus), and eligible studies were those conducted in humans.

**Results:** In most studies in which participants were first cooled, an increase in IRT-measured SST was noted. However, only 5 of 24 such studies also involved a nuclear technique that confirmed increased activity in BAT, and only 2 took into account the thickness of the fat layer when measuring SST by IRT.

**Conclusions:** More work is needed to understand the involvement of tissues other than BAT in determining IRT-measured SST; at present, IRT cannot determine whether any increase in SST is due to increased BAT activity.

**Introduction**

Brown adipose tissue (BAT) is a thermogenic tissue with an important role in murine metabolism (1). Cold exposure leads to norepinephrine release by the sympathetic nervous system, which activates brown adipocytes to increase uncoupling protein 1 activity, thus generating heat (1). In 2009, several studies demonstrated the presence of metabolically active BAT in humans via the measurement of $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) uptake as determined by positron emission tomography/computed tomography (PET/CT) scanning following exposure to cold (2-4). The high metabolic activity of BAT, and its ability to consume both glucose and fatty acids, suggest that it may have potential as a therapeutic target in the treatment of obesity (5).

$^{18}$F-FDG-PET/CT scanning is currently the technique most used for quantifying human BAT activity (6). However, it suffers the drawbacks of being expensive, invasive, and exposing individuals to radiation. Moreover, one of the biggest limitations of $^{18}$F-FDG is that it is based...
on the uptake of a glucose analogue, which is used to measure glucose uptake (quantitative measurement) or fractional FDG uptake in a reliable way (7). Alternative techniques for quantifying human BAT in vivo include PET/CT measurement of the uptake of $^{18}$F-fluoro-6-thia-heptadecanoic acid (8), $^{15}$O-O$_2$ (9), $^{11}$C-acetate (10), or adenosine (11), magnetic resonance imaging (12), and magnetic resonance spectroscopy (MRS) (13), but these are expensive, and some also expose individuals to radiation. A safe, inexpensive, reliable gold standard for human BAT quantification in vivo is thus lacking, as are alternative techniques (6).

Several studies have postulated that the suprascapular skin temperature (SST) can be used as a surrogate marker of BAT volume (14) and activity (14–16). SST is commonly measured using iButtons (Maxim, Dallas, Texas) (14,15,17) (small devices attached to the skin) or infrared thermography (IRT) (18), although alternatives exist. IRT assesses superficial temperature using the emitted infrared radiation, and it is widely used in mice to quantify skin temperature at the site of the interscapular BAT (19). Because IRT is a low-cost, noninvasive, easy-to-use method (and more importantly, it poses no hazard to patients), its use in human studies of SST as a marker of BAT activity is becoming more popular. Recent narrative reviews (18,20) that addressed the use of IRT as a means of measuring BAT activity in humans assumed the method to be valid for that purpose, though this has not been established. The present work therefore systematically reviews the state of the art of using IRT for analyzing SST as an indirect marker of BAT activity in humans.

Methods

This systematic review was conducted adhering to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (21) and was registered through the International Prospective Register of Systematic Reviews (PROSPERO registration number: CRD42018098837).

Search strategy

A literature search was conducted across the PubMed, Web of Science, and EBSCOhost (SPORTdiscus) databases from the time of their inception until April 20, 2019. The search terms used were “infrared thermography” OR “thermal imaging” OR “thermal camera” AND “brown adipose tissue” OR “BAT” OR “brown fat.” The reference lists of the initial search retrieved 95 articles, of which 24 manuscripts were selected after applying the inclusion and exclusion criteria. The first study to use IRT to estimate SST was published in 2011 (24). Ten studies were then published between 2011 and 2016 (25-34), six in 2017 (35-40), six in 2018 (41-44), and one until April 2019 (45).

Study quality

The literature search, data extraction, and assessment of paper quality were independently performed by two observers, and inconsistencies were resolved by consensus. The PEDro Methodological Quality Scale (22) was used to assess the quality of the studies included. This scale, which consists of 11 items, assesses the external validity, internal validity, and interpretability of studies and can detect potential bias with good reliability (22,23). The score is calculated as the sum of the scores for items 2 to 11. Scores of ≤3 were deemed to describe studies of low methodological quality, 4 to 6 those of moderate quality, and ≥7 those of high methodological quality.

Results

Overall results

Figure 1 shows the PRISMA flow diagram for the search strategy. The initial search retrieved 95 articles, of which 24 manuscripts were selected after applying the inclusion and exclusion criteria. The first study to use IRT to estimate SST was published in 2011 (24). Ten studies were then published between 2011 and 2016 (25-34), six in 2017 (35-40), six in 2018 (41-44), and one until April 2019 (45).

