Sexual Dimorphism of Brown Adipose Tissue Function

Authors

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~~**Short Title**~~ ~~Sexual dimorphism of brown fat function in children~~

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Abbreviations and acronyms

BAT – Brown adipose tissue

SCR – Supraclavicular region

T_{SCR} – Temperature of the supraclavicular region

ΔT_{SCR} – Change in supraclavicular temperature in response to cold-exposure

UCP1 – Uncoupling protein one

PET-CT – Positron emission-computed tomography

T_{mp} – Temperature of the anterior mandibular protuberance

FSH – Follicle-stimulating hormone

IRT – Infrared thermography

BMI – Body mass index

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Abstract

Objective

To determine whether BAT activity in school-age children differs between the sexes and to explore the impact of dietary intake, sedentary ~~behaviour~~behavior, and '"picky/fussy eating."~~.~~

Study design

~~Thirty six c~~Children aged 8.5-11.8 years of age (n=36) underwent infrared thermography (IRT) to determine the temperature of the skin overlying the main superficial BAT depot in the supraclavicular region ~~(SCR)~~ prior to, and following, 5 minutes' mild cold-exposure (single-hand immersion in cool tap water c. $20\,^{\circ}C$). Relationships between SCR temperature (T_{SCR}) and parental reports of food consumption, eating behaviour and inactivity were explored.

Results

T_{SCR} was higher in boys (n=16) at baseline, and following cold-exposure. Boys displayed a greater thermogenic response to cold. Strong negative correlations were observed between T_{SCR} and BMI centile, and differences in T_{SCR} between girls and boys persisted after adjustment for BMI centile. A negative linear relationship was observed between protein and vegetable intake and T_{SCR} in girls only, but did not persist after adjustment for multiple comparisons. There was no difference in adjusted T_{SCR} between active or inactive children, or picky/non-picky eaters.

Conclusion

These findings indicate sexual dimorphism in BAT thermogenic activity and a sex-specific impact of diet. Future studies should aim to quantify the contribution of BAT to childhood energy expenditure, energy imbalance, and any role in the origins of childhood obesity.

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Introduction

Globally, over 124 million school-age children are now classified as overweight or obese[1] and, as a consequence, are at risk of significant cardio-metabolic disease in adulthood[2, 3]. The individual components of childhood energy balance are poorly understood, and the impact of brown adipose tissue (BAT) on energy expenditure, has yet to be quantified.

BAT persists outside the neonatal period into childhood and beyond. Increasing evidence suggests that this highly metabolic, thermogenic tissue contributes to energy expenditure by oxidising lipids and glucose[4-7]. This is achieved by the dissipation of chemical energy as heat through the action of mitochondrial uncoupling protein (UCP)1. Histological evidence of BAT has been demonstrated in children in the neck, upper thorax, mediastinum[8, 9], and in the adipose tissue surrounding the heart[10] and kidneys[8, 11] as well as subcutaneously[11]. It is estimated that just 63g of adult BAT could combust the energy equivalent of 4.1kg of white adipose tissue over the course of a year[12]. As the prevalence and activity of BAT are higher throughout infancy[9, 13] and childhood[14], the relative contribution of BAT to bio-energetic metabolism may be even greater in infants and children than in adulthood.

Although associations between dietary intake and sedentary behaviours, such as TV viewing, on white adipose tissue (ie, obesity) have been widely reported, their effects on BAT has not. Methods for measurement of supraclavicular skin temperature using thermal imaging present an ethically acceptable, non-invasive, repeatable method for BAT assessment in children and have been repeatedly shown to reflect measurements of BAT activity as assessed on positron emission tomography/computed tomography (PET-CT)[15-17].

Sexual dimorphism in both BAT mass and its activity has been comprehensively reviewed in adults[18, 19] and, despite similar distribution of depots throughout the body, pre-menopausal women appear to have more BAT than men[20, 21] implicating a role for hormonal regulation. Data in infants and children are less clear[11, 14, 22-27]. Gilsanz et al report a higher BAT mass, increased BAT activity and more rapid increase in BAT during the pubertal growth spurt in boys, as compared with girls[14]. Although glucose is utilised during BAT

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thermogenesis, the primary substrates for brown adipocytes at the cellular level are fatty acids. Functional assessment of BAT beyond that of glucose uptake is integral to understanding how BAT may be regulated and its contribution to energy expenditure in early life.

Given the potential importance of BAT activity in metabolic health, our aim was to determine whether the sexual dimorphism observed in adults exists between girls and boys at baseline and in response to a brief mild cold stimulus[28, 29] when IRT of supraclavicular skin temperature is utilised as a measure of BAT thermogenic activity. In addition, as diet and habitual physical activity influence metabolic health, we sought to identify whether parental reports of dietary intake, picky eating behaviour, and TV viewing were associated with differences in BAT activity in a sex-specific manner.

