Obesity

Infrared Thermography for Estimating Supraclavicular Skin Temperature and BAT Activity in Humans: A Systematic Review

David Jimenez-Pavon [1,2,], Juan Corral-Perez [1,2,*], David Sánchez-Infantes [3,4], Francesc Villarroya [4,5], Jonatan R. Ruiz [6], and Borja Martinez-Tellez [6,7]*

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.

Obesity (2019) **27**, 1932-1949. doi:10.1002/oby.22635

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

[1] MOVE-IT Research Group, Department of Physical Education, Faculty of Education Sciences, University of Cádiz, Cádiz, Spain [2] Biomedical Research and Innovation Institute of Cádiz (INiBICA) Research Unit, Puerta del Mar University Hospital, University of Cádiz, Cádiz, Spain [3] Department of Endocrinology and Nutrition, Germans Trias i Pujol Research Institute, Badalona, Barcelona, Spain [4] Biomedical Research Center (Fisiopatología de la Obesidad y Nutrición) (CIBEROBN), ISCIII, Madrid, Spain [5] Department of Biochemistry and Molecular Biomedicine, Institute of Biomedicine, Barcelona, Spain [6] PROFITH (PROmoting FITness and Health through Physical Activity) Research Group, Department of Physical Education and Sports, Faculty of Sport Sciences, Sport and Health University Research Institute (iMUDS), University of Granada, Granada, Spain. Correspondence: Borja Martinez-Tellez (borjammt@gmail.com) [7] Department of Medicine, Division of Endocrinology, and Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Centre, Leiden, the Netherlands.

Funding agencies: This study was supported by the Spanish Ministry of Economy and Competitiveness (MINECO) via the Fondo de Investigación Sanitaria del Instituto de Salud Carlos III (PI13/01393), Retos de la Sociedad (DEP2016-79512-R) and European Regional Development Funds (ERDF), the Fundación Iberoamericana de Nutrición (FINUT), the Redes Temáticas de Investigación Cooperativa RETIC (Red SAMID RD16/0022), the AstraZeneca HealthCare Foundation, the University of Granada Plan Propio de Investigación 2016 Excellence actions: Unit of Excellence on Exercise and Health (UCEES), and Plan Propio de Investigación 2018 and the Junta de Andalucía, Consejería de Conocimiento, Investigación y Universidades (ERDF: SOMM17/6107/UGR). DSI is an Investigator of the Miguel Servet Fund from Carlos III National Institute of Health, Spain (CP15/00106). DJP is supported by grants from the Spanish Ministry of Science and Innovation-MINECO (RYC-2014-16938), MINECO/European Fund for Regional Development (FEDER) (DEP2016-76123-R), the Government of Andalusia, the Integrated Territorial Initiative 2014-2020 for the Province of Cádiz (PI-0002-2017), the European Union's ERASMUS+SPORT program (Grant Agreement 603121-EPP-1-2018-1-ES-SPO-SCP), and the EXERNET Research Network on Exercise and Health in Special Populations (DEP2005-00046/ACTI).

Disclosure: The authors declared no conflict of interest.

Author contributions: Conception and design of research: DJP, DSI, and BMT. Performed the searches: JCP and BMT. All authors interpreted the results. DJP, JCP, and BMT prepared the figures and drafted the manuscript; all authors critically revised the manuscript and approved the final version.

*David Jimenez-Pavon and Juan Corral-Perez are joint first authors.

Received: 23 May 2019; **Accepted:** 25 July 2019; **Published online 5 November 2019. doi:10.1002/oby.22635**

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 supraclavicular 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 retrieved studies were reviewed to identify additional studies.

Selection criteria

The inclusion criteria were (1) type of study (randomized controlled trials and longitudinal and cross-sectional observational studies) and (2) the use of IRT to quantify SST. The exclusion criteria were as follows: (1) studies written in languages other than English or Spanish, (2) studies published in non-peer-reviewed journals, (3) reviews, and (4) studies in animal models. No restrictions were placed on participant age or body composition.

Data extraction

The following data were collected from each included study: (1) study year, (2) participant characteristics (age, sex, and BMI), (3) cooling protocol followed, (4) IRT technology used, (5) IRT methodology used, (6) SST after cold intervention, (7) reference methods and comparisons, and (8) conclusions.

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 performed in 2011 (24). Ten studies were then published between 2011 and 2016 (25-34), six in 2017 (35-40), six in 2018 (16,41-44), 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

