BROWN ADIPOSE TISSUE, PHYSICAL ACTIVITY AND BODY COMPOSITION IN CHILDREN

INVESTIGATING COLD STIMULATED SUPRACLAVICULAR SKIN TEMPERATURE AS A MEASURE OF BROWN ADIPOSE TISSUE ACTIVITY AND ITS ASSOCIATION WITH PHYSICAL ACTIVITY AND BODY COMPOSITION IN 8-10 YEAR OLD BOYS

By SARAH KANJI BSc.

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TITLE: Investigating supraclavicular skin temperature as a measure of brown adipose tissue in children and its relation to physical activity and body composition in 8-10 year old boys

AUTHOR: Sarah Kanji, BSc

SUPERVISOR: Katherine Morrison

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ABSTRACT

BACKGROUND: Brown adipose tissue (BAT) is a thermoregulatory tissue that may have a positive influence on metabolic health by improving glucose homeostasis, reducing adiposity and increasing energy expenditure. It is enriched with uncoupling protein-1 (UCP1) and therefore produces heat by uncoupling oxidative phosphorylation from ATP production. It has long been known that infants are born with BAT, however, only recently has BAT been reported in children and adults. In humans, BAT is predominantly located in the supraclavicular (SCV) region, however there are smaller depots in the peri-renal and mediastinal areas. BAT has primarily been studied in humans using Positron Emission Tomography/Computed Tomography (PET/CT). Such studies have demonstrated that children appear to have a higher prevalence of BAT than adults, however this methodology is not suitable for widespread research in healthy children. Therefore non-invasive methods that accurately measure BAT are required. The factors influencing BAT activity are of interest as this tissue may act as a desirable therapeutic target for metabolic diseases such as obesity and diabetes.

PURPOSE: The purpose of this thesis project was two fold; Part 1 involved the examination of SCV skin temperature as a measure of BAT activity in children and the objective of Part 2 was to determine if SCV skin temperature had a relationship to body composition and physical activity in children. METHODS: We recruited 33 pre-pubertal boys (ages 8-10) to this cross-sectional study. The study included 3 visits, in which we measured lean mass and fat mass with dual-energy X-ray absorptiometry, resting energy expenditure (REE) before and after a 30 minute 12ºC cold exposure with indirect calorimetry, objective physical activity with an

accelerometer and SCV temperature (measured every 2.5 minutes during a 30 minute, 12ºC cold exposure) with an Infrared Thermal Camera. Lean mass and Fat mass were quantified as lean mass index (LMI) and fat mass index (FMI). Physical activity was quantified as total accelerometer counts per minute (CPM) and minutes of moderate to vigorous physical activity (MVPA) per day. For Part 1, we assessed the precision of IR-thermal imaging of SCV skin temperature by examining the reproducibility of eight skin temperature outcomes over two trials. Furthermore, we assessed the accuracy of these eight skin temperature outcomes by investigating their association with energy expenditure. For Part 2 we evaluated the relationship of FMI, LMI and physical activity (MVPA) with SCV skin temperature in a multivariate analysis. RESULTS: Following these analyses, post-cooling skin temperature had the highest reproducibility of the eight skin temperature outcomes (intraclass correlation coefficient of 0.95, $p<0.001$) and was also significantly correlated with energy expenditure (Pearson correlation=0.392 $p=0.032$). Therefore, we used this outcome measure when examining the relationship between SCV skin temperature, body composition and physical activity. Fat mass index (FMI) was inversely related to post-cooling SCV skin temperature ($\beta= -0.125$, $p<0.001$). Minutes of moderate to vigorous physical activity and lean mass index (LMI) were not related to post-cooling SCV skin temperature. CONCLUSION: This study determined that post-cooling SCV skin temperature may be useful for detecting BAT in children and it is inversely related to adiposity.

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CHAPTER I: LITERATURE REVIEW

1.1 Introduction

Obesity is a growing problem in Canada; Since 2003, the proportion of Canadians who were obese has increased by 17.5% (Navaneelan & Janz, 2014) and currently, it is estimated that 31% of Canadian children are overweight or obese (Statistics Canada, 2014). As childhood obesity increases the risk of obesity in adulthood and can contribute to early development of type 2 diabetes and heart disease (Public Health Agency of Canada, 2009), it is a pressing issue facing Canadians today. Studying the biological and lifestyle factors that influence metabolism is crucial for determining the factors contributing to obesity and for identifying possible interventions to reduce obesity. One potential avenue that has recently received attention is brown adipose tissue (BAT). Unlike white adipose tissue (WAT), which is well known for its role in energy storage and hormone production, BAT has a role in thermoregulation (Carpentier et al., 2010). Its function in thermoregulation directly impacts metabolism because BAT utilizes energy sources such as triglycerides and glucose to produce heat (Carpentier et al,. 2010). It has been suggested that 250 grams of BAT in humans can contribute 20% to whole body basal energy consumption (Carey & Kingwell, 2013). Although it has long been known that human infants are born with BAT, researchers believed that these stores disappeared into adulthood (Cypess et al,. 2009). However, recent evidence suggests that adult humans do carry BAT deposits (Cypess et al,. 2009, Lee et al., 2010., Yoneshiro et al,. 2011, Virtanen et al., 2009, van Marken Lichtenbelt et al., 2009). The natural function of BAT as a "fat burning furnace" is appealing to researchers who study metabolism as active BAT may improve glucose and lipid homeostasis by acting as a sink for these energy sources (Carey & Kingwell, 2013). This makes BAT an attractive

therapeutic target for metabolic disorders such as obesity and diabetes. In order to begin understanding BAT in the paediatric population, it is important to find a safe, feasible and non-invasive way to measure BAT. This will allow for a greater understanding of the role of BAT in humans throughout the lifespan and the factors that influence it.

1.2 Physiology of BAT

The physiological activity of BAT results in the production of heat for the body through a mechanism known as non shivering thermogenesis (Carpentier et al, 2010). It does so through exploiting the oxidative phosphorylation pathway. When energy sources such as carbohydrates and triglycerides are oxidized within mitochondria to create ATP, heat is produced as a byproduct. In BAT, the specialized cells allow for oxidation of energy sources without producing ATP (Betz et al,. 2011). Without ATP production, energy sources can continue oxidation without the usual negative feedback displayed when ATP is in excess. This is made possible through the action of a channel protein known as uncoupling protein 1 (UCP1) (Betz et al, 2011). As its name implies, its function is to separate oxidative phosphorylation from ATP production (Betz et al, 2011). This allows BAT to act as a furnace that burns energy sources to produce heat. BAT is distinct from WAT in several ways: BAT and WAT do not share the same cellular origin; rather brown adipocytes share their origin with myocytes (Seale et al, 2008, Cereijo et al 2014). Additionally BAT is abundant in mitochondria, is highly vascularized and is richly innervated with sympathetic afferents unlike WAT, which does not contain many mitochondria, is largely avascular and has little innervation from the nervous system (Carpentier, 2010). In adults, BAT is mainly found on both sides of the SCV/neck area and smaller deposits can be found around the

perirenal and mediastinal regions (Cypess et al,. 2009, Lee et al., 2010., Yoneshiro et al,. 2011, Virtanen et al., 2009, van Marken Lichtenbelt et al., 2009).

BAT is under the control of the sympathetic nervous system (Ponrartana et al., 2013). Binding of norepinepherine to β_3 adrenergic receptors stimulates the thermogenic activity of BAT (Ponrartana et al., 2013). Cold exposure, which triggers a response from the sympathetic nervous system, is known to activate BAT (Ponrartana et al., 2013). In accordance with this, human studies that visualized BAT using PET/CT found an increase in BAT activity after cold exposure and in the winter months (Yoneshiro et al., 2011, Ouellet et al., 2010).

In addition to the BAT described above, there is a type of tissue termed beige or brite (brown-in-white) (Wu & Spiegelman, 2013). Although these cells demonstrate UCP1 expression, thermogenesis, and are activated through β_3 adrenergic signalling like classical brown adipocytes, they are not of myogenic origin like classical BAT (Wu & Spiegelman, 2013). Although it is debated on where beige/brite adipocytes originate, it has been suggested that they develop during the maturation of white adipocytes exposed to various stimuli including norepinepherine (Broeders et a, 2014) or through transdifferentiation of WAT into BAT (Wu & Spiegelman, 2013). Studies of BAT in the SCV/neck region in humans have reported that this BAT deposit is actually a mosaic of WAT, classical BAT and beige/brite adipose tissue (Nedergaard & Cannon, 2013).

1.3 Studying Brown Adipose Tissue in Humans

To study BAT in humans, non-invasive technology is desirable. Currently, there are three types of technology that have been incorporated in human BAT studies and have been used to

detect the presence, activity, volume and mass of BAT. The majority of studies showing BAT in adult humans have used PET/CT imaging with the glucose analogue ^{18}F-FDG (Cypess et al,. 2009, Lee et al., 2010., Yoneshiro et al,. 2011, Virtanen et al., 2009, van Marken Lichtenbelt et al., 2009, Ouellet et al., 2010). This method is reliable for detecting BAT in humans (Zingaretti et al., 2009) and is considered the gold standard. Although ^{18}F-FDG PET/CT is a reliable and non-invasive technique for determining many characteristics of BAT, the exposure to radioactivity has limited the use of this technique for BAT research to certain populations. For example, many of the large-scale studies included patients with cancer (Lee et al, 2010), and studies conducted in healthy individuals have been limited by small sample size (Virtanen et al, 2009). Therefore a method that is applicable to a wider range of populations, specifically healthy children, is desired. More recently, Magnetic Resonance Imaging (MRI) has been used to study BAT morphology (Hu et al,. 2013) and activity (van Rooijen et al,. 2013). These studies have indicated that MRI can be used to distinguish BAT and WAT and allow for a dynamic picture of BAT activity following BAT stimulation (Hu et al,. 2013, van Rooijen et al,. 2013). However MRI imaging is expensive, which may limit the sample size in such studies. Additionally, MRI imaging requires subjects to lay still for a relatively long period of time in a small space, which could be difficult for children to do.

As BAT activity is believed to increase energy expenditure, resting energy expenditure (REE) measurements before and after cold exposure have also been performed in animal (Crane et al, 2014) and human studies (Boon et al, 2014) as an additional measure of BAT activity. In humans, indirect calorimetry is often used to measure REE (Boon et al, 2014, Chondronikola et al, 2014). Boon et al (2014) reported a significantly higher resting energy expenditure after cold

exposure in young healthy men who demonstrated BAT on PET/CT (BAT+) compared to individuals who did not have BAT (1493 ±52 vs. 1732 ±91). Similarly, Chondronikola et al (2014) reported a 15% increase in REE in BAT+ groups compared to no increase in BAT- groups.

Detecting skin temperature in the SCV region as a measure of BAT activity is another non-invasive means of detecting BAT activity and has recently been employed with promising results (Symonds et al,. 2012, Jang et al, 2014, Boon et al, 2014). Both skin temperature probes (iButtons) and IR thermal imaging have been used to measure skin temperature changes. Since it is believed the skin around an area of BAT would be significantly warmer when BAT is activated compared to non-BAT regions, it may be used as a measure of BAT activity/function (Symonds et al,. 2012). IR thermal imaging has been reported as a reliable method of measuring UCP1-mediated thermogenesis in rodents (Crane et al, 2014), however its usefulness in humans has not been fully established. Early studies employing IR thermography published by Symonds et al (2012) found a significant increase in temperature of about 0.2 degrees in the SCV/cervical region following a cold exposure in individuals aged 3-58 years old. They also compared the anatomical position, where an increase in temperature was observed, to age and sex matched PET/CT scans and found them to be comparable (Symonds et al,. 2012). However this study was limited for several reasons. First, the cold exposure was not consistent in all subjects. Secondly the study only had a population of 26 individuals across a large age span. Furthermore, the PET/CT images that were used to compare regions of interest were from different individuals who had likely been exposed to different conditions prior to imaging. Additionally it is not clear if the increase in temperature within the region of interest was due to BAT activity or another

physiological process. Subsequent studies have compared skin temperature changes in the SCV region using both iButtons (a small, wireless temperature sensing probe that can record temperature in real time) (Boon et, al, 2014) and IR-thermal imaging (Jang et al, 2014) to ^{18}F FDG PET/CT imaging. Boon et al (2014) recruited 24 lean healthy caucasian men and looked at the relationship between changes in SCV skin temperature measured with iButtons and BAT volume and activity measured by ^{18}FDG PET/CT. SCV skin temperature following a 2 hour individualized cooling protocol was found to have a positive correlation with ^{18}F FDG uptake ($R^2 = 0.52$ $p<0.001$) and BAT volume ($R^2=0.20$ $p=0.030$). Although the change in SCV skin temperature was significantly higher after cold exposure, this change did not correlate with BAT activity or volume. A similar study by Jang et al (2014) studied the relationship between SCV skin temperature measured by IR thermal imaging and BAT activity on ^{18}FDG PET/CT. They reported that the difference in temperature between the SCV region and the chest region (considered to be non-BAT) increased after cold exposure in those demonstrating BAT on PET/CT, but did not change in BAT-negative individuals. However they attributed this to a greater decrease in chest temperature observed in BAT positive individuals compared to BAT negative individuals; the physiological reason for this difference is unclear.

The results of these studies provide rational for continuing research into SCV skin temperature measurements to detect BAT activity, as it is a simple, non-invasive technique that can be used in a wide range of populations and could allow for monitoring of BAT dynamics over time.

1.4 Factors associated with brown adipose tissue

Several epidemiological studies in adults and children have indicated an association between certain factors and the presence, activity, mass and volume of BAT. These factors include weight, BMI, adiposity, age, sex, blood glucose homeostasis, musculature and outdoor temperature.

1.4.1 Adiposity and BMI

The metabolic role displayed by BAT suggests that it could be related to an individual's adiposity. Several studies have examined this relationship by using ^{18}F FDG/PET-CT to detect BAT and found an inverse relationship between adiposity and BAT. For example Chalfant et al (2012) studied the changes in subcutaneous and visceral adiposity in the abdominal region of pediatric cancer patients over an average interval of 4.7 months. They found that increases in subcutaneous adiposity were 3 times greater in individuals that did not demonstrate BAT activity and increases in visceral adiposity were 6 times greater in individuals that did not demonstrate BAT activity compared to those who did show BAT activity. Unlike the study conducted by Chalfant et al, Saito et al (2009) observed BAT in men who had a cold stimulus prior to imaging by ^{18}F FDG PET/CT. They found FDG uptake into the SCV regions on PET/CT scans was negatively correlated to total fat ($r= -0.56$) and visceral fat ($r= -0.68$) (Saito et al, 2009). These studies provide some evidence that BAT activity is related to adiposity, but it is not clear whether the BAT activity is influencing adiposity or vice versa. However, one study provided some evidence that BAT could be protective against age related accumulation of adipose tissue (Yoneshiro et al, 2011). This study looked at percent body fat, subcutaneous fat and visceral fat in individuals who demonstrated BAT and individuals who did not (based on PET/CT standard

uptake values). They did not observe differences between the BAT positive and BAT negative groups in younger individuals (ages 20-39) however in the group aged 40-49 there were significant differences for percent body fat (28.2% for BAT- vs. 19.9% in BAT+) and abdominal fat (261.5 cm^2 in BAT- negative vs. 128.5 cm^2 in BAT+). One limitation of this study is its cross-sectional design, which limits the ability to determine the cause-effect relationship between age, adiposity and BAT. Not all studies have shown a relationship between BAT activity and adiposity. Two pediatric studies could find no significant relationship. However one of the studies only looked at adiposity in the neck and buttocks areas (Gilsanz et al,. 2011, Gilsanz et al., 2012).

Several studies have demonstrated an inverse relationship between BMI (as a measure of adiposity) and BAT (van Marken Lichtenbalt et al 2009, Lee et al, 2010, Saito et al, 2009, Ouellet et al, 2010, Cypess et al, 2009). These retrospective studies reported on the activity of BAT (i.e SUV uptake) or probability of detecting BAT on a PET/CT scan (non-cold stimulated). For example van Marken Lichtenbalt et al (2009) and Saito et al (2009) and Ouellet et al (2009) all reported an inverse correlation between cold-stimulated BAT activity and BMI ($r= -0.6$, $r= -0.67$ and $\beta=-7.6$ respectively). When considering the probability of detecting BAT on a PET/CT (without any stimulation), Lee et al (2010) found the odds of demonstrating BAT on PET/CT were 2.8 times higher in individuals who had a BMI of less than 22.5. Similarly, In a review of over 8000 PET/CT scans, Ouellet et al (2010) found the probability of detecting BAT to be higher with lower BMI. Cypess et al (2009) did not find BMI to be an independent predictor of BAT presence in younger individuals, but this association became significant with increasing age.

Although there is less data for the paediatric population, one pediatric study (68 children aged 6-11) using IR thermography to detect BAT activity following cold exposure found a significant inverse association between BMI percentile and change in temperature in the SCV region after cold exposure (r=0.384) (Robinson et al, 2014). The cold exposure for this study involved cold-water bath (20°C) in which participants submerged their left hand for approximately 5 minutes. All together, this data suggest that BAT may have a positive influence on adiposity.

1.4.2 Musculature

Classical brown adipocytes are more closely related to myocytes than white adipocytes (Seale et al,. 2008). Evidence has accumulated showing the differentiation of brown adipocytes from myogenic 5 expressing myoblasts indicating that myocytes and brown adipocytes share the same origin (Seale et al, 2008). Additionally, brown adipocytes have a transcriptional and proteomic profile similar to that of myocytes and brown adipocytes contain muscle micro RNA, which is not seen in white adipocytes (Carpentier et al,. 2009, Betz et al,. 2011). This relationship inspired interest in determining if there is a relationship between brown adipocytes and musculature. Three pediatric studies have reported the volume of certain muscle groups was related with BAT. For example, in a study looking at pediatric patients aged 6-20 (during a cancer follow up), adolescents demonstrating BAT on ^{18}F FDG PET/CT had a 50% greater neck musculature and 33% greater gluteal musculature compared to individuals who did not show BAT on PET/CT (Gilsanz et al, 2012). However this correlation was only shown with specific muscle areas and it is unclear why they focused on these areas. In a separate study of similarly

aged individuals, the same authors found that trunk muscle volume was significantly greater in BAT positive individuals compared to BAT negative, additionally trunk muscle volume was significantly correlated with BAT volume (r= 0.64 in girls and r= 0.52 in boys) (Gilsanz et al,. 2011). Chalfant et al (2012) found abdominal muscle volume to be significantly greater in those who demonstrated BAT on PET/CT: 322 cm^3 in BAT positive vs. 251 cm^3 in BAT negative.[20] It is still unclear as to why this relationship exists, it could be the factors regulating muscle also influence BAT, or factors released from muscle could stimulate BAT. Further research is necessary to establish the relationship between muscle and BAT.