Methods

This study was approved by the University of Nottingham's School of Medicine Ethics Committee (E10012013 SCS ACH TREAT). Participants were recruited from two Nottingham City schools over a period of 3 weeks, and data collection was undertaken between April and June 2013. There were no specific eligibility criteria other than the requirement for parental written consent and receipt of children's verbal consent for anthropometry and imaging. Parents/guardians gave informed written consent for participation in this study, and all children gave additional verbal assent. Thirty-six children were imaged under free-living conditions within a primary school environment.

Infrared thermography of the neck and upper thorax was undertaken as previously described[28]. In brief, the reflective temperature, ambient room temperature and humidity were measured prior to each imaging session and entered into the thermal camera according to manufacturer's instructions. Supraclavicular (SCR) temperature (T_{SCR}) is also related to ambient temperature[28], and ambient room temperatures were stable during the study (Table 1). Each child sat 0.8m away from the thermal camera (FLIR B425FLIR Systems AB, Danderyd, Sweden) for 5 minutes wearing a sleeveless cotton vest. BAT in children responds to very mild cold stimulation[28-30] and participants next immersed their left hand in cool tap water (c. 20 ^{o}C) for 5 minutes, producing a similar decrease in hand temperature in girls and boys (Table 1). Images were

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taken at intervals throughout the study with comparable subject positioning. Thermograms obtained 1 minute prior to hand immersion, and 5 minutes post-hand immersion, were analysed to calculate T_{SCR}. Values were defined as baseline T_{SCR} and cold-exposed T_{SCR} respectively, and the thermogenic response to cold as ΔT_{SCR} (calculated as cold-exposed T_{SCR} minus baseline T_{SCR}). For the purposes of image analysis, the supraclavicular region was defined as the region of skin visible between the acromioclavicular joint, sternal notch and contour of the shoulder. Only thermograms where both left and right SCRs were completely exposed and unobstructed by clothing or hair, and where clavicles were positioned perpendicular to the camera lens, were regarded as acceptable for analysis.

The radiometric data obtained from thermograms were converted to temperature data within MATLAB (2017b, Mathworks, Natick, MA, USA) using a script adapted from Tattersall (2015). The thermal image was displayed on a graphical user interface, allowing identification of 5 points representing the apices of the SCR as defined above. T_{SCR} was calculated as the 95th percentile temperature value of the SCR of interest[15, 29, 31].

To determine baseline temperature of the skin not overlying BAT, a region away from the proximity of supraclavicular BAT visible in all children's thermograms was chosen as a comparator. This anatomical region, the centre of the anterior mandibular protuberance, was identified visually using FLIR's proprietary software, ResearchIR version 4 (FLIR Systems AB, Danderyd, Sweden). The mean temperature value of an 86-pixel area ellipse (T_{mp}) was calculated pre- and post-cold exposure (using the same thermograms as for the calculation of T_{SCR}). To confirm, and quantify, the degree of hand cooling, the temperature of the left hand prior to, and immediately after, cooling was calculated for each subject using the mean value of an individualised maximally-sized ellipse fitted to the anatomical area bound by the metacarpophalangeal joints and radial-ulnar styloid processes on the dorsum of the hand. Nine of the 36 children analysed for this study did not have thermograms of the left hand both prior to, and following, hand immersion. T_{mp} and hand temperature were calculated using the standard ellipse region-of-interest tool in ResearcherIR version 4 (FLIR Systems AB, Danderyd, Sweden).

The parents of each child completed questionnaires regarding their child's food intake, and were asked to rate their child's frequency of consumption of 151 foods (adapted from Wardle et al[32]) from 1 to 8 (1 'more than once a day' to 7 'less than once a month' and where 8 represented 'never' eaten). Responses were grouped into 7 standard food categories as described previously[33] and summed to create a score for each food group for each child. Each food group consisted of between 9 and 20 items.

Where consumption of a food was rated as 'never' in more than 25% of children, that food was excluded from grouped analysis[32]. Remaining foods were grouped into the following categories: i) carbohydrate; ii) dairy; iii) fruit; iv) protein; v) savoury; vi) sweet and vii) vegetable. Cronbach alpha was calculated for each group as a measure of the internal consistency of the scale. Reliability was acceptable (Cronbach's alpha > 0.7[34]) for all groups except dairy (0.54) for the food consumption questionnaires. 'Food consumption score', as a sum for each food group, was calculated for each child and compared with indices of supraclavicular temperature (i.e. baseline T_{SCR}, cold-exposed T_{SCR} and ΔT_{SCR}) derived from thermograms as described above.

To identify children with fussy eating behaviour, parents were asked if they would classify their child as a "picky eater". Parents were also asked to estimate their child's duration of TV exposure per day to the nearest half an hour. Those children reported as watching ≥2 hours a day were classed as sedentary, and those watching <2 hours TV a day as active.

To ensure responses were not contaminated by socially-desirable responding, parents also completed a short form of the Marlowe–Crowne Social Desirability Scale (MCSDS)[35]. Analysis of the MCSDS identified that food consumption scores were not contaminated by socially desirable responding. However, MCSDS scores were significantly correlated with the estimated number of hours of TV watched.