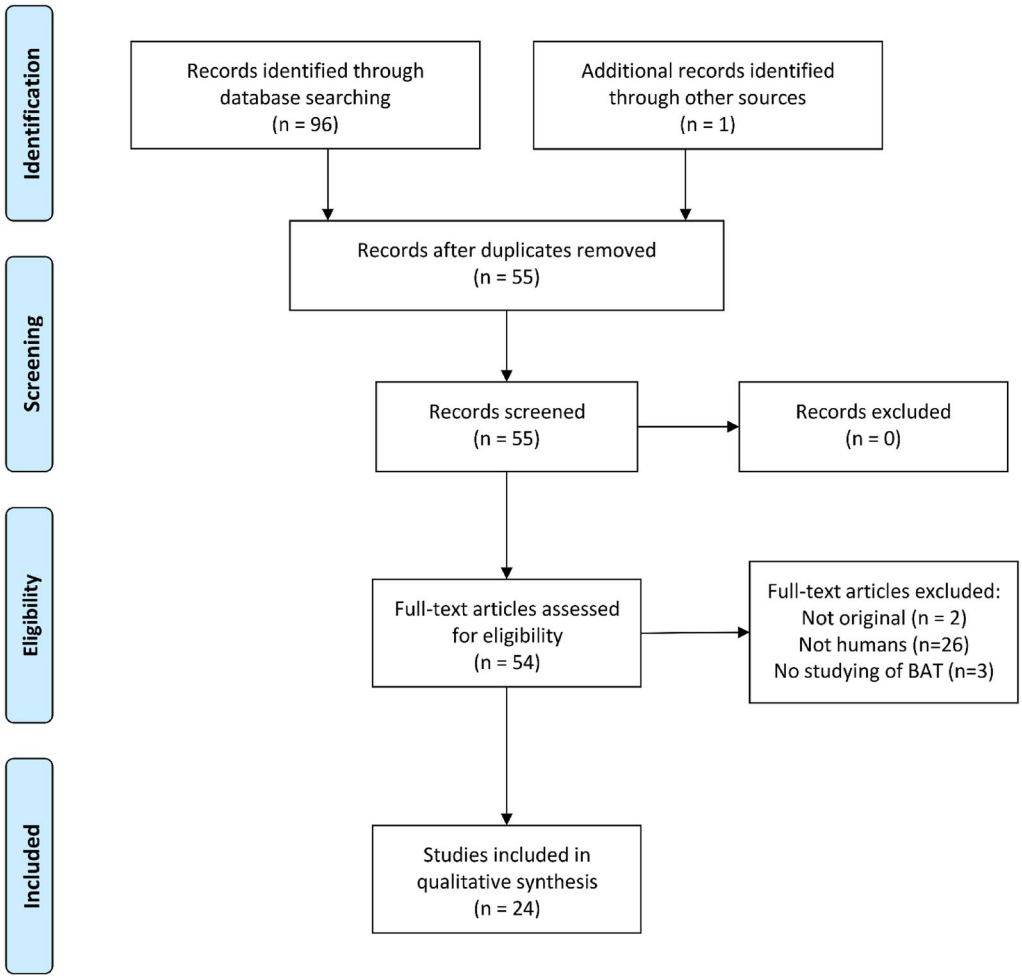

Figure 1 Flow diagram of the literature search/study selection procedure according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).

needs and interests (16,29,37-39,41), and two used software from other brands (ResearchIR and AMIDE) (32,36).

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 ^{18}F-FDG-PET/CT scan (16,26,31,32,34,42,45,46), $n=1$ 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 ^{18}F-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 ^{18}F-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 ^{18}F-FDG as measured by PET/CT. In another study comparing SST measured by IRT with ^{18}F-FDG-PET/CT, Law et al. (16) reported that SST relative to the sternal temperature correlated positively with BAT ^{18}F-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 ^{18}F-FDG-determined BAT volume ($r=-0.764$; $P=0.006$) but did not correlate at all with BAT activity as measured by ^{18}F-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

TABLE 1 Studies using IRT for analyzing SCV skin temperature as an indirect measurement of brown adipose tissue activity

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 **2nd Phase:** Two photos, before and after cold exposure **3rd Phase:** -SST -Mediastinal ST (control)	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/m^2 12 adolescents: 13-18 y, BMI: 22.6 ± 0.76 kg/m^2 7 adults: 35-58 y, BMI: 25.2 ± 1.45 kg/m^2	-**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 **2nd Phase:** 5 photos at 1-min intervals before and at other moments during immersion **3rd Phase:** ROI for SST encompassed upper thorax and neck, using anatomic landmarks of shoulder tips laterally, mandible superiorly, and nipple line inferiorly	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/m^2	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 **2nd Phase:** 2 photos, before and after MRI examination **3rd Phase:** -2 × 8-cm ROI for SST -2 × 8-cm ROI for suprasternal ST as control	ND	**M:** MRI **C:** No	Treatment with thyroid hormone increased SST

TABLE 1. (continued).

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/m^2	-**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 **2nd Phase:** 3 photos at baseline and at 60 min and 120 min of cooling **3rd Phase:** -2-cm-radius ROI for SST -2-cm-radius ROI for chest ST analyzed as control	-PET+ group did not change SST after cold exposure, whereas PET− did -Chest ST decreased after cold exposure only in PET+ group	**M:** ^{18}F-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 **2nd Phase:** Photos at baseline and after cooling protocol **3rd Phase:** Triangular ROI for SST using anatomic landmarks (sternocleidomastoid, trapezius, and clavicle)	Higher SST after cooling intervention	**M:** ND **C:** No	Negative relationship between BMI and SST at rest and in response to the cooling stimulus

TABLE 1. (continued).

