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Field and laboratory methods for quantifying brown adipose tissue thermogenesis

Stephanie B. Levy[1,2]

[1]Department of Anthropology, CUNY Hunter College, New York, New York

[2]Department of Anthropology, Yale University, New Haven, Connecticut

Correspondence
Stephanie B. Levy, Department of Anthropology, Yale University, 695 Park Ave. New York, NY 10065.
Email: stephanie.levy@hunter.cuny.edu

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Abstract

Non-shivering thermogenesis (NST) is a metabolic response to acute cold exposure that involves the liberation of chemical energy through physiological mechanisms that are separate from muscle shivering. Recent research suggests that the metabolic and endocrine action of brown adipose tissue (BAT) may play an important role in adult human NST. Thus, characterizing variation in BAT across human populations is of central importance to human biologists interested in human energetics and cardio-metabolic health. The gold standard for measuring BAT requires positron emission tomography (PET) and computed tomography (CT)—a technique that is expensive, exposes the participant to radiation, and is inaccessible to researchers working in many regions. Here, the author outline a noninvasive, portable alternative approach to quantifying BAT that modifies the protocols commonly used in PET/CT studies. The method consists of three components: (a) activating BAT thermogenesis using a mild cooling condition; (b) indirectly quantifying BAT thermogenesis by measuring the change in skin temperature where BAT is commonly stored using infrared thermal imaging; and (c) estimating NST by measuring the change in energy expenditure using open-circuit indirect calorimetry. The development of "field-friendly" methods will allow human biologists to better characterize population variation in BAT as well as its adaptive and health significance.

1 | INTRODUCTION: WHY MEASURE BAT?

Since the middle of 20th century, biological anthropologists have puzzled over how humans are able to adapt to an exceptional range of environmental conditions (Leonard, 2018). Thanks to methodological advancements, human biologists are beginning to uncover new answers to this classical research question. For instance, new strides have been made in the study of human biological adaptation to cold climates. Previous work indicates that indigenous circumpolar populations adapt to chronic cold stress through persistent elevations in basal metabolic rate (BMR) and seasonal shifts in thyroid hormone dynamics (Froehle, 2008; Levy et al., 2013; Rode & Shephard, 1995; Snodgrass, Leonard, Tarskaia, Alekseev, & Krivoshapkin, 2005). Recent research suggests that populations living in high-latitude regions may also adapt to cold exposure by increasing their non-shivering thermogenesis (NST) (see Table 1 for definitions of key terms) via the metabolic and endocrine action of brown adipose tissue (BAT) (Levy et al., 2018).

BAT is a specialized form of fat that functions to produce heat when the body feels cold by increasing NST. This tissue contains a high concentration of mitochondria, which gives it a brownish color. BAT mitochondria express uncoupling protein 1 (UCP1). When UCP1 is integrated into the mitochondrial membrane, it uncouples oxidative phosphorylation from ATP production, resulting in inefficient metabolism and

TABLE 1 Definitions of key terms

Term	Definition
Brown adipose tissue thermogenesis	The process whereby the mitochondria of brown adipocytes produce heat by using UCP1 within the mitochondrial membrane to dissipate the proton gradient generated from oxidative phosphorylation by bypassing ATP production.
Cold-induced thermogenesis	A change in energy expenditure in response to cold stress that is due to either shivering or non-shivering thermogenesis.
Emissivity	The ratio of the total radiant energy emitted by a body to the energy emitted by a full radiator (a.k.a. blackbody) at the same temperature (IUPS TC, 2001).
Infrared thermal image	An image generated using infrared thermal imaging that displays a color map depicting the range of temperatures of the objects in the image
Non-shivering thermogenesis	A heat-production mechanism through the liberation of chemical energy that does not involve muscle contractions (Janský, 1973).
Thermal sensitivity (in reference to infrared thermal imaging)	The smallest change in temperature that an instrument can detect (Kaplan, 2007).
Thermoneutral zone	The range of ambient temperatures at which regulation is achieved only by control of sensible heat loss, that is, without regulatory changes in metabolic heat production or evaporative heat loss (IUPS TC, 2001). While there is variation across individuals, the thermoneutral zone for humans is typically 25-30°C/77-86 °F

greater heat production (Lowell & Spiegelman, 2000). This process is referred to as BAT thermogenesis (Levy, 2017; Levy et al., 2018). Adult BAT is found primarily in the neck and above the clavicles, and occasionally around the spine, heart, kidneys, pancreas, liver, spleen, and scattered within white fat deposits of the greater omentum and mesocolon (Sacks & Symonds, 2013). While some adults completely lack BAT, it appears that most healthy young adults living in developed countries have some BAT (see Table 2). For additional resources regarding BAT physiology, regulation and measurement, see Table 3.

Preliminary research suggests that adult human BAT thermogenesis is an important biological adaptation to cold climates. Adults with greater BAT thermogenesis exhibit a larger increase in whole-body energy expenditure during cold exposure, suggesting a mechanistic link between BAT metabolism and NST (Chen et al., 2013; Hanssen et al., 2015; Levy et al., 2018; Otto Muzik, Mangner, Leonard, Kumar, & Granneman, 2017; van der Lans et al., 2013; van der Lans et al., 2016; van van Marken Lichtenbelt et al., 2015; Vosselman et al., 2012). Among the Yakut, a population that is indigenous to northeastern Siberia, adults with greater BAT thermogenesis expend more energy during acute cold stress (Levy et al., 2018). Additionally, populations living in temperate zones increase their BAT metabolism in response to repeated cold exposure and exhibit seasonal changes in BAT mass (Nirengi et al., 2018; van der Lans et al., 2013). By building on this work, future investigations of BAT may shed light on the evolution of human biological adaptations. Studies of human BAT may also produce insight into other fundamental research questions in biological anthropology, as well.

