# Brown Adipose Tissue Response to Cold Stimulation Is Reduced in Girls With Autoimmune Hypothyroidism

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**Objective:** The interaction between thyroid status and brown adipose tissue (BAT) activation is complex. We assessed the effect of autoimmune hypothyroidism (ATD) in female children on BAT activation, measured using infrared thermography.

**Design:** Twenty-six female participants (14 with ATD and 12 healthy controls) between 5 and 17 years of age attended a single study session. Thermal images were taken of the supraclavicular region before, and after, the introduction of a cool stimulus.

**Results:** Participants with ATD had lower resting (hypothyroid, 34.9 ± 0.7°C; control, 35.4 ± 0.5°C; $P = 0.03$) and stimulated (hypothyroid, 35.0 ± 0.6°C; control, 35.5 ± 0.5°C; $P = 0.04$) supraclavicular temperatures compared with controls, but there was no difference between groups in the temperature increase with stimulation. BAT activation, calculated as the relative temperature change comparing the supraclavicular temperature to a sternal reference region, was reduced in participants with ATD (hypothyroid, 0.1 ± 0.1°C; control, 0.2 ± 0.2°C; $P = 0.04$). Children with ATD were frequently biochemically euthyroid due to replacement therapy, but, despite this, increased relative supraclavicular temperature was closely associated with increased TSH ($r = 0.7$, $P = 0.01$) concentrations.

**Conclusions:** Girls with ATD had an attenuated thermogenic response to cold stimulation compared with healthy controls, but, contrary to expectation, those with suboptimal biochemical control (with higher TSH) showed increased BAT activation. This suggests that the underlying disease process may have a negative effect on BAT response, but high levels of TSH can mitigate, and even stimulate, BAT activity. In summary, thyroid status is a complex determinant of BAT activity in girls with ATD.



**Freeform/Key Words:** autoimmune, brown adipose tissue, cold-induced thermogenesis, hypothyroidism, infrared thermography, thyroid hormones

Abbreviations: ATD, autoimmune hypothyroidism; BAT, brown adipose tissue; BMI, body mass index; IQR, interquartile range; lnTSH, natural log–transformed TSH; PET, positron emission tomography; ROI, region of interest; SDS, SD score; SNS, sympathetic nervous system; TPO, thyroid peroxidase; $T_{Rel}$, relative supraclavicular temperature; $T_{SCV}$, supraclavicular temperature; UCP1, uncoupling protein 1; $\Delta T$, change in temperature.

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Since its rediscovery in adult humans in 2009 [1–4], interest in brown adipose tissue (BAT) has increased steadily. BAT has an integral role in adaptive thermogenesis due to its capacity to rapidly generate significant quantities of heat from fatty acids and glucose, facilitated by uncoupling protein 1 (UCP1) in the mitochondrial membrane, allowing the disassociation of ATP production from mitochondrial respiration [5–9]. As heat is eventually lost from the body, this represents a net loss of energy and has the potential to contribute to body weight management. Despite promising results in rodents demonstrating weight loss, improved metabolic profiles, and greater insulin sensitivity following BAT stimulation [10–15], translation to human studies has been slow [16]. Human studies have been limited by high ionizing radiation associated with positron emission tomography (PET)–CT, considered to be the gold standard for imaging BAT. Thermal imaging has been established as a valid alternative [17–20].

As a major regulator of energy expenditure, the thyroid axis can modulate the heat-generating capability of BAT [21, 22]. Thyroid hormones cross the blood–brain barrier [23] and act on the hypothalamus to increase sympathetic nervous system (SNS) activation [24]. Under SNS control, brown adipocytes express elevated iodothyronine deiodinase 2, which converts intracytoplasmic free T4, taken up from the systemic circulation, into its metabolically active form, T3 [25, 26], causing a localized intracellular hyperthyroid environment. Translocation and binding of T3 to its intranuclear thyroid hormone receptor-$\beta$ stimulates UCP1 transcription and translation [27], leading to heat generation. Reduced thyroid hormone concentrations may, therefore, affect BAT activity directly or by reducing SNS activation centrally. Despite this defined mechanistic pathway, *in vivo* studies in humans are limited and conflicting. In healthy volunteers, BAT activation is not associated with serum thyroid hormone concentrations [28, 29] but is with higher TSH concentrations [29]. BAT activity is increased in patients with hyperthyroidism and returns to normal after treatment [30]. In patients with hypothyroidism, BAT remains present, and indeed may become markedly hypertrophic in the absence of replacement therapy [31]. However, it is not clear whether treatment with T4 increases [32] or decreases [33] BAT activity, although a recent small study in healthy adults demonstrated a negative correlation between plasma free T4 and BAT volume [34].

Thyroid hormones are essential for brain and physical development in early life [35, 36] and continue to be critical through childhood when BAT activity is also increased [37, 38]. Adiposity patterns developed in this period can predict later obesity and metabolic health [39–43]. The influence of thyroid hormones on BAT activity, however, has not been examined in otherwise healthy children. Despite many patients achieving biochemical euthyroidaemia, physiological diurnal variation in TSH and thyroid hormone profiles is not achieved with hormone replacement therapy [44, 45].