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) (29)	$n=10$ (3 females) 22.5 y, BMI: ND	-**Type of cooling:** Feet to the level of the ankle joint in a water bath, before and after 6 wk of training -**T°:** 0°C -**RH:** ND -**RT:** ND -**Time:** 1 min	**Device:** Nec TH9100 (Nippon Avionics Co., Ltd., Tokyo, Japan) **Software:** Image Processor	**1st Phase:** -Distance: ND -Emissivity: ND -Height: ND **2nd Phase:** Photos during cooling protocol **3rd Phase:** 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) (32)	PET/CT: $n=6$ males, 22.7 ± 1.3 y, BMI: 22.0 ± 0.9 kg/m^2 IRT: $n=9$ (4 females), 22.7 ± 1.3 y, BMI: 21.8 ± 0.6 kg/m^2	-**Type of cooling:** Air temperature -**T°:** 16°C-17°C -**RH:** ND -**RT:** ND -**Time:** 120 min	**Device:** FLIR T650sc **Software:** ResearchIR version 4	**1st Phase:** -Distance: camera fixed 1 m away from participants -Emissivity: ND -Height: camera fixed at level of neck **2nd Phase:** Photos every 15 min during cooling protocol **3rd Phase:** Circular ROI for left and right SST	SST increased after cold in participants treated with glucocorticoids	**M:** ^{18}F-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) (30)	Normal weight: $n=14$, 29 ± 6 y, BMI: 22.9 ± 1.8 kg/m^2 With obesity: $n=16$, 24 ± 2.2 y, BMI: 33.3 ± 1.7 kg/m^2	-**Type of cooling:** Both hands immersed in cold water -**T°:** 5°C -**RH:** ND -**RT:** ND -**Time:** 20 min	**Device:** FLIR T450sc (FLIR Systems, Inc., Wilsonville, OR) **Software:** FLIR R&D	**1st Phase:** -Distance: camera fixed 1 m away from participants -Emissivity: ND -Height: camera fixed perpendicular to neck **2nd Phase:** Photos at baseline and after cooling protocol **3rd Phase:** -Triangular ROI for SST -Circular ROI for sternal ST as control	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

TABLE 1. (continued).

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/m^2	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 **2nd Phase:** 1 photo prior to FDG injection **3rd Phase:** -Triangular ROI limited by clavicle, sternocleidomastoid, and lateral neck contour for SST -Circular ROI for sternal and jugular ST as controls	PET+ scans returned higher SST values than did PET– scans	**M:** ^{18}F-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/m^2	-**Type of cooling:** Cooling vest -**T°:** 8°C -**RH:** ND -**RT:** ND -**Time:** 55 min	**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 **2nd Phase:** Video recordings at baseline, after start of cold exposure, and at end of trial **3rd Phase:** -ROI limited by acromioclavicular joint, cricoid prominence, and sternoclavicular joint for SST -For deltoid ST as control, ROI was limited by acromioclavicular joint to lateral extremity of deltoid	PET+ had higher SST compared with PET–	**M:** ^{18}F-FDG-PET/CT scan **C:** No	Only cold exposure induced an increase in SST, especially in PET+ patients

TABLE 1. (continued).

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/m^2	Participants not subjected to cold	**Device:** FLIR B425 (FLIR Systems, Danderyd, Sweden) **Software:** ThermaCAM Researcher Pro 2.10	**1st Phase:** -Distance: ND -Emissivity: ND -Height: ND **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	ND	**M:** ND **C:** No	Subtle changes in psychological stress, including anticipation, can stimulate the production of heat from BAT (SST)
Ang et al. (2017) (35)	*n* = 24 males 23 ± 0.4 y, BMI: 20.4 ± 0.3 kg/m^2	-**Type of cooling:** Both hands and feet in cold water -**T°:** 18°C -**RH:** ND -**RT:** ND -**Time:** 5 min	**Device:** ND **Software:** FLIR ResearchIR	**1st Phase:** -Distance: camera fixed at 1 m from participants -Emissivity: 0.98 -Height: ND **2nd Phase:** 5-min video at 30 f/s, with changes in position of head (turning to left and right) for 10 s on 3 different occasions **3rd Phase:** ROI created with seeded region growing algorithm for SST	SST higher after cold exposure in those considered BAT+	**M:** ND **C:** No	IRT is a promising tool for quantifying BAT
Scotney et al. (2017) (40)	*n* = 8 males 20 y, BMI: 23 kg/m^2	Participants not subjected to cold	**Device:** FLIR E60, 2.3 megapixels (FLIR Systems, Danderyd, Sweden) **Software:** FLIR QuickReport 1.2	**1st Phase:** -Distance: ND -Emissivity: ND -Height: ND **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	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

TABLE 1. (continued).

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) **Software:** Image Processor	**1st Phase:** -Distance: camera fixed at 3 m away from participants -Emissivity: ND -Height: camera fixed at 140 cm from floor **2nd Phase:** Photos recorded after 10 min of finished tests **3rd Phase:** ROI drawn on back and chest; ROI in SCV area not specified	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/m^2 Overweight: $n=5$ females, 32.2 ± 9.8 y, BMI: 27.5 ± 1.8 kg/m^2	-**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 **2nd Phase:** Photos every hour during glucose tolerance test and after cooling stimulation **3rd Phase:** -Square ROI (10 × 10 mm) for left SST	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/m^2	-**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 **2nd Phase:** 2 photos after acclimation and 2 photos after cold water immersion **3rd Phase:** Square ROI from clavicle to base of neck for SST	Mean SST change from control T°	**M:** ND **C:** No	BAT activity did not increase to fight against weight gain from overeating (SST)

TABLE 1. (continued).

