**The time course of changes in brown adipose tissue fat fraction during cooling and warming in adult males**

The time course of changes in brown adipose tissue fat fraction during cooling and warming in adult males

By Stephan Mark Oreskovich, B.HSc.

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**TITLE:** The time course of changes in brown adipose tissue fat fraction during cooling and warming in adult males

**AUTHOR:** Stephan Mark Oreskovich, B.HSc. (McMaster University)

**SUPERVISOR:** Dr. Katherine Morrison

**COMMITTEE:** Dr. Katherine Morrison, Dr. Gregory Steinberg and Dr. Zubin Punthakee

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**ABSTRACT**

**Background:** Brown adipose tissue (BAT) preferentially oxidizes stored triglycerides (TAGs) to generate heat during acute exposure to cold. However, the time course of its activation is not well described as we are currently limited to BAT measurements before and after an acute stimulus. Magnetic resonance imaging (MRI) is a preferred modality to uncover such evidence, as it estimates TAG content via fat fraction (FF), and permits repeat scans in the same subject. As such, serial FF measurements in a defined BAT region of interest during a uniform whole-body temperature challenge is warranted.

**Objectives:** The first objective of this study was to assess the pattern of change in supraclavicular (SCV) BAT and posterior neck subcutaneous adipose tissue (SAT; a region with an unestablished role in non-shivering thermogenesis) FF during a mild cold exposure in adult males. The second objective was to evaluate if indices of body composition were related to the pattern of cold-induced change in SCV BAT FF. The final objective was to assess the influence of warming immediately following cooling on these changes.

**Methods:** Twelve males between the ages of 19 and 28 were recruited to this cross-sectional study. Users of tobacco, nicotine, and/or alcohol, those with contraindications for magnetic resonance imaging (MRI), and diseases, surgeries, and/or medications associated with thermogenesis were excluded. There were two study visits in total. During the initial visit, anthropometric measurements were carried out in triplicate (i.e. height and weight to determine body mass index (BMI), and body composition measurements (i.e. % body total fat and lean mass (kg)) were obtained using Dual Emission X-Ray Absorptiometry. Within 30 days of this initial visit, subjects attended a time course MRI session. At this visit, participants underwent standardized cold (3-hours at 18°C) and subsequent warm (30 minutes at 32°C) exposures using a water-perfused suit while lying in a 3 Tesla MRI scanner, and the temperature of the water entering and leaving the suit was recorded throughout. FF in the SCV region and posterior neck SAT was measured at defined intervals during both temperature challenges. Separate time course plots of the mean reduction in FF from baseline were constructed for the cooling and warming phases. For the first objective, the rate and magnitude of FF changes in SCV BAT and posterior neck SAT over defined time intervals were determined through calculations of slope and area under the curve (AUC), respectively. Identification of the earliest point of change from baseline, and the point at which changes were no longer different from those measured after 3 hours of cooling, were accomplished through paired comparisons using a random-slope linear mixed model with measures at 0 minutes and 180 minutes used as the reference values, respectively. A random-intercept multilevel regression model was used to define the cold-induced change in FF over time. For the second objective, a Spearman rank-order correlation assessed the association between indices of body composition (i.e. BMI and % total body fat) and indices of BAT activity (i.e. AUC and FF reduction) at time points of interest as identified by objective 1.

**Results:** The mean±SD of BMI, LMI, and % total body fat were 24.7±2.8kg/m2, 17.6±1.6kg/m2 and 25.0±7.4%, respectively. Seven of the twelve subjects completed three hours of cold exposure (58.3%), and a further five endured at least one hour. A significant cold-induced reduction in SCV BAT FF was detected at 10 minutes following the onset of cold exposure (mean difference = -1.6%; *p*=0.005), and changes in FF beyond 30 minutes of cooling were similar to those measured after three hours (*p*<0.05). Meanwhile, the posterior neck SAT did not experience significant cold-induced changes in FF. A novel attempt at identifying a quadratic model to predict one’s BAT-specific response to a cold challenge was carried out, and the intercept, time, time2, and intraclass correlation coefficient (i.e. parameters which described the relationship between FF and time) were highly significant (*p*<0.001). Although every participant had a measurable decline in FF, those with a higher BMI and % body fat had a smaller magnitude of change throughout the time course. In particular, a strong negative correlation between BMI and AUC FF decline existed as soon as 10 minutes following the onset of cold (rho=-0.786), indicating that those with a lower BMI had a larger magnitude of change in SCV BAT FF at this point. Finally, warming did not visually influence the trajectory of SCV BAT FF.

**Limitations:** Only seven of the twelve participants completed the full 180 minutes of cold exposure, which further limited the already low statistical power of this study. Moreover, complementary measures of BAT activity, such as energy expenditure, and objective measurements of shivering, such as electromyography, could not be evaluated.

**Conclusions:** These findings suggest that significant cold-induced changes in BAT FF occur much sooner than three hours. Thus, a shorter duration of cold exposure may be considered in future studies using MRI to detect BAT activity, as this could increase the feasibility of gathering larger and younger sample populations.

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**LIST OF ABBREVIATIONS**

18F-FDG: 18-fluorodeoxy glucose

1H-MRS: proton magnetic resonance spectroscopy

ATP: adenosine triphosphate

AUC: area under the curve

BAT: brown adipose tissue

BMI: body mass index

BOLD: blood-oxygen-level dependent

DXA: dual emission x-ray absorptiometry

EE: energy expenditure

EMG: electromyography

FF: fat-fraction

FFA: free fatty acids

fMRI: functional MRI

iBAT: interscapular brown adipose tissue

IDEAL: iterative decomposition with echo-asymmetry and least squares estimation

IQR: interquartile range

IRT: infrared thermography

LMI: lean mass index

LMM: linear mixed model

LPL: lipoprotein lipase

MRI: magnetic resonance imaging

MST: mean skin temperature

NE: norepinephrine

NST: non-shivering thermogenesis

PDFF: proton-density fat-fraction

PET/CT: positron emission tomography – computer tomography

ROI: region of interest

SAT: subcutaneous adipose tissue

SCV: supraclavicular fossa

SD: standard deviation

SNS: sympathetic nervous system

TAGs: triglycerides

TRL: triglyceride-rich lipoprotein

UCP1: uncoupling protein-1

WAT: white adipose tissue

# LITERATURE REVIEW

## Introduction to Brown Adipose Tissue

### Background

Brown adipose tissue (BAT) contributes uniquely to whole-body metabolic homeostasis by catabolizing available substrates to generate heat1. Historically, the function and physiological significance of BAT has been discussed in the context of rodents and hibernating animals. Its potential relevance to humans was largely overlooked until a symmetrical depot in the supraclavicular (SCV) area of adult oncology patients undergoing fluorodeoxyglucose positron emission tomography/computed tomography (18F-FDG-PET/CT) was identified serendipitously as metabolically active BAT2. In subsequent investigations using PET/CT, BAT (i.e. presence and activity) was higher in paediatric populations (~50%) compared to adults (~5%)3. A potential explanation for this is the evolution of “classic” BAT (i.e. homogenous in morphology) from interscapular depots in infants to primarily the SCV in adults, with smaller amounts detected in the mediastinal, paravertebral, suprarenal, and upper abdominal regions4.

BAT activity is facultative and its evolutionary function is to maintain core body temperature via non-shivering thermogenesis (NST)5. NST is a physiological phenomenon whereby oxidative phosphorylation on the inner mitochondrial membrane is separated from adenosine triphosphate (ATP) synthesis via uncoupling protein 1 (UCP1). In short, UCP1 shuttles protons liberated during fuel oxidation across the inner mitochondrial membrane and the consequential loss of potential energy produces heat as a by-product6,7.The physiological process of substrate use during NST will be discussed in **Section 1.2** below.

### Mechanism of Non-Shivering Thermogenesis

The sympathetic nervous system (SNS) responds to a stimulus by secreting norepinephrine (NE), which preferentially binds to the β3 subset of adrenergic receptors on the surface of BAT cells6. A subsequent signaling cascade increases the activity of intracellular lipases, which function to release free fatty acids (FFAs) from stored triglyceride (TAGs)6. The resultant effect is twofold – FFAs are both the fuel for beta oxidation and the “switch” that activates UCP1 (the hallmark of NST in humans)2. As respiration is uncoupled from ATP synthesis, the energy produced from the combustion of these substrates is released as a functional byproduct – heat6.

#### BAT Stimulation in Humans

Cold exposure is the most potent and clinically relevant stimulator of BAT in humans, and therefore has been widely utilized to date. The physiological connection between cold and NST involves efficient communication between temperature sensors in the periphery and the SNS, resulting in -adrenergic receptor activation8. Currently, however, there is no cold stimulation protocol that defines an optimal temperature and duration for maximal NST9. The implications of this lack of consistency will be discussed in **Section 1.5** below.

Whether the exposure is acute or prolonged, there are quantifiable metabolic benefits to BAT activation and recruitment. Hanssen et al.10 examined the effects of a 10-day cold acclimation period (2-6 hours at 14-15°C) in ten obese adults and observed increased 18F-FDG uptake in BAT and skeletal muscle, both of which contribute positively to whole-body glucose homeostasis. Furthermore, Blondin et al. 11 used a 3 hour exposure at 18°C and in addition to showing similar increases in BAT-specific glucose uptake, they found a significant association between plasma FFA appearance rate (i.e. due to white adipose tissue (WAT) lipolysis) and BAT oxidative metabolism. Although this suggests a link between BAT activity and heightened glucose and lipid clearance, results of this study are limited by the use of lean and healthy young males.

The metabolic importance of prolonged BAT activation has been most evident in studies using animal models 12–15. For instance, knockout mice for PRDM16 or its coactivator EHMT1 (i.e. genes that stimulate BAT) develop concomitant obesity and hepatic steatosis12. Further, Bartelt et al.13 used a cold-activated mouse model to show that BAT activity can result in improved plasma TAG clearance through local lipoprotein lipase (LPL) activity. Complementary to this finding, murine models with BAT transplantation into abdominal visceral adipose tissue have displayed significantly improved glucose tolerance and insulin sensitivity14. Therefore, prolonged stimulation of BAT can potentially rescue an obese phenotype and its associated negative metabolic comorbidities.

## Fuels for BAT Non-Shivering Thermogenesis

Fuel selection during BAT NST is specific despite a diverse pool of available substrates. Ultimately, two macromolecules which are readily available throughout the human body are utilized – lipids and to a lesser extent, glucose.

### Lipids

Our current understanding is that BAT preferentially utilizes FFA stored within its many intracellular lipid droplets to fuel NST. In exposing rats to an environmental temperature of 6°C, Cameron and Smith16 observed a depletion of multilocular lipid material within the interscapular BAT (iBAT), a prominent depot in murine models, during the early periods of cold exposure (i.e. within 6 hours). A similar depletion of BAT intracellular TAG content was realized during necropsy of newborn infant and adult humans who died from hypothermia17. These early investigations sparked an interest among the research community, who have since used healthy human subjects and specialized imaging modalities to solidify this principle. For example, Ouellet et al.18 used PET with 11C-acetate (marker for tissue oxidative capacity, which increases during BAT activity) and 18FTHA (a fatty acid tracer) in healthy males exposed to 3-hours of 18°C cold and reported an insignificant utilization of plasma FFA despite a rapid increase in BAT radiodensity, as detected by CT (a surrogate measure of TAG content in a tissue). The authors concluded that BAT prefers intracellular stores of FFAs over exogenous sources during acute bouts of mild cold18. These findings have been repeated in subsequent work by this same group, further supporting the notion that intracellular TAGs are the predominant substrate for BAT NST in humans11,19,20.

In recent studies using animal models, the role of stored FFAs during BAT activity has been further clarified. In addition to serving as the substrate for beta oxidation, Fedorenko et al.21 showed that FFAs are essential for relieving UCP1 from the potent inhibition of purine nucleotides, such as GDP6,21,22. Expectedly, these effects are augmented by cold exposure, as Xian Yu et al.23 reported a coordinated up regulation of FFA synthesis (i.e. genes for fatty acid synthase (FAS)) and fuel combustion in the cold-activated iBAT of mice. Therefore, it is currently understood and accepted that NST is reliant on FFA uptake, synthesis, and combustion.

### Glucose

The discovery that BAT was present in appreciable amounts in adult humans was based on glucose uptake during PET/CT scanning24. At that point, glucose was presumed to be the primary fuel for NST in humans – however, in light of recent investigations (see “Lipids” section above), including the dissociation between glucose uptake and thermogenesis in UCP1-deficient mice25 and insulin-stimulated humans26, this notion has been questioned. Nevertheless, the involvement of glucose in BAT activity cannot be discounted entirely.

As reviewed by Festuccia et al.1, glucose has multiple fates once internalized by an active BAT cell. First, it can be oxidized in parallel with FFAs to fuel uncoupled respiration, though to a lesser extent27. Glucose can also be phosphorylated to glycerol-3-phosphate (G3P), which is the backbone for FFA esterification and subsequent *de novo* TAG synthesis1. These *de novo* FFA are rapidly compartmentalized into a distinct pool of TAG that are almost instantaneously oxidized25,28. Glucose is also presumed to play a complementary role in this process, as it can be catabolized (from its monomeric state or as glycogen) to generate energy for further TAG synthesis or other cell processes such as lipolysis1. In the context of BAT thermogenesis, these functions are indispensable – TAG stores need to be replenished once depleted and the uncoupling of ATP synthesis from oxidative phosphorylation will create a state of heightened energy demand. Considered together, the above notions feed back on the idea that FFAs are the primary source of fuel for BAT.

## Factors associated with brown adipose tissue presence and activity

To date, numerous studies in both adults and children have sought to identify the factors, both modifiable and non-modifiable, which might help to explain the observed variations in BAT prevalence and activity. Among those investigated, age, sex, body composition, and temperature have emerged as plausible predictors.

### Age

The inverse relationship between BAT (i.e. presence and activity) and age is well described29–33. As previously mentioned, there is a significantly higher prevalence of detectible BAT in pediatric compared to adult populations6. Among the latter group, Yoneshiro et al.32 found an age-related decrease in the prevalence of cold-activated BAT, wherein 50% of participants in their 20s versus less than 10% of those above the age of 50 had detectible 18F-FDG uptake. Similarly, Bahler et al.33 observed a reduced sympathetic drive to BAT in old (mean of 54 years) compared to young (mean of 25.5 years) men following a 2-hour cold exposure. However, much of the data concerning this relationship was derived from retrospective analyses and should therefore be interpreted with caution3.

### Sex

There is much debate as to whether or not BAT prevalence and activity is sex-specific. In a retrospective analysis of routine clinical PET/CT scans, both Ouellet et al.31 and Cypess et al.29 concluded that the prevalence of 18F-FDG uptake in BAT was significantly greater in women than men. However, a subsequent prospective study by the latter group did not observe a significant sexual dimorphism in any measure of cold-induced BAT function34. Given the heterogeneous nature of evidence to date9, no definitive conclusions can be made. What is clear is that hormonal changes, particularly during a female’s menstrual cycle, may introduce confounding effects on thermogenesis35 and should therefore be considered in the design of studies.

### Body Composition

To date, there is an established relationship between BAT activity and indices of body composition – namely body mass index (BMI) and % total body fat – in humans. In retrospective studies, a higher uptake of 18F-FDG in the SCV of subjects with lower BMI29–31,36 and less adiposity was observed30. Orava et al.37 furthered these initial findings in a prospective study wherein lean subjects exhibited heightened cold-induced BAT glucose uptake and blood flow compared to their obese counterparts. Finally, Deng and colleagues38 combined magnetic resonance imaging (MRI)-specific BAT measurements with an individualized cooling protocol (i.e. one hour at a temperature just above one’s threshold for self-reported shivering) in 15 male subjects under the age of 30. This group concluded that fat fraction (FF), a tissue-specific MRI property which will be discussed in **Section 1.4.2** below, was significantly higher in obese compared to lean subjects in both thermoneutral and cold-activated states. Furthermore, FF was negatively correlated with the volume of metabolically active BAT (i.e. 18F-FDG uptake) (*r*=-0.55), suggesting that those with greater adiposity had a blunted BAT-specific response to cold38. In closing, the relationship between BAT activity and indices of body composition has arguably been the most convincing and consistent among the available evidence.

### Environmental Factors

Ambient room and outdoor temperatures near the time of data collection have also been shown to influence BAT-specific outcomes39–41. In particular, Zukotynski et al.39 found that maintaining a constant room temperature of 24°C versus 21°C during 18F-FDG administration and PET scanning significantly decreased the intensity of radiotracer uptake by SCV BAT. Furthermore, Saito et al.30 identified a seasonal variation in the presence of detectible BAT activity between individuals, wherein those enrolled in the winter months exhibited heightened cold-induced 18F-FDG uptake compared to those whose measurements were obtained in the summer. Due to these initial findings, methodologies have since incorporated an acclimatization phase (i.e. a period of time spent in a warm/thermoneutral room before cold exposure and/or BAT measurements) which is thought to mitigate the effects of acute exposure to colder ambient temperatures prior to BAT measurements42.

## BAT imaging

There is currently a diverse array of imaging modalities based on the properties of BAT that undergo a measurable change during a state of activation, and these have all been instrumental in establishing the aforementioned predictors. A number of recent reviews have discussed each of these technologies in detail43–45 – however the most established in terms of accuracy and overall robustness are 18F-FDG PET/CT and MRI FF.

### 18F-FDG PET/CT

As evidenced by the numerous aforementioned reports using PET to detect 18F-FDG uptake by metabolically active BAT, it is currently the reference standard4. Static and dynamic approaches to this modality have been fundamental in triggering and advancing our knowledge of the identification, location, and nature of BAT in humans36. Furthermore, harmonizing 18F-FDG PET with CT radio-density (in Hounsfield units; HUs) offers anatomical localization of radiotracer uptake and a surrogate measurement of intracellular TAG consumption during BAT NST46,47.