Height was measured to the nearest 0.1cm using a stadiometer (Leicester height measure; Child Growth Foundation, Sutton Coldfield, United Kingdom), and weight to the nearest 0.1kg using a standard, calibrated weighing scale. BMI centile was calculated to adjust for sex and age using the National Health

Service Choices BMI calculator[36]. Height, weight and BMI centile for the children in this study are summarised in Table 1.

Statistical analyses

Data were analysed using SPSS V.24 (IBM, Armonk, New York). Data are mean±standard error and normally distributed (as assessed by the Shapiro-Wilk normality test) unless stated otherwise. Comparisons between left and right T_{SCR} were performed using the paired t-test. Percentages were compared using the chi-squared test. Pearson's product-moment correlation coefficients were calculated to determine the correlation between T_{SCR} and food exposure scores and T_{SCR} and BMI centile. A 2-way mixed ANOVA was conducted to examine the effects of sex and cold-exposure on T_{SCR}. Post-hoc comparisons were undertaken using the t-test. ANCOVA was performed to determine the effect of sex and cold-exposure on T_{SCR} after controlling for BMI centile. Two-way ANCOVA was conducted to examine the effects of sex and sedentary behaviour, and sex and picky eating behaviour, on T_{SCR} after controlling for BMI centile.

A sample size of 30 (15 boys and 15 girls) was calculated to detect a 25% difference in the supraclavicular skin temperature (i.e. ΔT_{SCR}) in response to cold-exposure, based on previously published data from healthy children of comparative age[28] (i.e. right ΔT_{SCR} 0.28±0.064˚C (mean±SD), β=0.2, α=0.05).

P values of <0.05 (2 tailed) were considered statistically significant. To account for multiple testing during calculation of Pearson's correlation coefficient, the false discovery rate (FDR) was controlled using the corrected method of Benjamini and Yekutieli[37] with the desired FDR of 5%.

Results

The skin temperature overlying supraclavicular BAT was higher in boys at baseline, following cold-exposure, and there was a greater change in temperature response to cold-exposure which was significant in boys but not girls (Figure 1). Consistent with this, sex had a statistically significant effect on the T_{SCR} response to cold-exposure (right T_{SCR}: $F(1,34)=6.37$, p=0.016, partial $\eta^2=0.16$; left T_{SCR} $F(1,34)=6.41$, $P = 0.016$, partial $\eta^2=0.16$).

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~~Since~~ Because a significant negative linear relationship was observed between T_{SCR} and BMI centile (r= -0.71 to -0.43, p<0.01), even after correction for multiple comparisons (data not shown).

Adjusted cold-exposed T_{SCR} was statistically higher in boys than girls (right T_{SCR} mean difference: 0.41˚C (95% CI 0.14 to 0.68˚C); left T_{SCR} mean difference: 0.41˚C (95% CI 0.12 to 0.70˚C)) but the similar trend in baseline T_{SCR} did not maintain statistical significance (right T_{SCR}: $F(1,33)=3.48$, p=0.07, partial η^2=0.10; left T_{SCR}: $F(1,33)=3.46$, p=0.07, partial η^2=0.10). However, thermogenic response to cold-exposure (ΔT_{SCR}) remained greater in boys (right T_{SCR} mean difference: 0.14˚C (95% CI 0.02 to 0.26˚C); left ΔT_{SCR} mean difference: 0.14˚C (95% CI 0.02 to 0.25˚C)). The adjusted means for T_{SCR} at baseline, following cold-exposure and for ΔT_{SCR}, are summarised in Table 2.

A significant negative linear relationship between T_{SCR} and parental report of vegetable and protein consumption was observed in girls. However, these relationships were no longer statistically significant following adjustment for multiple testing in our study sample. There were no associations between T_{SCR} and food consumption in any category in boys (Table 3) and no difference in vegetable and protein consumption scores between girls and boys (Table 1).

Sixty-one percent of children were reported to watch ≥2 hours of television per day, but there was no significant difference between boys and girls (Table 1). There was no effect of sex and sedentary behaviour (classified as ≥2h television per day) on T_{SCR} after controlling for the effect of BMI centile and no statistically significant ~~two~~2-way interaction between sex and sedentary behaviour, while controlling for BMI centile (Table 4; available at www.jpeds.com).

Therefore, an analysis of the main effects of sedentary behaviour and sex was performed. There was no statistically significant difference in unweighted marginal adjusted means for the main effect of sedentary behaviour on left, or right, T_{SCR} at baseline, or following cold-exposure (Baseline: right T_{SCR} mean difference: -0.25˚C (95% CI -0.54 to 0.05˚C), p=0.09; left T_{SCR} mean difference: -0.12˚C (95% CI -0.43 to 0.19˚C), p=0.44; Following cold-exposure: right T_{SCR} mean difference: -0.18˚C (95% CI -0.46 to 0.10˚C), p=0.19; left T_{SCR} mean difference: -0.10˚C (95% CI -0.39 to 0.22˚C), p=0.58).