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/m^2 Phase 2: *n*= 14 males, 20.9 y, BMI: 23.6 ± 3.2 kg/m^2	*Phase 1:* Visit 1: no IRT Visit 2: -**Type of cooling:** Air temperature and cooling blanket -**T°:** 32°C for air and 12° for blanket. -**RH:** ND -**RT:** ND -**Time:** 60+60 min Visit 3: -**Type of cooling:** Air temperature and cooling blanket -**T°:** 20°C-23°C for air and 12° for blanket -**RH:** ND -**RT:** ND -**Time:** 60+60 min Visit 4: -**Type of cooling:** Air temperature -**T°:** 20°C-23°C -**RH:** ND -**RT:** ND -**Time:** 120 min *Phase 2:* **Type of cooling:** Air temperature and cooling blanket -**T°:** 32°C for air and 12° for blanket -**RH:** ND -**RT:** ND -**Time:** 60+60 min	**Device:** ND **Software:** AMIDE	**1st Phase:** -Distance: camera fixed at 1 m from participants -Emissivity: 0.98 -Height: ND **2nd Phase:** Photos every 5 min **3rd Phase:** SST analyzed with rectangular ROI limited by mandible superiorly, clavicle inferiorly, and acromion and sternoclavicular joint medially	SST increased after cold exposure but not under thermoneutral conditions	**M:** ND **C:** No	BAT detection (SST) 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

TABLE 1. (continued).

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/m^2	-**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 **2nd Phase:** Photos after 5-s intervals **3rd Phase:** -Box-shaped ROI encompassing upper thorax and neck, using anatomical landmarks of shoulder tips laterally, mandible superiorly, and nipple line inferiorly for SST -10-pixel-diameter ROI in reference region (ND) as control	SST showed peak after 10 min of cold exposure	**M:** ^{18}F-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/m^2	-**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 **2nd Phase:** Photos at 0, 1, and 2 h of cooling. **3rd Phase:** -2 cm-radius ROI centered immediately above midclavicle points for SST -ROI for midsternal T° as control	SST slightly decreased after cold exposure	**M:** ^{18}F-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/m^2	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 **2nd Phase:** -Photo after 5 min at room temperature **3rd Phase:** -ROIs determined in 5 different muscle groups	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

TABLE 1. (continued).

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/m^2	**-Type of cooling:** 1 hand immersed in cool water **-T°:** 17°C to 18°C **-RH:** ND **-RT:** ND **-Time:** 5 min	**Device:** FLIR B60, 2.3 megapixels (FLIR Systems Australia Pty. Ltd., Melbourne, Australia) **Software:** FLIR QuickReport 1.2	**1st Phase:** -Distance: camera fixed at 1 from participants -Emissivity: ND -Height: camera fixed at level of neck **2nd Phase:** -Photos at baseline and after cooling intervention **3rd Phase:** ND	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/m^2 Participants with gestational diabetes mellitus: $n=73$, 31.6 ± 0.6 y, BMI: 26.24 ± 0.64 kg/m^2	Participants not subjected to cold	**Device:** FLIR B425, 3.1 megapixels (FLIR Systems Australia Pty. Ltd., Melbourne, Australia) **Software:** FLIR ResearchIR version 1.2	**1st Phase:** ND **2nd Phase:** ND **3rd Phase:** 2 cm-radius ROI for SST	ND	**M:** No **C:** No	SST as 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/m^2	**-Type of cooling:** Air temperature **-T°:** 19°C **-RH:** ND **-RT:** ND **-Time:** 180 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 **2nd Phase:** Photos at 0, 1, and 2 h of cooling **3rd Phase:** -For SST, 2-cm-radius ROI centered immediately above midclavicle points -ROI for midsternal T° as control	SST slightly decreased after cold exposure	**M:** ^{18}F-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)

TABLE 1. (continued).

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 **2nd Phase:** Photos before and after cooling protocol **3rd Phase:** -ROI manually drawn in SCV, sternal, hand, and forearm regions -ROI for midsternal T° as control	SST did not change after cold exposure	**M:** ^{18}F-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; ^{18}F-FDG-PET/CT, ^{18}F-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, supraclavicular; SST, supraclavicular skin temperature; ST, skin temperature; SUVmax, maximal standardized uptake value; T°, temperature; TITCH, thermal imaging technical conversion hub.