Overall, IRT has been used to assess SST in 792 individuals. Some 56% of these individuals were from four studies (n=164, n=102, n=88, n=86) (24,31,43,44) (Table 1). Twelve of the selected twenty-four studies included both sexes (24,26,28,29,31,32,37,41-43,45,46), three involved only females (27,33,44), and nine involved only males (16,25,30,34,36,38-40). One study took into account the phases of the menstrual cycle (32). A total of 25 cohorts were represented across the 24 studies, 21 composed of adults, 2 of adolescents (25,27), and 2 of children (25,43). Three of the studies did not report BMI (24,29,38).

Methodological quality of clinical trials

According to the PEDro scale criteria, 8.3% (n=2) (24,25) of the studies were of low quality, 62.5% (n=15) (20,26,27,30,31,33,36,39,41,43,44) of medium quality, and 29% (n=7) (32,34,35,40,42,45,46) of high quality (Table 2). The criteria least satisfied by the different studies were those of items 2 and 3 regarding allocation and 5, 6, and 7 regarding the blinding process. The best satisfied criteria were those of items 4, 8, 9, 10, and 11 regarding the design of randomization and the data displayed.

IRT device and software model

Two of the twenty-four studies did not indicate the device used (35,36). Seventeen used different models of FLIR cameras (FLIR Systems, Inc., Arlington, Virginia). Five studies used alternatives to FLIR cameras, such as FLK-Ti32 (Fluke Corp., Everett, Washington) (27), Nec TH9100 thermovision (Nippon Avionics Co., Ltd., Tokyo, Japan) (29,38), Varioscan 3021 ST ( Jenoptik, Jena, Germany) (31), and Fluke Ti9 Thermal Imagers (37). Three studies did not report the image analysis software used (24,27,31). Eleven used the software provided by FLIR (25,26,28,30,33,35,40,42,46), six employed special image processing software or custom-built software according to particular...
IRT for Estimating SST and BAT

Jimenez-Pavon et al.

Comparisons of SST measured by IRT as a marker of human BAT activity

Eighteen of the twenty-four studies interpreted the changes in SST as changes in human BAT activity (16,25,26,29,30,32-37,39,40,42-44), whereas six interpreted the results only as changes in SST (24,31,38,41,45,46). Ten studies took SST measurements by IRT and also quantified BAT activity via a nuclear medicine technique (n=8 static 18F-FDG-PET/CT scan (16,26,31,32,42,45,46), n=1 magnetic resonance imaging (27), n=1 MRS) (41) (Figure 2). Fourteen studies measured SST by IRT with no such quantification of BAT activity. Among the eight studies that measured SST by IRT and that quantified BAT activity via an 18F-FDG-PET/CT scan, four aimed to compare the two techniques (16,42,45,46); the remaining four did not examine the agreement between them. Thuzar et al. (42) correlated the changes in SST by IRT following an intervention with the changes in 18F-FDG uptake by BAT but reported no actual data regarding this finding. In another study by the same group (46), it was reported that SST measured by IRT after cold exposure positively correlated (r=0.62; P=0.03) with the BAT maximal standardized uptake of 18F-FDG as measured by PET/CT. In another study comparing SST measured by IRT with 18F-FDG-PET/CT, Law et al. (16) reported that SST relative to the sternal temperature correlated positively with BAT 18F-FDG uptake in eight lean adults. When analyses were performed with absolute SST values, however, no correlation was detected. In other work, Martinez-Tellez et al. (45) reported that SST at the end of the cold exposure correlated inversely with 18F-FDG-determined BAT volume (r = -0.764; P=0.006) but did not correlate at all with BAT activity as measured by 18F-FDG uptake after cold exposure. In addition, when they performed their analysis using values for SST relative to the sternal skin temperature, the former negative correlation they originally saw disappeared, and the latter lack of any significant correlation remained. One study compared SST as measured by IRT against SST as measured by MRS in lean adults (41) and found a positive correlation. Lastly, we observed that almost every study applied different cooling