Pediatric patients with hypothyroidism, for the most part, either have congenital hypothyroidism or autoimmune hypothyroidism, with the latter being more common in girls than boys. BAT is known to vary between sexes [46, 47], and so, to reduce heterogeneity, we compared girls with a diagnosis of autoimmune hypothyroidism (ATD) who, we hypothesized, would show reduced BAT activation in response to a cool stimulus compared with healthy age and sex-matched controls. We further hypothesized that those in the hypothyroid group who were relatively biochemically hypothyroid would have lower BAT activation than those who were relatively biochemically hyperthyroid.

## 1. Materials and Methods

### *A. Participants*

To determine the effect of ATD on the response of BAT to a cool stimulus, female children and adolescents (5 to 17 years of age) with a diagnosis of ATD [defined as a TSH level >10 mU/L and anti–thyroid peroxidase (TPO) antibodies levels of >60 IU/L at diagnosis] and no

associated major disease (n = 14) were compared with healthy, age-matched controls (n = 12). All participants successfully completed the study protocol.

Participants with ATD were recruited from the pediatric endocrinology clinic of Nottingham University Hospital NHS Trust (Nottingham, UK). Control participants were either healthy siblings of participants with ATD or were attending the pediatric ear, nose, and throat clinic at the Nottingham University Hospitals NHS Trust for unrelated simple surgical procedures (such as grommet insertion or tonsillectomy). The study was approved by the Nottingham-2 NHS Research Ethics Committee (13/EM/0102) and performed in accordance with the Declaration of Helsinki. Written, informed consent was provided prior to participation from the child or her parent or legal guardian as appropriate. If consent was provided by the parent or legal guardian, the child was invited to provide written assent.

### *B. Study Sessions*

Participants attended a 1-hour study session undertaken in the Academic Child Health Human Physiology laboratory in the Queen's Medical Centre campus of the University of Nottingham. Following informed consent, participants were required to wear a standard light cotton vest (0.06 Clo). A targeted medical history, current medications, and details of the last meal and physical activity during the preceding 24 hours were obtained from the child or her parent or caregiver. Basic anthropometric measurements of height and weight were made using a stadiometer (Seca, Hamburg, Germany) and class III digital weighing scales (Seca 899, Seca), respectively. The child or young person then sat upright, directly opposite a thermal imaging camera (FLIR B425, FLIR Systems, Danderyd, Sweden), which was level with her larynx [48] at a distance of ~1 m to ensure the shoulders remained within the frame while optimizing the proportion of the frame occupied by the region of interest (ROI). Skin temperature probes (M1024254, GE Healthcare, Fairfield, CT) were attached to the dorsum of the left hand and 2 cm below the midpoint of the left clavicle. Heart rate was recorded using pulse oximetry (Radical 7 pulse oximeter, Massimo, Irvine, CA) connected to the index finger of the left hand. The right hand was placed in an empty 5-L receptacle. Following an acclimatization period of at least 20 minutes, baseline images were acquired every 20 seconds for 1 minute followed by the introduction of cool water [median, 18.5°C; interquartile range (IQR), 18.0° to 18.9°C] into the receptacle to cover the right hand of the participant to the level of the distal forearm. A further 5 minutes of images were acquired at the same rate of three per minute. Children were free to stop at any time, but there were no reports of discomfort during the study imaging protocol and all sessions were completed.

### *C. Image Analysis*

Thermal images saved in FLIR's proprietary JPEG format were converted to an openly accessible 16-bit portable network graphics format [18] and the radiometric data were converted to temperature data within MATLAB (2017b, Mathworks, Natick, MA) using a script adapted from Tattersall [49], producing temperature data identical to those produced by FLIR software (ThermaCAM Researcher Pro 2.10; data not shown).

Data were analyzed within the MATLAB programming environment, as previously described [18]. In brief, the image was displayed on a graphical user interface allowing the user to identify five points representing the apices of the supraclavicular ROIs [18, 50]. The outline of the ROI was then calculated as (i) straight lines from the neck and shoulder apices to the central apex and (ii) the shoulder contour between the shoulder and neck apices. This method allowed the accurate, efficient, and reproducible identification of the ROIs (data not shown). Temperature points within the ROIs were then analyzed to identify the hottest 10%, corresponding to BAT [18]. The supraclavicular temperature ($T_{SCV}$) was defined as the median temperature of the hotspot (equivalent to the 95th percentile of the ROI). Additionally, the median temperature of a circular reference region on the sternum, directly inferior to the central apex, with a diameter of 10 pixels was calculated.

### *D. Hormone Analysis*

Plasma thyroid hormones and TSH were measured using Siemens ADVIA Centaur immunoassay systems with the following kits: FT3 assay no. 03154228 (119781) (reference ranges: 2 to 12 years, 5.1 to 7.4 pmol/L; 13 to 20 years, 4.7 to 7.2 pmol/L; intra-assay coefficient 2.6%), FT4 assay no. 06490106 (reference ranges: 2 to 12 years, 11.1 to 18.1 pmol/L; 13 to 20 years, 10.7 to 18.4 pmol/L; interassay coefficient 2.9%; intra-assay coefficient 2.7%), and TSH3-Ultra assay no. 06491080 (reference ranges: 2 to 12 years, 0.67 to 4.16 mU/L; 13 to 20 years, 0.48 to 4.17 mU/L; interassay coefficient 2.5%, intra-assay coefficient 2.3%). Anti-TPO antibodies were measured with the kit no. 10630887 (interassay coefficient 3.1%, intra-assay coefficient 4.1%).

