THE EFFECT OF COLD ACCLIMATION ON CHANGES IN MUSCLE ACTIVITY

By

Hans Christian Tingelstad

Thesis submitted to the

Faculty of Graduate and Post Doctoral Studies

University of Ottawa

in partial fulfillment of the requirements for the masters degree in the

School of Human Kinetics

Master's Degree (2013)
University of Ottawa
(Human Kinetics)

Title: The effect of four weeks of cold acclimation on changes in muscle activity.

Author: Hans Christian Tingelstad, B.Sc (Sports Biology)

Thesis Supervisor: Dr. Francois Haman, Ph.D., Department of Human Kinetics, Faculty of Health Sciences, University of Ottawa

Research funded by:

The Norwegian State Educational Loan Fund

SUMMARY:

Human beings have been exposed to different cold conditions throughout time, and have through cold acclimation developed mechanisms to survive in these conditions. Cold acclimation can be elicited through exposure to natural cold climates, or artificially induced in a laboratory to study the body's response to repeated cold exposures. Several studies looking at the effects of cold acclimation in humans have been conducted during the last 50 years, and have reported that cold acclimation can lead to a change in skin and core temperature, heat production and shivering. An accurate quantification of shivering thermogenesis (ST) during cold acclimation has not been done before, and most previous measurements of shivering during cold acclimation have been inaccurate and inadequate. In this study a Liquid Condition Suits (LCS) was used to elicit cold acclimation (10°C, 2hr daily, for 4 weeks) while an accurate measurement of the effect of cold acclimation on changes in muscle activity was conducted. In CHAPTER 2, results showed that four weeks of cold acclimation at 10°C did not change skin and core temperature, heat production or ST. The effects on shivering pattern and fuel selection were also analysed, but no effects of cold acclimation could be observed. These measurements were a part of a larger study, in which the effects of cold acclimation on changes in BAT were the main outcome measures. These data showed that an increase in BAT volume (45%) and activity (120%) were the only observed effects of cold acclimation. In CHAPTER 3, we set out to assess if changes in shivering from pre to post cold acclimation are associated with changes in BAT volume, and if the amount of BAT a participant possesses prior to cold acclimation can be used to predict changes in shivering intensity during cold acclimation. The interindividual variability in changes in thermal responses, heat production, shivering and BAT volume occurring between subjects during four weeks of cold acclimation was also addressed in this section.

ACKNOWLEDGEMENTS

First of all, I want to thank my thesis supervisor Dr. Francois Haman for taking me on as a master student and being my thesis supervisor. I have learned a lot from you during this year we have worked together, and your help and feedback has been invaluable for me finishing my master's degree. I also want to thank PhD candidate Denis Blondin for the excellent collaboration during data collection and for teaching me the techniques needed for data collection and data analysis, and also the research group at the Sherbrooke University Hospital for the collection of the BAT data. I want to say thank you to Dr. Mary-Ellen Harper and Dr. Ollie Jay for being on my thesis committee and all the useful feedback they have given me in the process of writing my thesis. I also want to thank Olivier Mantha for the great discussions we have had in the office and the feedback I have gotten from him on the written work in my thesis. I want to thank my parents Kari and Gudbrand Tingelstad and my grandparent Marit and Hans Tingelstad for the financial and mental support they have given me. Finishing my master thesis here in Ottawa would not have been possible without them. Last I want to thank my brother Lars Martin Tingelstad, my sister Hanne Tingelstad and all of my friends for the social support they have given me during the process of completing my master's degree.

Contents

LIST OF ABBREVIATIONS AND SYMBOLS:

Abbreviations

^{18}F-FDG – Fluordeoxyglucose

ATP – Adenosine Triphosphate

AUC – Area under the curve

BAT – Brown Adipose Tissue

BF – Body Fat

BMI – Body Mass Index

BMR – Basal Metabolic Rate

Ca^{2+} - Calcium

cAMP – cyclic Adenosine Monophosphate

CAST – Cold Air Stress Test

CHO – Carbohydrates

CNS – Central Nervous System

CT – Computed Tomography

ECG – Electrocardiography

EE – Energy Expenditure

EMG – Electromyography

FFA – Free Fatty Acids

H^+ - Hydrogen Proton

H_{prod} – Heat Production

LCS – Liquid Condition Suit

MU – Motor Unit

MR – Metabolic Rate

MVC – Maximal Voluntary Contraction

NA – Noradrenalin

NST – Non-shivering Thermogenesis

PET – Positron Emission Tomography

RMR – Resting Metabolic Rate

RMS – Root Mean Square

SCAT – Standard Cold Air Test

$Shiv_{WBI}$ – whole body shivering

SNS – Sympathetic Nervous System

SUV – Standard Uptake Value

ST – Shivering Thermogenesis

TAG – Triglycerides

T_{core} - Core Temperature

T_{skin} – Skin Temperature

UCP 1 – Uncoupling Protein 1

VO_{2max} – Maximal Oxygen Consumption

WAT – White Adipose Tissue

Units

˚**C** – degrees Celsius

g – gram

Hz – Hertz

kBq – kilo Becquerel

kg – kilo gram

kJ – kilo Joule

mL - millilitre

LIST OF TABLES AND FIGURES

Tables

Figures

CHAPTER 1: GENERAL INTRODUCTION

THE HUMAN BODY'S RESPONSE TO COLD

The acute effects of cold exposure in humans

Endotherms, like human beings, are dependent on a tightly controlled core temperature to maintain basic functions (Boyles et al. 2011; Mozo et al. 2005).The core temperature in humans is regulated within a narrow zone between 36-38°C (Mekjavic, Sundberg, and Linnarsson 1991; Schmidt-Nielsen 1997).Exposing human beings to an acute cold, activates different mechanisms to combat the cold stress. If no measures are taken, an acute cold exposure can cause a drop in core temperature, leading to reduced enzyme efficiency and diffusion capacity, a reduced cellular energy availability and decreased membrane ion fluxes (Morrison, Nakamura, and Madden 2008). The mechanism first activated when experiencing an acute cold exposure is vasoconstriction of peripheral blood vessels (Sessler et al. 1990). Vasoconstriction reduces blood flow to cutaneous areas of the skin, decreasing skin temperature and thereby the temperature gradient for heat loss between the skin and the environment. Vasoconstriction also helps reduce the return of cold blood from the periphery to central areas of the body, avoiding an even further cooling of the core. Sessler et al. (1990) found that vasoconstriction in mild cold (20°C) can decrease heat loss with as much as approximately 25%. Vasoconstriction is the body's immediate response when exposed to acute cold, because preserving heat is more efficient than generating heat (Sessler et al. 1990).

Non Shivering Thermogenesis

When limiting heat loss is not sufficeint to maintain homeostasis during an acute cold exposure, mechanisms to increase heat production are activated. The human body can increase its heat production through the activation of two distinct mechanisms: shivering (ST) and non-shivering thermogenesis (NST). NST is the first mechanism activated - in contrast to ST, NST does not inhibit locomotion (Stott and Slee 1985). NST is defined as heat producing processes liberating chemical

energy not including shivering (Janský 1973). BAT has a large heat producing potential and may be the biggest contributor of heat production to NST (Himms-Hagen 1984). A full section about the history, function and properties of BAT will follow later in this chapter. Two other mechanisms recognized to paricipate in NST are futile cycling in adipose tissue and muscles. In adipose tissue it is the cycling of free fatty acids (FFA) to triacylglycerides (TAG) that produces heat (Steinberg et al. 1964), whereas in muscle tissue it is the leakage of Ca^{2+} from sarcoplasmic reticulum and the re-pumping of Ca^{2+} that dissipates heat (Wijers, Saris, and Van Marken Lichtenbelt 2009).

The contribution of NST to total heat production in humans is hard to quantify, and the importance of NST has for a long time been believed to be minimal (Vallerand et al. 1999; Wolfe et al. 1990; Weber, Klein, and Wolfe 1990; Himms-Hagen and Ricquier 1998). Very few studies have attempted to measure the contribution of NST to total heat production, but Gosselin and Haman(2012) reported that green tea extract can be used to increase NST in the cold. Eight healthy adult male were exposed to cold (15°C water using an LCS) for 180 min with and without ingesting a green tea extract (1600 mg epigallocatechin gallate and 600 mg caffeine). Total heat production did not change, but ST decreased by 26% after the ingestion of green tea extract. These results suggest that the ingestion of green tea extract during an acute cold exposure might lead to a shift in the mechanisms of heat production, from ST to NST.

Shivering Thermogenesis

Shivering thremogenesis (ST) is the second step in cold induced thermogenesis. When humans (and other endotherms) are exposed to cold, different pathways are activated to decrease heat loss, and increase heat production. As described earlier, the first mechanism activated to increase heat production is NST. The increased heat production from the activation of NST is sufficient to maintain homeostasis during a mild, short term cold exposure. If the intensity of the cold exposure

is increased to moderate or severe, the heat producing capacity of NST is insufficient to maintain homeostasis. To compensate for the deficit in heat production, ST in skeletal muscles is activated. ST is defined as a heat production generated through asynchronous muscle contractions (Haman et al. 2004), and is under full control of the CNS. Shivering is initiated, terminated and adjusted by the CNS in response to an increased need for heat production (Hemingway 1963). Thermo-sensitive receptors in the skin senses a reduction in skin temperature, and relays the signal to the pre-optic area of the hypothalamus (Morrison, Nakamura, and Madden 2008). The hypothalamus stimulates neurons in the rostral medullary raphé, which again activates fusimotor fibers, leading to the onset of shivering (Sato 1981; Sato et al. 1990; McAllen et al. 2010). The heat producing capacity of ST is formidable compared to NST, mainly because of the volume of tissue involved. A healthy human being weighing ~80kg, has between 50 and 150g of BAT compared to ~30kg of skeletal muscles (Janssen et al. 2000). The activation of ST can increase metabolic rate (MR) to ~5 times resting metabolic rate (RMR) or ~40% of VO_2max (Eyolfson et al. 2001).

Shivering was earlier assessed by visual obersvation, but measuring electro physiological activity with EMG has been shown to be more precise and better for comparing changes within and between subjects, as well as quantifying the size of the changes. EMG can be measured in two different ways, through bipolar surface electrodes placed on the skin, or through implanted bipolar fine-wire electrodes to detect activity of deeper muscles (Soderberg and Cook 1984).
EMG recordings have identiyfied that shivering can occur in two distinct patterns, distinguished by differneces in intensity, rate of occurence and the type of muscle fibers recruited (Israel and Pozos 1989; Meigal 2002). Continous shivering occurs on a high rate of (8-10Hz) and with a low intensity (~2-5% of MVC) (Meigal, Lupandin Iu, and Kuz'mina 1993). Burst shivering occurs on a much lower frequency (0.1-0.2 Hz or 8-16 times/min), but with a much higher intensity (7-15% of MVC) (Israel and Pozos 1989). The continous low intensity shivering seems to be elicited through low threshold

motor units (MU), activating type 1, slow oxidative muscle fibers (highly fatigue resistant) which are fueled by lipid oxidation (Haman et al. 2004; Haman, Legault, and Weber 2004). Burst shivering results from activation of higher threshold MU's, activating type 2, fast-twich glycolytic fibers (more fatigable)(Petajan and Williams 1972; Meigal 2002) which are mainly fueled by CHO oxidation.

Cold acclimation

Human beings have been exposed to different environmental conditions throughout time depending on geographical location, and found ways to adapt to survive in these conditions. Different groups of people have adapted in different ways to cope with cold stress, according to the nature of the cold condition they are exposed to. In a reasearch paper written by Bittel in 1987, four types of cold adaptations were described: 1. Hypothermic adaptations, characterized by increased core cooling but less metabolic compensation (observed in Bushmen of the Kalahari dessert and cold-acclimated subjects under laboratory conditions), 2. Insulative adaptation, which is characterized by a lower mean skin temperature but no change in core temperature (observed in coastal tribes of tropical northern Australia and cold acclimation by cold water immersion), 3. Insulative hypothermic adaptation, which is described as an intermediate between insulative and hypothermic adaptations (observed in nomadic Lapps and people adapted to cold water immersion), and 4. Metabolic adaptations, characterized by an increased metabolic rate, higher skin temperature and a normal core temptrature (observed in Inuits and arctic first nations (reference to Eskimos and Arcitc indians, used in orginal publication) and caucasian subjects under cold laboratory conditions at rest and after physical exercise). Such adaptations can be provoked both under natural conditions and in a laboratory, and the nature of the cold exposure (duration of exposure,type of cold stress, physical fitness of participant etc.) seems to be one of the reasons for differences in adaptation. An example of the effect of duration of cooling was observed in divers,

diving in the arctic area. After three weeks, hypothermic adaptations were observed in the divers, whereas after 6-7 weeks this had changed to a more insulative adaptation (Skreslet and Aarefjord 1968).

Some of the first acclimation studies ever conducted were done in the 1950's on rodents and yielded surprisingly good information about how cold acclimation affects skin and core temperature, aswell as oxygen consumption and ST. In 1956, Hart, Heroux and Depocas conducted two separate but similar studies in which they investigated effects of cold acclimation on rats. In the first study, two groups of rats were exposed to 30°C (group 1) and 6°C (group 2) for 6 to 7 weeks. At the end of the acclimation period, both groups of rats were put through an experimental session of 45 min at 30°C and 60 min at 6°C. After being transfered to the cold of 6°C, both groups showed an abrupt increase in oxygen consumption. After 15 min of cold exposure, the warm acclimated rats also had a progressive increase in EMG activity in the two muscles from which EMG was recorded.The cold acclimated group, showed no change in EMG activity after being transfered to the cold. This showed that the cold acclimated rats relied more on NST, compared to the non acclimated rats which relied on ST to increase heat production and maintain homeostasis.

In the second cold acclimation study, two groups of rats were again kept at 6°C (group 1) and 30°C (group 2) for approximately 6 weeks. At the end of the acclimation period both the cold and warm acclimated rats where put through an experimental protocol, in which they were exposed to 30°C for at least 45 min, before spending 20-30 min at 20°C , 6°C and finally -6°C. Both EE and shivering intensity were measured during the experimental sessions. As seen in the previous study, the warm acclimated rats experienced an abrupt increase in EMG activity when exposed to 6°C cold, whereas the cold acclimated rats showed no change in EMG after being moved to 6°C. It was also evident that the increase in EMG was much smaller when the warm acclimated rats went from 30°C to 6°C,

compared to when they went from 6°C to -6°C. The cold acclimated rats showed no change in shivering after being transferred from 30°C to 6°C, but they had a marked increase in EE for the first 12 min, which plateaued and stabilized for the rest of the time spent at 6°C. After being transferred from 6°C to -6°C, a slight increase in muscle activity could be observered. In the group of cold acclimated rats, EMG measurements were also conducted during the acclimation period. During the first stage of cold acclimation the rats mainly depend on burst shivering to maintain heat production, but after a few days the character of shivering changed from burst shivering to continous shivering. The overall intensity of the shivering was high during the first weeks of acclimation before decreasing slowly. After four weeks, muscle activity was the same as in the warm acclimated rats at 30°C before being transfered to 6°C.

These studies from the early 1950's showed that cold acclimation can have an effect on EE, shivering intensity and shivering pattern in rats. This really sparked the interest for cold acclimation studies, and researchers started looking more towards humans and how cold acclimation would effect human beings and their thermoregulatory responses to cold.

Change in skin and core temperature.

Following these interesting findings in regards to the effects of cold acclimation in rats, several studies were undertaken to investigate if similar effects could be found in humans. Even though large changes in shivering and shivering intensity were observed during cold acclimation in rats, most of the human studies looked at changes in skin and core temperature, along with metabolic rate. If the effects on shivering was taken into consideration it was usually determined by visual obeservation.

In 1986, Young et al.(1996) conducted a study investigating the effects of repeated cold water immersion on human thermoregulatory responses to an acute cold air exposure. Seven volunteers

(~24 years old) were recruited for the study, and underwent five weeks of cold acclimation. The cold acclimation protocol consisted of 90 min of cold water immersion (18°C, stirred), five days a week for 5 weeks. Two days before and two days after the acclimation, a cold air stress test (CAST) was completed by each participant. The CAST consisted of a 30 min baseline period at 24°C, before 90 min of cold air exposure to 5°C in an environmental chamber. VO_2 consumption as well as skin and core temperature were measured during the baseline period, and then periodically (every 30 min) during the CAST. Cateocholamine levels in the blood were also measured for the last five minutes of both the baseline period and CAST.

The five weeks of cold acclimation led to a decrease in core tempeature during the CAST post acclimation compared to pre acclimation. A decrease in skin temperature during CAST post acclimation was also observed, although the initial skin temperature prior to CAST was the same pre and post acclimation. A significant difference in metabolic rate was only observed in the first 10 min of CAST between pre and post acclimation, but after correcting for body surface area this difference was negated. The blood catecholamine levels pre and post acclimation showed that cold acclimation led to a significant increase in norepinephrine levels during CAST, but no significant difference was observed in epinephrine levels.

The effect of cold acclimation on similar parameters as tested by Young et al., was investigated by Bittel in 1987. Ten healthy male participants were subjected to cold water immersion (10-15°C) for 1-3 hours daily (depending on cold tolerance), five consecutive days per week for two months. A cold test was performed before and after the acclimation period, where subjects were exposed to cold air (10°C) for two hours (1.5 hours of equilibration at 28°C prior to the cold test). During the cold test, measurements of skin and core temperature, metabolic heat production, EMG and ECG were recorded to determine the effects of cold acclimation. After the acclimation period a decrease in skin and core temperature was observed during thermoneutral conditions. During the cold test

the decrease in core temperature was smaller in the post acclimation test compared to the pre acclimation test. Shivering onset was also delayed by cold acclimation, and the core and skin temperature at shivering onset was lower post, compared to pre cold acclimation. Contradictory to the findings by Young et al., Bittel found that metabolic heat production was increased after cold acclimation.

Nine years after Bittel's study, a cold acclimation study was conducted by Jansky et al. (1996), yielding completely different results on the change in metabolic heat production, compared to Young et al. and Bittel. Jansky et al. investigated the change in thermoregulatory responses after cold acclimation in healthy adult sports men. The participants underwent cold water immersion (14±1°C, stirred) for one hour, three times a week for 4-6 weeks. Physiological measurements of core temperature, skin temperature and oxygen consumption were recorded from 20 min pre cold exposure to 15-20 min post cold exposure. The 4-6 weeks of repeated cold water immersion led to a small decrease in core temperature (~0.75°C), and a lowered skin temperature. The most surprising result found by Janksy et al., was the effect of cold acclimation on metabolic heat production. Their results showed that 4-6 weeks of repeated cold water immersions led to a decrease in metabolic heat production of ~20%.

As we can see from these studies, several attempts have been made to give a conclusive answer to how cold acclimation (through cold water immersion) affects thermoregulatory responses in humans. All of the previous studies reported that repeated cold water immersion leads to a decrease in both skin and core temperature. When it comes to the effects on metabolic heat production all three studies report quite contradictory results, ranging from a decrease, to no change, to an increase in heat production. The reasons for the variability in the results between these studies are unknown, but some of it could be explained by differences in the research

protocols applied. Each of the studies used cold water immersion, but the water temperature, duration of the cold exposure and duration of the acclimation period were different between all three studies.

The effect of cold acclimation on shivering measured with EMG

ST is an important mechanism for survival in the cold, because of its large heat producing capacity (Eyolfson et al. 2001). None of the previous studies described here have reported accurate data on the change in shivering during cold acclimation. When shivering was assessed in these previously mentioned studies, visual observation and/or the participant's subjective perception were used to determine shivering intensity.