Figure 1 Flow diagram of the literature search/study selection procedure according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).
| Study/year | Participants | Cooling protocol | IRT technology | IRT methodology | SST after cold intervention | Ref. method (M) | Comparison (C) | Interpretation of study by authors who published the work |
|------------|--------------|------------------|----------------|-----------------|-----------------------------|----------------|---------------|----------------------------------------------------------|
| Lee et al. (2011) (24) | n=88 (46 females) 31 ± 1 y, BMI: ND | - Type of cooling: Air temperature - T*: 17°C - RH: ND - RT: ND - Time: 30 min | Device: FLIR SC660 (Melbourne, Australia) Software: ND | 1st Phase: - Distance: ND - Emissivity: ND - Height: ND | Lesser reduction in SST than in mediastinal ST | M: ND | C: No | IRT is a promising, novel, noninvasive tool for BAT detection and monitoring |
| Symonds et al. (2012) (25) | n=26 7 children: 3-8 y, BMI: 16.9 ± 0.95 kg/m² 12 adolescents: 13-18 y, BMI: 22.6 ± 0.76 kg/m² 7 adults: 35-58 y, BMI: 25.2 ± 1.45 kg/m² | - Type of cooling: 1 hand put in cool tap water - T*: 19°C-20°C - RH: ND - RT: ND - Time: 15 min | Device: FLIR B60, 2.3 megapixels (ABD, Danderyd, Sweden) Software: FLIR QuickReport 1.2 | 1st Phase: - Distance: camera fixed 1 m from participants - Emissivity: ND - Height: camera fixed perpendicular to neck | Greater increase in T° in children compared with adolescents and adults | M: ND | C: No | IRT can quantify thermogenesis within the SCV area in different age groups |
| Kim et al. (2014) (27) | n=1 female 11.5 y, BMI: 18.62 kg/m² | Participants not subjected to cold | Device: FLK-Ti32 (60 Hz; Fluke Corp., Everett, WA) Software: ND | 1st Phase: - Distance: camera fixed at 30.48 cm from participant - Emissivity: 0.95 - Height: ND | ND | M: MRI | C: No | Treatment with thyroid hormone increased SST |
| Study/year | Participants | Cooling protocol | IRT technology | IRT methodology | SST after cold intervention | Ref. method (M) | Comparison (C) | Interpretation of study by authors who published the work |
|------------|--------------|------------------|----------------|-----------------|-----------------------------|----------------|---------------|----------------------------------------------------------|
| Jang et al. (2014) (26) | n = 17 (5 females) | 36 ± 8 y, BMI: 25.4 ± 5.9 kg/m² | -Type of cooling: Air temperature -T*: 19°C -RH: ND -RT: ND -Time: 120 min | Device: FLIR B425, 3.1 megapixels (FLIR Systems Australia Pty., Ltd., Melbourne, Australia) Software: FLIR ResearchIR version 1.2 | 1st Phase: -Distance: camera fixed 1 m from participants -Emissivity: 0.98 -Height: camera fixed perpendicular to neck | M: ¹⁸F-FDG-PET/CT scan | C: No | IRT is a promising technique for studying BAT |
| Robinson et al. (2014) (28) | n = 68 children (30 females) 8.42 y, BMI percentile: 58.8 ± 28.0 (n = 5 with overweight; n = 6 with obesity) | | -Type of cooling: 1 hand immersed in cold water -T*: 20.1°C -RH: ND -RT: ND -Time: 5 min | Device: FLIR B425 (FLIR Systems, Danderyd, Sweden) Software: FLIR Researcher Pro 2.10 | 1st Phase: -Distance: 0.8-0.9 m from participants -Emissivity: ND -Height: ND | M: ND | C: No | Negative relationship between BMI and SST at rest and in response to the cooling stimulus |
| | | | | | 2nd Phase: Photos at baseline and after cooling protocol | | | |
| | | | | | 3rd Phase: Triangular ROI for SST using anatomic landmarks (sternocleidomastoid, trapezius, and clavicle) | | | |
| Study/year | Participants | Cooling protocol | IRT technology | IRT methodology | SST after cold intervention | Ref. method (M) | Comparison (C) | Interpretation of study by authors who published the work |
|------------|--------------|------------------|----------------|----------------|----------------------------|----------------|--------------|--------------------------------------------------------|
| Yakushkin et al. (2014) | n = 10 (3 females) 22.5 y, BMI: ND | Feet to the level of the ankle joint in a water bath, before and after 6 wk of training | Nec TH9100 (Nippon Avionics Co., Ltd., Tokyo, Japan) | Image Processor | 1st Phase: -Distance: ND -Emissivity: ND -Height: ND | SST lower during cooling protocol after 6 wk of training | M: ND | C: No | BAT activity (determined as IRT-measured SST) appears not to be a universal homeostatic instrument |
| Ramage et al. (2016) | n = 6 males, 22.7 ± 1.3 y, BMI: 22.0 ± 0.9 kg/m², IRT: n = 9 (4 females), 22.7 ± 1.3 y, BMI: 21.8 ± 0.6 kg/m² | Air temperature | FLIR T650sc | ResearchIR version 4 | 1st Phase: -Distance: camera fixed 1 m away from participants -Emissivity: ND -Height: camera fixed at level of neck | SST increased after cold in participants treated with glucocorticoids | M: 18F-FDG-PET/CT scan | C: No | Acute ingestion of glucocorticoids increased SST after cooling intervention Chronic ingestion of glucocorticoids decreased SST |
| El Hadi et al. (2016) | Normal weight: n = 14, 29 ± 6 y, BMI: 22.9 ± 1.8 kg/m², With obesity: n = 16, 24 ± 2.2 y, BMI: 33.3 ± 1.7 kg/m² | Both hands immersed in cold water | FLIR T450sc (FLIR Systems, Inc., Wilsonville, OR) | FLIR R&D | 1st Phase: -Distance: camera fixed 1 m away from participants -Emissivity: ND -Height: camera fixed perpendicular to neck | SST increased after cold exposure in lean individuals but not in people with obesity | M: ND | C: No | In normal weight, IRT could be a simple technique to evaluate BAT In people with obesity, the degree of adiposity could limit the use of IRT |
| Study/year | Participants | Cooling protocol | IRT technology | IRT methodology | SST after cold intervention | Ref. method (M) | Comparison (C) | Interpretation of study by authors who published the work |
|------------|--------------|------------------|----------------|-----------------|----------------------------|----------------|---------------|----------------------------------------------------------|
| Gatidis et al. (2016) (31) | n=102 (44 females) 58 ± 17 y, BMI: 26 ± 5 kg/m² | Participants not subjected to cold | Device: Varioscan 3021 ST (Jenoptik, Jena, Germany) Software: ND | 1st Phase: -Distance: camera fixed 1 m from participants -Emissivity: ND -Height: ND | PET+ scans returned higher SST values than did PET− scans | M: 18F-FDG-PET/CT scan | C: No | SCV adipose tissue thickness affected SST, complicating the detection of activated BAT using single-use IRT |
| Salem et al. (2016) (34) | n=11 males 26.1 y, BMI: 22.5 kg/m² | Cooling vest -Type of cooling: 1st Phase: -Distance: camera fixed on tripod 1 m from participants -Emissivity: ND -Height: ND | Device: FLIR T440bc (FLIR Systems, West Malling, UK) Software: ThermaCAM Researcher Pro | 1st Phase: -Distance: camera fixed on tripod 1 m from participants -Emissivity: ND -Height: ND | PET+ had higher SST compared with PET− scans | M: 18F-FDG-PET/CT scan | C: No | Only cold exposure induced an increase in SST, especially in PET+ patients |