For instance, BAT may represent a useful focal point for exploring how evolutionary forces interact with social, economic, and cultural factors to shape biological variation across the life course. A suite of social, economic, and demographic factors, such as age, gender, occupation, and socioeconomic status, may shape the degree to which individuals are exposed to low temperatures in their everyday life, and thus likely influence the process of acclimatization to cold stress as well as seasonal variation in BAT activity (Mäkinen et al., 2006). In an era when global climate change threatens many lifeways, understanding the complex relationship between climate and human biology is perhaps more pressing than ever. Not only is BAT plasticity sensitive to cold exposure, environmental and lifestyle factors, such as diet, physical activity, immune function, and psychosocial stress, may also influence human BAT variation (Din et al., 2018; Townsend & Wright, 2018; van den Berg, van Dam, Rensen, de Winther, & Lutgens, 2016). Additionally, environmental factors during development may influence the trajectory of BAT growth across the life course. For example, maternal diet during gestation and lactation and childhood cold exposure may alter BAT development and have consequences for energy expenditure in adulthood (Argentato, de Cássia César, Estadella, & Pisani, 2018; Fan, Toney, Jang, Ro, & Chung, 2018; Levy, 2017).

Studies of human BAT also may reveal how environmental contexts and evolutionary forces influence population

TABLE 2 Body cooling techniques applied in ^{18}F-FDG PET/CT studies of adult human brown adipose tissue

Cooling method		Study[a]	Study location	Sample Size		Mean Age	BMI	% BAT detection
				Male	*Female*			
Cold Room	19°C room, 3 hours	Thuzar, Law, Dimeski, Stowasser, and Ho (2018)	Brisbane, Australia	2	8	28	24.4	60.0
	19°C room, 12 hours	Lee et al. (2013)	Bathesda, USA	14	10	28	22.5	25.0
	17°C room, 2 hours	Yoneshiro et al. (2013)	Sapporo, Japan	51	0	24.4	22	52.9
	17°C room, 2 hours	Admiraal et al. (2013)	Amsterdam, Netherlands	20	0	22.8	22.2	80.0
	17°C room, 2 hours	Schopman et al. (2014)	Amsterdam, Netherlands	9	0	22	22.4	100.0
	17°C room, 2 hours	Ramage et al. (2016)	Edinburgh, Scotland	6	0	22.1	22	100.0
	17°C room, 2 hours	Weir et al. (2018)	Edinburgh, Scotland	6	0	21.3	22.7	100.0
	16–18°C room, 2 hours	Vrieze et al. (2012)	Amsterdam, Netherlands	10	0	27.5	22.2	60.0
	16-18°C room, 2 hours	Bahler et al. (2016a)	Amsterdam, Netherlands	64	0	25.4	22.9	76.6
	16-17°C room, 2 hours	Bahler et al. (2016b)	Amsterdam, Netherlands	14	0	25.5	22	92.9
	15°C room, 1.5 hours	Muzik et al. (2013)	Detroit, United States	10	15	30	23.8	36.0
Cold Room and Feet on Ice Block	19°C room, 2 hours	Saito et al. (2009)	Changzhou, China	31	25	37.1	22.6	32.1
	19°C room, 2 hours	Yoneshiro et al. (2011)	Sapporo, Japan	13	0	22.8	20.8	50.0
	19°C room, 2 hours	Yoneshiro et al. (2013)	Sapporo, Japan	18	0	22.8	21.3	55.6
	19°C room, 2 hours	Matsushita et al. (2014)	Sapporo, Japan	184	76	26	21.6	67.9
	19°C room, 2 hours	Yoneshiro et al. (2016)	Sapporo, Japan	45	0	23.4	21.9	73.3
	19°C room, 2 hours	Yoneshiro et al. (2017)	Sapporo, Japan	15	0	23.1	21.4	60.0
Cooling Vest	19°C room, 17°C WP vest, 3 hours	Bredella et al. (2012)	Boston, USA	0	5	25	21.9	80.0
	15°C CP vest, 2 hours	Hwang et al. (2015)	New Haven, USA	10	0	24.6	21.6	80.0
	14°C WP vest, 2 hours	Cypess et al. (2015)	Boston, USA	15	0	22.2	22.7	80.0
Individualized Protocol	WP suit, 1.5 hours	van der Lans et al. (2013))	Maastricht, Netherlands	8	9	23	21.6	94.1
	Air-permeable tent, 1.5 hours	van der Lans, Vosselman, Hanssen, Brans, and van Marken Lichtenbelt (2016)	Maastricht, Netherlands	36	0	23.4	21.9	94.4
	Air-permeable tent, 2 hours	Vosselman et al. (2012)	Maastricht, Netherlands	10	0	22.5	21.6	100.0
	WP mattresses, 2 hours	Bakker et al. (2014)	Leiderdorp, Netherlands	24	0	24.1	21.75	95.7
	WP mattresses, 2 hours	Boon et al. (2014)	Leiderdorp, Netherlands	24	0	24.1	21.7	100.0
	20°C room, WP vest, 2 hours	Martinez-Tellez et al. (2017)	Granada, Spain	19	28	22	25	85.0

Abbreviations: CP, cold pack; WP, water-perfused.

[a]The ^{18}F-FDG PET/CT study was included in the table if the mean age was ≥18 years old and ≤40 years old, mean BMI ≤25.0, and participants were healthy.

variation in cardio-metabolic disease risk. Past work examining the relationship between BAT and body fatness have produced mixed results (Bahler et al., 2016a; Franssens, Hoogduin, Leiner, van der Graaf, & Visseren, 2017; Hanssen et al., 2015; Lee et al., 2013; Matsushita et al., 2014; Saito et al., 2009; Yoneshiro et al., 2013). Studies document a relationship between BAT mass and greater insulin sensitivity and lower fasting insulin and hemoglobin A1c levels (Matsushita et al., 2014; Zhang et al., 2013, p. 201). Thus, BAT thermogenesis may have protective effects against the development of cardio-metabolic diseases such as type II diabetes. Additionally, investigations of maternal diet and fetal BAT growth may reveal new developmental origins of adult disease risk.

