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Assessing the Reliability of an Infrared Thermography Protocol to Assess Cold-Induced Brown Adipose Tissue Activation in French Psychology Students

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Supplementary Materials: Data, Materials [see Index of Supplementary Materials]

Abstract

The authors use infrared thermography measurements of skin temperature to non-invasively assess the heat production of Brown Adipose Tissue (BAT). In species other than humans, BAT has been linked to maternal care, and may thus be crucial for understanding differences in attachment security. Whereas early BAT research measured its relative presence in the human body through radioactive tracers, researchers have recently used infrared thermography measurement of skin temperature in cold conditions to study BAT thermogenesis outside of medical facilities. Infrared thermography relies on comparing skin temperature in the supraclavicular region (where a BAT depot is located) with skin temperature in the sternal region (which contains no BAT depots) in cold conditions, when the supraclavicular BAT depot produces heat. We replicated an infrared thermography protocol, which previously reported an increase of 0.2 °C in supraclavicular (vs. sternal) skin temperature in cold (vs. control) conditions in only 7 adults, which probably led to overestimation of the effect. With a much larger sample size (N = 94 young adults) and a similar protocol, we did not find any significant variation in relative, Cohen's d = 0.10, 95% CI [-0.31, 0.50], or absolute supraclavicular skin temperature, Cohen's d = 0.11, 95% CI [-0.30, 0.52]. Using conditional random forests, we also excluded a variety of alternative explanations for why the

method failed to achieve an effect. This protocol of infrared thermography cannot measure BAT thermogenesis and is thus not recommended for future studies to study individual differences in attachment.

Keywords

social thermoregulation, infrared thermography, Brown Adipose Tissue, attachment, corelab

Highlights

- Infrared thermography was thought to constitute a non-invasive, low-cost, and risk-free alternative to study non-shivering thermogenesis' link with attachment as opposed to invasive CT scans.
- Through an independent replication of an established infrared thermography protocol with a larger sample size (N = 94) than the original (N = 7), we did not observe the expected heat produced by Brown Adipose Tissue, responsible for non-shivering thermogenesis.
- Comprehensive exploratory analyses, including conditional random forests, discounted alternative explanations for non-replication (such as participant sex, age or ambient room temperature).
- The studied infrared thermography protocol seems unreliable for assessing Brown Adipose Tissue activity and its potential association with attachment.

Brown Adipose Tissue (BAT) can transform energy from nutrients into heat in adult human beings. In species other than humans, BAT thermogenesis has been thought to be relevant for maternal care (e.g., Haig, 2008; Oelkrug et al., 2020). But if and how BAT is involved in human interpersonal interactions or adult human attachment is equivocal. The reason for BAT and its role in adult human social interaction being so ill-understood is that the most widely-used method to measure the amount and the activity of BAT relies on very intrusive radioactive tracers (through PET-CT scans) that can only be measured once per patient.

Symonds et al. (2012) proposed infrared thermography as a fast and non-invasive alternative method to measure BAT thermogenesis by measuring the increase of skin temperature above the clavicle in cold conditions (see also Brasil et al., 2020; Jimenez-Pavon et al., 2019). Given that we had originally presumed infrared thermography to provide a reliable measurement, our original goal was to study relative BAT thermogenesis and explore its relationship (if any) with attachment traits. However, as we ran our sequential analyses on the correlation between BAT thermogenesis and avoidant and anxious attachment, we failed to detect the effect (pre-registration and analyses available at https://osf.io/k86h7) and suspected a potential issue with the protocol. Thus, we instead used the sample to assess the reliability of the protocol of infrared thermography to measure BAT thermogenesis through a paradigmatic replication, by examining the

difference (or lack thereof) in supraclavicular (versus sternal) skin temperature in cold (vs. control) conditions that could indicate BAT thermogenesis.

Social Importance of Brown Adipose Tissue Thermogenesis

Newborn infants have higher amounts of BAT than adults and BAT is known to help infants regulate their core body temperature (Cannon & Nedergaard, 2004). Until 2003, it was generally accepted that adults did not have BAT depots. However, Cohade et al. (2003a) then discovered a significant amount of BAT (first called "USA-Fat") in adult humans (Cohade et al., 2003a). BAT can support the organism to produce heat whenever it experiences cold (Cohade et al., 2003b; Harms & Seale, 2013; Li et al., 2019) or after eating (i.e., dietary-induced thermogenesis; Heenan et al., 2020). Energy expenditure of BAT depends on the amount of BAT (Carpentier et al., 2018). Small variations in the amounts of BAT, which have been measured between adults (Carpentier et al., 2018), should thus logically result in differences in BAT thermogenesis.

We suspect that interindividual differences in the amount of BAT in adults is—at least partly—reliant on social experiences. An important way to decrease the metabolic costs of temperature regulation is to outsource it (i.e., by relying on social resources via *social thermoregulation,* for example). In species other than humans, social thermoregulation has been associated with metabolic energy savings of 6–53% (Gilbert et al., 2010; IJzerman, 2021). In humans, a few disparate findings linking social thermoregulation and metabolic energy savings exist. Ein-Dor et al. (2015) found that attachment avoidance is associated with higher fasting glucose levels, while the confidence that other people are available to socially thermoregulate is negatively associated with attachment avoidance (Dujols et al., 2022; see also Vergara et al., 2019). We thus suspect that BAT levels in human adults are positively associated with attachment avoidance (see also IJzerman et al., 2015). Because of the importance of social connection and loneliness for health (Holt-Lunstad et al., 2010), BAT may well form an entry-point to better understand why social relations are good for one's health and we regard the measurement of BAT as vital for better understanding these mechanisms.

