The Combined Effect of Hypoxia and Cold on Substrate Metabolism in Men at Rest

By

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A thesis submitted to the School of Graduate Studies in partial fulfillment of the requirements for the degree of

Master of Science in Kinesiology

School of Human Kinetics and Recreation

Memorial University of Newfoundland

February 2020

St. John's, Newfoundland

Abstract

This study was designed to examine the acute metabolic response of men to the combined exposure to cold and hypoxia at rest. As the individual effects of both conditions have been extensively studied, our research focused on their combined effects in an effort to develop a better understanding of a lesser known topic. Eight participants (age:28.9±11.8 y; weight:80.7±11.5 kg; height:175.8±0.067 m; BMI:29±11 kg·m^{-2}) were recruited to participate in three experimental sessions for two hours on separate days. The experiment conditions included cold (C), hypoxia (H), and both cold and hypoxia combined (CH). Participants were asked to fast for 12 hours prior to beginning each experimental session. Once they arrived at the laboratory, the participants were instructed to lay in a supine position on a bed and were placed underneath a canopy for 30 minutes to measure their basal metabolic rate (BMR). Once the BMR protocol was complete, participants were then fitted with 4 wireless Electromyography (EMG) electrodes [*trapezius* (TR), *pectoralis major* (PE), *rectus abdominis* (RA), and *rectus femoris* (RF)] and were instructed to complete a maximal voluntary contraction (MVC) protocol for all four muscles. Shivering activity throughout the experimental sessions was expressed as percentage of the MVC values for each muscle group. Upon the completion of the MVC protocol, participants were asked to self-insert a rectal thermocouple, used to monitor core-body temperature throughout the sessions. Next, 11 skin thermo-sensors, using a modified Hardy and Dubois 12-point system, were applied directly to their skin, before they were finally fitted with a liquid conditioned suit. Once all initial procedures were complete, they were then

transferred to an enclosed chamber to be exposed, in a random order while in a seated position, to C (FIO_2=0.2093, 10°C), H (F_iO_2=0.135 or ~2500m), or CH (10°C + F_iO_2=0.135). Mean skin temperature significantly dropped over time during exposure in the climatic chamber from 31.9±0.9°C to 26.9±0.9°C and from 32.6±0.8°C to 26.4±1.0°C for C and C+H respectively, with no change in H, from 32.5±0.7°C to 33.1±0.7°C. Energy production (EP) responded accordingly, as it significantly increased over time from 5.8±0.6kcal·min^{-1} to 10.6±1.9 kcal·min^{-1} and from 6.0±0.9 kcal·min^{-1} to 10.6±1.4 kcal·min^{-1} for C and CH, respectively. A slight non-significant effect was observed in H, from 5.8±0.4 kcal·min^{-1} to 6.7±0.7 kcal·min^{-1}. This effect was attributed to the seated position of the participants during exposure. The individual contributions of substrates to EP significantly differed between conditions over time, as glucose oxidation was higher during C when compared to H and CH, while lipid oxidation was higher during CH compared to C and H. To conclude, while the combination of CH did not impact energy production when compared to the other conditions, it did alter the contribution of individual substrates to energy production.

Acknowledgements

The completion of this thesis would not have been possible without the assistance of many important individuals. First and foremost, I would like to express my deepest gratitude to Dr. Fabien Basset, and Dr. Jonathan Power, as their tutelage and patience were instrumental in the completion of this thesis. Additionally, I would like to thank Dr. Jeanette Byrne, for lending her expertise throughout the data collection and analysis processes during this study. A very special thanks to Dr. Thamir Alkanani and Mr. Jason Blair, who spent countless hours in the lab, and were crucial members to this study's success. Ms. Erin Bradbury, who served not only as a mentor, but a true friend assisting me along the way. To all my friends and colleagues who sacrificed their time to participate in this experiment, your assistance was a vital part of this study's success, and I am indebted to each of you. Finally, and most important, my source of support, Brittany Bourgeois, for being a loving and supporting partner throughout this entire process. Without her constant encouragement and understanding, this thesis would not have been possible

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List of Equations

(eq.1) $\overline{T}_{SK} = (\overline{T}_1 \text{ x } 0.07 + \overline{T}_2 \text{ x } 0.088 + \overline{T}_3 \text{ x } 0.088 + \overline{T}_4 \text{ x } 0.14 + \overline{T}_5 \text{ x } 0.063 + \overline{T}_6 \text{ x } 0.065 + \overline{T}_7 \text{ x } 0.088 + \overline{T}_8 \text{ x } 0.088 + \overline{T}_9 \text{ x } 0.063 + \overline{T}_{10} \text{ x } 0.063\ \overline{T}_{11} \text{ x } 0.065)/0.88$

(eq.2) $G_{ox}\ (g\cdot min^{-1}) = 4.57\ \dot{V}CO_2 - 3.23\ \dot{V}O_2 - 2.60N$

(eq.3) $L_{ox}\ (g\cdot min^{-1}) = 1.69\ \dot{V}O_2 - 1.69\ \dot{V}CO_2 - 2.03N$

(eq.4) $Pro_{ox}\ (g\cdot min^{-1}) = 6.25N$

(eq.5) $\dot{V}O_2 = FR_i(F_iO_2) - FR_e(F_eO_2)$

(eq.6) $\dot{V}CO_2 = FR_e(F_eCO_2) - (FR_i(F_iCO_2)$

(eq.7) $EP\ (kcal\cdot min^{-1}) = EP = 3.74\ \ Gox + 9.46\ Lox + 4.32\ Pox$

List of Abbreviations

AMP - Adenosine monophosphate

AMPK - Adenosine monophosphate-activated protein kinase

ATP - Adenosine triphosphate

ANOVA - Analysis of variance

BMI – Body mass index

BMR – Basal metabolic rate

C - Cold condition

CH – Cold &hypoxic condition

CHO - Carbohydrate

EMG - Electromyography

EP – Energy production

Epo - Erythropoietin

EE – Energy expenditure

FiO_2 – Fraction of inspired oxygen

G_{ox} – Glucose oxidation

H - Hypoxic condition

HH – Hypobaric Hypoxia

HR - Heart rate

HREA – Health Research Ethics Authority

iEMG - Integrated electromyography

LCS – Liquid conditioned suit

L_{ox} – Lipid oxidation

MR - Metabolic rate

MVC - Maximum voluntary contraction

NH – Normobaric Hypoxia

O_2 - Oxygen

PAR-Q+ - Physical Activity Readiness Questionnaire

PE- Pectoralis major

Pr_{ox} – Protein oxidation

RA – Rectus abdominis

RF – Rectus femoris

RMS – Root mean squared

SD - Standard deviation

SpO_2 - Blood oxygen saturation

$\overline{T}_{SK}$ Mean skin temperature

TR - Trapezius

T_{re} -Rectal temperature

$\dot{V}CO2$ -Volume carbon dioxide output

$\dot{V}O2$ -Volume oxygen uptake

Chapter 1: Introduction

1.1 Background of the Study

This research project was designed to examine the metabolic responses of men to cold and hypoxic exposure. As the individual effects of both conditions have been extensively studied, our research focused on their combined effects. Exposure to cold and hypoxia will alter metabolic processes, which will impact the contributions of different fuel sources towards energy expenditure (Brooks et al., 1991; Matu, Deighton, Ispoglou & Duckworth, 2017). Currently, the mechanisms that control the alterations in metabolic processes, and the contributions from fuel sources, are not fully understood. A review of the available literature on the effects of cold exposure on humans would indicate that these environmental conditions will lead to changes in metabolic response.

