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# Feasibility of using infrared thermal imaging to examine brown adipose tissue in infants aged 18 to 25 months

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#### ABSTRACT

**Background:** Recent studies in adults indicate that cold-induced temperature change of supraclavicular skin corresponds with brown adipose tissue (BAT) thermogenesis.

**Aim:** This study examined the feasibility of using thermography to assess temperature changes in infants aged 18–25 months after mild cooling. Further, this study sought to evaluate whether cold exposure induces a thermal response suggestive of BAT activity underlying the supraclavicular region. **Subjects and Methods:** Changes in maximum skin temperature at the supraclavicular and interscapular regions were determined using thermal imaging following a mild 5-minute cooling condition (by removal of clothes in a climate-controlled room) in 67 Samoan infants. Temperature changes of the forehead and hand, known BAT-free regions, served as indicators of cooling efficacy.

**Results:** Infants with increased hand and forehead temperatures after cold exposure were excluded from analysis, reducing the effective sample size to 19 infants. On average, forehead (p < 0.001), hand (p < 0.001) and back (0.029) temperatures dropped significantly while supraclavicular temperatures remained constant. Participants with greater decreases in forehead temperature tended to exhibit greater supraclavicular thermogenesis (p = 0.084), suggesting potential BAT activity in this region.

**Conclusions:** While further work is necessary to develop a reliable cooling condition, this study provides proof-of-concept for non-invasive assessment of BAT activity in infants.

# Introduction

Despite comprising only 1-2% of body mass at birth, brown adipose tissue (BAT) plays a crucial role in facilitating the neonate's introduction to extrauterine life (Merklin 1974; Symonds and Lomax 1992). This is because new-born infants are unable to shiver in response to cold exposure. Instead, they depend primarily on BAT's thermogenic properties, which derive from a unique protein in the mitochondrial membrane called uncoupling protein 1 (UCP1) (Cannon and Nedergaard 1985). Over the first few weeks of life, white adipose tissue (WAT) replaces BAT as infants develop shivering thermogenic capacity (Aherne and Hull 1966). Although BAT was long thought to disappear completely after infancy, the recent identification of active BAT in adults has reinvigorated interest in deciphering its function throughout the lifespan (Cypess et al. 2009; Saito et al. 2009; Van Marken Lichtenbelt et al. 2009; Zingaretti et al. 2009).

The distribution of neonatal BAT in cervical/supraclavicular, perirenal/periadrenal, parasternal/pericardial and intrascapular depots was demonstrated in autopsies conducted decades ago (Aherne and Hull 1966; Merklin 1974; Poissonnet et al. 1984). Until the 2000s, post-mortem inspection was the principal method for assessing human BAT presence, with two notable exceptions. In 1972, Rylander et al. used the first commercially available thermal imaging system to demonstrate a gradual increase in interscapular skin temperature in cold-exposed neonates and confirmed the presence of subcutaneous BAT in this area via fine needle aspiration biopsy. In 1997, Oya et al. used thermography to assess the relationship between umbilical arterial blood gas concentrations and BAT activity in neonates receiving routine thermal care immediately after birth.

Due to advances in imaging technology, positron-emission tomography (PET) with <sup>18</sup>F-fluorodeoxyglucose and single-photon-emission computed tomography (CT) have since replaced autopsies as the gold-standard for assessing adult BAT activity. However, these techniques are invasive, expensive and inaccessible in many parts of the world. Radiation concerns also mean longitudinal studies of BAT in healthy paediatric populations are not feasible. Most BAT studies in paediatric populations are thus conducted among cancer patients undergoing PET/CT scans for staging purposes (Drubach et al. 2011; Gilsanz et al. 2011; 2012). These scans have consistently identified BAT in cervical/supraclavicular depots but much less frequently in other BAT depots

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Brown adipose tissue (BAT); infrared thermal imaging; infancy identified in earlier infant autopsy studies (Cypess et al. 2009; Van Marken Lichtenbelt et al. 2009; Drubach et al. 2011). Since all PET/CT BAT studies to date have excluded infants, among whom malignancies are exceedingly rare, the developmental changes in anatomical BAT distribution among this age group remain unknown.