However there are a number of limitations associated with this modality which include: 1) Injection of a radioactive analogue, such as 18F-FDG, may be harmful to healthy and young cohorts and thus strict radioactivity exposure guidelines must be observed48; 2) 18F-FDG considers only glucose utilization by BAT, which may lead to an underestimation of tissue mass and overall activity49; 3) In the absence of a sympathetic stimulus, such as cold exposure, PET/CT exhibits poor reproducibility36, and 4) The use of 18F-FDG assumes that glucose uptake reflects BAT activity; however it has been recently demonstrated that fatty acids from intracellular TAG lipolysis are the primary substrates for BAT-derived thermogenesis4,50,51.

Given these evident shortcomings that ultimately preclude the widespread use of PET/CT outside of the diagnostic setting, alternative modalities for BAT detection and quantification must be realized.

### MRI Fat Fraction

Chemical-shift MRI and its many sensitive and flexible contrast mechanisms can safely and non-invasively identify SCV BAT morphology and activity52. Namely, the Iterative Decomposition with Echo-Asymmetry and Least squares estimation (IDEAL) is an accurate and reproducible fat-water separation technique built within certain General Electric scanners that computes the proton density fat fraction (PDFF) of a region of interest (ROI) while accounting for various confounding variables53. In short, PDFF, or FF, is reflective of the relative amounts of fat and water within a given ROI, and is therefore capable of distinguishing adipose from non-adipose tissues54. It is also presumed that the multilocular nature of BAT would translate to consistently lower basal FF values compared to WAT – however the idea that morphologically distinct adipose tissue depots have unique FF signatures is debatable55–59. With regards to the utility of FF in between-subject analyses, Deng and colleagues38 used a receiver operating characteristic curve to demonstrate 100% sensitivity and specificity for the use of SCV BAT FF in differentiating between lean and obese subjects under non cold-stimulated conditions (i.e. higher SCV BAT FF with greater whole-body adiposity; FF cutoff =83% in their population of 6 normal and 9 overweight/obese young males). Furthermore, BAT is densely innervated by the SNS and upon activation, intracellular TAGs are rapidly mobilized and consumed, which MRI captures as a decrease in FF60. Therefore, the observed change in MRI FF is thought to be a measure of the primary fuel source for NST, which is unlike 18F-FDG PET/CT. Ultimately, given that FF might not effective at distinguishing between BAT and WAT, an additional MRI parameter is warranted.

### MRI T2\*

T2\* (or its reciprocal, R2\*), which is defined as the decay of transverse magnetization caused by inhomogeneities in the magnetic field (often presented as milliseconds (msec)), is a tissue-specific MRI property that can be used in combination with FF to further differentiate BAT from WAT (see **Figure 10** in **Appendix Section 7.1** for an excerpt from Hu et al.61 showing the unique MRI signatures of BAT-like tissue and WAT-like tissue)61,62. Basally, the abundance of iron-rich mitochondria in BAT accelerates T2\* decay to a greater extent than would WAT, translating into lower T2\* values62. During activation, there is a measured decrease in BAT T2\* due to the heightened oxygen exchange between saturated (i.e. oxyhemoglobin) and unsaturated (deoxyhemoglobin) iron-containing cells6. A larger concentration of the latter form of hemoglobin ultimately lowers T2\* values from baseline62. Since this MRI parameter overcomes a limitation in FF, T2\* serves as a complementary biomarker to improve the detection specificity of BAT and is therefore becoming more commonplace58,63,64.

### Strengths and Limitations Of MRI

MRI is a feasible method for assessing BAT activity in a healthy human cohort. Unlike alternatives, such as PET/CT, imaging of BAT using MRI does not require injection of radioactive tracers, does not deliver doses of radiation, and has no limitations in imaging penetration depth65. Furthermore, MRI FF offers a surrogate measure of fat tissue content within a specified region, which has a proven sensitivity to detect changes in BAT following a stimulus (i.e. a decrease in post-stimulus FF values from baseline levels is well correlated with 18F-FDG uptake, the current reference standard)66,67. Further, this imaging modality has been validated with histology and immunohistochemistry68 and is proven to be a reliable approach to BAT detection in both infant69 and adult57 humans.

Nonetheless, MRI does have limitations – namely, the spatial resolution of approximately 1mm3 is susceptible to “partial volume effects” as data from different types of cells (for example, in SCV BAT, where there is a mixture of WAT, BAT, muscle, and blood vessels) are often captured within the same voxel, leading to false positive and false negative findings65. Furthermore, MRI is extremely susceptible to motion artefacts as even the slightest movement between or during scans make image registration and delineation of anatomical borders less precise. That said, two images of the same individual taken minutes apart might appear entirely different if one’s position has changed. Lastly, whole-body acquisitions using the IDEAL sequence are not possible and therefore images at a given time point are limited to a predefined field of view.

## Current state of the literature in humans

As previously mentioned, mild cold exposure is the most potent activator of NST in humans. There are over 80 studies to date which have reported using cold exposure to activate BAT in human subjects. Among these, investigators have used various combinations of cooling methods and imaging modalities to capture the morphological and physiological changes in BAT. The potential implications of this lack of standardization was addressed in a review article by van der Lans et al.49 Despite these inconsistencies, MRI is becoming increasingly utilized to garner insight into the physiological changes associated with NST. So far, approximately 12 studies have combined cold exposure and MRI in humans, 9 of which have been published since 2015. Nonetheless, the notable heterogeneity among methodologies, particularly concerning cold exposure which has been delivered via a temperature-controlled room or a water-perfused garment at varying durations and intensities38,55,56,58,63,67,70–76, makes an approach to knowledge synthesis unfeasible. These differences aside, results have been unanimous in that cooling elicits a significant decrease in MRI-derived BAT FF (mean ΔFF between -0.4% and -5% in healthy adult populations)55,56,58,63,70.

However, few studies56,74 have taken advantage of arguably the most attractive feature of MRI – it’s safety. In other words, an absence of ionizing radiation or radioactive tracers permits repeat scanning in the same subjects. Rather than limiting one’s findings to pre- and post-cold measures of BAT, gathering data at defined intervals throughout the cold exposure can translate into a time course (or time series) plot of BAT-specific MRI measures. Time course studies using MRI are expected to inform about the physiological response of cold-induced BAT in the context of intracellular TAG use (i.e. decline in FF), and may assist in refining cold-exposure protocols according to when and how maximal BAT activity is captured by MRI.

### BAT Time Course Evidence to Date

As stated in a seminal review by Cannon and Nedergaard, early research measuring continuous NE turnover in rats suggested that “bouts” of BAT activity may be much shorter than several hours6. However, the time course of this process has still not been highlighted by the available literature. Given that intracellular TAGs are the primary fuel for BAT NST, MRI FF is a preferred modality to uncover such evidence. In 2013, Hu et al.77 challenged the research community to implement a MRI protocol that characterizes FF dynamically using a paradigm that stimulates BAT, such as cold. Deng et al.38 similarly recommended a continuous measurement of FF during a period of acute deactivation (i.e. warming) to investigate the potential restoration of these values, which is even less described than cooling. Though scarce, a continuous assessment of various aspects of BAT response to cold have been integrated in both human and animal research and these studies are summarized in **Appendix Section 7.1, Tables 7** (humans) and **8** (animals) on **page 60**.

#### Summary of Time Course Evidence

Although establishing the time course of BAT activity was not a primary objective of many of these studies, they have noted that BAT-specific responses in humans (i.e. time to nadir FF, 11C-acetate radioactivity, functional MRI (fMRI) signal in the hypothalamus, or SCV skin temperature) are immediate but transient (~ 60 minutes).42,56,73,78

Two independent studies have explored the time course of MRI-derived BAT FF during cooling in human subjects.56,74 Though each group contributed novel evidence to this field of research, there were notable limitations in their methodologies. Specifically, Stahl et al.56 selected a small square ROI in the interscapular region (ie. iBAT) of their subjects (5 males and 5 females under the age of 30, all with a normal BMI), an area not commonly associated with active BAT in human adults. Further, the researchers used distinct ROI selection criterion for each temperature phase (i.e. different FF thresholding for baseline, cold, and warm exposures), and were selective for only those FF voxels which produced a negative gradient during cooling. Though they scanned and presented data on the iBAT, WAT, and muscle of subjects every five minutes during cooling (90 minutes at 12°C) and subsequent warming (30 minutes at 37°C), they delivered these exposures using a water-perfused vest which does not cover the entire body53. McCallister et al.74 garnered SCV BAT FF measurements from seven subjects (males and females of all ages and BMI categories) during a 2.5-hour cold exposure distributed via water-perfused pads. ROI selection within the SCV was based on MRI-derived anatomical maps and areas of high intensity 18F-FDG uptake (i.e. to remove tissue that does not exhibit “BAT-like” properties), which was a notable strength. However, this group chose to scan at 30 to 40-minute intervals throughout the 2.5-hour cold exposure, resulting in only 4-5 time points. Lastly, changes in SCV BAT FF were not accompanied by comparator regions such as subcutaneous WAT or muscle, which would have added completeness to the results. Given this paucity of data, studies with added methodological rigor are warranted.

# STUDY RATIONALE AND OBJECTIVES

## Rationale and Significance

Our understanding of BAT activity in humans is currently limited to measurements of thermogenesis before and after a stimulus. In light of the evidence presented in **Appendix Section 7.1, Tables 7** and **8**, the research community might be neglecting key evidence concerning the time course of fuel utilization during NST. Since MRI provides a surrogate measurement of tissue TAG content that is sensitive to cold-induced thermogenesis, coupled with the ability to repeat scans in healthy and young human subjects, it is a preferred modality to uncover such evidence. In capturing these “real time” changes, findings might reveal that a cooling duration in the range of 2-3 hours is not necessary. Though a few groups have published evidence concerning this exact notion, there were notable shortcomings in each of their methodologies. As such, a protocol involving uniform whole-body mild cold exposure and subsequent warming, scanning at defined intervals to obtain measurements of FF, and a BAT ROI that is consistently reported in the literature will contribute to new knowledge.

## Overarching Purpose

To describe the patterns of change in SCV BAT FF during a 3-hour mild cold exposure (18°C) followed by a 30-minute warm exposure (32°C) in a cohort of adult males under the age of 30.

## Objectives and Hypotheses

### Objectives

1. To identify the pattern of change in SCV BAT FF during a 3-hour mild cold exposure (18°C).
   1. *What is the time course of change in SCV BAT FF during cooling*?
   2. *How do the rate and magnitude of change in SCV BAT FF vary over the period of cold exposure?*
   3. *What is the earliest time point at which a significant change in FF occurs in BAT? What is the earliest time point where changes no longer differ from the 180-minute value*?
   4. *How can the change in SCV BAT FF over the course of time be modeled mathematically?*
   5. *What is the pattern of change in an adipose tissue region with unknown thermogenic properties?*
2. To evaluate if indices of body composition are related to the pattern of cold-induced change in SCV BAT FF within this small cohort of male adults.
   1. *Are factors previously shown to be related to BAT activity correlated with the pattern of response to cold over time?*
3. To identify the patterns of change in SCV BAT FF during a 30-minute warm exposure (32°C).
   1. *What is the time course of change in SCV BAT FF during warming*?

### Hypotheses

1. I hypothesize cold exposure to elicit an immediate but transient decrease in SCV BAT FF. I also hypothesize the adipose tissue region which is presumably devoid of thermogenic function to exhibit no measurable cold-induced changes in FF.
2. I hypothesize BMI and % body fat to be negatively related to the magnitude of change in BAT FF throughout the time course.
3. I hypothesize that warm exposure (i.e. BAT deactivation) will recover FF towards the baseline value.

# METHODS

## Study Design and Population

Healthy adult males between the ages of 18 to 29 years old were prospectively recruited from McMaster University and the surrounding Hamilton Community (e.g. Mohawk college, businesses, recreation and fitness centers, and hospitals) using posters, internal media advertising, social media postings, and through referrals by active/past participants and/or fellow members of the research team. All participants completed two visits – (i) Initial Visit: Collection of anthropometric and body composition measurements; and (ii) Time Course MRI Session: Collection of BAT measurements. The study was approved by the joint Research Ethics Board of McMaster University and Hamilton Health Sciences and all aspects of the study were performed in accordance with that approval. Enrolled participants provided informed, signed consent. Exclusion criteria, subject preparation, and conditions used in the study can be found in **Table 1**.

Table 1 - Criteria and conditions used in the study

|  |  |  |
| --- | --- | --- |
| **EXCLUSION CRITERIA** | | |
| 1. Female sex 2. Under the age of 18 or over the age of 30 3. Self-reported alcohol intake greater than 7 drinks/week with no more than 3 drinks/day 4. Use of any of the following medications (β adrenergic, steatogenic, anti-hyperglycemic, antidepressant, anxiolytic, anti-psychotic, thyroid, antiemetic – 5HT3 antagonists or serotonergic drugs)† 5. Tobacco and/or nicotine use (smoking, nicotine patch, chew tobacco, nicotine gum, e-cigarette or cigar) 6. Any contraindications for MRI (claustrophobia, implanted metal, metallic injuries recent tattoo or weight>300lb) 7. Prior bariatric surgery or liver transplantation 8. Any conditions associated with brown adipose tissue, hepatic steatosis or liver disorders† | | |
| **CHARACTERISTICS** | **CRITERIA AND CONDITIONS IN STUDY** | |
| **Subject Preparation for Both Visits** | | |
| Meals 24 hours before visit | | Avoid the following serotonergic foods: tomato, plum, kiwi, avocado, banana, pineapple, walnuts |
| Caffeine before visit | | No caffeine 12 hours before visit |
| Fast duration before visit | | 8-12 hours |
| Strenuous activity within 48 hours of visit | | None |
| **Conditions (Time Course MRI Session)** | | |
| MRI room temperature | | 19-21°C |
| Time of visit | | Between 0745 and 1000 hours |
| Time of year | | Summer, fall, and winter |
| Outdoor temperature range | | -12.2°C to 18.9°C |
| MRI scanning frequency | | Cooling: Every 5 minutes during the first 60 minutes, and every 15 minutes thereafter; Warming: Every 5 minutes |
| **Cooling/Warming Protocol** **(Time Course MRI Session)** | | |
| Exposure paradigm | | Fixed |
| Exposure location | | MRI room |
| Exposure device | | Liquid-conditioned cooling garment (LCS; Two Piece, Allen-Vanguard, Ottawa, ON, Canada) |
| Cooling temperature | | 18°C |
| Warming temperature | | 32°C |
| Total duration of cooling | | 180 minutes |
| Total duration of warming | | 30 minutes |
| Method used to monitor inlet and outlet water temperatures | | Fiber-optic temperature probes (FO Temp Sensor, Polymide Tip, Neoptix, Qualitrol, Quebec, Canada) and data logger system (Model RFX273A, Neoptix, Quebec, Canada). |
| Method used to monitor skin temperature | | None |
| Method used to monitor shivering objectively | | None |

†*for a complete list of excluded medications and medical conditions, refer to* ***Appendix Section 7.2.1***

## Study Visits

### Initial Visit

Participants first consented to the study and were then re-screened for eligibility (initial screening was done during the recruitment phase). Anthropometrics (i.e. height, weight and waist circumference) and body composition using Dual Emission X-Ray Absorptiometry (DXA) were then measured, which are described in further detail below. See **Appendix Section 7.2.3** for the full visit timeline.

### Time Course MRI Session

The time course MRI session occurred within 30 days of the initial visit. This visit began with participants acclimatizing to room temperature (~21°C) for 30 minutes while wearing standardized clothing (light sleeveless t-shirt and shorts). Immediately following, participants changed into the liquid-conditioned suit and were instructed to lay in a supine position on the MRI scanning table. All subjects were provided with visual and audio entertainment throughout the cooling and warming paradigms using a MR-safe rear projection system. The fan which circulates air through the bore of the MR magnet was turned off as to avoid augmenting the intensity of the temperature challenge. Cooling began once the participant was in a comfortable position. Scans were acquired at 5-minute intervals during the first 15 minutes of cooling and every 15 minutes thereafter for the first participant. For the second participant, scanning occurred every 5 minutes during the first 30 minutes of cooling and every 15 minutes thereafter. Following the collection and analysis of data for these two subjects, a protocol was established in which scans were to be acquired every 5 minutes for the first 60 minutes of cold exposure, and every 15 minutes until the 180-minute time point was reached. Upon completion of this phase, participants were warmed for 30 minutes and neck MRI scans were again acquired every 5 minutes. See **Figures 12** and **13** in **Appendix Section 7.2.3** for the visit timeline and illustration of the equipment setup, respectively.

## Study Procedures

### Cold and Warm Exposures

A standardized, uniform, whole-body cold and warm exposure protocol was delivered using a specially designed liquid-conditioned cooling garment (LCS; Two Piece, Allen-Vanguard, Ottawa, ON, Canada) which covered a subject’s entire body except their hands, feet, and head. The same suit was used for all participants to ensure consistent tubing density and water flow. In brief, water was pre-cooled to 18°C using a self-regulating bath (IsoTemp 6200R28, Fisher Scientific, Ottawa, Canada) and delivered to the suit via insulated tubing extensions for up to 180 minutes. This cooling protocol is sufficient to detect BAT activity without significantly decreasing core body temperature19,20,78. The water initially increased in temperature (due to the subject’s “warmer” pre-cold skin temperature and the uncooled water within the suit’s tubing), however the exposure stabilized to 18°C after 10-15 minutes. Immediately following the cessation of cooling, the same water-perfused cooling garment was connected to a separate water bath set at 32°C (VWR, Avantor, Randor, USA) for a duration of 30 minutes.

#### Inlet-Outlet

Two fiber-optic temperature probes (FO Temp Sensor, Polymide Tip, Neoptix, Qualitrol, Quebec, Canada) were fixed to the tubing extension-suit interface (i.e. manifold system) and transmitted the inlet (i.e. water delivered to the suit) and outlet (i.e. water leaving the suit) temperatures to a data logger system (Model RFX273A, Neoptix, Quebec, Canada). Neolink software (Neoptix, Quebec, Canada) recorded temperature measurements throughout the cold and warm exposures in 15-second intervals. This measurement was obtained for six consecutive participants, and its purpose was to ensure standardization of the cold exposure protocol as the inlet-outlet difference is an indirect measurement of one’s whole-body thermogenic response to a temperature challenge.

### Primary Outcome Measure

SCV BAT FF (defined below) measured at defined intervals throughout the cooling and warming phases was the primary outcome of interest.