Only a few studies so far have made an actual attempt to measure changes in shivering intensity during cold acclimation. One of the first cold acclimation studies ever conducted on humans yielded some of the better measurements of the effects of cold acclimation on changes in ST. In 1961, Thomas Davis investigated changes in shivering during cold acclimation in two different groups. One group underwent cold acclimation in March (maximal cold acclimatization due to season) and the second group underwent cold acclimation between September and October (minimal seasonal effects). Each group consisted of 10 subjects, mean age of 25 years, who were exposed to cold air (11.8±0.5°C for group 1, and 13.5±1.5°C for group 2), 8 hours a day for 31 days (no testing on Sundays), and the subjects were not previously exposed to cold. To measure changes in muscle activity surface EMG electrodes were placed on the upper arms and thighs, and the recorded EMG values were averaged and presented as mV/min. Energy expenditure (EE) by O_2 consumption was measured and averaged over a 3-5 min period within the 2nd hour of a 2 hour period. Both skin and core temperature were monitored to look at changes during cold acclimation. Following the 31 days of cold acclimation Davis found that in the winter group, cold acclimation had no effect on EE. In the

summer group on the other hand, the initial EE (prior to acclimation) was higher than the winter group, and started decreasing, reaching approximately the same value as the winter group at the end of the acclimation period. In both groups no effect of cold acclimation on basal metabolic rate could be observed. Core temperature in both groups decreased until day 14, however even though there was some recovery, core temperature was lower on day 31 compared to day 1 and 8. Some fluctuations in skin temperature could be observed inside the winter group, but mean skin temperature showed no change between day 1 and day 31. In the summer group a small decrease in mean skin temperature was observed from day 1 to day 31. In both groups a significant decrease in the rate of shivering could be observed during the course of acclimation, and in seven subjects no shivering could be observed by day 21.

The study conducted by Davis in 1961, was the first to investigate changes in shivering during cold acclimation. His research showed that a decrease in shivering could be observed during cold acclimation. The limitation to this study is that the EMG signal was not filtered, analyzed or normalized for any of the subjects. This means the reliability and accuracy of the results are questionable. The results still give an idea about the effects of cold acclimation on shivering, which was studied more closely by Matthew et al. in 1981 . In 15 young healthy soldiers, they investigated the effects of cold acclimation on the thermoregulatory responses to a standard cold air test (SCAT). The SCAT consisted of a two hour cold exposure to 10°C in a cold chamber. The SCAT was performed on day 1, 6, 11, 16 and 21 of the cold acclimation period. Oxygen consumption, heart rate, EMG (6 subjects), skin and oral temperature were measured for each SCAT. The cold acclimation protocol consisted of a daily four hour exposures to cold air of 10°C, for 21 days. Their results showed that this cold acclimation protocol led to an increase in resting oxygen consumption from day 1 to day 21. In each SCAT an increase in oxygen consumption was observed, but the increase was significantly smaller on day 21 compared to day 1. Both skin and core temperature fell

during each SCAT, but the decrease was again significantly less on day 21, compared to day 1. The EMG recordings from 6 subjects showed that shivering was markedly decreased after the three weeks of cold acclimation, but how EMG signals were analyzed and the number of muscles from which EMG signals were recorded was not reported.

BROWN ADIPOSE TISSUE

Brown adipose tissue was for a long time considered absent and insignificant in human adults (Cunningham, Leslie et al. 1985; Jung, Leslie et al. 1988). Recent research has questioned these statements; having found that brown adipose tissue is highly present in human adults and could be of major significance for an increase in EE.

The human body consists of two types of adipose tissue, white adipose tissue (WAT) and brown adipose tissue (BAT). Although they are both adipose tissue, they differ both in function and to some extent in origin (Arbuthnott 1989). The main function of WAT is secretion of hormones, insulation and chemical energy storage (Albright and Stern 1998). The chemical energy is stored in WAT as triglycerides (TAG) in large unilocular droplets. The chemical energy in BAT is stored as multilocular droplets of TG, and in contrast to WAT, BAT has a high content of mitochondria (Nedergaard and Lindberg 1982) and is solely a heat producing organ (Barnard 1977). BAT is the only tissue in the in the body expressing the uncoupling protein 1 (UCP1) (Nedergaard et al. 2001), which has the ability to uncouple the proton gradient created by the electron transport chain, and dissipate energy as heat, instead of creating ATP (Klingenberg 1990; Cannon and Nedergaard 2004).

BAT plays an important role in thermogenesis of small mammals and human newborns. Fully activated, 50g of BAT has been estimated to account for 20% of total daily EE in rodents (Rothwell

and Stock 1983). Small mammals and newborn infants have a large surface to volume ratio, and BAT plays an important role in counteracting heat loss by increasing heat production. Newborn infants also lack the ability to shiver (Van Der Spek, Van Lingen, and Van Zoeren-Grobben 2009; Pursley 2008), which makes BAT an important organ for survival. BAT has now been identified in human adults as well, but the importance of the tissue in adults is still unknown. Calculations have estimated that 50g of BAT could account for 3-5% of BMR in humans, which is a significant amount (Vijgen et al. 2011), however there seems to be a great variability in the volume of active BAT human beings posses, varying from 0-130g (van Marken Lichtenbelt et al. 2009).

Function of Brown Adipose Tissue

BAT is under complete control of the SNS, and is activated *via* sympathetically released NA. The synaptic nerve endings surrounding BAT release NA into the synaptic cleft, which binds with β–adrenergic receptors on the BAT cell surface. A cascade of pathways is then activated, including the activation of G-protein, adenylyl cyclase, cAMP, lipolysis and hydrolysis of TAG into FFA in the BAT cell. The released FFA activates the UCP1 channel protein in the inner mitochondrial wall, leading to an influx of H^+-protons into the mitochondria. This influx of protons bypasses the ATP-synthase and the production of ATP, releasing energy as heat instead of creating ATP (Cannon and Nedergaard 2004).

History

Although BAT is considered a relatively new tissue when it comes to physiological relevance and prevalence in human adults, the discovery of brown adipose tissue in animals occurred already in 1553, by the Swiss naturalist Konrad Gessner (1551). He discovered the tissue in the interscapular region of the marmot, and described it as “neither fat nor flesh [nec pinguitudo, nec caro], but something in between” (Cannon and Nedergaard 2008). Since the first discovery, very little interest

was shown to investigate the properties of the tissue until the 20th century. In 1923-1924, Rasmussen discovered that BAT could also be found in 55 other mammalian species. True hibernators appeared to always have the tissue, but Rasmussen claimed it could also be found to a varying degree in non-hibernators such as humans (Rasmussen 1923). Several review papers in the 1950's and 60's stated that BAT was an extremely versatile metabolically active tissue (Smith and Hoijer 1962; Shapiro and Wertheimer 1956; Johansson 1959). In 1963, Smith and Roberts found that rats exposed to cold for 40-90 days, had 1.6 times the amount of BAT found in the control group exposed to thermoneutral conditions, and that the increase in O_2 consumption in cold exposed rats, corresponds to a heat production seven times higher than the control group. Since these first re-discoveries of BAT, several rat studies have been conducted to find ways to activate the tissue. The clinical limitations when it comes to studying rats are that the findings cannot be directly transferred to humans, due to the physiological differences between humans and rodents.

It was previously reported that human newborns possess BAT, because of their high surface to mass ratio and lack of ability to shiver (Pursley 2008; Van Der Spek, Van Lingen, and Van Zoeren-Grobben 2009), but the BAT was believed to diminish as the child grew older to eventually become insignificant when reaching adult age (Cannon and Nedergaard 2004). The lack of evidence for human adults possessing BAT before the 21th century could to a certain degree be accounted for by the deficiency of effective and non-invasive ways to measure the occurrence of BAT. In the late 1990's, medical doctors started using PET/CT scans to detect cancer tumors in patients (Pasquali et al. 1998; Beyer et al. 2000). Retrospective studies in the early 2000's (Hany et al. 2002; Cohade et al. 2003; Cohade, Mourtzikos, and Wahl 2003; Dorbert et al. 2004), from previous PET/CT scans found unusual symmetrical tracer uptake in the supraclavicular region of cancer patients. The CT scan showed that the tissue had the same density as adipose tissue, and it was hypothesized that it could be BAT. The latter was confirmed by Virtanen et al. (2009) and van Marken Lichtenbelt et al. (2009)

in two separate studies, where PET/CT scans were performed on healthy adult subjects, after a two-hour cold exposure. All the subjects (except one), showed tracer uptake in the supraclavicular region. To confirm the nature of the tissue, biopsies were taken, guided by the PET/CT images (Virtanen et al. 2009). The biopsies where analyzed for UCP1 content, and the results showed that the tissue had 1000 times the level of UCP1 mRNA compared to a sample of WAT taken from the same area. Based on these findings the researchers concluded that BAT is present in adult humans, but its physiological significance remains to be understood.

Origin of Brown Adipose Tissue

Human BAT was for a long time believed to have the same embryonic precursor as WAT. Although both BAT and WAT are named adipose tissue and contain droplets of TAG, the original BAT depots originate from a different precursor cell than WAT. Both cell lineages start off from a mesenchymal precursor cell, however brown adipocytes seem to share a common early transcription program with myocytes while white adipocytes contain another set of transcription factors differentiating it from mycocytes and brown adipocytes (Timmons et al. 2007; Seale et al. 2008). Research has found that the transcription factor PRDM16 acts as a switch turning pre myocytes into brown adipocytes, and that the absences of this transcription regulator leads to the development of muscle specific features in the particular cell, instead of adipose features (Kajimura, Seale, and Spiegelman 2010; Seale et al. 2008).

BAT can also arise from browning of existing WAT. Several transcription factors have been recognized to illicit this browning of white adipose tissue, such as the previously mentioned PRDM16 and bone morphogenic protein 7 (BMP7) (Schulz et al. 2011; Tseng et al. 2008; Seale et al. 2007). Recent research has also shown some evidence for the possibility of exercise playing a part in the turnover from white to brown-like adipose tissue. The newly discovered protein Irisin, released

by muscle cells during exercise, has shown a potential of possibly turning subcutaneous WAT into beige or brown like adipose tissue. Irisin has been identified in both human and mice, where it has been found to help counter obesity and type 2 diabetes in mice(Bostrom et al. 2012).

Interindividual Variability

The identification of BAT in adult humans is an important discovery, however a large variability in both the volume and activity of the tissue between individuals has also been observed. This variability has been related to age (young vs. old), genetics (sex, genes), adiposity (BMI, waist circumference, BF, %BF), and environmental temperature (summer vs. winter). Both the highest level of activity and the largest volume of BAT have been found in young adults (20-30 years old), while both mass and activity of the tissue seem to decline as an individual ages (Yoneshiro et al. 2011, Pfannenberg et al.2010). Females seem to have a higher prevalence of BAT mass and activity than males (Cypress et al. 2009; Pfannenberg et al. 2010; Au-Young et al. 2009), as well as a slower decline in mass with age (Pfannenberg et al. 2010). Adiposity is another factor influencing BAT activity. Studies investigating the relationship between obesity and BAT volume and activity have found a negative correlation between BAT and BMI, where people with a high BMI seem to have less BAT mass and lower activity. In a study conducted by van Marken Lichtenbelt et al. (2009) the lean subjects (BMI 23.2±1.2) had on average 4-times the level of BAT activity found in the obese subjects (BMI 30.3±4.2). A high BMI is a preindication of obesity, and research has also shown that other factors related to obesity, such as waist circumference, total body fat (BF), and %BF, have a negative correlation with BAT (Vijgen et al. 2011). Seasonality also seems to play a role in the level of BAT activity. Seasonal studies have shown higher BAT activity levels during winter in comparison to summer (Cohade, Mourtzikos, and Wahl 2003; Au-Young et al. 2009; Cypress et al. 2009; Saito et al. 2009) and some of the BAT depots identified during winter season were absent in scans

performed during the summer (Au-Young et al. 2009). A study conducted on Finish outdoor workers showed a higher prevalence of multilocular adipose tissue (i.e. BAT) compared to their indoor working counter parts (Huttunen, Hirvonen, and Kinnula 1981).

Although all of the factors mentioned above influence BAT activity, there are other unexplained factors causing variability between subjects. The phenomenon of interindividual variability was described for shivering in a study by Bell et al. (1992). Their research showed a large variability in shivering during acute cooling between lean subjects (n=5) after normalizing for age, height and adiposity. Comparable results were found by Haman et al. (2004), who after normalizing their subjects as much as possible for age, morphology (surface to volume ratio) and body composition (% body fat), found a difference in both shivering intensity and EE during a 90 min cold exposure. Between the eight subjects, VO_2 varied from 10.6 to 15.3 mL kg^{-1} min^{-1} (average values) and percent of peak shivering varied from 47.2% to 75.9%, for the same 90 min of a five degree cold exposure. Similar observations were later found by van Marken Lichtenbelt (2009) in subjects possessing BAT. In a group of 10 healthy young male subjects with similar anthropometric measures, mean BAT standard uptake value (SUV) of ^{18}F-FDG after cold exposure was 428 kBq, but the standard deviation was 394 kBq. This shows that although the subjects had quite similar anthropometric measures, the inter-individual variability was large. This was supported by Ouellet et al. (2012), who examined six healthy men, aged 23 to 42 years, BMI of 23.7 to 31.0 kg/m^2. The subjects underwent a two hour cold exposure using an individual cooling protocol, (to achieve the highest activation of BAT without shivering). Surprisingly, the variability in volume of active BAT varied from 31 mL to 329 mL. Although several determinants influencing size and activity of BAT have been recognized, there are still undiscovered factors playing a role in the activation of BAT.

SUMMARY AND PURPOSE OF THE INVESTIGATION

When the human body is exposed to acute cold, it responds by decreasing heat loss and increasing heat production to maintain homeostasis. Heat production can be increased by the activation of NST and ST, depending on the duration and the severity of the cold exposure. If the human body is subject to a series of repeated cold exposures, a cold acclimation occurs and more permanent adaptations take place. Cold acclimation has been reported to lead to changes in skin and core temperature, heat production and ST. Although attempts have been made to measure shivering during cold acclimation, an accurate quantification of change in shivering and shivering pattern during cold acclimation has not previously been conducted. The primary purpose of this master's thesis was to investigate the changes in muscle activity after four weeks of cold acclimation in healthy adult men. We hypothesized that four weeks of cold acclimation would lead to: **1)** A progressive decrease in shivering intensity, and an associated decrease in burst shivering, **2)** A progressive change in muscle recruitment, characterized by a relative decrease in the activity of distal muscles to total shivering intensity, and **3)** A progressive change in fuel selection associated with the reduction in burst shivering which will lead to a decrease in CHO oxidation. The experiments in CHAPTER 2 of my thesis sought to test these hypotheses and determine how cold acclimation affects ST.

The recent re-discovery of BAT in adult humans and its possible contribution to heat production is a major contribution to the scientific world. Even though BAT was found in almost 100% of the participants in recent research studies, large interindividual variability in the volume of BAT among the participants has been reported. BAT and ST are both mechanisms for heat production activated during a cold exposure, but the relationship between the two and how they change during cold acclimation has not yet been investigated. In CHAPTER 3, I will explore the relationship between change in BAT volume and change in shivering during cold acclimation, as well as identify to what

extent interindividual variability occur in changes in BAT volume and ST after cold acclimation among individuals.

REFERENCES:

Albright, Ann L., and Judith S. Stern. 1998. Adiopse Tissue. *Adipose tissue. In: Encyclopedia of Sports Medicine and Science*.

Arbuthnott, E. 1989. Brown adipose tissue: structure and function. *Proceedings of the Nutrition Society* 48:177-182.

Au-Young, Iain T.H., Natasha Thorn, Rakesh Ganatra, Alan C. Perkins, and Michael E. Symonds. 2009. Brown Adipose Tissue Seasonal Variations in Humans. *Diabetes* 58 (2583-2587).

Barnard, T. . 1977. Brown adipose tissue as an effector of nonshivering thermogenesis. *Generalia*.

Bell, D. G., P. Tikuisis, and I. Jacobs. 1992. Relative intensity of muscular contraction during shivering. *Journal of Applied Physiology* 72 (6):2336-2342.

Beyer, Thomas, Tony Brun Townsend, Paul E. Kinahan, Martin Carron, Raymond Roddy, Jeff Jerin, John Young, Larry Byars, and Ronald Nutt. 2000. A Combined PET/CT Scanner for Clincal Oncology. *Journal of Nuclear Medecine* 41:1369-1379.

Bostrom, Pontus, Jun Wu, Mark P. Jedrychowski, Anisha Korde, Li Ye, James C. Lo, Kyle A. Rasbach, Elisabeth Almer Bostrom, Jang Hyun Choi, Jonathan Z. Long, Shingo Kajimura, Maria Cristina Zingaretti, Birgitte F. Vind, Hua Tu, Saverio Cinti, Kurt Hojlund, Steven P. Gygi, and Bruce M. Spiegelman. 2012. A PGC1-[agr]-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 481 (7382):463-468.

Boyles, Justin G., Frank Seebacher, Ben Smit, and Andrew E. McKechnie. 2011. Adaptive Thermoregulation in Endotherms May Alter Responses to Climate Change. *Integrative and Comparative Biology* 51 (5):676-690.

Cannon, B, and J Nedergaard. 2004. Brown Adipose Tissue: Function and Physiological Significance. . *Physiology* 84:277-359.

Cannon, B, and J Nedergaard. 2008. Neither fat nor flesh. *Nature* 454.

Cohade, C., K.A. Mourtzikos, and R.L. Wahl. 2003. "USA-Fat": Prevalence Is Related to Ambient Outdoor Temperature—Evaluation with 18F-FDG PET/CT. *Journal of Nuclear Medecine* 44 (1267-1270).

Cohade, C., M. Osman, H.K. Pannu, and R.L. Wahl. 2003. Uptake in Supraclavicuar Area Fat ("USA-FAT"): Description on 18F-FDG PET/CT. *Journal of Nuclear Medecine* 44:170-176.

Cypress, A.M., L. Sanaz, G. Williams, I. Tal, E.L. Palmer, Y.H. Tsen, A. Doria, G.M. Kolodny, and C.R. Kahn. 2009. Identification and Importance of Brown Adipose Tissue in Adult Humans. *New England Journal of Medecine* 360 (1509-17).

Dorbert, N., C. Menzel, N Hamscho, N Wordehoff, W.T. Kranert, and F. Grunwald. 2004. Atypical thoracic and supraclavicular FDG-uptake in patients with Hodgkin's and non-Hodgkin's lymphoma. . *The Quarterly Journal of Nuclear Medecine and Molecular Imaging* 44:33-38.

Eyolfson, Douglas A., Peter Tikuisis, Xiaojiang Xu, Gillian Weseen, and Gordon G. Giesbrecht. 2001. Measurement and prediction of peak shivering intensity in humans. *European Journal of Applied Physiology* 84 (1-2):100-106.

Gessner, K. 1551. Conradi Gesneri medici Trigurine Historae Animalium: Lib. I De Quadredibus viviparis. .

Gosselin, C., and F. Haman. 2012. Effects of green tea extracts on non-shivering thermogenesis during mild cold exposure in young men. *Br J Nutr*:1-7.

Haman, F., S. R. Legault, and J. M. Weber. 2004. Fuel selection during intense shivering in humans: EMG pattern reflects carbohydrate oxidation. *J Physiol* 556 (Pt 1):305-13.

Haman, François, Stéphane R. Legault, Mark Rakobowchuk, Michel B. Ducharme, and Jean-Michel Weber. 2004. Effects of carbohydrate availability on sustained shivering II. Relating muscle recruitment to fuel selection. *Journal of Applied Physiology* 96 (1):41-49.