| Study/year                  | Participants                        | Cooling protocol                                                                 | IRT technology                      | IRT methodology                      | SST after cold intervention | Ref. method (M) | Comparison (C) | Interpretation of study by authors who published the work                                                                 |
|----------------------------|-------------------------------------|----------------------------------------------------------------------------------|-------------------------------------|---------------------------------------|-----------------------------|----------------|----------------|--------------------------------------------------------------------------------------------------------------------------|
| Robinson et al. (2016) (33) | n = 5 females 21-22 y, BMI: 22.2 ± 0.95 kg/m² | Participants not subjected to cold                                               | Device: FLIR B425 (FLIR Systems, Danderyd, Sweden) | 1st Phase: ND                         | ND                          | M: ND         | C: No          | Subtle changes in psychological stress, including anticipation, can stimulate the production of heat from BAT (SST) |
|                            |                                     |                                                                                  | Software: ThermaCAM Researcher Pro 2.10 | 2nd Phase: Photos at 20 and 10 min prior to test, right before test, and 10, 20, 30, 40, and 50 min after test. | 3rd Phase: For measuring SST, ROI was limited by left sternocleidomastoid muscle, clavicle, and lateral contour of neck |                                  |                |                                                             |
| Ang et al. (2017) (35)     | n = 24 males 23 ± 0.4 y, BMI: 20.4 ± 0.3 kg/m² | - Type of cooling: Both hands and feet in cold water | Device: ND Software: FLIR ResearchIR | 1st Phase: Distance: camera fixed at 1 m from participants | SST higher after cold exposure in those considered BAT+ | M: ND         | C: No          | IRF is a promising tool for quantifying BAT                                                                         |
|                            |                                     | - T°: 18°C                                                                       |                                      | - Emissivity: 0.98                       |                             |                |                |                                                             |
|                            |                                     | - RH: ND                                                                         |                                      | - Height: ND                            |                             |                |                |                                                             |
|                            |                                     | - RT: ND                                                                         |                                      |                                        |                             |                |                |                                                             |
|                            |                                     | - Time: 5 min                                                                    |                                      |                                        |                             |                |                |                                                             |
| Scotney et al. (2017) (40) | n = 8 males 20 y, BMI: 23 kg/m²        | Participants not subjected to cold                                               | Device: FLIR E60, 2.3 megapixels (FLIR Systems, Danderyd, Sweden) | 1st Phase: ND                         | ND                          | M: ND         | C: No          | Glucocorticoids modulate BAT thermogenesis and may represent an important physiological mechanism for maintaining human body T° at times of acute stress |
|                            |                                     |                                                                                  | Software: FLIR QuickReport 1.2       | 2nd Phase: Photos before and after first meal and 15 min before and 15, 40, and 50 min after infusion | 3rd Phase: ROI for SST - ROI for chest ST region close to xiphoid as control |                                |                |                                                             |
| Study/year | Participants | Cooling protocol | IRT technology | IRT methodology | SST after cold intervention | Ref. method (M) | Comparison (C) | Interpretation of study by authors who published the work |
|------------|--------------|------------------|----------------|-----------------|-----------------------------|----------------|---------------|----------------------------------------------------------|
| Kozlov et al. (2017) (38) | n=8 males 27.8 ± 4 y, BMI: ND | -Type of cooling: Feet immersed in cold water -T*: 0°C -RH: ND -RT: ND -Time: 1 min | Device: Nec TH9100SL (Nippon Avionics Co., Ltd., Tokyo, Japan) | 1st Phase: -Distance: camera fixed at 3 m away from participants -Emissivity: ND -Height: camera fixed at 140 cm from floor | SST increased after both Wingate and cooling protocols and decreased after breath holding | M: ND | C: No | Index of thermogenicity can be used for investigating mechanisms of thermography |
| Hartwig et al. (2017) (37) | Normal weight: n=5 females, 38.9 ± 10.3 y, BMI: 19.6 ± 2.3 kg/m² Overweight: n= 5 females, 32.2 ± 9.8 y, BMI: 27.5 ± 1.8 kg/m² | -Type of cooling: Left hand immersed in ice water -T*: 5°C to 9°C -RH: ND -RT: ND -Time: 1 min. | Device: Fluke Ti9 (Fluke Corp., Everett, WA) Software: IDL script specially designed | 1st Phase: -Distance: ND -Emissivity: 0.95 -Height: ND | High SST after cooling protocol in both groups, although increase was higher in lean individuals | M: ND | C: No | IRT may be a novel, noninvasive, radiation-free, easy to use, and low-cost method for monitoring SST |
| Peterson et al. (2017) (39) | n=14 males 24 ± 3 y, BMI: 24.5 ± 1.6 kg/m² | -Type of cooling: Feet immersed in ice water -T*: ND -RH: ND -RT: ND -Time: 2 min | Device: FLIR SC5500-M (FLIR Systems, Inc., St. Louis, MO) Software: MATLAB (version 8.5, MathWorks, Natick, MA) | 1st Phase: -Distance: 2 m away from participants -Emissivity: ND -Height: ND | Mean SST change from control T° | M: ND | C: No | BAT activity did not increase to fight against weight gain from overeating (SST) |