More recently, a dramatic increase in quality and reduction in cost of portable infrared technologies has revived interest in the application of thermography to assess BAT activity. Recent studies in adult populations indicate that change in skin temperature of the supraclavicular area after a cooling condition acts as an indirect biomarker of BAT thermogenesis (Symonds et al. 2012; Levy 2019). Comparison of infrared thermal and PET/CT imaging reveals significant anatomical overlap between regions with maximal surface temperature and glucose uptake, supporting the application of thermal imaging for BAT research (Law et al. 2018).

There are two main limitations of applying thermography to assess infant BAT activity. The first involves maximising infant compliance to a cold-exposure protocol that reliably activates BAT. To our knowledge, infant cold exposure for thermography was last attempted by Rylander et al. in 1972. The second challenge is ensuring infants remain still to facilitate high-quality image capture. While the limited motor skills of neonates are optimal for thermal imaging, as they develop the ability to roll over, crawl and walk, capturing still images becomes difficult until they grow old enough to reliably follow directions. Unsurprisingly, almost all thermographic BAT studies to date have been conducted among neonates within ten days of birth or children above age three (see review: Topalidou et al. 2019). Despite these challenges, BAT studies of older infants are necessary to fully understand the anatomy of BAT, its development, and its role in human metabolism. While no studies to date have assessed BAT activity in-vivo in children between 6 months and 3 years of age, several ex-vivo studies suggest that transition to infancy may represent a critical stage for changes in BAT morphology. For example, transcriptome analyses of epicardial adipose tissue demonstrated significant differences in expression patterns of thermogenic genes in biopsies obtained from older infants (aged 40 days to 12 months) compared to neonates (aged 6 to 24 days) (Ojha et al. 2016). Interestingly, a post-mortem analysis by Lean, et al. showed higher UCP1 concentrations in perirenal BAT biopsied from young children versus infants under age one (Lean et al. 1986). The development of a standardised thermal imaging protocol to assess BAT activity in infants is a necessary first step to understanding the significance of changes in BAT activity throughout early life.

Further, variation in BAT activity may have important implications for cardiometabolic disease risk. Both in-vitro and in-vivo studies suggest that BAT plays a significant role in systemic lipid metabolism, glucose homeostasis and insulin sensitivity (Chondronikola et al. 2014, 2016). While the relationship between body mass index and BAT remains controversial (Pfannenberg et al. 2010; Drubach et al. 2011), recent studies demonstrating a positive association between muscle mass and BAT activity in adolescents (Gilsanz et al. 2011) suggest a nuanced relationship between BAT and body composition. These studies support a putative role for BAT activation in musculoskeletal development, metabolic regulation and diabetes prevention.

The potential impact of BAT research is particularly significant for Pacific Islanders, who face a disproportionately high burden of obesity and related metabolic diseases (Hawley and McGarvey 2015). Average BMI among Polynesians is 32.2 kg/m<sup>2</sup> for females and 29.2 kg/m<sup>2</sup> for males, compared to global averages of 24.2 kg/m<sup>2</sup> and 24.4 kg/m<sup>2</sup> respectively (Di Cesare et al. 2016). The rate of increase in BMI in Oceania has surpassed the world average by more than threefold over the past 30 years (Finucane et al. 2011). For example, mean BMI in Samoa increased from 25.45 to 30.63 kg/m<sup>2</sup> among males and 27.64 to 34.34 kg/m<sup>2</sup> among females between 1975 and 2016 (Bentham et al. 2017). Currently, 80% of Samoan adults have either overweight or obesity (Hawley et al. 2014). Variation in BAT activity among Pacific Islanders has yet to be investigated, likely in part due to the assumption that BAT primarily evolved as a cold climate adaptation. However, the recent discoveries implicating BAT in metabolic regulation suggest that tropical populations should no longer be ignored. Of particular interest is elucidating how developmental changes in adipose tissue during infancy impact subsequent weight gain and metabolic health throughout the life course.