Hany, T.F., E. Gharehpapagh, E.M. Kamel, A. Buck, J. Himms-Hagen, and G.K. von Schulthess. 2002. Brown adipose tissue: a factor to consider in symmetrical tracer uptake in the neck and upper chest region. . *European Journal of Nuclear Medecine* 29 (10).

Hemingway, Allan. 1963. Shivering. *Physiological Reviews* 43 (3):397-422.

Himms-Hagen, Jean. 1984. Nonshivering thermogenesis. *Brain Research Bulletin* 12 (2):151-160.

Himms-Hagen, Jean, and D Ricquier. 1998. Brown adipose tissue. *Handbook of obesity*:415-441.

Huttunen, P., J. Hirvonen, and V. Kinnula. 1981. The occurrence of brown adipose tissue in outdoor workers. *Eur J Appl Physiol Occup Physiol* 46 (4):339-45.

Israel, D. J., and R. S. Pozos. 1989. Synchronized slow-amplitude modulations in the electromyograms of shivering muscles. *Journal of Applied Physiology* 66 (5):2358-2363.

Janský, L. 1973. NON-SHIVERING THERMOGENESIS AND ITS THERMOREGULATORY SIGNIFICANCE. *Biological Reviews* 48 (1):85-132.

Janský, L., H. Janáková, B. Uličný, P. Šrámek, V. Hošek, J. Heller, and J. Pařízková. 1996. Changes in thermal homeostasis in humans due to repeated cold water immersions. *Pflügers Archiv* 432 (3):368-372.

Janssen, Ian, Steven B. Heymsfield, ZiMian Wang, and Robert Ross. 2000. Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. *Journal of Applied Physiology* 89 (1):81-88.

Johansson, B. 1959. Brown fat: a review. *Metabolism* 8 (3):221-40.

Kajimura, Shingo, Patrick Seale, and Bruce M. Spiegelman. 2010. Transcriptional Control of Brown Fat Development. *Cell Metabolism* 11 (4):257-262.

Klingenberg, M. 1990. Mechanisms and evolution of the uncoupling protein of brown adipose tissue. *Trends in Biochemical Sciences* 15.

Mathew, Lazar, S. S. Purkayastha, A. Jayashankar, and H. S. Nayar. 1981. Physiological characteristics of cold acclimatization in man. *International Journal of Biometeorology* 25 (3):191-198.

McAllen, RobinM, Mutsumi Tanaka, Yoichiro Ootsuka, and MichaelJ McKinley. 2010. Multiple thermoregulatory effectors with independent central controls. *European Journal of Applied Physiology* 109 (1):27-33.

Meigal, A. 2002. Gross and fine neuromuscular performance at cold shivering. *Int J Circumpolar Health* 61 (2):163-72.

Meigal, AIu, V. Lupandin Iu, and G. I. Kuz'mina. 1993. [Electromyographic patterns of thermoregulatory activity of motor units in the process of body cooling]. *Fiziol Cheloveka* 19 (3):106-14.

Mekjavic, I. B., C. J. Sundberg, and D. Linnarsson. 1991. Core temperature "null zone". *Journal of Applied Physiology* 71 (4):1289-1295.

Morrison, Shaun F., Kazuhiro Nakamura, and Christopher J. Madden. 2008. Central control of thermogenesis in mammals. *Experimental Physiology* 93 (7):773-797.

Mozo, Julien, Yalin Emre, Frederic Bouillaud, Daniel Ricquier, and Francois Criscuolo. 2005. Thermoregulation: What Role for UCPs in Mammals and Birds? *Bioscience Reports* 25 (3-4):227-249.

Nedergaard, J., V. Golozoubova, A. Matthias, A. Asadi, A. Jacobsson, and B. Cannon. 2001. UCP1: the only protein able to mediate adaptive non-shivering thermogenesis and metabolic efficiency. . *Biochimica et Biophysia Acta* 1504:82-106.

Nedergaard, J., and O. Lindberg. 1982. The brown fat cell. *International Journal of Cytology* 74:187-286.

Ouellet, V., S. M. Labbe, D. P. Blondin, S. Phoenix, B. Guerin, F. Haman, E. E. Turcotte, D. Richard, and A. C. Carpentier. 2012. Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. *J Clin Invest* 122 (2):545-52.

Pasquali, Claudio, Domenico Rubello, Cosimo Sperti, Piero Gasparoni, Guido Liessi, Franca Chierichetti, Giorgio Ferlin, and Sergio Pedrazzoli. 1998. Neuroendocrine Tumor Imaging: Can 18F-Fluorodeoxyglucose Positron Emission

Tomography Detect Tumors with Poor Prognosis and Aggressive Behavior? *World Journal of Surgery* 22:588-592.

Petajan, J. H., and D. D. Williams. 1972. Behavior of single motor units during pre-shivering tone and shivering tremor. *Am J Phys Med* 51 (1):16-22.

Pfannenberg, C., M.K. Werner, S. Ripkens, I. Stef, A. Deckert, M. Schmadl, M. Reimold, H-U. Haring, C.D. Claussen, and N. Stefan. 2010. Impact of Age on the Relationships of Brown Adipose Tissue with Sex and Adiposity in Humans. . *Diabetes* 59 (7):1789-1793.

Pursley, DeWayne. 2008. Developmental Characteristics of Preterm Infants. *Pediatrics in Review* 29 (2):67-68.

Rasmussen, A. T. 1923. The so-called hibernating gland. *Journal of Morphology* 38 (1):147-205.

Rasmussen, A.T. 1923-1924. *Journal of Morphology* 38 (147).

Rothwell, N. J., and M. J. Stock. 1983. Luxuskonsumption, diet-induced thermogenesis and brown fat: the case in favour. *Clin Sci (Lond)* 64 (1):19-23.

Saito, M., Y. Okamatsu-Ogura, M. Matsushita, K. Watanbe, T. Yoneshiro, J. Nio-Kobayashi, I. Iwanaga, M. Miyagawa, T. Kameya, K. Nakada, Y. Kawai, and M. Tsujisaki. 2009. High Incidence of Metabolically Active Brown Adipose Tissue in Healthy Adult Humans. *Diabetes* 58:1526-1531.

Sato, Haruhiko. 1981. Fusimotor modulation by spinal and skin temperature changes and its significance in cold shivering. *Experimental Neurology* 74 (1):21-32.

Sato, Haruhiko, Takeshi Hashitani, Yoshiaki Isobe, Fujiya Furuyama, and Hitoo Nishino. 1990. Descending influences from nucleus raphe magnus on fusimotor neurone activity in rats. *Journal of Thermal Biology* 15 (3–4):259-265.

Schmidt-Nielsen, K. 1997. Animal Physiology: Adaptation And Environment. *Durham, Cambridge University Press*.

Schulz, Tim J., Tian Lian Huang, Thien T. Tran, Hongbin Zhang, Kristy L. Townsend, Jennifer L. Shadrach, Massimiliano Cerletti, Lindsay E. McDougall, Nino Giorgadze, Tamara Tchkonia, Denis Schrier, Dean Falb, James L. Kirkland, Amy J. Wagers, and Yu-Hua Tseng. 2011. Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat. *Proceedings of the National Academy of Sciences* 108 (1):143-148.

Seale, P., B. Bjork, W. Yang, Kajimura, S. Chin, S. Kuang, A. Scime, S. Deverakonda, H.M. Conroe, H. Erdjument-Bromage, P. Tempst, M.A. Rudnick, D.R. Beier, and B.M. Spiegelman. 2008. PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 454 (21).

Seale, Patrick, Shingo Kajimura, Wenli Yang, Sherry Chin, Lindsay M. Rohas, Marc Uldry, Geneviève Tavernier, Dominique Langin, and Bruce M. Spiegelman. 2007. Transcriptional Control of Brown Fat Determination by PRDM16. *Cell Metabolism* 6 (1):38-54.

Sessler, D. I., A. Moayeri, R. Støen, B. Glosten, J. Hynson, and J. McGuire. 1990. Thermoregulatory vasoconstriction decreases cutaneous heat loss. *Anesthesiology* 73 (4):656-660.

Shapiro, B., and E. Wertheimer. 1956. The metabolic activity of adipose tissue; a review. *Metabolism* 5 (1):79-86.

Skreslet, S., and F. Aarefjord. 1968. Acclimatization to cold in man induced by frequent scuba diving in cold water. *Journal of Applied Physiology* 24 (2):177-181.

Smith, R.E., and Roberts. 1963. Thermogenesis of brown adipose tissue in cold-acclimated rats. *Journal of Physiology* 206:143-148.

Smith, Robert E., and Dorothy Jared Hoijer. 1962. Metabolism and Cellular Function in Cold Acclimation. *Physiological Reviews* 42 (1):60-142.

Soderberg, Gary L, and Thomas M Cook. 1984. Electromyography in Biomechanics. *Physical Therapy* 64 (12):1813-1820.

Steinberg, Daniel, Paul J. Nestel, Elsworth R. Buskirk, and Ronald H. Thompson. 1964. Calorigenic Effect of Norepinephrine Correlated with Plasma Free Fatty Acid Turnover and Oxidation*. *The Journal of Clinical Investigation* 43 (2):167-176.

Stott, A. W., and J. Slee. 1985. The effect of environmental temperature during pregnancy on thermoregulation in the newborn lamb. *Animal Science* 41 (03):341-347.

Timmons, James A., Kristian Wennmalm, Ola Larsson, Tomas B. Walden, Timo Lassmann, Natasa Petrovic, D. Lee Hamilton, Ruth E. Gimeno, Claes Wahlestedt, Keith Baar, Jan Nedergaard, and Barbara Cannon. 2007. Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages. *Proceedings of the National Academy of Sciences* 104 (11):4401-4406.

Tseng, Yu-Hua, Efi Kokkotou, Tim J. Schulz, Tian Lian Huang, Jonathon N. Winnay, Cullen M. Taniguchi, Thien T. Tran, Ryo Suzuki, Daniel O. Espinoza, Yuji Yamamoto, Molly J. Ahrens, Andrew T. Dudley, Andrew W. Norris, Rohit N. Kulkarni, and C. Ronald Kahn. 2008. New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 454 (7207):1000-1004.

Vallerand, A. L., Zamecnik, J., Jones, P. J. H., Jacobs, and I. 1999. *Cold stress increases lipolysis, FFA R[a] and TG/FFA cycling in humans*. Vol. 70. Alexandria, VA, ETATS-UNIS: Aerospace Medical Association.

Van Der Spek, R. D. G., R. A. Van Lingen, and D. Van Zoeren-Grobben. 2009. Body temperature measurement in VLBW infants by continuous skin measurement is a good or even better alternative than continuous rectal measurement. *Acta Pædiatrica* 98 (2):282-285.

van Marken Lichtenbelt, W.D., J.W. Vanhommerig, N.M. Smulders, J.M.A.F.L. Drosseart, G.J. Kemerink, N.D. Bouvy, M.D., P. Schrauwen, and G.J. Jaap Teule. 2009. Cold Activated Brown Adipose Tissue in Healthy Men. *New England Journal of Medecine* 360:1500-8.

Vijgen, G.H.E.J., N.D. Bouvy, G.J.J. Teule, B. Brans, P. Schrauwen, and W.D. van Marken Lichtenbelt. 2011. Brown Adipose Tissue in Morbidly Obese Subjects. *PLoS ONE* 6 (2).

Virtanen, K.I., M.E. Lidell, J. Orava, M. Heglind, R. Westergren, T. Niemi, M. Taittonen, J. Laine, N-J. Savisto, S. Enerbak, and P. Nuutila. 2009. Functional Brown Adipose Tissue in Healthy Human Adults. *New England Journal of Medecine* 360:1518-25.

Weber, J. M., S. Klein, and R. R. Wolfe. 1990. Role of the glucose cycle in control of net glucose flux in exercising humans. *Journal of Applied Physiology* 68 (5):1815-1819.

Wijers, S. L. J., W. H. M. Saris, and W. D. Van Marken Lichtenbelt. 2009. Recent advances in adaptive thermogenesis: potential implications for the treatment of obesity. *Obesity Reviews* 10 (2):218-226.

Wolfe, R. R., S. Klein, F. Carraro, and J. M. Weber. 1990. Role of triglyceride-fatty acid cycle in controlling fat metabolism in humans during and after exercise. *American Journal of Physiology - Endocrinology And Metabolism* 258 (2):E382-E389.

Young, A. J., S. R. Muza, M. N. Sawka, R. R. Gonzalez, and K. B. Pandolf. 1986. Human thermoregulatory responses to cold air are altered by repeated cold water immersion. *Journal of Applied Physiology* 60 (5):1542-1548.

CHAPTER 2: CHANGES IN MUSCLE ACTIVITY DURING A FOUR WEEK COLD ACCLIMATION

INTRODUCTION

Human beings are endotherms and require a tightly regulated core temperature to function (Boyles et al. 2011; Mozo et al. 2005). The core temperature in humans is regulated within a narrow zone from 36-38°C (Mekjavic, Sundberg, and Linnarsson 1991; Schmidt-Nielsen 1997). A large fall in core temperature could lead to reduced enzyme efficiency, reduced cellular energy availability and reduced ion fluxes (Morrison, Nakamura, and Madden 2008). If no measures are taken, exposing the human body to acute cold causes a drop in core temperature. To avoid this from happening, the body responds by decreasing heat loss and increasing heat production. During an acute cold exposure, heat loss is reduced through vasoconstriction of blood vessels in the extremities and superficial areas of the body (Sessler et al. 1990), where the reduction in blood flow leads to a decrease in skin temperature and thereby the convective heat loss from the skin, and limits the return of cold blood to the core. Increase in heat production during an acute cold exposure is initiated through the activation of thermogenesis (Lowell and Spiegelman 2000; Jessen 1980). Thermogenesis has two distinct mechanisms for heat production, NST (Janský 1973) and ST (Haman et al. 2004). NST is defined as heat producing processes liberating chemical energy, not including shivering(Janský 1973). Examples of these processes are activation of futile cycles; FFA cycling in adipose tissue (Steinberg et al. 1964) and Ca^{2+} cycling in skeletal muscle sarcoplasmic reticulum (Wijers, Saris, and Van Marken Lichtenbelt 2009), and the process believed to have the largest heat producing potential, activation of BAT (Himms-Hagen 1984). During a mild short-term cold exposure, NST is the mechanism first activated to increase heat production. Thermoreceptors in the skin sense a fall in skin temperature, and relay this information to the hypothalamus, which again activates the different mechanisms of NST to increase heat production. If the cold exposure is prolonged or increased to moderate or severe, the heat production from NST is insufficient to maintain homeostasis, leading to an imbalance between heat production and heat loss. To meet the

elevated demand for heat production, ST is activated. ST is located in muscle tissue, and is defined as heat production generated through asynchronous muscle contractions (Haman et al. 2004). ST has a much larger capacity for heat production compared to NST, and can increase metabolic rate (MR) to five times basal metabolic rate (BMR) (Eyolfson et al. 2001). Shivering can occur in two distinct patterns; continuous low intensity shivering and high intensity burst shivering. These two patterns are separated by large differences in intensity and rate of occurrence. Continuous shivering has low intensity (2-5% of MVC) and occurs frequently (8-10Hz)(Meigal, LupandinIu, and Kuz'mina 1993), compared to burst shivering which is recognized by high intensity bursts (7-15% of MVC) occurring on a low frequency (0.1-0.2 Hz) (Israel and Pozos 1989). Continuous low-intensity shivering is linked to the activation of low threshold slow oxidative muscle fibers (type 1, fatigue resistant fibers, primarily dependent on lipid oxidation), compared to burst shivering which is associated with the recruitment of high intensity fast glycolytic muscle fibers (type 2, more fatigable fibers, dependent on CHO oxidation) (Meigal 2002; Petajan and Williams 1972).

The body's response to an acute cold exposure has been well studied, but accurate data on the responses to repeated cold exposures are limited. We know that when the human body experiences repeated cold exposures, more permanent adjustments occur to protect the body from cold stress, and these adjustments seem to be dependent on the cooling condition to which the individuals are exposed. Four different categories of adaptations have been recognized; hypothermic, insulative, metabolic or a combination of all strategies (Bittel 1987). Hypothermic adaptations are recognized by a natural fall in core temperature with less metabolic compensation; the body allows for a short term, uncompensated fall in core temperature. Such changes have been found in soldiers living in cold climates for a long period (LeBlanc 1956). Insulative adaptations are characterized by a decrease in skin temperature with no change in core temperature, and this have been observed in the coastal tribes living in the tropical northern Australia (Hammel et al. 1959).

Hypothermic insulative adaptations are an intermediate between the latter two adaptations, and have been found in Japanese and Korean divers (Hong 1963). Metabolic adaptations refers to an increase in metabolic rate and skin temperature with no change in core temperature, and have been found in Norwegians living under primitive conditions (Scholander et al. 1958). All of these adaptations can be provoked in a laboratory depending on the nature of the cold exposure: intensity of the cold, type of acclimation (cold water immersion vs. cold air) and length of the acclimation period (Skreslet and Aarefjord 1968; Adams and Heberling 1958).

The main focus of most cold acclimation studies so far has been the effect on skin and core temperature and changes in metabolic heat production, but the results, thus far, have been quite contradictory. Jansky et al. (1996), found that sports men exposed to cold through cold water immersion (14°C, 1h, 3 times per week for 4-6 weeks), exhibited a hypothermic response recognized by a decrease in skin and core temperature. The repeated cold water immersions also led to a decrease in heat production of 20%, compared to the control group at the end of cold acclimation. Similar results were found by Young et al. (1986), who exposed their participants to repeated cold water immersions, 5 days a week for 5 weeks at 18°C. Both skin and core temperature decreased after five weeks, but the cold acclimation had no effect on heat production. Hesslink et al., (1992) observed a small decrease in heat production, but no change in core temperature after 80 cold exposures of 30 min to 4.4°C (air temp). All of these studies yield important, although contradictory information about some of the human organism's responses to chronic cold exposures. However, very few studies have looked at muscle activity and changes in shivering during cold acclimation. Hesslink et al., (1992) and Young et al., (1986) used visual observation to determine the onset of shivering, and Bittel et al. (1987) used EMG to measure changes in shivering onset during cold acclimation. In Bittel's study only two muscles were included, and the signal was not analyzed to look at changes in shivering intensity from pre to post

acclimation. One of the few attempts to measure changes in ST during cold acclimation was done by Thomas Davis (1961). In 16 subjects, exposed for 8 hours daily to cold air (11.8-13.5°C) for 31 days, a decrease in shivering intensity was observed over the period of cold acclimation, but no data on the size of the decrease have been reported. ST is an important mechanism for heat production and survival in the cold, because of the large tissue mass being activated. Even though the importance of ST is well known, we know very little about how it is affected by repeated cold exposures. Could repeated cold exposures lead to a change in shivering intensity, fuel selection, shivering pattern and muscle recruitment to increase survivability in the cold?

The purpose of this study was to investigate the effect of cold acclimation on change in muscle activity in healthy adult men. Our hypothesis is based on the few studies conducted on change in shivering in humans during cold acclimation (Davis 1961; Mathew et al. 1981), but because there are several limitations to these studies, two cold acclimation studies conducted on rats described here, were also included to form our hypothesis. In two different studies in 1956, Hart, Hereoux and Depocas investigated changes in shivering during cold acclimation. In both studies, 16 rats where split in to two groups, one kept at 30°C the other kept at 6°C for 6 to 7 weeks. In the cold acclimated rats (6˚C) shivering was diminished after acclimation, whereas the warm acclimated rats (30˚C) experienced vigorous shivering when exposed to 6°C. In the second study, changes in shivering were measured during cold acclimation. In the first few days after the cold exposure started, the character of shivering changed from mainly burst shivering to more continuous low intensity shivering. Overall shivering remained high for the first week, before gradually decreasing during the weeks to follow. Even though these results are based on experiments on rats, it is the closest we have to research resembling our study. Based on the previous cold acclimation studies conducted on both humans and rats we hypothesized that four weeks of cold acclimation would lead to: A progressive decrease in shivering intensity, and an associated decrease in burst shivering, a

progressive change in muscle recruitment, characterized by a relative decrease in the activity of distal muscles to total shivering intensity, and a progressive change in fuel selection associated with the reduction in burst shivering which will lead to a decrease in CHO oxidation.