1.4.3 Age and pubertal status

Several studies using ^{18}F FDG PET/CT to detect BAT in adults have shown BAT is related to age. In general it appears that studies investigating brown fat in children without a cold stimulus demonstrated higher prevalence of BAT detection compared to similar studies in adults (Ponrartana et al., 2013). When looking only at the adult population, an inverse association between age and BAT is shown. For example in studies conducting a retrospective analysis of PET/CT images, the probability of detecting BAT decreases with age and the mean age of BAT positive individuals is lower than BAT negative individuals (Cypess et al, 2009, Lee et al, 2010). Due to the retrospective nature of these studies there was no intentional stimulation of BAT prior to imaging, therefore these data may not be representative of actual BAT presence. However, studies looking at BAT following a cold stimulus have also demonstrated an inverse relationship between age and BAT. For example, Yoneshiro et al (2011) found BAT in 50% of individuals in their 20s but only 10% in individual in their 50s.

In the paediatric population a different trend has been reported. Gilsanz et al (2012) found that BAT was present in 15% in prepubertal children compared to 75% of pubertal children. Additionally, when they compared the BAT volumes between prepubertal and pubertal children, they found the volume to be significantly greater in the final stages of puberty compared to the early stages. The reasons for this trend are currently unclear, however Symonds et al., (2012) suggested it could be due to the relative amounts of pubertal hormones that stimulate brown fat in children vs. adults.

Although not considered the gold standard method to detect BAT, thermal imaging to detect changes in temperature in the SCV area, which is attributed to BAT activity, have reported trends similar to the adult ^{18}F FDG PET/CT studies. Symonds et al (2012) found that the increase in temperature in the thermal area of the SCV region was significantly greater in children compared with adolescents and adults.

1.4.4 Sex

The relationship between sex and BAT presence, activity and mass/volume is unclear due to conflicting results. Cypess et al (2009) and Lee et al (2009) both report the prevalence of BAT activity to be higher in women than in men. Although Ouellet et al (2010) also found probability of detecting BAT to be higher in women, this association decreased with increasing age. It is important to note that these studies were retrospective analysis of PET/CT scans and did not use any stimulation procedures prior to BAT imaging. The reasons for these differences were not well explained, however it could be due to the differences in which men and women experience cold, i.e the threshold temperature at which women compared to men will have a physiological response to cold (Ouellet et al, 2010).

As the previously mentioned studies did not employ a BAT stimulus (i.e cold) prior to imaging, it is not clear if there are real differences in BAT expression between men and women. One study that employed cold exposure prior to imaging found no sex differences, however the sample size was significantly smaller compared to the studies demonstrating sex differences, (56 participant PET/CT scans vs. 3000-6000 retrospective PET/CT scans) (Saito et al, 2009).

Three paediatric studies employing a retrospective view of FDG PET/CT scans found no differences in prevalence of BAT between boys and girls (Gilsanz et al,. 2011, Gilsanz et al,. 2012, Chalfant et al,. 2012), however one of these studies, found the volume of BAT was significantly greater in boys during the last stages of puberty (Gilsanz et al,. 2012). This paper reported that the difference might be related to muscle volume as muscle or changes in growth factors and hormones during puberty that impact muscle (Gilsanz et al., 2012). However these studies all had much smaller samples sizes compared to those studies that demonstrated sex differences.

1.4.5 Diabetes/blood glucose/insulin

Since BAT is implicated as a potential therapeutic target for metabolic syndrome, the relationship between blood glucose, insulin levels and diabetes status and BAT has been studied. With regard to fasting plasma glucose, several studies have shown a significant negative correlation between BAT presence and plasma glucose level, however most of these relationships did not stay significant in a multivariate analysis including age, BMI/adiposity and sex as covariates (Cypess et al, 2009, Ouellet et al, 2010). Diabetic status may be associated with BAT: Ouellet et al (2010) reported that the probability of detecting BAT was approximately four times higher in non-diabetic individuals compared to diabetic individuals. With regard to insulin

sensitivity, Admiraal et al (2013) found that there was no correlation between BAT activity measured by ^{18}F FDG PET/CT and insulin sensitivity measured by hyperinsulinaemic-euglycaemic clamp. In contrast, Chondrondikola et al (2014) reported that whole body glucose disposal and insulin-induced glucose disposal increased after cold exposure in individuals demonstrating BAT on a PET/CT whereas this was not seen in BAT negative individuals. At baseline, insulin-induced glucose disposal was similar between BAT+ and BAT-, however baseline body glucose disposal (non insulin-stimulated) was higher in BAT+ group.

1.4.6 Exercise and BAT activity

Whether or not exercise can influence BAT activity in humans is contested, however several animal studies have indicated that exercise interventions can stimulate BAT. For example De Matteies et al (2013) exposed rats to a one-week or six-week regimen of motor treadmill running for 1 hour per day 5 days a week. Interscapular BAT was examined for morphology and mRNA. Sympathetic tone and vascularization was increased two fold in exercised rats (both one and six week exposures). These results suggest that exercise can influence BAT activity through effects on the sympathetic nervous system. De Matteies et al (2013) also found more intense UCP1 labelling in BAT tissue in exercised rats compared to sedentary rats, however, mRNA of UCP1 in BAT tissue was not different in exercised and sedentary rats. Another study exposed diet induced obese mice to a 7 day regimen of exercise (Durrant et al,. 2013). They found UCP1 mRNA in the BAT depot had a 2.1 fold increase in the exercise group (Durrant et al,. 2013). Seebacher et al (2010) found concentrations of UCP1 mRNA in the BAT depot increased significantly when both cold and exercise stimuli were present – almost 100 times more

expression compared to warm exercise conditions or cold non-exercise conditions. These animal studies suggest that exercise may have an influence on UCP1 in BAT and therefore it warrants investigation of exercise on BAT in humans.

1.4.7 Exercise and Beige/Brite Adipose Tissue

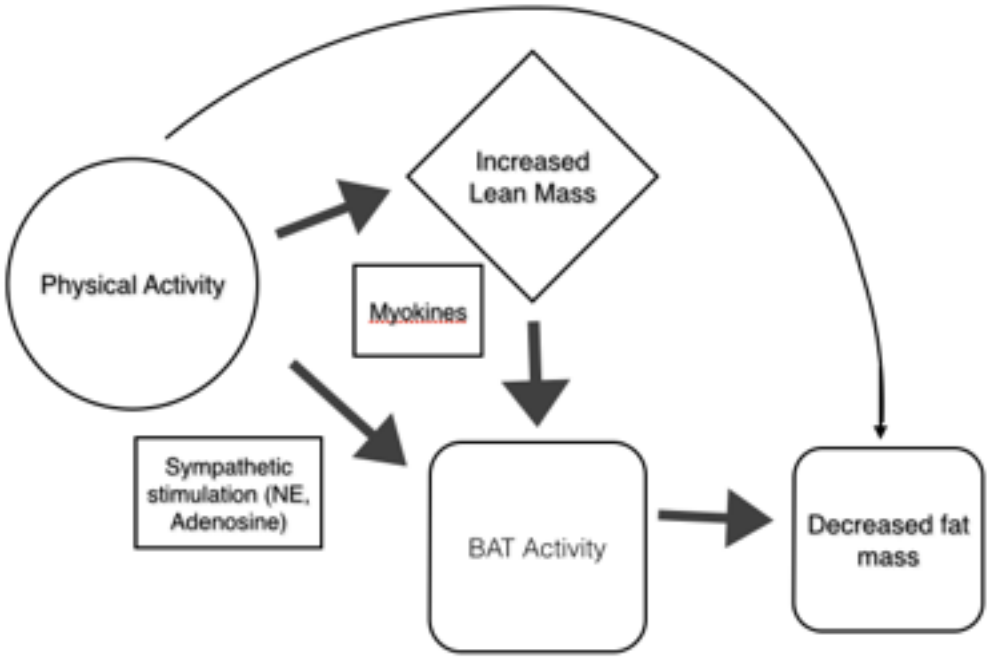
Exercise may also play a role in increasing thermogenesis through the activation of beige/brite adipose tissue. It is believed that physical activity and exercise can stimulate the release of certain factors that are known to up regulate beige adipose tissue expression. SNS stimulated release of catecholamines, which also stimulate classical brown adipocytes, has been noted to increase beige/brite cells in mice (Collins et al, 2010). Additionally certain myokines such as β-aminoisobutryic acid (BAIBA) and irisin, which are released into the blood stream during exercise, have been reported to cause browning (Roberts et al, 2014, Huh et al, 2012, Bostrom et al, 2012). Although irisin was initially reported to show increased levels in men after training (Bostrom et al, 2012), there are issues with the correct measurement of this peptide and therefore reports of the role of irisin in exercise induced browning in humans may not be reliable (Servik K, 2015). Lastly, cardiac peptides, such as natiuretic peptide, which are released during exercise, have been shown to induce lipolysis and UCP1 expression in human adipocytes (Ruiz et al, 2015). It is important to understand exercise in the context of beige adipose tissue as human adipose tissue could be mainly beige or a mosaic of classical brown and beige (Nedergaard et al, 2013). Therefore the influence of exercise on UCP1 mediated thermogenesis could be via classical brown adipocyte activation or expansion of the beige/brite adipose tissue.

CHAPTER II: STUDY RATIONAL AND STUDY DESIGN

2.1 PURPOSE AND SIGNIFICANCE

The purpose of this study is to investigate the response of the SCV skin temperature after cold exposure as a measure of brown adipose tissue activity in children and determine if there is a relationship between this measure and physical activity and body composition. Since BAT may have a beneficial effect on an individual's metabolic profile, it is important to elucidate a safe methodology to measure BAT. In doing so, one can begin to monitor BAT and understand how interventions influence BAT activity. Additionally, determining any relationship between body composition and BAT activity in childhood may help answer some of the questions regarding the development and persistence of obesity in childhood. Lastly, by understanding a modifiable lifestyle factor such as physical activity and its relationship with BAT activity (inferred from SCV skin temperature), we can determine if it is desirable to look into physical activity strategies to increase BAT activity. Further investigation of BAT in children may increase our knowledge of this tissue in humans and help to determine therapies targeting BAT for the improvement of metabolic diseases in childhood and adulthood.

2.2 MODEL



2.3 OBJECTIVES

PART 1

- To observe and document supraclavicular (SCV) skin temperature pattern with infrared (IR) thermal imaging during a cold exposure as a measure of brown adipose tissue (BAT) activity and to determine the accuracy and precision of this measurement in children.

PART 2

- To examine the association between physical activity and a measure of BAT activity (thermal imaging of SCV skin temperature) in children.
- To examine the association between body composition (lean mass and fat mass) and a measure of brown fat activity (thermal imaging of SCV skin temperature) in children.

2.4 HYPOTHESIS

- I hypothesize that SCV skin temperature will demonstrate an increase during the cold exposure and that this measurement will be both accurate and precise.
- I hypothesize that physical activity will have a positive correlation with brown adipose tissue activity whereby high active children will have a greater SCV temperature response to cold (inferring higher BAT activity)
- I hypothesize that individuals with higher lean mass and lower fat mass will have a greater SCV temperature response to cold (inferring higher BAT activity) and individuals with lower lean mass and higher fat mass will have a lower SCV temperature response to cold (inferring lower BAT activity)

2.5 STUDY OVERVIEW

This study was approved by the Hamilton Integrated Research Ethics Board in December 2013 (#13-849).

2.5.1 Study Design : Cross Sectional

2.5.2 Study Participants

Healthy, prepubertal boys between the ages of 8 and 10 years old were recruited from March 2013 to March 2014. Participants were recruited from three sources: 1) The Children's Exercise and Nutrition Centre at McMaster Children's Hospital, 2) The General Pediatrics Clinic at McMaster Children's Hospital and 3) Hamilton and Greater Hamilton Area. Clinic recruitment proceeded through a consent to contact process whereby clinicians obtained permission from patients to hear about the research study. Community participants were recruited through posters,

social media postings and word-of-mouth. Exclusion criteria included: 1) children younger than 8 or older than 10 at the time of recruitment, 2) females 3) children taking medication for asthma or mood disorders, 4) children exposed to cigarette smoke (subjective report), 5) children with a diagnosis of diabetes or muscular disorder. Boys between the ages of 8 and 10 were chosen to narrow the population in order to eliminate the influence of age, sex or puberty. Exposures such as medication for asthma or mood disorders and cigarette smoke were exclusionary criteria as these substances could influence BAT. Lastly, diagnosis of diabetes or muscular disorder were chosen as exclusion criteria to eliminate the influence of these diagnoses on BAT activity. A total of 33 participants completed all study visits (see Figure 1 below):

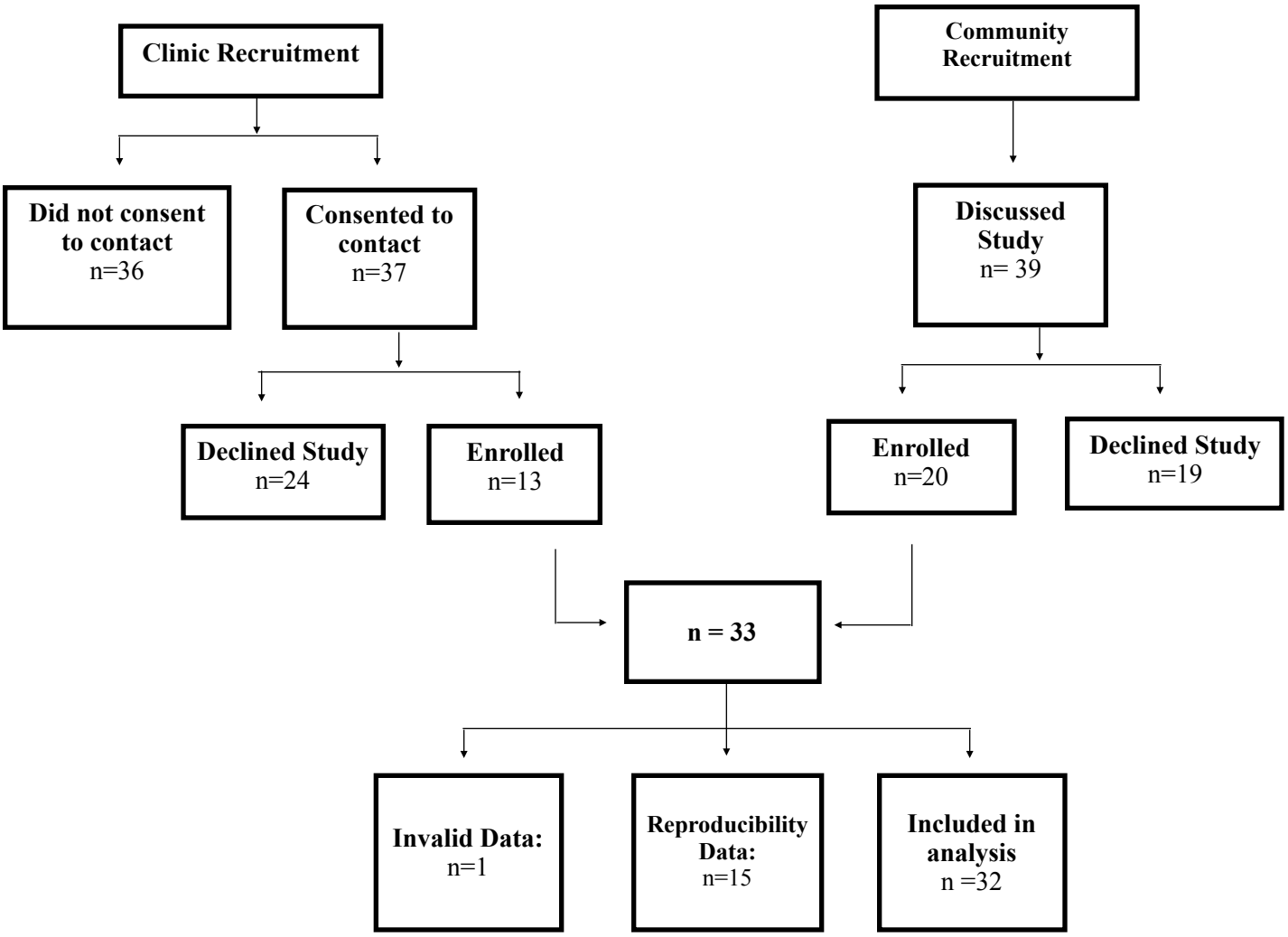


Figure 1: Recruitment flow chart. Invalid data due to inability to obtain appropriate SCV skin temperature or body composition measurements from participant.

2.5.3 Study Visits

Participants came in for a minimum of 2 and up to 3 study visits, scheduled between 7:00 am and 10:00 am approximately once per week. Starting in June 2014, a third visit was added to the protocol. Participants who were recruited starting in June 2014 were asked if they were willing to come in for this optional third study visit. Details regarding the conditions of each visit are included in the methods section of part 1 and part 2.

Visit 1:

During visit one, consent for the study was obtained from the parent/guardian and participant. Following this, anthropometric measures were taken. The resting energy expenditure (measured before and after cold exposure) was completed at this visit. A DXA scan was also completed at this visit when possible. Participants were sent home with an ActiGraph accelerometer, which was worn for the 7 days between the first and second visit.

Visit 2:

Within 1 to 2 weeks, participants returned for their second visit. During visit two, participants underwent a thermal imaging protocol to detect BAT thermogenesis. If the DXA scan could not be completed during the first visit it was completed during visit 2.

Visit 3:

23 participants were invited to return for visit 3 which occurred between 1 to 3 weeks after visit 2. The thermal imaging protocol was repeated at this visit. 15 out of these 23 participants completed visit 3.

CHAPTER III PART 1: EXAMINATION OF SCV SKIN TEMPERATURE AS A MEASURE OF BAT ACTIVITY: PATTERNS, RELATIONSHIP TO ENERGY EXPENDITURE, AND REPRODUCIBILITY

3.1.1 METHODS (PART 1)

3.1.1a Supraclavicular Skin Temperature

Measurement Protocol

To measure BAT activity, SCV skin temperature was monitored with an infrared (IR) thermal imaging camera (T650 sc, emissivity 0.98, FLiR systems, Wilsonville, Oregon) during a room temperature (22°C-23°C) acclimation period, a 12°C cooling period and a room temperature recovery period. IR thermal imaging has been used several times to detect BAT activity (Symonds et al. 2012, Jang et al, 2014) with one study demonstrating that the difference between the SCV and chest skin temperature was predictive of BAT activity as measured by ^{18}F FDG/PET CT (Jang et al, 2014). The IR thermal imaging camera has a precision of 0.02°C and is sensitive to temperatures ranging from -40°C to 650°C. Additionally, SCV skin temperature after cooling measured by iButtons was found to correlate significantly with BAT activity and BAT volume measured by ^{18}F FDG PET/CT (Boon et al, 2014).