#### MRI Acquisition and Segmentation of the SCV Region

All MRI scans were performed using a 3-Tesla whole-body MRI scanner (Discovery 750; GE Healthcare, Waukesha, WI, USA) located within the Imaging Research Centre at St. Joseph’s Healthcare Hamilton. BAT MRI scans were acquired with the IDEAL sequence in the axial plane using a Head/Neck/Chest coil. In brief, IDEAL generates FF and T2\* maps while accounting for T2\* decay and the multiple spectral peaks of fat66. This pulse sequence generates six distinct image contrasts: water-only, fat-only, in-phase, out-of-phase, FF and R2\* images. To ensure that the entire neck and SCV region were captured, image acquisition started at the C2/C3 disc and ended at the T4/T5 disc. MRI parameters specific to this procedure are included in **Appendix Section 7.2.4**.

Image analysis was performed on Analyze Pro (Version 1; Mayo Clinic, Biomedical Imaging Resource, AnalyzeDirect, Overland Park, KS, USA). All adipose tissue bound by the sternocleidomastoid medially, trapezius posteriorly, and clavicle inferiorly at each of the axial slices between the C5-C6 and T1-T2 discs were manually segmented77. Since the SCV region is heterogenous in morphology, a 30-100% FF threshold and 2-25msec T2\* threshold were applied to exclude “non-BAT-like” tissue from future analysis61. This approach is conceptually similar to fusing 18F-FDG PET images with MRI FF maps and segmenting only the SCV tissue within a defined FF range and with a high intensity of radiotracer uptake (i.e. two-stage thresholding)54,74. Further, the outer boarder of each ROI was eroded to mitigate any inherent partial volume effects. All voxels that fell within this set of criteria were considered to be SCV BAT, and the average FF value for each scan (i.e. time point) was considered going forward.

Changes to the posterior neck subcutaneous adipose tissue (SAT) FF, a region which is presumably devoid of thermogenic function in adult humans, was measured alongside SCV BAT79. The segmentation process of this tissue depot was similar to above, however a T2\* threshold was not applied (in line with the assumption that there was no active BAT in this region and therefore would have a homogenous morphology) and the ROI was defined as any superficial adipose tissue existing posterior to the trapezius at the following vertebral discs: C5-C6, C6-C7 and C7-T1. Stahl et al.56, Franssens et al.57, and Lundtrom et al.58 used a similar SAT ROI in their respective analyses, and Gifford et al.63 and Jones et al.59 also used distinct thresholding approaches for BAT and SAT.

A stepwise protocol for image segmentation is included in **Appendix Section 7.2.5.**

#### MRI Indices of BAT Activity

Quantification of SCV BAT FF throughout the time course allows for an indication of cold-induced BAT activity. For the purposes of this study, BAT activity was quantified as: 1) Reduction in FF from baseline; 2) Magnitude of change (i.e. area under the curve (AUC); and 3) Rate of change from baseline (i.e. slope of linear regression line).

**Reduction in FF from Baseline**

Reduction in SCV BAT FF and posterior neck SAT FF throughout the time course were calculated using the following formula:

Where time point 1 represented the pre-cooling FF measurement, which in turn normalized for baseline variability.

**Magnitude of Change in FF**

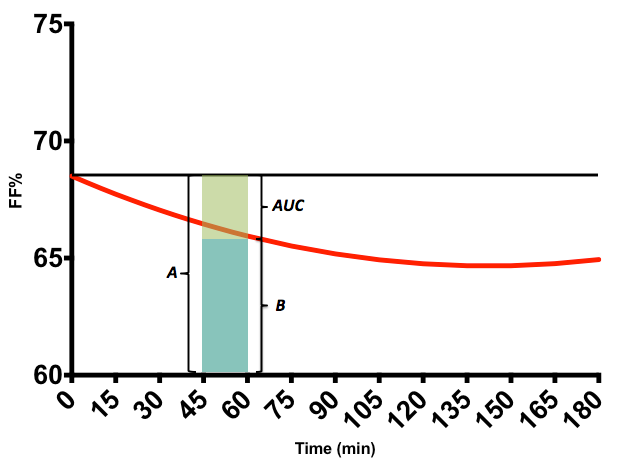
**** AUC with the trapezoidal rule was calculated using the following equation, which can be visualized in **Figure 1** below: where

Figure 1 - Calculation of AUC

**Rate of Change in FF**

The rate of change between two FF measures for a defined time interval was calculated using the “SLOPE” function in Microsoft Excel©. This function uses the following formula to derive a simple linear regression coefficient, or slope of a line between two specified time points with given y values:

Figure 2 - Calculation of slope

**Untitled:Users:stephanoreskovich:Desktop:Screen Shot 2018-03-12 at 9.03.00 PM.png**

Here, a negative value represented a decrease in FF, and a positive value represented an increase.

### Anthropometrics

Participants wore light clothing and were asked to remove shoes prior to their measurements. Weight (kg) was measured three times to the nearest 0.1kg using an electronic platform scale (BMI Scale Model 882; Seca, Hamburg, Deutschland). Height (to the nearest 0.1cm) was also obtained in triplicate using a wall-mounted stadiometer (Height Measuring Rod Model 240; Seca, Hamburg, Deutschland). The average of the three measurements were used for subsequent determination ofBMI (kg/m2).

### Body composition

Body composition was measured using DXA (GE Lunar Prodigy Advance, Model 8743). DXA accurately assesses body composition based on the unique X-ray attenuation parameters of various tissues, such as fat and bone minerals80. Total % body fat and lean mass (kg), the only measurements of interest for the present analysis, were automatically generated to the nearest 0.1% by the DXA scanner. Those with a % body fat greater than 25% were considered to have abnormal/excessive adiposity81,82. Further, total lean mass for each participant was adjusted for their current height by calculating lean mass index (LMI = total lean mass/height2 (kg/m2)). All scans were reviewed to ensure accurate delineation of the standardized ROIs.

### Environmental Factors

To ensure that ambient conditions during cooling and warming were consistent among subjects, the temperature inside the MRI scanning room (automatically displayed on the MRI control system) was recorded every 30 minutes. There were no deviations greater than ±0.5°C from the baseline-recorded temperature and therefore only the first recorded value was used for the present analysis.

To determine the influence of outdoor temperature on MRI findings, the average local temperature one hour before the time course session was obtained from historical data collected by the McMaster University Weather Station (http://geomedia.mcmaster.ca/muws/archives.html).

## Statistical Analysis

Analyses were performed on Microsoft Excel©, SPSS Statistics (version 23; IBM, North Castle, NY, USA), or GraphPad Prism (version 7; GraphPad Software, La Jolla, CA, USA), and a two-tailed *p-*value of<0.05 denoted significance. All study variables were tested for normality using a Shapiro-Wilk W-test. Participant demographics were presented as n or n (%) for categorical variables and mean (SD) for normally distributed continuous variables or median [quartile 1 (Q1), quartile 3 (Q3)] for non-parametric data. Values were also expressed as mean (SD) in the figures. The statistical approach(es) to each objective is/are outlined below:

**Objective 1 – Patterns of change in SCV BAT FF during cooling**

The sample size was based on a previous report by Blondin et al.19 which showed that *N*=6 subjects confers a power of >80% to detect a significant cold-induced effect in BAT (at a two sided alpha level of 0.05) with the same cooling protocol and in a similar cohort of males, albeit using a different imaging modality. A time course plot of the reduction in FF from baseline allowed for a descriptive analysis of the pattern of change over time. Pairwise comparisons using a random-slope linear mixed model (LMM) with FF reduction as the dependent variable, time as the independent variable, and measures at 0 minutes (for SCV BAT and posterior neck SAT) and 180 minutes (for SCV BAT only) as the reference values, was used to identify time points of significant change in FF during the time course83,84,85. This procedure was followed by Bonferroni’s multiple comparisons *post hoc* test. Finally, a random-intercept multilevel regression model was used to define the perceived quadratic relationship between SCV BAT FF (dependent variable) and time (independent variable).

**Objective 2 – Relating participant characteristics to the pattern of change in SCV BAT FF**

According to VanVoorhis et al.86, a sample size of no less than 50 subjects is required to make a definitive conclusion regarding the relationship between two variables; therefore, this analysis was underpowered. Nevertheless, a Spearman rank-order correlation was performed to assess the association between indices of body composition (i.e. BMI and % total body fat) and indices of BAT activity (i.e. AUC and FF reduction) at time points of interest as identified in objective 1.

**Objective 3 - Patterns of change in SCV BAT FF during warming**

The rationale used for determining an appropriate sample size to accomplish objective 1 was also applied to objective 3. Further, a descriptive analysis of the time course of change in FF from baseline was the only statistical approach used to accomplish this objective.

# RESULTS

## Project Recruitment

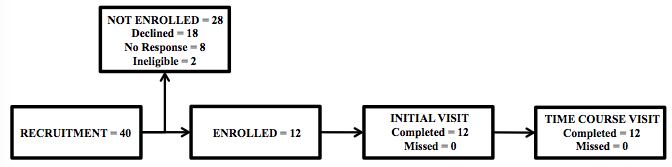
Between the months of June 2017 and February 2018, a total of 40 males under the age of 30 expressed interest in the study, and 12 were eventually enrolled (30% enrollment rate). The recruitment flow chart is presented in **Figure 3** below.

Figure 3 - Recruitment Flow Chart for the GETBAT Timecourse Substudy

## Participant Characteristics

The mean±SD of BMI, LMI, and % total body fat were 24.7±2.8kg/m2, 17.6±1.6kg/m2 and 25.0±7.4%, respectively. Of the six participants with an “overweight” BMI (25.0kg/m2), four had excess adiposity (>25%), and of the six subjects with a non-overweight BMI (<25.0 kg/m2), one had excess adiposity81,82. During the time course cold exposure session, 7 (58.3%) participants endured the full three hours of cold exposure, whereas the remaining 5 (41.7%) lasted at least one hour. Given the narrow dispersion of values for air temperature inside the MRI room (min,max = 19.2,21.0°C) and ΔOutlet-Inlet (min,max = 3.37,3.67°C), the intensity of cooling and resultant whole-body thermogenic response were comparable across the cohort (**Table 2** below).

**Table 2** – Variable Descriptives for the GETBAT Timecourse Substudy

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **VARIABLE** | **N (%)** | **MEAN (SD) OR MEDIAN [Q1,Q3)** | **MIN,MAX** |
| **PARTICIPANT CHARACTERISTICS** | **Age (years)** | 12 | 22.8 (2.6) | 19,28 |
| **BMI (kg/m2)** | 12 | 24.7 (2.8) | 19.8,28.9 |
| ***Normal*** | 6 (50) | 22.4 (1.7) | 19.8,24.4 |
| ***Overweight*** | 6 (50) | 27.1 (1.2) | 25.5,28.9 |
| **LMI (kg/m2)** | 12 | 17.6 (1.6) | 14.7,20.0 |
| **% Total Fat** | 12 | 25.0 (7.4) | 15.6,38.4 |
| **≤25%** | 7 (58.3) | 19.8 (2.7) | 15.6,22.2 |
| **>25%** | 5 (41.7) | 32.3 (5.3) | 26.0,38.4 |
| **TIME COURSE MRI SESSION** | **Ambient Temperature (**°**C)** | 10\* | 20.3 (0.5) | 19.2,21.0 |
| **Inlet Temperature (**°**C)** | 6† | 18.88 (0.17) | 18.60,19.03 |
| **ΔOutlet-Inlet (**°**C)** | 6† | 3.49 (0.11) | 3.37,3.67 |
| **Duration of Cold Exposure** | 12 | 180 [82.5,180] | 60,180 |
| ***=60 minutes*** | 3 (25) | - | - |
| ***>60 but <180 minutes*** | 2 (16.7) | - | - |
| ***180 minutes*** | 7 (58.3) | - | - |
| **Outdoor Temperature (**°**C)** | 12 | 2.9 (10.9) | (-12.2,18.9) |
| **Month of Visit** | 12 | - | - |
| ***June/July*** | 2 (16.7) | - | - |
| ***Oct/Nov*** | 4 (33.3) | - | - |
| ***Dec/Jan/Feb*** | 6 (50) | - | - |

†Data for 6 participants lost due to technical difficulties

\*Temperature of the MRI room was not recorded for the first two participants

Values are means (SD) for normally distributed data and median [Q1,Q3] for non-parametric data

## Data collection and analysis summary

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Table 3** - MRI Acquisition and Analysis Summary | | | | | | |
| **COOLING** | | |  | **WARMING** | | |
| **TIME POINT** | **SCANS COLLECTED** | **SCANS ANALYZED** |  | **TIME POINT** | **SCANS COLLECTED** | **SCANS ANALYZED** |
| 0 | 12 | 12 |  | 5 | 11 | 11 |
| 5 | 12 | 11 |  | 10 | 11 | 11 |
| 10 | 12 | 12 |  | 15 | 11 | 10 |
| 15 | 12 | 12 |  | 20 | 11 | 10 |
| 20 | 11 | 11 |  | 25 | 11 | 10 |
| 25 | 11 | 10 |  | 30 | 11 | 11 |
| 30 | 12 | 12 |  |
| 35 | 10 | 10 |  |
| 40 | 10 | 9 |  |
| 45 | 12 | 12 |  |
| 50 | 10 | 10 |  |
| 55 | 10 | 9 |  |
| 60 | 12 | 11 |  |
| 75 | 9 | 9 |  |
| 90 | 9 | 9 |  |
| 105 | 8 | 8 |  |
| 120 | 8 | 8 |  |
| 135 | 8 | 8 |  |
| 150 | 7 | 7 |  |
| 165 | 7 | 7 |  |
| 180 | 7 | 7 |  |

The number of scans acquired and analyzed are shown in **Table 3**. Twelve scans were not collected at each time point due to change of protocol after the first two subjects and differential ability to withstand the cold challenge. In total, five scans collected during cooling of the participants could not be analyzed due to excessive motion artifacts that made delineation of anatomical borders impossible (n=3), or a technical limitation in post-image processing termed fat-water swapping (n=2)61. One of the twelve subjects did not proceed with warming, and a further three scans were lost due to fat-water swapping61.

## Identifying the time course of change in SCV BAT FF with cooling

The first objective of this project was to identify the pattern of change in SCV BAT FF during 3-hour cold exposure at 18°C. All participants exhibited a measurable decrease in FF regardless of the duration of cooling. In fact, the nadir FF was detected before the end of cooling in all but one participant (Subject 10 in **Table 4**). Individual time course plots can be found in **Appendix Section 7.3.1.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Table 4 -** Summary of individual cold-induced changes in SCV BAT FF | | | | | |
| **SUBJECT** | **PRE-COLD FF (%)** | **MAX FF REDUCTION (%)** | **TIME TO MAX FF REDUCTION (min)** | **FF REDUCTION @ END OF COLD (%)** | **DURATION OF COLD (min)** |
| **1** | 74.69 | 4.69 | 105 | 1.80 | 180 |
| **2** | 71.73 | 2.89 | 60 | 0.65 | 180 |
| **3** | 74.52 | 4.03 | 150 | 3.34 | 180 |
| **4** | 75.60 | 6.14 | 50 | 6.07 | 60 |
| **5** | 74.15 | 2.11 | 20 | 0.30 | 60 |
| **6** | 67.75 | 6.97 | 105 | 3.06 | 135 |
| **7** | 70.90 | 5.42 | 135 | 4.02 | 180 |
| **8** | 68.86 | 7.43 | 45 | 7.16 | 60 |
| **9** | 62.22 | 6.44 | 150 | 3.19 | 180 |
| **10** | 59.35 | 4.34 | 90 | 4.34 | 90 |
| **11** | 63.78 | 7.71 | 135 | 5.90 | 180 |
| **12** | 66.85 | 8.51 | 135 | 6.48 | 180 |

The cold-induced reduction in FF was immediate and increased in magnitude over the first 90 minutes, after which values were largely maintained until the end of cooling (**Figure 4A,B**). Further, the mean slope (i.e. rate of change in SCV BAT FF) throughout the time course appeared to shift from being predominantly negative (i.e. declining FF) to a period of variability around the x-axis (i.e. little change in FF) at roughly 90 minutes (**Figure 4C**). These observations were maintained when those who did not complete the full duration of cold exposure were removed from the analysis (n=7, see **Figure 16** in **Appendix Section 7.3.4**). Overall, these findings suggested that there was little change in FF beyond the 90 minutes of cold exposure.

As an exploratory outcome of the present study, SCV BAT T2\* was measured concomitantly with FF. Depicted in **Figure 15** in **Appendix Section 7.3.3**, T2\* showed an immediate cold-induced decrease (i.e. a greater amount of DeoxyHb relative to OxyHb as a consequence of oxygen consumption in the BAT tissue) which was sustained throughout the duration of cooling. Considering FF and T2\* together, it can be postulated SCV BAT responds immediately to a cold challenge by liberating and oxidizing substrate, resulting in the production of heat.

Figure 4 – Changes in SCV BAT FF during cooling. Time course plots of FF reduction (A), AUC for 30 minute intervals (B), and slope for 30 minute intervals (C). Data are presented as mean±SD.

**Identifying Time Points of Interest**

Given that cold-induced changes in SCV BAT FF appeared to be rapid and transient, we were interested in determining the earliest point at which a significant reduction from baseline had occurred. Further, we were curious as to whether or not there became a point during the time course at which changes no longer differed from those measured after 3 hours of cooling (the usual post-cold time point).

A significant reduction in FF was detected as soon as 10 minutes following the onset of cold exposure (mean difference = -1.60%; *p*=0.007). This analysis was repeated with “180 minutes” used as the reference value and it was found that beyond 35 minutes, reductions in FF no longer differed from those measured at the end of the cooling protocol (*p>*0.05). Tabular output from this analysis can be found in **Appendix Section 7.3.5**.