MATERIALS AND METHODS

Subjects

Eight healthy, non acclimated adult men volunteered to participate in this study. All participants signed a written consent form, stating possible risks involved with participating in this study, all meeting the standards of the Helsinki Declaration and the Ethics Board's at the University of Ottawa and the University of Sherbrooke. The exclusion criteria for participant recruitment were: cold acclimated (outdoor workers etc.), age outside the range of 18 to 35 years, participants using any type of medication or having a prior history of cardiovascular or respiratory disease, and a BMI outside the range of 18 to 24.9 kg/m^2. During the acclimation period the participants were asked to refrain from caffeine and alcohol and limit physical activity to five hours per week or to the same level as they regularly exercise. No restrictions were given on the participants' diets, except for instructing the participants to be fasted for at least 12 hours prior to each testing and acclimation session. Participants were also given a standardized meal to eat the night before the experimental sessions (3220kJ or 770kcal, 42% CHO, 28% fat, 30% protein), to ensure all of the participants had approximately the same energy stores before each experimental session.

Experimental Design

Each participant completed four weeks of cold acclimation, which included 15 acclimation sessions of 120 min at 10°C and five experimental sessions of 150 min at 4°C. A liquid condition suit (LCS, three-piece high density, Allen-Vanguard Inc., Ottawa, ON) was used to cool the participants during both the acclimation and experimental sessions. All the acclimation sessions were held in a laboratory at Montpetit Hall at the University of Ottawa, while the experimental sessions were conducted in the Nutrition and Metabolism Research Unit at the Montfort Hospital. All acclimation and experimental session were held at the same time of the day (in the morning) to avoid an effect

of changes in circadian rhythms and daily thermal stress. The experimental sessions were held on day one (pre) of the study and on each of the following four Fridays (week 1, week 2, week 3 and post).

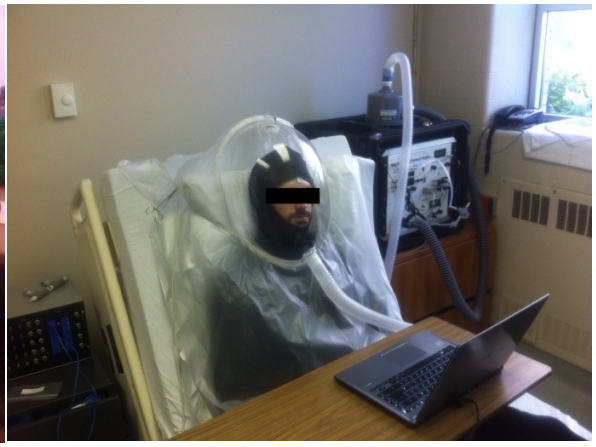
Experimental Session

For each of the experimental sessions, the participants met in the lab at the Montfort hospital in a post-absorptive state. After swallowing a telemetric pill (Jonah™ Ingestible Core Temperature Capsule, Mini Mitter CO., Inc., Bend, OR, USA) to measure core temperature (T_{core}), the participants were instrumented with surface EMG electrodes (Delsys EMG System, USA) to measure shivering intensity ($Shiv_{int}$). The electrodes were placed on the following 12 muscles: *m. trapezius superior* (TRA)*, m. latissimuss dorsi* (LAT)*, m. sternocleidomastoid*(SCM), *m. pectoralis major* (PEC)*, m. deltoideus* (DT)*, m. biceps bracchii* (BICEP)*, m. triceps bracchii* (TRI)*, m. rectus abdominis* (RA)*, m. vastus lateralis* (VL)*, m. vastus medialis* (VM)*, m. rectus femoris* (RF)*,* and *m. biceps femoris* (BF). After placing the electrodes, three maximum voluntary contractions (MVC) were recorded for each of the selected muscles. The participants were verbally encouraged to exert maximal isometric force for five seconds, and were given a one minute pause between each contraction. To measure skin temperature (T_{skin}), wireless temperature sensors (Thermocron iBUTTONS® model DS1922H, Maxim) were placed on 12 sites around the body. After completing the placement of EMG electrodes and iBUTTONS the participants put on the LCS, before sitting down in a semi-recumbent position on a hospital bed. A canopy was placed over the participant's head to sample air for indirect calorimetry, before baseline measurements of oxygen consumption and shivering EMG signals were recorded for 30 minutes in ambient air (~24 ˚C). After the baseline period, the participants were prepared to start the cold exposure. The LCS was connected to a temperature- and flow-controlled circulation bath (Endocal, NESLAB Model 200-00, Micropump, Vancouver, WA,

USA), and perfused with 4°C water. During the 150 min of cold exposure, shivering EMG activity was measured continuously, while O_2 consumption and CO_2 production were measured for 20 minute intervals, with 10 minute bouts of ambient air measurements in-between. The participants were encouraged to abstain from any voluntary muscle contraction during the cold exposure, to avoid contaminating the EMG recording of shivering. The cold exposure was terminated after 150 min, and the participants were stripped for all the instruments.

Acclimation Session

For the acclimation sessions, participants arrived in the morning in a fasted state. Upon the arrival in the laboratory at Montpetit Hall, the participants put on the three piece LCS and sat down in an upright position. The LCS was connected to the water bath, before being perfused with water (10°C) for 120 min, with no restrictions on movement. At the end of the cold exposure the suit was disconnected from the water bath, and removed from the participant.

Thermal Responses

Core temperature was measured using a telemetric pill (Jonah™ Ingestible Core Temperature Capsule, Mini Mitter CO., Inc., Bend, OR, USA) and recorded on a Vital Sense Integrated Physiological Monitor. The position of the telemetric pill after a certain time period was determined

during PET/CT scans in the beginning of the cold acclimation period. This timeline was used to determine the time point for ingestion of the pill to know the exact location core temperature was measured from. Skin temperature was measured using iBUTTONS previously validated for human research (van Marken Lichtenbelt et al. 2006), placed on the following 12 skin sites; forehead, upper back, lower back, upper abdominal area, quadriceps, hamstrings, front calf, back calf, chest, biceps, forearm, and hand. Each skin sight was weighted to calculate overall mean skin temperature ($\overline{T}$skin) using the following proportions: head 7%, hand 4%, upper back 9.5%, chest 9.5%, lower back 9.5%, biceps 9%, forearm 7%, quadriceps 9.5%, front calf 8.5% and back calf 7.5% (Hardy, Du Bois, and Soderstrom 1938; Du Bois D 1916).

Whole-body Heat Production

Whole-body heat production (H_{prod}), carbohydrate and lipid oxidation were determined from indirect calorimetry, using the measured values for VO_2 consumption and VCO_2 production. VO_2 consumption and VCO_2 production were measured using a flow-through open-circuit respiratory system (FoxBox Field Gas Analysis System, Sable systems, Las Vegas, NV, USA). The following equations (Livesey and Elia 1988) was used to calculate total carbohydrate (CHO_{ox}), and lipid (FAT_{ox}) oxidation rates:

(1) $CHO_{ox}(g.min^{-1}) = 4.59VCO_2\,(l.min^{-1}) - 3.23VO_2(l.min^{-1})$

(2) $FAT_{ox}(g.min^{-1}) = -1.70\,VCO_2\,(l.min^{-1}) + 1.70\,VO_2(l.min^{-1})$

The values for VCO_2 ($l.min^{-1}$) and VO_2 ($l.min^{-1}$) were corrected for the volumes of O_2 and CO_2 corresponding to protein oxidation (1.010 and 0.843 $l.g^{-1}$, respectively). Protein oxidation rate was estimated at 66 mg/min based on previously published urinary urea extraction measurements made on 12-hour postabsorptive men with normal CHO reserves (Haman, Legault, and Weber 2004; Haman et al. 2002). To calculate the relative contribution of different fuels to total heat production,

energy potentials of 16.3kJ g^{-1} (%CHO oxidation), 40.8 kJ g^{-1} (%lipid oxidation) and 19.7 kJ g^{-1} (% protein oxidation) were applied (Elia 1991; Péronnet and Massicotte 1991).

Shivering Intensity

Shivering EMG activity was measured using a wired EMG system (Delsys EMG System, USA). EMG surface electrodes were placed on the following 12 muscles: *m. trapezius superior* (TRA)*, m. latissimuss dorsi* (LAT)*, m. sternocleidomastoid* (SCM) *m. pectoralis major* (PEC)*, m. deltoideus* (DT)*, m. biceps bracchi* (BICEP)*, m. triceps bracchi* (TRI)*, m. rectus abdominis* (RA)*, m. vastus lateralis* (VL)*, m. vastus medialis* (VM)*, m. rectus femoris* (RF)*,* and *m. biceps femoris* (BF). These muscles were selected to represent large proximal and distal muscle groups, and are also known to represent more than 90% of all shivering muscles (Bell, Tikuisis, and Jacobs 1992). Each skin site was shaved and prepared using 3M Red Dot Trace Prep (3M Canada, London, ON, Canada) and ethanol swabs (Alcohol Prep Pad, Dukal Corporation). The EMG electrodes were placed on the muscles on the right side of the body, directly over the muscle belly. After the electrodes were placed, MVC's were recorded for each of the muscle groups included in the study. The participants were asked to perform three isometric MVC's, which lasted approximately five seconds. A one minute pause was given between each contraction to avoid any effects of muscle fatigue. The resistance force for the MVC's was applied by a research assistant and the participant was verbally encouraged to achieve maximum effort. During the cold exposure, muscle activity was recorded in 10 min intervals, from 0 to 150 min. Raw EMG signals were collected at 1000 Hz, and the signal was filtered to remove spectral components below 20 Hz and above 500 Hz, as well as 60 Hz contamination and related harmonics. All of the analyses of the EMG signals were performed using a custom designed MATLAB algorithm (Mathworks, Natick, MA). To quantify shivering intensity of individual muscles, root-mean-square (RMS) values were calculated from raw EMG signals using a 50-ms overlapping window (50%). Baseline RMS values ($RMS_{baseline}$: 15 min RMS average measured prior to cold

exposure) was subtracted from shivering RMS (RMS_{shiv}) values and RMS values obtained from MVC of individual muscles (RMS_{MVC}). Shivering intensity was normalized to RMS_{MVC} using the following equation (Haman et al. 2004):

(3) $$EMG_{shiv}\ (\%MVC) = \frac{RMS_{shiv} - RMS_{baseline}}{RMS_{MVC} - RMS_{baseline}} \times 100$$

Shivering intensity of individual muscles, EMG^{m}_{shiv} (where the index *m* denotes the muscle) for the entire experimental session was determined from area under the curve using the Riemann sum:

(4) $$EMG^{m}_{shiv} = \sum EMG_{shiv}\Delta t, m = 1\text{-}12$$

Where the summation was taken over the average of three 10 min periods and change in time (Δt) was = 30 min. To calculate the relative contribution of each muscle to whole body shivering ($\%shiv^{m}_{total}$), the EMG^{m}_{shiv} was divided by the sum of shivering activity of all the muscles included in the study (Haman et al. 2004).

(5) $$\%shiv^{m}_{total} = EMG^{m}_{shiv} / \sum EMG^{1\text{-}12}_{shiv}$$

To calculate whole body shivering index ($Shiv_{WBI}$) each shivering muscle was assigned to a muscle group: upper trunk (UT), upper extremities (UE), lower trunk (LT) or lower extremities (LE). Each muscle group was weighted depending on their muscle mass and then summed based on the following formula adapted from Bell et al. (1992):

(6) $$Shiv_{WBI} = (UT*0.34)+(UE*0.085)+(LT*0.19)+(LE*0.34)$$

The value for UT was calculated taking the average of the EMG_{shiv} for TRA, LAT, SCM, PEC and DT, for UE the average of BICEP and TRI, for LT the value for RA and for LE the average of VL, VM, RF and BF.

Burst shivering ($burst_{TOTAL}$) was calculated using a MATHLAB algorithm previously constructed in our lab. A shivering burst is defined as an EMG interval having a duration of >0.2 seconds, an interburst interval >0.75 seconds and an amplitude higher than the amplitude threshold for the 10 minute recording period. The amplitude threshold used for the identification of shivering bursts was calculated by: 1) averaging the shivering intensity for the full recording period ($\bar{A}_{EMG}$), 2) averaging all the remaining values above $\bar{A}_{EMG}$ ($\bar{B}_{EMG}$). The amplitude threshold was then set to $\bar{B}_{EMG}$, and all values ranging above this line was determined to be shivering bursts.
Average burst duration ($burst_{DUR}$) and burst intensity ($burst_{INT}$) was also calculated using the MATHLAB algorithm.

Statistical Analysis

The results are presented as mean±SEM. To analyze the week to week changes in T_{core}, $\overline{T}_{skin}$, $Heat_{prod}$ and $Shiv_{WBI}$ a repeated measures ANOVA was applied with a Bonferroni correction, to determine where the differences occur. A two-tailed paired t-test was applied to determine significant difference between the pre and post measurements for muscle recruitment, shivering pattern and fuel selection. Statistical differences were considered significant when $p \leq 0.05$.

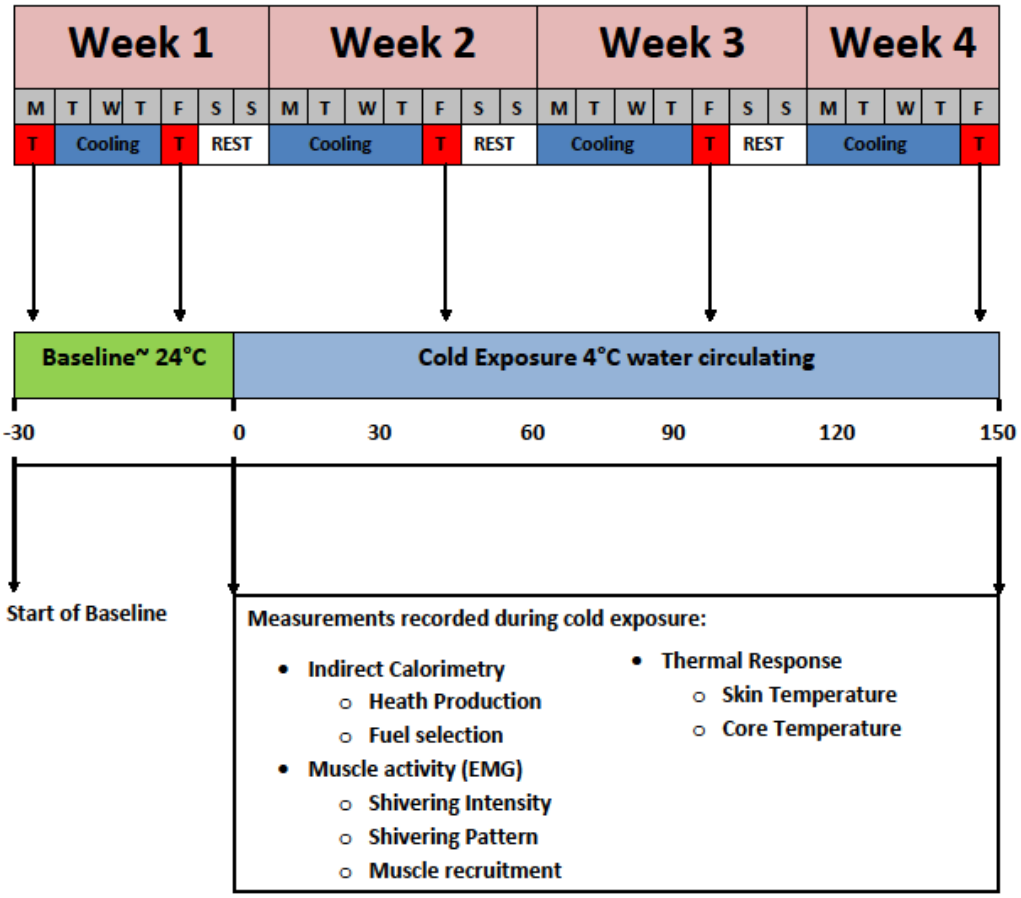


Figure 2.1: Steps of the experimental protocol and an overview of the measurements recorded during each experimental session.

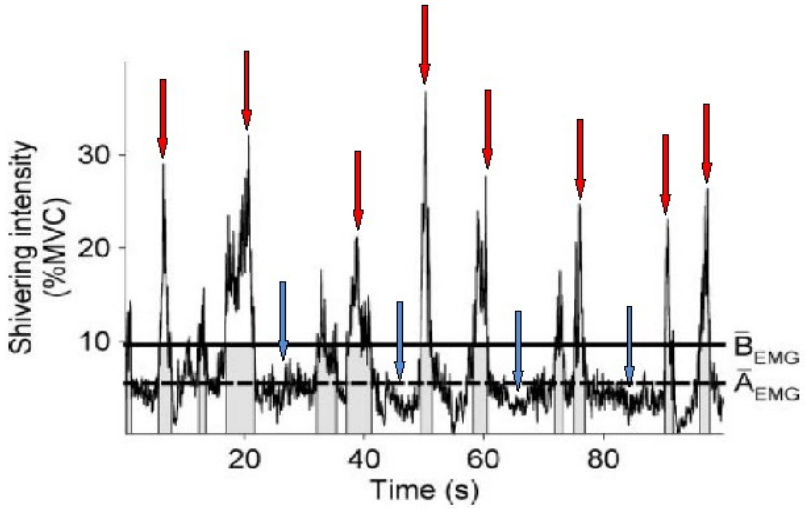


Figure 2.2: A representative recording of the average of total shivering intensity ($\bar{A}_{EMG}$) and the average of the values above $\bar{A}_{EMG}$ are calculated to determine burst shivering threshold ($\bar{B}_{EMG}$). The amplitude above $\bar{B}_{EMG}$ (red arrows) is spikes of burst shivering, and amplitude bellow $\bar{B}_{EMG}$ (blue arrows) are considered continuous low intensity shivering.

RESULTS

Thermal Responses

Changes in $\overline{T}_{skin}$ and T_{core} are presented in figure 2.3 A and B. No change in T_{core} was observed during the 150 min cold exposure in any of the five experimental sessions, and there was no change in core temperature between any of the experimental sessions. $\overline{T}_{skin}$ decreased 24% from 0 min (32.6±0.1°C) to 150 min (24.8±0.0°C), but no significant difference in $\overline{T}_{skin}$ was observed between experimental sessions.

Heat Production

$Heat_{prod}$ measured from 0 min to 150 min for each experimental session is shown in Figure 2.3 C. Heat production increased 93% from 0 min to 150 min (7.8±0.04 kJ/min vs. 15±0.28kJ/min), but no change was observed between any of the experimental sessions.

Shivering Intensity

Week to week changes in $Shiv_{WBI}$ are presented in Figure 2.3 D. The average increase in $Shiv_{WBI}$ was 11 fold (0.27±0.01 %MVC vs. 2.92±0.21 %MVC) from 0 min to 150 min, for the five experimental sessions, but no significant changes in $Shiv_{WBI}$ were observed between experimental sessions. Pre-post results for $Shiv_{WBI}$ and $\sum shiv_{All\ Muscles}$ (the sum of %MVC of all muscles) during the last 30 min are presented in Figure 2.4 A and B. $Shiv_{WBI}$ decreased from 317.8±36.9 to 253±44.8 (AUC) but the decrease was not significant. Changes in shivering seemed to be greatest during the last 30 min of cold exposure, but no change in $\sum shiv_{All\ Muscles}$ could be observed during the last 30 min.