### *E. Dietary and Physical Activity Analysis*

Nutritional data were analyzed for nutrient composition (energy, carbohydrate, fat, and protein content) using online nutritional software (Cronometer, Revelstoke, BC, Canada; https://cronometer.com/).

Participants were asked to rate their involvement in a number of activities in three recent time periods (before school, during school, and after school) as either "None," "A little," or "A lot" using a standardized questionnaire [51]. Activities were classified as physical (such as running) or sedentary (such as watching television), and the number of activities in each category was summed.

### *F. Statistical Analysis*

Anthropometric data [height, weight, and body mass index (BMI)] were analyzed both as absolute values and as age- and sex-normalized SD scores (SDSs). TSH results were natural log transformed (lnTSH) for further analysis to reduce data skew (Fig. 1A and 1B).

The relative supraclavicular temperature ($T_{Rel}$) was calculated by the difference between the supraclavicular and reference-region temperature ($T_{Rel} = T_{SCV} - T_{Ref}$). Resting temperatures were defined as the mean of the images in the minute prior to the introduction of the cool stimulus. A rolling average of 1-minute duration was calculated for the stimulation period, and the stimulated temperatures were defined as the maximum rolling average temperature during stimulation. Change in temperature ($\Delta T$) was the difference between resting and stimulated temperatures. Summary measures are presented as the mean of each group with the difference and 95% CI of the difference unless otherwise stated. Independent Student *t* tests were used to compare BAT activation, as the change in relative temperature in response to the cool stimulus ($\Delta T_{Rel}$), between the groups. Percentages were compared using a $\chi^2$ test. Adjusted *P* values were calculated using linear regression. Statistical analysis was carried out using *R: A Language and Environment for Statistical Computing*, version 3.4.3 (R Core Team).

## 2. Results

There was no significant difference between participants in the ATD group and the control group in terms of baseline characteristics, although there was a trend toward participants in the ATD group being shorter for their age, as previously reported [52], and having a higher BMI (Table 1). Four (29%) of the participants were newly diagnosed (<1 month) and/or had not yet started treatment, and 10 (71%) had an existing diagnosis. The median (IQR) length of diagnosis was 61.5 (4.4 to 133) weeks and the age at diagnosis was 12 (11.2 to 13.8) years, which reflects the typical age of onset of ATD [53]. All children in the study group were treated with, or due to start, levothyroxine [median (IQR) dose: 50 (50 to 100) μg, 1.3 (0.7 to 1.7) μg/kg], and most children did not have significantly abnormal TSH, free T3, or free T4 on the day of testing (Fig. 1C). The median (IQR) TSH was 4.1 mU/L (3.5 to 15.7; normal range, 0.3 to