BAT is an advantageous focal point for a range of research questions sprouting from biocultural and evolutionary perspectives in biological anthropology. How much variation is

TABLE 3 Additional resources

Literature/online tools	Article/Website	
Thermal physiology and infrared thermal imaging in human research	Chen et al. (2016)	
	Fernández-Cuevas et al. (2015)	
	IUPC (2001)	
	Martinez-Tellez et al. (2017))	
	Moreira et al. (2017)	
Cellular biology and physiology of BAT thermogenesis	Cannon and Nedergaard (2008))	
	Devlin (2015)	
	Symonds, Pope, & Budge (2015)	
Measuring energy expenditure	Fullmer et al. (2015)	
	Leonard (2012)	
Body Cooling and Infrared Thermal Imaging Systems	**Producer**	**Estimated Cost (US$)**
Water-perfused vest with water chiller reservoir	Polar Products, Cool Flow Vest System	1345
Water-perfused suit and connectors for tubing	Med-Eng, BCS4 Body Cooling System	2000
Submersible pumps and water cooler sold separately	EcoPlus, 396 GPH	70-100
Water-perfused suit with leg-mounted cooling system	Med-Eng, BCS4 Body Cooling System	3700
Infrared thermal imaging camera	Flir, e60bx	7225
Camera tripod	Sirui T-025x	200
Tripod adapter	Sirui TY-C10 Quick Release Plate	20
iButtons	Maxim Integrated, High-Resolution Thermochron iButton	20/iButton
iButton reader	USB iButton Reader	30

there in BAT across adults and what shapes that variation? What is the adaptive significance of BAT for contemporary populations and across human evolution? What are the implications of BAT for human energetics, and how does BAT change across the life course? What are the consequences of BAT thermogenesis for other biological processes and for disease risk? How do social, cultural, and economic contexts get under the skin by altering plasticity in BAT?

Answering these questions requires a method for quantifying BAT thermogenesis that is minimally invasive and can be applied in a wide variety of research settings. The gold standard method for quantifying BAT thermogenesis is positron emission tomography (PET)/computed tomography (CT), a technique that exposes participants to radiation and is inaccessible to researchers in many regions. In this article, the author describes a "field-friendly" approach, which modifies the protocol commonly used in PET/CT studies and utilizes infrared thermal imaging as an alternative for quantifying BAT thermogenesis.

The protocol consists of three components: (a) activating BAT thermogenesis by exposing the participant to a standardized, mild cooling condition; (b) quantifying the change in skin temperature of the supraclavicular (SCV) area as an indirect measure of BAT thermogenesis, and; (c) calculating the percent change in energy expenditure as an estimate of NST. Building on the approach commonly used in PET/CT studies, such as the protocol outlined by Chen et al. (2016), Table 4 lists recommendations for standardizing the process of activating and quantifying BAT thermogenesis and NST using a combination of infrared thermal imaging and indirect calorimetry. This approach represents a noninvasive, inexpensive, portable method, and, thus, may be advantageous for human biologists interested in investigating population variation in human BAT and its adaptive and health significance.

2 | ACTIVATING BAT THERMOGENESIS

When the Swiss naturalist Konrad Gessner first described BAT in *Historiae animalium* in 1551, he remarked that the tissue was "neither fat, nor flesh – but something in between" (Cannon & Nedergaard, 2008; Gessner, 1551). Brown adipocytes share developmental origins with myocytes and exhibit overlapping exomes with white adipocytes. For these reasons, distinguishing BAT mass from white adipose tissue (WAT) and muscle mass under thermoneutral conditions is difficult using most in vivo imaging techniques. Furthermore, the

TABLE 4 Protocol recommendations for activating and quantifying BAT thermogenesis and estimating NST for adult participants

Category	Recommendation
Participant characteristics	• Healthy with no known acute conditions. • Exclude pregnant and lactating women. • Between 18 and 45 years old; the protocol recommendations may not be appropriate for participants outside of this age range.
Study equipment set-up	• Infrared thermal imaging camera on a tripod positioned ~1 m away from participant. • Room temperature must be between 21°C and 28°C. • Set up should be away from objects that may emit or reflect interfering infrared light such as windows, walls, or a radiator.
Participant preparation	• ≥ 7 hours prior to study, participants should refrain from eating, consuming caffeine, smoking, and exercising. • Participants should wear light-weight clothing; report thermal insulation value (clo) of clothing. • Outfit participants with a heart rate monitor, water-perfused garments, face mask/canopy for metabolic measurements.
Infrared thermal imaging camera parameters	• Emissivity = 0.98. • Set ambient temperature and humidity to the temperature and humidity of the room. • Distance to target = 1 m. • Confirm that the image is in crisp focus to maximize measurement accuracy.
Thermoneutral condition	• Monitor heart rate. • Measure energy expenditure for 20 minutes. • Capture thermal images of the shoulder and neck area on the right and left sides of the body at the end of the thermoneutral condition.
Cooling condition	• Monitor heart rate. • Pump cold water (~10°C) through the water-perfused suit. • If the participant shivers, stop the pump, allow the participant to rewarm, record the sternum temperature and time that shivering starts and ends. • Measure energy expenditure for 30 minutes. • Capture thermal images of the shoulder and neck area on the right and left sides of the body at the end of the cooling condition.
Data processing and statistical analysis	• Calculate the change in maximum skin temperature of the supraclavicular area (ΔSCV). • Eliminate the first 5 minutes of metabolic rate data for each temperature condition. • Select CIT data based on a steady state (CV ≤ 10%). • In analyses of ΔSCV, control for percent body fat, age, sex, and change in skin temperature of a point on the sternum.
Publications should record the following information	• Cooling equipment system used. • Cooling protocol. • Infrared thermal imaging camera model and metabolic cart model. • Time, date, and location of data collection.

metabolic significance of variation in BAT mass under thermoneutral conditions is currently ambiguous (Gatidis et al., 2016; Hwang et al., 2015; U Din et al., 2016). Thus, in vivo studies of BAT typically quantify variation in BAT thermogenesis after it has been triggered by a mild cooling condition.

The amount of heat produced by BAT depends on the time length and intensity of the cooling condition. PET/CT studies of human BAT have used a variety of cooling protocols and equipment, thus making comparisons across studies difficult. Table 2 provides a list of various cooling protocols employed in published ^{18}F-FDG PET/CT studies of human BAT. The table is limited to displaying studies that recruited healthy adults with a mean age between 18 and 40 years old and a sample mean BMI of 25.0 kg/m^2 or less. Below, the author provides a description of various cooling protocols for activating BAT thermogenesis and their benefits and limitations.