Muscle Contribution

Values for changes in EMG^{m}_{shiv} and $\%shiv^{m}_{total}$, from pre to post cold acclimation are presented in Table 2.2. The only significant change in EMG^{m}_{shiv} was found in RF, where shivering (AUC) decreased 72.2% from pre to post cold acclimation (468.8±142.4 vs. 128.2±56.5). As mentioned earlier,

changes in $\%shiv^{m}_{total}$ were calculated based on the change in each muscles contribution to total shivering. The only muscle that showed a significant change in contribution was RF, which had a decrease in contribution to total shivering from 11.1±0.7% pre to 3.6±0.7% post cold acclimation.

Burst Shivering

Burst shivering was distinguished from continuous low-intensity shivering based on large differences in intensity and rate of occurrence (Figure 2.2). All the burst shivering data were measured during the last 30 min of cold exposure, but no change in burst shivering could be observed in the week to week experimental sessions. The results for changes from pre to post cold acclimation are presented in Figure 2.5 A, B and C. Figure 2.5A shows the $burst_{TOTAL}$ (sum of shivering bursts) during the last 30 min of cold exposure pre and post cold acclimation, averaged for all muscles for all participants. The results show that no change in total burst shivering could be observed (128.2±56.5 vs. 132.8±5.8). Neither in $burst_{INT}$ or $burst_{DUR}$ (Figure 2.5 B and C) could a change be observed from pre to post acclimation ($burst_{INT}$ 9.9±1.1 vs. 10.4±3.2 and $burst_{DUR}$ 0.13±0.01 vs. 0.13±0.01).

Individual muscle burst shivering data is presented in Table 2.3. LAT and BICEP were the only muscles showing significant changes, with an increase in $burst_{TOTAL}$ from 138±9.0 to 159.4±14.4 for LAT and an increase from 75±9 to 126±18 for BICEP. None of the individual muscles showed any change in $burst_{DUR}$, but $burst_{INT}$ of PEC decreased from 13.8±2.6 %MVC to 9.7±1.8 %MVC post cold acclimation.

Fuel Selection

The values for change in fuel selection during four weeks of cold acclimation are presented in Table 2.4. There was no change in CHO oxidation (20.2±3.0 g/min vs. 23.1±4.6 g/min) or lipid oxidation

(34.5±1.6 g/min vs. 33.6±2.9 g/min) from pre to post cold acclimation, and there was also no change in the contribution of CHO (17.3±2.6% vs. 20.1±4.5%) and lipids (73.5±2.6% vs. 70.6±4.7%) to total heat production.

Table 2.1: Participant characteristics measured pre cold acclimation

Participant Characteristics	Average		SEM
Age (years)	23	±	1
Height (cm)	182	±	2
Weight (kg)	81.6	±	3.0
BMI (kg/m2)	24.7	±	1.1
BSA*(m2)	2.0	±	0.0

***Body surface area**

Table2.2: Data for changes in individual muscle shivering and % change in individual muscle contribution to total shivering measured pre and post cold acclimation.

	% MVC (AUC)			% Muscle contribution		
	Pre	Post	P-value	Pre	Post	P-value
Trapezius	832 ± 267	730 ± 158	0.72	20 ± 5	23 ± 3	0.41
Lateralis	503 ± 153	455 ± 114	0.67	12 ± 3	16 ± 5	0.34
Sternocleidomastoid	279 ± 103	235 ± 98	0.42	6 ± 2	6 ± 2	0.95
Pectoralis	578 ± 187	438 ± 124	0.14	15 ± 4	14 ± 4	0.89
Deltoid	43 ± 16	58 ± 21	0.61	1 ± 1	2 ± 1	0.49
Bicep	114 ± 36	71 ± 18	0.29	3 ± 1	3 ± 1	0.63
Tricep	139 ± 46	173 ± 100	0.72	4 ± 2	4 ± 3	0.73
Rectus Abdomins	169 ± 25	148 ± 49	0.73	5 ± 1	5 ± 1	0.94
Vastus Lateralis	459 ± 161	207 ± 63	0.19	11 ± 3	6 ± 1	0.12
Vastus Medialis	290 ± 54	270 ± 61	0.58	8 ± 2	9 ± 2	0.46
Rectus Femoris	469 ± 142	128 ± 57	0.04*	11 ± 3	4 ± 1	0.02*
Biceps Femoris	173 ± 36	178 ± 50	0.91	5 ± 1	7 ± 2	0.20

Table 2.3 Burst data for individual muscles measured during the last 30 min of cold exposure, pre and post cold acclimation.

	Nr of burst			Burst Intenisty (%MVC)			Burst Duration (sec)		
	Pre	Post	P-value	Pre	Post	P-value	Pre	Post	P-value
Trapezius	154 ± 19	167 ± 17	0.12	11.8 ± 2.7	11.3 ± 2.9	0.89	0.2 ± 0.0	0.2 ± 0.0	0.99
Lateralis	138 ± 9	159 ± 14	0.05*	14.4 ± 4.4	10.9 ± 2.6	0.49	0.2 ± 0.0	0.2 ± 0.0	0.60
Sternocleidomastoid	119 ± 18	149 ± 11	0.08	9.2 ± 3.0	6.5 ± 2.5	0.14	0.1 ± 0.0	0.1 ± 0.0	0.45
Pectoralis	157 ± 11	176 ± 18	0.14	13.8 ± 2.6	9.7 ± 1.8	0.05*	0.2 ± 0.0	0.2 ± 0.0	0.97
Deltoid	64 ± 9	76 ± 16	0.55	3.3 ± 0.9	2.9 ± 0.9	0.78	0.1 ± 0.0	0.1 ± 0.0	0.36
Bicep	75 ± 9	126 ± 18	0.03*	5.6 ± 1.6	2.4 ± 0.7	0.07	0.1 ± 0.0	0.2 ± 0.0	0.15
Tricep	87 ± 7	86 ± 13	0.94	4.7 ± 1.2	2.8 ± 1.3	0.23	0.1 ± 0.0	0.1 ± 0.0	0.92
Rectus Abdomins	138 ± 28	138 ± 34	0.99	7.7 ± 1.6	12.0 ± 7.2	0.61	0.1 ± 0.0	0.1 ± 0.0	0.49
Vastus Lateralis	102 ± 7	114 ± 19	0.48	15.4 ± 3.9	20.4 ± 10.8	0.69	0.1 ± 0.0	0.1 ± 0.0	0.94
Vastus Medialis	124 ± 10	104 ± 27	0.33	10.2 ± 2.4	16.5 ± 5.5	0.15	0.1 ± 0.0	0.1 ± 0.0	0.44
Rectus Femoris	114 ± 10	129 ± 16	0.45	16.0 ± 4.0	21.0 ± 15.3	0.77	0.1 ± 0.0	0.1 ± 0.0	0.92
Biceps Femoris	128 ± 12	130 ± 19	0.95	6.9 ± 1.5	8.0 ± 3.6	0.81	0.1 ± 0.0	0.1 ± 0.0	0.68

Table 2.4: Change in heat production, CHO and lipid oxidation from pre to post cold acclimation, and the specific fuels contribution to total heat production.

Fuel Selection				
		Pre	Post	P-value
H_{prod} (kJ/min)		2147 ± 42	2166 ± 69	0.77
CHO Oxidation				
	g/min	20.2 ± 3.0	23.1 ± 4.6	0.55
	kJ/min	329 ± 49	376 ± 76	0.55
	% H-prod	17.3 ± 2.6	20.1 ± 4.5	0.54
Lipid Oxidation				
	g/min	34.5 ± 1.6	33.6 ± 2.9	0.75
	kJ/min	1409 ± 63	1371 ± 120	0.75
	% H-prod	73.5 ± 2.6	70.6 ± 4.7	0.55

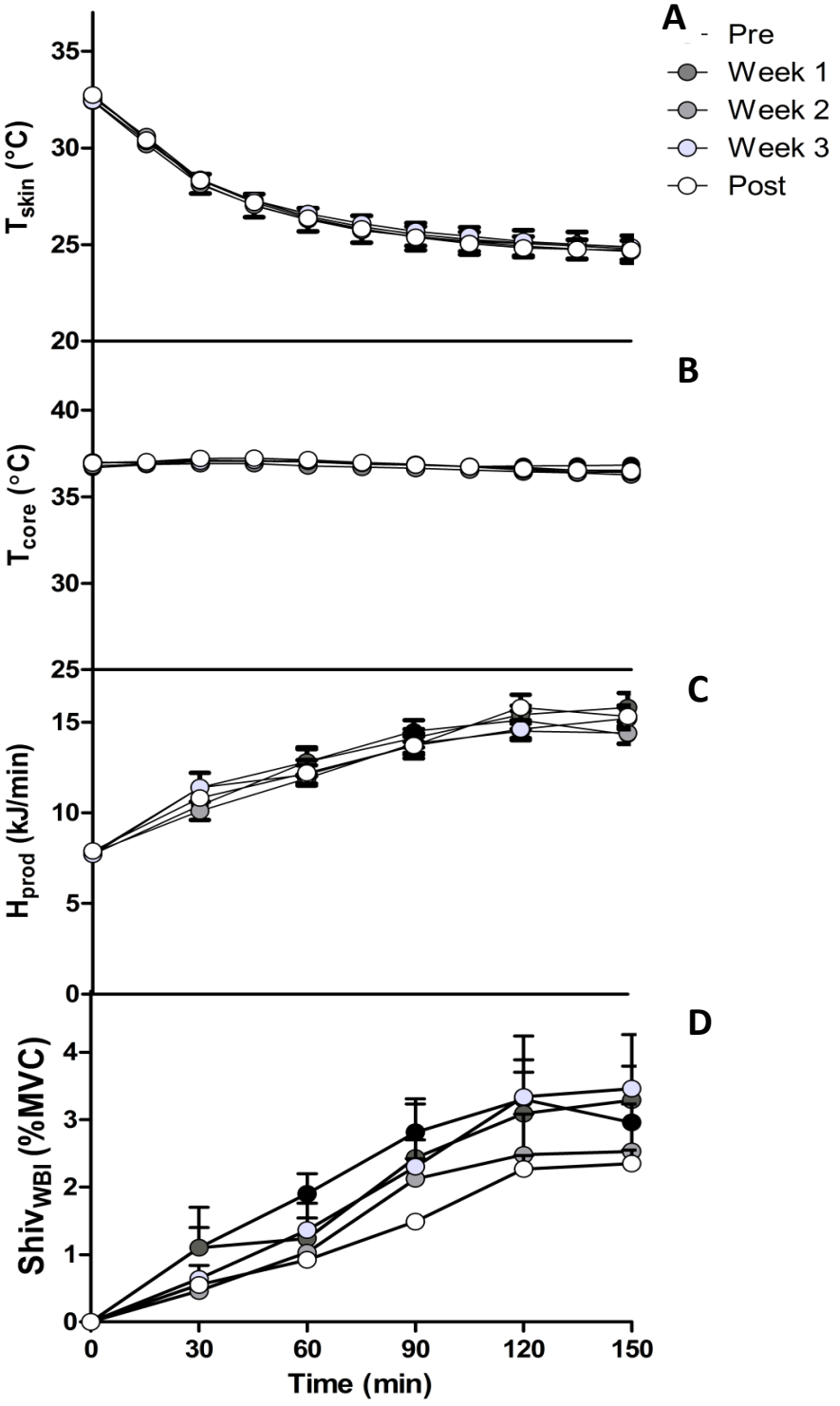


Figure 2.3: Core temperature (A), skin temperature (B), heat production (C) and whole body shivering (D) measured over 150 min of cold exposure (4°). Values presented for pre, week 1, 2 and 3, and post cold acclimation.

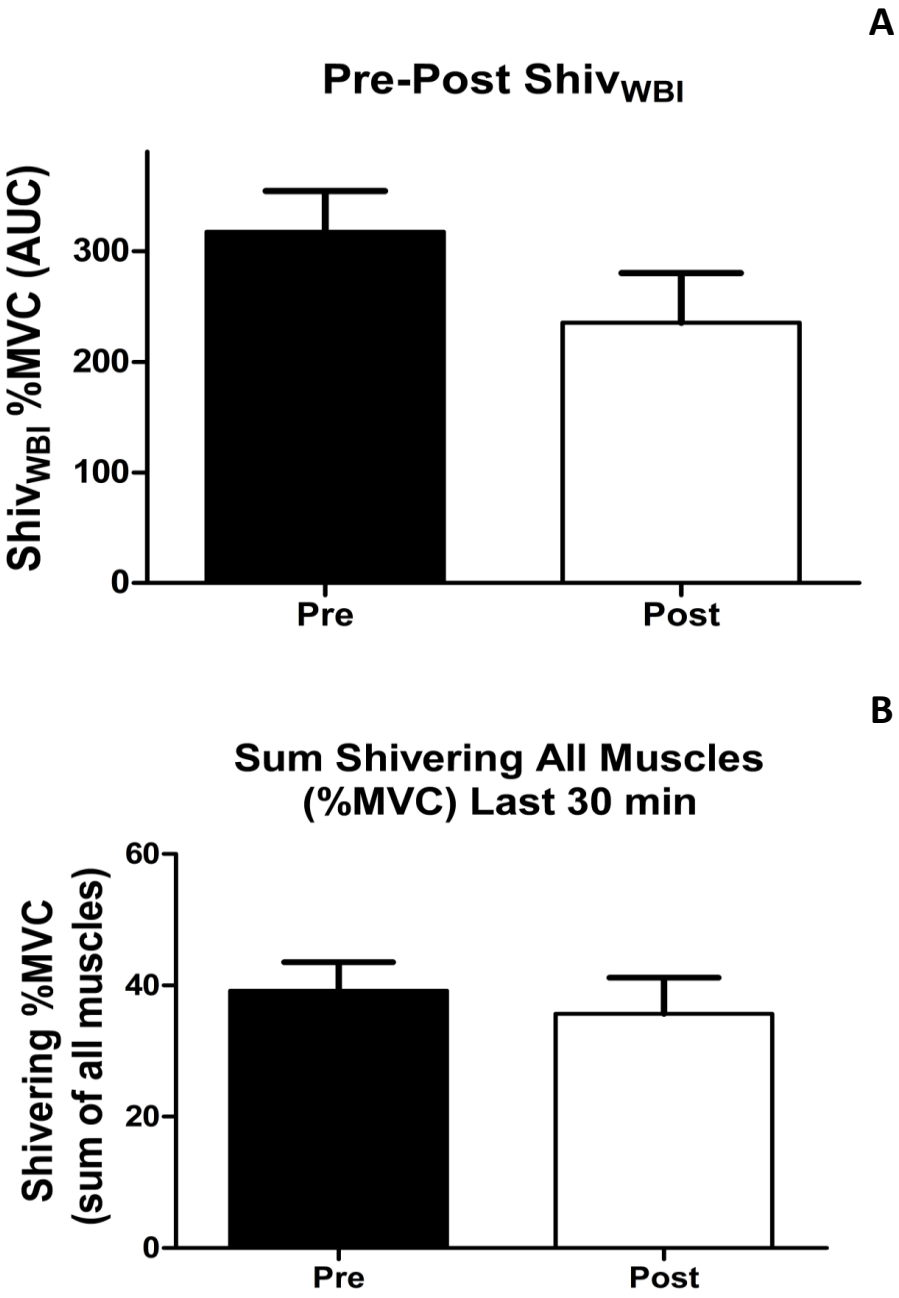


Figure 2.4: Shivering intensity measured pre and post cold acclimation. Whole body shivering index was measured during the 150 min of cold exposure expressed as AUC (A) and % MVC for all muscles summed was measured for the last 30 min of cold exposure (B).

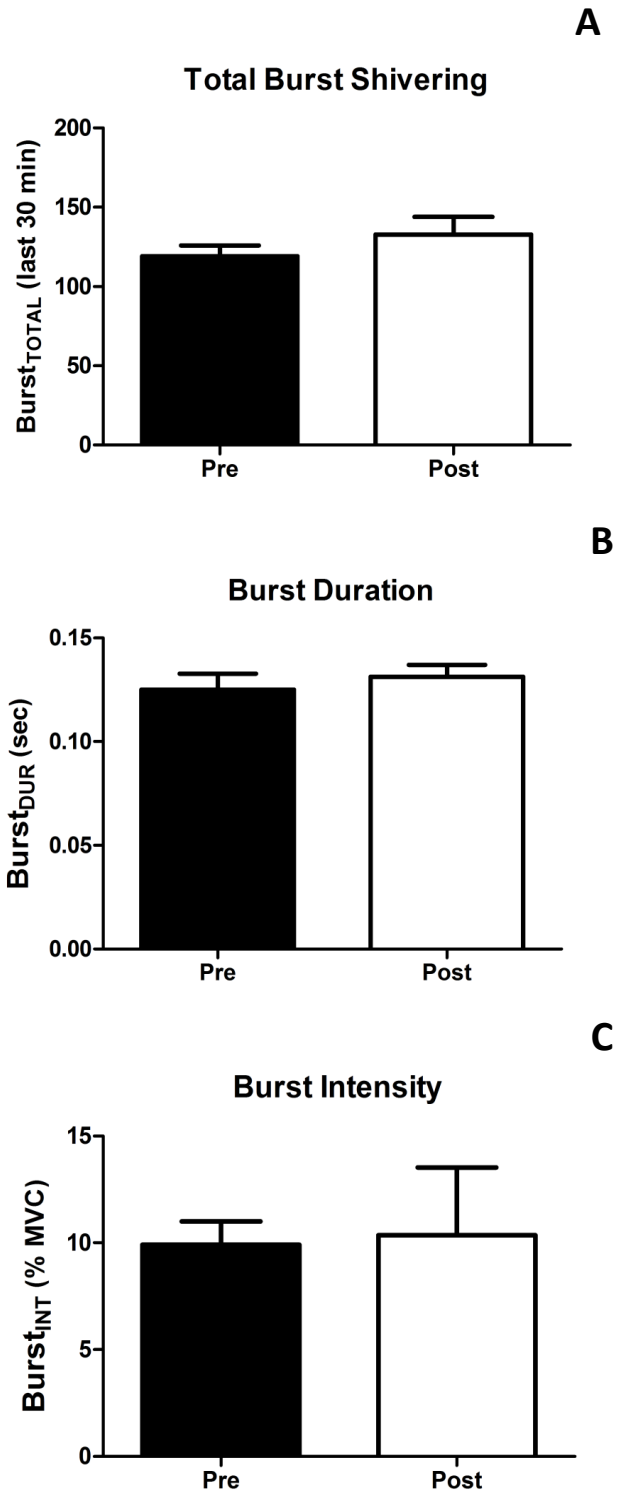


Figure 2.5 Values for total number of shivering bursts (A), average burst intensity (B) and average burst duration (C) for the last 30 min of the cold exposure, measured pre and post cold acclimation.