Low intensity shivering induces thermogenesis, or heat production, that is primarily fuelled by lipids (Haman, Legault & Weber, 2004). As the intensity of shivering increases, fuel selection begins to favor carbohydrate (CHO) sources, (Haman, Legault & Weber, 2004). Conversely, as individuals return to a normothermic state, fuel preferences shift from CHO to lipids (Haman, Scott & Kenny, 2007). It is important to note that none of the participants in any of the aforementioned studies experienced any form of hypothermia during or after cold exposure (Gosselin & Haman, 2012). Research conducted on hypoxic exposure has indicated a metabolic shift towards CHO will occur during acute hypoxic exposure (Clanton, Hogan & Gladden, 2013). This response occurs because CHO

sources generate more ATP per litre of oxygen than lipid sources; a more oxygen-efficient state in conditions of low partial pressure of oxygen.

1.2 Purpose of the Study

The current literature dealing with the effects of cold and hypoxic exposure on metabolic response in humans has been predominantly focused on exposure to each of the conditions separately. At this time, there has been very little research examining the combined effects of both cold and hypoxia, creating a gap in the literature. Therefore, the premise of this study was to examine the cumulative effects of acute hypoxia and cold exposures on the metabolic response on humans at rest, particularly substrate partitioning. The results from this study may prove useful for future researchers exploring the effects of cold and hypoxia on substrate metabolism. In fact, the metabolic adjustments induced by hypoxia and by the cold exposure have a major impact on the contribution of substrates to ATP production, mechanisms not fully understood. If in the former environmental condition CHO contributes the most to energy metabolism, in the latter, fat oxidation provides the major part of it. However, little is known about their combine effect on metabolic response. Therefore, the current project might shed some lights on the mechanisms underlying the contribution of substrates to ATP production in these combined conditions and might lead to applications in nutrition, pharmacology and environmental medicine.

1.3 Research Hypotheses

1) Hypoxic vasodilation is a physiological response to low partial pressure of oxygen that increases blood supply to match metabolic demands (Totzeck et al. 2012). In contrary, cold-induced vasoconstriction (Stocks et al. 2012), a mechanism that protects body core temperature, hinders blood supply. According to these two opposite acute responses to environmental stress, it is hypothesized that exposure to both cold and hypoxic conditions simultaneously will trigger an increase in the rate of deep-body cooling due to increased hypoxia-induced vasodilation, leading to earlier onset of shivering thermogenesis than during exposure to cold alone. The compensable cold exposure paradigm (i.e., no change in body core temperature) should favor vasodilation.

2) As a consequence of early onset of shivering thermogenesis, substrate partitioning, that is the contribution of substrates to ATP production, will shift towards CHO sources to maximize energy production during low partial pressure of oxygen, induced by hypoxic condition.

1.4 Significance of the Study

Currently, a gap in this area of research exists, and a need for further research is present. It is with this project that we will begin to fill this gap, to gain a better understanding of substrate utilization when humans are exposed to both cold and hypoxia. Once we begin to better understand how the body's metabolic system responds during exposure to these conditions, we can then explore how to maximize the body's energy

efficiency during exposure. One practical application for this research would be a better understanding of human survival time when exposed to cold and hypoxia. How the body utilizes its fuel sources, and for how long will be a major contributor to survival during exposure. Once these factors are better understood, we can potentially better plan to optimize energy production during situations where the potential for exposure to this type of environment exists. This information may be very useful for select groups such as military, and mountaineers, who may find themselves in these situations.

Chapter 2: Review of Literature

2.1 Background

Human beings, as a species, are one of the most unique of all mammals on the planet. Our highly developed, and complex brain has allowed us to evolve, and unquestionably become the most dominant species. With our complex cognitive abilities, comes a series of traits that are unique to any other species. One trait that is only present in humans, is our willingness to subject ourselves to extreme environmental conditions for reasons other than survival. As a form of training and exercise, or for pure entertainment, humans will climb the highest peaks, and expose themselves to some of the harshest conditions imaginable. Two common conditions humans find themselves exposed to in their environment are cold, and hypoxia. Exposure to these two conditions will elicit changes in physiological processes (i.e., respiratory and circulatory systems) and as a result, will alter metabolic pathways (that is, contribution of substrate to energy metabolism). Various metabolic processes occur in the human body primarily for the purpose of energy production and storage. Exposure to extreme environmental conditions will cause changes in the body's energy requirements, and therefore will elicit changes in metabolic responses. How and why these changes occur has been a topic of interest for many researchers in nutrition, pharmacology and environmental medicine in recent history. This review will examine the literature dealing with the body's fuel selection processes that occur during environmental exposure to cold, hypoxia and the combination of both. More specifically, the main focus of this review will be on the utilization of lipid, carbohydrates, and protein

sources, as these are the three energy substrates involved in the body's energy producing processes. These substrates are oxidized to provide energy to cells within the body. As per the 1st law of thermodynamics, energy within the human metabolic system is transferred, primarily via ATP synthesis, and the storage of free energy produced by substrate oxidation (Bonora et al., 2012). Although the term "energy production" is not correct in a technical within human physiological processes, it has become synonymous with ATP synthesis, and therefore will be used throughout this paper. The majority of the literature examined will focus on the roles of lipids and carbohydrates, as they are responsible for the majority of the body's energy yielding processes. Protein, while not as extensively examined for this particular topic, remains an important metabolic substrate, and thus will be included. The intent of this review is to examine the metabolic effects caused by exposure to cold and hypoxia individually, as well as both conditions simultaneously.

2.2 Cold Exposure

Before discussing the effects of cold exposure on the body's metabolic processes, we must first understand the physiological responses that occur when humans are exposed to cold. Body temperature is controlled through a fine balance of heat accumulation and dissipation, designed to regulate core body temperature at an optimal level for physiological function. This optimal level falls within a very narrow range of 35° - 40°Celsius, with normal body temperature considered to be ~37° Celsius (Stocks, Taylor, Tipton & Greenleaf, 2004). As per the clinical definition, hypothermia is said to occur when core body temperature is decreased to 35°Celsius (Pozos & Danzl, 2001). However, this is said to only be a rough estimate, as variances between individuals exists. Hypothermia occurs

in humans in two stages: primary, and secondary (Pozos & Danzl, 2001). Primary hypothermia occurs when normal thermoregulatory responses are ineffective in maintaining core body temperature. The cold exposure will eventually overcome the body's physiological defense to cold, and hypothermia will begin. Secondary hypothermia occurs when physiological mechanisms are altered due to illness or fatigue, which leads to impaired thermoregulation. Secondary hypothermia is more dangerous than primary, as the consequences are more severe with a decreased chance of survival (Pozos & Danzl, 2001). While this review does not focus on literature involving secondary hypothermia, it is important to have an understanding of the potential impacts of cold exposure when dealing with humans.

As with all organisms, the human body is bound to the laws of thermodynamics. Heat, is energy in transfer and it flows from an area of high to low energy, in an attempt to establish an equilibrium. Therefore, when a human is placed in a cold environment, where the surrounding temperature of the system is colder than skin temperature, the body will lose heat to its surroundings. Alternatively, when a human is placed in a warm environment, the inverse will occur. However, unlike some organisms, the human body has physiological mechanisms in place to resist major fluctuations in core body temperature. These physiological mechanisms are designed to maintain optimal body temperature for the body's physiological processes to function.