Fifty-three percent of children were reported to be picky eaters by their parents. There was no significant difference between the eating behaviour of girls or boys (Table 1). A ~~two~~2-way ANCOVA was conducted to examine the effects of sex and picky eating behaviour on T_{SCR}, while adjusting for the effect of BMI centile and there was no statistically significant two-way interaction between sex and picky eating behaviour while controlling for BMI centile (Table 5; available at www.jpeds.com).

Therefore, an analysis of the main effects of picky eating behaviour and sex was performed. The main effect of eating behaviour showed no statistically significant difference in unweighted marginal adjusted means of the left or right T_{SCR} at baseline or following cold-exposure (Baseline: right T_{SCR} mean difference: 0.04˚C (95% CI -0.26 to 0.34˚C), p=0.79; left T_{SCR} mean difference: -0.07˚C (95% CI -0.38 to 0.23˚C), p=0.63; Following cold-exposure: right T_{SCR} mean difference: 0.01˚C (95% CI -0.27 to 0.29˚C), p=0.93; left T_{SCR} mean difference: -0.01˚C (95% CI -0.31 to 0.29˚C), p=0.96).

Discussion

We found a sexual dimorphism in the skin temperature overlying supraclavicular BAT in young children which may be related to dietary intake. Boys exhibit higher BAT activity under resting and cold-stimulated conditions than girls of a similar age and BMI, indicating that boys may have not only higher basal BAT activity under free-living conditions but also BAT that is more responsive to cold stimulation.

Previous investigations of the impact of sex on BAT in healthy young children are currently limited, with the majority of studies confined to clinical imaging in the context of paediatric malignancy. Furthermore, as the purpose of such scanning is to determine disease recurrence/progression, these scans are undertaken under environmental conditions designed to minimise BAT uptake of radio-labelled FDG (i.e. warm room temperatures) and do not measure BAT activity in response to physiological BAT substrates. Despite these limitations a small number of studies support our findings. Gilsanz et al found no sex-specific differences in the numbers defined as BAT positive on PET-CT (57% girls vs 60% boys), however multiple regression analysis identified an independent effect of sex on BAT volume after adjustment for pubertal stage, BMI, season and exposure to previous glucocorticoid treatment[14]. Chalfant et al identified BAT in 65% of boys

and only 50% of girls (using PET-CT following successfully treated malignancy) and, although not statistically significant, their study was not specifically powered to explore sex-differences[27].

Similarly, Drubach et al reported no difference in overall BAT activity in boys and girls on PET-CT. However, when stratified by age group, median BAT activity was 2.4 times higher in boys than girls aged between 11 and 13 years[26]. Deng et al identified sex as a significant covariate of the MRI properties of BAT in 28 young healthy children aged 9-15 years, reporting increased markers of tissue perfusion (ADC - apparent tissue diffusion coefficient and D* - perfusion coefficient) on diffusion-weighted imaging, and a higher T2* using Dixon MRI[25] findings, suggestive of higher BAT activity in boys in this age group. Even fewer studies have directly examined childhood BAT histologically, but those that have report either no sex-specific differences[11], or did not look for them[10, 11].

Differences between pre-pubertal girls and boys which are contrary to our findings have been reported[30]. However, these differences were principally identified when "area" of the SCR ($Area_{SCR}$) was used as a thermal index. This measure, defined as the number of pixels over an arbitrary threshold of 35.5˚C, has yet to be validated against other assessments of BAT, such as PET-CT in the same way as T_{SCR}.

The children taking part in this study were between the ages of 8.5 and 11.8 years. The UK median age of pubertal onset is 11.3[38] and 11.6 years for girls and boys respectively, meaning our study population may be a heterogeneous group in terms of pubertal stage. It is, therefore, likely that they exhibited a wide range of gonadotrophin and sex steroid hormones which could explain, in part, variation in basal and cold-stimulated T_{SCR}. Given our study was undertaken in the primary school environment, physical assessment of pubertal stage was not considered appropriate. Future study under laboratory conditions utilising self-reported pubertal stage in combination with non-invasive measures of sex steroid hormones (e.g. saliva or urinary assays) presents a practical and ethical solution to the assessment of T_{SCR} in relation to pubertal status for future studies.

Our univariate analyses suggest BAT thermogenesis may also be influenced by dietary intake in a sex-specific manner. In girls, higher vegetable and protein consumption was associated with a lower T_{SCR} (lower

BAT activity). However, the sample size of our study was limited and it was underpowered to detect significant relationships between T_{SCR} and food consumption after correction for multiple testing. Parental reports of dietary intake may be subject to bias. Hours of screen time are commonly used as a simple assessment of sedentary behaviour[39] but may falsely classify some children as active, and can also be associated with dietary intake[40]. The use of accelerometry in future studies could provide a comprehensive assessment of movement duration and intensity.