*T* = temperature measured by infrared thermography.
| Study/year | Participants | Cooling protocol | IRT technology | IRT methodology | SST after cold intervention | Ref. method (M) | Comparison (C) | Interpretation of study by authors who published the work |
|------------|--------------|------------------|----------------|-----------------|-----------------------------|----------------|----------------|---------------------------------------------------------|
| Haq et al. (2017) (36) | Phase 1: n = 28 males, 23.9 ± 5.9 y, BMI: 25.2 ± 3.9 kg/m² | Phase 1: Visit 1: no IRT | Device: ND | 1st Phase: | SST increased after cold exposure but not under thermoneutral conditions | M: ND | C: No | following the 32°C-cold protocol and using IRT is unlikely to be affected by environmental T° and subcutaneous neck fat metabolic activity in adults |
|             | Phase 2: n = 14 males, 20.9 y, BMI: 23.6 ± 3.2 kg/m² | Visit 2: | Software: AMIDE | 2nd Phase: | Photos every 5 min | | | |
|             | Phase 1: Visit 1: no IRT | | | | | | | |
|             | Visit 2: | | | | | | | |
|             | | | | | | | | |
|             | | | | | | | | |
|             | | | | | | | | |
| Study/year | Participants | Cooling protocol | IRT technology | IRT methodology | SST after cold intervention | Ref. method (M) | Comparison (C) | Interpretation of study by authors who published the work |
|------------|--------------|------------------|----------------|----------------|-----------------------------|----------------|---------------|----------------------------------------------------------|
| Law et al. (2018) (16) | n=8 males 23.5 y, BMI: 22.0 kg/m² | - Type of cooling: Cooling vest - T*: 8°C - RH: ND - RT: ND - Time: 10 min | Devices: FLIR T440bx (FLIR Systems, West Malling, UK) Software: Custom-built TITCH, a Raspberry-based device | 1st Phase: - Distance: ND - Emissivity: ND - Height: ND | SST showed peak after 10 min of cold exposure | M: ¹⁸F-FDG-PET/CT scan | C: Yes | IRT can provide a safe, credible, and quantifiable alternative to PET/CT |
| Thuzar et al. (2018) (42) | n=13 (7 females) 28 ± 2 y, BMI: 24 ± 1 kg/m² | - Type of cooling: Air temperature - T*: 19°C - RH: ND - RT: ND - Time: 120 min | Device: FLIR B425, 3.1 megapixels (FLIR Systems Australia Pty. Ltd., Melbourne, Australia) Software: FLIR ResearchIR version 1.2 | 1st Phase: - Distance: camera fixed at 1 m from participants - Emissivity: ND - Height: camera fixed at level of neck | SST slightly decreased after cold exposure | M: ¹⁸F-FDG-PET/CT scan | C: Yes | Prolonged glucocorticoid suppressed the function of human BAT |
| Sarasniemi et al. (2018) (41) | n=20 (15 females) 48 ± 5 y, BMI: 28.1 ± 5.3 kg/m² | Participants not subjected to cold | Device: FLIR A325, 3.2 megapixels (FLIR Systems Australia Pty. Ltd., Melbourne, Australia) Software: MATLAB-based Biosignal Scientists software | 1st Phase: - Distance: camera fixed at 1 m from participants - Emissivity: 0.98 - Height: camera fixed at level of neck | ND | M: MRS | C: Yes | SST as measured by IRT correlated positively with MRS temperature in lean adults; however, SST measured by IRT correlated negatively with MRS temperature in adults with obesity |
| Study/Year | Participants | Cooling Protocol | IRT Technology | IRT Methodology | SST After Cold Intervention | Ref. Method (M) | Comparison (C) | Interpretation of Study by Authors Who Published the Work |
|------------|--------------|------------------|----------------|-----------------|-----------------------------|----------------|--------------|----------------------------------------------------------|
| Malpique et al. (2019) (43) | n = 86 (47 females) 8.5 ± 0.1 y, BMI: 17.5 ± 0.4 kg/m² | Type of cooling: 1 hand immersed in cool water | Device: FLIR B60, 2.3 megapixels (FLIR Systems Australia Pty., Ltd., Melbourne, Australia) | 1st Phase: Distance: camera fixed at 1 from participants | SST slightly increased after cold exposure; females had higher baseline and post-cold SST | M: No | C: No | SST measured by IRT seems to be similar in healthy children born either small-for-gestational age or appropriate-for-gestational age |
| Zhou et al. (2018) (44) | Nondiabetic pregnant participants: n = 91, 30.0 ± 0.6 y, BMI: 24.38 ± 0.87 kg/m² Participants with gestational diabetes mellitus: n = 73, 31.6 ± 0.6 y, BMI: 26.24 ± 0.64 kg/m² | Participants not subjected to cold | Device: FLIR B425, 3.1 megapixels (FLIR Systems Australia Pty., Ltd., Melbourne, Australia) | 3rd Phase: SST measured by IRT seems to be reduced in pregnant women with gestational diabetes mellitus |
| Thuzar et al. (2019) (46) | n = 2 males and 8 females 28 ± 1 y, BMI: 24.4 ± 1.2 kg/m² | Type of cooling: Air temperature | Device: FLIR B425, 3.1 megapixels (FLIR Systems Australia Pty., Ltd., Melbourne, Australia) | 1st Phase: Distance: camera fixed at 1 m from participants | SST slightly decreased after cold exposure | M: ¹⁸F-FDG-PET/CT scan | C: Yes | SST measured by IRT seems to be reduced upon cold exposure; positive correlation between SST and BAT activity (SUVmax) |
| Study/year          | Participants                                      | Cooling protocol | IRT technology | IRT methodology | SST after cold intervention                                                                 | Ref. method (M) | Comparison (C) | Interpretation of study by authors who published the work |
|--------------------|---------------------------------------------------|------------------|----------------|-----------------|------------------------------------------------------------------------------------------------|----------------|----------------|----------------------------------------------------------|
| Martinez-Tellez et al. (2019) (45) | n=2 males and n=10 females | 21.9 ± 2.2 y, BMI: 23.5 ± 4.8 kg/m^2 | -Type of cooling: Air temperature + cooling vest. -T°: 19°C -RH: ND -RT: ND -Time: 180 min | Device: FLIR E60 (3200 X 240, FLIR Systems, Inc., Wilsonville, OR) Software: FLIR ResearchIR version 14.40.6.24 | 1st Phase: -Distance: camera fixed at 1 m from participants -Emissivity: 0.98 -Height: camera fixed at level of neck | SST did not change after cold exposure | M: 18F-FDG-PET/CT scan C: Yes | SST measured by IRT negatively correlated with BAT volume, but not with BAT activity (SUV mean and peak); same results were observed when SST was represented relative to the sternal ST |