There is no standardized acclimation or cooling period to measure BAT activity using skin temperature methods. Therefore, the current study implemented the temperature and timing of exposures based on pilot studies by our research team. Participants arrived to their study visit after an overnight fast. Participants were instructed to sit upright in a chair with a headrest specially designed for this study. They wore a standard cotton undershirt revealing the SCV and upper chest region. A researcher located the participant's sternal notch, left and right clavicle bone, left and right acromion joint, and jaw line. This was done by palpating the clavicular region until the clavicle bone was located, and following the clavicle bone to the sternal notch and acromion joints (this was completed for both the left and right clavicle bones). The jawline

was located by palpating the upper neck region and chin. Several markers were placed around the participant's sternal notch, on the left and right acromion joints and on the left and right side of the jawline (Figure A1 Appendix 1, Page 73). The distance between the camera and the participant was one metre. For the first 15 minutes, participants sat at room temperature (22°C-23°C) and thermal photographs of the SCV/cervical and upper chest region were taken at every five minutes. Following this, a water infused cold blanket (Blanketrol II, Cincinatti sub zero, Cincinnati, Ohio), set at a temperature of 12°C was wrapped around the participant's torso (chest to hips). The participants were instructed to sit with the cold blanket for 30 minutes as thermal photographs were taken every two and a half minutes. After this 30-minute period, the blanket was removed and participants remained at room temperature for ten minutes as thermal pictures were taken every two and a half minutes.

Analysis of Thermal Images

The IR thermal imaging camera yielded JPEG images which were converted to CSV files using the FLiR imaging software (FLIR Research IR Version 3.1.432235.2002)**.** CSV files were subsequently converted to RAW files with a command code using MATLAB software. (MATLAB Version 7.5.0.342 (R2007b). RAW files were used to determine skin temperature from image voxels using AMIDE medical imaging software (Version 1.0.4, 2000-2012). RAW images were imported into the AMIDE software (modality: pet, data format: Float little endian 32 bit, dimensions: 480X, 640Y 1Z, voxel side: 1mm, scale factor: 1). In the AMIDE software, regions of interest (ROIs) were created for the left SCV area, right SCV area, sternum and chin regions. Sternum and chin ROIs were set to an area of 121 mm^2 whereas left and right SCV ROIs were drawn horizontally from the sternal notch markers, to the acromion marker and

vertically to the jawline marker to create a rectangle (Figure 1, Appendix 1, Page 73). Temperature in the left and right SCV regions was determined by taking the mean of the top 10% voxels within the ROI. Left and right SCV region temperatures were averaged to give the SCV skin temperature. Control region temperatures were quantified by taking the mean of all voxels within the ROI.

Skin temperature outcomes

As there is no standard protocol to analyze skin temperature in the SCV region to quantify BAT activity, several outcomes were investigated:

1. *Area Under the Curve of change in SCV skin temperature from baseline* ***(AUC)***

- Change in temperature from baseline at each time point during cooling and recovery was plotted. The area under this curve was quantified using the trapezoidal rule (Samaras, 2013). This measure allows for determination of the magnitude of skin temperature response over the cooling and recovery period.

2. *Change in temperature at 30 minutes of cooling from baseline* ***(Delta T)***

- The final temperature in each region of interest was determined by averaging the five temperature points obtained after 20-30 minutes of cold exposure. From this value, the baseline temperature was subtracted to yield the change in temperature from baseline. This measure allowed for the quantification of the change in skin temperature due to cooling.

3. *Baseline SCV Skin Temperature*

- The absolute baseline skin temperature after acclimating for 15 minutes at room temperature was taken in each region of interest

4. *Post Cooling SCV Skin Temperature*

- The skin temperatures recorded between 20-30 minutes of cooling were averaged to yield the final "post-cooling" temperature in each region of interest.

5. *Adjusting for control region*

- The sternum region was used as the control (or non BAT area). Jang et al (2014) found a predictive relationship between the differential temperature of the SCV and chest and BAT activity on PET/CT. Therefore all of the above outcomes were adjusted for the control region (i.e the equivalent sternum temperature was subtracted from the BAT temperature for each of the above outcomes).

3.1.1b Pattern: Characterization of Temperature Response

As many reports of BAT in humans classify participants as responders (showing a BAT positive PET/CT scan) and non responders (showing a BAT negative PET/CT scan) (Gilsanz et al, 2011, Lee et al, 2009, Ouellet et al, 2009), the current study looked into the skin temperature response patterns to classify responses. Participants were characterized based on the magnitude of the change in skin temperature in the SCV region. Participants having an a Delta T and an AUC value of greater than zero were considered responders. Participants having a Delta T **or** an AUC value of zero or less were considered non responders.

3.1.1c Precision: Reproducibility of IR Thermal imaging of SCV Skin Temperature During Cold Exposure

The protocol for monitoring SCV skin temperature during cold exposure was repeated between 1 and 3 weeks after the first session. This repeated session occurred at the same time of

day (fasting) and at the same indoor temperature exposures as the participant's first thermal imaging procedure.

3.1.1d Accuracy: Relationship of SCV skin temperature to Energy Expenditure

Resting energy expenditure (REE) was measured at rest and after a 20-30 minute cold exposure to evaluate the accuracy of SCV skin temperature quantification of BAT activity. Energy expenditure was used as the comparison because cold is believed to increase resting energy expenditure through stimulation of BAT. For example, individuals demonstrating BAT on a PET/CT often show an increase in REE (kcal/day) after a cold exposure (Saito et al, 2012, Boon et al, 2014). Indirect calorimetry is often used as a reliable measure of energy expenditure in humans (Goran M, 1998). It is based on the assumption that total energy expenditure is reflected in total oxygen consumption and carbon dioxide production by tissues (Goran et al, 1998).

In the current study, REE was measured using a ventilated canopy attached to a Metabolic Cart (Vmax Encore 29, Viasys Respiratory Cre, Palm Springs, CA). The flow sensor was calibrated prior to each measurement. Participants arrived for the test after an overnight fast and relaxed in a supine position for the duration of the test. A video was shown throughout testing to help children relax/remain still because the timing of the test ranged from 40-60 minutes. REE was determined at room temperature after a 15-20 minute resting period and again following a 20-30 minute exposure to a 12 degree cold blanket worn around the torso. REE at room temperature was established when there had been 3 consecutive minutes when energy expenditure measurements were within 5% variability. REE values (Kcal/day) were averaged

over this three minute period to obtain the baseline REE. At this point a 12 degree cold blanket was applied to the torso and worn for a minimum of 20 minutes. After 20 minutes, energy expenditure was monitored until a steady state had been reached (based on criteria above).

3.1.1e Data Analysis

Pattern: Magnitude of Skin Temperature Response

Skin temperature measurements taken in the SCV region, sternum and chin, during cooling and recovery phases were compared to the baseline temperature taken after 15 minutes of acclimation. This delta measurement was plotted on a graph of change in temperature vs. time and averaged for all participants. This allowed for the observation of the skin temperature response pattern in each region of interest. The AUC and Delta T were calculated for each region. These measures were compared for each region with a one-way ANOVA and Tukey's Post Hoc to determine if the SCV skin temperature response was different from the sternum and chin skin temperature responses.

Precision: Reproducibility of Skin Temperature Outcomes

The reproducibility of all 8 skin temperature outcomes reported above was determined by computing intraclass correlation coefficients (ICC) and constructing Bland-Altman Plots. For the ICC, a two-way mixed model was chosen to calculate the ICC as the conditions were kept consistent for each trial (i.e we controlled for variability between trials) but the subjects were random.

Accuracy: Skin temperature vs. Energy expenditure

The 8 skin temperature outcomes listed above were compared with corresponding resting energy expenditure outcomes using the Pearson correlation method. Specifically, changes in skin temperature (Delta T, AUC) were compared with changes in REE, baseline skin temperature was compared with baseline REE and post-cooling skin temperature was compared with post-cooling REE.

3.1.2 RESULTS (PART 1)

3.1.2a Description of skin temperature outcomes and energy expenditure measures for the study population:

32 of the boys who completed the study were included in the analysis; their skin temperature responses and energy expenditure data are reported in Table 1. One participant was excluded due to inability to complete the thermal imaging protocol or DEXA scan. Out of 32 individuals, 30 successfully completed the REE measurement and their data is included in Table 1. Two participants were unable to obtain a steady state REE measurement after 30 minutes at rest and therefore did not undergo cooling as they would not tolerate the test any longer. 15 out of 32 participants returned to repeat the thermal imaging protocol.

The SCV skin temperature was analyzed several different ways. Each outcome is outlined in Table 1 below. AUC and Delta T values after adjusting for the sternum region were greater than when not adjusting for the sternum region. This is likely due to a decrease in sternum temperature during the cold exposure. The AUC and Delta T were very variable between participants. On average, the post-cooling SCV skin temperature was higher after cooling

compared to baseline and the difference between the SCV region and the sternum was also greater after cooling compared to baseline.

Table 1: Description of Study Measures Included in Part 1

Variable	n	Mean (SD)	(Min, Max)
Age	32	9.43 (1.0)	(8.0, 11.0)
SCV Skin Temperature Outcomes			
AUC SCV Temperature	32	6.35 (7.7)	(-8.3, 22.5)
AUC SCV - AUC Sternum	32	11.37 (11.6)	(12.31, 46.3)
Delta T SCV temperature (C)	32	0.23 (0.24)	(-0.17, 0.79)
Delta T SCV - Delta T Sternum (C)	32	0.41 (0.4)	(-0.32, 1.54)
Baseline SCV (C)	32	34.3 (0.7)	(32.95, 35.29)
Baseline SCV - Baseline Sternum (C)	32	1.91 (0.5)	(0.88, 3.4)
Post-Cooling SCV (C)	32	34.54 (0.7)	(33.15, 35.70)
Post-Cooling SCV - Post Cooling Sternum (C)	32	2.32 (0.5)	(1.33, 3.50)
Energy Expenditure Measurements			
REE before cold (kcal/day/kgFFM)	30	45.28 (4.5)	(36.6, 54.8)
REE after cold (kcal/day/kgFFM)	30	46.76 (6.9)	(28.2, 59.5)
Change in REE (Kcal)	30	36.27 (138.5)	(-337.0, 275.0)

3.1.2b Pattern of Skin Temperature Response to Cold Exposure in the SCV Sternum and Chin

The change in skin temperature from baseline in the SCV, sternum and chin regions were observed during the 30 minute cooling period and 10 minute recovery period (Figure 2). Based on the average response of 32 boys included in the study, there was a steady increase in SCV

skin temperature and the Delta T peaked at 0.25ºC at 25 minutes of cooling. During the recovery period, SCV skin temperature began to decline, but on average, remained higher than baseline. The chin and the sternum regions were observed as control regions as they are not believed to contain BAT. On average, the sternum temperature demonstrated a decline in temperature after introducing the cold stimulus and a slight increase during the recovery period. The chin region demonstrated a slight increase during the introduction of the cold stimulus and a gradual decline, especially during the recovery period. However, the chin had a very variable response depending on the individual being tested, as demonstrated by the large standard errors. An ANOVA revealed differences in AUC and Delta T for the three regions ($F = 7.02$ $p=0.001$ and $F=6.6$ $p=0.002$). A Tukey's post hoc analysis revealed that the SCV region had a significantly higher AUC and Delta T compared to both control regions, which did not differ from each other (Table 2).

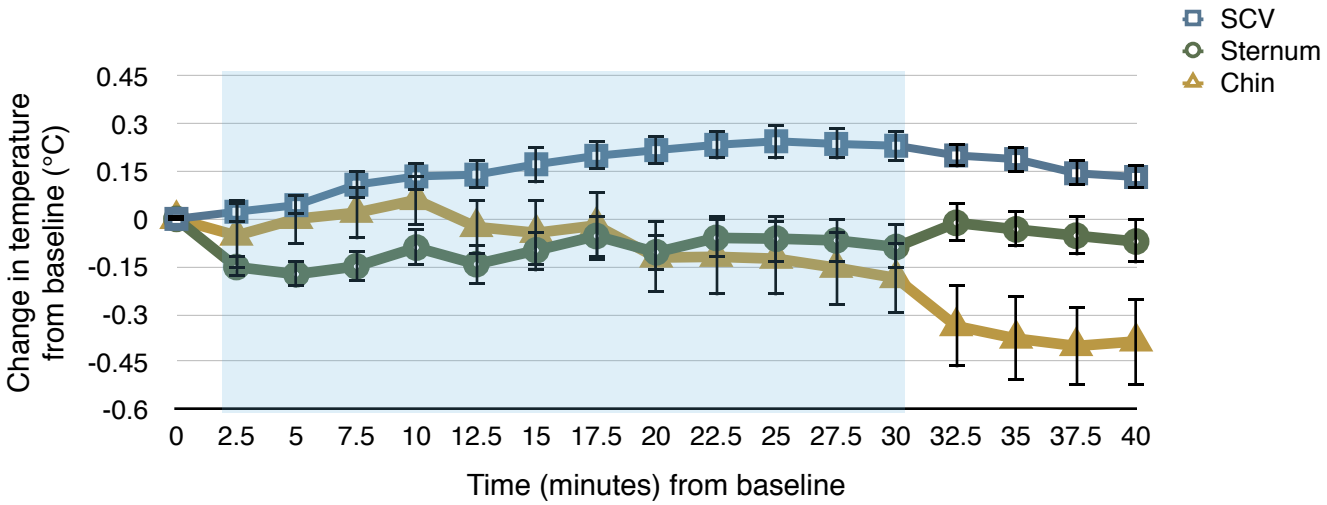


Figure 2: Average change in skin temperature in the the SCV region (most probable to contain BAT in humans), sternum (control 1) and chin regions (control 2). Values are the average of 32 participants (+/- SEM). Cold exposure was 12°C

Table 2: AUC and Delta T values for skin temperature in the SCV, Sternum and Chin regions demonstrated in Figure 2 (n=32)

	Mean AUC (SD)	Mean Delta T (SD)
SCV	6.3 (7.8)*	0.23 (0.25)*
Sternum	-5.75 (11.9)	-0.17 (0.4)
Chin	-3.97 (19.7)	-0.10 (0.63)

**Significantly different from sternum and chin based on one-way ANOVA and Tukeys Post-Hoc*

3.1.2c "Responders" vs. "Non Responders"

A closer look at the skin temperature responses in the SCV region revealed two types of responses. A subset of the group did not demonstrate an increase in their SCV skin temperature, defined as an AUC of ≤0 or a Delta T of ≤0. These individuals were considered "non-responders" as they did not demonstrate the expected pattern of skin temperature change that would reflect active BAT activity (heat production after cold exposure resulting in increased SCV skin temperature). Individuals who demonstrated an increase in SCV skin temperature, defined as having an AUC of ≥0 and a Delta T of ≥0, were considered "responders". Responders had a skin temperature response expected of active BAT (increase in SCV skin temperature after cold exposure). Based on this, the skin temperature responses in each region (SCV, sternum and chin) were compared in responders and non responders (Figure 3a and 3b). A one way ANOVA (and Tukey's post-hoc test) revealed no differences in AUC and Delta T between SCV, sternum and chin regions in non-responders, whereas responders had a significantly higher AUC and Delta T in their SCV region compared to the two control regions (F=4.9 and 4. 8, p = 0.01 and 0.01) (Table A2 and A3, Appendix 2, Page 76). The characteristics of responders and non responders can be seen in Table A1, Appendix 2 Page 75.

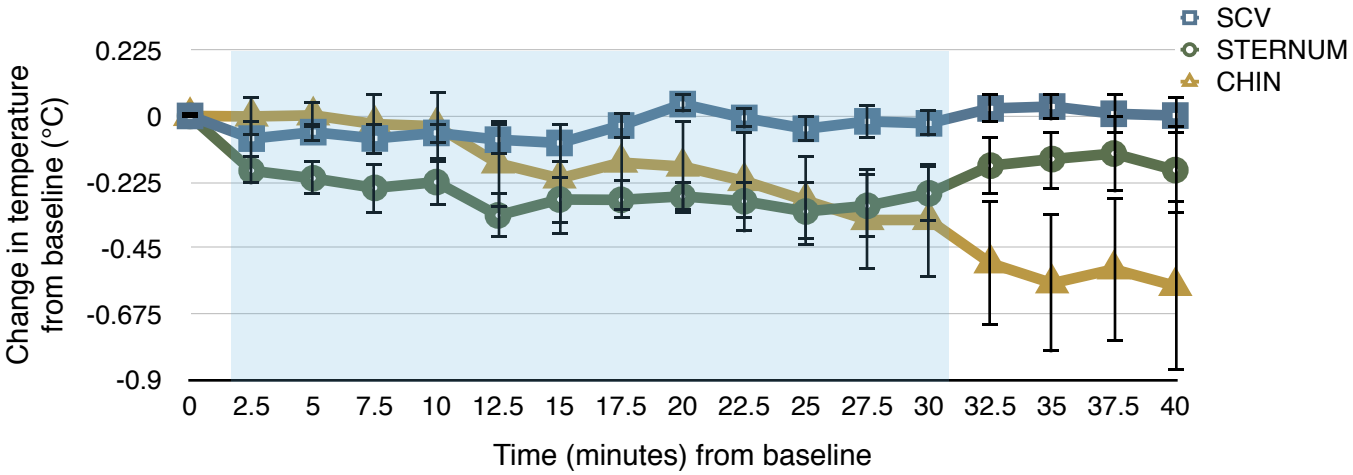


Figure 3a: Average change in skin temperature of non responders in the the SCV region (most probable to contain BAT in humans), sternum (control 1) and chin regions (control 2). Values are the average of 12 participants (+/- SEM) demonstrating an overall negative AUC and Delta T.