The insulative effect of overlying subcutaneous adipose tissue (SCAT) has not been quantified. Gatidis et al suggest SCAT significantly impacts T_{SCR} however, their measurements were defined as the minimal distance between the vascular compartment and the skin of the neck[41] (rather than the white fat layer that lies immediately below the dermis). Biopsy of adipose tissue surrounding the supraclavicular vasculature clearly demonstrates UCP1[42] indicative of capacity for thermogenesis. Therefore, the assumption that this layer is purely insulative is too simplistic. As the white fat layer immediately below the skin in the posterior triangle of the skin is minimal in healthy subjects in our studies we have been unable to obtain reproducible and robust measures with either ultrasound or magnetic resonance imaging. We have however identified that the relationship between T_{SCR} and BMI persists in a non-obese group following adjustment for SCAT in close proximity to the SCR (dorsal aspect at T1) (unpublished data). The dynamic response of T_{SCR} correlates well with FDG uptake in BAT on PET-CT in a non-obese adult cohort with proven FDG detected BAT[15], the children in this study were similarly non-obese, and all had a BMI centile of $\leq 85^{th}$ centile. These findings suggest that the insulative effect of SCAT is minimal, in a non-obese population at least.

Given the contribution of BAT to energy balance and metabolic health, further exploration of these findings will provide a better understanding of how BAT may be activated, and recruited, in early life. This helps to delineate how BAT dysfunction may predispose to childhood obesity.

Acknowledgements

Figures and Tables

Table 1. Demographic and anthropometric variables in healthy girls and boys aged 8-11 years (n=36). *Contaminated by socially desirable responding; [#]Food consumption score: higher numerical value = lower consumption

Figure 1. Change in supraclavicular skin temperature following 5 minutes single hand immersion in cool water. A) representative anterior thermal images of a girl and boy (matched for age and BMI centile) at baseline and following cold exposure and B) left sided change in supraclavicular temperature following 5 minutes cold exposure in healthy girls and boys aged 8-11 years (n=36). Post-hoc pair-wise comparisons - Right T_{SCR}: $F(1,15)=11.38$, $p=0.004$, partial $\eta^2 =0.43$; left T_{SCR}: $F(1,15)=15.16$, $p=0.007$, partial $\eta^2=0.50$, *P <0.05.

Table 2 Unadjusted means (SEM) for left and right supraclavicular skin temperatures (T_{SCR}) and means (SEM) adjusted for BMI percentile[¥] in healthy girls and boys prior to, and following, 5 minutes cold-exposure (n=36). [¥]ANCOVA, BMI percentile; *P < 0.05, boys compared ~~to~~ with girls; [$]P < 0.05, left compared ~~to~~ with right; [#]P < 0.05, baseline compared ~~to~~ with cold-exposed.

Table 3. Relationship between supraclavicular skin temperature (T_{SCR}) measured by infrared thermography and food consumption score in healthy volunteer boys and girls aged 8-11 years (n=36). Pearson'~~s~~ r, 95% confidence intervals, R2 and p value. *P < 0.05; bold type indicates statistical significance following adjustment for multiple comparisons.

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Table 4 online. Means (SEM) for left and right supraclavicular skin temperature (T_{SCR}), unadjusted and adjusted for BMI percentile, in sedentary and active healthy children at baseline, and following 5 minutes cold-exposure (n=36). (ANCOVA: baseline left T_{SCR} F=0.25, partial η^2=0.008, p= 0.62, right T_{SCR} F= 1.66, partial η^2= 0.051, p=0.28; cold stimulated left T_{SCR} F=0.04, partial η^2=0.001, p=0.84 cold stimulated right T_{SCR} F=0.06, partial η^2=0.002, p=0.81).

Table 5 online. Means (SEM) for the left and right supraclavicular skin temperature, unadjusted and adjusted for BMI percentile, in children classified as picky or non-picky eaters at baseline and following 5 minutes cold-exposure (n=36).(ANCOVA: baseline left T_{SCR} F=0.11, partial η^2=0.004, p= 0.77, right T_{SCR} F=0.01, partial η^2=0.0004, p=0.92; cold stimulated left T_{SCR} F=0.12, partial η^2=0.004, p=0.73, cold stimulated right T_{SCR} F=0.13, partial η^2=0.004, p=0.72).

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Table 1

Table 1.