2.1 | Cooling hands or feet

One technique for activating BAT thermogenesis that can be easily employed in a variety of research settings is to cool the participants' hands or feet. Some studies combine this approach with lowering the room temperature to around 15°C (Matsushita et al., 2014; Ramage et al., 2016; Saito et al., 2009; Yoneshiro et al., 2013, 2016). Table 2 includes a list of studies that placed participants' feet on an ice block intermittently in addition to lowering the room temperature. Several studies have replicated the cooling protocol of

Symonds et al. (2012), which involves submerging either one hand or both feet in water that is 19°C to 20°C for 5 minutes, and then quantifying the change in BAT thermogenesis between the thermoneutral condition and the end of the cold condition (Ang et al., 2017; Ramage et al., 2016; Robinson, Ojha, Symonds, & Budge, 2014). In a sample of children, Symonds et al. (2012) found that BAT thermogenesis (measured by the change in skin temperature of the SCV area) peaked after 5 minutes of submersion and remained constant for the following 10-15 minutes. Additionally, the degree of BAT thermogenesis that was activated by submerging one hand was similar to submerging both feet (Symonds et al., 2012).

2.2 | Benefits and limitations to cooling hands or feet

The primary advantage of the approach used by Symonds et al. (2012) is its utility for a wide range of research settings and participant characteristics. For instance, this approach has been applied to several studies of BAT in children, and it may be appropriate for a study sample that is sensitive to cold stress or has a restricted thermoneutral zone. A limitation of this technique, however, is that cooling the hands or feet may not be a sufficiently robust stressor to activate BAT thermogenesis in all participants with BAT stores, especially young adults that may be acclimated to cold stress and have a lower thermoneutral zone. PET/CT studies with a cooling protocol that combined a cold room with cooling the participants' feet on an ice block appear to have a lower rate of BAT detection than studies that use water-perfused garments and individualized protocols (see Table 2).

2.3 | Water-perfused garments

Water-perfused clothing items are lined with tubing and are connected to a water source. Cold water is pumped through the tubing of the clothing item, thus cooling the participant. See Figure 1 for examples of a water-perfused suit and equipment setup. Water-perfused garments can be purchased with or without a water pumping and temperature regulation system. It is possible to construct a water-pumping system for the garment at a low cost using small submersible pumps and a water cooler; however, the researcher must manually monitor and maintain the water temperature by adding ice to the water reservoir. Purchasing a garment that includes a water pumping and/or temperature regulation system allows the researcher to more accurately manipulate the temperature of the cooling condition, and, thus, reduces the burden of the protocol for the researcher.

2.4 | Benefits and limitations of water-perfused garments

Based on a comparison of the studies listed in Table 2, water-perfused garments, such as a vest or suit, present several advantages over other cooling techniques for anthropological fieldwork. Studies that used water-perfused suits typically have a higher rate of BAT detection than other techniques, such as manipulating room temperature using air conditioning. This is likely because water-perfused suits expose participants to lower temperatures. While water-perfused suits do not necessitate an air-conditioned room, they require access to electricity and ice.

2.5 | Individualized protocols

PET/CT studies of BAT often utilize an individualized cooling protocol. The goal of an individualized protocol is to expose the subject to a temperature that is low enough to ensure activation of BAT thermogenesis but above the shivering threshold. In this approach, the water temperature of the cooling garment is decreased at a steady rate until the participant begins to shiver. This process takes anywhere from 30 to 90 minutes depending on the rate at which the water temperature decreases and the participants' shivering threshold (Martinez-Tellez, Sanchez-Delgado, Garcia-Rivero, et al., 2017; van der Lans et al., 2013). The subject is then rewarmed in order to halt shivering. Then, the water is maintained at a temperature that is two to four degrees above

FIGURE 1 Examples of water-perfused suit, water pump, indirect calorimetry and thermal imaging set-up

the shivering threshold for a predetermined period, typically 2 hours, and BAT thermogenesis is quantified toward the end of the cooling condition.

2.6 | Benefits and limitations of individualized protocols

The advantage of using an individualized cooling protocol is that the chances of activating any BAT deposits that are present are higher than using a standard temperature across all subjects. An individual's thermoneutral zone depends on their body size, body composition, and history of cold exposure, and it may be independent of BAT mass and activity (Martinez-Tellez, Sanchez-Delgado, Garcia-Rivero, et al., 2017). Thus, the ambient temperature that will stimulate BAT thermogenesis will differ between participants and study conditions.

An individualized protocol, however, is not always appropriate or feasible for anthropological fieldwork. This approach exposes participants to a total of 1.5 to 3 hours of mild cold exposure (Martinez-Tellez, Sanchez-Delgado, Garcia-Rivero, et al., 2017; van der Lans et al., 2013). For many human biologists, a cooling condition of this length is too great a burden on their participants' time and comfort. Additionally, an individualized protocol does not prevent shivering for all participants. Sanchez-Delgado et al. (2018) found that even after performing a shivering threshold test, 17 of 63 participants shivered during the 1-hour cooling condition. This is problematic if the researcher is simultaneously quantifying the energy expenditure associated with NST (see Estimating NST below). Finally, a lengthy cooling protocol may not be necessary for generating a standardized measurement of BAT thermogenesis.

2.7 | Semi-individualized protocols

An alternative option is to use a semi-individualized protocol that also aims to maximize NST and minimize shivering. Levy et al. (2018) used a 30-minute semi-individualized cooling protocol to examine the relationship between BAT thermogenesis and cold-induced changes in energy expenditure among Yakut adults, a population indigenous to northeastern Siberia. During the cooling condition, cold water (mean temperature: 10.3°C ± 2.9°C) was pumped through a water-perfused suit (Med-Eng) so that the internal temperature of the suit was maintained at ~15°C. If the participant began to shiver, the researcher immediately measured and recorded the skin temperature of the sternum and the time at which the shivering started. The water pumps were shut off so that the subject would rewarm, and the researcher recorded the time at which the subject stopped shivering. If the skin temperature increased 2°C above the temperature at which shivering began, the pumps were turned back on. Using this technique, 6 of 74 participants shivered. Table 5 shows that after just 20 minutes of mild cooling, there was a significant positive association between BAT thermogenesis and percent change in energy expenditure. These data suggest that a semi-individualized protocol can be applied to activate BAT thermogenesis.