**Modeling the change in FF over time**

It was also of interest to mathematically describe the relationship between FF (dependent variable) and time (dependent variable) using a random-effects multilevel regression model. Relevant parameters and a visual representation of the model are included in **Table 5** and **Figure 5** below, respectively:

Table 5 - Random-effects multilevel regression model describing the relationship between FF (dependent variable) and time (independent variable)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **PARAMTER** | **ESTIMATE** | **STD. ERROR** | **P-VALUE** | **95% CI LL** | **95% CI UL** |
| Intercept | 68.50 | 1.62 | <0.001\* | 65.33 | 71.66 |
| Time | -0.054 | 0.0058 | <0.001\* | -0.065 | -0.042 |
| Time2 | 0.00019 | 0.000033 | <0.001\* | 0.00013 | 0.00026 |
| rho | 0.96 | 0.017 | <0.001\* | 0.91 | 0.98 |
| Overall model: | | | | | |

CI = confidence interval; LL = lower limit; UL = upper limit

The coefficients (i.e. slopes) for time (linear parameter; β=-0.054) and time2 (quadratic parameter; β=0.00019) were statistically significantly different from 0 (*p* <0.001 for all), indicating that each had a meaningful impact on FF%. Further, a significant rho (or intraclass correlation (ICC)) value of 0.96 suggests that 96% of the variance in FF% was explained by accounting for individual variation (i.e. random intercepts, where each subject is a distinct cluster) in the model. Since the exposure conditions were kept constant across all subjects (e.g. overnight fast, time of day, acclimation period, and cooling intensity), much of the observed variability in response can in turn be linked to an individual’s innate ability to generate heat, which is dependent on the following equation: Hprod = shivering thermogenesis (ST) + NST. Factors that could potentially implicate the latter variable within this equation (i.e. NST) were explored in the subsequent section. In general, the model was considered to be of good fit based on the highly significant *p*-values corresponding to all parameters which described the relationship between FF and time.

Finally,deriving an equation to predict the SCV BAT FF at a given time point increases the generalizability of the results of this study. In an analogous population of males who underwent a cold challenge that was similar in intensity but varied in duration, SCV BAT FF findings of past and future studies can be compared. Though preliminary, this is a novel attempt at using a model to predict one’s BAT-specific response to a cold challenge.

Figure 5 - Graphical representation of the quadratic model (red) for the absolute change in SCV BAT FF (black)

**The time course of change of posterior neck SAT FF**

Measurements of the posterior neck SAT were obtained in parallel with the SCV BAT. Visually, the FF of this adipose tissue depot did not considerably change throughout the time course (**Figure 6A-C**). Although mean values were consistently below reference time point 0, suggesting that the posterior neck SAT was metabolically active during cooling and in turn opposing my *a priori* hypothesis, these changes were not statistically significant (**Tables 15** and **16** in **Appendix Section 7.3.6**). Furthermore, in some subjects, the pattern of change in posterior neck SAT mirrored that of the SCV BAT (**Appendix Section 7.3.1**), which might also allude to the unique thermogenic properties of this tissue depot.

Figure 6 – Changes posterior neck SAT FF during cooling. Time course plots of FF reduction (A), AUC for 30-minute intervals (B), and slope for 30 minute intervals (C). Data are presented as mean±SD.

## Investigating if participant characteristics are related to the pattern of cold-induced change

Since not all participants withstood the entire duration of cooling, covariates of BAT activity were compared between those who completed the full cold exposure and those who did not. In brief, no discernible group-specific patterns for temperature (i.e. outdoor and ambient)30,31,41 or indices of body composition (i.e. BMI, LMI, and % body fat)29–31,36,87,88 were present. A summary of these findings are included in **Appendix Section 7.3.7.**

Figure 7 - Individual patterns of FF reduction among those who completed the full cooling protocol (n=7)

As evidenced by **Figure 7** above, there was notable variability in the patterns of change in SCV BAT FF among those who sustained the full 180 minutes of cold exposure. Therefore, we were interested in exploring the relationships between expected covariates of BAT activity (based on the literature) and an individual’s BAT-specific response to cold exposure. Given the abundance of evidence suggesting an association between BMI and % body fat and BAT activity12,38,73,89–93, these indices of body composition were correlated with indices of BAT activity (i.e. FF reduction from baseline and AUC from baseline, the latter of which accounts for all measurements within a time interval of interest) at 10 minutes (i.e. the earliest point at which significant changes occurred); 60 minutes (i.e. the first hour of cold exposure); 90 minutes (i.e. the point at which FF appeared to reach a nadir), and 180 minutes (i.e. end of exposure).

BMI and % total body fat were at least moderately related to indices of BAT activity at all time points of interest (**Figure 8A**;corresponding *p-*values are included in **Table 18** in **Appendix Section 7.3.7**). In fact, a strong negative correlation between BMI and AUC existed as soon as 10 minutes following the onset of cold (rho=-0.786), indicating that those with a lower BMI had a larger magnitude of change in FF at this point. These relationships were also visually apparent according to **Figure 8B**.

Figure 8 – Correlation matrix identifying the Spearman’s rho value for each combination of BMI and % body fat and indicator of BAT activity at 10 minutes, 60 minutes, 90 minutes, and 180 minutes (A). Time course of FF reduction according to BMI and % fat classifications (B). \*denotes statistical significance at *p*<0.05. N=7 in each analysis.

As an exploratory analysis, a plot of the strength of these correlations at each cold-induced time point was constructed (see **Figure 20** in **Appendix Section 7.3.7**). Unlike FF reduction, the associations between AUC and indices of body composition appeared to follow a discernible time-dependent pattern until 75 minutes, after which rho values remained consistent until the end of the cold challenge. Not only does this finding corroborate the time course of change in SCV BAT FF, it also suggests that AUC might be a more robust indicator of BAT activity than FF reduction.

## Identifying the time course of change in SCV BAT FF with warming

The final objective of this thesis was to identify the time course of change in SCV BAT FF during a period of warming at 32°C for 30 minutes. In performing this phase immediately following cooling, the effect of BAT “deactivation” could be quantified.

Figure 9 - Time course of FF reduction during warming in the SCV BAT. Data are mean±SD.

The pre-cold FF measurement was used as the reference value, and therefore **Figure 9** represents FF reduction relative to that point. It was hypothesized that warming would induce a recovery of FF towards baseline (i.e. the x-axis) since the sympathetic stimulus, and hence the driver of BAT-specific NST, would be absent. However, neither the pattern nor the magnitude of change in FF was influenced by a whole-body exposure to warming, as FF appeared to be maintained at or around the last measured cold-induced value. Whether or not this phenomenon was due to a lack of substrate to replenish intracellular TAG stores, or a concomitant increase in perfusion and lipid droplet anabolism, is uncertain56,69. To offer insight into the latter notion, changes in T2\* were recorded and a similar effect was seen in that this measurement was not influenced by 30 minutes of warming at 32°C (see **Figure 21** in **Appendix Section 7.3.8**).

# DISCUSSION

The first objective of this project was to identify the patterns of change in SCV BAT FF during a 3-hour mild cold exposure. Second, we were interested in seeing if expected covariates of BAT activity were related to indices of BAT activity at various points throughout the time course. Finally, we wanted to measure the trajectory of SCV BAT FF during a transition from cooling to 30 minutes of warming (i.e. BAT “deactivation”). The results of these three objectives will be discussed in sequence below.

## Objective 1: Time Course of Change in SCV BAT FF over cooling

**Pre- and post- cold measurements of SCV BAT FF**

The distribution of pre-cold SCV BAT FF values (range: 59.35%-75.60%) within this small sample of male adults was comparable to what has been reported for similar populations 38,63. In particular, Gifford et al. (2016) identified a slightly wider interval of 51.6-78.7% in a cohort of 17 normal and overweight subjects (11 females and 6 males) under the age of 30 using a combined MRI and PET/CT thresholding approach (CT radiodensity between -200 and -2 HU, MRI FF between 50-100%, MRI R2\* <150 s-1 (T2\* >7 msec), and no limits on FDG uptake) 63. Deng et al.38 studied 11 normal and overweight males under the age of 30 and used a stepwise SCV BAT segmentation algorithm (subject-specific R2\* and FF thresholding values) to report a mean (standard deviation) of 70% (8%) under non-cold stimulated conditions. The notable differences in ROI selection procedures between the present study and those conducted previously likely contribute to the slight variability in findings.

Further, all subjects, regardless of the duration of cooling, had a measurable reduction in FF (**Table 4**). Among the seven males who completed the full 180 minutes, the mean reduction in FF from baseline was 3.62% (range: 0.65%-6.48%). This finding is comparable to Ong et al. (unpublished), who utilized the same cold exposure protocol and reported a mean FF decrease of 2.97% in a cohort of males and females between the ages of 18 and 50. To date, no published study has combined a standardized, whole-body cold exposure using a water-perfused suit with MRI-derived FF measurements. Of those who have reported a mean change in FF during cooling, the magnitude of response was variable (range: 0.4%-5.0%)56,58,63,70–72,74,88.

**Pattern of change in SCV BAT FF during cooling**

In this study, a standardized, whole-body cold exposure protocol was used and acquisition of SCV BAT FF measurements at frequent, predefined intervals in healthy young men was carried out. We concluded that cold-induced changes in SCV BAT FF were immediate and transient, as values decreased rapidly in the first 10 minutes, and as a group declined more gradually to the 90-minute time point, after which FF stabilized (**Figure 4** above). These findings were maintained even when removing those who did not complete the full duration of cooling from the analysis (**Appendix Section 7.3.4**).

As aforementioned, two independent studies have presented individualized time course plots of BAT FF changes during cold activation56,74. Stahl et al.56 used a water-perfused vest to deliver a 12°C exposure for 90 minutes in a cohort of 10 normal weight individuals (5 males and 5 females) under the age of 30. The individual time course plots of changes in FF within iBAT, a region not associated with active BAT in adults, present with noticeable intersubject variability that makes generalization difficult. Nonetheless, there was an apparent decrease in FF, which was more pronounced in some individuals, over the course of the 90-minute cold exposure. Most of these changes appeared to occur within the first hour, after which FF values stabilized (see **Appendix Section 7.1**, subjects S01, S03, S04, S09 for examples). McCallister et al.74 included a similar population in their study design – however SCV BAT FF was measured over the course of a 150-minute cold exposure delivered via water-perfused pads. Individual time course plots of FF (**Appendix Section 7.1**) are also difficult to interpret as cold exposure duration and scanning intervals varied across participants. Of those presented, 2 participants experienced a gradual decline in FF throughout (subjects A and E), 2 exhibited an initial decline followed by a recovery towards baseline (subjects B and F), and two underwent no apparent cold-induced changes (subjects C and G)74. In the present study, a measurable decline in the SCV BAT FF during the early phases of cooling was visually apparent for all subjects (see **Appendix Section 7.3.1** for individual time course plots). However, these attempts at identifying a time course of change in BAT FF during cooling are particularly heterogeneous with respect to segmentation of the BAT ROI (i.e. interscapular versus SCV region, FF thresholds, and presence/absence of a secondary threshold such as T2\* or FDG uptake; see **Section 1.5.1.1** above), and therefore such comparisons should be interpreted with caution.

A meaningful reduction in FF was detected as soon as 10 minutes following the onset of cold exposure (mean difference = -1.6%; *p*=0.005), and various independent research groups using multiple modalities of detection have also observed a rapid BAT-specific response to a sympathetic stimulus. In humans, Haq et al.42 observed a maximal increase in SCV skin temperature, as measured using infrared thermography (IRT), after only 10 minutes of a 12°C cold exposure in a cohort of young adult males. Garretson et al.94 also reported a significant temperature change in the skin overlying the iBAT of Siberian hamsters within 5 minutes of CL-316,243 injection. Regardless of the chosen method of stimulation, this finding has been consistent among other studies which measured BAT-specific temperature changes95–97. At the molecular level, Motillo and colleages98 used animal models and *in vivo* experiments to observe the appearance of ligands for PPAR-gamma (i.e. prefacing intracellular lipolysis and eventual UCP1 action) at BAT lipid droplet surfaces within minutes of stimulation. Therefore, the connection between substrate mobilization and oxidation appears almost instantaneously. Time-reactivity curves of 11C-acetate (a marker of BAT oxidative metabolism) obtained by Ouellette et al.78 and Blondin et al.20 (**Appendix Section 7.1**) during cold exposure in human adults further illustrate the prompt response of BAT thermogenic machinery to sympathetic stimuli.

Beyond 30 minutes, the magnitude of change in BAT was similar to those measured after 3 hours of cooling, a similar finding among studies using animal models exposed to either acute cold exposure or sympathomimetics. Khanna et al.99 injected wild type (WT) mice with NE and reported an iBAT temperature increase (direct measurement via insertion of a probe beneath the fat pad) of 5.5°C at 50 minutes, which began to regress at 60 minutes, eventually returning to pre-injection levels around 140 minutes. This finding was furthered by Branca et al.65, who stimulated mice with a β-adrenergic agonist and mirrored a rapid but short-lived increase in iBAT temperature (measured the same as above) with brief changes in a BAT-specific dynamic T2\* signal (i.e. indirect surrogate of tissue oxygen exchange). A similar pattern of change in whole-body energy expenditure was identified in β-adrenergically activated (i.e. no shivering) WT mice by a separate group of investigators100. Xian Yu et al.23 investigated the trajectory of BAT gene expression during a prolonged 4°C cold exposure in WT mice, and reported an acute (~60 minute) increase in the expression of genes implicated in FA synthesis and TAG lipolysis. This was in line with findings from a later study, which indicated that levels of FFAs within the serum of WT mice reached a maximum within 30 minutes of cold exposure, after which there was a slight decrease101. In humans, Ouelette et al.78 exposed a small sample of healthy males to an identical cold exposure as the present study and observed a rapid increase in whole-body energy expenditure (EE) that occurred independently of shivering. This pattern was not sustained, however, as EE recorded between 60 and 80 minutes amounted to 80% of that recorded during the final 10 minutes of cold78. More recently, Leitner et al.102 demonstrated in a population of lean and young males and females that 80 minutes of cold exposure (cooling vest set between 128-16.1°C) elicited similar 18F-FDG uptake as 120 minutes, indicating that SCV BAT is recruited sooner than previously thought. Furthermore, given that BAT activity has a proposed inverse association with skeletal muscle-derived shivering thermogenesis50,103, and that findings from both Hanssen et al.104 and Haman et al.105 suggest that observed shivering is increased after 60 minutes of mild cold exposure, NST might indeed be a transient process that largely contributes to whole-body EE during the early phases of a cold challenge. Therefore, results of previous investigations in both humans and animals are complementary to the present study in that BAT measurements consistently show an immediate, yet temporary response to a stimulus. Thus, a shorter duration of cold exposure may be considered in future studies using MRI to detect BAT activity, as this could increase the feasibility of gathering larger and younger sample populations.

Since FF is dictated by the relative amounts of fat and water within a tissue, an increase in the latter following BAT stimulation could contribute to the present findings. The unique morphology of BAT includes a dense system of vasculature to both deliver substrate and dissipate heat upon activation6. Therefore an increase in tissue perfusion (i.e. increase in water content) and concomitant decrease in intracellular fat content are likely to be both implicated in the observed decrease SCV BAT FF. Panagia et al.106 used blood-oxygen-level dependent (BOLD) imaging (based on changes in T2\* signaling, which is a tissue-specific MRI property that is sensitive to the relative amounts of deoxyHb and oxyHb in a tissue where an increase in the former, such as during BAT activation, decreases T2\* values53) to measure the response in the iBAT of mice subjected to a beta-adrenergic agonist. Briefly, there was an immediate but transient spike in BOLD signal, suggesting that the initial BAT response to stimulation might be dominated by increased blood flow rather than lipid consumption106. This finding was repeated by Reeder et al.107 and Van Rooijen et al.67, who both noted a brief spike in oxyHb levels within the SCV BAT of a small cohort of human subjects during acute bouts of cold exposure, which furthers the previous analysis by showing the speed at which oxygen is consumed by BAT. Individual variation in cardiac output and heart rate could also implicate the supposed acute increase in blood flow to BAT – however, this relationship is currently unclear108. Therefore, these findings in combination with the time course of change in SCV BAT T2\* (**Figure 15** in **Appendix Section 7.3.3**) deduced by the present study suggests that FF could be influenced by water more so than fat during the initial phases of cold exposure, but likely not after prolonged stimulation.

**Rate of change in SCV BAT FF during cooling**

The rate of change in SCV BAT FF during the time course transitioned from a net negative slope (i.e. decrease in FF) to stabilization around the x-axis (i.e. no change in FF) at around 90 minutes from the onset of cold exposure. This finding is in line with the above in that changes in BAT were transient, and therefore no further discussion is warranted.

To the best of my knowledge, Stahl et al.56 was the only time course study in humans that reported the rate of cold-induced change in BAT FF. In particular, Figure 5 from this publication presented slopes (FF%/hour) for five males under the age of 30 that were included in their analysis. Among these individuals, FF decreased by ~0.8 to ~6%/hour, which is within the range of values for this analysis (**Table 6** below):

Table 6 - Rate of change in FF from 0-60 minutes of cold exposure (%/hour)

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **SUBJECT** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| **SLOPE** | -3.7 | -4.2 | -1.9 | -8.1 | -0.9 | -5.0 | -1.2 | -8.3 | -6.1 | -1.6 | -6.0 | -4.6 |

Notable differences between the methodologies of Stahl et al.56 and the present study, namely the BAT depot of interest (i.e. interscapular versus SCV) and cold exposure protocol (i.e. water-perfused vest at 12°C and whole-body water-perfused suit at 18°C), should be considered when interpreting the above notion. Altogether, the rate of change in a BAT-specific measurement during acute activation is absent within the literature and should therefore be highlighted by future studies.

**Mathematically describing the change in FF over time**

A preliminary model describing the change in SCV BAT FF over time was deduced from this small dataset of male subjects under the age of 30, which enhances the generalizability of the present findings. In particular, one can use this equation to predict the measured FF value at any given time point – however it is important to remain cautious of the study population of concern, the BAT depot of interest, and the cooling protocol used when interpreting the results.

Stahl et al.56 proposed a mean FF reduction of 2.9% after one hour of cold exposure, which is comparable to the 2.6% that was mathematically derived from our quadratic formula. This similarity exists despite large variations in cooling protocols and BAT ROI selections. Furthermore, Gifford et al.63 and Holstila et al.70 measured a mean SCV BAT FF decrease of 2.2% and 0.4%, respectively, after two hours of cooling. Both are considerably less than what would be predicated using our model (i.e. 3.74%), which might again be attributed to the inherent differences between methodologies (i.e. older versus younger populations, lean versus obese populations, whole-body versus targeted cold exposure, etc.)49.