Thus, the two aims of this preliminary study were to 1) assess the feasibility of using thermal imaging to assess temperature changes in infants after a mild cooling condition, and 2) evaluate whether cold exposure induces a thermal response in the supraclavicular region suggestive of underlying BAT activity in Samoan infants aged 18–25 months.

# Subjects and methods

#### Study population

The participants in this study were originally recruited as part of a prospective birth cohort consisting of 160 Samoan mother-infant dyads (Arslanian et al. 2021). Briefly, mothers were recruited at 35–40 weeks gestation at the Tupua Tamasese Meaole (TTM) Hospital in Apia. Only women above 18 years of age with singleton pregnancies and no complications were eligible for the study. At approximately age 21 months, 67 infants from this cohort were thermally imaged at the Obesity, Lifestyle, and Genetic Adaptations (OLaGA) research space in the Samoan Ministry of Health building and their data used here.

Mothers of the infant participants gave their written informed consent during pregnancy for participation in the first three infant assessments and consented separately to participation in the 21-month assessment. Protocols were approved by both the Yale University Institutional Review Board (HIC #2000021076) and the Health Research Committee of the Samoa Ministry of Health.

# **Body size**

Infant height was measured to the nearest 0.1 cm using a portable GPM anthropometer (Pfister imports, New York, NY) and weight to the nearest 0.1 kg using a Tanita HD 351 digital weight scale (Tanita Corporation of America, IL). Both traits were measured in duplicate, and average values were used for analysis. In the event of significant discrepancies between duplicate measurements (>0.1 kg for weight, > 0.5 cm for height), a third reading was taken and the average between the two closest readings used for analysis. Weightfor-height z-scores were calculated using WHO standards and account for the approximately 0.7 cm discrepancy between height and length in children under 2 years old (WHO Multicentre Growth Reference Study Group 2006).

# Thermal imaging

While cooling conditions in adult populations often involve wearing a suit perfused with cold water ( $\sim 10^{\circ}$ C), cooling challenges in paediatric populations tend to be milder. For example, Symonds et al. asked children to place one hand in cold water (19-20 °C) (2012). In the same study, it was found that changes in supraclavicular skin temperature reached a maximum within 5 minutes of placing one hand into cool water (19-20°C) and remained unchanged for the next 10-15 minutes in a cohort of 7 children, ages 3-8 years of age. Because infants are 1) unlikely to tolerate placing one hand into water for 5 minutes, and 2) lose heat guickly due to a high surface area to volume ratio (Lidell 2019), we decided to test whether removal of all clothing (except the diaper) in an air-conditioned room temperature of 25-26 °C for five minutes would activate infant BAT. The thermoneutral zone ranges from 33.8–35.5 °C in neonates depending on birthweight (Hey and Katz 1970) and declines throughout development before stabilising at 28.5-32.0 °C in adulthood (Hardy and Dubois 1937). While the limited empirical data on the human thermoneutral zone suggests remarkable variation between individuals (Pallubinsky et al. 2019), 25-26°C remains considerably lower than the expected thermoneutral zone of a 21-month-old infant.

Infants were in the temperature-controlled research centre for  $\sim$ 25 minutes undergoing other evaluations as part of the larger study before commencing the cooling protocol. During this period, efforts were made to prevent infants from moving around because muscle activation generates heat directly, via energy production, and indirectly, via dilation of surrounding blood vessels (Armstrong and Laughlin 1984; González-Alonso et al. 2000). We dimmed the laboratory lights, played movies and encouraged mothers to sing lullabies and rock their infants to sleep. Non-essential personnel were asked to vacate the research space when infants were present in order to reduce unnecessary stimulation. Mothers were instructed to limit physical contact with their infants starting 5 minutes before commencement of the cooling condition to avoid heat transfer during the study protocol.