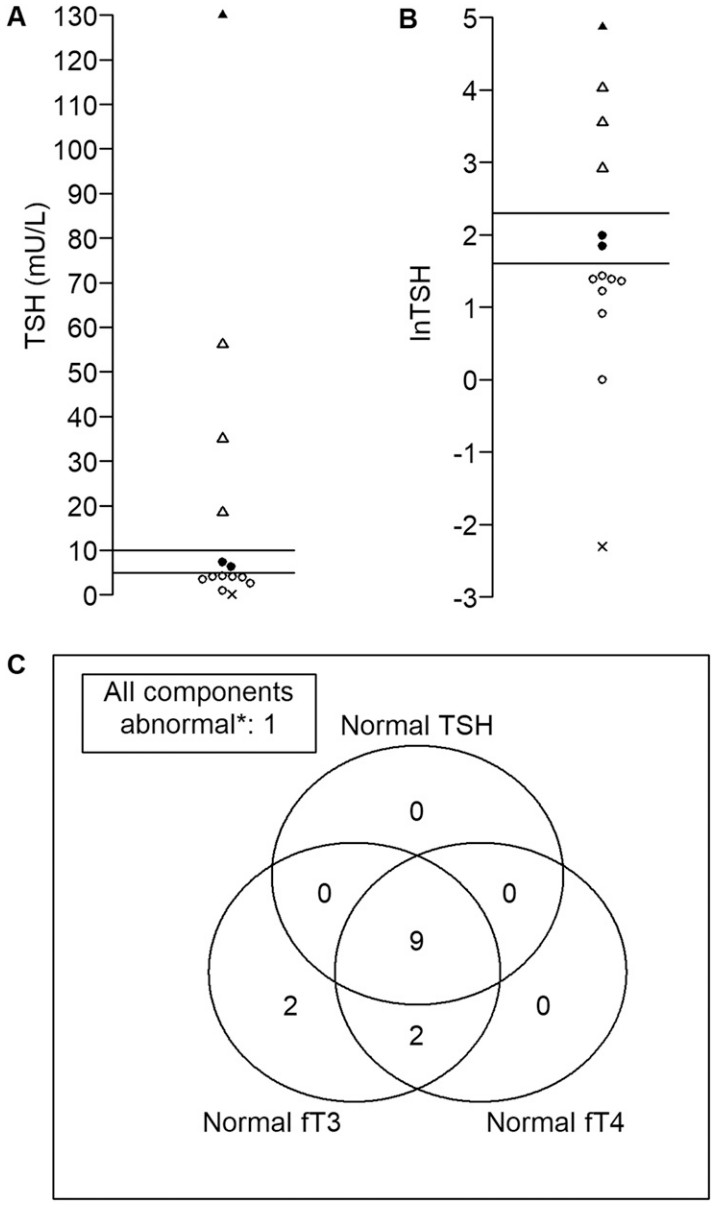


**Figure 1.** (A) TSH concentrations in participants with ATD. (B) Natural lnTSH concentrations in participants with ATD. ×, low; ○, normal; ●, borderline high; △, high; ▲, very high. (C) Venn diagram results of thyroid function tests of ATD group participants. In two cases where only a TSH was available (both in the normal range), the T3 and T4 were presumed to be normal; in three further cases where only a T3 result was not available, the T3 was presumed to be in the same category as the T4 result (one normal with a normal T4 and TSH, one normal with a normal T4 and high TSH, *one low with a low T4 and very high TSH); and two cases with borderline TSH concentration (5.6 to 10.0 mU/L) were classified as normal as both T3 and T4 levels were normal.

5.5), free T4 was 15.9 pmol/L (11.2 to 17.5; normal range, 10.0 to 19.8), and free T3 was 5.6 pmol/L (5.0 to 5.8; normal range, 3.5 to 6.7). Anti-TPO antibodies were raised in all patients at diagnosis (>1300 IU/mL in nine participants, 90.2 to 273.7 IU/mL in the other five participants).

There was no difference in the study conditions between the groups, including stimulation temperature, outside temperature, room temperature, time since the last meal, or in the number of physical activity sessions or sedentary sessions (Table 2). Energy and carbohydrate

**Table 1. Characteristics of Participants**

| | ATD | Control | Difference (95% CI) | *P* |
|---|---|---|---|---|
| n | 14 | 12 | | |
| Age, y | 13.20 | 11.48 | −1.72 (−3.88 to 0.44) | 0.11 |
| Height, cm | 152.1 | 150.6 | −1.4 (−14.8 to 12.0) | 0.83 |
| Height SDS | −0.33 | 0.71 | 1.03 (−0.01 to 2.08) | 0.05 |
| Weight, kg | 55.5 | 47.2 | −8.3 (−21.4 to 4.8) | 0.20 |
| Weight SDS | 0.94 | 0.83 | −0.11 (−1.09 to 0.86) | 0.82 |
| BMI, kg/m$^2$ | 23.7 | 20.0 | −3.6 (−7.3 to 0.0) | 0.05 |
| BMI SDS | 1.26 | 0.65 | −0.61 (−1.54 to 0.33) | 0.19 |
| Core temperature, °C | 36.8 | 36.8 | 0 (−0.4 to 0.4) | 0.98 |

*P* values are results of *t* tests.

content of the last meal was higher in the control group, but this did not reach statistical significance. Protein content was similar but fat content trended toward being significantly higher in the control group ($P < 0.1$; Table 2). Heart rate was similar between the groups and did not change significantly following stimulation (Table 3).

Absolute $T_{SCV}$ was lower in participants with ATD than in control participants in both the resting and stimulated state. There was less separation between the groups in resting and stimulated $T_{Rel}$ than $T_{SCV}$ and the difference was not significant. Age, BMI and BMI SDS, and weight and weight SDS were negatively associated with $T_{SCV}$, but not $T_{Rel}$, $\Delta T_{SCV}$, or $\Delta T_{Rel}$, and they were closely correlated with each other (Fig. 2). Neither height nor height SDS was associated with $T_{SCV}$ or $T_{Rel}$. Because BMI SDS correlated with $T_{SCV}$, trended toward being significantly different between the groups, and there is a known relationship between BMI and BAT activity [4, 54–57], a *P* value adjusted for BMI SDS was calculated using linear regression (Table 4).

The mean response to cold water stimulation (mean $\Delta T_{SCV}$) was 0.15°C (95% CI, 0.12° to 0.18°; $P < 0.001$). $\Delta T_{SCV}$ was similar between groups, but the relative response to cold water stimulation ($\Delta T_{Rel}$) was significantly reduced in participants with ATD compared with healthy controls (Table 4). Although the ATD group also had a lower resting and stimulated $T_{SCV}$, after controlling for BMI SDS, the difference was no longer statistically significant (resting $T_{SCV}$, $F_{2,23} = 6.5$, $P = 0.10$; stimulated $T_{SCV}$, $F_{2,23} = 7.1$, $P = 0.12$). There was no difference in the resting or stimulated $T_{Rel}$ between the groups, but there was a significant positive correlation in the ATD group with lnTSH (Fig. 3; resting $T_{Rel}$, $r = 0.7$, $P = 0.009$; stimulated $T_{Rel}$, $r = 0.7$, $P = 0.01$) and negative correlation with T4 (resting $T_{Rel}$, $r = -0.6$, $P = 0.04$; stimulated $T_{Rel}$, $r = -0.6$, $P = 0.04$), with a similar pattern seen at all time points