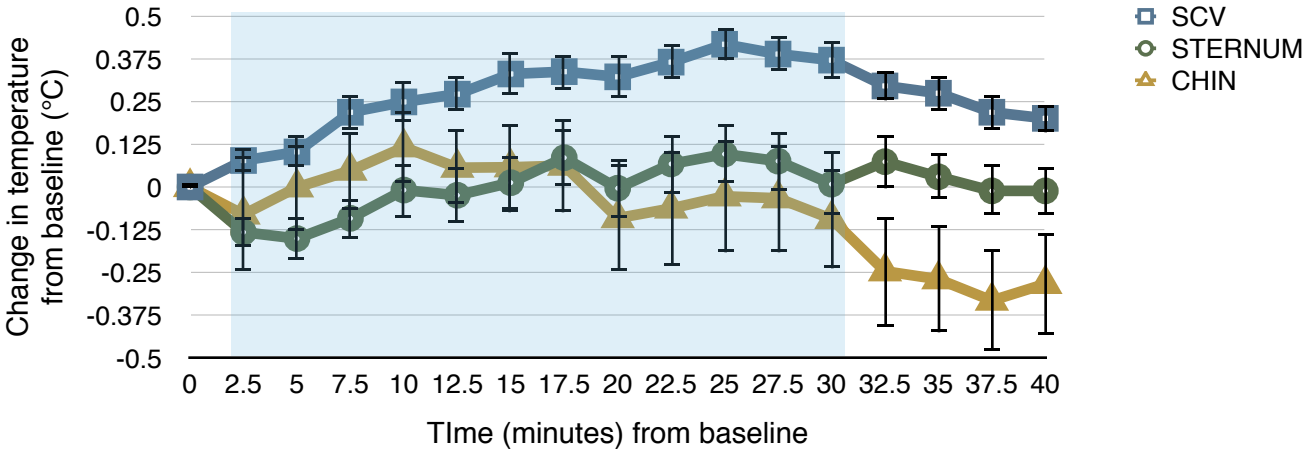


Figure 3b: Average change in skin temperature in responders in the the SCV region (most probable to contain BAT in humans), sternum (control 1) and chin regions (control 2). Values are the average of 20 participants (+/- SEM) demonstrating an overall positive AUC and Delta T.

3.1.2d Precision: Reproducibility of IR imaging to detect skin temperature change in response to cold.

An important step when employing a new diagnostic measurement tool is to determine the precision and accuracy of that tool (Taverniers et al., 2004). As IR thermal imaging to detect skin temperature in the SCV region has not yet been established as a tool to detect BAT in humans, we completed a reproducibility analysis to estimate its precision.

Supraclavicular skin temperature

The reproducibility of Delta T and AUC for the SCV region was poor between trial 1 and trial 2 as demonstrated by insignificant ICC values (Table 3). In contrast, baseline SCV and post-cooling SCV skin temperature were very reproducible, evidenced by significant ICC values approaching 1. Reproducibility is also demonstrated visually in a series of Bland-Altman plots (Figure 4). Although most values fall within the limits of agreement (mean difference ±2 standard deviations) for each skin temperature outcome, these limits are very wide for SCV AUC and SCV Delta T due to high variability in the measures.

Supraclavicular skin temperature adjusted for sternum

The reproducibility of thermal imaging was also completed for SCV skin temperature after adjusting for the sternum control region. When adjusting for the sternum control region, the Delta T is somewhat reproducible, with an ICC value on the lower end of the threshold considered to be reproducible, however the AUC is still not reproducible. Similar to the non adjusted values, baseline SCV temperature adjusted for baseline sternum temperature and post-cooling SCV temperature adjusted for post-cooling sternum temperature are very reproducible. Reproducibility is also demonstrated visually in a series of Bland-Altman plots (Figure A2,

Appendix 2, Page 77). The post-cooling SCV temperature adjusting for the sternum shows smaller differences between Trial 1 and Trial 2 for higher mean values.

Table 3: Reproducibility of skin temperature outcomes from Trial 1 and Trial 2 of thermal imaging.

Outcome Measure	ICC (Absolute Agreement)	ICC (Consistency)	Significance
SCV AUC	0.104	0.099	0.424
AUC SCV - AUC Sternum	0.599	0.599	0.05
Delta T of SCV	0.019	0.029	0.479
Delta T SCV - Delta T Sternum	0.739	0.736	0.009
Baseline SCV T	0.957	0.961	<0.001
Baseline SCV T - Baseline Sternum T	0.681	0.668	0.024
Final SCV T	0.952	0.955	<0.001
Final SCV T - Final Sternum T	0.938	0.936	<0.001

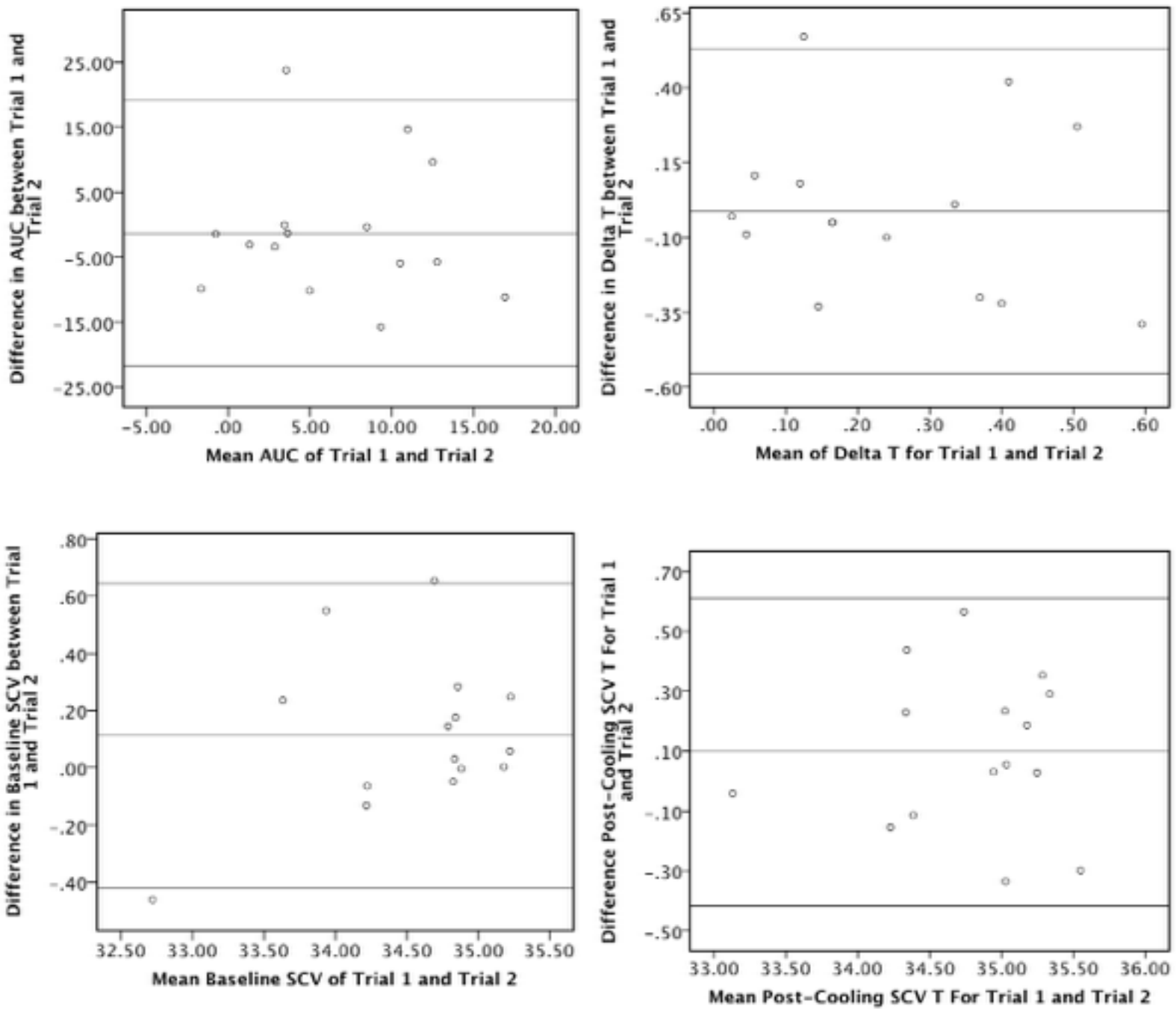


Figure 4: Bland-Altman plots demonstrating the reproducibility of (A) SCV AUC, (B) SCV Delta T, (C) Baseline SCV and (D) Post-cooling SCV skin temperatures.

3.1.2e Accuracy: Energy Expenditure vs. Supraclavicular Skin Temperature Response to Cold

As mentioned above, it is important to establish precision and accuracy when testing a new measurement tool. In order to determine the accuracy of IR thermal imaging to detect BAT activity, we compared skin temperature response to energy expenditure at baseline and after the same cold exposure.

Supraclavicular skin temperature vs. REE

Based on Pearson correlation calculations, there was no relationship between change in energy expenditure and changes in SCV skin temperature (AUC and Delta T). A positive correlation between baseline SCV skin temperature and baseline REE (kcal/day/KgFFM) was seen, but only approaching significance ($p=0.07$). Additionally, there was a significant and positive correlation between energy expenditure (kcal/day/KgFFM) after cold exposure and SCV skin temperature after cold exposure ($p=0.032$). (Table 4 and Figure 5)

Supraclavicular skin temperature adjusted for sternum vs. REE

It was also of interest to determine if skin temperature adjusted for the sternum region was related to energy expenditure. Similar to the unadjusted SCV skin temperature, there were no relationships between changes in REE and changes in SCV skin temperature (AUC and Delta T adjusted for the sternum). Although only approaching significance, the post-cooling SCV temperature after adjusting for the sternum was inversely correlated with the post cooling REE (kcal/day/KgFFM) (Table 4 and figure 6).

Table 4: Correlation between REE and skin temperature outcomes

Variables Tested	n	Pearson Correlation	Significance
Change in REE vs. SCV AUC	30	0.034	0.859
Change in REE vs. SCV Delta T	30	-0.015	0.937
Change in REE vs. SCV AUC adjusted for sternum	30	-0.047	0.807
Change in REE vs. SCV Delta T adjusted for sternum	30	-0.309	0.097
Baseline REE/KgFFM vs. Baseline SCV temperature	30	0.332	0.073
Baseline REE/KgFFM vs. Baseline SCV temperature adjusted for sternum	30	-0.007	0.969
Post cooling REE/ KgFFM vs. Final SCV Temperature	30	0.392	0.032*
Post cooling REE/ KgFFM vs. post-cooling SCV temperature adjusted for sternum	30	-0.336	0.069

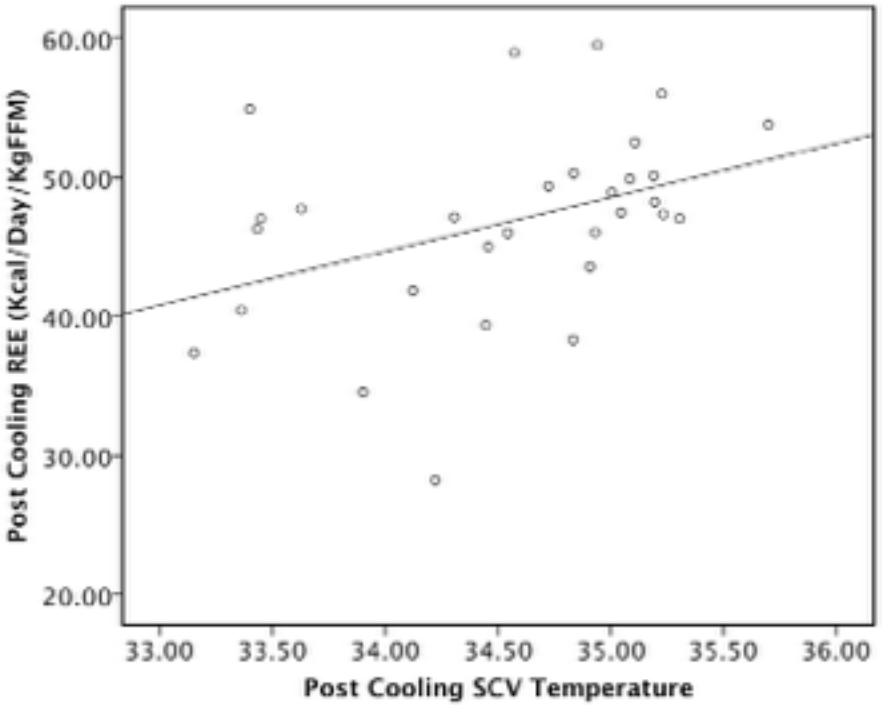


Figure 5: Correlation plot of energy expenditure after cold exposure (adjusted for fat free mass) and SCV skin temperature after cold exposure (r= 0.392, p=0.032, Rsquare 0.154)

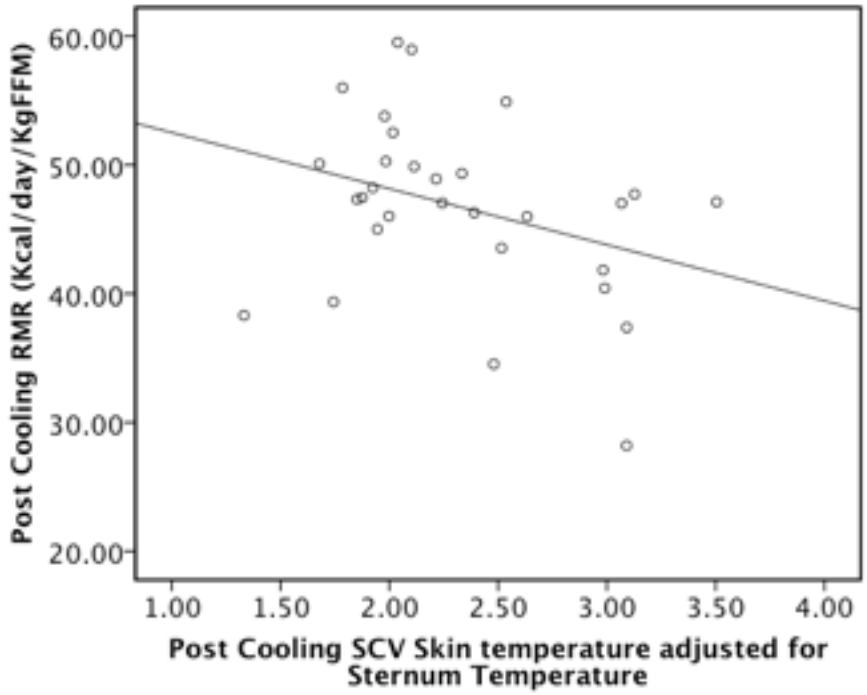


Figure 6: Correlation plot of energy expenditure after cold exposure (adjusted for fat free mass) and SCV skin temperature adjusted for sternum after cold exposure (r= -0.336, p=0.069, rsqaure 0.113)

3.1.2f Summary

Based on the reproducibility and validity analysis of several SCV skin temperature outcomes, it was determined that post-cooling SCV skin temperature was the best measure of BAT thermogenesis. The post-cooling SCV skin temperature and the baseline SCV skin temperature were found to have the highest reproducibility and therefore were considered the most precise of all 8 measures. However only the post-cooling SCV skin temperature was significantly related to energy expenditure, so it was considered the most accurate of all 8 skin temperature outcomes.

We chose not to control for the chin region because it was highly variable between participants. Additionally, all outcome measures adjusting for the sternum region may be unreliable estimates of BAT activity as they did not have a significant relationship with energy expenditure. In fact, adjusting for the sternum temperature may result in an overestimation of BAT activity due to the decrease in sternum temperature after cooling. As the sternum region was quite close to the cooling blanket, this decrease in temperature may be because of the proximity between the cooling blanket and the sternum region. As a control, it wouldn't be appropriate as it is not subjected to the same conditions as the SCV region. Having the sternum temperature decrease due to its proximity to the cold exposure would bias the overall SCV skin temperature responses (when adjusting for the sternum) to a more positive value (i.e the difference between the SCV region and sternum region would be greater due to a decrease in sternum temperature and not due to an increase in SCV temperature).

For these reasons, the post-cooling SCV skin temperature will be used to address the relationship between BAT activity, body composition and physical activity.

CHAPTER III (PART 2): EXAMINATION OF THE RELATIONSHIP BETWEEN POST-COOLING SCV SKIN TEMPERATURE (AS A MEASURE OF BAT ACTIVITY), PHYSICAL ACTIVITY, AND BODY COMPOSITION

3.2.1 METHODS (PART 2)

Objectives:

1. To examine the association between physical activity and a measure of BAT activity (thermal imaging of SCV skin temperature) in children.
2. To examine the association between body composition (lean mass and fat mass) and a measure of brown fat activity (thermal imaging of SCV skin temperature) in children.

Study Measures

3.2.1a Adiposity

Fat mass and fat percentage were determined by Dual X-Ray Absorptiometry (DXA) (Lunar Prodigy model #8743, GE Healthcare, United Kingdom). DXA is considered an accurate tool to determine percent fat (Gutin et al, 1996). In the current study, children were instructed to wear light clothing without any metal or plastic pieces prior to the scan. They were asked to lie flat on their back with their arms at their sides and a researcher positioned them to ensure they were within the confines of the scan. Total body percent fat was automatically calculated by the Lunar Prodigy software following the scan. Additionally, Fat Mass Index (FMI) was calculated by taking the absolute fat mass in Kg and adjusting for a participant's height in meters squared as recommended by VanItallie et al. (1990).

High adiposity and low adiposity groups were identified to compare skin temperature outcomes. High adiposity groups were defined as greater than the 75th percentile for body fat and low adiposity groups were defined as less than 75th percentile for body fat. This percentile reference was readily available from the NHANES population study which reported body fat percentages measured by DEXA in 8-10 year old boys (Kelly et al, 2009). Although there are no

standard references to split individuals into high risk or low risk groups based on percent body fat as there is with BMI measurements, the 75th percentile was used as this level of adiposity has been reported to be associated with several risk factors for metabolic syndrome. For example, Going et al (2011) reported that in boys ages 6-11, a total percent fat greater than 20% and especially greater than 30% increased the odds (by 2-3 times) of being in placed in an high-risk group for several factors associated with metabolic syndrome (blood pressure, cholesterol, C-reactive protein, fasting glucose and insulin). The 75th percentile used in this study constitutes body fat greater than or equal to 32.5%, therefore these individuals may be considered a high risk group.

3.2.1b Lean Mass

Lean mass was also quantified using the DXA method as outlined above. Lean mass was calculated automatically by the Lunar Prodigy software following the scan and used to generate the Lean Mass Index (LMI), also known as Fat Free Mass Index (FFMI) which accounts for height similar to the FMI and is recommended by (VanItallie et al, 1990).

3.2.1c Physical Activity

Physical activity was measured objectively through the use of an ActiGraph accelerometer (GT3X model, ActiGraph, Pensacola FL). The accelerometer can detect an individual's gross motor activity to determine how much time is spent at different levels of physical activity ranging from mild to vigorous (Riddoch et al,. 2004). It is commonly used in epidemiological studies in children to reliably gauge physical activity level (Dencker et al,. 2006, Riddoch et al., 2004). The accelerometer was worn for 7 days (except when sleeping and showering). The criteria for valid data include a minimum of 3 weekday days and 1 weekend day

with each day having at least 10 hours of wear time. These thresholds were based on reports by Rowlands (2007). The accelerometer was analyzed at an epoch length of 15 seconds. The procedure for calculating wear time is reported in section A1.1, Appendix 1, Page 73. As wear time for each accelerometer was cross-checked with participant logs, participants who did not return a completed log were not included in the physical activity analysis.