Immediately after clothing removal, thermal images were captured of the dorsal surface of the hand, forehead, back and both sides of the neck using an infrared thermal imaging camera (FLIR E60bx; FLIR Systems, Wilsonville, OR). Images were captured from approximately 1 m from the infant at an angle perpendicular to the plane of the skin being imaged (Fernández-Cuevas et al. 2015). Infants were positioned away from heat-emitting objects (other than the researcher or mother) in the centre of the study room. After 5 minutes, the same images were obtained.

# Image processing

Following the best practices for infrared thermal imaging camera parameters and data processing established by Levy (2019), maximum skin temperature of the intrascapular and supraclavicular area of the thermoneutral and cold condition images were determined using FLIR Tools software (FLIR E60bx; FLIR Systems, Wilsonville, OR) box tool with emissivity set to 0.98. The supraclavicular area was defined as the triangle delineated inferiorly by the clavicle, medially by the sternocleidomastoid and laterally by the anterior border of the trapezius (Figure 1). The interscapular area was bounded superiorly by the transverse axis containing cervical vertebra seven and inferiorly by the axis connecting the inferior angle of the scapula bilaterally. Of note, there exists debate regarding whether comparing maximum temperatures or average temperatures of a region of interest (ROI) should be considered best practice for detecting BAT thermogenesis (Fernández-Cuevas et al. 2015). While maximum temperatures were used here to avoid the possibility of introducing error during manual ROI selection, Ludwig et al. did not find significant differences between the two analytical approaches (Ludwig et al. 2014). As human BAT is not found on the hand or forehead, temperature changes in these regions between thermoneutral and cold conditions were used as indicators for efficacy of the cooling condition. As in Blondin et al. (2017, 2015) and Yoneshiro et al. (2011, 2016), hand and forehead temperatures serve not as surrogates for core temperature, but as points of comparison to skin overlying areas likely to contain BAT. Infants who exhibited increases in temperature at either area after the cooling condition were considered not effectively cooled and were thus excluded from analyses.

#### Statistical analyses

Shapiro-Wilk tests were used to assess normality of all variables. Depending on normality, two-tailed paired t-tests or Wilcoxon signed-rank tests were used to determine whether maximum supraclavicular temperature, maximum intrascapular temperature, forehead temperature and hand temperature changed significantly between temperature conditions. *A priori* calculations indicated that given Cohen's d = 0.5, commonly considered moderate effect size (Cohen 1988; Sawilowsky 2009), a sample size of 34 is necessary to detect significant changes in skin temperature with 80% power. At Cohen's d = 0.8, a sample size of 14 is sufficient to detect



Figure 1. Sample thermal and corresponding digital images of the supraclavicular (a, b) and intrascapular regions (c, d). Red border marks region of interest in relation to visible surface anatomy. Target marks maximum temperature within the region determined via box tool. Images were captured prior to cold exposure.

	Whole Sample ( $N = 67$ )	Cooled Infants ( $N = 19$ )	Not Cooled Infants ( $N = 48$ )
Sex			
Boys (n, %)	37 (55.2)	10 (52.6)	27 (56.3)
Girls (n, %)	30 (44.8)	9 (47.4)	21 (43.7)
Age (months)			
Mean (SD)	20.92 (1.42)	20.94 (1.33)	20.91 (1.47)
Range	18.71–24.53	19.23-24.00	18.71–24.53
Height (cm)			
Mean (SD)	81.78 (3.48)	81.29 (3.01)	81.98 (3.66)
Range	73.45-90.15	73.5–85.4	73.45–90.15
Weight (kg)			
Mean (SD)	12.02 (1.60)	11.89 (1.53)	12.08 (1.64)
Range	7.95–17.80	9.2–14.95	7.95–17.80
Weight-for-height Z-score			
Mean (SD)	1.29 (1.06)	1.43 (1.22)	1.23 (1.00)
Range	-2.38-3.62	-0.48-3.46	-2.38-3.62

the effects of cooling on skin temperature at 80% power. Two-tailed t-tests were used to identify any significant differences in skin temperature change by sex. Depending on normality, Spearman or Pearson correlation tests were used to identify relationships among skin temperature changes across different regions and body size. The threshold for statistical significance was set at  $\alpha = 0.05$ .