For the purpose of this review, we will be examining three different forms of cold exposure: air, water and exposure through a Liquid Conditioned Suit (LCS). When the human body is exposed to a cold environment, there is a cascade of events that are triggered

in response. The initial response to cold exposure is vasoconstriction, with cutaneous vasoconstriction, being the most prominent response to cold exposure with regards to circulation (Flouris, Westwood, Mekjavic & Cheung, 2008).Vasoconstriction causes a constriction of the blood vessels to decrease peripheral blood flow, and redistribute blood towards the core and vital organs. Vasoconstriction is an autonomic response triggered in part by cutaneous cold receptor afferences coming from the region of the body that is being cooled. It is also controlled in part by the cold blood returning from the peripheral circulation to the central core, stimulating the temperature regulating center in the preoptic area of hypothalamus (Morrison, 2016; Sawasaki, Iwase & Mano, 2001). As long as body temperature can be regulated through this method, the sympathetic nervous system's control of vascular tone will predominate over the body's other regulatory responses to cold exposure (Sawasaki, Iwase & Mano, 2001). As heat loss continues, shivering will begin as a means of increasing heat production, to prevent a dramatic drop in core temperature (Stocks, et al., 2004). Shivering is involuntary contractions, or "shaking" of skeletal muscles, designed to generate heat. The intensity of shivering that will occur is dependent on several other factors. Initial low intensity shivering is elicited by the peripheral thermoreceptors as skin temperature declines. If heat loss exceeds heat generated through low intensity shivering, this will lead to a decrease in core body temperature, which will in turn induce an increase in shivering intensity (Stocks et al., 2004). The body's thermogenic response will depend greatly on whether the cold exposure is compensable or uncompensable (Haman & Blondin, 2017). If an increase in shivering intensity and subsequent endogenous heat production can combat surface heat loss and maintain core temperature, the cold exposure is said to be compensable (Haman & Blondin, 2017).

During compensable cold exposure, there will be a measurable decrease in skin temperature resulting from surface heat loss, however core temperature will remain stable. When the body experiences uncompensable cold exposure, surface heat loss surpasses endogenous heat production and will lead to a decrease in both skin and core temperature. As a decrease in core temperature can be detrimental to the body's overall function, the body will reach maximum shivering intensity in a final effort to prevent further heat loss (Haman & Blondin, 2017). For the purpose of this review, and our experimental design, we will focus mainly on compensable cold exposure, and the metabolic response these conditions will elicit.

The effect of cold exposure and fuel utilization has been the topic of many studies over the years (Stocks et al., 2004). The interest surrounding this topic stems from the more general desire to understand what metabolic changes will occur in response to cold exposure (Stocks et al., 2004). A better understanding of the metabolic responses, and fuel selection, will give great insight to human survival during prolonged cold exposure. From this, we can determine if there are factors such as diet and substrate availability, that can be manipulated to optimize the metabolic processes and improve survival.

Shivering thermogenesis is an increased metabolic rate, with fuel sources varying depending on additional factors such as diet and substrate availability. The general assumption with regards to fuel selection during shivering, is that shivering thermogenesis is mainly fueled by CHO and lipids, with minimal contributions from proteins (Haman 2006). However, in the past, there has been some debate as to which substrate plays a greater role in fueling shivering thermogenesis. For example, Vallerand and Jacobs (1989)

examined substrate utilization rates during acute cold air exposure of fasted men. The results from their experiment showed energy production more than doubled during the cold trials when compared to the warm control trials. From this increase in energy expenditure, there was a 588% increase in CHO oxidation, 63% increase in lipid oxidation, while no change occurred with protein contribution. They also calculated that 51% of the total energy production for thermogenesis during cold exposure was fueled from CHO sources. From these results, the researchers concluded that carbohydrates played a much larger role in fueling thermogenesis during cold exposure than lipids. Alternatively, Weller, Greenhaff & Macdonald (1998) reported opposing findings, as they found that an increase in fat oxidation was responsible for fueling shivering thermogenesis. Fasted men were cooled for 90 minutes using an LCS, for approximately two hours following an exhaustive exercise protocol. Results from the trials showed an increase in heat production, and fat oxidation, with no changes in CHO oxidation from baseline values. At first read, it may seem that these two studies reported conflicting results. Vallerand and Jacobs (1989) found carbohydrates as the main thermogenic fuel source during cold exposure, while Weller, Greenhaff & Macdonald (1998) reported an increase in fat oxidation was the main fuel source for shivering thermogenesis. While both studies used fasted males as their subjects, Weller, Greenhaff & Macdonald (1998) also included an exercise protocol prior to exposure as an additional variable. These two studies are a perfect example of the multitude of factors at play in determining substrate utilization during cold exposure, and how the manipulation of individual variables can generate opposing conclusions. Determining which fuel source will be more predominant in heat production is largely dependent on substrate availability, as well as shivering intensity.

Variations in available substrate levels will affect fuel selection during shivering thermogenesis. Haman, Leagault, Rakobowchuck, Ducharme & Weber (2004) questioned what effect circulatory glucose availability during sustained shivering would have on the utilization of other fuel sources with respect to heat production. For this study, participants were exposed to cold for two hours using a LCS under two experimental conditions. The first condition had participants follow a low CHO diet, with heavy exercise, in an attempt to lower CHO stores; while the second condition had participants follow a diet high in CHO with no exercise. In order to isolate the contribution of circulatory glucose from glycogen stores throughout the body, they employed stable isotope tracer methods. Participants ingested a glucose compound (U-13C) prior to exposure, and every 30 minutes throughout the experiment. The isotopic composition of plasma glucose was isolated using double-bed ion exchange chromatography by taking samples of expired gas throughout exposure. This method was used to differentiate between oxidation of exogenous glucose, and glucose from glycogen stores. Plasma glucose and lactate concentrations were measured via blood sampling and were measured using spectrophotometric techniques post-acquisition. Oxidation of glucose from glycogen stores was determined directly through both indirect calorimetric methods, and the lactate shuttle and was subtracted from the oxidation of plasma glucose to isolate the true value of glycogen utilization. Indirect respiratory calorimetry was used before and during the exposure to cold to measure alterations in heat production and the individual contributions of substrates to heat production. CHO stores manipulation did not have any effect on heat production but revealed a fuel dependent effect. In the high CHO trials, CHO oxidation accounted for 65% of heat production, but fell to 27% when CHO was depleted. The loss of contribution from CHO towards heat

production in the low CHO trials was largely compensated through an increase in lipid oxidation of 53%, compared to 23% in the high CHO trials. In addition, a minor increase 19% in protein oxidation was observed in the low CHO trials, compared to 12% in the high CHO trials. These results show the body's ability to utilize multiple fuel sources to ensure heat production is not comprised despite the reduced level of other substrates. This information is very valuable to researchers who are interested in strategies to prolong the survival of individuals exposed to cold for extended periods.

In addition to indirect calorimetry, EMG activity of shivering muscles was also measured during the experiment presented in the previous study. Results from the EMG measurements were presented in a companion study (Haman, Leagault & Weber, 2004). The purpose for including EMG measurements was to determine how shivering activity is affected when changes occur in substrate utilization. EMG activity was measured at eight of the major muscles that are responsible for the bulk of shivering muscles. The results from the EMG measurements indicated that despite the alterations in fuel selection that was shown between high and low CHO availability, there was no alteration in the EMG activity of the shivering muscles. Additionally, burst shivering, which makes up approximately 10% of total shivering activity, remained the same between the low and high CHO trials. From both the indirect calorimetric and EMG measurements, the researchers were able to conclude that by using mixtures of varying fuels sources within the same muscle fibres, humans are able to maintain thermogenesis during low-intensity shivering.