TABLE 2 Methodological quality of clinical trials using PEDro Scale

Study/year	1[a]	2	3	4	5	6	7	8	9	10	11	Score
Lee et al. (2011) (24)	N	N	N	N	N	N	N	Y	N	N	Y	2
Symonds et al. (2012) (25)	Y	N	N	N	N	N	N	Y	N	Y	Y	3
Kim et al. (2014) (27)	N	N	N	Y	N	N	N	Y	Y	Y	Y	5
Jang et al. (2014) (26)	N	N	N	Y	N	N	N	Y	Y	N	Y	4
Robinson et al. (2014) (28)	Y	N	N	Y	N	N	N	Y	Y	N	Y	4
Yakushin et al. (2014) (29)	Y	N	N	Y	N	N	N	Y	Y	Y	N	4
Ramage et al. (2016) (32)	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	10
El Hadi et al. (2016) (30)	N	N	N	Y	N	N	N	Y	Y	Y	Y	5
Gatidis et al. (2016) (31)	Y	N	N	Y	N	N	N	Y	Y	Y	Y	5
Salem et al. (2016) (34)	Y	Y	N	Y	Y	N	N	Y	Y	Y	Y	7
Robinson et al. (2016) (33)	Y	N	N	Y	N	N	N	Y	Y	Y	Y	5
Ang et al. (2017) (35)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	10
Scotney et al. (2017) (40)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	10
Kozlov et al. (2017) (38)	N	Y	N	Y	N	N	N	Y	Y	Y	N	5
Hartwig et al. (2017) (37)	N	N	N	Y	N	N	N	Y	Y	Y	Y	5
Peterson et al. (2017) (39)	N	N	N	Y	N	N	N	Y	Y	Y	Y	5
Haq et al. (2017) (36)	Y	N	N	Y	N	N	N	Y	Y	Y	Y	5
Law et al. (2018) (16)	Y	N	N	Y	N	N	N	Y	Y	Y	Y	5
Thuzar et al. (2018) (42)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	10
Sarasniemi et al. (2018) (41)	N	N	N	N	N	N	N	Y	Y	Y	Y	4
Malpique et al. (2019) (43)	Y	N	N	Y	N	N	N	Y	Y	Y	Y	5
Zhou et al. (2018) (44)	Y	N	N	Y	N	N	N	Y	Y	Y	Y	5
Thuzar et al. (2019) (46)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	10
Martinez-Tellez et al. (2019) (45)	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	9

1: Eligibility criteria specified; 2: Individuals randomly allocated; 3: Allocation concealed; 4: Groups similar at baseline regarding most important prognostic indicators; 5: "Blinded" participants; 6: "Blinded" therapist; 7: "Blinded" assessors; 8: Measures of at least one key outcome obtained from more than 85% of participants; 9: Data analyzed by "intention to treat"; 10: Statistical comparisons between groups; 11: Point measures and measures of variation.
[a]Item number 1 not used to calculate PEDro score because it influenced external validity but not internal or statistical validity of trial.
N, criteria not satisfied; Y, criteria satisfied.

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 (16,24-26,28-30,32,35-39,41-43,45) (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