**Table 2. Study Conditions of Each Group**

| | ATD | Control | Difference (95% CI) | *P* |
|---|---|---|---|---|
| Room temperature, °C | 24.5 | 24.5 | −0.0 (−1.3 to 1.2) | 0.98 |
| Outdoor temperature, °C | 15.2 | 15.7 | 0.5 (−2.2 to 3.2) | 0.71 |
| Water (stimulus) temperature, °C | 18.3 | 18.5 | 0.2 (−0.5 to 0.9) | 0.56 |
| Time since last meal, h | 5.5 | 4.4 | −1.2 (−6.1 to 3.7) | 0.63 |
| Energy of last meal, kcal | 304 | 401 | 97 (−41 to 236) | 0.16 |
| Carbohydrate content of last meal, g | 41 | 52 | 11 (−7 to 29) | 0.22 |
| Fat content of last meal, g | 9 | 14 | 6 | 0.06[a] |
| Protein content of last meal, g | 9 | 11 | 1 | 0.62[a] |
| Number of physical activities | 10.4 | 10.8 | 0.5 (−4 to 4.9) | 0.83 |
| Number of sedentary activities | 5.4 | 5.7 | 0.3 (−1.7 to 2.3) | 0.75 |

Values are means, and *P* values are the results of *t* tests (except as noted).
[a]Value is the median, and *P* value is the result of a Mann–Whitney test.

**Table 3. Summary of Heart Rate by Group Before, and During, Cold Stimulation**

| | ATD | Control | Difference (95% CI) | *P* |
|---|---|---|---|---|
| Resting HR, bpm | 77 | 83 | 7 (−2 to 15) | 0.11 |
| Stimulated HR, bpm | 79 | 87 | 8 (−2 to 17) | 0.13 |
| Difference, bpm (95% CI) | 2 (−12 to 7) | 3 (−12 to 5) | | |
| *P* | 0.62 | 0.41 | | |

*P* values are results of *t* tests.
Abbreviations: bpm, beats per minute; HR, heart rate.

(data not shown). In the nine participants for whom data were available, there was no correlation between T3 and resting, stimulated, or change in $T_{SCV}$.

To further define the relationship between thyroid function and $T_{Rel}$, participants with ATD were categorized as either biochemically hypothyroid or biochemically euthyroid/hyperthyroid. A TSH cutoff of >10 mU/L was used to define the biochemically hypothyroid group. Compared with girls who were biochemically euthyroid or hyperthyroid (n = 10), biochemically hypothyroid girls (n = 4) had a significantly greater resting $T_{Rel}$ [high TSH group, 1.7°C; low TSH group, 2.5°C; difference, 0.8°C (95% CI, −1.6° to 0.0°); $P = 0.04$; Fig. 3C] and a trend toward a greater stimulated $T_{Rel}$ [high TSH group, 2.6°C; low TSH group, 1.8°C, difference 0.8°C (95% CI, 0.0° to 1.5°), $P = 0.05$]. There was no difference in change in temperature ($\Delta T_{Rel}$) or in $T_{SCV}$.

*Post hoc* analysis did not show any meaningful association between $T_{SCV}$ or $T_{Rel}$ and sedentary behavior, physical activity, or nutritional composition of the last meal (energy, carbohydrate, fat, and protein content), once removal of a single influential outlier in sedentary behavior was excluded and fat content was corrected for BMI SDS. Increased carbohydrate content in the preceding meal was associated with lower $\Delta T_{Rel}$, but visual inspection of the results failed to indicate a valid relationship.

## 3. Discussion

We demonstrate that female children and adolescents with ATD have a reduced thermogenic response to cold stimulation, as measured by the change in relative supraclavicular temperature ($\Delta T_{Rel}$), which has been shown to be closely correlated with BAT activity measured using fludeoxyglucose ($^{18}F$) PET-CT [17–20]. However, contrary to expectation, those children with ATD who were currently biochemically hypothyroid (defined as TSH >10 mU/L) demonstrated greater resting and stimulated $T_{Rel}$ than did their biochemically euthyroid counterparts, and a strong positive correlation was seen between TSH and BAT thermogenesis in the ATD group, indicated by resting and stimulated $T_{Rel}$. These apparently contradictory findings may help to explain the contrary and conflicting information previously published on this subject [28–30, 32–34]. Unfortunately, plasma TSH and thyroid hormone levels were not available for the control group, as this would have helped delineate further between the effect of the underlying disease and the effect of the TSH *per se*. However, in the subset of the group with ATD who did not have raised TSH, the relative supraclavicular temperature closely mirrors the distribution seen in a healthy population, indicating that the raised $T_{Rel}$ is a consequence of the biochemical pathology, rather than the underlying autoimmune process, and that, given appropriate replacement therapy, this difference can be moderated. That the change in $T_{Rel}$ in response to cold stimulation remains different between the study group and healthy controls suggests that a subtler difference in the thermogenic response of BAT remains, potentially independent of thyroid hormone levels.