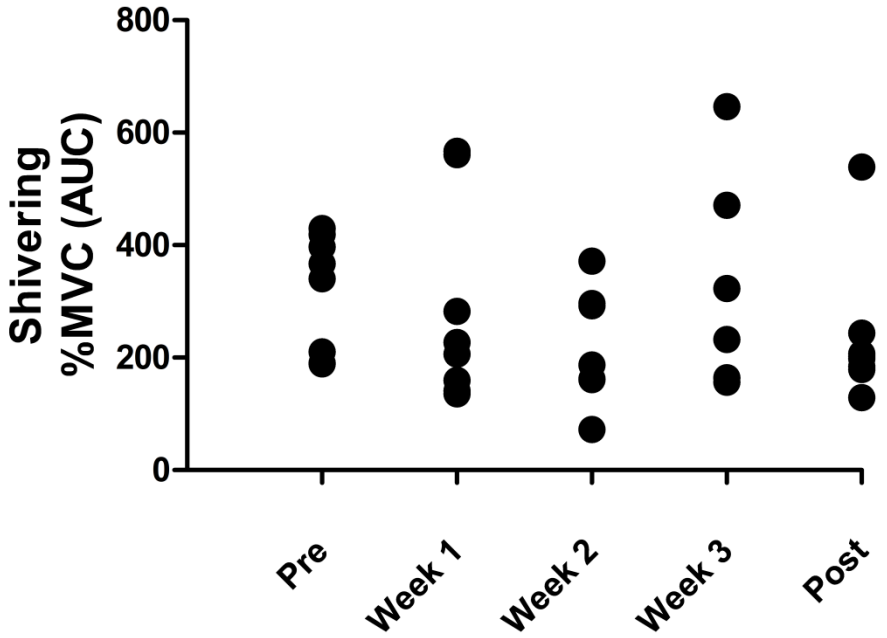


Figure 2.6: Individual values of whole body shivering presented as area under the curve for each experimental session.

DISCUSSION

This study sought to investigate the changes in muscle activity during four weeks of cold acclimation. The measurements of changes in muscle activity were a part of a larger study in which the effect of cold acclimation on BAT was the main outcome measure. The cold acclimation did not lead to a change in thermal responses (skin and core temperature) or heat production, which is in contradiction to what has been reported in previous cold acclimation studies (Bittel 1987; Janský et al. 1996; Young et al. 1986; Hesslink et al. 1992). It did on the other hand increase both BAT volume and activity (unpublished data from our lab). Although vague attempts have been made previously to measure shivering during cold acclimation, precise quantification of the effect of cold acclimation on change in muscle activity and whole body shivering has never been done before. EMG measurements from 12 muscles were used to investigate changes in muscle activity during cold acclimation, but no change in whole body shivering or shivering pattern was found. This was in contradiction to what we hypothesized, and the only effect of cold acclimation observed was an increase in BAT volume and activity. Is it possible that a change in BAT due to cold acclimation can be sufficient to change ST?

Cold Acclimation

Changes in skin and core temperature and heat production to maintain homeostasis have been recognized as adaptations to cold acclimation. After four weeks of repeated cold exposure, we were unable to observe a change in any of these parameters. As predicted, the 150 min cold exposure resulted in a 24% decrease in skin temperature, but no difference could be observed between experimental sessions (Figure 2.3A). Based on the absence of change in skin temperature, this cold acclimation did not affect vasomotor responses. Core temperature remained unchanged during the cold exposures, and did not differ between experimental sessions (Figure 2.3B). Heat production

almost doubled during the 150 min cold exposure, reaching similar levels in all experimental sessions (Figure 2.3C). Unchanged heat production, skin and core temperature following cold acclimation show an absence of change in thermal conductance of the studied organisms, therefore showing that the flow of endogenously produced heat was not affected. A change in skin and core temperature with the same heat production could have occurred if there was a change in body composition (increase or decrease in body fat or muscle mass) or a change in skin blood flow. In 1987, Bittel established heat debt (difference between heat loss and heat production) as an index for cold acclimation. In our study we could not observe any change in core temperature or heat production meaning heat loss was not altered, and based on this heat debt was not changed by repeated cold exposures. Based on the findings in previous studies (Hesslink et al. 1992; Janský et al. 1996; Bittel 1987; Young et al. 1986) and the lack of change observed in thermal responses and heat production it seems that in our study four weeks of repeated cold exposure did not elicit a cold acclimation.

As mentioned above, the measurements of thermal responses, heat production and muscle activity was a part of a broader study in which the goal was to investigate the effect of cold acclimation on changes in BAT volume and activity. Cold exposure has been shown to be the most potent activator of BAT (Cypess et al. 2012), and a mild short-term cold exposure can elicit a large change in activity of the tissue (van Marken Lichtenbelt et al. 2009). The use of PET/CT technology allowed us to quantify change in BAT volume and activity after the cold acclimation (data collected by our lab). BAT volume and activity was measured in six of eight subjects and showed an increase in volume from 65.5±29.7 to 95.2±27.8 mL (45%) and increase in activity from 0.72±0.3 to 1.59±0.33 volume of BAT(mL)*oxidative metabolism index (sec^{-1}) (120%) from pre to post cold acclimation (unpublished data). No study has ever investigated the effect of cold acclimation on change in BAT in humans, but these findings are supported by animal studies showing a similar increase in BAT

volume and activity (Smith and Roberts 1963). BAT is believed to be the most important contributor to NST (Cannon and Nedergaard 2004), and based on this large increase in BAT volume and activity, an increase in NST should have occurred.

Shivering Thermogenesis

Total heat production in humans is the sum of NST and ST (H_{prod}=NST+ST) when no external work is performed (van Ooijen et al. 2005). Based on the lack of change in heat production, and relatively large increase in BAT volume and activity (NST), there should be an overall decrease in ST. However, contrary to what we expected no change in whole body shivering was observed after four weeks of cold acclimation (Figure 2.4). Several other calculations (sum %MVC for the whole experimental session, AUC for last 30 and 60 min, etc) were also performed to try to detect a change in ST. Still all calculations led to the same conclusion, that whole body shivering was not changed by cold acclimation. To investigate if cold acclimation had an effect on individual muscles not seen during the analysis of whole body shivering, changes in individual muscle shivering were also analyzed. Overall there was no change in individual muscle shivering or individual muscle contribution to total shivering from pre to post cold acclimation (Table 2.2). The only muscle that showed a significant change from pre to post acclimation was RF. Even though the change in shivering intensity was large (72%), RF is a small muscle and even a large change is not sufficient to affect whole body shivering.

The absence of change in ST was in contradiction to our hypothesis and to previous studies. Where previous studies (Davis 1961; Mathew et al. 1981) have found that cold acclimation leads to a decrease in ST, a similar change could not be observed in our study. This could be addressed to the approach we used to determine whole body shivering. While previous studies only assessed change in the raw EMG signal or the change in EMG signal from single muscles, we normalized our EMG

signal to MVC and used an equation (6) to determine whole body shivering. This equation was based on the mass of the muscles involved, allowing us to accurately quantify changes in whole body ST. Our study also measured shivering from 12 different muscles, which is more that any study has done before. Most of the previous cold acclimation studies based their results on changes in shivering in single muscles, and concluded that changes in single muscles indicated a change in total shivering. Our study showed that even though changes in shivering in single muscles occurred, this did not dictate a change in whole body shivering.

In this study, it was decided to use an experimental temperature of 4°C while the acclimation sessions were conducted at 10°C. This was done to assure the occurrence of shivering in every experimental session. Previous studies on rodents show that six weeks of cold acclimation at 6°C abolished shivering, while some shivering still occurred when the same rodents were exposed to -6°C (Hart, Heroux, and Depocas 1956; Heroux, Hart, and Depocas 1956). Unlike humans, rodents rely heavily on NST for heat production (Mineo et al. 2012), while ST is the main contributor to heat production in humans exposed to cold. Based on this, it is possible that a decrease in whole body shivering did occur at 10°C, but that this change could not be observed when the participant was exposed to 4°C. Another explanation for the lack of change in ST is the choice of duration and method of cooling used to elicit cold acclimation. Our approach to elicit a cold acclimation in healthy adult men using an LCS has not been attempted before. The three cold acclimation studies described in the introduction by Young et al., Jansky et al., and Bittel used cold water immersion with a temperature between 10-18°C, lasting from four to six weeks to elicit cold acclimation, and all of the studies were able to alter skin and core temperature and heat production. The studies by Davis and Matthews et al., used a cooling protocol lasting 4-8 hours, daily, for 21-31 days with cold air exposure (10-13°C) to elicit cold acclimation, and was able to evoke a change in skin and core temperature and heat production, and also a decrease in shivering. Considering no change was

observed in any of the parameters we measured, it is possible that when using an LCS to elicit cold acclimation, four weeks at 10°C is not sufficient to have an effect on any of these mechanisms. Overall the four weeks of cold acclimation did not change whole body or individual muscle shivering, and based on these results it seems that even a relatively large change in NST is insufficient to have an effect on ST. This again confirms the notion that ST is the main contributor to heat production for humans in the cold. To be sure that cold acclimation had no effect on ST; an analysis of shivering pattern was also conducted.

Burst Shivering

After finding no effect of cold acclimation on changes in whole body or individual muscle shivering, a detailed analysis of shivering pattern was performed. This was done to identify if more subtle effects of cold acclimation on changes in ST occurred, not uncovered by the analysis of whole body shivering. The two shivering patterns, high intensity burst shivering and continuous low intensity shivering was distinguished by large differences in intensity (7-15% vs. 2-5% MVC) and rate of occurrence (0.1-0.2Hz vs. 8-10Hz) (Meigal 2002; Israel and Pozos 1989). The effect of cold acclimation on shivering pattern has never been investigated before, and our analysis shows that shivering pattern was not affected by four weeks of cold acclimation. No change could be observed in total burst shivering, burst duration or burst intensity (Figure 2.4A, B and C). Burst shivering and continuous low intensity shivering have been linked to recruitment of specific muscle fiber types. Continuous low intensity shivering has been related to activation of type-1 muscle fibers while burst shivering has been related to activation of type-2 muscle fibers (Petajan and Williams 1972; Meigal 2002). The lack of change in both continuous shivering and burst shivering suggests that four weeks of cold acclimation did not lead to a change in muscle fiber recruitment to sustain heat production. The only significant change in burst shivering was observed in single muscles. A small increase in total burst shivering for LAT (138±9 vs. 159±14) and BICEP (75±9 vs. 126±18) and a small decrease in

burst intensity for PEC (13.8±2.6 vs. 9.7±1.8) were the only changes observed in shivering pattern. These changes are small and are occurring only in single muscles, making them insufficient to have an effect on whole body shivering. None of the other individual muscles showed any change in total burst shivering, burst intensity or burst duration. Overall, cold acclimation had no effect on shivering pattern.

Fuel Selection

Our study was the first to investigate changes in fuel selection during cold acclimation. The results showed that no effect of cold acclimation on fuel selection could be observed during four weeks the study lasted (Table 2.4). Rate of CHO and lipid oxidation did not change, and the distinct fuels contribution to total heat production remained unchanged from pre to post acclimation. This is counter to our hypothesis, but it is in accordance with the absence of change in shivering pattern. As mentioned in the previous section burst shivering is linked to the recruitment of type-2 muscle fibers fueled by CHO oxidation, while continuous low intensity shivering is linked to the recruitment of type-1, fatigue resistant muscle fibers predominately fueled lipid oxidation (Haman et al. 2004). Based on the increase in both BAT volume and activity, it is surprising to see the absence of change in lipid oxidation. BAT is primarily fueled by lipids, and a 45% increase in BAT volume, accompanied by a 120% increase in activity, with no change in shivering pattern, should have had an effect on lipid oxidation. The reason why we didn't see the expected change in lipid oxidation could be related to the fact that the volume of active BAT is relatively small compared to other metabolically active tissues. Compared to other highly active tissues like liver (~1.8kg), brain (~1.4kg) and skeletal muscle(~28kg) (Gallagher et al. 1998), a change in BAT volume from 0.065 to 0.095 kg (the average of unpublished data from our lab) is probably not sufficient to affect total lipid oxidation or fuel selection in general. The absence of change in fuel selection, confirms the absence of change in burst shivering, which again confirms the absence of change in whole body shivering.

Interindividual Variability

In figure 2.6, each participants individual shivering responses (presented as AUC) is plotted for every experimental session. Differences in anthropometric measures (weight, height, adiposity, etc.) can have an effect on the size of an individual's shivering response in the cold, so to avoid large effects of this we normalized as much as possible for anthropometric measures during our participant recruitment. Still, based on the results seen in figure 2.6, there is unexplained large interindividual variability in the amount of shivering observed among our participants. Similar interindividual differences have been observed among participants during an acute cold exposure (Haman et al. 2004; Bell, Tikuisis, and Jacobs 1992), and the interindividual differences seen among participants during cold acclimation will be discussed closer in CHAPTER 3.

CONCLUSION

Judging from the lack of change in thermal responses, heat production and shivering, and an increase in BAT volume and activity, it could be possible that we are seeing a type of cold acclimation that has not been described in humans before. The only parameter showing a significant change in our study was BAT volume and activity, so there is a possibility that during the acclimation, the body's heat production was sufficient to maintain homeostasis, but the mechanism responsible for heat production shifted from ST more towards NST. We don't know the reason for why this would happen, but we do know NST does not inhibit locomotion in the same was ST does (Stott and Slee 1985). These are only speculation, and further research on this topic is needed.

From the results yielded in this study we conclude that four weeks of cold acclimation at 10°C did not lead to a change in skin and core temperature, heat production or ST. There was neither an effect on shivering pattern or fuel selection, and the only observed effect of cold acclimation was an increase in BAT volume and activity.

Based on the results presented in figure 2.6 it could seem like assessing the effect of cold acclimation looking only at changes in mean values is not the perfect approach. Large interindividual differences seem to occur in the effects of cold acclimation on thermoregulatory responses, and this will be assessed and discussed more closely in CHAPTER 3.

CHAPTER 3: INTERINDIVIDUAL VARIABILITY & THE EFFECTS OF CHANGE IN BAT ON CHANGES IN MUSCLE ACTIVITY

INTRODUCTION

The interindividual variability in human adult responses to cold is an interesting but not well described phenomenon. During our preliminary analysis of the individual responses in shivering to cold acclimation (figure 2.6) in CHAPTER 2, we observed a large interindividual variability among individuals that cannot be explained by differences in anthropometric measures. Previous research has also shown that large interindividual differences can occur especially in shivering responses, but also in the amount of active BAT observed during an acute cold exposure. One of the first studies reporting evidence of the interindividual variability in shivering during acute cold exposures was conducted by Bell et al. (Bell, Tikuisis, and Jacobs 1992). Two groups of five participants (LEAN and NORM) were exposed to cold air (15°C) for 120 min, and shivering activity was measured from six muscles, together with heat production. A scatter plot was made to compare changes in heat production and whole body shivering at 17 time points during the cold exposure. The scatter plot showed that within the NORM group, but especially in the LEAN group, large differences in the amount of shivering for the same change in heat production occurred. This indicates that when participants with similar anthropometric measures are exposed to the same type of cold for a certain period of time, their shivering response to increase heat production is very different. Similar differences were found by Haman et al. (2004), looking at changes in fuel selection during intensive shivering. After exposing eight healthy, physically active adult male participants to 5°C cold for 90 min, large differences in the changes in both peak shivering and fuel selection were found. Even after normalizing as much as possible for age, morphology (surface to volume ratio), body composition and diet, peak shivering showed variability from 47.2% to 75.9% among individuals. The changes in CHO oxidation during the 90 min of cold exposure varied from 1.9 fold to an 8.3 fold increase, and while some of the participants showed no change in lipid oxidation other had a 23.4 fold increase.

Large variability in BAT volume and activity has also been shown among participants in recent research studies. Although it has been established that BAT volume is dependent on body composition (Vijgen et al. 2011), age (Yoneshiro et al. 2011; Pfannenberg et al. 2010) and season (Au-Young et al. 2009; Huttunen, Hirvonen, and Kinnula 1981), large individual differences occur after normalizing for theses parameters. An example of this was given in the study conducted by Van Marken Lichtenbelt et al.(2009). They investigated the effects of an acute cold exposure on the activation of BAT in 10 lean subjects (BMI 23.2±1.2 kg/m^2, BF 16.2±5.2%). After 120 min of exposure to cold air (16°C), active BAT was found in all 10 participants. Average activity of BAT (SUV) was measured to be 428 kBq, but the standard deviation was 394 kBq. The average volume of active BAT was found to be 130 cm^3, but the standard deviation was as large as 98 cm^3. This indicates that even between participants having similar anthropometric measures, large interindividual differences in both activity and volume of BAT are present. Similar findings were reported by Ouellet et al.(2012) in six healthy male adults (aged 23 to 42 years, BMI from 23.7 to 31.0 kg/m^2) exposed to 18°C cold using an LCS for 120 min. After measuring BAT volume using PET/CT scans, large differences were found among participants varying from 31 mL to 329 mL. These previous research studies has shown that large initerindividual variability in shivering and BAT volume occur among participants during acute cold exposures, but what remains to be explored is: “Can similar interindividual variability be found during four weeks of cold acclimation?”

BAT is an important heat producing organ necessary for the survival of small mammals and human infants. Both small mammals and human infants have a large surface to volume ratio (Pursley 2008) and human infants lack the ability to shiver (Van Der Spek, Van Lingen, and Van Zoeren-Grobben 2009; Pursley 2008) meaning they rely heavily on heat production from BAT. Recent research has suggested that BAT is also present in human adults and could play a part in increasing heat production. Some of the first studies proposing adult humans possessing BAT were done by van

Marken Lichtenbelt et al., in 2009. Using PET/CT scans to detect BAT, they found that after a 120 min mild cold exposure (16˚C), active BAT could be observed in 10 out of 10 healthy lean male subjects. Ever since this discovery, several studies have been conducted yielding results supporting the findings of van Marken Lichtenbelt et al., (Virtanen et al. 2009; Cypess et al. 2012; Cypress et al. 2009; Saito et al. 2009). Very few of these studies took into account the effects of shivering on change in heat production. Research has shown that change in heat production is related to a change in shivering during acute cold exposure (Haman et al. 2004) and during long term cold acclimation (Davis 1961; Mathew et al. 1981). Both BAT and ST are heat producing mechanisms found to be activated during a cold exposure, but what is yet to be explored is the relationship between BAT and shivering. Can the amount of BAT a participant possesses predict the change in shivering during four weeks of cold acclimation, and is the change in shivering correlated with the change in amount of BAT during cold acclimation?

PURPOSE/HYPOTHESIS

The purpose of this third chapter was to investigate if similar interindividual variability found during acute cold exposure studies, also occur during four weeks of cold acclimation. The second purpose was to explore the possibility of using BAT as a predictor for changes in shivering during cold acclimation, and also to explore if a relationship exists between changes in shivering and changes in BAT during four weeks of cold acclimation. Based on the fact that BAT plays a significant part in NST (van Marken Lichtenbelt and Schrauwen 2011) and that NST+ST= $heat_{prod}$ when no external work is performed (van Ooijen et al. 2005), we hypothesize that a negative relationship exists between changes in BAT and changes in ST.

METHODOLOGY

Participants

Data from the same group of participants described in CHAPTER 2 were used in the current chapter. Only six of the eight participants underwent PET/CT scans to measure BAT volume and activity, while individual shivering data was collected from all eight participants.

Shivering data collection.

The procedures for measurements of thermal responses, heat production, and shivering have been described in CHAPTER 2. The experimental sessions and acclimation sessions were the same between the two studies.

BAT data collection.

The methodology of BAT data collection is described in detail in Ouellet et al. (2012) experimental protocol A, and just a brief overview will be given here. BAT data was collected at the Sherbrooke University Hospital Center, by PhD candidate Denis Blondin in collaboration with the research unit at the hospital. A total of six participants underwent PET/CT scans pre and post cold acclimation (cold acclimation protocol described in CHAPTER 2). The participants were exposed to 18° C cold using an LCS, for 180 min. The volume of active BAT was measured after the 180 min of cold exposure, using the radioactive tracer ^{18}F-FDG.

Statistical Analysis

To test if any relationship could be established between changes in shivering and BAT volume, a Pearson's correlation was applied. A relationship was confirmed if the p-value was lower or equal to 0.05, and the strength of the correlation between the two variables was determined based on the r-value given by the correlation calculation.