Phase 1 refers to all methodological aspects in preparation for IRT. Phase 2 corresponds to use of IRT. Phase 3 describes all procedures followed for quantitative analysis of images. BAT, brown adipose tissue; 18F-FDG-PET/CT, 18F-fluorodeoxyglucose, positron emission tomography/computed tomography; f/s, frames per second; IDL, interactive data language; IRT, infrared thermography; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; ND, not described; RH, relative humidity; ROI, region of interest; RT, reflected temperature; SCV, supradacicular; SST, supradacicular skin temperature; ST, skin temperature; SUVmax, maximal standardized uptake value; T°, temperature; TITCH, thermal imaging technical conversion hub.
protocols, which makes the comparison between studies even more difficult (Table 1).

**Cooling protocols**

Cold exposure is the main activator of BAT in humans (1). Most, but not all, of the identified studies (n=18; 75%) included cooling protocols, although the duration, temperature, and the way of inducing cooling differed between studies (16,24,26,32,34,38,43,45) (Figure 2). Nine out of these eighteen studies used hands/feet immersion in cold water (temperature 10.8°C ± 8.7°C, duration: 6.1 ± 6.8 minutes) (25,28-30,35,37-39). Seven of these nine studies (28,30,35,37-39,43) used a cold exposure of < 5 minutes. In contrast, 8 of the 18 studies (16,24,26,32,34,42,45) cooled their participants via air cooling or through the use of cooling vests (temperature 17.9°C ± 1.3°C and 8°C; duration 97.5 ± 45.0 minutes and 35.5 ± 31.8 minutes, respectively). One study combined air cooling and cooling blankets at 12°C for 120 minutes (36). In four studies, the duration of cooling was 120 minutes or longer (26,32,36,42,46). Only one study followed a personalized cooling protocol (45) (Table 1).