# Results

# Feasibility

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Table 1 describes the anthropometric characteristics of our study population. There were no differences in weight

(p = 0.20), height (p = 0.99), or weight-for-height z-score (p = 0.70) by sex. Average weight-for-height z-score was more than one standard deviation greater than the WHO 50<sup>th</sup> percentile. Based on WHO cut-offs, 3 infants qualified as having obesity (weight-for-height Z-score >3), 14 infants had overweight (weight-for-height Z-score >2) and 25 were "atrisk" (weight-for-height Z-score >1). Only 19 infants exhibited decreased temperatures of both hand and forehead after the cooling condition. This was likely because, despite the valiant efforts of both mothers and laboratory staff, many infants remained very active immediately prior to and during the cooling phase. Infants were bewildered by the unfamiliar lab space, and it proved very difficult to prevent infants from moving around or crying without mothers using physical

Table 2. Mean skin temperatures at baseline and post-cold exposure.

	Baseline (°C)	Post-Cold Exposure (°C)	Change (°C)	V-value <sup>B</sup>	p Value
Whole Sample ( $N = 67$ )					
Supraclavicular	36.42 (0.51)	36.40 (0.69)	-0.01 (0.62)	994.5	0.48
Back	35.05 (0.83)	34.96 (1.17)	-0.09 (1.14)	921.5	0.87
Hand	32.97 (1.01)	33.03 (1.12)	0.05 (1.00)	936.5ª	0.66
Forehead	34.48 (1.19)	34.42 (1.37)	-0.07 (1.55)	1036	0.85
Cooled Infants (N = 19)					
Supraclavicular	36.37 (0.29)	36.26 (0.51)	-0.11 (0.29)	118 <sup>b</sup>	0.17
Back	35.00 (0.72)	34.30 (1.56)	-0.70 (1.29)	146 <sup>c</sup>	0.04
Hand	33.07 (0.95)	32.35 (0.92)	-0.72 (0.58)	171 <sup>d</sup>	$2.1*10^{-4}$
Forehead <sup>A</sup>	34.74 (0.98)	33.48 (1.50)	-1.26 (1.18)	153	$3.2^{*}10^{-4}$
Not Cooled Infants ( $N = 48$ )					
Supraclavicular	36.42 (0.53)	36.45 (0.75)	0.02 (0.71)	469	0.15
Back	35.04 (0.89)	35.19 (0.88)	0.15 (0.98)	338.5	0.11
Hand	32.93 (1.03)	33.27 (1.11)	0.34 (0.98)	290	$6.3^{*}10^{-3}$
Forehead	34.38 (1.26)	34.79 (1.14)	0.41 (1.42)	47 <sup>e</sup>	8.3*10- <sup>3</sup>

<sup>A</sup>The removal of the outlier resulted in temperatures of 34.74 (1.01) at baseline and 33.65 (1.34) post-cold exposure, an average change of -1.09 (0.94). This change was significant (v-value = 136, p-values =  $2.4 \times 10^{-4}$ )

<sup>B</sup>For ease of comparison, V-values for paired two-sided Wilcoxon signed-rank tests are provided because the majority of variables were nonnormal. For normally distributed variables, T-test results are printed with alphabetical superscripts.

<sup>a</sup>T-value –0.42, *p*-values 0.66

<sup>b</sup>T-value 1.63, *p*-values 0.12

<sup>c</sup>T-value 2.37, *p*-values 0.029

<sup>d</sup>T-value 5.41, *p*-values 3.9\*10<sup>-5</sup>

<sup>e</sup>T-value – 1.97, *p*-values 0.054

touch to provide comfort. Thermal images often included a signature striated heat pattern depicting heat transfer from the mother's fingers to the infant's skin. These compliance challenges greatly limited our ability to tightly control cooling condition settings. The high exclusion rate, despite a mild cooling condition that was purposefully designed to maximise compliance, suggests that infants aged 18-25 months may be a particularly difficult age-range to study. There were no significant differences in anthropometric characteristics between infants who were effectively cooled versus those who were not.