RESULTS

Interindividual variability

Individual values for changes in shivering, heat production, thermal responses and BAT volume, from pre to post cold acclimation are presented in Table 3.1. The results showed that changes in skin and core temperature did not vary among participants, and seven out of eight participants had no change in heat production. Participant 1 was the only one experiencing a change in heat production, with an increase of 24.2% (from 1982 kJ to 2388 kJ). Among the other measured variables, large individual differences occurred. Changes in BAT volume varied from a decrease of 10.5% (from 65.7 ml to 58.9 ml) in participant 1, to an increase of 539.6% (from 6.2 to 39.9 ml) in participant 3. The variability in changes in shivering intensity among participants varied from an increase of 35.8% (from 396.6 %MVC-AUC to 538.9 %MVC-AUC) in participant 1 to a decrease of 44.8% (from 366.7 %MVC-AUC to 202.4 %MVC-AUC) in participant 4. Changes in burst shivering diverged from an increase of 47.4% in participant 5, to a decrease of 18.9% in participant 7. Large differences also occurred in fuel selection, where changes in CHO oxidation varied from an increase of 163.4% to a decrease of 49.7%, and changes in lipid oxidation varied from an increase of 35.2% to a decrease of 43.4%

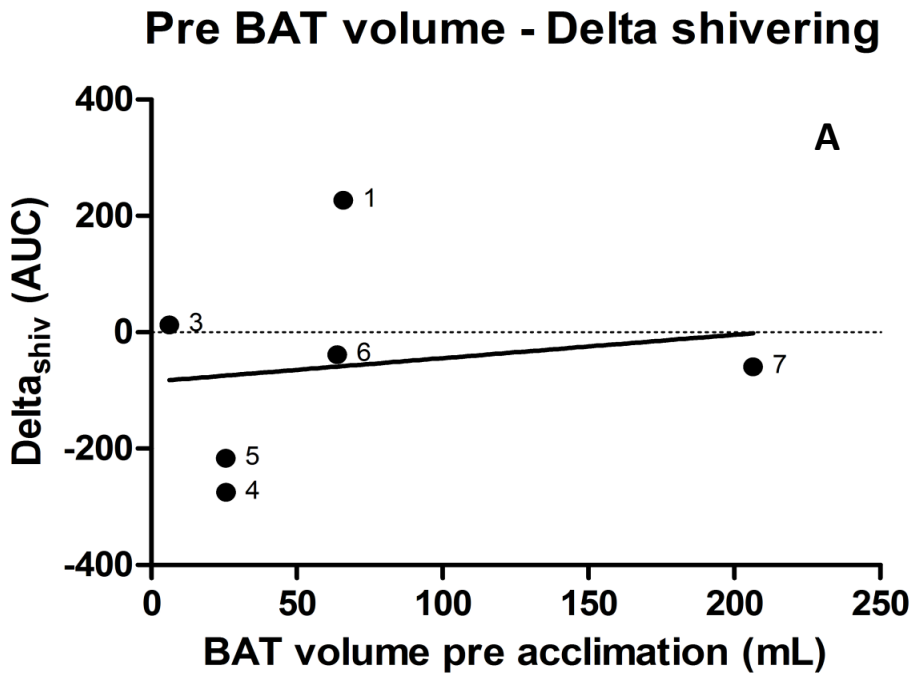
Changes in BAT vs. Changes in Shivering

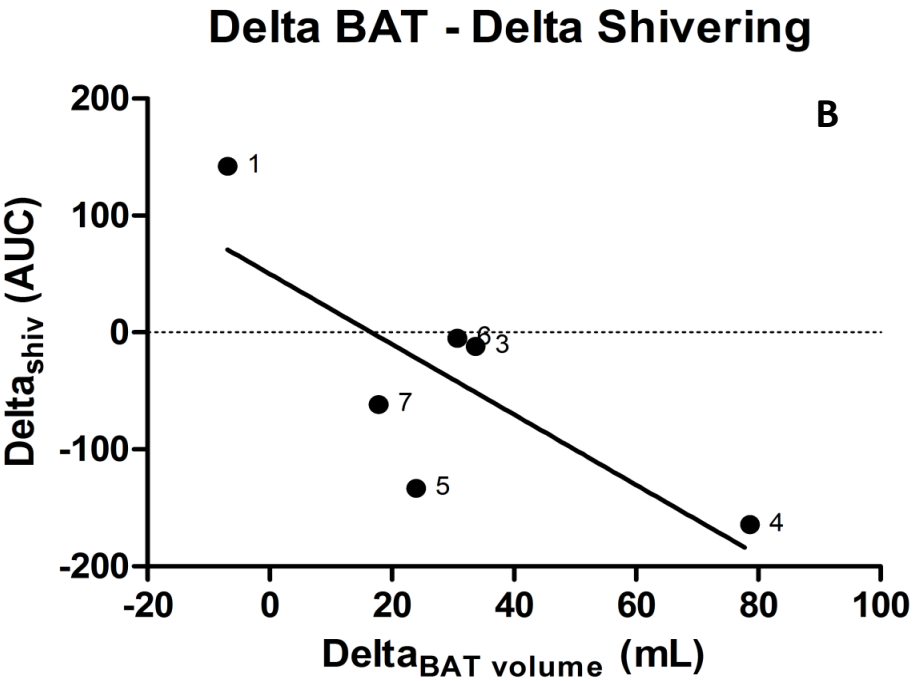
The data for changes in BAT volume and shivering from pre to post cold acclimation are presented in Table 3.1. Based on these values several correlation calculations were attempted, but none of them showed a significant relationship between BAT and ST. The two graphs presented in Figure 3.1 A and B are chosen to best answer the questions posed in the introduction. Figure 3.1 A shows that no relationship exists between the amount of BAT a participant possess prior to cold acclimation and the change in ST ($r=0.16$, p-value=0.75).

The relationship between change in BAT volume and change in shivering are presented in Figure 3.1B. A trend towards strong negative correlation between changes in BAT volume and changes in shivering are seen here, but the correlation was not significant (r=-0.77, p-value=0.07).

Table 3.1 Shows the change from pre to post cold acclimation of all the measured parameters for each individual participant.

Individual Changes Pre-Post Acclimation								
Participant	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Heat Prod. Mech.								
Δ BAT								
volume (mL)	-6.88		33.67	78.63	23.94	30.72	17.81	
volume (%)	-10.5		539.6	306.2	93.8	48.2	8.6	
Δ Shiv								
AUC	142	-251	-12	-164	-133	-5	-62	-175
AUC (%)	35.8	-58.3	-5.7	-44.8	-39.2	-2.6	-32.3	-41.8
Δ Burst								
total	3.0	15.5	16.2	41.6	48.6	-9.0	-20.6	13.8
total (%)	2.3	14.1	16.0	26.9	47.4	-6.8	-18.9	12.5
Thermal Responses								
Δ Heat prod								
AUC	366	104	-82	30	-104	-120	-48	-8
AUC (%)	24.2	6.1	-5.4	1.8	-6.0	-7.4	-3.0	-0.4
Δ Core Temp								
°C	0.0	-0.2	0.3	0.4	-0.5	-0.3	-0.2	-0.2
°C (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-0.5
Δ Skin Temp								
°C	1.5	0.2	-0.1	0.9	1.8	-1.4	1.8	-0.7
°C (%)	0.0	0.0	0.0	0.0	0.1	-0.1	0.1	0.0
Fuel Selection								
Δ CHO								
(g/min)	-1.5	3.5	-5.5	-14.1	14.0	24.4	11.0	-8.9
(kJ/min)	-25	56	-89	-229	228	398	179	-145
Δ(%)	-6.5	49.1	-24.6	-49.7	61.2	98.7	163.4	-33.5
Δ % contribution	-5.1	2.4	-4.3	-11.9	13.5	25.3	10.1	-7.0
Δ Lipids								
(g/min)	10.5	1.2	0.0	6.8	-9.0	-13.9	-6.2	3.0
(kJ/min)	426	49	2	277	-366	-568	-251	122
Δ(%)	35.2	2.9	0.2	20.7	-24.9	-43.4	-15.7	8.6
Δ % contribution	7.0	-1.9	3.7	12.1	-14.1	-26.2	-10.5	6.9


Pre BAT volume - Delta shivering
A
400
200
0
-200
-400
0
50
100
150
200
250
Delta$_{shiv}$ (AUC)
BAT volume pre acclimation (mL)
1
3
6
7
5
4


Delta BAT - Delta Shivering
B
200
100
0
-100
-200
-20
0
20
40
60
80
100
Delta$_{shiv}$ (AUC)
Delta$_{BAT\ volume}$ (mL)
1
6
3
7
5
4

DISCUSSION

Large, unexplained interindividual differences in both shivering and BAT volume have been reported during acute cold exposures (Ouellet, xE et al. 2012; Haman et al. 2004; Bell, Tikuisis, and Jacobs 1992; van Marken Lichtenbelt et al. 2009), but investigating the interindividual variability occurring among participants during cold acclimation has not been done before. In this third chapter, we explored if similar interindividual difference found during an acute cold exposure, also occurred during four weeks of cold acclimation. What we found was a surprisingly large amount of variability among participants in how they responded to cold acclimation. Only two parameters, change in skin and core temperature showed no differences between individuals, while large individual differences were found for changes in BAT volume, shivering intensity and burst shivering.

Interindividual Variability in Heat Production

Heat production was not changed in seven of eight participants after four weeks of cold acclimation, except for in participant 1. In this particular case, heat production increased by 24.2% (from 1982 kJ to 2388 kJ) after cold acclimation. Based on the absence of change in core temperature and the increase in heat production of 24.2%, it seems like participant 1 also suffered from an increase in heat loss not seen in any of the other participants. The reason for participant 1 suffering from an increased heat loss is hard to explain. Average skin temperature increased with 1.5°C from pre to post cold acclimation, but this is not sufficient to explain an increase of 24.2% in heat production. Respiratory heat loss was not measured, so it is possible that some of the heat lost could be caused by an increase in respiratory heat loss.

Interindividual Variability in Shivering and BAT

After analyzing the individual results from using an LCS to elicit cold acclimation over four weeks, a large variability in the changes in BAT volume from pre to post cold acclimation were found. Five participants had an increase in BAT volume ranging from 8.6-539.6% (i.e. from 206.3 to 224.1 ml (8.6%) in participant 7 and from 6.2 to 39.9 ml (539.6%) in participant 3). Conversely, participant 1 had a decrease in BAT volume of 10.5% (from 65.7 ml to 58.9 ml) after four weeks of cold acclimation. As seen in the results section, although not significant a change in BAT volume showed a trend toward a correlation with change in ST. The five participants showing an increase in BAT volume also showed a decrease in shivering intensity after four weeks of cold acclimation. Participant 1, who had a decrease in BAT volume, had in contradiction to the rest of the participants an increase in shivering intensity of as much as 35.8% (from 396.6 %MVC-AUC to 538.9 %MVC-AUC). Based on these results it seems like there is a trend towards participants 3,4,5,6 and 7 adapting to cold by a shift of the responsibility of heat production from ST to NST (increase in BAT). Participant 1 has an opposite response, adapting to cold by decreasing NST and increasing ST to maintain homeostasis.

Interindividual Variability in Shivering Intensity and Pattern

An interesting case to look closer at is participants 4 and 5. Change in BAT volume (Table 3.1) is different between the two participants, where participant 4 has an increase in BAT volume of 306.2% (from 25.7 ml to 104.3 ml) and participant 5 has an increase of only 93.8% (25.5 ml to 49.5 ml). Even with large differences in the change in BAT volume, change in ST is quite similar for both participants, and they both had a negligible change in heat production. A possible explanation for the difference in change in BAT volume, for a similar change in ST could be explained by a change in shivering pattern. Participant 5 had a smaller decrease in burst shivering than participant 4, and this could account for the difference in change in BAT volume. Instead of having a similar increase in

BAT volume as participant 4, participant 5 relied in part on retaining a high amount of burst shivering to maintain heat production.

Relationship between changes in BAT volume and changes in ST.

BAT has a great heat producing potential and is believed to be the most important contributor to NST (Cannon and Nedergaard 2004) . Based on the equation NST+ST= $Heat_{prod}$ when no external work is performed (van Ooijen et al. 2005) , having a large amount of BAT would mean less shivering during cold exposures, and that change in ST during four weeks of cold acclimation should be small due to the heat produced by BAT. The results from six participants presented in Figure 3.1A, shows that effect of cold acclimation on change in ST cannot be predicted by the volume of BAT the participant possess prior to cold acclimation. Large changes in thermogenesis seem to occur during four weeks of cold acclimation, and the volume of BAT the participants had prior to cold acclimation could not predict their change in ST. The participant possessing the largest volume of BAT prior to cold acclimation had a similar change in ST as the participant possessing the smallest volume of BAT. Even though BAT has a great heat producing potential, volume of BAT possessed prior to cold acclimation did not show a correlation with changes in ST during four weeks of cold acclimation.

Change in BAT volume might be a more reliable predictor for the change in shivering resulting from cold acclimation. In Figure 3.1 B we can see that although not significant, a strong negative trend towards a correlation between change in BAT volume and change in shivering during four weeks of cold acclimation can be observed. This means that among the individuals in this study, as the volume of BAT increases, there seems to be an accompanying decrease in ST.

CONCLUSION

After exploring the interindividual differences occurring among participants after four weeks of cold acclimation, it is evident that some type of cold adaptation took place in all participants. Some of the participants responded with an increase in BAT volume accompanied by a decrease in ST, some responded with a change in shivering pattern, while one participant responded by increasing ST to sustain sufficient heat production to maintain homeostasis. What seems apparent is that even though some type of acclimation occurs in every participant, a large variability is present in the pathways activated to compensate for the cold stress of repeated cold exposures.

The results from CHAPTER 3 showed that the volume of BAT possessed prior to cold acclimation could not serve as a predictor for change in ST. Our hypothesis was not confirmed, but it seems possible that a relationship exists between changes in BAT volume and changes in ST. Although there was no overall change in ST during cold acclimation as reported in CHAPTER 2, the individual participants that had an increase in BAT volume experienced a similar decrease in ST.

CHAPTER 4: GENERAL CONCLUSSION

Human beings have been exposed to different cold conditions throughout time, and have through cold acclimation developed mechanisms to survive in these conditions. Cold acclimation can be elicited through exposure to natural cold climates, or artificially induced in a laboratory to study the body's response to repeated cold exposures. Several studies looking at the effects of cold acclimation in humans have been conducted during the last 50 years, and they have shown that cold acclimation can lead to changes in skin and core temperature, heat production and shivering. An accurate quantification of ST during cold acclimation has not been done before, and most previous measurements of shivering during cold acclimation have been inaccurate and inadequate. CHAPTER 2 in this study sought to investigate the changes in muscle activity during four weeks of cold acclimation. The question, "Can cold acclimation help increase survivability in the cold?" was posed in the introduction of CHAPTER 2, and the results from this study do not give a conclusive answer to this question. There were no overall changes in thermal responses, heat production or shivering, and the increase in NST (BAT) seems to be insufficient to have an effect on ST. The results from this study seem to indicate that four weeks of cold acclimation at 10°C using an LCS under our experimental conditions, is not sufficient to have an effect on thermal responses, heat production or ST.

Although no effect was found on any of the previously mentioned parameters, these measurements were a part of a larger study, where the effect of cold acclimation on changes in BAT was the main outcome measure. The results from the measurements of changes in BAT showed an increase in BAT volume (45%) and activity (120%) after four weeks of cold acclimation.
Previous research have found large interindividual differences in BAT and ST during an acute cold exposure, and the possibility of similar differences occurring during four weeks of cold acclimation

were examined in CHAPTER 3. The only two parameters showing no difference among individuals were change in skin and core temperature. While only one participant had a change in heat production, large interindividual differences were found in changes in BAT volume, shivering intensity and burst shivering. Although all participants showed some type of adaptation to cold, the pathways taken to adapt to cold was widely different among individuals.

While exploring the relationship between change in shivering and BAT volume, we found that the amount of BAT a participant possess prior to cold acclimation could not be used as predictor for change in shivering during four weeks of cold acclimation. On the other hand, although not significant there seems to be a trend toward a relationship between changes in shivering and changes in BAT volume, where changes in BAT volume shows a non-significant negative relationship with changes in ST.

The overall conclusion of this study is that the novel approach of using an LCS is to elicit cold acclimation led to an increase in BAT volume and activity, but had no effect on changes in muscle activity.

REFERENCES:

Adams, Thomas, and Earl J. Heberling. 1958. Human Physiological Responses to a Standardized Cold Stress as Modified by Physical Fitness. *Journal of Applied Physiology* 13 (2):226-230.

Albright, Ann L., and Judith S. Stern. 1998. Adiopse Tissue. *Adipose tissue. In: Encyclopedia of Sports Medicine and Science*.

Arbuthnott, E. 1989. Brown adipose tissue: structure and function. *Proceedings of the Nutrition Society* 48:177-182.

Au-Young, Iain T.H., Natasha Thorn, Rakesh Ganatra, Alan C. Perkins, and Michael E. Symonds. 2009. Brown Adipose Tissue Seasonal Variations in Humans. *Diabetes* 58 (2583-2587).

Barnard, T. . 1977. Brown adipose tissue as an effector of nonshivering thermogenesis. *Generalia*.

Bell, D. G., P. Tikuisis, and I. Jacobs. 1992. Relative intensity of muscular contraction during shivering. *Journal of Applied Physiology* 72 (6):2336-2342.

Beyer, Thomas, Tony Brun Townsend, Paul E. Kinahan, Martin Carron, Raymond Roddy, Jeff Jerin, John Young, Larry Byars, and Ronald Nutt. 2000. A Combined PET/CT Scanner for Clincal Oncology. *Journal of Nuclear Medecine* 41:1369-1379.

Bittel, J. H. 1987. Heat debt as an index for cold adaptation in men. *Journal of Applied Physiology* 62 (4):1627-1634.

Bostrom, Pontus, Jun Wu, Mark P. Jedrychowski, Anisha Korde, Li Ye, James C. Lo, Kyle A. Rasbach, Elisabeth Almer Bostrom, Jang Hyun Choi, Jonathan Z. Long, Shingo Kajimura, Maria Cristina Zingaretti, Birgitte F. Vind, Hua Tu, Saverio Cinti, Kurt Hojlund, Steven P. Gygi, and Bruce M. Spiegelman. 2012. A PGC1-[agr]-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 481 (7382):463-468.

Boyles, Justin G., Frank Seebacher, Ben Smit, and Andrew E. McKechnie. 2011. Adaptive Thermoregulation in Endotherms May Alter Responses to Climate Change. *Integrative and Comparative Biology* 51 (5):676-690.

Cannon, B, and J Nedergaard. 2004. Brown Adipose Tissue: Function and Physiological Significance. . *Physiology* 84:277-359.

Cannon, B, and J Nedergaard. 2008. Neither fat nor flesh. *Nature* 454.

Cohade, C., K.A. Mourtzikos, and R.L. Wahl. 2003. "USA-Fat": Prevalence Is Related to Ambient Outdoor Temperature—Evaluation with 18F-FDG PET/CT. *Journal of Nuclear Medecine* 44 (1267-1270).

Cohade, C., M. Osman, H.K. Pannu, and R.L. Wahl. 2003. Uptake in Supraclavicuar Area Fat ("USA-FAT"): Description on 18F-FDG PET/CT. *Journal of Nuclear Medecine* 44:170-176.