As important a role substrate availability plays in determining substrate utilization during shivering thermogenesis, shivering intensity is also a major contributor to

determining fuel selection. The effect of low intensity shivering was explored by Haman et al. (2002) where the researchers examined the utilization of muscle glycogen, plasma glucose, and lipids during cold exposure in order to quantify the respective contributions of each substrate to heat production. As with the aforementioned study (Haman, et al, 2004a) stable isotope tracer methods (U-13C), and blood sample analysis were used to distinguish oxidation of exogenous glucose and endogenous glycogen sources. Six men underwent acute cold exposure for a two-hour period via a LCS, while indirect calorimetric methods were used to determine oxidation rates of individual substrates. During the cold exposure protocol, there was an increase in heat production by 2.6 times the normal rate. Oxidation rates of plasma glucose, muscle glycogen, and lipids increased by 138%, 109%, and 376% respectively. However, despite a major increase in the oxidation rates of all three substrates, their contribution to heat production did not follow the same trend. Results showed that during low-intensity shivering, lipids were responsible for 50% of heat generated, followed by muscle glycogen (30%), and plasma glucose and proteins only contributing 10% each. From these values, the researchers were able to conclude that lipids are the predominant fuel source contributing to heat production during low-intensity shivering.

While lipid sources are used primarily for shivering thermogenesis at low intensity, a switch in preferred fuel will occur with an increase in shivering intensity. Haman et al. (2004a) examined fuel selection during high intensity shivering, as well as exploring any potential link with muscle activity. In this study, the group investigated if EMG pattern could provide insight into fuel selection during intense shivering. During the experimental

protocol, participants were exposed to cold for 90 minutes via LCS, with simultaneous recording of EMG activity, metabolic rate and substrate oxidation rates. While the changes measured in metabolic rates were similar for all the participants, the contribution from lipid and carbohydrate oxidation rates varied between participants. Total heat production from CHO sources ranged from 33 to 78% and from 14 to 60% for lipids. The researchers believe that the variance in fuel selection between participants was not caused by changes in metabolic rates, instead, resulted from differences in burst shivering rates. Burst shivering is described as short bursts of high intensity shivering during cold exposure, used to optimize heat production. Burst shivering relies on activation of type II muscle fibers, which contain high glycolytic capacity, and utilize carbohydrates as their main fuel source. Because of their reliance on substrate level phosphorylation, type II fibers will fatigue quickly (Fitts, 1994). Alternatively, type I muscle fibers, which are activated during low intensity shivering, are oxidative fibres and more reliant on lipid sources. Due to their characteristics, they are better suited for prolonged exercise and activity (Fitts, 1994). As a result, the researchers postulated that fiber type recruitment plays an integral role in determining fuel selection during high intensity shivering.

While the resulting conclusions from this study Haman et al. (2004a) may hold true for high intensity shivering, the role of fiber type recruitment on fuel selection may differ during sustained shivering. Haman et al. (2004b) in a separate study examined fuel selection during sustained shivering, while controlling for carbohydrate availability. Based on previous research, they hypothesized that any changes in fuel selection during sustained shivering would be achieved through recruitment of different fiber types. Participants in

this study underwent cold exposure via LCS under two separate conditions; 1) low glycogen as a result of low-carb diet and exercise, and 2) high glycogen induced by high-carb diet and no exercise. The two groups, with differing state of glycogen stores or reserves were used to determine any differences when substrate availability is altered. Contrary to their original hypothesis, there were no changes in fiber type recruitment between the low and high glycogen groups. Burst shivering was measured, and only represented 10% of the total shivering activity; therefore, the majority of the heat generated was through type I fiber activity. This led to conclusion that changes in fuel selection may occur within the same muscle fibers, and that other mechanisms may play a role in the selection of fuel source for shivering. The main factor to be considered is fuel availability, specifically glycogen reserves, as this was manipulated in the study. Because it has been shown that fuel selection between fiber types, and individual fiber type recruitment varies during different intensities, it is likely that alternate factors and mechanisms, such as shivering time and intensity must be present.

Differences in fuel selection during high and low intensity shivering has been shown in the previous studies. High intensity shivering has been shown to be dependent on recruitment of type II fibers during burst shivering, while low intensity shivering triggers oxidation of different fuel sources within the same muscle fibers. A further understanding of both shivering intensities was provided in the findings of Haman, Scott & Kenny (2007). The design of their study involved a rewarming protocol, where participants were submerged and then removed from a cold-water bath. The participants' EMG and metabolic activity were then monitored as they rewarmed to normal core body temperature. Initially,

as the participants were cooled, shivering intensity was much higher in reference to peak shivering intensity. Fuel selection during the period of high intensity shivering was shown to favour CHO for heat production, as a result of burst shivering and type II fiber recruitment. As core body temperature increased, shivering intensity began to decrease, leading to a switch from type II to type I fibers, as well as a switch from CHO to lipid dominance in fueling heat production. It is interesting to note that the crossover point from CHO to lipid dominance occurred around 50% of peak shivering intensity. From this, we can infer that high intensity shivering can be classified as higher than 50% of peak shivering, and low intensity below 50%. During the rewarming phase, CHO oxidation rate decreased 10-fold, while lipid oxidation rate remained constant throughout. In comparison, findings from Haman et al. (2005) stated that lipid oxidation rates were shown to never exceed ~165 $mg \cdot min^{-1}$ regardless of increase in shivering intensity. From the information presented in both studies, it can be concluded that maximum lipid oxidation is achieved at low intensity shivering, and any increase in substrate utilization is controlled by alterations in the rate of CHO oxidation. This conclusion is supported by the findings from Haman et al. (2004a) which stated that "differences in burst shivering were directly related to CHO oxidation rate". It would seem that the rate of CHO oxidation is a major determinant of overall substrate utilization for heat production during shivering thermogenesis.

The body's ability to fluctuate CHO oxidation rate throughout exposure to cold, is believed to be a mechanism designed to protect CHO stores, and ultimately prolong survival. By sustaining low intensity shivering during prolonged cold exposure, the body will rely heavily on the large lipid stores, while sparing CHO reserves. Recent findings

have suggested that during cold air exposure, switching fuel sources from CHO to lipids during long term shivering can extend the time to muscle glycogen depletion as much as fifteen days, instead of the original prediction of 30-40 hours (Haman et al., 2016). One major question this study posed, as mentioned by the research team, is what impact would modification of nutritional conditions have on survival time? Previous research has suggested that modification of CHO reserves will impact fuel selection during shivering. Individuals with high glycogen reserves due to their diet, have been shown to favour CHO as a main fuel source during shivering thermogenesis. Alternatively, individuals with low glycogen reserves from low carb diets would seem to favour lipid sources during shivering (Haman, 2006). These results are consistent with the belief that fuel selection during shivering will adjust to preserve glycogen reserves. The question we are then left with, will modifications to diet and glycogen loading protocols impact long term shivering thermogenesis? It will be imperative in the future to investigate this question, to determine if modifications in diet can help prolong survival time. This information would be useful for certain populations who may find themselves exposed to cold for extended periods. Such populations could include military personnel, as well as workers in offshore and arctic environments. Individuals who work in these areas may find themselves in a situation where they are exposed to cold for an extended period, whether purposefully or accidentally. Any knowledge we can bring to these populations that can prolong survival time would be of great benefit to everyone involved.

In addition to cold exposure, there are other environmental conditions that exist in our world affecting the metabolic processes within the human body. On other such

condition that humans may encounter is a reduction of alveoli oxygen partial pressure , or "hypoxia", which we will explore next.