Further, a relationship that is quadratic in nature assumes that at some point, values begin to trend in the opposite direction (i.e. there is a point of inflection). One can observe that in the time course of SCV BAT FF (**Figure 4**), measurements appeared to inflect towards baseline within the last hour of cold exposure, which the quadratic formula identified as 150 minutes. In addition to the physiological implications of substrate availability and use (discussed below) , this pattern might be related to a homeostatic mechanism in which thermal receptors become desensitized to a constant stimulus109. Following an initial, strong, discharge at the beginning of a stimulus, the response progressively weakens and eventually ceases altogether109.

Lastly, obtaining a rho value (i.e. ICC) of 0.96 when considering each individual as a unique cluster suggests that the overall variability in FF over time depends largely on between-subject differences. These differences may pertain to one’s relative dependency on shivering and non-shivering thermogenesis for whole-body heat production11,50,78, which will be addressed in **Section 5.2** below.

**A hypothesized physiological explanation for the time course of fuel utilization during BAT NST**

Though the pattern of change in FF may in part be attributed to the small sample size and random noise that is inherent in MRI signals, it might provide insight into the interplay between fuel sources during BAT NST. In brief, a seminal review by Cannon and Nedergaard suggested that bouts of BAT activity, as measured by continuous NE turnover, may be much shorter than several hours, and that the degree of recruitment is limited by the amount of BAT tissue one has at baseline6. Given that Motillo et al.98 suggested a time lag of mere minutes between the formation of lipolysis products and induction of oxidative genes in BAT, and that *in vitro* experiments identified lipogenesis from exogenous FFAs28 and endogenously derived FFAs (i.e. from glucose)25,28,110–112 as a rapid and frequent process (i.e. minutes), it is reasonable to postulate that a three hour cold exposure would present multiple transitions between intracellular TAG depletion and resurgence.

A well-supported notion is that BAT NST is fueled preferentially by FFAs liberated from intracellular TAG pools27,78,113,114– however the time course of this process is not well described. Given that in the present study there was an initial decrease in FF over the 90 minutes of cold exposure, this might suggest that stored TAGs were used during this time period. Since FF appeared to be maintained beyond this point, BAT might indeed be drawing on exogenous substrates to fuel thermogenesis during prolonged activation. Glucose is one such substrate that can directly (i.e. oxidation during uncoupled respiration27) or indirectly (i.e. conversion to G3P to replenish the TAG reserves in BAT) contribute to BAT NST. With regards to the latter idea, animal studies suggest that glucose-derived *de novo* FA are compartmentalized rapidly into a distinct pool of TAG that are almost instantaneously oxidized25,27,28. The high turnover of this distinct pool of TAGs might explain the lack of observed change in FF during the latter phases of cold exposure. Furthermore, glucose uptake into BAT is controlled via negative wherein an increase in lipolysis and a decrease in intraceullar TAG content promotes exogenous substrate utilization20,28. In addition to glucose, there is evidence suggesting that activated BAT draws directly on exogenous FFAs to sustain thermogenesis6. Though dietary FFAs are one source of such fuel114,115 and therefore could be implicated in the pattern of change in FF, the participants in the present study were exposed to cold in a fasted state. As such, the subjects might have relied more on FFA liberated from circulating triglyceride rich proteins (TRLs)1 and/or sympathetically activated WAT depots94,115. Specifically, Bartelt et al.13 found increased LPL in the iBAT vasculature of cold-exposed mice, which translates to two important outcomes: 1) local LPL activity in BAT is required for exogenous FFA uptake, and 2) LPL activity increases vascular permeability and permits the internalization of TRLs116. There is also an abundance of evidence suggesting that whole-body WAT lipolysis is essential for sustained BAT activity7,11,115,117,118. Among these studies, Shin et al.115 and Schreiber et al.118 were the most convincing as they independently showed reduced cold tolerance in mice with global deactivation of lipolysis compared to animals with defective lipolysis only in BAT. Furthermore, Lynes et al.119 introduced 12,13-diHOME as a “batokine” that acts in an autocrine-paracrine manner to further augment FA uptake into BAT during cold activation. These exogenous FA, unlike glucose-derived TAGs, are distinctly stored in a larger and less transient pool of TAGs,28 potentially contributing to the slight recovery of FF towards the end of cold exposure that was observed in the present study (see **Figure 22** in **Appendix Section 7.4** for a proposed model summarizing the time course of fuel use during BAT activation). Nonetheless, whether or not the observed signal fluctuations are due to the inherent limitations in MRI or the dynamic nature of BAT fuel use is certainly up for debate.

**Time Course of Change in Posterior Neck SAT FF Over Cooling**

The posterior neck SAT, a region not generally referred to as a source of significant NST in adult humans67,120, was measured alongside SCV BAT FF during cooling. As seen in **Figure 6A-C** above, cold did not induce considerable changes in FF throughout the time course in this adipose depot. However, there was a measurable, albeit non-significant, decrease from baseline as time proceeded (mean±SD of maximal FF reduction = 1.1±2.0%). This was particularly observable in some participants (namely 071, 072, and 074, **Appendix Section 7.3.1**), as the pattern of change in posterior neck SAT FF mirrored SCV BAT FF. Coincidentally, these participants had a lean BMI and low % body fat (range: 19.8-22.4 kg/m2 and 15.6-21.2%, respectively), a phenotype which is known to be associated with an augmented whole-body lipolytic response as a result of a sympathetic stimulus, such as cold121. Upon WAT SNS stimulation, vascular permeability is increased which allows liberated FFA to leave the interstitial space and remove the negative feedback on lipolysis121, which would translate to a measurable decrease in tissue fat content (i.e. MRI FF).

Given that the posterior neck SAT is proximal to the interscapular region, which is where BAT is abundant and active in human infants6, this fat pad might have an augmented capacity for NST under acute cold exposure compared to more distal subcutaneous depots, such as the abdomen122. For instance, the range of pre-cold posterior neck SAT FF intersected with the range of pre-cold SCV BAT FF (66.9%-89.4% and 59.4%-75.6%, respectively) despite differences in thresholding techniques, suggesting that these two anatomically, and presumably functionally,55 distinct fat pads might not have unique MR signatures. In line with this notion, Jones et al.59 recently attempted to identify an optimal FF threshold to differentiate between SCV BAT and posterior neck SAT, but could not specify a universal cut-off that exhibited high sensitivity and specificity. Finally, Koskensalo et al.55 used proton magnetic resonance spectroscopy (1H-MRS) to measure the FF of SAT within upper posterior thoracic region (i.e. posterior neck SAT) in 10 healthy subjects aged 25-45. Despite reporting significantly larger pre-cold FF values within SAT (mean±SD of 88.3±4.8%), which may in part be attributed to the technical differences between 1H-MRS and chemical-shift MRI, this group found a significant decrease in SAT FF after a 2-hour individualized cooling protocol (mean±SD of 83.9±6.6%)55. This was paralleled by an insignificant cold-induced change in SCV BAT FF, and researchers attributed their findings to increased lipolysis94 and perfusion106 in the posterior neck SAT. These findings are substantiated by Muzik et al.91 who quantified lower tissue radiodensity (i.e. CT HU’s), higher glucose uptake, and a higher metabolic rate of oxygen in WAT proximal to the SCV region compared to the abdomen. Considering that a consensus has not been reached, future investigations should consider using larger cohorts and standardized approaches to cold stimulation and BAT detection in order to clarify the role of the posterior neck SAT within whole-body thermogenesis.

## Objective 2: Relationship between indices of BAT activity at time points of interest and covariates of BAT activity.

In this study, moderate-to-strong associations between indices of body composition and BAT activity throughout the time course were identified (i.e. |*rho|*=0.393-0.964); consistent with our current understanding of BAT in humans. For example, Din et al.123 investigated the SCV BAT of a heterogeneous study population (n=66, 45F/21M, 25-50 years, all BMI categories) using CT and found significant inverse relationships between measures of adiposity (i.e. BMI, waist circumference, and hip circumference) and cold-activated BAT radiodensity (i.e. HUs – an indirect surrogate of fat tissue content), which remained significant after adjustment for age (*r*=-0.71, -0.75, and -0.62, respectively). In recording MRI-derived FF before and after cooling (individualized protocol using a water-perfused blanket), Deng and colleages.38 found that male subjects under the age of 30 with a greater BMI and % body fat had a higher SCV FF at both time points (*r*>0.7 for all). However, this same study38, among others55,63,91 reported no observed correlations between PET-derived BAT activity (i.e. 18F-FDG or 15O2 uptake (BAT oxidative metabolism)) and body composition, which might be attributed to the inherent technical limitations of this imaging modality49. Further, since lean mass is associated with an increased basal metabolic rate which in turn alters the lower critical temperature at which cold-induced thermogenesis is activated124, those with a higher LMI might not have been as “cold” as those with a comparatively low LMI. However, given that inlet-outlet temperature differences were comparable across the cohort, and that no associations between LMI and indices of BAT activity were observed (**Figure 20** in **Appendix Section 7.3.7**), this participant characteristic was likely not implicated in the results of this study. Overall, our findings that individuals with a higher BMI and % body fat had a blunted BAT-specific response to cold are well supported.

An interesting result of the present study was the strong inverse correlation between BMI and AUC at ten minutes following the onset of cold exposure, suggesting that those with a lower BMI had a greater magnitude of change in FF at this time point (*rho*=-0.786*;* **Figure 8** above). To the best of my knowledge, Rachid et al.73 offers the only other evidence of this proposed relationship in humans. This group used hypothalamic functional MRI to detect BAT activity, which assumes that BAT is regulated solely by hypothalamic-generated sympathetic signals. In summary, Figures 5A and D from this publication (**Appendix Section 7.1**) showed that lean compared to obese females (n=12 in each group) displayed heightened hypothalamic activity within 5 minutes of a 16°C cold exposure. Although this is in line with the present findings, correlation analysis revealed no association between BAT activity (as determined by 18F-FDG uptake) and hypothalamic responsiveness to cold and therefore these results should be interpreted with caution.73 Nonetheless, the notion that obese versus lean individuals have a blunted BAT-specific response to cold can be reasoned by the fact that human SCV BAT exhibits characteristics of “beige” fat, an inducible form of WAT6,8,49,67,125. As one accretes more body fat, SCV BAT loses its “brown” phenotype and undergoes a shift in functionality from energy expenditure to storage, ultimately blunting one's BAT-specific thermogenic machinery90,92,93,125. Furthermore, in addition to impairing whole-body insulin sensitivity, increasing adiposity also reduces the sympathetic drive and overall catabolic activity of SAT depots12,20,121, thereby adversely impacting the utilization of glucose and FFA as exogenous fuels for NST. Despite this phenotype, Schreiber et al.118 observed sustained UCP1 protein content and mitochondrial function in a lipolysis-deficient mouse model with induced whitening of BAT. This suggests that upon continued activation by a stimulus, such as cold, the thermogenic response in BAT tissue can be rescued6,122,126. As such, a smaller magnitude of change in FF among those with a higher BMI/% body fat as compared to leaner individuals can be partially explained by the beige nature of SCV BAT. In other words, a “whiter” BAT might have a reduced capacity for NST during acute exposures and might in fact take longer to “wake up” before the thermogenic machinery becomes fully active. This concept also alludes to one’s *a priori* state of BAT activation, which ties into the succeeding discussion section.

**Other participant characteristics that could implicate BAT activity**

There are many factors that can influence an individual’s BAT-specific response to cold exposure, and one’s *a priori* acclimation state is a notable predictor. Various independent research groups have examined the effects of cold acclimation on BAT activity in healthy human cohorts. Seminal investigations have observed heightened 18F-FDG uptake by BAT in subjects studied in the winter versus the summer months4,127,128. In addition, Lee et al.129 examined the effects of a 4-month crossover study (24°C 🡪 19°C 🡪 24°C 🡪 27°C) of overnight temperature acclimation on five healthy men and found increased BAT activity (i.e. augmented 18F-FDG uptake and radiodensity) after the cold month, which was muted after subsequent warm exposures. This early knowledge shed light on the notion that BAT activity is facultative, as it is only functional when acutely activated59,113,126. A few research groups have since recruited small, homogenous, samples of human subjects and measured clinical and BAT-specific outcomes before and after a cold acclimation protocol of several weeks in duration8,10,89,104,114,130. Van der Lans et al.130 exposed a group of lean adults under the age of 30 to an ambient temperature of 15-16°C for 2-6 hours on 10 consecutive days and reported increased 18F-FDG uptake and whole-body EE in parallel with decreased self-reported shivering. Hanssen et al.10,104 used the same acclimation protocol to repeat these findings in overweight/obese populations. Similarly, Blondin et al.114 studied the effects of a four-week (3 hours at 18°C, five times per week) cold sensitization in a homogenous population of lean males and indicated a 2.6-fold increase in BAT-oxidative metabolism without alterations in objectively measured shivering intensity. These results were furthered by Yoneshiro et al.89 who subjected 12 males to 2 hours of 17°C daily for 6 weeks and observed heightened BAT recruitment, increased EE, and decreased % body fat even among those with unobserved 18F-FDG uptake at baseline. Therefore, the frequency and intensity of exposure to cold environments could certainly be implicated in one’s time course of change in SCV BAT FF. Although we saw no influence of outdoor temperature on MRI findings (**Figure 17** in **Appendix Section 7.3.7**), we did not control the extent to which subjects were exposed to their ambient environment (e.g. being outside and wearing a jacket of a certain thermal insulation value) and therefore numerous external factors likely confounded this relationship.

Individual variation in cold-induced BAT activity could also be explained by differences in cardiac output, which would presumably enhance substrate delivery and subsequent distribution of generated heat118,123. Din et al.123 correlated cold-induced BAT radiodensity (i.e. TAG use) with local blood volume, and Schreiber et al.118 suggested that impaired cardiac reserve contributes to cold intolerance in mice with defective BAT machinery. Although Flynn et al.108 disconnected BAT blood flow with total cardiac output, they were trialing a novel modality for BAT detection (i.e. contrast-enhanced ultrasound) and therefore this notion warrants further investigation.

In summary, this study did not account for various factors that could influence MRI measures of BAT activity. Nonetheless, the goal of this project was not to identify the physiology behind cold tolerance but rather to present and identify potential predictors of the time course of change in FF during cooling.

**AUC as an indicator of BAT Activity**

We elected to use both FF reduction from baseline, a common MRI-derived indicator of BAT activity which describes a change in lipid fat content between two points, and AUC from baseline, which accounts for all measurements between two points to derive a magnitude of change over a defined time interval. As depicted by **Figure 20** in **Appendix Section 7.3.7**, associations between AUC and indices of body composition were less variable than those produced by FF reduction, and ultimately did not change beyond 90 minutes of cooling. Further, the combination of AUC and BMI consistently produced moderate-to-strong Spearman correlation coefficients at each point over the time course of cooling. Therefore, this exploratory analysis substantiates earlier findings in that associations with established covariates of BAT activity can be detected soon after the onset of a cold stimulus, and also alludes to the potential utility of AUC as an indicator of BAT activity when more than two cold-induced measurements are obtained.

## Objective 3: Time course of FF during warming

It was hypothesized that upon cessation of a sympathetic stimulus (i.e. BAT deactivation), FF would begin to recover towards baseline114. In line with this presumption, a recent report by Weir et al.131 indicated persistent 18F-FDG uptake by SCV BAT during warming (ambient temperature of ~25°C), but that much of this internalized glucose is used for *de novo* lipogenesis. However, as presented in **Figure 9** above, FF was maintained at or around the last measured cold-induced value in the current study.

Four independent groups have measured SCV FF in both cooling to warming conditions38,55,56,58. Stahl et al.56 implemented a similar warming phase of 30 minutes at 37°C and reported comparable subject-specific patterns of change in FF reduction despite notable differences in methodologies. According to Figure 4 in this publication (**Appendix Section 7.1**), warming induced a mild increase in iBAT FF in one subject, a continuing decrease in five subjects, and no change in the remaining four56. Considered together, the five female participants had a net slope of -1.9±11.8%/hour and the five males had a net slope of -0.9±4.4%/hour, indicating a slight decrease in iBAT FF during warming56. Though not a time course representation, Deng et al.38 obtained MRI scans following 60-minutes of individualized cooling and 20-minutes of warming (30°C) in 15 young male participants. Their descriptive analysis indicated variability in the direction and degree of FF changes during the latter phase, as seven subjects showed a trend of recovery towards baseline, and six had continued decreases38. They also provided the following group-wise summary to show a lack of change in FF after transitioning from cold to warm conditions: FF of normal/overweight males during cooling = 67±8% and warming = 67±9%; FF of obese males during cooling = 84±4%, warm = 83±4%38. However, warming appeared to restore R2\* (inverse of T2\*) to pre-cold levels in the majority of participants38. Lundstrom and colleagues58 mirrored this finding (i.e. BAT FF remained decreased at the post-cooling level (-1.92%), whereas R2\* showed a weak trend towards normalization) in a similar population of subjects, which might imply that reheating has a greater impact on lowering perfusion in the BAT tissue. Though not a primary outcome of the present study, changes in T2\* (i.e. indirect surrogate of tissue perfusion) were measured alongside FF and **Figure 21** in **Appendix Section 7.3.8** appears to show no influence of warming on the trajectory of T2\*. Given that FF also remains stable during this phase, a balance between lipid consumption and oxidation must persist in order to maintain the relative amounts of fat and water in this tissue. The findings from the present study are corroborated by a recent report which indicated persistent, albeit significantly reduced, 18F-FDG uptake in SCV BAT after 180 minutes of 23°C warming which immediately succeeded 120 minutes of ~14°C cooling102. In contrast to above, Koskensalo et al.55 reported a significantly lower 1H-MRS-derived BAT FF in cold (2 hours at individualized temperature) compared to warm (not specified) conditions (76.0±5.6% and 80.9±5.6, respectively) in a small, heterogeneous sample of 10 healthy adults. However, these results are controversial given that measurements were obtained on separate days. Taken together, these data demonstrate that human SCV BAT activity may persist for an extended period after removal of a sympathetic stimulus, such as cold exposure.