Infrared Thermography: A Non-Invasive Way to Measure BAT Thermogenesis

Assessing BAT thermogenesis directly is invasive and costly: Therefore researchers and medical professionals typically detect BAT thermogenesis through PET-CT imaging (Cohade et al., 2003a; Saito et al., 2009). Because of the risks associated with ionizing radiation during PET-CT imaging, researchers have sought to develop alternative methods to estimate BAT activity, such as infrared thermography (Symonds et al., 2012). Infrared thermography provides a non-invasive, low-cost, and risk-free method to evaluate BAT thermogenesis: this method does not require the assistance of healthcare professionals

and is therefore potentially usable in psychology research settings. Infrared thermography measures superficial temperature to assess variations of skin temperature in the supraclavicular area where the largest human BAT depots are located (Saito et al., 2009). While most of the heat produced by BAT contributes to internal temperature, some of the heat might be leaked toward the skin. Heat is typically produced by BAT through a cold (versus control) manipulation (Harms & Seale, 2013). Symonds et al. (2012) found an increase in supraclavicular relative skin temperature (as compared to the sternal area, where no BAT depots are present) in cold-experimental (versus control) conditions and that the effect decreased with age (N = 26 including the critical 7 adults). They were the first to introduce infrared thermography as a method to evaluate BAT activity.

Heterogeneity in BAT Measurement and Statistical Power Problems

Since then, two systematic reviews evaluated the results of different methods of infrared thermography to measure BAT activity in humans (Brasil et al., 2020; Jimenez-Pavon et al., 2019). Unfortunately, both reviews reported considerable levels of heterogeneity in the protocols, with seven showing a positive and significant association between infrared thermography measures and PET/CT scans on the same day (Gatidis et al., 2016; Law et al., 2018; Ramage et al., 2016; Salem et al., 2016; Symonds et al., 2012; Thuzar et al., 2018), relying on 6–13 healthy adults and no pre-registration (with the exception of Gatidis et al., 2016; N = 102, but no pre-registration and BAT detected in only N = 9). Two studies detected no relationship between infrared thermography measures of supraclavicular skin temperature and PET-CT scan results (Jang et al., 2014; Martinez-Tellez et al., 2019), but again, with small sample sizes (N ranging from 11–17 healthy adults, with no pre-registration). Finally, two other studies reported a positive correlation between infrared thermography measures of supraclavicular skin temperature and PET-MRI, a different invasive method (N = 12–20; Andersson et al., 2019; Sun et al., 2019).

Overall, infrared thermography measures of supraclavicular skin temperature seemed to be a valid proxy of BAT uptake in lean adults. In hindsight however, the sample sizes in past studies were too modest, no close replications have been conducted, and no assessment of publication bias has been made. Further, if one were to study social-thermoregulation-based attachment, developing reliable measurements is crucial. After all, psychology has been confronted with a sort-of measurement crisis (see, e.g., Flake & Fried, 2020; Hussey & Hughes, 2020), making the evaluation of measurement vital. Finally, like most of the scientific literature suffering from such shortcomings, effect sizes are likely overestimated due to small-sample bias (Kühberger et al., 2014). While infrared thermography is a thought-provoking method, protocols to assess BAT seem not to have been rigorously tested.

Selecting a Protocol of Infrared Thermography

As we were interested in assessing individual differences in attachment and/or interpersonal interactions, infrared thermography seemed promising, affording us with a safe and accessible method to evaluate BAT thermogenesis in a large number of psychology students. Originally, Symonds et al. (2012) reported an increase of 0.20°C, 95% CI [0.14, 0.26] in supraclavicular skin temperature in 7 healthy adults of healthy body-mass index in cold (versus control) conditions. They did not report any effect-size; based on their design we estimated a Cohen's d = 2.5, 95% CI [-0.08, 4.98]. We established that the reason for why our estimation of the effect size was very large and non-significant was because Symonds et al. had a small sample size. We, therefore, wanted to use the same paradigm as Symonds et al. (2012) with a large number of psychology students to evaluate individual BAT thermogenesis on a single day and have a better estimation of the effect and the reliability of the protocol.

We conducted a so-called "paradigmatic replication" of their study however, "modifying an original (to-be-replicated) study's method, design, or materials, and potentially correcting flaws in the methods, design, or materials, so that the new study is diagnostic of the original study's claims as our best-faith effort interpretation of the original studies" (p. 8, Adetula et al., 2021; see also Vohs et al., 2021). We used Symonds et al.' protocol, using the same procedure but we lowered the water temperature used for the cooling intervention, because one of the co-authors, Costello, managed to replicate Symonds's study using 15°C water instead of 19–20°C water and the lower water temperature would increase the chances for BAT activity.