2.3 Hypoxia

As with cold exposure, a brief discussion on hypoxia, and the body's response to hypoxic exposure is essential before delving into specific effects on substrate utilization and energy production. Hypoxia occurs in humans when the body is deprived of sufficient oxygen to properly supply body tissues (Ostergaard & Gassmann, 2016). Hypoxia exposure can occur in one of two environmental conditions. The most common form of hypoxic exposure occurs at altitude, which is known as hypobaric hypoxia (HH). Contrary to popular belief, the oxygen content of air does not decrease with an increase in altitude. Rather, the partial pressure of oxygen (PO_2), along with atmospheric pressure and air density all decrease with an increase in altitude (Ostergarrd & Gassman 2016). The reductions to partial pressure of oxygen, atmospheric pressure and air density will result in an insufficient transfer of oxygen from the lungs to the bloodstream. Consequently, this will lead to less than favourable levels of oxygen for all of the tissues and cells found within the body. The second mechanism of hypoxic exposure, known as normobaric hypoxia (NH), does involve a reduction in the oxygen content of the air (Ostergarrd & Gassman 2016). However, this type of environment is not commonly present in the real-world and is typically created in a laboratory setting. NH is achieved one of two ways. The first method, shown in Basset et al., (2006) involves placing participants in an enclosed chamber with a continuous flow of hypoxic air in order to reduce the percentage of O_2 they will inspire. The second method, as used by Kelly and Basset (2017) requires participants to be

fixed with a breathing apparatus that provides them with a gas mixture containing a reduced level of oxygen saturation.

Whether or not variations in physiological response will occur between NH and HH exposure is debated within the literature. An increase in breathing frequency, and a reduction in both tidal volume and minute ventilation was shown in HH when compared to NH (Savourey, Launay, Besnard, Guinet & Travers, 2003).These differences are present even when PO_2 is the same and have been attributed to an increase of dead space ventilation resulting from a reduction in barometric pressure. This phenomenon has been termed "the specific response to hypobaric hypoxia" (Savourey et al., 2003). These findings are supported in the review by Millet, Faiss, & Pialoux (2012), who report that ventilation is lower in HH than NH, with a lower tidal volume and a higher respiratory frequency. They also agree that HH might induce a higher alveolar physiological dead space (Millet, Faiss & Pialoux, 2012). In a counterargument, Mounier, & Brugniaux (2012) argues that the physiological responses elicited by NH and HH are equivalent and "no robust hypothesis could reasonably be proposed to explain the putative physiological differences between these two modalities of hypoxia". One argument they present is with regards to red blood cell production, as initiated by secretion of erythropoietin (Epo). They report from their review that the increase in Epo is of similar magnitude in response to hypobaric or normobaric hypoxia. These opposing viewpoints highlight the complexity of metabolic response to hypoxic exposure.

Much like most metabolic processes, the body's ability to maintain oxygen homeostasis is crucial for survival. When the body is exposed to insufficient oxygen partial

pressure, physiological responses must occur to optimize oxygen supply and utilization within the body's tissues. The body's acute response to HH is outlined in Hochachka, & Somero (2002). Firstly, hypoxic ventilatory response is initiated, which is an increase in ventilation to compensate for oxygen shortage. Next, a constriction of the pulmonary vasculature will occur. This response is designed to increase the transfer of oxygenated blood to deprived tissues, while pulling the blood away from areas of poor ventilation. Metabolic responses involving the attenuation of oxidative and glycolytic pathways has also been shown to occur (Hochachka, & Somero 2002). A switch from oxygen-dependent respiration, to oxygen-independent glycolysis, also known as the "Pasteur effect", will occur within the cells. The switch in metabolic processes is regulated by the activation of adenosine monophosphate, (AMP), activated protein kinase, (AMPK), which is responsible for upregulating adenosine triphosphate, (ATP), generating pathways, and downregulating pathways consuming ATP. AMPK activation will stimulate translocation of GLUT-4 to the plasma membrane, increasing glucose uptake, while inhibiting fatty acid and triglyceride synthesis (Michiels, 2004). The shift towards glucose as primary fuel source may seem counterintuitive, as the absolute production of ATP will be lower than with other substrates. However, in terms of oxygen consumption, glucose is the more efficient fuel source, producing 6-6.3 mol of ATP per mol of O_2, compared to 5.6 mol of ATP from lipid (Lundby & Hall 2002). As oxygen preservation becomes paramount, it would seem that the body chooses to switch metabolic processes to utilize the most oxygen efficient fuel source.

The idea that hypoxic exposure will lead to an increase in CHO utilization was explored by Roberts et al. (1996). The research group examined the effects of CHO

metabolism at rest and during exercise while exposed to acute HH. As shown by Brooks et al. (1991) exposure to altitude will activate the sympathoadrenal system, which controls the release of epinephrine, and consequently increase reliance on CHO fuel sources. To evaluate the role of epinephrine in glucoregulation, the researchers decided to administer a β-blockade, which is designed to block receptor sites for epinephrine, to one group of participants. Results from the control group were compared to β-blocked participants, to determine if a β-blockade would mediate the effects of any increase in CHO utilization. Participants were all given standardized diets a week before their exposure, mimicking the diet that would maintain their weight at sea level. Participants were transported to an altitude of 4300 m, during which they were breathing 100% O_2 from an oxygen tank until they reached their destination. During acute exposure, rate of appearance of glucose was significantly higher at rest, and during exercise when compared to control measurements at sea level. During the exercise protocol, glucose uptake in the leg increased, with an 80% rate of disappearance for the control group, and 97% for the β-blocked group. After analyzing the results from the study, it was concluded that acute altitude exposure does in fact increase glucose utilization during rest and exercise. Additionally, despite the researcher's initial belief, the β-blockade caused an even greater increase in glucose utilization, rather than mitigating the response. In their final remarks, the research group added their belief that the shift towards glucose sources will occur during acute hypoxia, is a result of glucose being the more O_2 efficient fuel source. This assertion concurs with the previously mentioned studies, (Lundby and Hall 2002; Michiels 2004), which all agree the switch in substrates to be advantageous in response to decreased O_2. An increase in CHO utilization during acute hypoxic exposure is supported by Clanton, Hogan & Gladden

(2013). Their review outlines a shift in metabolic substrate usage as a response for dealing with lowered levels of oxygen. As stated, an elevation of glucose transport, along with a shift away from fatty acid oxidation towards glucose oxidation has shown to be the body's response to acute hypoxic exposure. Once again, this review supports the notion that the shift towards glucose utilization represents the body's need for oxygen efficiency. Utilization of glucose sources has shown a ~14% advantage in ATP/mole over lipid sources (Clanton, et al., 2013).

In addition to affecting fuel selection, acute hypoxic exposure has also been shown to alter the body's energy production. During hypoxic exposure, infant humans have been shown to have a decreased metabolic rate, which is a common strategy of many animal species. However, adult humans do not utilize this strategy of decreased metabolic rate with hypoxic exposure, which is similar to many larger animal species (Mortola, 2004). In order to explore metabolic rate during hypoxia, Butterfield et al. (1992) measured BMR of seven men taken to an altitude of 4300 m. BMR was collected during initial exposure, at day two, as well as throughout their 21-day stay. These measurements were then compared to control measurements collected at sea level prior to hypoxic exposure. BMR measurements at day two indicated a 27% increase from the control values. An increase in energy expenditure was also reported by Workman and Basset (2012). Their study was designed to examine the effects of passive NH exposure, in both acute and short-term conditions, on sedentary overweight men. Acute exposure consisted of a single 3-hour session exposed to the NH gas mixture, while short-term exposure consisted of a total of seven sessions exposed to the same conditions. For the purpose of this review, the focus of the results will be on the

acute group. A significant increase in energy expenditure of 16% was observed in the acute group, compared to 12.5% in the control group during the 3-hour session. Fuel utilization response showed a significant decrease in glucose oxidation by 31% in the acute group, with an increase of 35% in the control group. Fat oxidation was found to increase significantly in the acute group by 44%, compared to a 4% decrease in the control group. Workman and Basset (2012) concluded that passive acute normobaric hypoxic exposure will impact energy expenditure and substrate utilization in sedentary overweight men.