2.8 | Benefits and limitations of semi-individualized protocols

The primary advantage of this approach is that the researcher generates a standardized measurement of BAT thermogenesis with a shorter cooling condition. One limitation of the semi-individualized cooling protocol is that it relies on participants reporting whether they are shivering or visual confirmation from the researcher. Differences in the ability to detect shivering across participants or researchers may introduce error into the measurements of BAT thermogenesis and NST. Martinez-Tellez, Sanchez-Delgado, Acosta, et al. (2017), however, report strong agreement between shivering detection using EMG and subject self-report.

TABLE 5 Relationships between percent change in energy expenditure after cooling[a] and change in the supraclavicular skin temperature and possible confounding variables after 15, 20, 25, and 30 minutes of cooling. Table reproduced from Levy (2017)

	Model 1:15 minutes. Adj. $R^2 = 0.01$		Model 2:20 minutes. Adj. $R^2 = 0.16$		Model 3:25 minutes. Adj. $R^2 = 0.18$		Model 4:30 minutes. Adj. $R^2 = 0.15$	
Measure	***β Coef.***	***P-value***	***β Coef.***	***P-value***	***β Coef.***	***P-value***	***β Coef.***	***P-value***
Δ SCV (°C)	10.34	0.422	28.61	0.013*	26.24	0.005**	24.455	0.012*
Propensity score[b]	6.75	0.615	−14.494	0.097	−7.824	0.422	0.293	0.983

Note: Multiple regression analyses: relationship with percent change in energy expenditure were significant at: $*P \leq .05$; $**P \leq .01$; $***P \leq .001$.

Abbreviations: change in supraclavicular temperature (Δ SCV).

[a]Cooling protocol was an individualized protocol using a water-perfused suit (Med-Eng).

[b]Propensity score controlling for age, sex, fat-free mass, percent body fat, trial start time, and change in sternum temperature.

3 | CURRENT METHODOLOGIES FOR MEASURING BAT

3.1 | The gold standard: ^{18}F-FDG PET/CT scans

PET/CT is an imaging technique in which a subject is injected with a positron-emitting radioactive tracer. The scanner detects the tracer and produces images at multiple angles in order to generate a three-dimensional image. The most commonly used radiotracer in PET/CT scans of BAT metabolic activity is ^{18}F-flourodeoxyglucose (^{18}F-FDG), which is a glucose analog in which a hydroxyl group has been replaced with a positron-emitting radionuclide-18. When BAT thermogenesis is activated by norepinephrine in response to cold stimulation, glucose is taken out of the bloodstream into brown adipocytes. Thus, ^{18}F-FDG PET/CT quantifies BAT's glucose metabolism by reporting the amount of ^{18}F-FDG in a tissue in standard uptake value (SUV) units. Individuals with SUVs above a set threshold (typically 1.5 or 2.0 SUV) in the SCV area are considered to be "BAT positive," while scans that fail to detect metabolic activity above the threshold are considered "BAT negative." SUV values, a measure of BAT metabolic activity, can be converted into BAT volume using an auto-contouring function. PET/CT allows researchers to precisely quantify BAT volume and glucose metabolism; however, they are expensive, difficult to access, invasive, and they expose participants to radioactive tracers.

3.2 | Skin temperature of the SCV area as a biomarker of BAT thermogenesis

Multiple ^{18}F-FDG PET/CT studies have demonstrated that the skin temperature of the SCV area can act as a noninvasive biomarker of BAT thermogenesis after it has been activated by mild cooling. The frequency of BAT detection is highest in the SCV area compared to other BAT depots (Becker, Nagel, Wolfrum, & Burger, 2016). In this region, brown adipocytes are located between the anterior neck muscles in the SCV fossa posterior to the brachial plexus, under the clavicles, and around the common carotid arteries (see Figure 2) (Sacks & Symonds, 2013). Since these brown adipocytes are located close to the surface of the body, they likely influence skin temperature.

Table 6 lists studies that compared SCV with BAT SUV from ^{18}F-FDG PET/CT scans after activation by a mild cooling condition. This table compares various approaches to quantifying BAT thermogenesis using SCV by displaying the relationship between various skin temperature variables and BAT SUV. The studies included in this table used a variety of cooling protocols. Additionally, some studies quantified temperature using iButtons (see *Measuring BAT Thermogenesis with iButtons* below) while others used infrared thermal imaging (IR) (see *Measuring BAT Thermogenesis with Infrared Thermal Imaging* below). The column labeled "Skin Temp. Variable" describes the skin temperature variables that were measured by the study and the conditions under which they were measured. The column labeled "Correlation with BAT SUV?" signifies whether there was a statistically significant relationship ($P \leq .05$) between the skin temperature variable and BAT SUV, while the column labeled R provides the correlation coefficient for the association. The final column presents the results of Student's *t*-tests examining whether there was a significant difference in the skin temperature variable between individuals designated BAT-positive vs BAT-negative based on PET/CT scans.

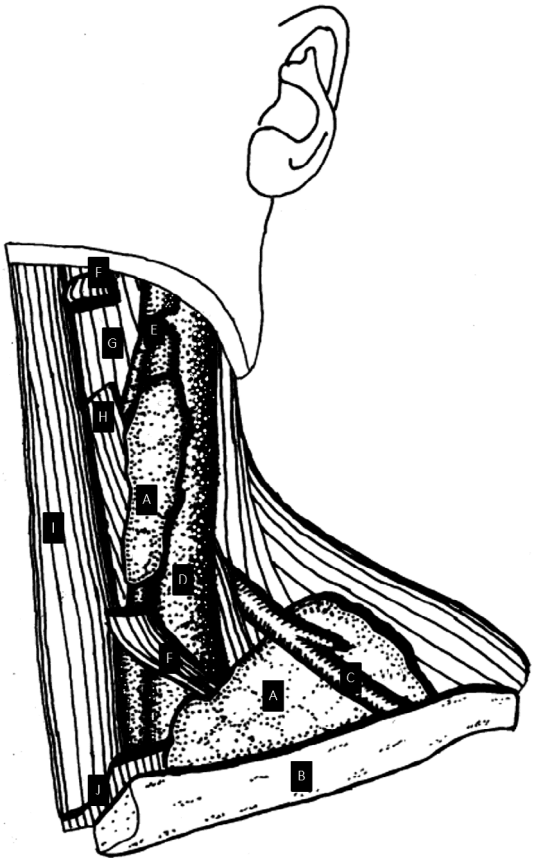

FIGURE 2 BAT depots of the neck and supraclavicular area. A, Brown adipose tissue (BAT); B, clavicle; C, Brachial plexus; D, Internal jugular vein; E, Common carotid artery; F, Omohyoid muscle (cut); G, Thyrohyoid muscle; H, Sternothyroid muscle; I, Sternohyoid muscle; J, Sternocleidomastoid muscle (cut)