Cypess, Aaron M., Yih-Chieh Chen, Cathy Sze, Ke Wang, Jeffrey English, Onyee Chan, Ashley R. Holman, Ilan Tal, Matthew R. Palmer, Gerald M. Kolodny, and C. Ronald Kahn. 2012. Cold but not sympathomimetics activates human brown adipose tissue in vivo. *Proceedings of the National Academy of Sciences* 109 (25):10001-10005.

Cypress, A.M., L. Sanaz, G. Williams, I. Tal, E.L. Palmer, Y.H. Tsen, A. Doria, G.M. Kolodny, and C.R. Kahn. 2009. Identification and Importance of Brown Adipose Tissue in Adult Humans. *New England Journal of Medecine* 360 (1509-17).

Davis, Thomas R. A. 1961. Chamber cold acclimatization in man. *Journal of Applied Physiology* 16 (6):1011-1015.

Dorbert, N., C. Menzel, N Hamscho, N Wordehoff, W.T. Kranert, and F. Grunwald. 2004. Atypical thoracic and supraclavicular FDG-uptake in patients with Hodgkin's and non-Hodgkin's lymphoma. . *The Quarterly Journal of Nuclear Medecine and Molecular Imaging* 44:33-38.

Du Bois D, Du Bois E. F. 1916. Clinical calorimetry: Tenth paper a formula to estimate the approximate surface area if height and weight be known. *Archives of Internal Medicine* XVII (6_2):863-871.

Elia, M. 1991. Energy equivalents of CO2 and their importance in assessing energy expenditure when using tracer techniques. *American Journal of Physiology - Endocrinology And Metabolism* 260 (1):E75-E88.

Eyolfson, Douglas A., Peter Tikuisis, Xiaojiang Xu, Gillian Weseen, and Gordon G. Giesbrecht. 2001. Measurement and prediction of peak shivering intensity in humans. *European Journal of Applied Physiology* 84 (1-2):100-106.

Gallagher, Dympna, Daniel Belmonte, Paul Deurenberg, Zimian Wang, Norman Krasnow, F. Xavier Pi-Sunyer, and Steven B. Heymsfield. 1998. Organ-tissue mass measurement allows modeling of REE and metabolically active tissue mass. *American Journal of Physiology - Endocrinology And Metabolism* 275 (2):E249-E258.

Gessner, K. 1551. Conradi Gesneri medici Trigurine Historae Animalium: Lib. I De Quadredibus viviparis. .

Gosselin, C., and F. Haman. 2012. Effects of green tea extracts on non-shivering thermogenesis during mild cold exposure in young men. *Br J Nutr*:1-7.

Haman, F., S. R. Legault, and J. M. Weber. 2004. Fuel selection during intense shivering in humans: EMG pattern reflects carbohydrate oxidation. *J Physiol* 556 (Pt 1):305-13.

Haman, François, Stéphane R. Legault, Mark Rakobowchuk, Michel B. Ducharme, and Jean-Michel Weber. 2004. Effects of carbohydrate availability on sustained shivering II. Relating muscle recruitment to fuel selection. *Journal of Applied Physiology* 96 (1):41-49.

Haman, François, François Péronnet, Glen P. Kenny, Denis Massicotte, Carole Lavoie, Chris Scott, and Jean-Michel Weber. 2002. Effect of cold exposure on fuel utilization in humans: plasma glucose, muscle glycogen, and lipids. *Journal of Applied Physiology* 93 (1):77-84.

Hammel, H. T., R. W. Elsner, D. H. Le Messurier, H. T. Andersen, and F. A. Milan. 1959. Thermal and metabolic responses of the Australian aborigine exposed to moderate cold in summer. *Journal of Applied Physiology* 14 (4):605-615.

Hany, T.F., E. Gharehpapagh, E.M. Kamel, A. Buck, J. Himms-Hagen, and G.K. von Schulthess. 2002. Brown adipose tissue: a factor to consider in symmetrical tracer uptake in the neck and upper chest region. . *European Journal of Nuclear Medecine* 29 (10).

Hardy, James D., Eugene F. Du Bois, and G. F. Soderstrom. 1938. The Technic of Measuring Radiation and Convection: One Figure. *The Journal of Nutrition* 15 (5):461-475.

Hart, J. S., O. Heroux, and F. Depocas. 1956. Cold Acclimation and the Electromyogram of Unanesthetized Rats. *Journal of Applied Physiology* 9 (3):404-408.

Hemingway, Allan. 1963. Shivering. *Physiological Reviews* 43 (3):397-422.

Heroux, O., J. S. Hart, and F. Depocas. 1956. Metabolism and Muscle Activity of Anesthetized Warm and Cold Acclimated Rats on Exposure to Cold. *Journal of Applied Physiology* 9 (3):399-403.

Hesslink, R. L., M. M. D'Alesandro, D. W. Armstrong, and H. L. Reed. 1992. Human cold air habituation is independent of thyroxine and thyrotropin. *Journal of Applied Physiology* 72 (6):2134-2139.

Himms-Hagen, Jean. 1984. Nonshivering thermogenesis. *Brain Research Bulletin* 12 (2):151-160.

Himms-Hagen, Jean, and D Ricquier. 1998. Brown adipose tissue. *Handbook of obesity*:415-441.

Hong, S. K. 1963. Comparison of diving and nondiving women of Korea. *Fed Proc* 22:831-3.

Huttunen, P., J. Hirvonen, and V. Kinnula. 1981. The occurrence of brown adipose tissue in outdoor workers. *Eur J Appl Physiol Occup Physiol* 46 (4):339-45.

Israel, D. J., and R. S. Pozos. 1989. Synchronized slow-amplitude modulations in the electromyograms of shivering muscles. *Journal of Applied Physiology* 66 (5):2358-2363.
Janský, L. 1973. NON-SHIVERING THERMOGENESIS AND ITS THERMOREGULATORY SIGNIFICANCE. *Biological Reviews* 48 (1):85-132.
Janský, L., H. Janáková, B. Uličný, P. Šrámek, V. Hošek, J. Heller, and J. Pařízková. 1996. Changes in thermal homeostasis in humans due to repeated cold water immersions. *Pflügers Archiv* 432 (3):368-372.
Janssen, Ian, Steven B. Heymsfield, ZiMian Wang, and Robert Ross. 2000. Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. *Journal of Applied Physiology* 89 (1):81-88.
Jessen, K. 1980. An Assessment of Human Regulatory Nonshivering Thermogenesis. *Acta Anaesthesiologica Scandinavica* 24 (2):138-143.
Johansson, B. 1959. Brown fat: a review. *Metabolism* 8 (3):221-40.
Kajimura, Shingo, Patrick Seale, and Bruce M. Spiegelman. 2010. Transcriptional Control of Brown Fat Development. *Cell Metabolism* 11 (4):257-262.
Klingenberg, M. 1990. Mechanisms and evolution of the uncoupling protein of brown adipose tissue. *Trends in Biochemical Sciences* 15.
LeBlanc, J. 1956. Evidence and Meaning of Acclimatization to Cold in Man. *Journal of Applied Physiology* 9 (3):395-398.
Livesey, G, and M Elia. 1988. Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: evaluation of errors with special reference to the detailed composition of fuels. *The American Journal of Clinical Nutrition* 47 (4):608-28.
Lowell, B. B., and B. M. Spiegelman. 2000. Towards a molecular understanding of adaptive thermogenesis. *Nature* 404 (6778):652-660.
Mathew, Lazar, S. S. Purkayastha, A. Jayashankar, and H. S. Nayar. 1981. Physiological characteristics of cold acclimatization in man. *International Journal of Biometeorology* 25 (3):191-198.
McAllen, RobinM, Mutsumi Tanaka, Yoichiro Ootsuka, and MichaelJ McKinley. 2010. Multiple thermoregulatory effectors with independent central controls. *European Journal of Applied Physiology* 109 (1):27-33.
Meigal, A. 2002. Gross and fine neuromuscular performance at cold shivering. *Int J Circumpolar Health* 61 (2):163-72.
Meigal, Alu, V. Lupandin Iu, and G. I. Kuz'mina. 1993. [Electromyographic patterns of thermoregulatory activity of motor units in the process of body cooling]. *Fiziol Cheloveka* 19 (3):106-14.
Mekjavic, I. B., C. J. Sundberg, and D. Linnarsson. 1991. Core temperature "null zone". *Journal of Applied Physiology* 71 (4):1289-1295.
Mineo, P. M., E. A. Cassell, M. E. Roberts, and P. J. Schaeffer. 2012. Chronic cold acclimation increases thermogenic capacity, non-shivering thermogenesis and muscle citrate synthase activity in both wild-type and brown adipose tissue deficient mice. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 161 (4):395-400.
Morrison, Shaun F., Kazuhiro Nakamura, and Christopher J. Madden. 2008. Central control of thermogenesis in mammals. *Experimental Physiology* 93 (7):773-797.
Mozo, Julien, Yalin Emre, Frederic Bouillaud, Daniel Ricquier, and Francois Criscuolo. 2005. Thermoregulation: What Role for UCPs in Mammals and Birds? *Bioscience Reports* 25 (3-4):227-249.

Nedergaard, J., V. Golozoubova, A. Matthias, A. Asadi, A. Jacobsson, and B. Cannon. 2001. UCP1: the only protein able to mediate adaptive non-shivering thermogenesis and metabolic efficiency. . *Biochimica et Biophysia Acta* 1504:82-106.

Nedergaard, J., and O. Lindberg. 1982. The brown fat cell. *International Journal of Cytology* 74:187-286.

Ouellet, V., S. M. Labbe, D. P. Blondin, S. Phoenix, B. Guerin, F. Haman, E. E. Turcotte, D. Richard, and A. C. Carpentier. 2012. Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. *J Clin Invest* 122 (2):545-52.

Ouellet, V., xE, ronique, Labb, S., M. bastien, Denis P. Blondin, Serge Phoenix, Gu, Brigitte rin, Fran Haman, ois, Eric E. Turcotte, Denis Richard, Andr Carpentier, and C. 2012. Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. *The Journal of Clinical Investigation* 122 (2):545-552.

Pasquali, Claudio, Domenico Rubello, Cosimo Sperti, Piero Gasparoni, Guido Liessi, Franca Chierichetti, Giorgio Ferlin, and Sergio Pedrazzoli. 1998. Neuroendocrine Tumor Imaging: Can 18F-Fluorodeoxyglucose Positron Emission

Tomography Detect Tumors with Poor Prognosis and Aggressive Behavior? *World Journal of Surgery* 22:588-592.

Péronnet, F., and D. Massicotte. 1991. Table of nonprotein respiratory quotient: an update. *Canadian journal of sport sciences = Journal canadien des sciences du sport* 16 (1):23-29.

Petajan, J. H., and D. D. Williams. 1972. Behavior of single motor units during pre-shivering tone and shivering tremor. *Am J Phys Med* 51 (1):16-22.

Pfannenberg, C., M.K. Werner, S. Ripkens, I. Stef, A. Deckert, M. Schmadl, M. Reimold, H-U. Haring, C.D. Claussen, and N. Stefan. 2010. Impact of Age on the Relationships of Brown Adipose Tissue with Sex and Adiposity in Humans. . *Diabetes* 59 (7):1789-1793.

Pursley, DeWayne. 2008. Developmental Characteristics of Preterm Infants. *Pediatrics in Review* 29 (2):67-68.

Rasmussen, A. T. 1923. The so-called hibernating gland. *Journal of Morphology* 38 (1):147-205.

Rasmussen, A.T. 1923-1924. ?? *Journal of Morphology* 38 (147).

Rothwell, N. J., and M. J. Stock. 1983. Luxuskonsumption, diet-induced thermogenesis and brown fat: the case in favour. *Clin Sci (Lond)* 64 (1):19-23.

Saito, M., Y. Okamatsu-Ogura, M. Matsushita, K. Watanbe, T. Yoneshiro, J. Nio-Kobayashi, I. Iwanaga, M. Miyagawa, T. Kameya, K. Nakada, Y. Kawai, and M. Tsujisaki. 2009. High Incidence of Metabolically Active Brown Adipose Tissue in Healthy Adult Humans. *Diabetes* 58:1526-1531.

Sato, Haruhiko. 1981. Fusimotor modulation by spinal and skin temperature changes and its significance in cold shivering. *Experimental Neurology* 74 (1):21-32.

Sato, Haruhiko, Takeshi Hashitani, Yoshiaki Isobe, Fujiya Furuyama, and Hitoo Nishino. 1990. Descending influences from nucleus raphe magnus on fusimotor neurone activity in rats. *Journal of Thermal Biology* 15 (3–4):259-265.

Schmidt-Nielsen, K. 1997. Animal Physiology: Adaptation And Environment. *Durham, Cambridge University Press*.

Scholander, P. F., H. T. Hammel, K. Lange Andersen, and Y. L⊘yning. 1958. Metabolic Acclimation to Cold in Man. *Journal of Applied Physiology* 12 (1):1-8.

Schulz, Tim J., Tian Lian Huang, Thien T. Tran, Hongbin Zhang, Kristy L. Townsend, Jennifer L. Shadrach, Massimiliano Cerletti, Lindsay E. McDougall, Nino Giorgadze, Tamara Tchkonia, Denis Schrier, Dean Falb, James L. Kirkland, Amy J. Wagers, and Yu-Hua Tseng. 2011.

Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat. *Proceedings of the National Academy of Sciences* 108 (1):143-148.

Seale, P., B. Bjork, W. Yang, Kajimura, S. Chin, S. Kuang, A. Scime, S. Deverakonda, H.M. Conroe, H. Erdjument-Bromage, P. Tempst, M.A. Rudnick, D.R. Beier, and B.M. Spiegelman. 2008. PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 454 (21).

Seale, Patrick, Shingo Kajimura, Wenli Yang, Sherry Chin, Lindsay M. Rohas, Marc Uldry, Geneviève Tavernier, Dominique Langin, and Bruce M. Spiegelman. 2007. Transcriptional Control of Brown Fat Determination by PRDM16. *Cell Metabolism* 6 (1):38-54.

Sessler, D. I., A. Moayeri, R. Støen, B. Glosten, J. Hynson, and J. McGuire. 1990. Thermoregulatory vasoconstriction decreases cutaneous heat loss. *Anesthesiology* 73 (4):656-660.

Shapiro, B., and E. Wertheimer. 1956. The metabolic activity of adipose tissue; a review. *Metabolism* 5 (1):79-86.

Skreslet, S., and F. Aarefjord. 1968. Acclimatization to cold in man induced by frequent scuba diving in cold water. *Journal of Applied Physiology* 24 (2):177-181.

Smith, R.E., and Roberts. 1963. Thermogenesis of brown adipose tissue in cold-acclimated rats. *Journal of Physiology* 206:143-148.

Smith, Robert E., and Dorothy Jared Hoijer. 1962. Metabolism and Cellular Function in Cold Acclimation. *Physiological Reviews* 42 (1):60-142.

Soderberg, Gary L, and Thomas M Cook. 1984. Electromyography in Biomechanics. *Physical Therapy* 64 (12):1813-1820.

Steinberg, Daniel, Paul J. Nestel, Elsworth R. Buskirk, and Ronald H. Thompson. 1964. Calorigenic Effect of Norepinephrine Correlated with Plasma Free Fatty Acid Turnover and Oxidation*. *The Journal of Clinical Investigation* 43 (2):167-176.

Stott, A. W., and J. Slee. 1985. The effect of environmental temperature during pregnancy on thermoregulation in the newborn lamb. *Animal Science* 41 (03):341-347.

Timmons, James A., Kristian Wennmalm, Ola Larsson, Tomas B. Walden, Timo Lassmann, Natasa Petrovic, D. Lee Hamilton, Ruth E. Gimeno, Claes Wahlestedt, Keith Baar, Jan Nedergaard, and Barbara Cannon. 2007. Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages. *Proceedings of the National Academy of Sciences* 104 (11):4401-4406.

Tseng, Yu-Hua, Efi Kokkotou, Tim J. Schulz, Tian Lian Huang, Jonathon N. Winnay, Cullen M. Taniguchi, Thien T. Tran, Ryo Suzuki, Daniel O. Espinoza, Yuji Yamamoto, Molly J. Ahrens, Andrew T. Dudley, Andrew W. Norris, Rohit N. Kulkarni, and C. Ronald Kahn. 2008. New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 454 (7207):1000-1004.

Vallerand, A. L., Zamecnik, J., Jones, P. J. H., Jacobs, and I. 1999. *Cold stress increases lipolysis, FFA R[a] and TG/FFA cycling in humans*. Vol. 70. Alexandria, VA, ETATS-UNIS: Aerospace Medical Association.

Van Der Spek, R. D. G., R. A. Van Lingen, and D. Van Zoeren-Grobben. 2009. Body temperature measurement in VLBW infants by continuous skin measurement is a good or even better alternative than continuous rectal measurement. *Acta Pædiatrica* 98 (2):282-285.

van Marken Lichtenbelt, W.D., J.W. Vanhommerig, N.M. Smulders, J.M.A.F.L. Drosseart, G.J. Kemerink, N.D. Bouvy, M.D., P. Schrauwen, and G.J. Jaap Teule. 2009. Cold Activated Brown Adipose Tissue in Healthy Men. *New England Journal of Medecine* 360:1500-8.

van Marken Lichtenbelt, Wouter D., Hein A. M. Daanen, Loek Wouters, Rolf Fronczek, Roy J. E. M. Raymann, Natascha M. W. Severens, and Eus J. W. Van Someren. 2006. Evaluation of

wireless determination of skin temperature using iButtons. *Physiology & Behavior* 88 (4–5):489-497.

van Marken Lichtenbelt, Wouter D., and Patrick Schrauwen. 2011. Implications of nonshivering thermogenesis for energy balance regulation in humans. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 301 (2):R285-R296.

van Ooijen, A. M., W. D. van Marken Lichtenbelt, A. A. van Steenhoven, and K. R. Westerterp. 2005. Cold-induced heat production preceding shivering. *Br J Nutr* 93 (3):387-91.

Vijgen, G.H.E.J., N.D. Bouvy, G.J.J. Teule, B. Brans, P. Schrauwen, and W.D. van Marken Lichtenbelt. 2011. Brown Adipose Tissue in Morbidly Obese Subjects. *PLoS ONE* 6 (2).

Virtanen, K.I., M.E. Lidell, J. Orava, M. Heglind, R. Westergren, T. Niemi, M. Taittonen, J. Laine, N-J. Savisto, S. Enerbak, and P. Nuutila. 2009. Functional Brown Adipose Tissue in Healthy Human Adults. *New England Journal of Medecine* 360:1518-25.

Weber, J. M., S. Klein, and R. R. Wolfe. 1990. Role of the glucose cycle in control of net glucose flux in exercising humans. *Journal of Applied Physiology* 68 (5):1815-1819.

Wijers, S. L. J., W. H. M. Saris, and W. D. Van Marken Lichtenbelt. 2009. Recent advances in adaptive thermogenesis: potential implications for the treatment of obesity. *Obesity Reviews* 10 (2):218-226.

Wolfe, R. R., S. Klein, F. Carraro, and J. M. Weber. 1990. Role of triglyceride-fatty acid cycle in controlling fat metabolism in humans during and after exercise. *American Journal of Physiology - Endocrinology And Metabolism* 258 (2):E382-E389.

Yoneshiro, T., S. Aita, M. Matsushita, Y. Okamatsu-Ogura, T. Kameya, Y. Kawai, M. Miyagawa, M. Tsujisaki, and M. Saito. 2011. Age-Related Decrease in Cold-Activated Brown Adipose Tissue and Accumulation of Body Fat in Healthy Humans. *Obesity* 19:1755-1760.

Young, A. J., S. R. Muza, M. N. Sawka, R. R. Gonzalez, and K. B. Pandolf. 1986. Human thermoregulatory responses to cold air are altered by repeated cold water immersion. *Journal of Applied Physiology* 60 (5):1542-1548.