We reported both relative and absolute measures of supraclavicular skin temperature to compare our results with Symonds's study and quantify variations of temperature of the supraclavicular/BAT area compared to a control area.

Method

This research was conducted in line with the CO-RE Lab Lab Philosophy v5 (Silan et al., 2021).

Participants

We recruited 114 participants (M_{age} = 20.6, SD_{age} = 4.2, 102 identified as women, 12 as men) among psychology students directly on the official website of the lab where they could access the web page of the experiment (see https://sites.google.com/view/etude-lip-21-22-220). We ran a Bayesian Sequential analysis (as pre-registered, see Supplementary Materials) but instead of stopping recruitment when the Bayesian Sequential analysis was over, we stopped recruiting when we realized the method did not have the same effect as Symonds et al. (2012). Before stopping data

collection, we conducted a sensitivity analysis to evaluate the minimum effect size our design could have captured: our design could have detected a Cohen's d = .29, which is smaller than the Cohen's d = 2.5 we estimated to represent the effect size Symonds et al. measured (more information about the Bayesian Sequential analysis and the sensitivity analysis are available at https://osf.io/avdzf).

Procedure

Pre-Experimental Indications

On the experiment's website, participants received information about the two requirements of the study: having completed questionnaires in a so-called "test-week" conducted by the "laboratoire"[1] earlier in the year and being a current psychology student. They were informed that they would have to remove the clothes or bra strap covering the measured areas (i.e., the neck and the left shoulder). Given that they had to remove clothing, in the registration form, participants were also asked if they preferred a female experimenter (Blandine Ribotta), a male experimenter (Nathan Vidal), or if they had no preference.

Procedure

Test-Week — Participants answered the test-week questionnaires a few weeks before the BAT measurement. All questions were included by researchers from the "laboratoire" and were thus not under our control. The test-week included a multitude of questionnaires, but we only used two of them, the Social Thermoregulation and Risk Avoidance Questionnaire (STRAQ-1; Vergara et al., 2019), the Experience in Close Relationships Questionnaire Revised (ECR-R; Fraley et al., 2000) and self-reported demographics (for a full list of questionnaires, see https://osf.io/j5yzr). The order of the questionnaires, the question blocks, and the question order within each questionnaire were randomized.

Experimental Procedure — Participants came to the laboratory for a 1-hour visit. We asked them not to eat 30 minutes prior to testing to prevent dietary-induced thermogenesis. Upon arrival, participants were informed of the procedure of the experiment and were asked to fill out a consent form to provide informed consent. We recorded information relevant to their ability to (socially) thermoregulate, such as mass, height, age, relationship status, smoking status (and, if yes, how many cigarettes per day), whether they use medication (and if so, which kinds of medication), and their last menstrual onset (for the full social thermoregulation protocol, see IJzerman et al., 2021; Sarda et al., 2021). We reported if participants had any potential cardiovascular, respiratory, and

1) In France, "laboratoire" is used to indicate a research department.

neurological disorders before starting the study. We asked participants not to use their phone during the experiment.

We scraped the average temperature and photoperiod of the day (i.e., number of hours of sunlight) from the open dataset of the collaborative project OpenstreetMap France to be used as control variables. During the measurement, we used an air-conditioning unit to maintain room temperature at 19.5°C, 95% CI [19.3, 19.7] (Brasil et al., 2020; Symonds et al., 2012), but also measured the temperature of the room via a BlueMaestro TempoDisc to measure any variations. Index finger temperature was measured using the ISP131001, a wireless temperature sensor that showed good validity and reliability in both warm and cold conditions (Sarda et al., 2021). Following the installation of the sensors, participants then went through the following steps:

1. The experimenters asked them to take off their clothes and bra strap (if applicable) covering the measured areas (i.e., the neck and the left shoulder). The infrared thermography camera was then fixed on a level tripod in front of the participant. The height of the camera was adjusted to the height of the participant's supraclavicular area (between 1 and 1.2 m). The camera was powered at least 10 minutes before the first measure to ensure it reached a stable internal temperature (Grgić & Pušnik, 2011). The experimenters instructed participants to sit in an upright position for the rest of the experiment.
2. The experimenters then asked the participants to sit for 30 minutes so that the body would get accustomed to the room temperature.
3. The experimenters then requested participants to put their right hand up to the elbow in a cold-water bath for 15 minutes to activate BAT thermogenesis (Van der Lans et al., 2014). Fifteen minutes represented the midpoint between times used in existing BAT research (Ramage et al., 2016). Water was kept at 15°C using a Cold pressor Techne RU 200[2]. The experimenters then asked participants to keep moving fingers to disrupt the insulation of still water.
4. Finally, the experimenters asked participants to remove their right hand from the bath for a rewarming period of 15 minutes.