Although underwhelming, the findings that FF is not influenced by warming suggests that an increase in temperature prior to post-cooling MRI scans would not mask the changes in FF but could instead be used as a standardized methodological tool for limiting the prevalence of motion artifacts due to shivering. Furthermore, a brief time lag between the cessation of cooling and acquisition of SCV BAT FF scans, as often reported in studies performing a cold exposure *outside* of an MRI, would not impact cold-stimulated measures. Therefore, regardless of the outcome, this investigation was highly warranted.

# CONCLUSION

## Summary of Findings

In this study, we quantified SCV BAT FF during cooling (3 hours at 18°C) and warming (30 minutes at 32°C) and concluded the following: (i) the cold-induced reductions in SCV BAT FF were rapid and transient, as a significant reduction in FF was detected as soon as 10 minutes following the onset of cold exposure (mean difference = -1.6%; *p*=0.005), and changes in FF beyond 30 minutes of cooling were similar to those measured after three hours (*p*<0.05); ii) the posterior neck SAT did not experience significant cold-induced changes in FF; iii) although every participant had a measurable decline in FF, those with a higher BMI and % body fat had a smaller magnitude of change throughout the time course; and iv) warming did not appear to influence the trajectory of SCV BAT FF. These findings reinforce the feasibility of implementing a mild cold exposure protocol of a short duration to capture changes in MRI measures of SCV BAT in humans.

## Limitations and Recommendations for Future Studies

This study has notable limitations. First, only twelve subjects were included in the present analysis, and seven were able to complete the full duration of cold exposure while inside the MRI. Though potential covariates of BAT activity such as age32,33 and sex31,132 were taken into consideration by recruiting only males under the age of 30, statistical analyses were limited as there was insufficient power to perform a multiple regression analysis (i.e. determine independent predictors of one’s change in FF at time points of interest)86.

Furthermore, despite the strengths of MRI, it does have inherent shortcomings. Participants have to remain perfectly still while images are being obtained, which made tolerating the cold exposure increasingly difficult. Furthermore, the resolution of the IDEAL sequence used to generate FF values is insufficient for differentiating between multiple cell types within a single voxel53,133. Since the human SCV region is heterogeneous in nature, especially among those who are overweight or obese (i.e. abundance of WAT interspersed within BAT)61, it is particularly susceptible to these “partial volume effects”. Although the chosen FF and T2\* thresholds were chosen in an attempt to separate “BAT-like” tissue from “non-BAT-like” tissue, partial-volume effects are often beyond the sensitivity of water-fat MRI61. Subsequent studies should consider reducing the voxel size (i.e. increase resolution)63 or experiment with intermolecular zero quantum coherence nuclear magnetic resonance spectroscopy alongside chemical-shift MRI66 to determine if this reduces partial volume effects.

Regardless of these technical limitations, numerous groups have commented on the difficulty of manually segmenting the SCV adipose tissue as its shape exhibits tremendous intersubject variability69,134 and therefore makes standardization so challenging. Recent attempts at validating an automated segmentation tool for BAT in mice135 and humans64 have produced inconsistent findings, and therefore manual approaches are still the most reliable to date.

Unlike the brain, where registration of a series of images is common practice to ensure consistent segmentation of a ROI, the appearance of the SCV region is extremely sensitive to even a slight change in position. In the present study, subjects would subconsciously shift between repeated scans, which removed the possibility of registration and necessitated manual segmentation of each individual image. Lastly, MRI does not offer a whole-body assessment of FF and is instead limited to a desired field of view. If this was not the case, the time course of other BAT (i.e. perirenal)12 and SAT (i.e. abdominal) depots could have been simultaneously investigated.

Before subjects began the cold exposure protocol, they were acclimatized to a ~21°C room wearing a tank top and shorts for 30 minutes. Though thermal comfort was obtained by offering blankets when a participant felt “cool”, true thermoneutrality before cold exposure was likely not achieved. The implications of not reaching thermoneutrality before cold exposure on subsequent BAT outcomes has been investigated by a number of independent groups42,95,112,118. In brief, Haq et al.42 discovered that SCV skin temperature, as measured by IRT, gradually increased over the final 15 minutes of a 60-minute room temperature acclimation (20-23°C), suggesting that BAT was activated during this time. By using an acclimation temperature of 32°C to mask these effects, cold-induced increases in SCV skin temperature were concomitantly augmented42. This is in accordance with Boon et al.136, who reported a similar finding after one-hour acclimation at 32°C. Using MRI, Stahl et al.56 measured the pre-cold changes in iBAT FF during 20 minutes of 23°C exposure and derived a rate of change of -1.3±0.9%/hour among 5 male subjects. Given this, cold-induced changes in the SCV BAT FF of subjects in the present study may have already begun during acclimation resulting in an underestimation of changes in FF. Subsequent investigations should consider sufficient warming (i.e. 32°C for 30-60 minutes) beforehand. With regards to more prolonged exposures, Sanchez-Guraches et al.112 observed differential gene expression in the iBAT of WT mice housed at 30°C versus 22°C and 4°C, wherein those acclimatized to 22°C have an upregulation of thermogenic genes compared to those at 30°C, but not at 4°C. This notion alludes to the potential BAT-specific effects of a subject’s *a priori* acclimatization state (discussed in **Section 5.2**)61,118, which might confound results and should therefore also be considered in future investigations.

Given the apparent interplay between skeletal muscle and BAT-derived thermogenesis18,91,108,137,138113,139, an objective quantification of shivering using EMG would have added to the present findings. In other words, using EMG in parallel with BAT measurements would provide a quantification of thermogenic mechanisms occurring alongside NST during cold exposure. However, a commercially available MRI-safe EMG system was not available at the time of this study. Furthermore, the apparent dichotomy between shivering and non-shivering thermogenesis might not be as simplistic as current evidence presents it to be. Unlike Ouellett et al.18, Blondin et al.11 reported a non-significant relationship between shivering intensity and PET/CT-markers of BAT activity. Instead, this group suggested that deep muscles proximal to the spinal cord, which are not easily recorded by EMG, are preferentially recruited by thermogenesis11. Furthermore, Vijgen et al.140 found a high individual variation in the onset of shivering as detected by EMG, even among morphologically similar subjects. Lastly, the emerging idea that muscles themselves participate in NST adds another dimension to our already convoluted understanding of cold tolerance141. Beta-adrenergic agonists and sympathomimetics could overcome this limitation, but their use in humans presents added systemic complications34,142,143. Regardless, even participants matched based on age and body composition can differ in their response to cold48, and it is therefore important that, in addition to shivering, cold-induced measures of whole-body heat production (e.g. indirect calorimetry and/or monitoring of the inlet and outlet temperature of a liquid-perfused garment, the latter of which was performed for 6 participants), and changes in mean skin temperature (MST) be made concurrent with BAT measurements 47. With this holistic assessment of cold-induced thermoregulation, BAT’s relevance in the realm of whole-body systemic thermogenesis can be evaluated based on the following relationship: Hprod (via indirect calorimetry or inlet-outlet) = ST (via EMG) + NST (via BAT). Therefore, should MR-safe approaches to whole-body heat production and MST be available, they should certainly be taken into consideration by researchers hoping to standardize this investigation before employing larger and more heterogeneous cohorts.

Finally, considering one’s state of physical endurance in addition to their thermal history might have helped to explain the observed intersubject variation in BAT FF changes6,144. This study also did not control for the type of movie or TV show that subjects watched over the course of cooling, which might have created unwanted variability in the amount of sympathetic stimuli one received. Lastly, combined PET-MRI would permit quantification of FF and PET-specific radiotracers (i.e. BAT oxidative metabolism using 11C-acetate78, perfusion using 15O-H2O145, or FFA uptake using 18F-FTHA20) during the same cold exposure session. However, since ionizing radiation is a notable limitation of PET, and that these tracers are largely inaccessible in most research centers, the general feasibility of this approach in a time course context is limited. Applicability of similar approaches using MRI and hyperpolarized substrates, such as 129Xe (i.e. tissue perfusion) and 13C (i.e. oxidation), are currently being explored as a means to overcome this barrier146,147.

## Next Steps

First, this study design should be extended to larger and more heterogeneous cohorts to develop a time course that is representative of a diverse population of individuals. In accomplishing this, the predictive value of a mathematical model describing the change in FF over time would inherently be improved. However, in order for a study of this nature to be feasible, a reliable and reproducible automatic segmentation algorithm for SCV BAT must be developed. Second, the effects of a warmer acclimation period (i.e. 30 minutes at 32°C)42 should be compared against what was performed in the current study to determine if the magnitude of change in FF was indeed masked. Third, all variables in the thermogenesis equation (i.e. Hprod = ST + NST) should be accounted when making BAT-specific conclusions. Fourth, the potentially unique thermogenic properties of the posterior neck SAT should be explored by future trials (i.e. using the same FF and T2\* thresholds as the SCV region). Finally, studies that wish to measure MRI-derived changes in BAT with a shorter duration of cold exposure should further investigate the utility of AUC by reproducing previous associations with established covariates of BAT activity.

# APPENDIX

## Supplementary Material (Introduction)

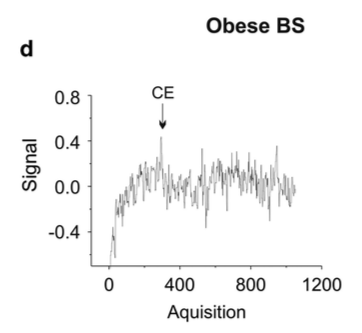
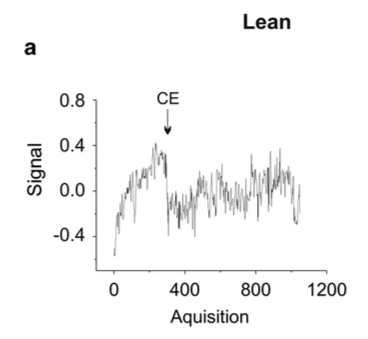
Figure 10 - Figure 6 from Hu et al. (2013) showing FF and T2\* signatures from SCV BAT (gray) and subcutaneous WAT (black) for a lean 18 year old-female participant.

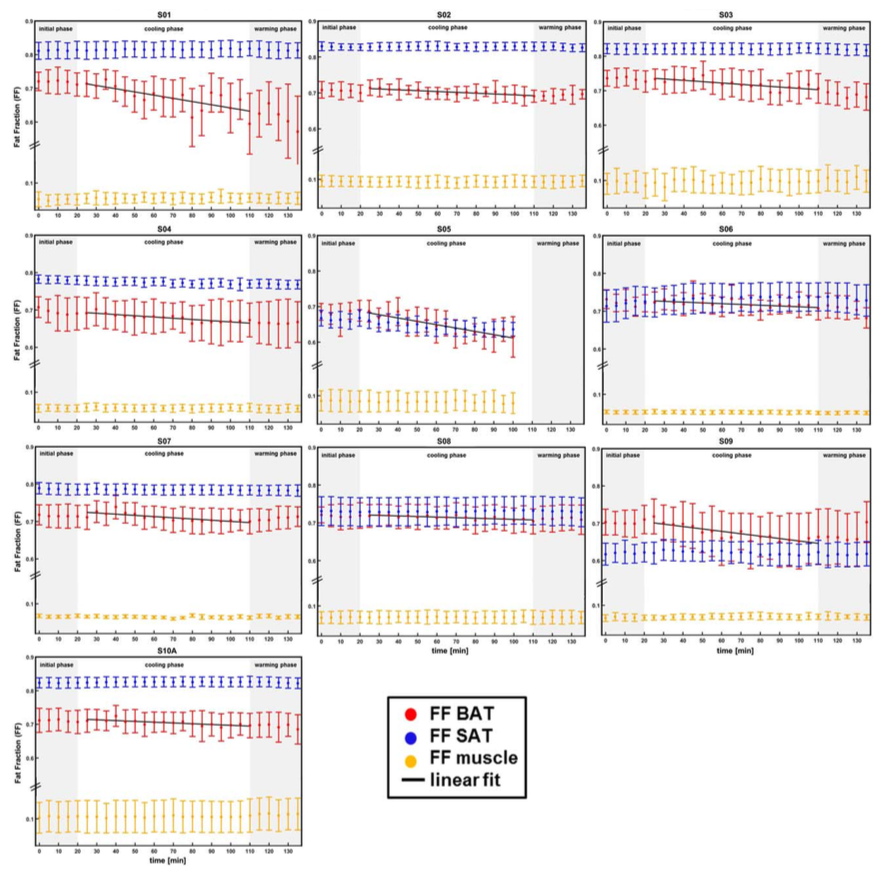
Table 7 - Time Course Measurements (Humans)

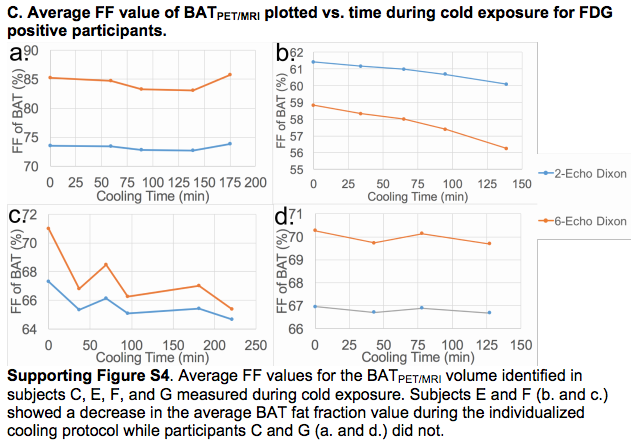
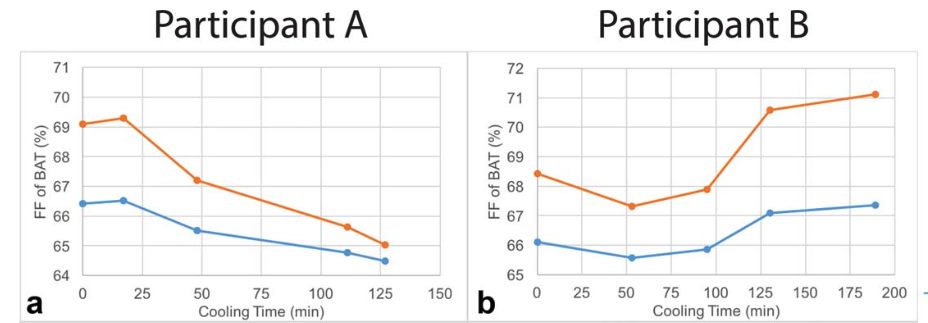
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Reference** | **Population** | **BAT Stimulation** | **Modality for BAT Detection** | **Findings** |
| Ouelette et al. (2012)18 | - Healthy males (n=6, 23-42 years, “normal” BMI) | - Acute cold exposure (180 minutes at 18°C) | - 18F-FDG, 11C-acetate, and 18FTHA PET/CT  - Dynamic PET acquisition | - Figure 4B is the time-radioactivity curve of 11C-acetate in BAT, which shows immediate uptake and maximal radioactivity at 60 seconds, after which it normalizes |
| Van Rooijen et al. (2013)67 | - Healthy adults (n=11, 7F, 4M, 21-28 years, “normal” BMI, “overweight” BMI) | - Acute cooling paradigm (two bouts of 10 minutes at NST) | - 2-Deoxy-2- 18F-FDG PET/CT  - Dynamic T2\* MRI | - Temporal fluctuation in T2\* signal that correlates with the cooling paradigm (figure 4D in paper) |
| Hanssen et al. (2015)104 | - Overweight males with type 2 diabetes (T2D) (n=8, 50-70 years) | - Cold acclimation (10 consecutive days of 2-6 hours of ambient temperature set at 14-15°C) | - 18F-FDG PET/CT | - Supplemental Figure 1 shows that after 60 minutes of “no” reported shivering, there is a gradual increase to “tense muscle” and “sometimes” |
| Rachid et al. (2015)73 | - Healthy females (n=12 “obese” BMI, n=12 “normal” BMI)  \*No age information | - Acute cold exposure (25 minutes at 18°C) | -18F-FDG PET/CT  - fMRI (hypothalamic activity) | - Figure 5A/D shows an abrupt reduction in fMRI signal (i.e. heightened hypothalamic activity) in lean but not obese subjects in response to cold |
| Annamalai et al. (2015)148 | - Healthy adults (n=23)  \*No other information available | - Acute cold exposure (2-6 hours total, including NST) | - 2-Deoxy-2-D- 18F-FDG PET/CT | - Figure 2D shows the respiratory capacity of human SCV BAT tissue by addition of substrates, inhibitors, and uncouplers, which suggests maximal BAT activation within ~5 minutes |
| Stahl et al. (2016)56 | - Healthy adults (n=10, 5F, 5M, 23-30 years, “normal” BMI) | - Acute cold exposure (90 minutes at 12°C) | - MRI | - Figure 4 shows time to nadir FF in BAT: S01: 90 mins; S02: 85 mins; S03: 70 mins; S04: 60 mins; S06: 60 mins; S07: 60 mins; S08: 60 mins; S09: 55 mins; S10: 75 mins (average = ~60 minutes)  - Only 2 subjects recovered FF during warming (S07 and S09), whereas S01 and S03 showed decreases |
| Haq et al. (2017)42 | - Healthy males (n=62, 18-39 years, all BMI categories) | - Acute cold exposure (60 minutes at 12°C) | - Infrared Thermography (IRT) | - Figure 3 shows that cold elicits a rapid change in SCV skin temperature, however this increase plateaus at 15-20 minutes and declines steadily until the end of the 60 minute exposure (top and middle lines in Figure 3) |
| McCallister et al. (2017)74 | - Healthy adults (n=16, 7F, 9M, 21-49 years, all BMI categories) | - Acute cold exposure (2-2.5 hours at NST) | - 18F-FDG PET/MRI | - Figure 9 and Supplementary Figure 4 presents BAT FF time course descriptions as follows:  🡪 Subject A (lean 24 year old M): Initial increase at around 20 minutes, then steady decrease until end (no time to nadir FF)  🡪 Subject B (overweight 30 year old M: Slight decrease until 50 minutes (nadir FF), and then increase until end of exposure  🡪 Subject C (lean 30 year old F): Slight decrease until around 80 mins (nadir FF), increased to above baseline FF at end  🡪 Subject E (lean 25 year old M): Gradual decrease throughout (no time to nadir FF)  🡪 Subject F (lean 20 year old M): Up and down pattern throughout (time to nadir FF around 50 mins)  🡪 Subject G (lean 25 year old F): Up and down (no net FF change, time to nadir FF before 50 mins)  🡪 Average time to nadir FF, if it did appear, occurred around 60 mins |
| Blondin et al. (2015)20 | - Healthy young adults (n=6; 22-26 years; normal/overweight BMI), type 2 diabetics (n=6; 56-64 years; all BMI categories)), age-matched controls (n=7; 56-62 years; normal/overweight BMI) | - Acute cold exposure (180 minutes at 18°C) | 11C-acetate PET/CT | - Figure 3B shows a time reactivity curve of BAT 11C over for healthy young controls, who had an initial increase over the first ~80 seconds, after which it fell towards baseline |
| Reber et al. (2018)107 | - Healthy humans (n=3)  \*No other details provided | - Acute cold exposure (13°C) | - Multi-spectral optoacoustic tomography (MSOT) | - Figure 5I shows a time course of VO2 and HbO2 (oxygenated Hb, the MSOT signal), which peak within minutes of stimulation |