The studies that involved cold stimulus (n=18/24) reported conflicting results, with increases (16,25,28,30,32,34-38,43), no change (26,45), or slight reductions (24,42,46) in SST. Those studies that found an increase or no change after cold exposure were performed in lean adults or children (16,25,28,30,32,34-38,43,45), whereas those that observed a reduction were conducted in individuals with obesity (24,42) or lean individuals (46). In one study that reported increases in SST in children after cold exposure (43), greater increases were seen in girls than in boys. However, comparisons between studies should be made with caution because different cold stimuli were used and different physiological responses may therefore have been elicited.

**Discussion**

The present work reviews all human studies that have assessed IRT-measured SST as a proxy of BAT activity. Only 5 out of 24 studies directly compared IRT-measured SST against BAT activity determined via a nuclear technique. Most of the studies in which some type of cold stimulus was applied reported an increase in SST although, in some studies, no change in SST was seen when reporting data as absolute values. All the studies found cold to have a great effect on SST in lean adults, independent of sex. Individuals with obesity seemed to have a
Figure 2 Type of cooling protocols (T.cooling) used in the studies included in this review. Supraclavicular skin temperature (SST) columns show the effect of cold exposure on SST. Red arrows mean an increase of SST after the cold exposure, whereas blue arrows mean a decrease of SST. Equal symbols (”=” mean that SST did not change upon cold exposure. Gray boxes mean those studies that compared SST measured by IRT with brown adipose tissue (BAT) measured by a nuclear medicine technique. Yellow boxes mean those studies that measured BAT with a nuclear medicine technique but that did not compare with SST measured by IRT. White boxes mean those studies that used only SST measured by IRT as a proxy of BAT activity. CB, cooling blankets; CV, cooling vest; IRT, infrared thermography; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; 18F-FDG-PET/CT, 18F-fluorodeoxyglucose, positron emission tomography/computed tomography; ND, not described by the study.
different response. Most studies assumed that the increase, or lack of change, in SST upon cold exposure involved BAT activation.

In 1987, Kellman et al. (47) performed a thorough study in which they analyzed the tissue composition of the supraclavicular fossa in humans and found it to be composed of the subclavian vessels, the brachial plexus, the omohyoid and scalene muscles, fat, and (to a lesser extent) lymph nodes and the posterior lung apex. This anatomical area is known to contain adipose tissue with a morphology and gene expression pattern indicative of the presence of brown or beige fat (48,49). However, the amount of active BAT present in humans is still questioned (50,51). Blondin et al. (52) observed that the deep and cervical muscles showed a greater 18F-FDG uptake than other skeletal muscles after cold exposure, with the Longus colli and sternocleidomastoid muscles returning the highest values in the upper body and after applying a fixed cooling protocol. In addition, Hanssen et al. (53) found 18F-FDG uptake by the scalene muscle to be higher than that seen in other skeletal muscles both before and after a 10-day cold-acclimation intervention and after applying an individualized cooling protocol. Using 15O-2-O2-PET/CT scanning, U Din et al. (9) showed the deep and cervical muscles to be the main contributors toward the increase in energy expenditure upon cold exposure in humans. They also showed that the supraclavicular and cervical BAT contributed toward this increase in energy expenditure by only ~2%. These results indicate that skeletal muscles contribute toward human cold-induced thermogenesis more than BAT does (54-56). Therefore, when skeletal muscles located in the supraclavicular fossa shiver and generate heat during cold exposure, they might be related to the increase in SST, although further studies are needed to confirm this hypothesis. Even after applying different cooling protocols, both Blondin et al. (52) and Hanssen et al. (53) observed that 18F-FDG uptakes by skeletal muscles were different. Whether these different cooling protocols activate muscle shivering thermogenesis in different manners, and therefore could be contributing differently to SST, remains unknown.

The supraclavicular subcutaneous fat layer acts as an insulation tissue that, depending on its thickness, may influence the value of SST as measured by IRT (31). Indeed, IRT-measured SST following cold exposure may represent the sum of different tissues’ features, including the thermogenic activity of skeletal muscles and of BAT, the temperature of vessels associated with the extent of blood flow, and insulation from the supraclavicular fat layer. Of note is that IRT is able to measure only skin temperature, not blood flow or heat production by the tissue (57). At best, IRT measures a reflection of the heat leakage, which is only a part of the heat transported from BAT. Therefore, from a theoretical point of view, probably at best, with IRT one can discriminate between no BAT activity and BAT activity. Thus, it remains unknown whether a direct link between changes in SST and BAT activation can be accurately detected. Because other thermogenic tissues are located in the same area, their possible contribution toward SST cannot be ignored.