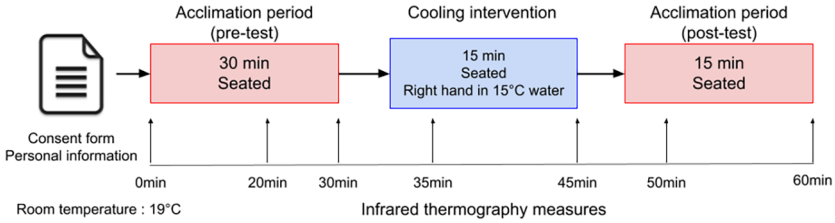
For a visualization of the full procedure, see Figure 1. Our procedure followed the guidelines for using infrared thermology imaging in sports and exercise medicine (Moreira et al., 2017). We used an infrared camera from Thermovision (TG297, FLIR Systems, Sweden) and a FLIR Thermal Studio[3] to measure skin temperature. Constant skin emissivity (i.e., the proportion of radiation emitted by an object compared to an ideal black body) for the infrared thermography camera was set at 0.98 (Steketee, 1973).

2) http://www.techne.com/product.asp?dsl=199

3) THERMAL STUDIO STARTER, Teledyne FLIR LLC, Revision 02/22

Figure 1

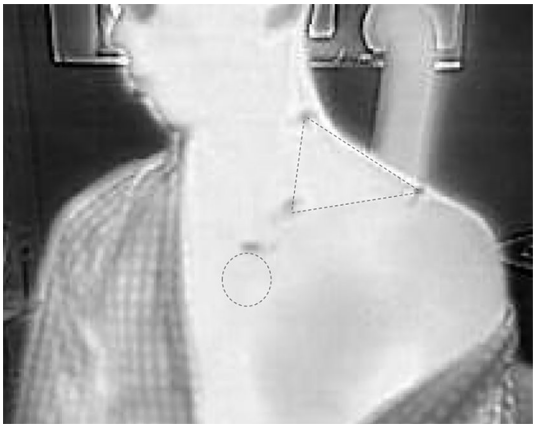
The Experimental Procedure



We calculated skin temperature from 3 consecutive infrared measures of the regions of interest. The region of interest of the control temperature was the sternal region, at the manubrium level (Van der Lans et al., 2014). This area reported no thermal response to the cooling of the hand (Symonds et al., 2012). The region of interest of the supraclavicular skin temperature was the supraclavicular area defined as in previous research and Symonds et al.'s (2012) study by the triangle area between the left sternocleidomastoid, trapezius, and clavicle (see Figure 2; Brasil et al., 2020).

Figure 2

Infrared Thermography Image of One Participant



Note. The triangle with the dashed lines represents the supraclavicular region, delimited by 3 small pieces of wood taped to the skin of the participant. Supraclavicular area was delimited by the clavicular head, the SCV fossa, and the apex of the trapezius and neck. The circle with the dashed line corresponds to the control area (i.e., the sternal region). Photo of the participant is included with their consent (email exchange available on a private OSF component at https://osf.io/xv4ec/).

Both regions of interest were delimited by small pieces of wood (insulating material) taped to the skin before any measurement. We took measures 0, 20, 30, 35, 45, 50 and 60 minutes after the beginning of the experiment (see also Figure 1). Relative supraclavicular skin temperature was the mean of the three consecutive measures of supraclavicular skin temperature minus the mean of the three consecutive measures of the temperature at the control area (Symonds et al., 2012). During the procedure, we noted any technical problems and any self-reported shivering or feeling of discomfort of the participant, as advised when studying non-shivering thermogenesis (Van der Lans et al., 2014).

Data Analyses

Data Preprocessing

We first downloaded room temperature data from the TempoDisc Data via an app called Tempo Plus 2. We then downloaded finger skin temperature data from the ISP131001 directly from our lab's OVH server. We cleaned finger and room temperature data with a Python 3.9 script to merge finger and room temperature at each measure of BAT activity for further analyses. We coded demographic qualitative variables numerically: sex (binary, 0 = female, 1 = male), tobacco use (continuous, number of cigarettes per day), alcohol consumption (continuous, number of day(s) per week the participant consumes alcohol), and shivering (binary, 0 = no shivering, 1 = shivering).

Outliers and Unusable Data

We removed infrared thermography data of the first four participants from the study because the infrared measurements did not capture the proper regions of interest, leaving 110 participants. We then defined outliers by the Interquartile Range (IQR) method (Jones, 2019; more information available at https://osf.io/avdzf). Data from 16 participants were considered outliers and removed from the analysis.

Manipulation Check: Statistical Analysis

Analysis scripts are available at https://osf.io/tjc9y/. Statistical analyses were conducted using R-4.2.1 for Windows (R Core Team, 2013) with the R packages *ggplot2* (Wickham, 2016), *party 1.3-10* (Hothorn et al., 2006), *lattice 0.20-45* (Sarkar, 2008), and *plyr 1.8.7* (Wickham, 2011). We (a) validated the experimental setup with descriptive statistics and (b) evaluated the effect of the cooling intervention on relative and absolute supraclavicular skin temperature. We ran a paired *t*-test on relative and absolute supraclavicular skin temperature as within-participants between the two conditions (presence or not of the cooling intervention). Doing so allowed us to compare our effect with the effects of Symonds et al.'s (2012) study. We then (c) assessed the variations of relative and absolute supraclavicular skin temperature during the experiment through a one-way

repeated-measure ANOVA on relative and absolute supraclavicular skin temperature as within-participants between time points.