Several studies document a significant correlation between the change in SCV (ΔSCV) and the maximum SUV of this area and found that BAT-positive adults had a larger ΔSCV (ie, maintained warmer skin temperatures) when compared to BAT-negative adults (Chondronikola et al., 2016; van der Lans et al., 2016; Yoneshiro et al., 2011, 2016). Boon et al. (2014) and Yoneshiro et al. (2016) note that there is a significant association between the

TABLE 6 Studies that compare ^{18}F-FDG PET/CT scans of brown adipose tissue with skin temperature measurements of the supraclavicular area

Study	Sample Size		Mean Age	Mean BMI	Cooling Protocol	iButton or IR?	Skin Temp. Variable[a]	Correlation with BAT SUV?[b]	R[c]	BAT+/BAT— Difference?[d]
	Males	Females								
Boon et al. (2014)	24	0	24.1	21.7	Water-perfused mattress, individualized, 2 hours	iButtons	SCV, Cold	Yes	0.57	…
	…	…	…	…		…	Chest, Cold	No	…	…
Chondronikola et al. (2016)	18	0	46.9	29.5	Water-perfused vest and blanket, individualized, 6 hours	iButtons	ΔSCV	Yes	0.58	Yes
Jang et al. (2014)	12	5	36	25.4	19°C room, 2 hours	IR	SCV, TN	No	…	Trend
	…	…	…	…	…	…	SCV, Cold	No	…	Trend
	…	…	…	…	…	…	Chest, TN	…	…	No
	…	…	…	…	…	…	Chest, Cold	…	…	Yes
Thuzar et al. (2018)	2	8	28	24.4	19°C room, 2 hours	IR	SCV, Cold	Yes	0.62	…
van der Lans et al. (2016)	36	0	23.4	21.9	Air-conditioned tent, individualized, 1.5 hours	iButtons	SCV, TN	No	…	…
	…	…	…	…		…	SCV, Cold	No	…	…
	…	…	…	…		…	Chest, TN	No	…	…
	…	…	…	…		…	Chest, Cold	No	…	…
	…	…	…	…		…	ΔSCV	Yes	0.48	…
	…	…	…	…		…	ΔChest	No	…	…
Yoneshiro et al. (2011)	13	0	22.8	20.8	19°C room, feet on ice intermittently, 2 hours	iButton	ΔSCV	…	…	Yes
						…	ΔChest	…	…	No
Yoneshiro et al. (2016)	45	0	23.4	21.7	19°C room, feet on ice intermittently, 2 hours	iButtons	SCV, TN	…	…	No
	…	…	…	…		…	SCV, Cold	Yes	0.33	Yes
	…	…	…	…		…	ΔSCV, Summer	…	…	No
	…	…	…	…		…	ΔSCV, Winter	…	…	Yes
	…	…	…	…		…	ΔChest, Summer	…	…	No
	…	…	…	…		…	ΔChest, Winter	…	…	No

[a]Anatomical location of the skin temperature measurement and the conditions in which it was measured.
[b]Linear regression analysis of the relationship between the skin temperature variable and the maximum standard uptake value (SUV) for the supraclavicular region of interest; significance level: $P \leq .05$.
[c]Pearson's correlation coefficient for the associations listed in the column to the left.
[d]Student's t-tests were used to compare the skin temperature variable between individuals designated positive vs negative for active BAT based on a predetermined SUV threshold. Yes: $P \leq .05$; Trend: $P \leq .1$ No: $P > .1$.

temperature measured by an iButton of a point in the SCV area after a cooling condition and the maximum SUV of this area. Similarly, Thuzar et al. (2018) found that the mean temperature of the SCV area after cooling was significantly associated with BAT SUV. Jang et al. (2014) demonstrate that the difference between the SCV skin temperature and the chest temperature after cooling was significantly associated with maximum SUV.

Table 6 highlights the cross-study variability in the types of skin temperature variables that are chosen to act as biomarkers of BAT thermogenesis as well as their relationship with BAT SUV. This variability is likely due to the fact that the studies in Table 6 do not control in their analyses for variation in participant characteristics that may confound the relationship between skin temperature and BAT thermogenesis. For instance, the skin's response to cooling varies with

age, body fatness, and sex (Inoue et al., 2016; Salamunes, Stadnik, & Neves, 2017). Differences in vasoconstriction of the core may also confound the relationship between ΔSCV and BAT thermogenesis (Levy et al., 2018). Thus, it is recommended that analyses using ΔSCV as an indirect, noninvasive biomarker of BAT thermogenesis control for percent body fat, age, and sex, as well as the change in skin temperature of a point on the sternum to control differences in vasoconstriction of the core.

Readers should note that PET/CT studies have not specifically examined the utility of ΔSCV as a biomarker of BAT thermogenesis among participants with percent body fat or BMI values in the overweight or obese categories. Adults with a higher percent body fat exhibit lower skin temperatures (Alexander et al., 2015; Salamunes et al., 2017). Large WAT deposits in the neck may prevent the heat produced by BAT thermogenesis from radiating toward the skin. Additionally, it is likely that ΔSCV is influenced by the presence of large blood vessels close to the skin in this area (Martinez-Tellez, Sanchez-Delgado, Acosta, et al., 2017). BAT deposits co-locate these with major blood vessels, such as the common carotid artery and the internal jugular vein (Sacks & Symonds, 2013). For these reasons, it is difficult to disentangle the contribution of BAT thermogenesis to shifts in SCV skin temperature from changes in blood temperature that may result from thermogenesis generated elsewhere in the body. Despite these limitations, quantifying ΔSCV is an efficacious alternative to PET/CT for assessing variation in BAT thermogenesis.