BAT has been shown to interact with immunomodulation, potentially helping to identify self and prevent autoimmunity developing. Adipectomy of the intrascapular BAT in rats increases autoimmune processes [58, 59] and, in humans, lipodystrophy is associated with autoimmune diseases [60, 61]. This raises the possibility that the reduced BAT

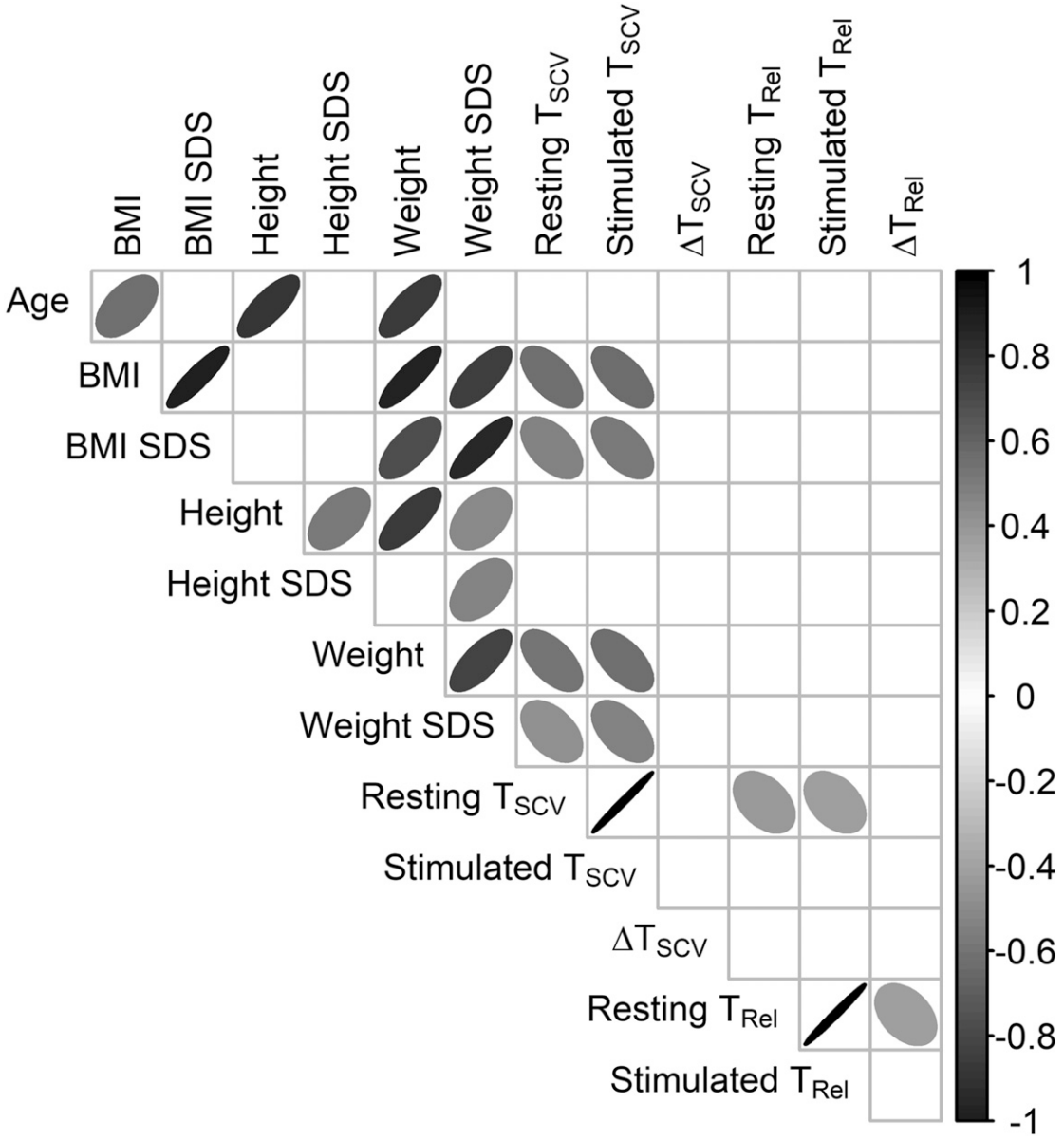


**Figure 2.** Correlogram of association between participant characteristics (age, BMI and BMI SDS, height and height SDS, and weight and weight SDS) and thermographic BAT measurements [resting, stimulated, and change ($\Delta$) in $T_{SCV}$ and resting, stimulated, and change in $T_{Rel}$]. Only significant ($P < 0.05$) associations are shown. Ellipse size and shading indicate the absolute value of the Pearson correlation coefficient ($r = 0$, white; $|r| = 1$, black); ellipse direction indicates the sign of the coefficient [down (\), negative; up (/), positive].

thermogenesis seen is, in fact, due to reduced BAT activity predisposing to autoimmune disease, rather than being a consequence of it, and that those with autoimmune disease may have less BAT prior to the onset of the later autoimmune pathology. However, there is no direct evidence for this in humans, and the animal models of near-total adipectomy represent a much greater insult than the difference that might be expected to occur between individuals naturally. The effects, if present, would therefore probably be subtle and likely to require larger studies to be detected.