Total wear time was then broken down into four activity types (Sedentary, Light, Moderate and Vigorous) based on previously determined ranges of accelerometer counts. There are several reported cut points for accelerometer counts, however the cut points reported by Evenson (2008) were used as they were developed based on a paediatric population (5-8 years old) and the accelerometer output was tested for sensitivity and specificity (using an ROC curve) to determine level of physical activity (sedentary, light, moderate and vigorous). The Evenson (2008) study determined actual level of physical activity by measuring oxygen consumption and heart rate (with a portable breath-by-breath metabolic unit) during standardized activities including treadmill walking and running, stair climbing, basketball dribbling, jumping jacks and cycle ergometry. The sensitivity was 68%, 77% and 95% for vigorous, moderate and sedentary activity respectively and the specificity was 89%, 81% and 93% for vigorous, moderate and sedentary activity respectively (Evenson et al, 2008). Total MVPA was weighted for wear time according to the formula published by Katapally et al (2014) (Formula 1, Appendix 1, Page 74). An additional weighted analysis was completed based on weekend and weekday days according to (Formula 2, Appendix 1, Page 74).

The shortcomings of accelerometry may include an overestimation of physical activity as participants are aware they are being monitored. It is important to note that the accelerometer

does not provide feedback to participants which may mitigate the motivation for participants to perform more physical activity than normal. Additionally, since the device is only being worn for 7 days, it is possible that the week of wear was not representative of a typical week of physical activity. Additionally, the accelerometer can only pick up step based physical activity and it is not water-resistant, therefore swimming and other aquatic activities cannot be measured (Welk et al, 2000). However, for measuring regular physical activity in children outside of the laboratory, the accelerometer is superior to the other available methods such as self-report questionnaire.

High physical activity groups and low physical activity groups were identified based on the recommendation of 60 minutes of MVPA per day set by the Canadian Society for Exercise Physiology (CSEP) for 8-10 year old boys. High active individuals were defined as having greater than or equal to 60 minutes of MVPA and low active groups were defined as having less than 60 minutes of MVPA. (CSEP, 2015)

3.2.1d Anthropometry

Weight was determined through the use of an electronic platform scale (Healthweigh, Rice Lake Weighing Systems, Rice Lake WI) and reported in Kg. Participants were asked to remove their shoes and any heavy clothing prior to the measurement. Weight was measured three times and these three measures were averaged to give the participant's actual weight. Height was measured using a wall mounted stadiometer (Perspective Enterprises, Portage, MI). Participants were asked to remove their shoes and stand against the stadiometer as the slider was adjusted for their height. This was repeated three times and recorded as the average of each measurement in centimetres to the nearest 0.5 cm. Height and weight were used to calculate BMI and BMI Z-score (for 8-10 year old boys) according to the WHO growth standards using the WHO

AnthroPlus software (Version 1.0.4; based on reference from 2007 anthropometric data for ages 5-19).

3.2.1e Outdoor Temperature

Outdoor temperature was recorded from the the Government of Canada's historical climate data website (climate.weather.gc.ca). The temperature approximately 1 hour prior to the scheduled study visit was recorded for Hamilton RBG area (climate ID 6153301).

3.2.1f Supplementary outcome measures

Heart Rate

To determine the extent of sympathetic stimulation in response to the 12 degree cold exposure, heart rate (HR) was monitored throughout the thermal imaging session. This is based on the idea that cold stimulates norepinepherine release, which may result in an increased heart rate (Goldberg et al, 1960). With this measurement, it may be possible to get an objective measure to how participants experience the cold stimulus.

In a subset of participants, heart rate was measured with an electronic-optical sensor continuous heart rate watch worn on the wrist (MIO Alpha, Physical Enterprises Inc). Measurement began during the acclimation period and continued throughout the cooling period and recovery period. HR in beats per minute was recorded every second. These measures were averaged for each minute and plotted on a temperature vs. time graph. Average HR for each period (acclimation, cooling and recovery) was also computed.

Oral Temperature

Oral temperature was measured as a surrogate to estimate how internal temperature responded to the cold stimulus. In a subset of individuals, oral temperature was taken with a

digital thermometer (SureTempPlus 690, WelchAllyn, Skaneateles Falls, NY) by placing the temperature sensing probe under the tongue. Temperature was measured at baseline after the 15 minute acclimation period, after 15 minutes of cold exposure, after 30 minutes of cold exposure and once more, 10 minutes after removing the cold exposure.

3.2.1g Data Analysis

Relationship of Post-cooling SCV skin temperature with Physical Activity, Fat mass, Lean Mass and Outdoor temperature:

A univariate analysis was completed to determine the relationship of post-cooling SCV skin temperature with the following continuous outcome measures: physical activity , lean mass, fat mass and outdoor temperature. Physical activity was assessed as minutes of moderate to vigorous physical activity (MVPA) and as counts per minute (CPM).

Following this, a multivariate analyses was completed for independent variables that had a $p<0.10$ in the univariate analysis. Co-linearity of each of these independent variables was assessed with VIF scores; as there were no independent variables showing a VIF score of 10 or greater, all variables were deemed appropriate to include in a single multivariate model.

Physical activity and Adiposity Groups:

The post-cooling skin temperature was compared for groups of high adiposity and low adiposity and high physical activity and low physical activity (as defined above). A chi square statistic was calculated to determine any relationship between physical activity and adiposity. Following this, adiposity groups were stratified by physical activity level (either high active or low active). The post-cooling skin temperature was compared amongst these groups using a one-

way ANOVA and Tukey's Post-Hoc. If the data did not meet the assumptions of a one-way ANOVA (i.e. not normally distributed or containing outliers) the Kurskal-Wallis Test was used instead.

Sample Size Calculation

Sample size was calculated based on the multivariate analysis investigating the influence of MVPA, Lean Mass Index, Fat Mass Index and outdoor temperature on post-cooling SCV skin temperature. A general rule of thumb is 10 participants per independent variable. As 4 variables have been included in the regression analysis, a sample size of 10 x 4 = 40 was required for sufficient power (80%). The current study had only 27 participants included in the multivariate analysis, therefore the results of these analysis are underpowered.

3.2.2 RESULTS PART 2

3.2.2a Characteristics of Study Population

All participants were between the ages of 8 and 10 at the time of recruitment, however one participant turned 11 before completing all study visits. As participants were recruited from a weight management clinic and the community, there were 13 normal weight individuals, 8 overweight individuals and 11 obese individuals (based on BMI Z scores calculated with WHO guidelines). DXA scans were completed for all 32 participants. Based on adiposity percentile guidelines published by the NHANES study (Kelly et al, 2009), there were 13 participants over the 75th percentile for adiposity and 18 individuals below the 75th percentile for adiposity. Out of 32 individuals, 27 had valid physical activity data. The five individuals who were not included in the physical activity analysis either did not complete the accelerometer log book (1/5) and/or did not meet the criteria for valid days (4/5). Eight out of 27 individuals with valid physical activity data had at least 60 minutes of MVPA per day and 19 out of 27 had less than 60 minutes of MVPA per day. See Table 5 below.

Table 5: Description of study population

Variable	n	Mean (SD)	(Min, Max)
Age	32	9.43 (1.0)	(8.0, 11.0)
Weight (Kg)	32	41.69 (18.9)	(20.1, 107.9)
Height (m)	32	139.51 (8.9)	(123.4, 160.3)
BMI (kg/m	32	20.78 (6.7)	(11.1, 42.0)
BMI Z score	32	1.36 (2.0)	(-1.27, 5.75)
Normal Weight (Z<1)	13	-0.53 (0.61)	(-1.27, 0.66)
Overweight (1<Z<2)	8	1.42 (1.1)	(1.07, 1.77)
Obese Z>2	11	3.57 (1.2)	(2.04, 5.75)
Adiposity (%Fat)	32	27.03 (14.0)	(7.5, 55.3)
Low/Normal Fat (<75th percentile)	19	16.86 (6.1)	(7.5, 29.3)
"High Fat" (>75th percentile)	13	41.87 (6.8)	(33.2, 55.3)
Fat mass (Kg/m	32	6.15 (5.0)	(1.0, 20.6)
Lean mass (Kg/m	32	13.7 (1.5)	(11.7, 18.6)
Physical Activity Data			
Total Valid Accelerometers	27	-	-
Total Invalid Accelerometers	5	-	-
Incomplete Log Book	1	-	-
Insufficient wear time	4	-	-
Total Wear Time (hours)	27	71.6 (14.9)	(44.5, 97.7)
Minutes MVPA (average min/day)	27	49.66 (22.6)	(17.0, 97.7)
"High Active" (MVPA>60 min/day)	8	76.84 (15.6)	(60.0, 97.7)
"Low Active" (MVPA<60 min/day)	19	38.2 (13.4)	(17.0, 55.0)
Counts Per Minute (CPM)	27	602.75 (225.2)	(285.3, 1340.2)
Outdoor temperature (C)	32		
Thermo Visit	32	3.1 (11.8)	(-22.0, 18.10)
RMR visit	32	2.90 (11.1)	(-15.7, 18.20)

3.2.2b Relationship between physical activity, body composition and outdoor temperature with post-cooling SCV skin temperature

Post-cooling SCV skin temperature was observed in relation to LMI, FMI and physical activity. To look at the influence of temperature/season, outdoor temperature was included in the models. In a univariate analysis, post-cooling SCV skin temperature was inversely related to LMI and FMI, but there was no association seen with physical activity or outdoor temperature (Table 6). Note that scatter plots demonstrating the relationship for each variable with post-cooling SCV skin temperature are included in Appendix 2 (Figure A3-A7, Pages 78-80). After running the multivariate model, post-cooling SCV temperature, demonstrated a significant inverse relationship with FMI (Table 7a and 7b). For every unit increase in FMI, post-cooling SCV skin temperature decreases by 0.125ºC ($p<0.001$). Post-cooling SCV skin temperature did not have a relationship with lean mass, physical activity (minutes of MVPA) or outdoor temperature in the multivariate analysis. Note that a correlation matrix for all independent variables is included in Appendix 2 (Table A4, Page 78).

Table 6: Unadjusted univariate regression analysis for body composition, physical activity and outdoor temperature measures in relation to post-cooling SCV T.

	n	Unstd. β	Std. error	Std. β	R	Significance
Lean mass index	32	-0.290	0.061	-0.653	0.427	<0.001
Fat mass index	32	-0.119	0.012	-0.883	0.779	<0.001
MVPA	27	0.010	0.006	0.324	0.324	0.099
Counts/ Minute	27	0.001	0.001	0.289	0.083	0.144
Outdoor Temperature (C)	32	-0.018	0.010	-0.313	0.098	0.081

Table 7a: Multivariate regression analysis observing the relationship between LMI, FMI, Physical Activity and Outdoor temperature with Post-cooling SCV T (n=27)

Variable	B (unstandardized)	SEB	Beta (standardized)	P value
Constant	35.65	0.818		<0.001
Lean Mass Index (kg/m	-0.022	0.072	-0.047	0.758
Fat Mass Index (kg/m	-0.125	0.024	-0.881	<0.001
PA (MVPA)	0	0.004	-0.009	0.945
Outdoor temperature	0.004	0.006	0.074	0.489

Table 7b: Model Summary

R	R Square	Adjusted R Square	Significance
0.888	0.789	0.750	<0.001

3.2.2c Comparison of skin temperature outcomes in groups differing in physical activity and adiposity

It was of interest to compare groups with high adiposity (>75th percentile adiposity) and normal adiposity (<75th percentile adiposity) as well as groups with high physical activity (>60 minutes MVPA) and low physical activity (<60 minutes MVPA). A chi square test demonstrated that physical activity was not independent of adiposity (Pearson Chi Square 6.62, P=0.01) therefore high adiposity and normal adiposity groups were stratified by physical activity level. The characteristics of each group are outlined in Table 8.

Post Cooling SCV Skin temperature

The post-cooling SCV was lower in the high adiposity group (with no differences seen between groups differing in physical activity) (see figure 7). This is reflective of the multivariate analysis.

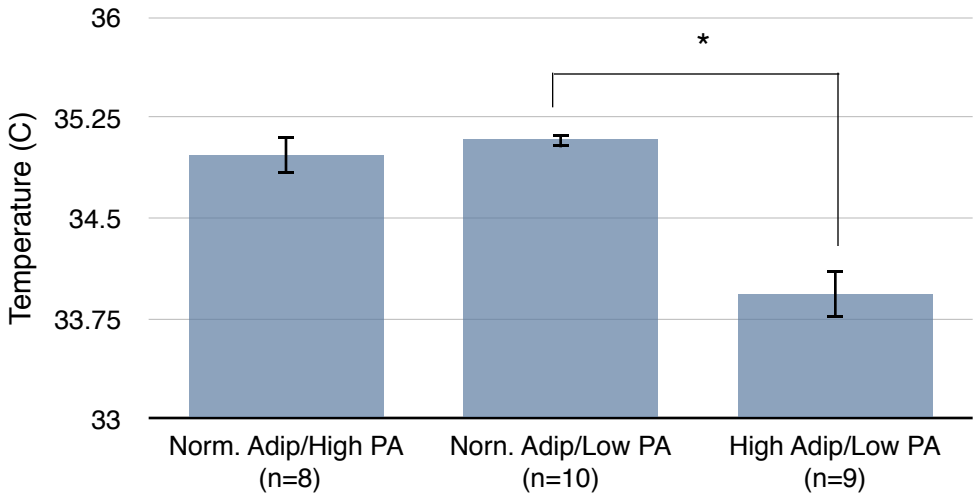


Figure 7: Post-Cooling SCV skin temperature in groups differing in PA and Adiposity. Values are average temperature in degrees celsius (+/- SEM). High PA Low Adiposity n=8, Low PA Low adiposity n=10, Low PA High Adiposity n=9. *p<0.001

Table 8: Characteristics of groups differing in adiposity stratified by physical activity level

	Normal Adiposity/ High PA (n=8)	Normal Adiposity/ Low PA (n=10)	High Adiposity/Low PA (n=9)	Significance
Age	9.13 (0.79)	9.14 (0.98)	9.64 (1.13)	0.446
Height (cm)	136.92	134.50	141.78	0.147
Weight (Kg)	31.77 (10.0)	28.80 (12.0)	46.27 (17.7)	<0.001
Percent Fat	15.15 (10.0)	14.20 (13.0)	38.50 (9.0)	<0.001
Lean Mass/height (m)	13.68 (2.0)	12.79 (0.96)	14.13 (1.66)	0.167
Fat Mass/height (m)	2.40 (2.23)	2.12 (2.53)	8.89 (4.80)	<0.001
MVPA	70.32 (29.24)	37.96 (21.69)	33.02 (30.38)	<0.001

Values are reported as median (IQR); significance level based on Kurskal-Wallis Test completed for all variables except age as assumptions for one-way ANOVA were not met. † Mean (SD) reported; significance level based on one way ANOVA

CHAPTER IV: DISCUSSION

The results of both Part 1 and Part 2 of this thesis will be discussed in the following sections

PART 1: Pattern, Accuracy, and Precision of SCV skin temperature response to cold as a measure of BAT activity (4.1.1-4.1.3)

4.1.1 Pattern of skin temperature response in SCV, sternum and chin region

In the current study, a steady increase in SCV skin temperature was observed during the cooling period, followed by a slight decline after removal of the cold stimulus. This was not observed in the average response curve of the control regions (the sternum and the chin), providing some evidence that the cold exposure is activating SCV BAT. It is important to note that the chin was not considered a reliable control region as there was high variability in the temperature response between participants at each time point and even between temperatures recorded at each time point as seen in Figure 2 (Results part 1). This suggests that many external factors could be influencing the skin temperature change pattern in the chin. Additionally, the sternum was not considered a reliable control region due to its proximity to the cold stimulus.

Upon exposure to cold, temperature sensing neurons in the periphery send signals to the pre-optic area of the hypothalamus. In a homeostatic response, afferent neurons release norepinepherine on the β3 adregernic receptors located on brown adipose tissue (Blondin et al, 2014), resulting in increase UCP1 activity and thermogenesis (Carpentier et al, 2012). As heat is released and dissipated around BAT during this process, it is hypothesized that the skin above the BAT area (SCV area in humans) will warm up (Symonds et al, 2012, Jang et al, 2014).

Therefore, the observation of an increase in SCV skin temperature during cold exposure is what would be expected during BAT activation.

On average, the maximum change in SCV skin temperature was 0.25°C observed at 25 minutes after administering the cold stimulus. This peak temperature is comparable to the change in SCV skin temperature observed by Symonds et al (2012) in 12 individuals aged 13-18 years old (0.25°C +/- 0.08°C). The change observed in all participants is lower than what Symonds et al reported in 7 children aged 3-8; 0.62°C +/- 0.14°C. The higher value reported in the younger group could be due to greater BAT in the very young children, as BAT in humans is highest in infancy and is shown to decrease gradually with age (Heaton, 1972). Furthermore, this population were all of normal body mass index whereas 12 out of 32 participants in our population had high BMI.

In contrast to the current study, Symonds et al. (2012) reported that the maximum increase in skin temperature is observed after 5 minutes of cold exposure, whereas this study found the maximum increase to occur between 20-30 minutes of cooling. This could be due to different acclimation periods; the current study used a 15 minute acclimation period whereas Symonds et al (2012) did not report their acclimation period. Additionally, Symonds et al. (2012) had participants place their hands or feet in 19-20 degree cold water for an unknown period of time prior to the thermal imaging; the cold exposure in this study was 30 minutes and was continuous. It is important to note that the population in the current study had 12 individuals demonstrate a decrease in the SCV skin temperature during the cold exposure. When looking only at individuals showing a positive change in skin temperature, the average peak change in SCV skin temperature from baseline was 0.42°C.

Two adult studies have reported skin temperature response patterns in the SCV region to cold. In adult males, a 1 hour cold exposure using a cold-water infused blanket resulted in a significant increase in SCV skin temperature (35.2 vs. 35.5) (Boon et al, 2014) which is comparable to the increase observed in the current study. Conversely, Jang et al (2014) measured SCV skin temperature after cold exposure using IR thermal imaging but did not see an increase in this region during cold exposure. This could be attributed to their cold exposure; they used a air conditioned room (19°C), therefore the SCV region was also exposed to the cold. Boon et al (2014), Symonds et al (2012) and the current study employed cold exposures that did not directly cover the SCV region.