**Table 8 -** Time Course Measurements (Animals)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Reference** | **Population** | **BAT Stimulation** | **Modality for BAT Detection** | **Findings** |
| Xian Yu et al. (2002)23 | - Healthy mice (n=?) | - Cold (48 hours at 4°C) | - RT-PCR for iBAT gene expression | - Figure 6 presents the expression of various BAT-related genes, including the following:  🡪 Cluster 1: GLUT1-3 inhibited by acute (1-24hours) but not chronic (24-48 hours) cold  🡪 Cluster 2: UCP1 and LPL expression increased in response to cold (6-24 hours)  🡪 Cluster 3: FAS increase during first hour, then drops until 6 hours  🡪 Cluster 5: MG Lipase increases during first hour, then decrease until 6 hours |
| Motillo et al. (2012)98 | - Healthy mice (n=11) | - B3-adrenergic agonist (CL-316,243) | - Live cell fluorescent reporter assay of PPAR activation | - Ligands for PPAR alpha and delta (receptors that modulate induction of oxidative genes) are detected at the lipid droplet surface within minutes of PKA activation (i.e. lipolysis) and can transcriptionally activate PPAR alpha and gamma over a period of hours (i.e. expansion of oxidative capacity to match growing FFA supply) |
| Chen et al. (2012)149 | - Healthy rats (n=9) | - B3-adrenergic agonist (CL-316,243) | - MION fMRI  - 18F-FDG PET/CT | - Figure 6 shows an immediate increase in iBAT blood volume upon BAT stimulation which is sustained for the duration of measurement |
| Khanna et al. (2012)99 | - Healthy mice (n=10) | - Intraperitoneal injection of NE | - BOLD fMRI  - iBAT temperature | - Figure 1: iBAT temperature increases a maximum of 5.5°C at 50 minutes after NE injection, and begins to decrease after 60 mins, reaching pre-injection levels around 140 mins  - Figure 2B: iBAT is “fully stimulated” 1-2 minutes after NE injection (maximum BOLD signal change after roughly 40 minutes), and the signal slowly recovers over 60 minutes as NE is degraded in the blood |
| Branca et al. (2013)65 | - Healthy mice (n=4) | - B3-adrenergic agonist (CL-316,243) | - BATSCI MRI | - Figure 6A shows the iBAT temperature following stimulation, which reaches a peak at 60 minutes and falls steadily thereafter  - Figure 6B shows that iBAT BATSCI signal decreases immediately after stimulation, reaches a maximal signal change at around 50-60 minutes, and begins to rise |
| Panagia et al. (2016)106 | - Healthy mice (n=5) | - B3-adrenergic agonist (CL-316,243) | - BOLD fMRI | - Figure 2C shows a transient change in iBAT BOLD signal following stimulation, forming a sharp “spike” that lasts 4-6 “time points” |
| Olsen et al. (2017)100 | - Healthy WT mice (n=?) | - B3-adrenergic agonist (CL-316,243) | - 18F-FDG PET/CT  - Metabolic chamber | - Figure 1C shows that upon stimulation, oxygen consumption increases transiently and appears to be reaching a maximum by 50 minutes post-injection |
| Garretson et al. (2016)94 | - WT Siberian Hamsters (n=36) | - B3-adrenergic agonist (CL-316,243) | - iBAT temperature | - Figure 6C shows that iBAT temperature appears to increase within the first 15 minutes of stimulation, after which it stabilizes |
| Simcox et al. (2017)101 | - WT male mice (n= 5) | - Cold exposure (6 hours at 4°C) | - Serum measurements | - Figure 4 shows a time course of the following: FFAs increase to 1.1mM within 30 minutes of cold, whereas increases in serum LCACs occur at 3 hours (same with hepatic Cpt1b, which produced LCACs) |
| Shin et al. (2017)115 | - WT mice (n= 5) | - B3-adrenergic agonist (CL-316,243) | -BAT temperature  - Oxygen consumption | - Figures 3F and G show an immediate increase in core body temperature and oxygen consumption in response to injection in control animals, respectively |

Figures 5A and D from Rachid et al. (2015)73 which presents a reduction in fMRI signal within the first 5 minutes of cold exposure (CE) in lean but not obese subjects

Figure 4 from Stahl et al. (2016)56 which presents the time course of FF changes during thermoneutrality, cooling (white box), and warming in 10 subjects

Figure 9 and Supplementary Figure 4 from McCallister et al. (2017)74

## Supplementary Material (Methods)

### Exclusion Criteria

Table 9 - List of excluded medications

|  |  |
| --- | --- |
| **Class of Drugs** | **List** |
| **Drugs affecting β-adrenergic receptor** | **β blockers**   * Acebutolol (Sectral) * Atenolol (Tenormin) * Bisoprolol (Zebeta) * Metoprolol (Lopressor, Toprol-XL) * Nadolol (Corgard) * Propranolol (Inderal LA, InnoPran XL)   **Asthma/COPD beta-adrenergic agonists**   * Bambuterol (Bambec, Oxeol) * Bitolterol mesylate (Tornalate) * Clenbuterol (Dilaterol, Spiropent, Ventipulmin) * Fenoterol (Berotec N) * Formoterol (Foradil, Zenhale, Symbicort, Forpack Discair, Oxeze/Oxis) * Isoprenaline/ Isoproterenol (Isuprel) * Levosalbutamol (Levalbuterol, Xopenex) * Metaproterenol (Alupent) * Olodaterol (Striverdi) * Pirbuterol (Maxair) * Procaterol * Salbutamol (Albuterol, Ventolin) * Salmeterol (Serevent Diskus) * Terbutaline (Bricanyl) * Vilanterol (Breo Ellipta, Relvar Ellipta)   **Others**   * Mirabegron (Myrbetriq) |
| **Drugs associated with hepatic steatosis** | **Corticosteroids**   * Betamethasone (Celestone) * Budesonide (Pulmicort, Entocort EC) * Cortone Acetate (Cortone) * Cotolone * Dexamethasone (Decadron) * Fludrocortisone (Florinef Acetate) * Methylprednisolone (Medrol, Methylpred-DP) * Prednisone (Bubbli-Pred, Deltasone, Prednicot, Prelone, Pediapred 5, Pms-prednisolone) * Triamcinolone (Aristocort)   **Tetracycline**   * Demeclocycline (Declomycin) * Doxycycline (Doryx, Vibramycin) * Minocycline (Dynacin, Minocin, Monodox) * Oxytetracycline (Terramycin) * Tetracycline (Achromycin) * Tigecycline (Tygacil)   **Other**   * Amiodarone (Cordarone, Nexterone, Pacerone) * L-asparaginase (Elspar) * Methotrexate (Rheumatrex, Trexall) * Tamoxifen(Nolvadex) * Valproic acid (Depakote, Depakote ER, Depakote Sprinkle, Depakene, Depacon, Stavzor) |
| **Anti-hyperglycemic drugs** | **Alpha-Glucosidase Inhibitor**   * Acarbose (Precose) * Miglitol (Glyset)   **Biguanides**   * Metformin (Glucophage, Glucophage XR, Glumetza, Fortamet, Riomet) * Metformin combination drugs   + Actoplus Met   + Avandamet   + Duetact   + Glucovance   + Janumet   + Jentadueto   + Komboglyze   + Metaglip   + PrandiMet   **Dipeptidyl peptidase-4 (DPP-4) inhibitor**   * Alogliptin (Nesina) * Canagliflozin (Invokana) * Dapagliflozin (Farxiga) * Linagliptin (Tradjenta) * Saxagliptin (Onglyza) * Sitagliptin (Januvia)   **Glucagon-like peptide**   * Exenatide (Exendin-4, Byetta) * Liraglutide (Victoza) * Lixisenatide (Lyxumia)   **Meglitinides**   * Repaglinide (GlucoNorm, Prandin, NovoNorm) * Nateglinide (Starlix)   **Insulin**  **Sulfonylurea**   * Chlorpropamide (Diabinese) * Glimepiride (Amaryl) * Glipizide (Glucotrol, Glucotrol XL) * Glyburide (DiaBeta, Glynase PresTab, Micronase) * Tolbutamide * Yolazamide   **Thiazolidinediones**   * Pioglitazone (Actos) * Rosiglitazone (Avandia) |
| **HIV drugs** | * HAART |
| **Antidepressants, anxiolytic drugs, anti-psychotic drugs** | **5-HT2 Receptor Antagonists**   * Trazodone (Desyrel, Oleptro, Trazorel, Trialodine, Trittico)   **5-HT3 Receptor Antagonists**   * Vortioxetine (Brintellix, Trintellix)   **Dopamine Reuptake Blocker**   * Bupropion (Wellbutrin)   **MAOIs (Monoamine oxidase inhibitors)**   * Isocarboxazid (Marplan) * Phenelzine (Nardil) * Selegiline (Emsam) * Tranylcypromine (Parnate)   **SNRIs (Serotonin and norepinephrine reuptake inhibitors)**   * Desvenlafaxine (Pristiq) * Duloxetine (Cymbalta) * Venlafaxine (Effexor XR)   **SSRIs (Selective serotonin reuptake inhibitor)**   * Citalopram (Celexa) * Escitalopram (Lexapro) * Fuoxetine (Prozac) * Fuvoxamine (Luvox) * Paroxetine (Paxil) * Sertraline (Zoloft)   **Tetracyclic Antidepressant**   * Maprotiline (Teva-Maprotiline) * Mirtazapine (Tera-Mirtazapine)   **Tricyclic medication**   * Amitriptyline (Elavil) * Amoxapine (Asendin**)** * Clomipramine (Anafranil) * Desipramine (Norpramin) * Doxepin (Silenor) * Imipramine (Tofranil) * Nortriptyline (Pamelor) * Protriptyline (Vivactil) * Trimipramine (Surmontil) |
| **Thyroid drugs** | **Anti-thyroid**   * Methimazole (Tapazole) * Propylthiouracil (Propyl-Thyracil or PTU)   **Thyroid**   * levothyroxine (T4) (Levothroid, Levoxyl, Synthroid, Tirosint, Unithroid) * liothyronine (T3) (Cytomel) * liotrix (T3 and T4) (Thyrolar) |
| **Antiemetic (5HT3 antagonists)** | * Dolasetron (Anzemet) * Granisetron (Granisetron Hydrochloride) * Ondansetron (Zofran) * Palonosetron (Aloxi) |
| **Drugs associated with serotonin metabolism** | * Amphetamine * Dextromethorphan * Metoclopramide |

**Table 10 -** List of excluded conditions

|  |  |
| --- | --- |
| **Conditions** | **List** |
| **Diseases associated with brown adipose tissue dysfunction** | * Adrenal gland disorder (i.e. pheochromocytoma) * Hibernoma |
| **Diseases associated with hepatic steatosis and liver disorders** | * Abetalipoproteinemia * Celiac disease * Cystic fibrosis * Galactosemia * Glycogen storage disease * Hemochromatosis * Hepatitis B or C * Hepatocellular carcinoma (HCC) * Homocystinuria * Inflammatory bowel disease * Lipodystrophy * Polycystic liver disease * Tyrosinemia * Weber-Christian syndrome * Wilson’s disease |

### List of serotonergic foods

Foods to avoid 24-hours before each visit included:

1. Banana
2. Walnut
3. Pineapple
4. Plum
5. Kiwi
6. Tomato
7. Avocado

### Visit Timelines

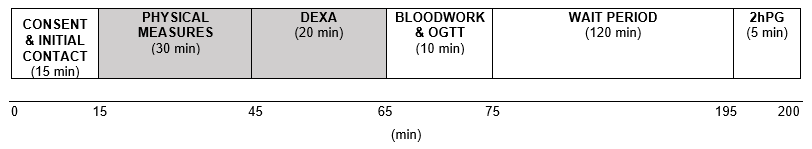
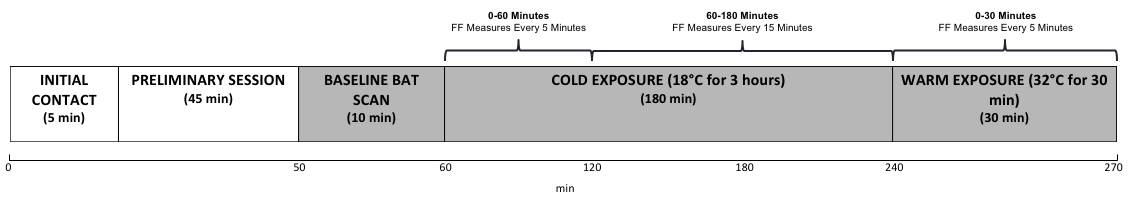
Data presented in this thesis are from the shaded regions in **Figures 1 and 2** below.

Figure 11 – Initial Visit (McMaster University Medical Centre)

Figure 12 – Time Course Visit (St. Joseph's Healthcare Hamilton)

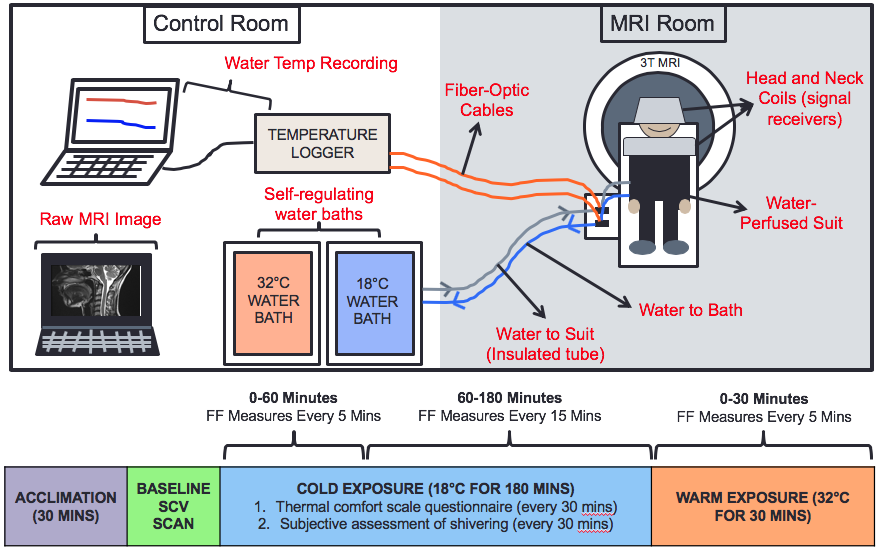


Figure 13 - Visual representation of the time course MRI session. Water was transported from the temperature-controlled baths to the water-perfused suit using insulated tubing. At the same time, fiber-optic temperature sensors recorded the temperature of the water entering and leaving the suit. All subjects wore a head and neck coil throughout the cold exposure in order to transmit the signal back to a computer which produced a raw image. Before they entered the scanner, all subjects were acclimatized at room temperature for 30 minutes and immediately following baseline FF measurements, cooling was started and scans were acquired every 5 minutes during the first 60 minutes of cooling, and every 15 minutes thereafter. Scans were then acquired every 5 minutes during the subsequent warming phase.

### MRI Parameters

**Table 11 -** MRI protocol parameters for IDEAL-IQ

|  |  |  |
| --- | --- | --- |
| **Pulse Sequence: IDEAL-IQ** | | |
| **Parameter** | **SCV** | **Liver** |
| Patient Entry | Head First | Feet First |
| Patient Position | Supine | |
| Coil | HNS Head/Neck/Chest | NeoCoil 32 Channel Torso Array |
| Orientation | Axial | |
| Flip angle | 4 | 3 |
| TE | Min Full | |
| Number of echoes | 6 | |
| Echo Train Length | 3 | |
| Number of shots | 2 | |
| Bandwidth | 111.11 | |
| Frequency axis | Bottom/Up | |
| Phase axis | Right/Left | |
| Spatial resolution (mm) | 1.48 x 1.48 | 1.33 x 1.33 |
| Acquired slice thickness (mm) | 4 | 8 |
| Imaging Options | EDR, Fast, IDEAL, ARC | |

### Stepwise procedure for image segmentation

**SCV BAT**

The following segmentation protocol for SCV BAT, which exhibited excellent inter-rater reliability (intraclass correlation coefficient of agreement >0.9 between three raters for both SCV BAT FF and segmentation volume) compared to previous studies57,69, was established by Ong et al. (unpublished) and is described accordingly:

Segmentation was performed in the axial plane using semi-automated and manual segmentation tools available within AnalyzePro (AnalyzeDirect, Overland Park, KS). Semi-automated techniques were used to circumvent processes that would normally be affected by operator bias or subjectivity, such as delineation of anatomical borders. The final protocol for SCV BAT consisted of five steps, which were repeated for each individual image:

**1. Application of fat mask:** A fat mask, which was based on an image-specific threshold that was established via observed differences in MR intensities, was applied directly to the FF map (a post-processing product of the IDEAL sequence) at the C7-T1 disc. This process was used to isolate “adipose-like” tissues and exclude background noise from future consideration. The image at the C7-T1 disc was used as the reference for all participants as this anatomical level contains a relatively large amount of fat in the SCV region and consistently displayed reasonable fat-water contrast which is imperative for the accurate separation of adipose from non-adipose tissues.