In contrast, the actual capturing of thermal images proved remarkably straightforward, as the FLIR E60bx is capable of rapid image capture. When necessary, mothers simply held their infants in position at the abdomen and upper arm, allowing images to be captured consistently from the standardised 1 m distance and perpendicular angle. Thus, while compliance challenges during the cooling condition inevitably introduced irregularities in methodology, the resulting thermal images likely represent reasonably accurate skin temperature readings.

# Supraclavicular Temperature change as proxy for BAT activity

Table 2 summarises temperature changes in all four regions for infants who exhibited decreased temperatures on both the hand and forehead, and thus deemed effectively cooled (n = 19), and those who did not (n = 48). Within the subset of 19 infants deemed properly cooled, supraclavicular temperatures did not change significantly after cold exposure while temperatures of the hand  $(p = 3.9*10^{-5})$ , forehead  $(p = 3.2*10^{-4})$  and back (p = 0.029) decreased (see Supplementary Figure 1). Despite the exclusion of 48 infants, the reduced sample size exceeded 14, the number needed to achieve 80% power at Cohen's d = 0.8. The mean

observed Cohen's d for temperature changes of the hand and forehead was 0.95, exceeding the benchmark for large effect size employed in the *a priori* power calculations. The reduced sample size of 19 was thus sufficiently large to detect cooling effects on skin overlying areas without BAT.

After removal of one highly influential (Cook's distance greater than 4/sample size) outlier with change in forehead temperature greater than two standard deviations below the mean, there was a trending negative relationship between supraclavicular area and forehead temperature change (p = 0.084, adjusted r<sup>2</sup>=0.12) (Figure 2). Supraclavicular temperature change was not significantly related to back (p = 0.63) or hand (p = 0.26) temperature changes. Nor was supraclavicular temperature change related to sex (p = 0.57) or body size (p = 0.84).

# Discussion

Only 19 of 67 infants exhibited a decrease in hand and forehead temperature, indicating that the cooling condition was not adequate for most infants. Their high activity levels and heat transfer from mothers presented logistical challenges to the strict implementation of a consistent cooling condition. Despite the exclusion of 48 infants, our study was sufficiently powered to detect the impact of cooling of the scale observed at skin overlying areas unlikely to contain BAT. Consequently, the lack of significant change in supraclavicular temperature after cold exposure suggests potential evidence of BAT activity. Among those who exhibited decreases in hand and forehead temperatures, a trending negative relationship between change in forehead and supraclavicular temperatures was observed, suggesting potential physiological differences in heat dissipation between these locations. In other words, infants more affected by cooling, as



Figure 2. Relationship between temperature changes of neck and forehead after cold exposure in effectively cooled infants (N = 18). Although 19 infants exhibited decreases in both hand and forehead temperatures after cold exposure, 1 outlier with forehead temperature change >2 standard deviations from the mean was removed from this analysis. Simple linear regression demonstrated that neck temperature change =  $-0.13^*$ (forehead temperature change) -0.24. Overall model fit was  $r^2=0.12$ , p = 0.084.

indicated by sharper declines in forehead temperature, may be more likely to activate BAT thermogenesis.

Notably, we did not observe temperature changes suggestive of underlying BAT in the interscapular region. Our finding is supported by a recent MRI/CT post-mortem study of a three-month-old infant (Hu et al. 2012). While it is possible that the interscapular BAT depot is already significantly diminished in toddlers, the lack of supporting imaging data does not necessarily imply that BAT is non-existent in these locations. It does, however, raise the possibility that current thermal imaging techniques alone are not yet sensitive enough to detect isolated depots of BAT. BAT often exists in small clusters of 5-100 cells surrounded by large numbers of white adipocytes and may contain lipid vacuoles of varying sizes (Aherne and Hull 1966). It is possible that the mingling of brown and white adipocytes may disproportionately hinder the identification of BAT activity in individuals with large WAT depots. While this hypothesis remains to be tested, it is particularly important to consider in our cohort, which included 17 infants categorised with overweight or obesity and another 25 "at-risk" according to WHO guidelines.