Variable	Value		P value
	Boys (n=16)	Girls(n=20)	
Age (decimal years)	9.9 (0.24)	8.5 (0.23)	0.62
Height (cm)	138.8 (2.0)	139.0 (1.7)	0.94
Weight (kg)	31.81 (1.33)	34.89 (1.72)	0.12
BMI percentile	48.36 (8.00)	59.57 (6.63)	0.28
Ambient temperature (°C)	22.01 (0.20)	22.10 (0.14)	0.71
T_{SCR} pre cold exposure (°C)	36.02 (-0.14)	35.63 (-0.12)	0.03
T_{SCR} post cold exposure (°C)	36.17 (-0.14)	35.62 (-0.12)	0.004
T_{mp} pre cold exposure (°C)	32.96 (-0.25)	33.13 (-0.31)	0.65
T_{mp} post cold exposure (°C)	33.36 (-0.22)	33.09 (-0.27)	0.46
Hand temperature drop (°C, n=28) (F: n=16; M: n=12)	-8.85 (-0.30)	-8.00 (-0.57)	0.20
TV watching (hrs)	2.12 (0.26)	2.20 (0.24)	0.84
Picky eater (yes)	8/16	11/20	0.76
Food consumption score (Likert-item group sum)[#]			
Protein	61.69 (2.67)	63.60 (2.13)	0.57
Vegetable	84.94 (4.66)	86.45 (2.85)	0.77

[#] food consumption score: higher value = lower consumption

T_{SCR}: supraclavicular skin temperature; T_{mp}: mandibular prominence temperature

Table 2

Table 2.

		Supraclavicular skin temperature (°C)					
		Baseline		Cold exposure		ΔT_{SCR}	
		Boys	Girls	Boys	Girls	Boys	Girls
Unadjusted means (SEM)	Left T_{SCR}	35.88[*$] (0.13)	35.47[$] (0.13)	36.06[*$#] (0.14)	35.51[$] (0.13)	0.18[*$] (0.05)	0.03[$] (0.04)
	Right T_{SCR}	36.02[*] (0.14)	35.63 (0.12)	36.17[*#] (0.14)	35.62 (0.12)	0.15[*] (0.04)	-0.002 (0.04)
Adjusted means (SEM)[¥]	Left T_{SCR}	35.81 (0.11)	35.53 (0.10)	35.98 (0.11)	35.57 (0.10)	0.17 (0.04)	0.04 (0.04)
	Right T_{SCR}	35.95 (0.11)	35.68 (0.10)	36.10 (0.10)	35.68 (0.09)	0.14 (0.04)	0.02 (0.04)

Table 3

Table 3.

			Boys						Girls					
			Left			Right			Left			Right		
			Pre T$_{SCR}$	Post T$_{SCR}$	ΔT$_{SCR}$	Pre T$_{SCR}$	Post T$_{SCR}$	ΔT$_{SCR}$	Pre T$_{SCR}$	Post T$_{SCR}$	ΔT$_{SCR}$	Pre T$_{SCR}$	Post T$_{SCR}$	ΔT$_{SCR}$
Correlation to consumption score (Pearson's product-moment coefficient)		**Carbohydrate**	-0.05	-0.02	0.10	-0.09	-0.09	0.00	0.21	0.16	-0.19	0.20	0.20	0.01
		Dairy	-0.23	-0.25	-0.09	-0.29	-0.31	-0.04	0.11	0.13	0.06	0.09	0.16	0.19
		Fruit	-0.33	-0.25	0.21	-0.25	-0.26	-0.01	-0.07	-0.07	0.00	-0.09	-0.08	0.03
		Protein	-0.02	-0.08	-0.19	0.07	-0.03	-0.31	**0.48***	**0.48***	0.00	**0.47***	**0.51***	0.09
		Savoury	-0.19	-0.26	-0.23	-0.20	-0.27	-0.24	0.32	0.28	-0.12	0.39	0.39	-0.01
		Sweet	-0.39	-0.42	-0.12	-0.43	-0.46	-0.07	-0.19	-0.15	0.13	-0.12	-0.08	0.14
		Vegetable	0.11	0.13	0.06	-0.01	0.03	0.14	**0.53***	0.46*	-0.23	**0.52***	**0.48***	-0.13

Table 4 online only

Table 4.

			Left T_{SCR} (˚C)				Right T_{SCR} (˚C)			
			Boys		Girls		Boys		Girls	
			Sedentary (n=10)	Active (n=6)	Sedentary (n=12)	Active (n=8)	Sedentary (n=10)	Active (n=6)	Sedentary (n=12)	Active (n=8)
Baseline T_{SCR}		**Mean**	35.82	35.97	35.50	35.44	35.88	36.26	35.64	35.60
		SE	0.20	0.11	0.20	0.13	0.20	0.11	0.17	0.18
		Mean adjusted	35.73	35.93	35.51	35.56	35.79	36.22	35.66	35.72
		SE	0.14	0.18	0.13	0.16	0.13	0.17	0.12	0.15
Cold exposed T_{SCR}		**Mean**	36.06	36.06	35.51	35.51	36.11	36.27	35.61	35.65
		SE	0.22	0.09	0.20	0.13	0.22	0.08	0.17	0.16
		Mean adjusted	35.96	36.01	35.53	35.64	36.01	36.23	35.63	35.78
		SE	0.14	0.18	0.12	0.15	0.13	0.16	0.11	0.14

Table 5 online only

Table 5.