Alternatively, Oltmanns et al. (2006) found that a decrease in resting energy expenditure (REE) occurred after acute hypoxic exposure relative to normoxic conditions. In their study, thirteen men underwent 30 minutes of hypoxic exposure. Hypoxia was induced by reducing hemoglobin oxygen saturation from 96% to 75%) after which REE was measured for 150 minutes. REE measurements from hypoxic exposure were compared to a normoxic control condition at a minimum of four weeks later. Results from the hypoxic condition showed a decrease in REE after acute exposure, from 1656 ± 80 to 1564 ± 97 $kcal \cdot day^{-1}$, compared to a rise in REE during the normoxic control condition from 1700 ± 82 to 1749 ±79 $kcal \cdot day^{-1}$. These data would indicate the body's acclimation process of lowering energy production when oxygen supply is limited. One explanation for this decrease in energy production can be explained by the increase in efficiency of oxidative phosphorylation when there is a decrease in oxygen availability (Gnaiger, Mendez & Hand, 2000). *In vitro* measurements of mitochondria suggest that when oxygen partial pressures are low, there is a depression of both proton leak and uncoupled respiration are depressed (Stuart, Brindle, Harper & Brand, 1999). As proton leak can be responsible for up to 20%

of standard metabolic rate in rats, limiting this waste would prove to be advantageous towards metabolic efficiency. However, these results have yet to have been replicated in human participants but remains as a possible explanation for the decreased REE that was measured during hypoxia.

2.4 Hypoxia and Cold

As shown in this review, it has been well documented that exposure to both hypoxic and cold environments will have an impact on human metabolic processes, causing a shift in substrate utilization towards differing fuel sources. However, presently there is limited literature present examining the effects of exposure to cold and hypoxia simultaneously. From the previous discussion on cold and hypoxic exposure, it is evident that combined exposure to both conditions will create a compromising situation for the body. It is evident that the body's metabolic processes will rely on the large lipid stores to preserve CHO during low intensity shivering to maintain heat production. Alternatively, a decrease in oxygen levels may lead to a shift towards processes involving CHO sources, which is the more oxygen efficient of the two substrates.

Past studies have been conducted on hypoxic and cold exposure using animals. During exposure to cold air and NH, mice and dogs have been shown to experience a loss in temperature control, and experience a decrease in body temperature (Kottke, Phalen, Taylor, Visscher & Evans, 1948). Additionally, hypoxia was shown to inhibit shivering in the animals when exposed to cold. Rats were also shown to experience a decrease in

shivering and non-shivering thermogenesis, as well as a decrease in body temperature when exposed to cold and hypoxia, (Gautier, Bonora, M'Barek & Sinclair, 1991; Cadena & Tattersall, 2014). It is reasonable to speculate that the addition of hypoxia during exposure to cold in humans will affect body temperature control by inhibiting thermogenesis. By limiting the body's natural thermogenic responses, there will be less reliance on lipid sources to fuel shivering. It is possible that this response occurs in order to shift metabolic process towards oxygen efficient CHO sources.

While animal studies seem to generate similar conclusions, human studies have produced mixed results thus far. In addition to mice and dogs Kottke et al. (1948) also utilized an unlisted number of human participants in their NH experiments. As with the animals, humans were also shown to have inhibited shivering, loss of body temperature control and an increase in heat loss from the skin with NH. Results from this study would indicate animals and humans will have similar metabolic response during cold and hypoxia, however, more recent studies would suggest this is not the case. Robinson and Haymes (1990) examined the effects of NH and cold both separately and together, during rest and at exercise. Participants were exposed to four different conditions (normoxia + neutral temperature, normoxia + cold, NH + neutral temperature & NH + cold) to determine if any changes in metabolism would occur. Hypoxia was achieved by breathing 12% O_2, while the cold environment was produced using cold air with a temperature of 8°C. Exercise intensity was set at 50% HR reserve, in order to allow participants to complete 30 minutes of exercise while breathing 12% O_2. Robinson and Haymes (1990) reported increases in cardiac work and energy requirements. This was a result of increased cardiac output from

NH exposure, combined with increased peripheral resistance from vasoconstriction response to cold exposure. An increased reliance on shivering at rest was observed and was believed to occur as result of the inhibition of non-shivering thermogenesis. During exercise, heat loss was accelerated, and level of perceived exertion increased when compared to control trials. Increases in shivering during exposure to cold and NH was also shown in the work by Reading, Robert, Hogdon & Pozos (1996). Eight men and two women participants were subjected over two hours to NH of 15% O_2, and a cold air exposure of 4.4°C. During the exposure, an increase in shivering was measured, despite the reduction in inspired O_2. However, the increase in shivering was not sufficient to combat heat loss, and a decrease in core temperature was measured throughout the duration of the trials. When compared to animal studies, the results from these two studies are, in fact, opposite. However, as mentioned previously, the results of human trials have been mixed, resulting in a need for further analysis. We hypothesize that increased shivering activity elicited by exposure to cold and hypoxia will cause energy production to favour CHO sources, as the most efficient fuel source.

Kottke et al. (1948) reported that exposure to hypoxia resulted in a decrease in shivering in their experiment. In addition, there was no measured increase in skin temperature, which would suggest shivering thermogenesis was able to compensate for heat lost during cold exposure. These results would conflict with findings of Lim and Luft (1962) who examined the effects of cold air exposure on six participants at an altitude of 5300m, with results that conflicted with findings from the previously cited research. One major finding they reported was an insignificant effect of hypoxia on shivering

thermogenesis, which was indicated by unchanged O_2 uptake and heat production. Kottke's claims that heat loss at the skin increases with hypoxic exposure are also challenged by Cipriano and Goldman (1975). Six unclothed men underwent exposure to three air temperatures of 15.5°C, 21°C and 26.5°C at three different altitudes of sea level, 2500m and 5000m. Rectal temperatures for the participants were shown to be lower at altitude than at sea level. In addition, mean skin temperature was still shown to decrease, but not as much at altitude when compared to exposure at sea level. However, Cipriano and Goldman (1975) reported that these changes do not indicate increased heat loss as a result of hypoxia. There were no measured increases in heat production, as well as no loss in body heat storage. This would lead to the assumption that the cold exposure in their study was compensable by the body's thermoregulatory response.

The effect of HH on thermoregulatory response was examined by Blatteis and Lutherer (1976). Twelve unacclimated participants were exposed to cold air in temperature-controlled rooms at three different altitudes in the Central Andes Mountains of Peru of 150m, 3350m and 4360m, along with five individuals native to each altitude. All participants were also tested at sea level for control purposes. Acute exposure, upon arrival at each altitude level was tested, along with an examination of the changes that will occur as a result of chronic exposure for six weeks. Results for response to acute and chronic exposure yielded several interesting findings. The participants were not able to attain the same resting metabolic rate at altitude as they could at sea level, which is not surprising considering the impact of altitude on oxygen delivery. The metabolic rate did not recover during chronic exposure but was restored to normal levels once participants returned to sea

level. Shivering was present during cold exposure at all altitudes and seemed to progress to more vigorous levels with an increase in altitude. Increased shivering intensity was evident throughout the entire six-week exposure, but the rates of shivering decreased once the participants returned to sea level the rates decreased. Rectal temperature decreased at all altitudes, while skin temperature fell rapidly during initial exposure, then stabilized. From these results, the researchers concluded that exposure to altitude will reduce human calorigenic response to cold, which was indicated by a decrease in metabolic rate. However, because there was a visible increase in shivering, despite lower thermogenic response, it is believed that there was a reduction in non-shivering thermogenesis.