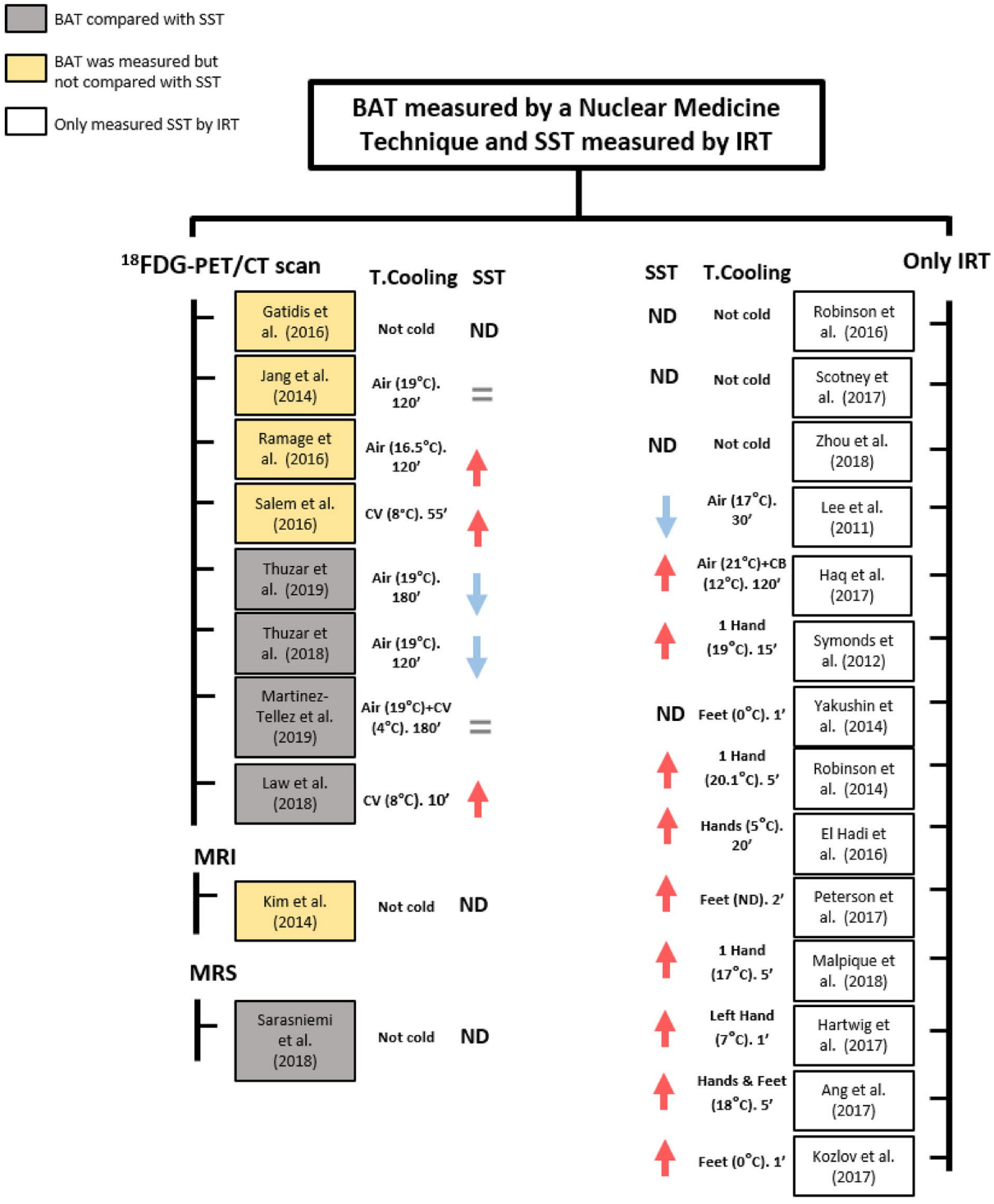

Figure 2 Type of cooling protocols (T.cooling) used in the studies included in this review. Supraclavicular skin temperature (SST) columns show the effect of cold exposure on SST. Red arrows mean an increase of SST after the cold exposure, whereas blue arrows mean a decrease of SST. Equal symbols ("=") 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; ^{18}F-FDG-PET/CT, ^{18}F-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 ^{18}F-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 ^{18}F-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 ^{15}O-O_2-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 ^{18}F-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 ^{18}F-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 ^{18}F-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 ^{18}F-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 ^{18}F-FDG uptake as did 120 minutes of cold exposure. Whether shorter times induce similar BAT ^{18}F-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 ^{18}F-FDG-PET/CT scanning (60). It is important to understand that this measures the amount of ^{18}F-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, deserve 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 compared. Finally, further studies are needed to identify the biological factors that determine the temperature of the supraclavicular area. O

Acknowledgments

The authors thank Adrian Burton for language and editing assistance.