4.1.2 ***Responders vs. Non Responders***

12 participants demonstrated a decrease in SCV skin temperature (Delta T <0 or AUC<0) whereas 20 participants had an increase in SCV skin temperature during the cold exposure (Delta T >0 and AUC>0). Consistent with the notion of responder and non responder reported in studies using ^{18}F FDG PET/CT to study BAT, we identified responders and non responders based their SCV skin temperature response. By choosing 0 as the cut off value, we are being liberal in that any increase, even a small one, is considered a response. It is important to note that the cold exposure was the same for all participants, therefore it is possible that individuals classified as "non-responders" were not as sensitive to the cold exposure. Boon et al (2014) used an individualized cooling protocol, whereby the cold exposure temperature was steadily decreased until shivering was reported and then increased by a few degrees until shivering stopped. In this way, they optimized the cooling temperature to the lowest possible before inducing shivering thermogenesis (but only as precisely as the participant could identify shivering). In future

studies, it might be helpful to implement a similar procedure in order to ensure all individuals have a comparable cooling experience. One limitation to this method is the fact that it is not known how the initial induction of shivering thermogenesis may influence non-shivering thermogenesis of BAT. Therefore it may be appropriate to establish the optimal cooling temperature for each participant on a day separate from the day BAT activity is measured. An additional limitation to this method is the possible variability in BAT response due to different temperature exposures between participants.

Responders and non-responders were compared based on the baseline SCV skin temperature (Table A1 Appendix 2, Page 75). The median baseline SCV skin temperature was significantly lower in responders compared to non responders (34.28°C vs. 34.79°C). As all subsequent temperature measurements are compared to this baseline temperature, it is possible that individuals were classified as "responders" because they happened to have a lower baseline temperature. As the acclimation period was only 15 minutes, it is possible that this wasn't long enough for all participants to reach a stable baseline. For example, the group classified as "responders" may not have fully warmed up to the room temperature after 15 minutes and therefore their SCV skin temperature could have continued to increase during the cooling period because they were equilibrating to the room temperature. For example, preliminary SCV temperature data over a one hour acclimation period in adult men demonstrate that SCV skin temperature does not begin to level out until around 40 minutes sitting at room temperature (Haq, 2015) (Figure A8, Appendix 2 Page 81).

4.1.3 Comparing Different skin temperature outcomes

As previously stated, there is no standardized method to determine BAT activity from SCV skin temperature outcomes, therefore several skin temperature outcomes were investigated. In terms of understanding change in SCV skin temperature response, the AUC and Delta T were thought to be a good starting place. However, change in skin temperature may not be the best measure of BAT activity if BAT is already active; if BAT is already activated and warming up the SCV region prior to the cold stimulus, (i.e during the winter months), then changes in SCV skin temperature due to a standard cold exposure may not be detectable. For example in non cold-stimulated studies measuring BAT in children using ^{18}F-FDG PET/CT, approximately 1/3 of children have detectable BAT (Ponrartana et al, 2013), suggesting that many children may have BAT active even without a prior cold stimulus. Because of this, baseline SCV skin temperature was measured. Boon et al (2014) reported post-cooling skin temperature in the SCV region as having a positive correlation with both BAT activity and BAT mass measured by ^{18}F-FDG PET/CT, therefore the current study was interested in understanding this measurement as well. We found that, on average, post-cooling SCV skin temperature was higher than baseline SCV skin temperature; a finding that is in agreement with the steady increase in SCV skin temperature seen during cold exposure.

Measuring the SCV region without adjusting for other factors that could influence the temperature in this region make it difficult to determine if the temperature patterns are due to BAT activity or some other physiological mechanism. Therefore, all SCV skin temperature outcomes (AUC, Delta T, Baseline and Post-cooling) were adjusted for the sternum temperature response. The sternum was chosen over the chin as it was less variable between participants.

Additionally, Jang et al (2014) found the difference between the SCV region and chest to be predictive of BAT activity measured by PET/CT. Specifically, in the BAT positive groups, the difference between the SCV and chest on the left and right were 1.2°C and 1°C whereas in the BAT- groups, the differences in the left and right regions were 0.6°C and 0.5°C. Although we did not have chest region as a control, the sternum is more comparable to the chest region than the chin is. In the current study, all participants had a baseline difference between SCV and sternum of 1.91°C and a post-cooling difference of 2.32°C. These differences are much higher than what is reported by Jang et al (2014), which could be because of the age difference in study populations (pre-pubertal boys vs. adults) or because of the different cooling protocols. However as previously stated, the sternum region was very close to the cooling blanket and demonstrated a decrease in temperature. Because of this, actual BAT activity may not be represented when adjusting SCV skin temperature for this region. In future studies, a better "control" may be the SCV skin temperature change overtime without a cold exposure. This can be done by repeating the thermal imaging protocol on a separate day without introducing a cold exposure. Although there may be variability due to different testing days, replicating the conditions in combination with a longer acclimation may help reduce some of this variability.

4.1.4 Evaluation of skin temperature outcomes

Precision: Reproducibility of SCV skin temperature outcomes

Reproducibility of the 8 potential skin temperature outcomes was observed to determine the precision of each measurement. The current study found baseline and post-cooling SCV skin temperatures (both unadjusted and adjusted for the sternum) had high and significant ICC values,

suggesting good reproducibility. The skin temperature measures of change in skin temperature response pattern overtime demonstrated poor reproducibility. Overall these data suggest that IR thermal imaging to detect skin temperature is reproducible when measuring absolute temperatures (at baseline and after cooling), but temperature response patterns/changes in temperature are highly variable in repeated trials in the same participant. One reason for this could be due to the scale of each outcome measure. The absolute temperatures were between 32-34°C and did not vary by more than a couple tenths of a degree between visits, whereas the AUC and Delta T measures were all less than 1. Therefore, small differences (i.e tenths of a degree) in AUC and Delta T values between trials would constitute a relatively large difference. It may be necessary to have a larger sample size with more than 2 reproducibility sessions to confirm the reproducibility of the Delta T and AUC values. It is possible that the variability in skin temperature change over time (AUC and Delta T) is due to the short acclimation period of 15 minutes. As the population of interest was young children, a protocol longer than 1 hour may have been difficult for participants to comply with. As previously mentioned, data in adult men observing SCV skin temperature (measured by IR thermal imaging) during a 1 hour acclimation show that SCV skin temperature takes longer than 15 minutes to settle (Figure A8, appendix 2, Page 81).

Few studies have reported on the reproducibility of BAT imaging by methods such as ^{18}F FDG PET/CT. Lee et al (2010) reported that the the probability of detecting BAT in the same individual on two occasions was 13.3%. However, this was based on analysis of non-cold stimulated scans obtained at various times of day and in several conditions. Symonds et al (2012) reported that SCV skin temperatures were consistent and reproducible during a control

period (no cold stimulus), with the coefficient of variation between control periods of 1.57% and within control periods of 0.46%-0.67%. However the control period was only 5 minutes long and they did not report the time between control periods nor did they assess reproducibility of the skin temperature response to a cold stimulus.

Precision of methods to measure BAT may be limited due to the plasticity of BAT (van Marken Lichtenbelt, 2012). For example it is known that BAT detection by PET/CT can be influenced by the season (Ouellet et al,. 2010) and it has also been shown to change in patients after undergoing bariatric surgery patients (Vijgen et al,. 2012). The time frame and exposures that cause BAT plasticity are not yet clear. However, Jang et al (2014) performed repeated PET/CT scans in a subset of their participants 2-3 weeks after their first scan. Interestingly, they decided to treat each measurement (including repeated measurements in the same individual) as a separate case because of possible variability in BAT activity within each patient. This suggests that there was high variability in ^{18}FDG PET/CT scans to detect BAT activity even within a 2-3 week time frame.

Another possible reason to explain the high variability in skin temperature response patterns during cooling (AUC and Delta T) could be variability in heat conductance to the skin from BAT. IR-thermography and iButtons have been criticized in their ability to detect thermogenesis because of the relatively low amount of heat released to the skin compared to the core (Anouk et al, 2014). Therefore even if BAT activity is similar between two trials, it is not clear how similar a BAT related skin temperature response would be.

Accuracy: Relationship between REE and SCV skin temperature outcomes

At baseline, there was a correlation approaching significance (p=0.07) between energy expenditure adjusted for FFM (at baseline) and absolute SCV skin temperature. Furthermore, after cold exposure, energy expenditure adjusted for FFM was significantly correlated with absolute skin temperature in the SCV region (p=0.032). One odd finding was that post-cooling SCV skin temperature adjusting for the sternum temperature had an inverse correlation, approaching significance, with energy expenditure adjusted for FFM after cold exposure (p=0.06). This suggests that the greater the difference between the SCV and sternum, the lower the energy expenditure. Because of the decrease in sternum skin temperature during cold exposure, this skin temperature response may not be reflective of BAT thermogenesis. Overall, this data suggest that post-cooling SCV skin temperature may be the most representative of BAT activity since it was the only skin temperature outcome that was significantly correlated with energy expenditure.

Changes in energy expenditure were not related to changes in SCV skin temperature or changes in SCV skin temperature adjusted for the sternum temperature (AUC and Delta T outcomes). There are several reasons why this may be. One reason is the already established high variability of changes in SCV skin temperature (AUC, Delta T and AUC and Delta T adjusted for sternum). Additionally, changes in REE were also very variable. This may be due to the challenges associated with performing this procedure in children. For example, Symonds et al (2012) reported that young children are less likely to remain in the same position for prolonged periods of time. This is one of the reasons for the shorter duration of their cold stimulus prior to recording SCV skin temperature (Symonds et al, 2012). Furthermore, these measurements were

completed in two separate study visits, therefore if BAT activity is highly variable from day to day, it is possible to see differences between the energy expenditure changes and skin temperature changes due to cold. Lastly, this lack of relationship may have been due to basally active BAT contributing to energy expenditure which was addressed by looking at the relationship between baseline SCV skin temperature and baseline energy expenditure.

As the current study could not compare SCV skin temperature with FDG PET/CT imaging of BAT, energy expenditure was used as the "standard"comparison. BAT activity is known to increase energy expenditure through oxidative metabolism (Ouellet et al, 2012). Additionally, in rodent models measuring energy expenditure with a metabolic cage, UCP1 knockout mice do not demonstrate an increase in energy expenditure after cold exposure (Crane et al,. 2014). Therefore it is thought that increases in energy expenditure in response to cold stimulus can reflect BAT activity. Although there aren't any human studies reporting a relationship between changes in energy expenditure and SCV skin temperature, in rodents, change in oxygen uptake was significantly related to change skin temperature (overlaying the BAT region) after cold exposure (Crane et al, 2014).

It is important to note that energy expenditure changes with cold exposure may not be the best measurement for BAT activity. Some note that it may be difficult to distinguish the possible contribution of shivering thermogenesis to the increase in energy expenditure noted after cold exposure (Anouk et al, 2014). For example, there may be involuntary muscle movements that do not visibly show as shivering, and may contribute to the increase in energy expenditure (Anouk et al, 2014, Ouellet et al, 2014).

PART 2: Relationship of post-cooling SCV skin temperature with Body Composition and Physical Activity (4.2.1-4.2.3)

4.2.1 Adiposity and Post-cooling SCV Skin Temperature

SCV skin temperature after cold exposure was inversely related to adiposity (measured with DXA). Additionally, children with adiposity classified as 75th percentile or greater had a significantly lower post-cooling SCV skin temperature compared to children with adiposity less than the 75th percentile. These results extend the observations by Robinson et al (2013) who reported a significant inverse correlation between BMI percentile and change in SCV skin temperature after a 5 minute cold exposure involving an extremity placed in cold water ($r=0.384$, $p=0.004$). Several studies measuring BAT with ^{18}F FDG PET/CT have also demonstrated an inverse relationship between BAT and adiposity. For example, Saito et al (2009), Yoneshiro et al (2011) and Hwang et al (2015) all reported an inverse relationship between total adiposity, subcutaneous adiposity, and visceral adiposity and BAT activity and/or presence in adults. Although fewer studies have looked at adiposity in children, Chalfant et al (2011) reported that BAT negative children had a greater increase in adiposity between baseline and a follow up visit (4.7 ±2.4 month interval) compared to BAT positive children (based on 18 FDG PET/CT).

Although causation cannot be established in this cross-sectional study, it provides evidence that low BAT activity or absent BAT may contribute to higher adiposity in young children. One of the short comings of this technique is that it is based on skin temperature and not direct BAT temperature, therefore it is uncertain how the layer of subcutaneous fat under the skin influences surface skin temperature in individuals with higher adiposity. However, Robinson et al (2013) reported that their unpublished thermography studies in adults suggest that high BMI

would not be a major factor in limiting the ability to detect a thermal response of BAT by measuring SCV skin temperature. Therefore as BMI is considered to be representative of adiposity (Flegal et al, 2010), it is possible that the lower SCV temperature seen in the higher adiposity group is representative of absent BAT activity and not due to excess subcutaneous fat. Additionally, a study looking at surface skin temperature patterns in overweight and normal weight adult women reported no difference in chest skin temperature (Chudecka et al, 2014) between overweight and normal weight women. However these data are based on adult studies, and although the chest is in proximity to the SCV region, it is not exactly the same. Future studies should look into the relationship between subcutaneous fat in the SCV region and skin temperature to determine if there is a need to correct for subcutaneous fat thickness. This could be done by measuring SCV skin fold thickness and relating it to surface skin temperature.

4.2.2 Lean Mass and Post Cooling SCV Skin Temperature

No relationship between our measure of BAT activity and lean mass was found. As SCV skin temperature is a potential measure of BAT activity, this could indicate no relationship between lean mass and BAT activity in children, which is contradictory to what is currently reported in adults and adolescents. For example Gilsanz et al (2011, 2012) reported that children between the ages of 6-20 who demonstrated BAT on a PET/CT had a significantly larger muscle volume in the gluteal region and neck. In adults, Hwang et al (2015) reported that the uptake of novel tracer -norepinepherine transporter ligand- on PET/CT imaging was higher in individuals with higher lean mass (measured by bioimpedance).

There are several reasons that could explain why we did not find a relationship between SCV skin temperature and lean mass. One reason could be the age of the study population in this study compared to the previously mentioned studies. The current study had a very narrow age group - boys between the ages of 8-10- whereas the other paediatric study reported the relationship in a population of children between the ages of 6-20. As muscle changes dynamically with age with respect to muscle area and fibre type (Lexell et al,. 1992) it is possible that the relationship between muscle and BAT does not become apparent until puberty. This would be in line with reports by Gilsanz et al (2011) stating that BAT was more prevalent in children in tanner stages 3, 4 and 5 vs. Tanner stages 1 and 2. This was suggested to be due to greater muscle volume in the later stages of puberty. Additionally, in infants, BAT is highly prevalent and decreases during the transition into early childhood (Heaton, 1972) whereas this time is characterized by an accretion in lean mass (Lexell et al,. 1992). Another reason for this contradiction could be due to different methodologies employed to measure BAT across studies. The current study measured SCV skin temperature as an indication of BAT activity, however Gilsanz et al (2011 and 2012) looked at BAT presence or absence based on retrospective review of PET/CT data without a standardized cold exposure. Hwuang et al (2015) measured BAT activity on a PET/CT with the standard 18F-FDG tracer as well as a novel tracer that binds to a norepinepherine transporter. They found the relationship between lean mass and BAT activity measured with the novel tracer but did not report a relationship between lean mass and FDG uptake in BAT. Additionally, the methods used to assess lean mass were different in all of these studies. Our study used DXA, which provides total lean mass in Kg, Hwuang et al (2015) used

bioelectrical impedance technology to measure total lean mass and Gilsanz et al (2011, 2012) reported muscle volume measured with a CT scan.

4.2.3 Physical Activity and Post-Cooling SCV Skin Temperature

No relationship was observed with SCV skin temperature outcomes and minutes of MVPA or total counts/minute measured over 7 days with an accelerometer. As SCV skin temperature is used as the measure of BAT activity, these results may suggest no association between regular physical activity and BAT activity in children. Exercise is believed to influence BAT as demonstrated by animal studies (De Matteis et al, 2013), however there is limited evidence in human studies. The mechanism by which exercise could potentially influence BAT in humans has not been fully elucidated, however researchers have reported that exercise may work to stimulate BAT through sympathetic stimulation or myokine release from muscle (Ruiz et al, 2015). In humans, these mechanisms may work through the induction of beige/brite adipose tissue rather than through the stimulation of classical BAT. For example, the myokine B-amino-butyric-acid (BAIBA) was demonstrated to induce a brown adipocyte like phenotype in human induced pluripotent stem cells in vitro (Roberts et a, 2014). Our results are in contradiction to the one other study that has reported on the relationship between habitual physical activity and BAT activity (Dinas et al, 2014). Dinas et al (2014) found that habitual physical activity in METs-minutes/week had a positive relationship with BAT activity measured by ^{18}F-FDG uptake on PET/CT scan in adults. However, this study employed a subjective physical activity questionnaire (IPAQ) which is not as reliable as measuring physical activity objectively (Arem et al,. 2015) like what was done in our study.

There are several reasons why we may not have observed a relationship between physical activity and SCV skin temperature in this study. One reason is that physical activity may not influence SCV BAT depots in children in the same way as adults. As there are no other published studies looking at physical activity in children in relation to BAT activity, this relationship is currently not established. If physical activity or exercise works to influence BAT through the muscle, we may not have observed a relationship for the same reasons why there was no relationship between lean mass and SCV skin temperature; children's muscle is still developing and growing and therefore different than adult muscle (Lexell et al,. 1992). Furthermore, there was no relationship seen between physical activity (MVPA) and lean mass index in this population (Figure A9, Appendix 2, Page 82), therefore, if physical activity influences BAT though muscle, this study would not have observed a relationship. Another potential reason why no relationship was seen is the fact that children's physical activity patterns are different than adult physical activity patterns. Children's physical activity is characterized by many intermittent bouts of MVPA in contrast the single bouts of continuous activity that may be seen in adults (Welk et al, 2000). It is possible that sustained or continuous activity patterns are necessary to influence BAT activity. For example, in the animal studies reporting a positive influence of exercise on BAT, rodents were exercised one hour a day (continuous treadmill running) every day for 5-7 days. (De Matteis et al, 2013, Durant et al, 2013).

4.3 Limitations

There are several limitations to this study involving the methodologies and study population. Using SCV skin temperature as a measure of BAT activity is limited in that it has not been established as a way to measure BAT reliably in humans. Although there have been reports that cold-induced SCV skin temperature is positively correlated with both BAT clavicular volume ^{18}F-FDG uptake on PET/CT imaging of BAT ($R^2 = 0.20$, $p=0.03$ and $R^2=0.27$, $p=0.01$ respectively), the current study was unable to compare SCV skin temperature in the population with ^{18}F-FDG PET/CT imaging. Additionally, all participants were exposed to the same cold exposure, however it is possible that different individuals respond differently to cold; this may have influenced the activation of BAT.

We are also limited with our protocol because it had to be designed in a way that is tolerable by 8-10 year old boys. For example, the acclimation and cooling periods had to be set at a timing that would allow an 8-10 year old boy to remain relatively still, therefore we were limited in the amount of time of each exposure. Even with the current timing it was still difficult for participants to remain still. This also applies to the length of the REE measurement. Boys were to remain still for 40-50 minutes, since the cold exposure was between 20-30 minutes, the amount of time in acclimation could not be extended anymore. Furthermore, it was difficult and uncomfortable for many children to stay still underneath the clear ventilated hood for the duration of the measurement and it is unknown how this could have influenced the REE measurements.