Similarly, Johnston et al. (1996) determined that acute NH exposure was able to lower thermoregulatory responses, which leads to acceleration of core cooling. Participants were tested on two separate days, with a normoxic control condition, and a NH trial in which participants inspired 12% O_2. An important note for this study was that participants completed a bout of exercise on a recumbent bike, in order to increase core temperature and elicit sweating. Participants then cooled passively while sitting in water, breathing either normal room air for control, or a gas mixture containing 12% O_2 for the NH condition. Variations in core cooling between the normoxic and NH conditions was of interest with respect to our experimental design. Results from the trials showed an increase of core cooling rate by 33% during the NH trials, ($1.83 \pm 0.72°C \cdot h^{-1}$), when compared to the control condition, ($1.38 \pm 0.85°C \cdot h^{-1}$). Fifty minutes post exercise, T_{es} was 0.44 ± 0.3 °C lower in the hypoxic condition ($p < 0.05$). The researchers suggested several possible reasons for increased core cooling during NH exposure. Hypoxia may limit O_2 supply to

organs responsible for shivering thermogenesis or may lead to a decreased efferent output to these organs. However, the authors state that these possible mechanisms are likely not responsible for the increase in core cooling that was measured. Alternative possibilities which were considered to be more likely by the authors include increased heat loss from respiration, along with an increased cardiac output and blood flow to the peripheries.

While the aforementioned studies lay the groundwork for research on the combined effects of cold and hypoxia, there is still much work to be done. Measurements of substrate utilization during exposure to cold and hypoxia are lacking thus far, even though its implication in survival strategies in altitude and its applications in nutrition and pharmacology need to be addressed. Previous research on exposure to both stressors individually have determined CHO sources will be favoured during acute hypoxic exposure, while lipid sources are favoured during cold exposure. The question then remains: what response will occur with regards to substrate utilization when both occur simultaneously? One theory could be that the addition of cold during hypoxic exposure will lead to a reliance on CHO utilization for its oxygen efficiency. However, until significant results are produced involving the measurement of substrate utilization, we can only speculate the physiological responses during both cold and hypoxia.

2.5 Conclusion

As discussed, there has been ongoing research with regard to metabolic response to both hypoxic and cold exposure. However, what occurs with substrate utilization when

acutely exposed to both conditions is yet to be fully explored. It is important that research in this area is conducted, as it will further our understanding of both conditions as well as creating new discoveries. The premise of this study was to begin bridging the current gap in the literature, by examining the cumulative effects of exposure to cold and hypoxia on the body's various metabolic responses. The major interest for the research team was the effects of exposure on energy production, and more specifically the acute effects on substrate utilization. As shown in the previous literature, hypoxia and cold elicit very different responses with regards to substrate utilization. With the two response in direct competition for energy sources, it is hypothesized that substrate utilization will favour CHO sources, in order to maximize EP and help maintain body temperatures during hypoxia and cold exposure.

Chapter 3: Methodology

3.1 Participants

Eight healthy men individuals aged 18-50 years old were recruited for this study that consisted of a total of four sessions, including a familiarization session, and 3 experimental sessions. Ethical approval for this study was obtained from The Health Research Ethics Authority of Newfoundland & Labrador (HREA #2017.096). Participants were screened for eligibility during a familiarization session that occurred at least 48 hours prior to beginning the study. When all procedures were explained and understood, participants were asked to read and sign the informed consent form. During the familiarization session, participants were informed of all experimental procedures, and were asked to complete a Physical Activity Readiness Questionnaire (PAR-Q+), for the purpose of screening the participants for any medical conditions that would deem them ineligible for participation in physical activity, which would lead to their exclusion from the study. The participant's tolerance to breathing a nitrogen diluted normobaric hypoxic gas mixture at an arterial O_2 saturation (SpO_2) of 85 % was assessed during a 20-min hypoxic breathing tolerance test. Participants were excluded from the study if they experience increased physiological stress to breathing the hypoxic gas mixture, score greater than three on the Lake Louise Mountain Sickness Score (Appendix A), or reach a systolic blood pressure > 140 mmHg, or a diastolic blood pressure > 95 mmHg. In addition, height and weight were also recorded during the familiarization session.

3.2 Study Timeline

For all three experimental sessions, participants were asked to arrive at the lab at 7:00 a.m. (via public transportation or vehicle) at least 48 hours after the familiarization session. Participants were asked to fast for 12 hours and refrain from physical activity for 24 hours prior to arriving at the lab. The reason for these restrictions was to ensure accuracy in collection of BMR. Prior to beginning the session, participants were asked to void their bladder. Once participants were ready, their BMR was then collected for 30 minutes. Following completion of BMR, participants were prepped for MVC's, which included shaving and cleaning of skin sites with alcohol wipes, and placement of electrodes. Once prepped, participants completed three MVC's for each of the four muscle groups. Next, 11 Thermochron temperature loggers (DS1921G-F5#, iButtonLink, Whitewater WI, USA) used to record skin temperature, were secured with hypoallergenic tape to various sites on the participant's body (see section 3.5.2). Participants were then given instructions for self-insertion of the rectal thermocouple, used to record core temperature; a procedure done in the privacy of the washroom located in the lab. Once preparation was complete, the first urine sample was collected from participants. Participants were then placed seated into a sealed hypoxic chamber and exposed to one of the three experimental conditions between 9:00 and 11:00 a.m. A second urine sample was taken immediately after the experimental condition. Once the experimental sessions completed, researchers – after participants had a one-hour rewarming period – ensured participants had returned to their pre-testing state, prior to giving permission to leave the lab. The experimental sessions occurred on separate

days, with a minimum wash-out period of 48 hours between sessions to ensure no carry over effect occurred.

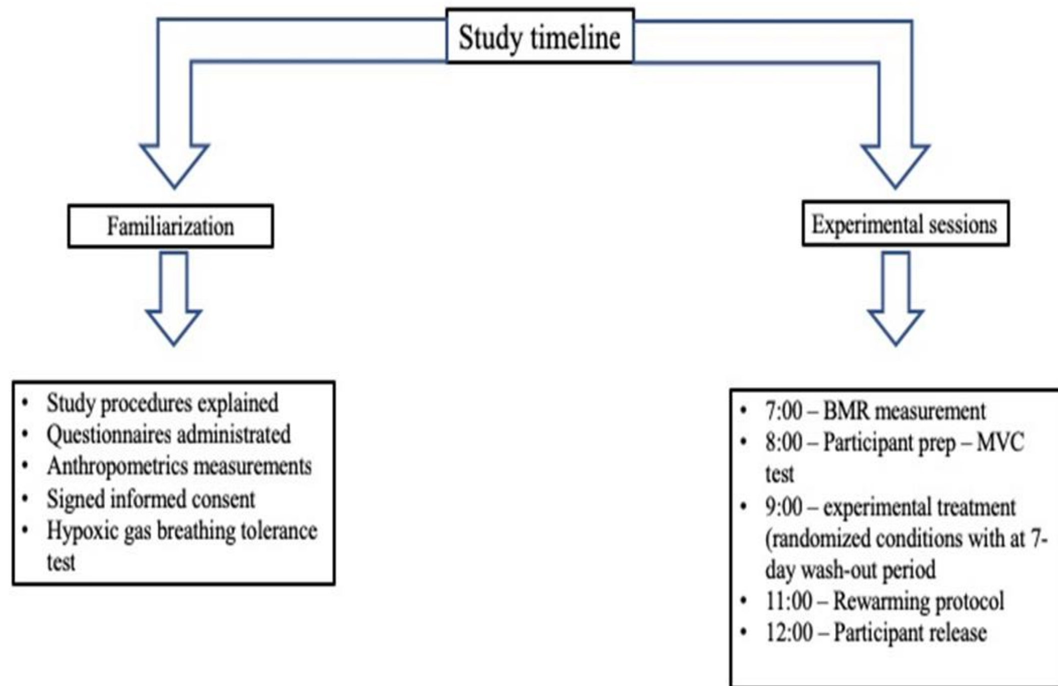

Figure 3-1: Study Timeline

3.3 Experimental Protocol

The experiment encompassed three five-hour experimental sessions of three conditions: hypoxic exposure (H), cold exposure (C), and combination of the two (CH). Prior to treatment, the participant's BMR was determined. The BMR was measured under a canopy for 30-min in a thermoneutral environment 22-24°C with dimmed lights. Participants were then randomly assigned to one of the three treatments of H, C, or CH.