		Left T_{SCR} (˚C)				Right T_{SCR} (˚C)			
		Boys		Girls		Boys		Girls	
		Picky (n=8)	Not picky (n=8)	Picky (n=11)	Not picky (n=9)	Picky (n=8)	Not picky (n=8)	Picky (n=11)	Not picky (n=9)
Baseline T_{SCR}	**Mean**	35.96	35.79	35.49	35.46	36.07	35.98	35.57	35.69
	SE	0.19	0.19	0.16	0.20	0.22	0.18	0.17	0.17
	Mean adjusted	35.82	35.80	35.59	35.46	35.93	35.98	35.67	35.70
	SE	0.16	0.16	0.14	0.15	0.16	0.16	0.13	0.15
Cold exposed T_{SCR}	**Mean**	36.12	36.00	35.49	35.53	36.27	36.07	35.55	35.71
	SE	0.20	0.19	0.16	0.21	0.22	0.17	0.16	0.18
	Mean adjusted	35.96	36.00	35.60	35.54	36.12	36.08	35.66	35.72
	SE	0.16	0.15	0.13	0.14	0.15	0.14	0.12	0.14
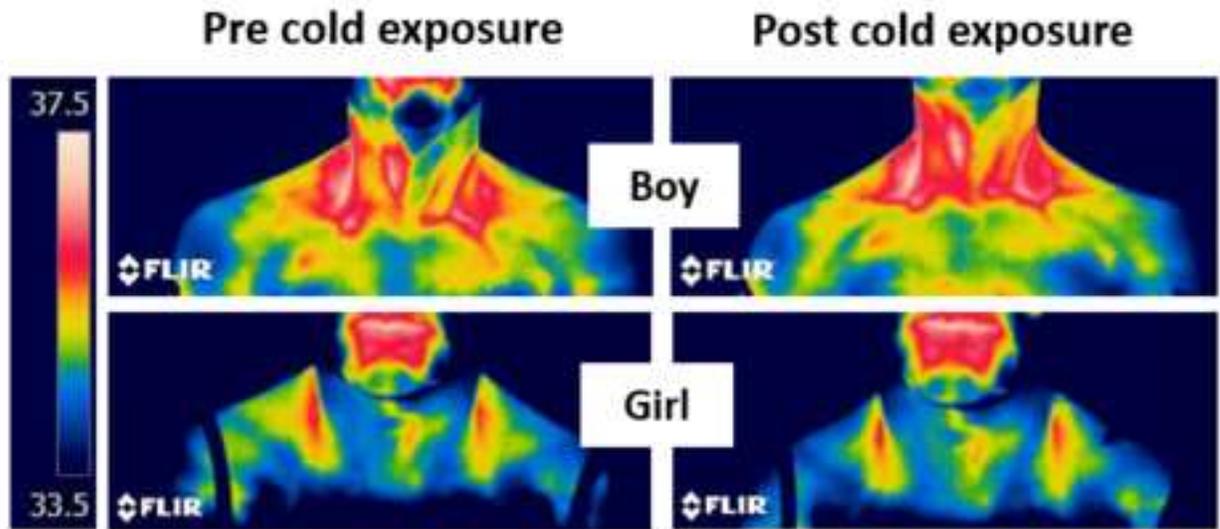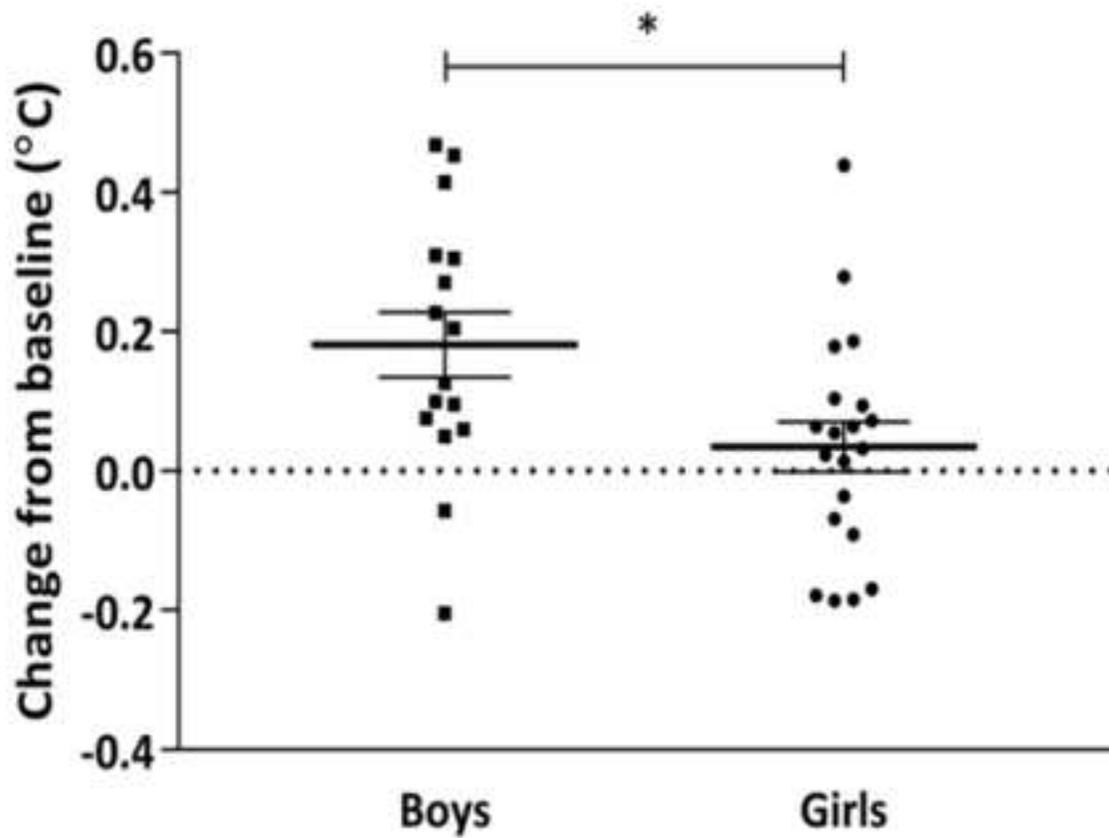
Statistical analysis: online only content.