Finally, we (d) searched for possible alternative explanations for the lack of effect with a conditional random forest (Breiman, 2001; Strobl et al., 2008). Conditional random forest is a non-parametric supervised learning method that accounts for the effect of each independent variable without a priori predictions of the effect. For instance, the conditional random forest can assess non-linear relationships, and does not presume direction. This method is particularly useful when multiple predictor variables are included to test multiple hypotheses reducing the possibility of overfitting. Conditional random forest is an efficient method in exploratory analysis when the pre-registered hypothesis fails and could be a first step towards building a more specific model for the dependent variable. In our case, we included as many possible predictors as we could to explore all possible explanations. Note that, in contrast to the traditional linear model, conditional random forests benefit from having additional predictors in the dataset for accuracy of prediction as it has fewer problems with collinearity. Conditional random forest evaluated if independent variables could predict relative supraclavicular skin temperature in cold conditions. Conditional random forest relies on bagging, which involves building a decisional tree to predict the dependent variable based on a random part of the dataset.

We set the bagging process to be repeated for 500 iterations (ntree), which should lead to sufficiently stable results (Latinne et al., 2001). Given that our total number of variables were 16 and 17, we fixed the number of variables to sample (mtry) at 4 or 5. We ran the program twice with a mtry of 4 and twice with a mtry of 5, so a total of 4 trials. Finally, we set the seed at 100, giving us a total of 400 variable importance lists, which we averaged into one. This averaged variable importance list identifies which are the best predictors of the dependent variable and which of the predictors differ from random noise (Wittmann et al., 2022).

Results

Description of the Sample

Our final sample included 94 participants (M_{age} = 20.5, SD_{age} = 4.1, 84 identified as women, 10 as men, see Table 1 and Table 2 for demographics). The body mass index of our sample (M_{bmi} = 21.5 kg/m^2, SD_{bmi} = 3.2) is considered "normal" by the World Health Organization (WHO Expert Consultation, 2004). More than 80% of participants did not smoke, were not on medication that can impact BAT, or had any health condition. More than half of the participants did not consume alcohol at all. In our sample, 59% of participants reported shivering at least once during the experiment. For 30 participants, the experiment was run by a female experimenter and 64 by a male experimenter. All participants went through the whole 1-hour experiment. One participant asked to stop

the cooling intervention for 1 minute because it was very uncomfortable before wanting to resume the experiment. We included this participant in the analyses.

Table 1

Description of the Sample (1)

Category	*n*	*M*	*SD*	Min	Max
Gender					
Female	84 (89%)				
Male	10 (11%)				
Age	93	20.5	4.1	18	45
Height (cm)	93	165.4	7.2	150	183
Weight (kg)	93	58.8	9.4	39	95
BMI (kg/m^2)	93	21.5	3.2	15.4	35.3
LMC (days)	66	17.6	14.8	0	98

Note. Reported over 94 participants. n refers to the number of participants who responded to the question. BMI = Body-Mass Index; LMC = Last Menstrual Cycle's onset.

Table 2

Description of the Sample (2)

Category	n_0	n_1
Tobacco	78 (83%)	15 (16%)
Medication	80 (85%)	13 (14%)
Alcohol	55 (59%)	37 (39%)
Health condition	84 (89%)	9 (10%)
Shivering	36 (38%)	55 (59%)

Note. Reported over 94 participants. n_0 is the number of participants that do not smoke, or take no medication, or do not consume alcohol, or present no health conditions, or have not shivered during the experiment. Reversely, n_1 is the number of participants that smoke, or take medication, or consume alcohol, or present health conditions, or shivered during the experiment. NAs Missing data are not displayed here. "Medication" refers to any use of a medication that is known to influence Brown Adipose Tissue. "Health condition" refers to any cardiovascular, respiratory or neurological condition of the participant.

Manipulation Check

Validation of the Experimental Conditions

We measured an average room temperature of 19.5°C, 95% CI [19.3, 19.7], which is similar to the ambient temperature in Symonds et al.'s (2012) study which was between 19 and 20°C. Index finger skin temperature significantly decreased in our study, 0.75°C, 95% CI [0.56, 0.95], $t(86) = 7.63$, $p < .001$, in the first 5 minutes of hand immersion. It differs

from Symonds et al., who measured a non-significant decrease of 0.14°C in mean skin temperature outside the area of interest.

Evaluation of the Effect of the Protocol

We checked whether the cooling intervention produced the same effects as in Symonds et al.'s (2012) studies (i.e., an increase in relative supraclavicular skin temperature). In Symonds et al.'s (2012) study, supraclavicular relative skin temperature significantly increased by 0.20°C, 95% CI [0.14, 0.26], estimated Cohen's d = 2.5, 95% CI [-0.08, 4.98], when the hand was immersed (cooling intervention).

We conducted a paired t-test to compare relative supraclavicular skin temperature during the cooling intervention (i.e., the measures taken at 35 and 45 minutes) and when there was no intervention (i.e., the measures taken at 0, 20, 30, 50 and 60 minutes). We found no significant variation in relative supraclavicular skin temperature, +0.08°C, 95% CI [-0.09, 0.24], $t(93) = 0.967$, $p = .34$, $d = 0.10$, 95% CI [-0.31, 0.50], in the cooling (versus the control) condition (see Figure 3A). We found a significant decrease of 0.20°C, 95% CI [-0.32, -0.08], $t(91) = -3.39$, $p = .001$, in absolute supraclavicular skin temperature in the cooling (versus the control) condition.