References

1. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev* 2004;84:277-359.
2. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, et al. Cold-activated brown adipose tissue in healthy men. *N Engl J Med* 2009;360:1500-1508.
3. Cypess AM, Lehman S, Williams G, et al. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* 2009;360:1509-1517.
4. Virtanen KA, Lidell ME, Orava J, et al. Functional brown adipose tissue in healthy adults. *N Engl J Med* 2009;360:1518-1525.
5. Ruiz JR, Martinez-Tellez B, Sanchez-Delgado G, Osuna-Prieto FJ, Rensen PCN, Boon MR. Role of human brown fat in obesity, metabolism and cardiovascular disease: strategies to turn up the heat. *Prog Cardiovasc Dis* 2018;61:232-245.
6. Chondronikola M, Beeman S, Wahl RL. Non-invasive methods for the assessment of brown adipose tissue in humans. *J Physiol* 2018;596:363-378.
7. Hoeke G, Kooijman S, Boon MR, Rensen PCN, Berbée JFP. Role of brown fat in lipoprotein metabolism and atherosclerosis. *Circ Res* 2016;118:173-182.
8. Blondin DP, Tingelstad HC, Noll C, et al. Dietary fatty acid metabolism of brown adipose tissue in cold-acclimated men. *Nat Commun* 2017;8:14146. doi:10.1038/ncomms14146
9. U Din M, Raiko J, Saari T, et al. Human brown adipose tissue [(15)O]O2 PET imaging in the presence and absence of cold stimulus. *Eur J Nucl Med Mol Imaging* 2016;1878-1886.
10. Blondin DP, Labbé SM, Noll C, et al. Selective impairment of glucose but not fatty acid or oxidative metabolism in brown adipose tissue of subjects with type 2 diabetes. *Diabetes* 2015;64:2388-2397.
11. Lahesmaa M, Oikonen V, Helin S, et al. Regulation of human brown adipose tissue by adenosine and A_{2A} receptors - studies with [^{15}O]H_2O and [^{11}C]TMSX PET/CT. *Eur J Nucl Med Mol Imaging* 2019;43:743-750.
12. Gifford A, Towse TF, Walker RC, Avison MJ, Welch EB. Characterizing active and inactive brown adipose tissue in adult humans using PET-CT and MR imaging. *Am J Physiol Endocrinol Metab* 2016;311:E95-E104.
13. Koskensalo K, Raiko J, Saari T, et al. Human brown adipose tissue temperature and fat fraction are related to its metabolic activity. *J Clin Endocrinol Metab* 2017;102:1200-1207.
14. Boon MR, Bakker LEH, van der Linden R, et al. Supraclavicular skin temperature as a measure of 18F-FDG uptake by BAT in human subjects. *PLoS One* 2014;9:e98822. doi:10.1371/journal.pone.0098822
15. van der Lans AA, Vosselman MJ, Hanssen MJ, Brans B, van Marken Lichtenbelt WD. Supraclavicular skin temperature and BAT activity in lean healthy adults. *J Physiol Sci* 2016;66:77-83.
16. Law J, Morris DE, Izzi-Engbeaya C, et al. Thermal imaging is a noninvasive alternative to PET/CT for measurement of brown adipose tissue activity in humans. *J Nucl Med* 2018;59:516-522.
17. Martinez-Tellez B, Garcia-Rivero Y, Sanchez-Delgado G, et al. Supraclavicular skin temperature measured by iButtons and 18F-fluorodeoxyglucose uptake by brown adipose tissue in adults. *J Therm Biol* 2019;82:178-185.
18. Law J, Chalmers J, Morris DE, Robinson L, Budge H, Symonds ME. The use of infrared thermography in the measurement and characterization of brown adipose tissue activation. *Temperature (Austin)* 2018;5:147-161.
19. Carter EA, Bonab AA, Paul K, Yerxa J, Tompkins RG, Fischman AJ. Association of heat production with 18F-FDG accumulation in murine brown adipose tissue after stress. *J Nucl Med* 2011;52:1616-1620.
20. Law J, Morris DE, Budge H, Symonds ME. Infrared thermography. *Handb Exp Pharmacol* 2019;251:259-282.
21. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med* 2009;6:e1000100. doi:10.1371/journal.pmed.1000100
22. de Morton NA. The PEDro scale is a valid measure of the methodological quality of clinical trials: a demographic study. *Aust J Physiother* 2009;55:129-33.
23. Maher CG, Sherrington C, Herbert RD, Moseley AM, Elkins M. Reliability of the PEDro scale for rating quality of randomized controlled trials. *Phys Ther* 2003;83:713-721.
24. Lee P, Ho KKY, Lee P, Greenfield JR, Ho KKY, Greenfield JR. Hot fat in a cool man: Infrared thermography and brown adipose tissue. *Diabetes Obes Metab* 2011;13:92-93.
25. Symonds ME, Henderson K, Elvidge L, et al. Thermal imaging to assess age-related changes of skin temperature within the supraclavicular region co-locating with brown adipose tissue in healthy children. *J Pediatr* 2012;161:892-898.
26. Jang C, Jalapu S, Thuzar M, et al. Infrared thermography in the detection of brown adipose tissue in humans. *Physiol Rep* 2014;2:e12167. doi:10.14814/phy2.1216
27. Kim MS, Hu HH, Aggabao PC, Geffner ME, Gilsanz V. Presence of brown adipose tissue in an adolescent with severe primary hypothyroidism. *J Clin Endocrinol Metab* 2014;99:E1686-E1690.
28. Robinson L, Ojha S, Symonds ME, Budge H. Body mass index as a determinant of brown adipose tissue function in healthy children. *J Pediatr* 2014;164:318-322.e1. doi:10.1016/j.jpeds.2013.10.005
29. Yakushkin AV, Akimov EB, Andreev RS, et al. Effect of training on treadmill performance, aerobic capacity, and body responses to acute cold exposure. *Hum Physiol* 2014;40:422-432.
30. El Hadi H, Frascati A, Granzotto M, et al. Infrared thermography for indirect assessment of activation of brown adipose tissue in lean and obese male subjects. *Physiol Meas* 2016;37:N118-N128.
31. Gatidis S, Schmidt H, Pfannenberg CA, Nikolaou K, Schick F, Schwenzer NF. Is it possible to detect activated brown adipose tissue in humans using single-time-point infrared thermography under thermoneutral conditions? Impact of BMI and subcutaneous adipose tissue thickness. *PLoS One* 2016;11:e0151152. doi:10.1371/journal.pone.0151152
32. Ramage LE, Akyol M, Fletcher AM, et al. Glucocorticoids acutely increase brown adipose tissue activity in humans, revealing species-specific differences in UCP-1 regulation. *Cell Metab* 2016;24:130-141.
33. Robinson LJ, Law JM, Symonds ME, Budge H. Brown adipose tissue activation as measured by infrared thermography by mild anticipatory psychological stress in lean healthy females. *Exp Physiol* 2016;101:549-557.
34. Salem V, Izzi-Engbeaya C, Coello C, et al. Glucagon increases energy expenditure independently of brown adipose tissue activation in humans. *Diabetes Obes Metab* 2016;18:72-81.
35. Ang QY, Goh HJ, Cao Y, et al. A new method of infrared thermography for quantification of brown adipose tissue activation in healthy adults (TACTICAL): a randomized trial. *J Physiol Sci* 2017;67:395-406.
36. Haq T, Crane JD, Kanji S, et al. Optimizing the methodology for measuring supraclavicular skin temperature using infrared thermography: implications for measuring brown adipose tissue activity in humans. *Sci Rep* 2017;7:11934. doi:10.1038/s41598-017-11537-x
37. Hartwig V, Guiducci L, Marinelli M, et al. Multimodal imaging for the detection of brown adipose tissue activation in women: a pilot study using NIRS and infrared thermography. *J Healthc Eng* 2017;2017:5986452. doi:10.1155/2017/5986452
38. Kozlov AV, Sonkin VD, Yakushkin AV. Method to estimate activity of subcutaneous thermogenic structures on exposure to stimuli of different modalities. *Hum Physiol* 2017;43:719-728.
39. Peterson CM, Orooji M, Johnson DN, Naraghi-pour M, Ravussin E. Brown adipose tissue does not seem to mediate metabolic adaptation to overfeeding in men. *Obesity (Silver Spring)* 2017;25:502-505.
40. Scotney H, Symonds ME, Law J, Budge H, Sharkey D, Manolopoulos KN. Glucocorticoids modulate human brown adipose tissue thermogenesis in vivo. *Metabolism* 2017;70:125-132.