Two-way mixed ANOVA

A two-way ANOVA was conducted to examine the effects of sex and cold-exposure on T_{SCR}. Residual analysis was performed to test for the assumptions of the two-way ANOVA. Outliers were assessed by inspection of a box plot, normality was assessed using Shapiro-Wilk's normality test for each cell design and homogeneity of variances was assessed by Levene's test. There were no outliers, residuals were normally distributed (P>0.05) and there was homogeneity of variances (p>0.05).

ANCOVA

ANCOVA was performed to determine the effect of sex and cold-exposure on T_{SCR} after controlling for BMI centile. There was a linear relationship between BMI centile and T_{SCR} in girls and boys both at baseline and after cold-exposure, as assessed by visual inspection of a scatterplot. There were homogeneity of regression slopes as the interaction terms were not statistically significant. Standardised residuals for the sex ,and for the overall model, were normally distributed, as assessed by Shapiro-Wilk's test (p > 0.05). There was homoscedasticity and homogeneity of variances, as assessed by visual inspection of a scatter plot and Levene's test of homogeneity of variance (p>0.05), respectively. There were no outliers in the data, as shown by no cases with standardised residual greater than ±3 standard deviations. All assumptions for the multivariate analyses were met, except for the assumption for homogeneity of

regression slopes – this was violated for the left and right ΔT_{SCR}.
Therefore, two-way ANCOVA was only undertaken for values of baseline
and cold-exposed T_{SCR}.

Two-way ANCOVA

A two-way ANCOVA was conducted to examine the effects of sex and
inactivity, and sex and eating behaviour on T_{SCR} (after controlling for BMI
centile). There was a linear relationship between BMI centile and TSCR for
each group, as assessed by visual inspection of scatter plots. There was
homogeneity of regression slopes as determined by comparison between
the two-way ANCOVA models with, and without, interaction terms. There
was homoscedasticity within groups, as assessed by visual inspection of
the studentised residuals plotted against the predicted values for each
group, and there was homogeneity of variances, as assessed by Levene's
test of homogeneity of variance (p>0.05). There were no outliers in the
data, as shown by no cases with studentised residuals greater than ± 3
standard deviations. There were no leverage or influential points, as
assessed by leverage values and Cook's distance, respectively.
Studentised residuals were normally distributed, as assessed by Shapiro-
Wilk's test (p>0.05).

The assumption for homogeneity of regression slopes was violated for the
left and right ΔT_{SCR}. Therefore, two-way ANCOVA was only undertaken for
values of baseline and cold-exposed T_{SCR}.

As summed food consumption scores were obtained using an 8-level item scale, and food groups consisted of more than 9 items, this was treated as continuous interval data. Pearson's product-moment correlation coefficients (r) were calculated to determine the size of linear relationships between two variables (e.g. food consumption score and baseline T_{SCR}).

STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,4
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5-7
Objectives	3	State specific objectives, including any prespecified hypotheses	6-7
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	na
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-10
Bias	9	Describe any efforts to address potential sources of bias	8, 13-15
Study size	10	Explain how the study size was arrived at	10
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10 + Appendix
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10 appendix
		(*b*) Describe any methods used to examine subgroups and interactions	10 appendix
		(*c*) Explain how missing data were addressed	na
		(*d*) If applicable, explain how loss to follow-up was addressed	na
		(*e*) Describe any sensitivity analyses	na
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	na
		(c) Consider use of a flow diagram	na
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	na
		(c) Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	Report numbers of outcome events or summary measures over time	10-11 + Tables 1-

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-12
		(*b*) Report category boundaries when continuous variables were categorized	10-11 + Tables 1-3 and Fig 1
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	na
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	na

Discussion

Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13,15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15

Other information

| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 2 |

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.