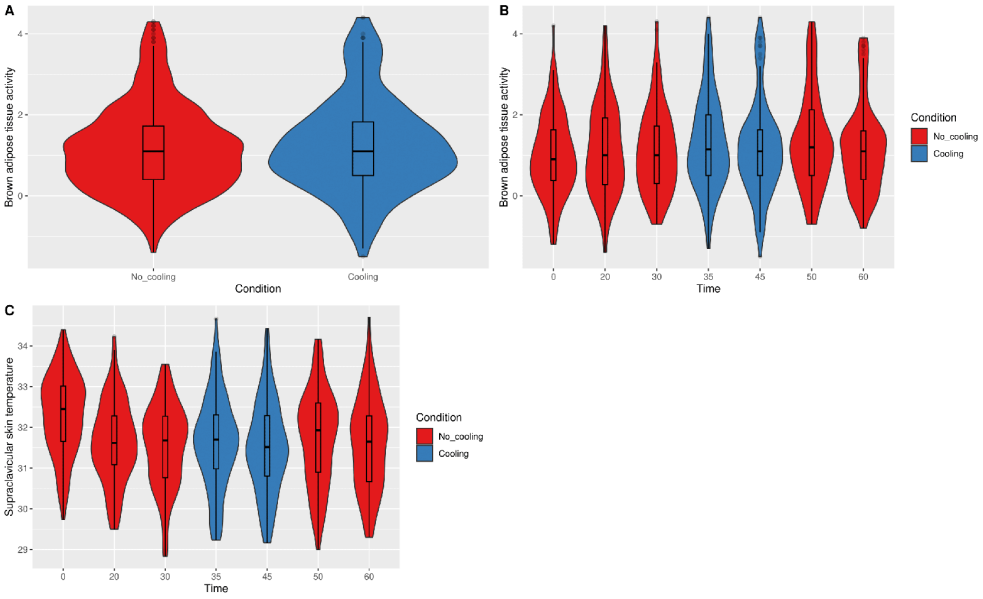
However, we suspected this difference in absolute supraclavicular skin temperature to be due to the measures of temperature taken at minute 0. Skin temperature at minute 0 should be higher because participants had just removed the clothing covering the measured areas. Indeed, when we excluded measures taken at 0 minutes from the analysis, the significant decrease in absolute supraclavicular skin temperature became smaller and non-significant, -0.07°C, 95% CI [-0.19, 0.06], $t(91) = -1.06$, $p = .29$, $d = 0.11$, 95% CI [-0.30, 0.52]), while the relative supraclavicular skin temperature also remained non-significant, +0.05°C, 95% CI [-0.12, 0.22], $t(93) = 0.59$, $p = .56$, $d = 0.06$, 95% CI [-0.34, 0.46].

To assess if and how relative and absolute supraclavicular skin temperature varied during the whole experiment, we checked the effect of time on relative supraclavicular skin temperature with a repeated-measures ANOVA (within-participants, across timepoints). Relative supraclavicular skin temperature was not significantly correlated to time, $F(6, 93) = 0.84$, $p = .54$ (see Figure 3B). However, absolute supraclavicular skin temperature significantly varied with time, $F(6, 93) = 6.46$, $p < .001$.

We again suspected that supraclavicular skin temperature measured at 0 minute (before the body becomes accustomed to the room temperature) was responsible for the significant effect of time on absolute supraclavicular skin temperature (see Figure 3.C). Pairwise comparisons showed that supraclavicular temperature significantly decreased between t = 0 minute and t = 20, 30, 35, 45, 50 or 60 minutes, $p < .001$ for the 6 pairwise comparisons). Only measures taken at 0 minutes were significantly different from measures taken at other timepoints (other p-values were close to 1). When measures taken at 0 minutes were removed from the ANOVA analyses, the effect of time on absolute supraclavicular skin temperature disappeared, $F(5, 93) = 0.52$, $p = .764$.

Figure 3

Mean Relative and Absolute Supraclavicular Skin Temperatures During the Experiment



Note. A: Relative supraclavicular skin temperature i.e., supraclavicular skin temperature minus sternal skin temperature (°C) (named Brown Adipose Tissue activity) in the two conditions (n = 94). B and C: Relative (n = 94) and absolute (n = 107) supraclavicular skin temperature (°C) across time (minutes).

Investigating Possible Explanations for Non-Replication

There are a host of reasonable alternative explanations for *why* the manipulation failed to achieve the desired effect. For instance, perhaps our sample differed on BMI, tobacco use, alcohol use, or that some of our participants shivered (amongst many other explanations). Given that we had data to investigate these alternative explanations, we were able to test them within our dataset. Using Null-Hypothesis Significance Testing would be an inefficient way to test such alternative hypotheses, as, given the sheer number of potential tests, it is nearly impossible not to data-dredge towards a significant effect.