3.3 | Measuring BAT thermogenesis with infrared thermal imaging

Infrared thermal imaging is a noninvasive, inexpensive approach to measuring ΔSCV and represents a "field-friendly" alternative to using PET/CT scans for quantifying BAT thermogenesis. Infrared cameras detect mid to long wave infrared radiation emanating from objects. Since the amount of infrared radiation emitted by an object increases with the object's temperature, infrared data can be converted to temperature data and used to create a digitized image or a high-speed video rendering of a thermal map in false color (Tattersall, 2016).

One advantage of infrared thermal imaging is that many cameras have a high degree of thermal sensitivity and, therefore, can detect small differences in temperature within an image. For example, the FLIR E60bx has an advertised thermal sensitivity of <0.045°C. Given that changes in BAT thermogenesis might result in subtle shifts in SCV skin temperature, a high degree of thermal sensitivity is advantageous in this context. Manufacturers such as FLIR advertise that many of their infrared cameras, such as the E60bx, have an absolute accuracy of 2% or ± 2.0°C; however, the accuracy can be improved by capturing an image under standardized conditions (Moreira et al., 2017). With proper calibration and attention to measurement parameters, the possible margin of error can be less than 1°C (see Table 4) (Fernández-Cuevas et al., 2015).

For instance, the researcher should rely on an external thermometer and hygrometer in order to accurately set the camera's ambient temperature and humidity parameters. The camera should be placed 1 m away from the participant at an angle that is perpendicular to the plane of the SCV area (Fernández-Cuevas et al., 2015). The emissivity should be set to 0.98, which is the emissivity value of dry skin. Additionally, objects from the surrounding environment can emit or reflect infrared light and introduce error. Thus, images should be captured away from other objects such as windows, walls, or a radiator (Moreira et al., 2017).

The maximum SCV skin temperature can be calculated from the infrared thermal images using a software package such as Flir Tools Software (FLIR) (see Figure 3). The box tool can be used to determine the maximum skin temperature of the SCV in each image. The maximum skin temperature of a point on the sternum can also be determined using either the box or the spot tool in Flir Tools.

3.4 | Measuring BAT thermogenesis with iButtons

An alternative approach to quantifying SCV skin temperature is to use Thermochron iButtons (Maxim Integrated). Thermochron iButtons are small (17.35 mm diameter) metal canisters that contain a semiconductor temperature sensor, a computer chip with a real-time clock and memory, and a 3 V Lithium battery. The iButton is taped to the area above the clavicle with the sensor side flush against the skin. The manufacturer advertises that the iButton DS1291H has an accuracy of ±1°C and a thermal sensitivity of ±0.5°C. iButtons are well suited for field research in human biology as they are noninvasive, inexpensive, and highly portable. However, van Marken Lichtenbelt et al. (2006) performed a validation study investigating the utility of iButtons for measuring changes in skin temperature by comparing iButton and thermocouple measurements. When the room temperature was decreased to 15°C (59°F), measurements taken from the iButton were significantly warmer than the thermocouple measurements for the entire 30-minute cooling period. Thus, when ambient temperature changes quickly and dramatically, iButton skin temperature errors are likely to be greater than 1°C due to thermal inertia (van Marken Lichtenbelt et al., 2006). Given that the difference in ΔSCV between a person with active BAT and a participant without BAT can be 1°C or less, this study calls into question the applicability of iButtons for accurately quantifying BAT thermogenesis.

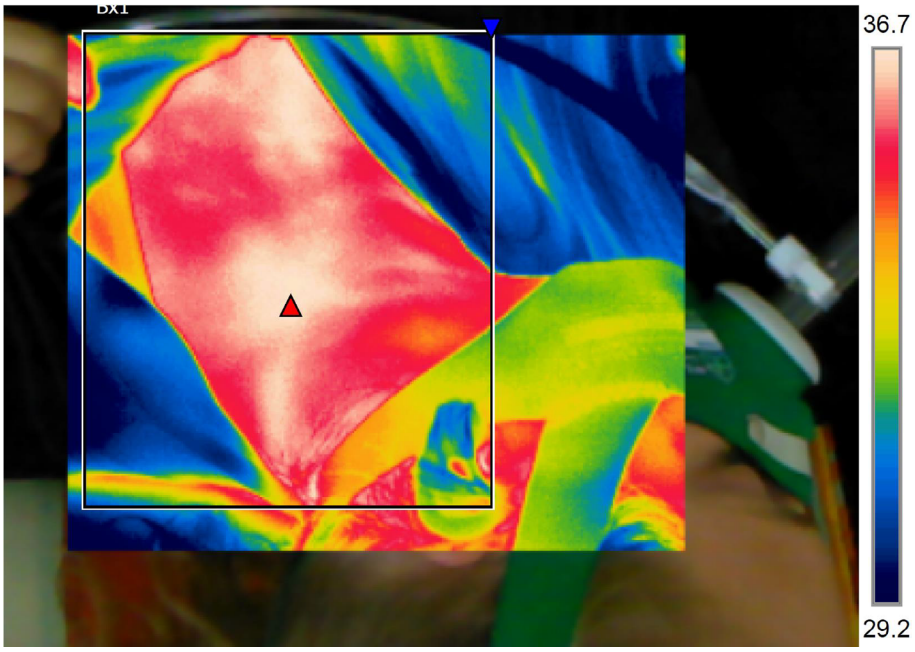

FIGURE 3 Example of a thermal image of the supraclavicular area

4 | ESTIMATING NST

As mentioned above, adults with greater BAT thermogenesis exhibit a larger increase in energy expenditure during cold exposure independent of shivering, suggesting a mechanistic link between BAT metabolism and NST (Chen et al., 2013; Hanssen, Hoeks, et al., 2015; Levy et al., 2018; Otto Muzik et al., 2017; van der Lans et al., 2013, 2016; van van Marken Lichtenbelt et al., 2015; Vosselman et al., 2012). However, estimates of the tissue-specific contribution of BAT metabolism to whole-body energy expenditure based on the rate of oxygen use within BAT are lower than expected—just 12 kcal/day (Muzik et al., 2013). Additional research is needed in order to clarify the physiological mechanisms that link BAT thermogenesis and energy expenditure during NST.