Moreover, it is certainly possible that infants possess BAT depots in other regions. We chose to focus our analyses on the detection of BAT in the supraclavicular depot, where the likelihood of BAT detection is highest, and the interscapular depot, the largest BAT depot in neonates. However, the presence of perirenal and para-aortic deposits in the neck and mediastinum have been confirmed in adult populations (Enerbäck 2010). We noticed significant heat activation in the posterior cervical region among many infants. However, we did not attempt to quantify temperature changes in this region due to the common presence of fat folds, which can

trap heat and interfere with temperature measurements using infrared thermography.

Besides the challenges of ensuring infant compliance to the cooling procedure and resulting low sample size discussed previously, there are several other important limitations to this study. First, we did not capture serial images throughout cold exposure. While we set cold exposure duration to 5 minutes based on protocols validated for children ages 3-8 (Symonds et al. 2012), additional time course data may have identified maximal BAT activation at a different timepoint. Second, if the study procedures caused discomfort due to cold exposure or unfamiliar positioning, it is possible that heat generation in the supraclavicular region occurred due to activation of the stress response rather than BAT activation. Third, we were unable to measure metabolic rate, which would have allowed us to link metabolic rate changes during cold exposure to BAT activation. Although our study protocol originally included an assessment of resting metabolic rate using a metabolic cart, it guickly became obvious that infants would not tolerate the infant canopy given their poor compliance with the thermal imaging protocol. Finally, BAT thermogenesis was not directly quantified, rather a change in the supraclavicular temperature relative to hand and forehead change in temperature was used as a probable signal of BAT thermogenesis. However, while peripheral vasodilation could increase surface temperatures, it is unlikely that thermographic change in the supraclavicular area is simply associated with circulatory changes (Deng and Liu 2004). Cooling would be expected to promote local vasoconstriction, thereby reducing regional blood flow and decreasing skin surface temperature.

In conclusion, despite the challenges of ensuring participant adherence to an efficacious cooling condition in this study, thermal imaging provides a promising technique for investigating BAT activity during infancy. While recent PET/ CT studies have uncovered the persistence of active BAT into adulthood, neonatal autopsy studies conducted over four decades ago comprise the bulk of our knowledge of BAT activity in infancy. We still know startlingly little about how BAT activity and distribution changes throughout early life. Once a standardised protocol for assessing BAT activity with thermal imaging is established, longitudinal thermographic studies could fill this important knowledge gap. As the cooling condition implemented in this study clearly requires further modifications, several alternatives are proposed here. One method could involve wrapping infants with cooling blankets, potentially allowing mothers to continue holding their infants during the cooling condition without affecting its efficacy. Another alternative that infants may find more enjoyable could involve bathing in water. The use of iButtons (Thermochron, Dallas, TX), small thermocouples that can be applied directly to the skin, could mitigate the difficulty of capturing infant still shots and potentially improve the accuracy of temperature readings. While the present study does not provide definitive proof of successful BAT activation and identification in infants using infrared technology, increasing appreciation for negative results indicate that our methodology and the alternatives outlined above can inform future research and drive the development of innovative methods (Mlinarić et al. 2017; Porter et al. 2017; Nimpf and Keays 2020).

Portable thermography equipment provides a novel opportunity to assess population-level BAT variation and expand our understanding of BAT function beyond cold climate adaptation. Recent evidence suggesting a putative role for BAT in metabolic regulation, combined with a growing body of literature supporting the developmental origins of health and disease (Barker 1990), highlight the importance of better characterising BAT in early life. Variation in the rate at which BAT activity decreases throughout infancy may uncover important clues for predicting obesity risk and other metabolic comorbidities in adulthood. To that end, the importance of further work to develop a reliable cooling condition for thermographic assessment of BAT activity in infants cannot be understated.

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The authors report no conflict of interest.

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#### Data availability statement

Access to the dataset may be granted upon reasonable request to the corresponding author.

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