Sarasniemi et al. (41) compared IRT-measured SST with the temperature of the supraclavicular depot as measured by MRS and observed a positive and significant correlation in lean adults but a negative correlation in adults with obesity. Moreover, Thuzar et al. performed two independent studies (42,46) in which they compared IRT-measured SST against BAT 18F-FDG uptake as measured by a PET/CT scan on the same day, reporting a positive and significant association between them. In contrast, Law et al. (16) made the same kind of measurements on separate days and using different cold exposure times. Comparing the latter two studies is, therefore, not easy. It seems that IRT-measured SST might, however, be related to BAT 18F-FDG uptake mostly in lean adults, although this positive association was seen only when SST results were presented relative to the sternal skin temperature. When absolute values were used, no association was seen at all. The present authors performed a similar study in which correlations between IRT-measured SST (45) (absolute and relative to the sternal skin temperature) and BAT 18F-FDG uptake were sought; an inverse correlation was seen. Sarasniemi et al. (41) reported the same thing. Recently, Leitner et al. (58) observed that 80 minutes of cold exposure (using a cooling vest) induced similar levels of BAT 18F-FDG uptake as did 120 minutes of cold exposure. Whether shorter times induce similar BAT 18F-FDG uptakes is unknown; the one study that did use a 10-minute cold exposure did not employ a nuclear technique to measure BAT activation. Furthermore, the cooling technique used (applying cold to the hands) could have activated BAT via a side pathway rather than through the classical cold pathway (59). More studies are needed to determine which protocol is the most appropriate for activating human BAT (5).

**General limitations of studies discussed**

**Measurement of human skin temperature by IRT.** Moreira et al. (57) published a consensus statement on the measurement of human skin temperature by thermography imaging in sports and exercise settings but did not focus on its use as a potential surrogate for BAT activity. They created a checklist of recommendations to be taken into account before starting any IRT measurement of skin temperature. The studies discussed in the present review did not follow these methodological recommendations, which may have affected the SST results obtained, especially in studies involving cold stimuli. For instance, almost no study reported how long the skin was exposed to the air temperature totally uncovered before performing the measurements to allow stabilization in skin temperature. Moreira et al. (57) also postulated relative humidity to be an important parameter that should be taken into account in line with thermography principles to properly correct the measures of temperature. Again, none of the studies discussed here recorded and corrected for this (Table 1). However, these recommendations themselves need to be revised and adapted for studies in which SST is measured to provide joined guidelines on both thermography and SST related to BAT.

**Lack of gold standard.** Currently, the method most used in the field of BAT quantification is 18F-FDG-PET/CT scanning (60). It is important to understand that this measures the amount of 18F-FDG (a glucose analogue) that a tissue consumes. It is well known, however, that BAT also consumes fatty acids; in fact, they are this tissue’s preferred substrate for thermogenesis (61). The lack of a valid gold standard for measuring BAT activity therefore hampers the use of any PET/CT scan-based data as a means of validating IRT-based measurements. Radiolabeled fatty acids would more closely mimic the actual metabolic activity of BAT, but to date, this idea has been poorly developed. Thus, based on current knowledge, IRT-measured SST should be understood as complementary data that may represent the thermogenic activity of other tissues in addition to BAT. An accurate method for specifically measuring BAT activity in vivo in humans is, therefore, still needed.

**Cooling protocols.** There are almost as many cooling protocols as there are studies, which hinders the making of comparisons. The use of different cooling protocols could induce different BAT activities, and the SST response may therefore differ. Certainly, the lack of any changes in
IRT-measured SST when employing 5-minute cooling times may reflect that such short cold stimuli are insufficient to induce BAT activation (it should be noted that the majority of studies did not report the temperature of other zones as controls). However, extremely long cooling protocols might activate BAT via pain rather than cold pathways (59). Basically, different cooling protocols could induce different responses, and the contribution of the tissues in nonshivering and shivering thermogenesis could be dissimilar. It is important that future studies use longer cooling protocols and report the temperatures of different areas.

IRT-measured SST in different human populations

The aforementioned limitations in IRT use for assessing SST may manifest themselves differently in different human cohorts, and certainly the thickness of the subcutaneous fat layer might lead to different results being recorded in individuals with obesity and lean individuals. The relative value of IRT-based measurements, and their validation in pediatric populations, deserves further research.

Conclusion

IRT-measured SST is still to be unequivocally validated as a tool for indirectly quantifying human BAT activity. It remains to be seen whether there is a direct link between changes in IRT-measured SST and BAT activation. Because other thermogenic tissues are located in the same area, their contribution to SST cannot be ignored. Future human studies must take into account the thickness of the subcutaneous fat layer if SST in individuals with different body compositions is to be identified. Finally, further studies are needed to identify the biological factors that determine the temperature of the supraclavicular area.

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