NST is typically estimated by calculating the percent difference between resting metabolic rate (RMR) and the rate of energy expenditure during cold exposure using open-circuit indirect calorimetry. There are limitations to interpreting estimates of NST due to uncertainty regarding the possible contribution of muscle shivering that is imperceptible to the participant or researcher; thus, researchers often refer to the percent change in energy expenditure as cold-induced thermogenesis (CIT). Measurements of CIT vary greatly across individuals, with some adults experiencing a modest decline in metabolic rate, while others exhibiting an increase of over 70% (Levy et al., 2018; Sanchez-Delgado et al., 2018).

Leonard (2012) provides a useful overview of the methods used to quantify energy expenditure for human biologists, including open-circuit indirect calorimetry using metabolic carts. Prior to metabolic measurements (and infrared thermography of BAT thermogenesis), participants should be fasted for at least 7 hours and should refrain from consuming caffeine (Fullmer et al., 2015). Participants should also refrain from physical activity, cold exposure, and smoking prior to data collection. As described in Leonard (2012), participants should be outfitted with a heart rate monitor that is interfaced with the metabolic equipment in order to track anxiety. Participants also wear a facemask, mouthpiece, or a canopy that is connected to sensors that will measure the ventilatory rate (V_E), oxygen consumption (VO_2), and carbon dioxide production (VCO_2), as well as calculate the respiratory quotient (RQ). After the participants have adjusted to breathing through the face mask or other equipment and the oxygen and carbon dioxide analyzers have been calibrated, V_E, VO_2, VCO_2, and RQ are monitored for the entire thermoneutral and cooling condition. Metabolic rate is calculated by converting VO_2 to kcal/day based on the respiratory quotient using the modified Weir (1949) formula (McArdle, Katch, & Katch, 2010).

Estimates of NST are sensitive to the methods used to select and analyze metabolic rate data (Sanchez-Delgado et al., 2018). Sanchez-Delgado et al. (2018) recommend excluding the first 5 minutes of the cooling condition data as the participant adjusts to the cooling sensation. When using a semi-individualized cooling protocol, care should be taken to exclude the data points that were collected during the time periods when participants were shivering. Similar to the recommended protocol for quantifying RMR, Sanchez-Delgado et al. (2018) also advise that researchers select data based on a steady state period, or a stretch of data in which the coefficient of variation (CV) is $\leq$10%. The purpose of discarding data to achieve a steady state period is to minimize artifact, or nonmetabolic variation in gas exchange, in the measurement (Fullmer et al., 2015).

5 | LIMITATIONS FOR THE FIELD

Infrared thermal imaging has opened the door for human biologists to explore variation in BAT thermogenesis across populations in a variety of laboratory and field settings; however, there are of course limitations to this approach. In order to obtain accurate measurements of RMR, CIT, and BAT thermogenesis using infrared thermal imaging and indirect calorimetry, participants must be fasted and abstain from moderate or rigorous physical activity, smoking, and exposure to low temperatures. Depending on the study population and research context, these requirements may unduly inconvenience or burden participants. Researchers should consider individuals' daily routines when constructing the data collection schedule and location in order to limit the inconvenience for the participants and maximize the likelihood of meeting protocol requirements.

As previously described, quantifying BAT thermogenesis requires that the researcher first stimulate BAT metabolic activity by exposing participants to a mild cooling condition. This of course presents several obstacles for fieldwork (Gatidis et al., 2016). Researchers that are collecting data at multiple locations must transport the necessary equipment, and they must ensure that the study settings have access to water and electricity and are comparable in temperature. Additionally, researchers who choose to manually manipulate and monitor the water temperature of cooling garments must also have easy access to a large amount of ice.

Recent work suggests that BAT metabolism may contribute to diet-induced thermogenesis; thus, consuming a standardized meal may be an alternative to mild cooling for activating BAT thermogenesis (Din et al., 2018). However, the degree to which food consumption activates BAT thermogenesis varies depending on the nutritional composition of the standardized meal and the characteristics of the study sample (Hibi et al., 2016; Martinez-Tellez et al., 2018; Peterson, Orooji, Johnson, Naraghi-Pour, & Ravussin, 2017). Additionally, the physiological mechanisms that link the consumption of various nutrients with BAT thermogenesis remain unclear.

As is the case with all research in human biology, investigators must balance the desire to carry out a standardized protocol with the imperative need to adjust to the specific conditions of the research setting and the circumstances surrounding the participants. Researchers interested in applying these techniques should consider the nature of their relationship with the community, the community's history of involvement in research, and the degree to which BAT-related research questions are aligned with the interests and goals of the community. Investigators should regard the climate and season of the study locale when considering the community's comfort with exposure to mild cold stress. Cooling protocols may not be appropriate for research in communities living in regions where the ambient temperature rarely drops below the thermoneutral zone and where swimming in cool water is not a common practice. Thus, developing and carrying out a protocol that is appropriate for a specific study population demands a breadth of knowledge of the local context, a long-standing relationship with the community, and buy-in from participants.

Finally, additional research is needed to further validate the use of infrared thermal imaging for quantifying BAT thermogenesis. Future work should examine the validity of the data collection and analysis recommendations outlined here in a variety of geographic contexts and diverse populations. Given the infancy of the application of this technique, additional research is likely to generate new methodological advancements and protocol recommendations.

6 | CONCLUSION

This article provides an overview of the tools used to quantify BAT thermogenesis, including a noninvasive, "field-friendly" method that can be applied by human biologists in a variety of research contexts, as well as a discussion of the benefits and limitations of these methods. The study of BAT thermogenesis will bring new insight to the field of human energetics. The contribution of NST to total energy expenditure is highly variable; in response to the same degree of cold exposure, some individuals experience modest declines in metabolic rate, while others experience an increase of 70% or more. Understanding the determinants of this broad degree of variation and its relationship with BAT will shed light on population patterns in energy allocation and cardiometabolic health. Thus, BAT plasticity represents an advantageous focal point for investigating human biological adaptation. New tools such as these will broaden our understanding of human variation and its social, ecological, and evolutionary underpinnings.

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ORCID

Stephanie B. Levy https://orcid.org/0000-0003-2828-2014

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