**2. Application of FF threshold:** A FF threshold from 30 to 100% was applied to the FF image. This ensured that only voxels within that defined range were included in the segmentation process (i.e. exclusion of “non-adipose tissue”). Ong et al. (unpublished) based this approach on published data in humans which suggests that SCV BAT occupies a wide range of FF values, and also through a series of protocol trials comparing this broad threshold (i.e. 30 to 100%) to a narrower threshold (i.e. 50 to 80%), wherein the former was more conservative in capturing cold-induces changes in FF.

**3. Manual delineation of the SCV:** A ROI was manually drawn over the SCV region using the “free-hand draw” tool (AnalyzeDirect, Overland Park, KS). Any adipose tissue between the C5/C6 and T1/T2 discs that was bound by the trapezius muscle posteriorly, the sternocleidomastoid muscle medially, and the clavicle bone inferiorly was included (see **Figure 13** below).77

**4. Erosion of ROI:** The ROIs were then post-processed using a one-time 2D-dimensional 1x3 erode (jack structural element) to correct for any inherent partial volume effects in the images.

**5. Application of T2\* mask:**As the FF mask failed to discriminate between different types of “adipose-like” tissues within the SCV, which is heterogeneous in nature, a T2\* mask was applied to the FF map. In particular, a T2\* threshold between 2 and 25 msec isolated WAT-like voxels from BAT-like voxels. The upper bound of this T2\* range was selected based on previous reports that identified ≥ 26 msec as being characteristic of muscles, fluids and white adipocytes61. Whereas, the lower bound was selected based on the inherent limitations in IDEAL, which is unreliable at quantifying T2\* values below 2 msec.

Once SCV BAT FF values were generated for each image, results were combined on a spreadsheet and a time series plot was generated for each participant (Prism 7; GraphPad Software, La Jolla, CA, USA).

**Posterior Neck SAT**

The segmentation procedure for the posterior neck SAT was identical to what has been described for SCV BAT above, with two notable exceptions. First, posterior neck SAT was identified as a superficial tissue which was bound by the trapezius muscle anteriorly (**Figure 13** below). Second, a T2\* mask was not used as this tissue was presumed to be homogenous with respect to white adipocytes. Since this was not a primary outcome measure, only slices at the C5/C6, C6/C7 and C7/T1 discs were considered.

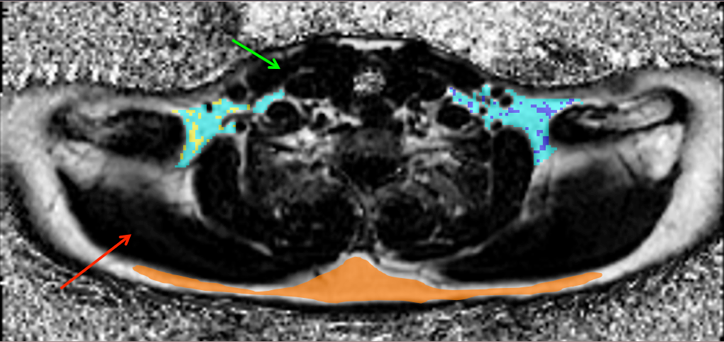
****In **Figure 13**, tissue bound by the sternocleidomastoid medially (green arrow) and trapezius posteriorly (red arrow) from the C5/C6 to T1/T2 discs was identified as SCV adipose tissue. “BAT” (teal) was then differentiated from “non-BAT” (yellow and blue) using a T2\* mask. Cold-induced changes in SCV BAT were compared to the posterior neck SAT (orange).

Figure 14 - Axial Slice of the Lower Cervical Region

### Pairwise comparisons for identifying time points of interest

Pairwise comparisons using a linear mixed model (LMM) with random slopes was selected due to its flexibility, robustness against missing data and unequally spaced time points, and ability to control for the inherent variations due to random effects (i.e. between subjects) when determining how much variation is due to a specified fixed effect, such as time83. First-order autoregressive (AR1) repeated covariance structure provided the lowest absolute Bayesian Information Criteria value (BIC) (see **Table 12**). BIC was used as a reference due to the fact that it penalizes a model fit based on the total number of parameters and number of subjects included in the analysis84,85. Two separate analyses were conducted using the above model specifications for SCV BAT: one with time point 1 (i.e. 0 minutes) as the reference, and one with time point 21 (i.e. 180 minutes) as the reference. The same analysis procedure was repeated for the posterior neck SAT, but only time point 1was used as the reference. A Bonferroni correction was employed to minimize the implications of multiple comparisons error.

**Table 12 -** Linear mixed model selection summary

|  |  |  |
| --- | --- | --- |
| **FF REDUCTION** | | |
| **REPEATED COVARIANCE TYPE** | **RANDOM COVARIANCE TYPE** | **BIC RESULT** |
| COMPOUND SYMMETRY |  | 810.492 |
|  |
|  |
| ARH1 |  | 790.819 |
|  |
|  |
| ARH1 HETEROGENEOUS |  | 1021.902 |
|  |
|  |
| TOEPLITZ |  | 917.038 |
|  |
|  |

### Quadratic Modeling of SCV BAT FF

A random-intercept multilevel regression model with the lowest BIC value (as above) was used to evaluate the relationship between BAT FF (dependent variable) and time (independent variable). Such model accounts for the intra-class correlation (ICC) due to multiple measurements within subjects, and for random effects between subjects150. Due to the perceived quadratic relationship between BAT FF and time, the following model was used: where is the model intercept, is the slope for the linear component, is the slope for the quadratic component, and is unobserved random error.

## Supplementary Material (Results)

### Individual Time Course Plots (FF reduction)

|  |  |  |
| --- | --- | --- |
| **COMPLETED 180 MINUTES OF COLD EXPOSURE** | | |
|  |  | |
|  | |  |
|  | |  |
|  | |  |
|  | | |

|  |  |
| --- | --- |
| **COMPLETED > 60 TO < 180 MINUTES OF COLD EXPOSURE** | |
|  |  |

|  |  |
| --- | --- |
| **COMPLETED 60 MINUTES OF COLD EXPOSURE** | |
|  |  |
|  | |

### Individual Time Course Plots (Absolute Change)

|  |  |
| --- | --- |
| **ALL** | |
|  |  |

|  |  |
| --- | --- |
| **COMPLETED 180 MINUTES OF COLD EXPOSURE** | |
|  |  |
|  |  |
|  |  |
|  | |

|  |  |
| --- | --- |
| **COMPLETED > 60 TO < 180 MINUTES OF COLD EXPOSURE** | |
|  |  |

|  |  |
| --- | --- |
| **COMPLETED 60 MINUTES OF COLD EXPOSURE** | |
|  |  |
|  | |

### Time course of change in T2\* during Cooling

Figure 15 - T2\* reduction during cooling

### Time course of change among individuals who completed the full duration of cooling (n=7)

Figure 16 – Changes in SCV BAT FF (left) and posterior neck SAT FF (right) during cooling among those who completed the full 3-hour exposure. Time course plots of FF reduction (A,D), AUC for 30 minute intervals (B,E), and slope for 30 minute intervals (C,F). Data are presented as mean±SD. N=7.

### Tabular Output from the Pairwise Comparisons

**SCV BAT FF**

Table 13 - Pairwise comparisons using LMM with 0 minutes used as the reference (SCV BAT)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **REFERENCE POINT (J)** | **COMPARISON POINT (I)** | **N** | **MEAN DIFFERENCE (I-J)** | **P-VALUE** |
| 0 MIN | 5MIN | 11 | -1.26 | 0.097 |
| 10MIN | 12 | -1.60 | 0.007\* |
| 15MIN | 12 | -1.92 | 0.001\* |
| 20MIN | 11 | -1.88 | 0.002\* |
| 25MIN | 10 | -2.29 | <0.001\* |
| 30MIN | 12 | -1.82 | 0.004\* |
| 35MIN | 10 | -2.54 | <0.001\* |
| 40MIN | 9 | -1.92 | 0.008\* |
| 45MIN | 12 | -2.93 | <0.001\* |
| 50MIN | 10 | -2.73 | <0.001\* |
| 55MIN | 9 | -3.18 | <0.001\* |
| 60MIN | 11 | -3.21 | <0.001\* |
| 75MIN | 9 | -3.86 | <0.001\* |
| 90MIN | 9 | -4.56 | <0.001\* |
| 105MIN | 8 | -4.45 | <0.001\* |
| 120MIN | 8 | -3.97 | <0.001\* |
| 135MIN | 8 | -4.76 | <0.001\* |
| 150MIN | 7 | -4.76 | <0.001\* |
| 165MIN | 7 | -4.28 | <0.001\* |
| 180MIN | 7 | -3.81 | <0.001\* |

\*Denotes statistical significance at the 0.05 level

**Table 14 -** Pairwise comparisons using LMM with 180 minutes used as the reference (SCV BAT)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **REFERENCE POINT (J)** | **COMPARISON POINT (I)** | **N** | **MEAN DIFFERENCE (I-J)** | **P-VALUE** |
| 180 MIN | 0MIN | 12 | 3.81 | <0.001\* |
| 5MIN | 11 | 2.54 | 0.004\* |
| 10MIN | 12 | 2.21 | 0.019\* |
| 15MIN | 12 | 1.89 | 0.081 |
| 20MIN | 11 | 1.92 | 0.074 |
| 25MIN | 10 | 1.52 | >0.100 |
| 30MIN | 12 | 1.98 | 0.042\* |
| 35MIN | 10 | 1.26 | >0.100 |
| 40MIN | 9 | 1.88 | 0.084 |
| 45MIN | 12 | 0.88 | >0.100 |
| 50MIN | 10 | 1.08 | >0.100 |
| 55MIN | 9 | 0.62 | >0.100 |
| 60MIN | 11 | 0.59 | >0.100 |
| 75MIN | 9 | -0.06 | >0.100 |
| 90MIN | 9 | -0.75 | >0.100 |
| 105MIN | 8 | -0.64 | >0.100 |
| 120MIN | 8 | -0.16 | >0.100 |
| 135MIN | 8 | -0.95 | >0.100 |
| 150MIN | 7 | -0.95 | >0.100 |
| 165MIN | 7 | -0.48 | >0.100 |

\*Denotes statistical significance at the 0.05 level

### Time course of change in posterior neck SAT FF

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Table 15 -** Summary of individual cold-induced changes in posterior neck SAT FF | | | | | |
| **SUBJECT** | **PRE-COLD FF (%)** | **MAX FF REDUCTION (%)** | **TIME TO MAX FF REDUCTION (min)** | **FF REDUCTION @ END OF COLD (%)** | **DURATION OF COLD (min)** |
| **1** | 85.10 | 2.73 | 150 | 0.91 | 180 |
| **2** | 77.99 | 0.26 | 75 | -0.47 | 180 |
| **3** | 87.19 | 0.71 | 120 | 0.3 | 180 |
| **4** | 89.00 | 1.4 | 45 | 0.97 | 60 |
| **5** | 89.43 | 0.89 | 55 | 0.52 | 60 |
| **6** | 69.34 | 2.67 | 15 | 0.23 | 135 |
| **7** | 83.4 | 3.14 | 180 | 3.14 | 180 |
| **8** | 76.42 | 2.42 | 20 | 0.89 | 60 |
| **9** | 69.53 | 0.07 | 150 | -0.7 | 180 |
| **10** | 80.54 | 3.31 | 15 | 2.71 | 90 |
| **11** | 66.88 | 3.1 | 55 | -0.06 | 180 |
| **12** | 75.00 | 3.52 | 180 | 3.52 | 180 |

Table 16 - Pairwise comparisons using LMM with 0 minutes used as the reference (posterior neck SAT)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **REFERENCE POINT (J)** | **COMPARISON POINT (I)** | **N** | **MEAN DIFFERENCE (I-J)** | **P-VALUE** |
| 0 MIN | 5MIN | 11 | 0.14 | >0.100 |
| 10MIN | 12 | -0.10 | >0.100 |
| 15MIN | 12 | -0.50 | >0.100 |
| 20MIN | 11 | -0.43 | >0.100 |
| 25MIN | 10 | -0.67 | >0.100 |
| 30MIN | 12 | -0.38 | >0.100 |
| 35MIN | 10 | -0.52 | >0.100 |
| 40MIN | 9 | -0.63 | >0.100 |
| 45MIN | 12 | -0.43 | >0.100 |
| 50MIN | 10 | -0.45 | >0.100 |
| 55MIN | 9 | -0.76 | >0.100 |
| 60MIN | 11 | -0.44 | >0.100 |
| 75MIN | 9 | -0.66 | >0.100 |
| 90MIN | 9 | -0.97 | >0.100 |
| 105MIN | 8 | -1.26 | 0.074 |
| 120MIN | 8 | -1.18 | >0.100 |
| 135MIN | 8 | -0.67 | >0.100 |
| 150MIN | 7 | -1.11 | >0.100 |
| 165MIN | 7 | -0.92 | >0.100 |
| 180MIN | 7 | -1.16 | >0.100 |

\*Denotes statistical significance at the 0.05 level

### Investigating if participant characteristics are related to the pattern of cold-induced change

**Comparing those who withstood the entire duration of cooling and those who did not**

*Environmental Factors*



Figure 17 - Jitter plots of ambient temperature during the time course cold exposure visit (left) and outdoor temperature one hour before the time course cold exposure visit (right) in individuals who completed <60 minutes, >60,<180 minutes, and 180 minutes of cold

MRI room temperature, which can augment the effects of a cold exposure delivered via a water-perfused suit, was similar among all 12 participants (left panel). Furthermore, outdoor temperature might allude to an individual’s *a priori* state of cold acclimation151. However given the large variability of outdoor temperature recorded near the time course acquisition visits, especially among those who endured the full duration of cold, this was also likely not a contributing factor to the observed findings.

*Body Composition*

Figure 18 - Jitter plots of BMI (left), LMI (middle) and % total body fat (right) in individuals who completed <60 minutes, >60,<180 minutes, and 180 minutes of cold. Data are presented as median±IQR.



Although BMI was comparable between groups (left panel), % body fat of those who endured only the first hour of cold exposure were either outside or approaching the upper IQR of the other two groups (right panel). In other words, those with a higher % total body fat appeared to tolerate cold the shortest. Complementary to this finding, those who withstood cold the longest tended to have a higher LMI which might allude to a heightened ability to undergo shivering thermogenesis, a dominant source of cold-induced heat production11,114.

*Indices of BAT Activity*

AUC calculations within the first 60 minutes of cold exposure were visually compared between groups.



Figure 19 - Jitter pots summarizing total AUC at 10 minutes (left) and 60 minutes (right)

**Figure 18** presents an expected increase in AUC over the first hour of cold exposure regardless of length of cold exposure endured. However, the distribution of AUC within and between groups did not appear to change over this time, suggesting that it might not feasible to differentiate between individuals who endured varying lengths of cold based solely on patterns of SCV BAT FF.

Table 17 - Tabular output comparing those who completed the full duration of cooling and those who did not. Values presented as median (min,max)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **60 MINS (n=3)** | **>60, <180 MINS (n=2)** | **180 MINS (n=7)** |
| **COVARIATES** | **BMI (kg/m2)** | 27.0 (24.4, 28.9) | 22.7 (19.8, 25.5) | 23.7 (21.0, 28.0) |
| **% TOTAL FAT** | 37.1 (21.7, 38.4) | 21.7 (21.2, 22.2) | 21.1 (15.6, 30.9) |
| **AMBIENT TEMP** | 20.5 (20.1, 20.6) | 20.5 (20.3, 20.6) | 20.4 (19.2, 21.0) |
| **OUTDOOR TEMP** | -0.4 (-10.6, 9.6) | 2.6 (1.9, 3.2) | 9.6 (-12.2, 18.9) |
| **INDICES** | **10 MIN AUC** | 8.95 (3.18, 22.08) | 12.14 (4.48, 19.8) | 7.0 (-3.6, 27.78) |
| **25 MIN AUC** | 41.63 (22.5, 62.95) | 38.18 (7.93, 68.43) | 25.48 (5.55, 57.10) |
| **35 MIN AUC** | 63.58 (31.2, 142.1) | 63.26 (18.7, 107.83) | 37.5 (18.9, 79.85) |
| **45 MIN AUC** | 87.43 (40.8, 205.35) | 88.35 (23.68, 153.03) | 57.2 (41.78, 116.03) |
| **60 MIN AUC** | 169.5 (58.5, 258.6) | 126.5 (36.9, 216.0) | 110.4 (72.9, 181.0) |

**Relating indices of BAT activity with indices of body composition at time points of interest**

**Table 18 -** P-values corresponding to each Spearman rho value identified in Figure 8 above

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **AUC** | **BMI** | **% BODY FAT** |  | **FF REDUCTION** | **BMI** | **% BODY FAT** |
| **180 MIN AUC** | 0.048\* | 0.034\* |  | **180 MIN REDUC** | 0.003\* | 0.110 |
| **90 MIN AUC** | 0.048\* | 0.034\* |  | **90 MIN REDUC** | 0.167 | 0.088 |
| **60 MIN AUC** | 0.007\* | 0.034\* |  | **60 MIN REDUC** | 0.139 | 0.066 |
| **10 MIN AUC** | 0.048\* | 0.396 |  | **10 MIN REDUC** | 0.200 | 0.200 |

**Relating indices of BAT activity with indices of body composition at all time points**

****

Figure 20 - Time course of Spearman correlation coefficients for the relationships between indices of body composition (i.e. BMI (orange), % body fat (blue), and LMI (green)) and FF reduction (A) and AUC (B). The magnitude and direction of each rho value is presented. \*denotes statistical significance at *p*<0.05. N=7 in each association unless specified otherwise.

### Time course of T2\* reduction during warming

Figure 21 - T2\* reduction during warming

## Supplementary Material (Discussion)

Figure 22 - Proposed model for time course of fuel use during BAT activation

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