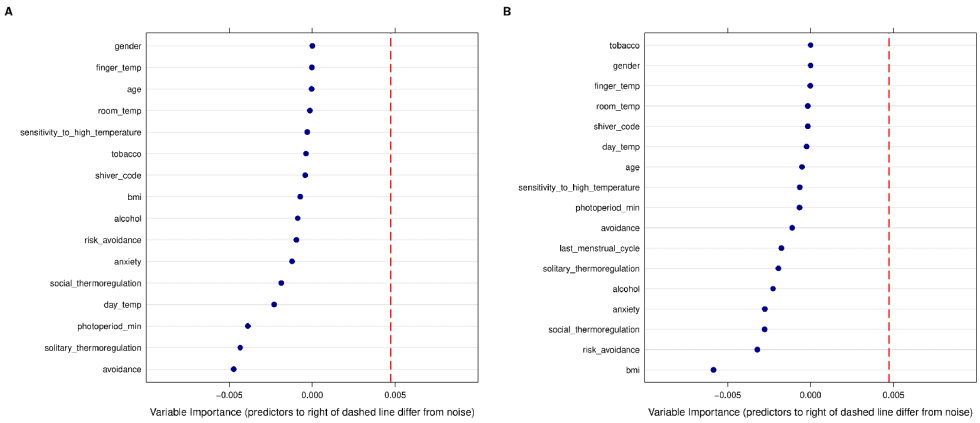
We therefore investigated if the variation of relative supraclavicular skin temperature during the cooling intervention could be explained by one or several variables of our study by running a set of conditional random forests with the difference between relative supraclavicular skin temperature at t = 30 min and at t = 45 min, as dependent variable. Our predictors were age, gender, average temperature and photoperiod of the day, individual scores of the two questionnaires of particular interest (scores in the two subscales of the ECR and the four subscales of the STRAQ-1), BMI, tobacco use, alcohol consumption, shivering, average finger temperature, and average room temperature. The

number of days since the last menstrual onset was added in a second model because it contained missing data for 28 participants.

Some of our predictors were known to be correlated with BAT activity (such as age, gender, body mass index, environmental temperature and photoperiod) and some were not (such as tobacco use and alcohol consumption). We ran each model four times (twice with 4 variables to sample and twice with 5). In the first (16 predictors, n = 90) and the second model (17 predictors, n = 62), none of the predictors differed from random noise, given that all variables were to the left of the red lines (see Figure 4). We investigated if the rank of a predictor in the full list of predictors was stable over the four (average of 100) trials and found a Spearman r = .98 for the first model and r = .97 for the second model. Given the stability of the model, the conditional random forest excluded a variety of possible explanations at once.

Figure 4

Results of the Conditional Random Forest Analyses



Note. The values on the graph should not be considered absolute or compared as an effect size to other data. Variable importance was averaged over four iterations of the program. A: results were based on n = 90 participants. Tested predictors were temperature of the day ("day_temp"), photoperiod of the day ("photoperiod_min"), attachment anxiety score of the ECR scale (see Methods) ("anxiety"), attachment avoidance score of the ECR scale ("avoidance"), gender ("gender"), sensitivity to high temperature, social thermoregulation desire, solitary thermoregulation and risk avoidance scores of the STRAQ-1 scale (see Methods) ("sensitivity_to_high_temperature"), age ("age"), body mass index ("bmi"), tobacco use ("tobacco"), alcohol consumption ("alcohol"), shivering during the experiment ("shiver_code"), index finger temperature ("finger_temp") and room temperature ("room_temp"). B: The number of days since the onset of the last menstrual cycle ("last_menstrual_onset") was added to the previous list of tested predictors. Results were based on n = 62 participants.

Discussion

We initially sought to investigate the link between attachment avoidance and anxiety and interindividual differences in BAT activity in response to a cooling stimulus with a protocol that could be applied in most social psychology research labs. When we suspected measurement error, we evaluated BAT activation in the supraclavicular area. With a much larger sample than Symonds et al.'s (2012) study (n = 7), we found no effect in relative, n = 94, Cohen's d = 0.10, 95% CI [-0.31, 0.50], or absolute supraclavicular skin temperature, n = 94, Cohen's d = 0.11, 95% CI [-0.30, 0.52], despite relying on a larger sample than Symonds et al. (2012). What could be the reason for this non-replication?

Could Differences Between Studies Explain the Differences in Results?

Conditions of the experiment in Symonds et al.'s (2012) study and in our replication were similar. The room temperature, our infrared image sampling, and the thermal sensitivity of the camera were approximately the same as in Symonds et al.'s (2012) study. We measured the supraclavicular area for 1 hour and used a 15-minute cooling intervention while Symonds et al. (2012) measured the supraclavicular area for 7 minutes and used a 5-minute cooling intervention. Therefore, our design could have captured variations of BAT thermogenesis longer before and longer after the cooling intervention than the original study, but Symonds et al. reported infrared measurements once every minute, thus with greater measurement accuracy.[4]

The index finger skin temperature decreased more during the protocol in our study than in Symonds et al.'s (2012) study. There are a few potential reasons for why this effect may have differed: A decrease of 0.75°C is probably not detectable in Symonds et al.'s 7 adult participants, while water temperature in our study was lower (around 15°C in our study vs. 19–20°C in the original study).