Alternatively, there may be an autoimmune process reducing BAT thermogenesis directly. Anti-TPO antibodies have little cross-reactivity outside of the thyroid gland, except for breast tissue [62, 63] and possibly myeloperoxidase [64], so it is unlikely that they directly affect

**Table 4. Absolute and Relative Supraclavicular BAT Temperatures (°C) of Each Group**

| | ATD | Control | Difference (95% CI) | *P* | Difference (Adjusted) | *P* (Adjusted) |
|---|---|---|---|---|---|---|
| Resting $T_{SCV}$ | 34.9 | 35.4 | 0.5 (0.1 to 1.0) | 0.03 | 0.4 | 0.10 |
| Resting $T_{Rel}$ | 1.9 | 1.6 | −0.4 (−0.9 to 0.2) | 0.20 | −0.3 | 0.27 |
| Stimulated $T_{SCV}$ | 35.0 | 35.5 | 0.5 (0.0 to 0.9) | 0.04 | 0.3 | 0.13 |
| Stimulated $T_{Rel}$ | 2.1 | 1.8 | −0.3 (−0.8 to 0.3) | 0.33 | −0.2 | 0.42 |
| $\Delta T_{SCV}$ | 0.2 | 0.1 | −0.0 (−0.1 to 0.0) | 0.29 | −0.1 | 0.20 |
| $\Delta T_{Rel}$ | 0.1 | 0.2 | 0.1 (0.0 to 0.2) | 0.04 | 0.1 | 0.03 |

*P* values are results of *t* tests. Adjusted values are the result of a linear model adjusting for BMI SDS.

BAT. However, the presence of one autoimmune antibody increases the likelihood of the presence of another [65–69]. As only anti-TPO antibodies were measured in the study group, whether anti–TSH receptor antibodies or other antibodies were present is not known, and future research may want to consider testing a wider panel of autoantibodies to explore this further.

An alternative explanation may lie in the lack of physiological diurnal variation in thyroid hormones observed in patients on mono T4 replacement therapy. In the absence of thyroid disease, TSH levels are lower in the day, with a nadir in the late afternoon (between 3:00 and 5:00 PM) and 140% higher at night, peaking at 11:00 PM in children (and 2:00 to 4:00 AM in adults), along with a shorter 30-minute pulsatile cycle overlying the diurnal rhythm [44, 45]. There is a mirroring, but subtler, rise in both T4 and T3 levels, which increase ~7% and 15%, respectively, thought to be in response to TSH pulses, but also a rise in the morning independent of TSH, resulting in the highest levels in the morning and lowest in the late afternoon, along with a similar overlying short pulsatile cycle [44, 45]. Replacement therapy is typically (and for all participants in the study group) with levothyroxine (T4), taken once a day and usually in the morning. On replacement therapy, the TSH variation remains but the T3 and T4 levels rise to a maximum 3 hours after ingestion, in the middle of the day, before falling until the following dose the next morning [70], and there is no short pulsatile cycle overlying the diurnal cycle. BAT is known to exhibit a circadian rhythm [20] and, given the critical role of thyroid hormones in the cellular biochemistry of the brown adipocyte, the diurnal thyroid hormone variation may be important to the proper function of BAT and its absence may diminish the ability of BAT to respond maximally to a cold challenge. Additionally, levothyroxine monotherapy does not always fully resolve symptoms of hypothyroidism, and quality of life scores remain below those of healthy controls [71, 72], possibly due to a lack of euthyroid status at the tissue level [73–75]. A TSH in the normal range may be insufficient to drive the local conversion to T3 within the brown adipocyte, where T3 is not being produced by the thyroid gland (normally thought to account for 20% of circulating levels [76]).

The TSH receptor, previously thought only to be expressed by thyroid follicular cells, has now been shown to be expressed on many other extrathyroidal cells, including BAT [77–79], at least in small mammals. Furthermore, the insertion of an activating TSH receptor mutation to human orbital preadipocytes increases UCP1 gene expression [80]. Our results suggest a similar potential mechanism for activation of classical BAT depots *in vivo* in humans, but an indirect mechanism, either due to increased sensitivity to cold or by changes in plasma T3 and T4, should also be considered. Additionally, changes in thyroid hormone concentrations may affect BAT directly or via a central effect on the SNS, and the response to thyroid replacement therapy can vary between tissues [81], raising the possibility that the tissue-level thyroid status was not fully reflected by serum TSH concentrations, despite the buffering action of deiodinases.

Even in the presence of frank biochemical hypothyroidism, our results would suggest that TSH is able to exert a marked thermogenic influence on BAT, either directly or indirectly, raising the resting activity level while still allowing a similar response to the cold challenge,