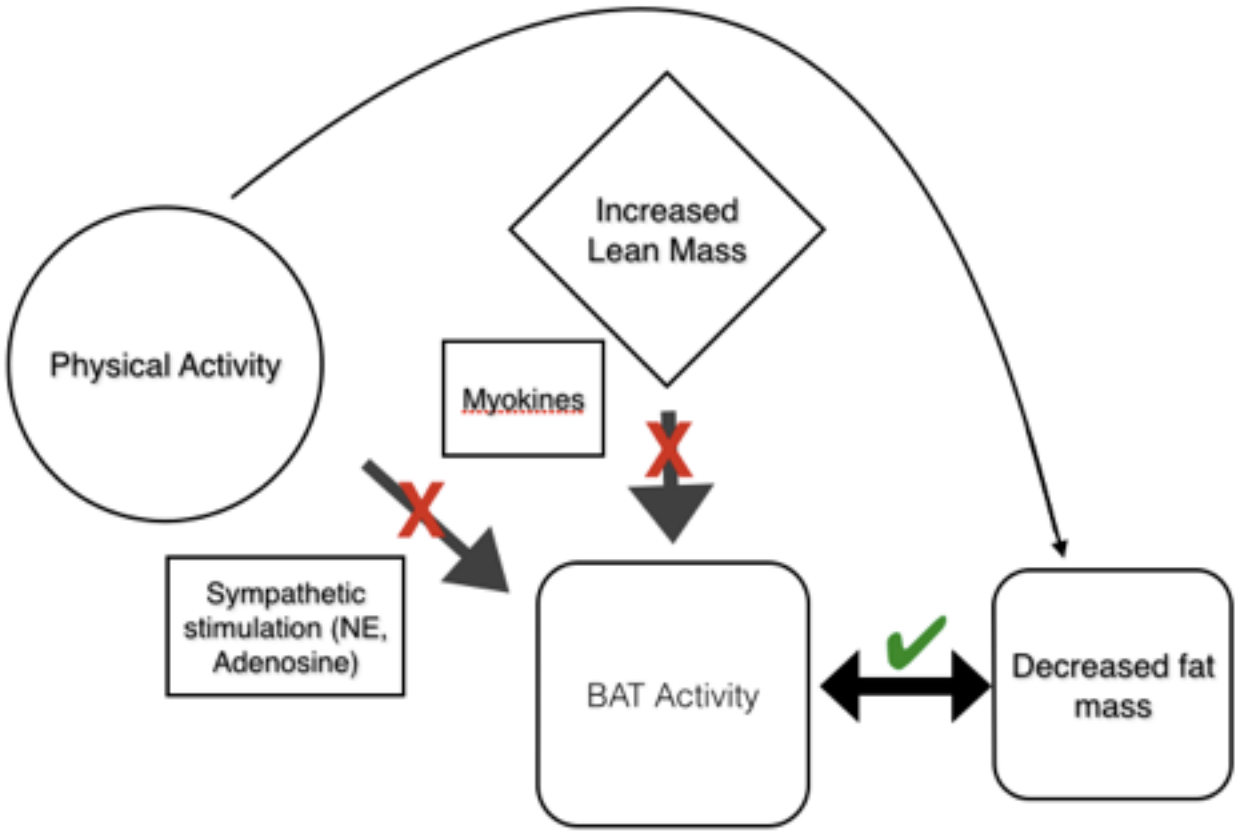
The sample size of this population was also underpowered to examine multiple variables and their relationship to BAT activity. For example the regression analysis only included 27

individuals whereas the sample size needed for the number of independent variables was 40. However, even with this smaller sample size, a significant relationship ($p<0.001$) was seen with adiposity. Those variables that did not show a relationship (PA, lean mass and outdoor temperature), did not appear to have an independent relationship, not even one approaching significance, with SCV skin temperature. Therefore it is possible that the same trends would have been seen with a larger sample size.

The measure of physical activity used was an accelerometer. Although this is superior to a self-report questionnaire, it still has limitations. For example, the children are aware that they are wearing a device that is recording their physical activity, therefore they may not have behaved in a way representative of their regular physical activity patterns. In addition, the accelerometer was only monitoring activity for one week, therefore if the week was not representative of a normal week for any reason (i.e sickness, vacation etc.) the child may have been misclassified as over or under active. Regardless of these limitations, this is the first study to employ an objective measure of physical activity to determine its association with BAT activity.

4.4 Conclusion

Based on the investigation of several skin temperature outcomes obtained using IR thermal imaging of SCV/neck/chest region during cold exposure, post-cooling absolute skin temperature may be the best measure of BAT activity as this measure was highly reproducible and was related to energy expenditure after cold exposure. Using this method to quantify BAT activity, we observed an inverse relationship between BAT activity and adiposity in 8-10 year old boys, but no relationship to lean mass or objectively measured habitual physical activity. Based on these findings, the initial model has been modified:



4.5 Future Directions

SCV skin temperature as a measure of BAT activity should be further evaluated to determine its validity in humans. In future studies, a longer acclimation period is recommended to ensure stable baseline SCV skin temperature. The current study did not find the sternum or the chin to be adequate control regions, therefore a more appropriate control region should be sought in a non BAT region of the body. Alternatively, the sternum may be an appropriate control region if the cold exposure is modified to ensure the the SCV region and sternum are influenced in the same way by the cold. This would require testing several different positions in which a cold blanket or other cold stimulus is placed. Furthermore, it may be important to implement an individualized cooling protocol to ensure all individuals have a similar cold exposure experience. Although, when introducing such changes, the reproducibility and accuracy of SCV skin temperature would have to be reassessed for each different condition (i.e longer acclimation, modified cold exposure, individualized cooling protocols). Implementing these measures may reduce the influence of external factors controlling the SCV skin temperature response to cold.

Lastly, as there was a relationship between adiposity and SCV skin temperature, it is important to establish the influence of subcutaneous fat tissue on the emission of heat in the SCV region during cold exposure. This could be done by measuring skin fold thickness in the SCV region and how it relates to the surface skin temperature. As IR thermal imaging is feasible based on its safety and relative ease, it should not be dismissed as a measure of BAT activity in the human population. Currently, all studies are limited by the lack of consensus around a reliable

methodology to measure BAT activity. Given the potential of BAT as a therapeutic target for metabolic disease, efforts in this area are highly desirable.

<u>Appendix 1:</u> Figures and Calculations related to Methods

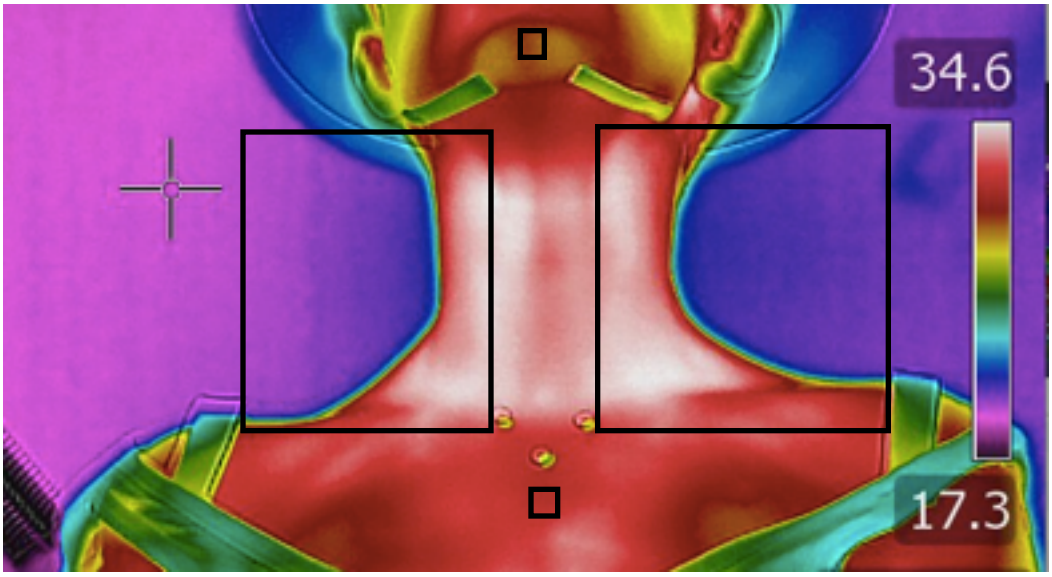


Figure A1: Example of a thermal image taken to measure SCV skin temperature, chin skin temperature and sternum skin temperature. Notice the 7 anatomical markers placed at the jawline, the left and right acromion joints, and surrounding the sternal notch region. The 4 boxes mark the regions of interest (ROI) for the chin, the left and right SCV region and the sternum. Chin and Sternum ROIs were 121 mm^2 and the left and right SCV ROIs were drawn based on the anatomical markers as shown.

A1.1 Wear time Calculation

Actual wear time was determined based on a combination of ActiGraph software analysis and participant self report. First the accelerometer wear time was validated in ActiLife software (Version 6, ActiGraph, Pensacola FL) based on the following criteria:

- Minimum Length- 10 minutes
- Activity threshold- 0 counts per minute
- Spike tolerance- 1

Appendix 1

Following initial automated wear-time analysis in the ActiLife software, participant accelerometer logs were cross checked with automated wear time output. For example, if the log indicated that the accelerometer was on the participant, but the program indicates the time period as "non-wear time" this time period was included in the wear time. Additionally, if the participant noted a time period in which the accelerometer was not worn (i.e showering/sleeping etc) but the program indicates activity, this period was excluded from the wear time.

Formula 1: The following formula obtained from (Katapally et al, 2014) was used to adjust MVPA according to wear time. Note that WT_{CON} = 10 x ∑Valid Days and WT_{UNCON} = Total wear time for all valid days

$$\text{Mean Standardized MVPA (minutes)} = \frac{(Wt_{CON}) \times \text{Unstd MVPA (minutes)}}{Wt_{UNCON} / \sum \text{Valid Days}}$$

Formula 2: The following formula was used to weight MVPA and CPM according to whether they were attained on weekends or weekdays.

$$\frac{[\text{Std. Weekday MVPA (min)} \times \#\text{weekdays}] + [\text{Std. Weekend MVPA (min)} \times \#\text{weekend days}]}{(\#\text{Weekdays} + \#\text{Weekend Days})}$$

Appendix 2: Supplementary data related to results

A2.1 Comparison of characteristics and skin temperature outcomes in "Responders" vs. "Non Responders"

Responders and non responders were compared based on characteristics including body composition, physical activity, energy expenditure and skin temperature outcomes. All characteristics were similar in mean or median for each group except for baseline SCV temperature, which demonstrated a lower median for the responders group (p=0.029)

Table A1: Comparison of characteristics between responders and non responders

Variable	n included in analysis	Mean (SD) of Responders (n=20)	Mean (SD) of Non Responders (n=12)	Significance
% Fat	32	31.25 (24.5)	16.05 (21.7)	0.076
BMI Z Score	32	1.72 (2.04)	0.78 (1.74)	0.194
Weight (Kg)	32	39.10 (17.2)	33.80 (16.4)	0.136
LMI	32	13.55 (1.43)	13.32 (2.09)	0.632
FMI	32	6.23 (6.27)	2.55 (5.78)	0.116
MVPA	27	40.49 (26.04)	58.08 (30.9)	0.114
Change in RMR after cold	30	26.0 (198.5)	69.0 (321.0)	0.692
Baseline RMR (kcal/day/ KgFFM)	32	45.05 (4.7)	45.63 (4.25)	0.730
Post cooling RMR (kcal/ day/KgFFM)	32	46.26 (8.30)	47.52 (4.25)	0.632
Outdoor temperature	32	4.63 (11.22)	0.55 (12.70)	0.350
Baseline SCV	32	34.28 (1.19)	34.79 (0.87)	0.029
Final SCV	32	34.56 (0.97)	34.88 (0.99)	0.326

Values are reported as Mean (SD), significance values based on independent samples T Test (students T test)
†Values are reported as Median (IQR); significance values based on Mann Whitney U Test as variables did not meet assumptions of the independent samples T test.

A2.2 Comparison of change in temperature (AUC and Delta T) between the sternum, SCV and chin in responders and non responders

Table A2: AUC and Delta T for skin temperature in SCV, Sternum and Chin for "non responders" as demonstrated in figure 2a (n=12)

	Mean AUC (SD)	Mean Delta T (SD)
SCV	-1.09 (3.60)	0 (0.10)
Sternum	-11.13 (8.2)	-0.37 (0.25)
Chin	-7.21 (18.3)	-0.21 (0.56)

Table A3: AUC and Delta T for skin temperature in SCV, Sternum and Chin in "responders" as demonstrated in figure 2b. (n=20)

	Mean AUC (SD)	Mean Delta T (SD)
SCV	10.98 (5.7)*	0.38 (0.19)*
Sternum	-1.26 (13.03)	0 (0.41)
Chin	-1.94 (20.2)	-0.05 (0.67)

A2.3 Supplementary figures demonstrating reproducibility for skin temperature outcomes controlling for the sternum region

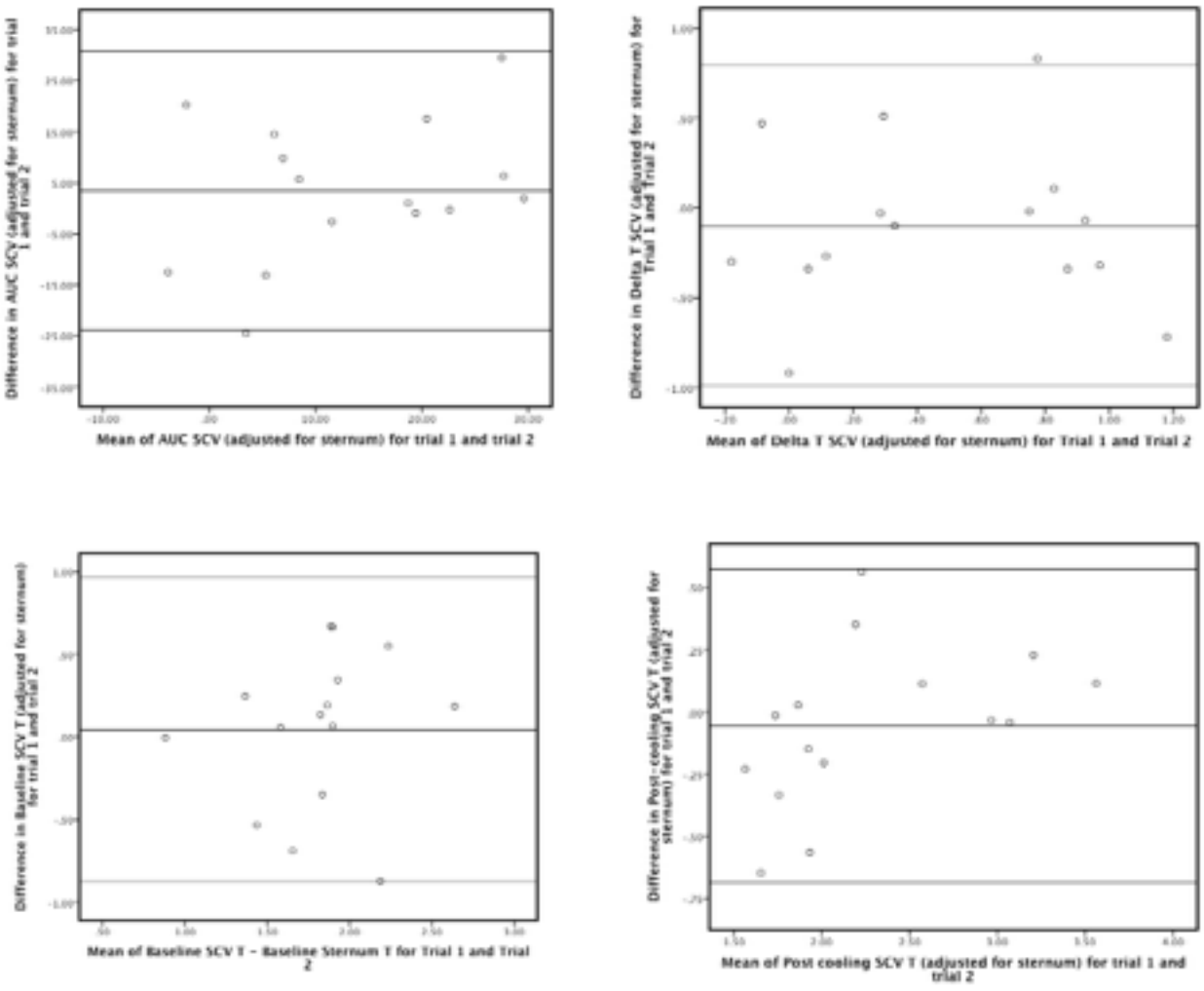


Figure A2: Bland-Altman plots demonstrating the reproducibility of SCV AUC, SCV Delta T, Baseline SCV and Post-cooling SCV skin temperatures adjusted for sternum temperature.