The most probable explanation however can be found in Symonds et al. (2012) estimated effect size, which was extremely large, effectively non-significant, and with a wide confidence interval, Cohen's d = 2.5, 95% CI [-0.08, 4.98]. For comparison, this effect is larger than the effect size for height differences between men and women (d = 1.86), that US liberals like Michelle Obama more than US conservatives do (d = 1.26), or that older people expect to retire sooner than younger people do (d = 1.48; Simmons, 2014). Crucially, we were able to exclude a host of other explanations due to our exploratory analysis techniques.

4) We also asked participants not to eat 30 minutes prior to the experiment while Symonds et al. (2012) asked for at least 1 hour between the experiment and the last meal. Asking not to eat for 1 hour before the experiment would have deterred students from coming in the early afternoon or morning and we would have ended up with fewer participants. Therefore, we chose 30 minutes but it is unlikely to have impacted the results as eating *increases* BAT heat production.

Excluding Alternative Explanations Through Exploratory Analyses

A priori, we found it reasonable that one can expect differences due to known predictors of BAT activity, such as age, gender, photoperiod, medication use, or last menstrual cycle onset (amongst others), as these have all been linked to BAT activity measured by PET/CT scans (Cohade et al., 2003b; Cypess et al., 2009; Symonds et al., 2015).

Our sample allowed us to test for some factors to some extent, with a rather restricted range for age (M_{age} = 20.5, SD_{age} = 4.1), reasonable variation in height (M_{height} = 165.4cm, SD_{height} = 7.2cm) and weight (M_{weight} = 58.8kg, SD_{weight} = 9.4kg), and reasonable variation in last menstrual cycle onset (M_{lmc} = 17.6 days, SD_{lmc} = 14.8 days). We were somewhat limited in the number of men (10) versus women (84), however women are thought to have more active BAT than men (Cypess et al., 2009).

Further, the number of participants that shivered was considerable (n = 55), thus it is reasonable to presume that we studied shivering—and not non-shivering thermogenesis—which would preclude us from observing BAT activity. However, not only did the variable "shivering" not predict BAT activity in our conditional random forests, the sample that did not shiver (n = 36) was still larger than the original (n = 7), and it was sufficiently large to detect the original effect size. Still, we advise future studies to rely on a higher temperature than 15°C for water, or to adapt water temperature to the thermoneutral zone of the participants (Van der Lans et al., 2014), to prevent shivering in most participants and efficiently assess non-shivering thermogenesis.

Concluding Remarks

Although we were hopeful to use infrared thermography to study the link between BAT activity and individual differences in attachment, we conclude that the protocol is not sufficiently reliable to detect individual differences in BAT activity. At this stage, we do not know why the protocol fails. Perhaps the cooling protocol does not lead to sufficient BAT activity, perhaps the measure of infrared thermography cannot assess BAT activity in the supraclavicular area, or perhaps BAT activity can only be detected through infrared thermography in a subset of the population (e.g., men). Whatever may be, for psychologists it is—for now—insufficiently usable to understand attachment security.

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Competing Interests: Hans IJzerman has a conflict of interest as he wrote a popular science book about social thermoregulation (Heartwarming; IJzerman, 2021) and is the director of a company to ensure accuracy in applied behavioral science (https://fr.linkedin.com/company/annecy-behavioral-science-lab). The other co-authors report no conflict of interest.

Author Contributions: *Nathan Vidal*—Idea, conceptualization | Data management (storage, curation, processing, etc.) | Data analysis | Funding to conduct the work | Data collection | Design planning | Project coordination, administration | Resource provision (materials, participants, etc.) | Research implementation (software, hardware, etc.) | Validation, reproduction, checking | Visualization (data presentation, figures, etc.) | Writing | Feedback, revisions. *Joseph T. Costello*—Idea, conceptualization | Design planning | Supervision, mentoring | Validation, reproduction, checking | Feedback, revisions. *Blandine Ribotta*—Data collection | Feedback, revisions. *Lilas Gurgand*—Data analysis | Research implementation (software, hardware, etc.). *Hans IJzerman*—Idea, conceptualization | Data analysis | Funding to conduct the work | Design planning | Project coordination, administration | Resource provision (materials, participants, etc.) | Research implementation (software, hardware, etc.) | Supervision, mentoring | Validation, reproduction, checking | Visualization (data presentation, figures, etc.) | Feedback, revisions.

Data Availability: Our data and analysis scripts are available in the Supplementary Materials (see Vidal & IJzerman, 2021).

Supplementary Materials

The supplements include the preregistration report of the project (which describes the prior literature, the original objectives, the method of the study, and power analysis), the anonymized dataset and the scripts used for data cleaning and processing and the final results, sensitivity analyses showing we had enough power to detect the effect, and detailed information regarding the outlier detection strategy. As auxiliary analyses, we include the originally pre-registered Bayesian Sequential analysis, with the results accessible online. Finally, we provide the full questionnaire administered to participants (see Vidal & IJzerman, 2021).

Index of Supplementary Materials

Vidal, N., & IJzerman, H. (2021). *Understanding the role of Brown Adipose Tissue in people's self-reported individual differences* [Data, scripts, materials, analyses]. OSF. https://osf.io/c9ys3/

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