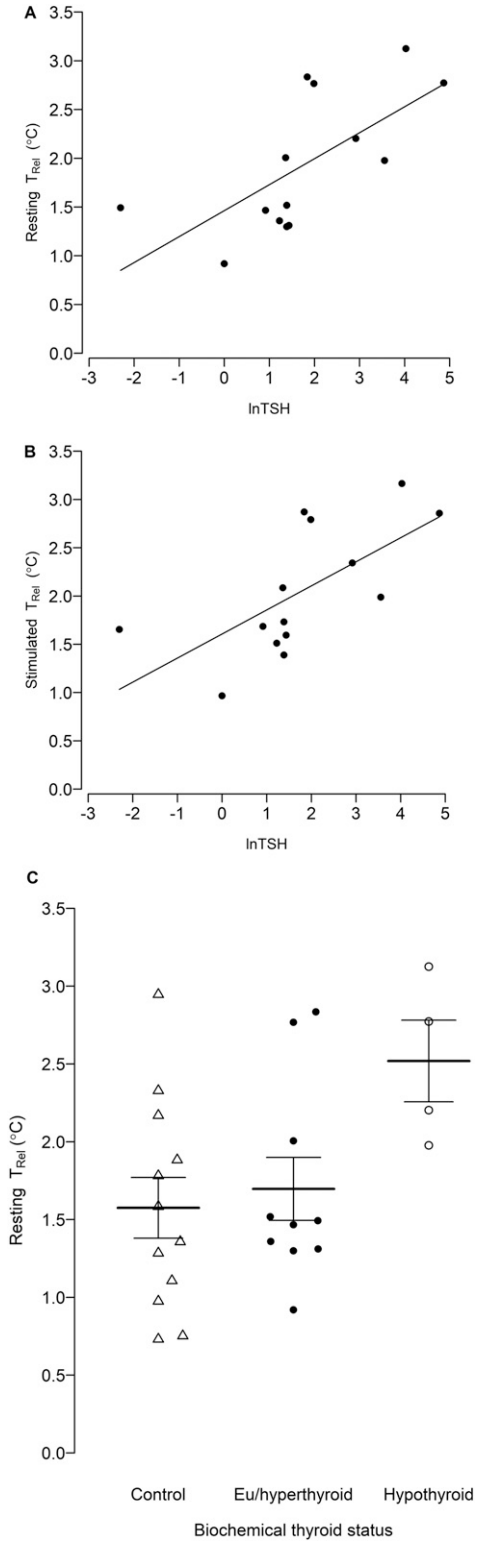


**Figure 3.** Association between lnTSH concentration of participants in the ATD group and (A) resting $T_{Rel}$ ($r$ = 0.7, $P$ = 0.01) and (B) stimulated $T_{Rel}$ ($r$ = 0.7, $P$ = 0.01). (C) Resting $T_{Rel}$ of girls in the control group (△) compared with those with ATD who were biochemically euthyroid/hyperthyroid (TSH ≤10 mU/L, ●) and those who were biochemically hypothyroid (TSH >10 mU/L, ○). Bars are mean ± SEM.

even in the near absence of thyroid hormones. Via upregulation of iodothyronine deiodinase 2, the relatively abundant T4 can be converted intracellularly to the biologically active T3, potentially maintaining intracellular concentrations. This points to the critical importance of the BAT for survival.

Clearly, the relationship between thyroid status and BAT activity is complex, but the close association between TSH with both resting and stimulated relative BAT temperatures further adds to the concept that thyroid status is an important determinant of BAT activity in humans [22].

To further elucidate this, future studies should consider longitudinal repeat imaging of participants with a new diagnosis of hypothyroidism to assess the changes in BAT activity as they return a biochemical euthyroid status following initiation of thyroid replacement therapy.

Although the age difference was not statistically significant, the group participants with ATD were slightly older than those in the control group. Given the age of the participants, it is likely that there is a degree of heterogeneity in their pubertal status and, in that context, the age difference between the groups may have had an influence on the results. In our study setting, assessment of pubertal stage was not considered appropriate, but the lack of data is a potential weakness and prevents any adjustment for pubertal status. However, although sex hormones appear to affect BAT activity in rodents [82] via FSH [83], it is not known whether human BAT expresses FSH receptors in childhood. Additionally, we did not show any meaningful relationship between dietary intake immediately preceding imaging, or recent physical activity levels, and thermogenic measurements. However, this was not the focus of this study nor was it designed to rigorously investigate these variables, so caution should be applied before drawing any firm conclusions from the lack of association here.

We show that BMI and BMI SDS, weight and weight SDS, and age are all negatively associated with $T_{SCV}$ (resting, maximal, and at all time points). There was a high degree of intercorrelation between these variables, and the size of our study means that it is not possible to distinguish between the relative effects of each variable. However, all of these variables potentially have a common factor in increasing the insulation between the superficial skin and BAT depot, either by a relative abundance of adipose tissue or by simply an increased size, suggesting this as a mechanism for reduced skin temperature rather than necessarily reduced BAT activity. Consistent with this assertion, when $T_{SCV}$ is compared with a sternal reference point, the association between these variables is no longer significant, emphasizing the suitability of $T_{Rel}$ to control for potential confounders. In contrast, height and height SDS do not correlate with any outcome measure of IR thermography and are also less likely to be associated with increased insulation between BAT and the skin surface, further reinforcing the proposed mechanism. Also, note that despite thermal imaging producing a two-dimensional image, as opposed to the three-dimensional image of PET-CT, there is a close relationship between each different measure of BAT function [18].

In summary, BAT activation was reduced in girls with a diagnosis of ATD compared with healthy controls. The unexpected finding of increased relative BAT temperature in subjects with suboptimal biochemical control now requires confirmation in larger studies.

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***Disclosure Summary:*** The authors have nothing to disclose.

***Data Availability:*** The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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