A2.4 Supplementary tables and figures with respect to Univariate and Multivariate analysis

Table A4: Correlations Between Independent Variables in Multivariate Regression

		1	2	3	4	5
1	Lean Mass Index	-	0.665*	0.081	0.227	0.094
2	Fat Mass Index	-	-	-0.381*	0.332*	-0.294
3	MVPA	-	-	-	0.011	0.741*
4	Outdoor Temp	-	-	-	-	0.150
5	Counts per Minute	-	-	-	-	-

* ($p<0.05$)

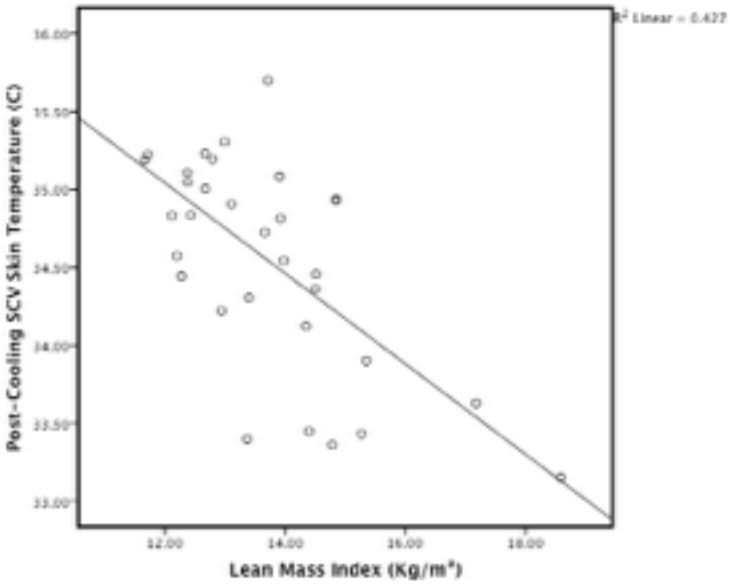


Figure A3: Correlation plot showing relationship with post-cooling SCV skin temperature and LMI (n=32)

Appendix 2

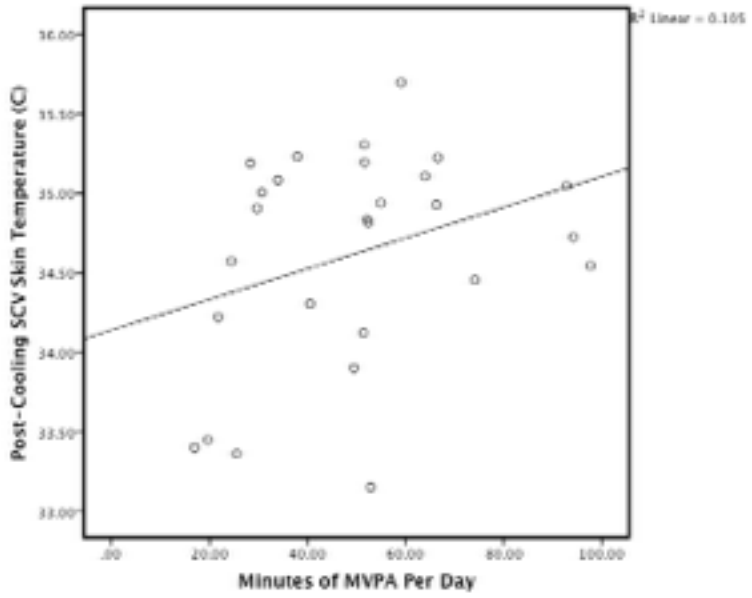


Figure A4: Correlation plot showing relationship with post-cooling SCV skin temperature and MVPA (n=27)

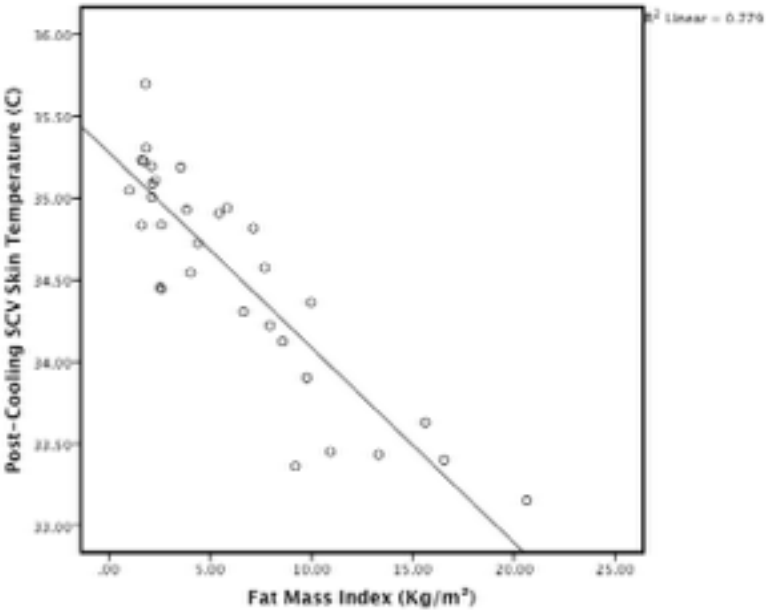


Figure A5: Correlation plot showing relationship with post-cooling SCV skin temperature and FMI (n=32)

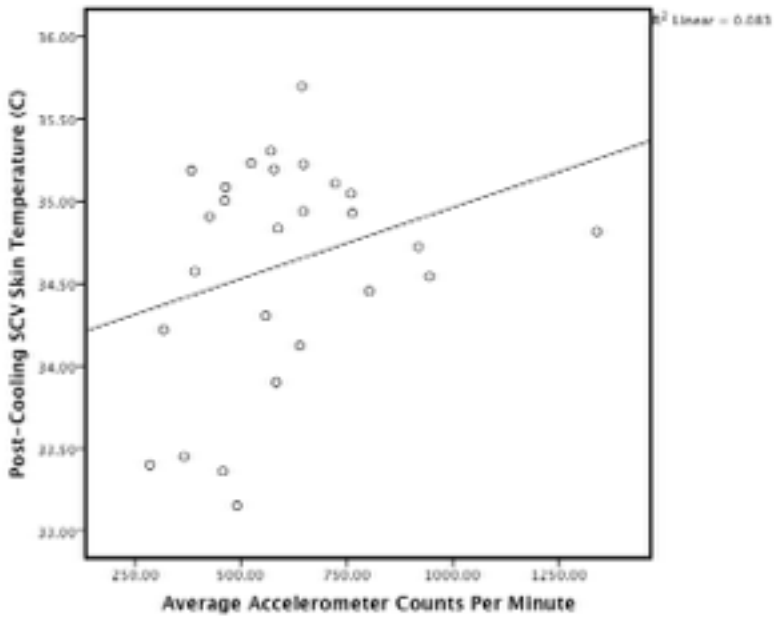


Figure A6: Correlation plot showing relationship between post-cooling SCV skin temperature and average accelerometer counts per minute (n=27)

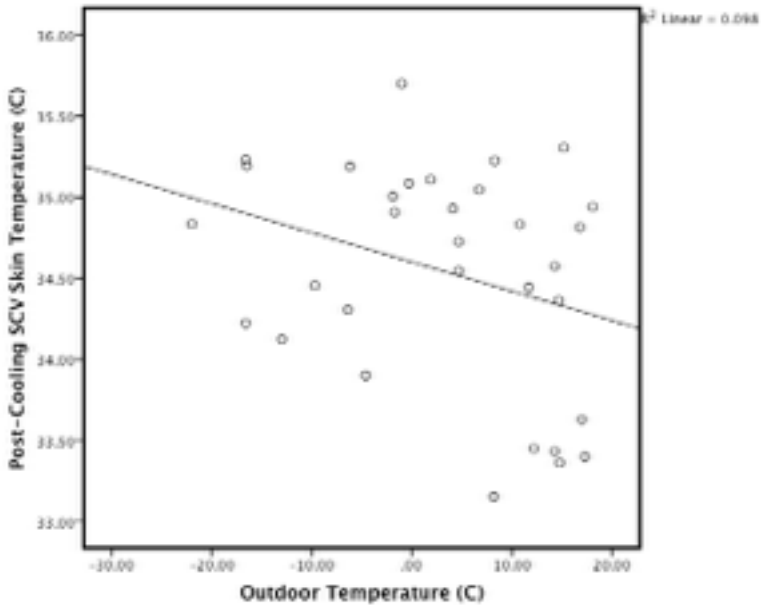


Figure A7: Correlation plot demonstrating relationship between post-cooling SCV skin temperature and outdoor temperature (n=32)

A2.5 Acclimation of SCV skin temperature over 1 hour exposure at room temperature

In figure 2, the SCV skin temperature changes over a one hour period at room temperature are outlined. It appears that SCV skin temperature rises during the first 40 minutes of acclimation before beginning to level out. This data suggest that a longer acclimation period is needed before exposing participants to cold in order to get a reliable estimate of the changes in SCV skin temperature due to cold exposure.

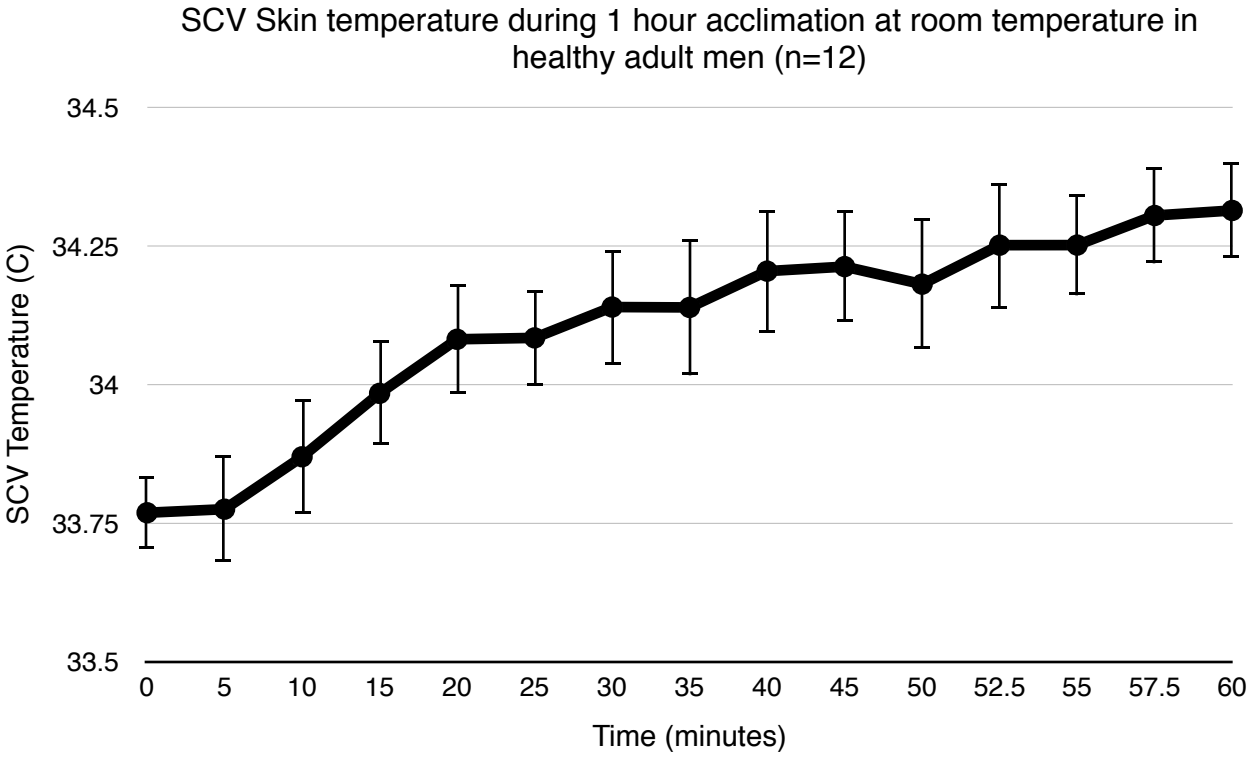


Figure A8: Average SCV skin temperature for 12 healthy men during a one hour acclimation period at room temperature (between 22-24 °C). Values are mean (+/- SEM).

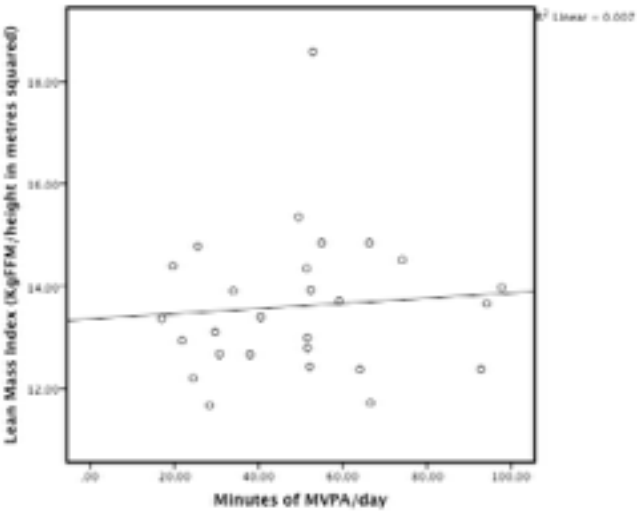


Figure A9: Correlation plot showing no relationship between lean mass index and MVPA. Pearson correlation is 0.081 (p=0.689).

A2.6 Heart Rate and Oral Temperature response to cold

Heart rate was monitored before, during and after cold exposure in 7 participants and oral temperature (OT) was monitored in 9 participants. HR and OT did not change in response to the 30 minute 12 degree cold exposure as demonstrated in Figure A10 and Figure A11 below.

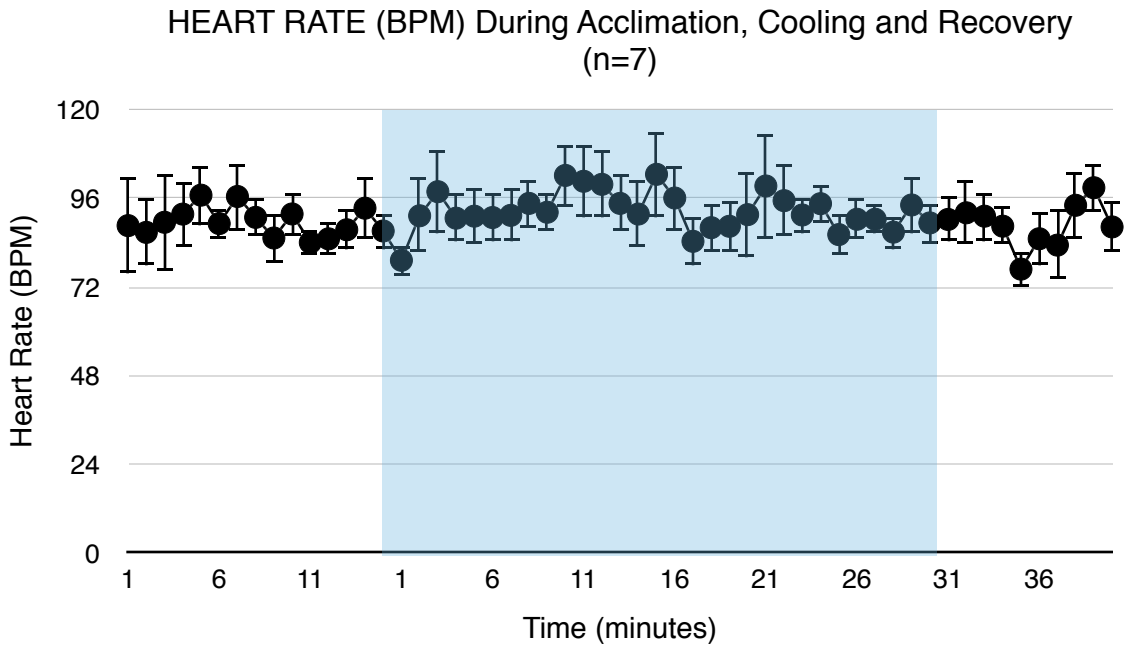


Figure A10: Average heart rate (BPM) during acclimation, cooling and recovery in 7 healthy boys. Values are mean (+/- SEM).

Table A5: Average HR at each time period

	Acclimation	**Cooling**	**Recovery**
Average HR (SD)	89.55 (3.92)	92.5 (5.3)	88.79 (6.1)

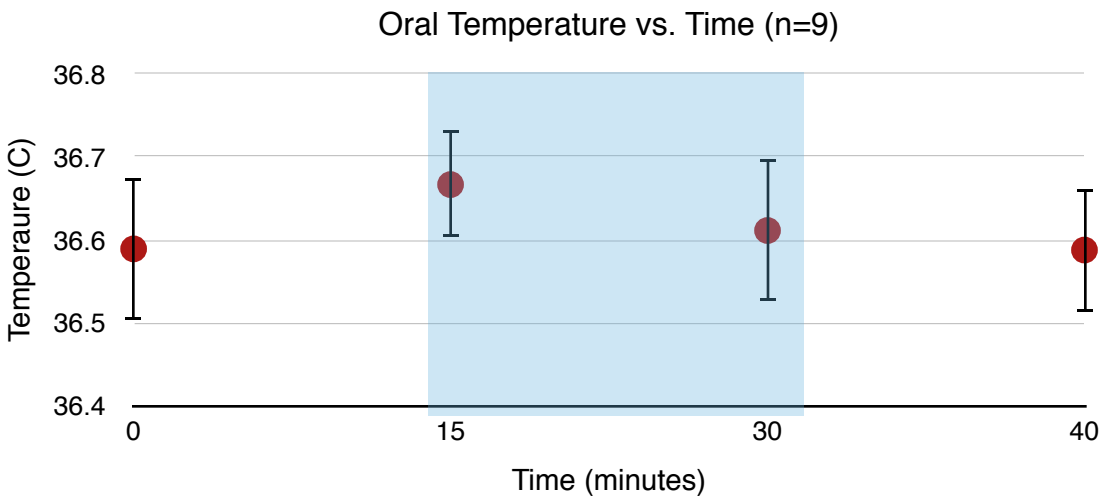


Figure A11: Average Oral Temperature (C) taken at baseline after 15 minutes of acclimation, twice during cold exposure and 10 minutes after removal of the cold exposure. Values are average (+/- SEM).

Table A6: Average Oral Temperature (mean +/- SEM) at baseline, during cooling and 10 minutes after cooling in 9 participants

	Baseline	**15 minutes**	**30 minutes**	**10 minutes post cooling**
Average Oral Temp (SD)	36.6 (0.25)	36.7 (0.20)	36.7 (0.18)	36.6 (0.21)

Anova model insignificant for any differences in oral temperature at each time point.

Discussion of Oral Temperature and Heart Rate outcomes

Heart rate response to cold

Heart rate was monitored during acclimation, cooling and recovery, to get an objective measure of how sensitive participant's were to the cold exposure. As catecholamines act to increase heart rate (Goldberg, 1960), and cold is thought to stimulate BAT through catecholamine release (Blondin et al, 2014), it was thought that an increase in HR would provide evidence that the cold was in fact inducing a sympathetic response. For example Orava et al (2011) found a significant increase in plasma NE in participants after they spent 2 hours in a 17

degree room and intermittently placed their feet in an 8 degree cold water bath. After observing HR in a subset of the study population (n=7), no significant increase in HR was observed. Although the expected response was not seen, it does not necessarily mean that participants did not respond to the cold. It is possible that subtle changes in HR due to cold exposure may not be detectable as there are many other factors that influence heart rate including environmental stress. Additionally, increases in HR due to cold may not manifest after a longer mild cold exposure. For example, Korhonen (2006) studied HR in men exposed to a 10 degree cold chamber for 2 hours. During the first 10 minute there was no change in HR and after one hour there was a slight decrease in HR. Based on this, heart rate after cold exposure may not increase due to NE release and may therefore not be the best measure of NE release due to cold exposure.

Oral Temperature response to cold

Oral temperature was not observed to change during cold exposure in a subset of 9 participants. This is consistent with findings by Boon et al (2014) who reported no difference in mean core body temperature (measured by an ingested telemetric pill) during their cold exposure, however, all participants in the Boon et al study demonstrated BAT activity. Chondronikola et al (2014) reported a slightly lower core body temperature during cold exposure in BAT- individuals (assessed by FDG PET/CT). As the current study was unable to characterize the presence of BAT, it is unknown if all participants who had their temperature measured were BAT+ or not.

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