41. Sarasniemi JT, Koskensalo K, Raiko J, et al. Skin temperature may not yield human brown adipose tissue activity in diverse populations. *Acta Physiol* 2018;32-34.
42. Thuzar M, Law WP, Ratnasingam J, Jang C, Dimeski G, Ho KKY. Glucocorticoids suppress brown adipose tissue function in humans: A double-blind placebo-controlled study. *Diabetes Obes Metab* 2018;20:840-848.
43. Malpique R, Gallego-Escuredo JM, Sebastiani G, et al. Brown adipose tissue in prepubertal children: associations with sex, birthweight, and metabolic profile. *Int J Obes (Lond)* 2019:43:384-391.
44. Zhou J, Wu N-N, Yin R-L, et al. Activation of brown adipocytes by placental growth factor. *Biochem Biophys Res Commun* 2018;504:470-477.
45. Martinez-Tellez B, Perez-Bey A, Sanchez-Delgado G, et al. Concurrent validity of supraclavicular skin temperature measured with iButtons and infrared thermography as a surrogate marker of brown adipose tissue. *J Therm Biol* 2019;82:186-196.
46. Thuzar M, Law WP, Dimeski G, Stowasser M, Ho KKY. Mineralocorticoid antagonism enhances brown adipose tissue function in humans: a randomized placebo-controlled cross-over study. *Diabetes Obes Metab* 2019;21:509-516.
47. Kellman GM, Kneeland JB, Middleton WD, et al. MR imaging of the supraclavicular region: normal anatomy. *AJR Am J Roentgenol* 1987;148:77-82.
48. Jespersen NZ, Larsen TJ, Peijs L, et al. Short article a classical brown adipose tissue mRNA signature partly overlaps with brite in the supraclavicular region of adult humans. *Cell Metab* 2013;17:798-805.
49. Cypess AM, White AP, Vernochet C, et al. Anatomical localization, gene expression profiling and functional characterization of adult human neck brown fat. *Nat Med* 2013;19:635-639.
50. Martinez-Tellez B, Sanchez-Delgado G, Boon MR, Rensen PCN, Llamas-Elvira JM, Ruiz JR. Distribution of brown adipose tissue radiodensity in young adults: implications for cold [18F]FDG-PET/CT analyses (published online May 30, 2019). *Mol Imaging Biol* 2019. doi:10.1007/s11307-019-01381-y
51. Martinez-Tellez B, Nahon KJ, Sanchez-Delgado G, et al. The impact of using BARCIST 1.0 criteria on quantification of BAT volume and activity in three independent cohorts of adults. *Sci Rep* 2018;8:8567. doi:10.1038/s41598-018-26878-4
52. Blondin DP, Labbé SM, Phoenix S, et al. Contributions of white and brown adipose tissues and skeletal muscles to acute cold-induced metabolic responses in healthy men. *J Physiol* 2015;593:701-714.
53. Hanssen MJW, Hoeks J, Brans B, et al. Short-term cold acclimation improves insulin sensitivity in patients with type 2 diabetes mellitus. *Nat Med* 2015;21:6-10.
54. Bahler L, Holleman F, Booij J, Hoekstra JB, Verberne HJ. Hot heads & cool bodies: the conundrums of human BAT activity research. *Eur J Intern Med* 2017;40:26-29.
55. Martinez-Tellez B, Sanchez-Delgado G, Boon MR, Rensen PCN, Ruiz JR. Activation and quantification of human brown adipose tissue: methodological considerations for between studies comparisons: Comment on: Hot heads & cool bodies: the conundrums of human BAT activity research. *Eur J Intern Med* 2017;40:e19-e21.
56. Tan CL, Knight ZA. Regulation of body temperature by the nervous system. *Neuron* 2018;98:31-48.
57. Moreira DG, Costello JT, Brito CJ, et al. Thermographic imaging in sports and exercise medicine: a Delphi study and consensus statement on the measurement of human skin temperature. *J Therm Biol* 2017;69:155-162.
58. Leitner BP, Weiner LS, Desir M, et al. Kinetics of human brown adipose tissue activation and deactivation. *Int J Obes (Lond)* 2019;43:633-637.
59. Mischkowski D, Palacios-Barrios EE, Banker L, Dildine TC, Atlas LY. Pain or nociception? Subjective experience mediates the effects of acute noxious heat on autonomic responses- corrected and republished. *Pain* 2019;160:1469-1481.
60. Chondronikola M, Porter C, Ogunbileje JO, Sidossis LS. Identification and quantification of human brown adipose tissue. *Methods Mol Biol* 2017;1566:159-176.
61. Schilperoort M, Hoeke G, Kooijman S, Rensen PCN. Relevance of lipid metabolism for brown fat visualization and quantification. *Curr Opin Lipidol* 2016;27:242-248.