The normobaric hypoxic condition (H) simulated moderate altitude of ~2500 meters above sea-level corresponding to a fraction of inspired oxygen (F_IO_2) of ~0.14 induced by a hypoxic air generator, (8850 SUMMIT 3in 1, Altitudetech, Ontario, Canada), continuously pumping 75 $L \cdot min^{-1}$ of hypoxic air into a 1350 L plethysmograph chamber (V62J Vmax Autobox, Sensormedics Yorba Linda CA, USA), modified for the purpose of the study. Gas concentrations were monitored by an oxygen sensor (Maxtech O_2+, Maxtech, Salt Lake City Utah, USA), to ensure (F_IO_2) was maintained at ~0.14. Researchers also monitored temperature, barometric pressure, and humidity in the chamber during the experimental sessions

The cold condition was induced by fitting the participants with a liquid conditioned suit (three-piece high density; Med-Eng Holdings, Inc., Ottawa, ON), flowing 10°C water from a circulation bath (custom built, Memorial University of Newfoundland, St. John's, NL). The water temperature was maintained at 10.0 ± 0.1°C while a water pump circulated the water at a rate of 1 $L \cdot min^{-1}$. Cold exposure trials were run under normal sea level conditions (F_IO_2 = 0.2093). The hypoxic and cold condition was induced by combining both exposure conditions listed above.

3.4 Data Collection

3.4.1 Anthropometrics

Anthropometrics were taken during the familiarization session. Height and weight, measurements were obtained from all participants. Height was measured using a stadiometer, (± 0.1 cm – Perspective Enterprises, Portage, Michigan, USA) and body

weight was measured using a weight scale, (± 50.00g or 0.02% of the maximum scale – LifeSource, model UC-321, A&D Company, Tokyo, Japan).

3.4.2 Metabolic (Respirometry)

Indirect calorimetry was used to measure metabolic rate and to determine energy production and substrate partitioning. The expired gas was collected using a mass flow generator and controller (FK-500 Flow Kit; Sable Systems International, Las Vegas, NV) with an airflow rate of 75 $L\cdot min^{-1}$. A sub-sample of expired air was pull at 150 $mL\cdot min^{-1}$ by a sub-sampler pump (SS-4; Sable Systems International) set in an excurrent fashion. The aliquot of air flowed into a water vapour analyser (RH-300; Sable Systems International), a carbon dioxide analyser (Model CA- 10; Sable Systems International) and a paramagnetic oxygen analyser (Model PA-10; Sable Systems International), which measured the fractional amount of these gases at 1 Hz. Fractions of gases in the room were recorded before and after each measurement for baseline references. Prior to testing, the oxygen and carbon dioxide analysers were calibrated with room air and reference gases of 100% nitrogen and 1% carbon dioxide gases. Water vapour pressure was zeroed by drying it by passing through a column of magnesium percolate and the sub-sampler pump was calibrated using a flow meter (Gilmont Rotameter). Additionally, to ensure accurate performance of both metabolic systems, a propane gas verification was performed with a gas mass flow meter prior to testing. The verification technique that was used followed the methods outlined by Ismail et al. (2019).

3.4.3 EMG

Shivering EMG signals were recorded from the skin surface overlying four muscles located on the left side of the body: *trapezius* (TR), *pectoralis major* (PE), *rectus abdominis* (RA), *rectus femoris* (RF). Wireless surface electrodes (Trigno IM Sensor, Delsys, Nantick Massachusetts, USA) were positioned over the bellies of each muscle and secured with transparent tape. The surface electrodes were connected using pre-amplified and grounded EMG wires to a signal amplifier at a sample rate of 1 kHz. Shivering activity of the four individual muscles were recorded for one minute, every five minutes throughout each exposure protocol. Voluntary muscle activity was minimized as much as possible throughout all conditions. Before treatment, MVC's were determined by producing three 10-sec maximal contractions in a standardized position for each of the muscle groups with a 30-sec rest interval for the purpose of determining shivering intensity post-data acquisition.

3.4.4 Core Temperature

Rectal temperature (T_{re}) was measured using a Philips 400 series thermistor (model 21090A, Philips Medical Systems; Andover, MA) that was participant-inserted 15 cm into the rectum. To minimize movement of the rectal temperature sensor, a t-bandage was used to secure the sensor once inserted. The probe is designed for insertion into oesophagus or rectum and made of an electrically insulated thermistor permanently secured within a PVC tube that provides atraumatic insertion. The accuracy of the thermistor is ±0.1°C from 25°C to 45°C.

3.4. 5 Ambient temperatures

The ambient air and water temperature of the testing environment were measured with 400 series thermistors (model 21090A, Philips Medical Systems; Andover, MA). The air temperature was measured inside the plethysmograph chamber. The water temperature of the tank was measured 1 meter below the water's surface.

3.4.6 Mean skin Temperature ($\overline{T}_{SK}$)

$\overline{T}_{SK}$ (°C) was measured using Thermochron temperature loggers (DS1921G-F5#, iButtonLink, Whitewater WI, USA) located at eleven (11) sites on the body, (forehead, chest, abdominals, forearm, anterior thigh, shin, upper back, lower back, buttocks, posterior thigh, calf), using a modified Hardy and Dubois 12-point system (Appendix B) (Hardy & DuBois, 1938). Thermochron temperature loggers has a temperature range of -40°C to +85°C, with an accuracy of ±1°C in the range of -30° to +70°C and an accuracy of ±1.3°C outside of that range. The resolution of this device is 0.5°C. It can also read up to 2048 values at a logging rate of 1 minute to 255 minutes (https://www.ibuttonlink.com/products/ds1921g).

3.4.7 Heart Rate

Heart rate (HR) values were recorded for the entirety of each session with a heart rate monitor (Suunto, model Ambit2, Suunto OY, Vantaa, Finland) and uploaded to MovesCount (www.movescount.com) and transferred to Igor Pro 6.3 (WaveMetrics Inc,

Lake Oswego, Ore, USA) for determination of HR changes during the three experimental conditions.

3.4.8 SpO_2

Blood oxygen saturation (S_PO_2) was recorded via a finger sensor, using a pulse oximeter (Model Rad-8, Massimo Corporation, Irvine, CA), to control for hypoxia-induced blood desaturation. The pulse oximeter was synced through U12 (Sable Systems International), and data were collected via Expedata-P (Acquisition System, Sable Systems International). S_PO_2 was used monitor metabolic stress induced by the experimental conditions. For instance, if S_PO_2 fell below 80%, the test was terminated.

3.4.9 Urine samples

Participants were required to provide urine samples throughout the study to determine amino acid (protein) oxidation through analysis of urinary urea nitrogen. All urine samples were kept frozen at -20°C until further analyses. Prior to analysis, however, samples were thawed once and were then aliquoted to avoid constant freezing and thawing. Urinary urea nitrogen was determined using an enzyme-based colorimetric assay (Bio Scientific Corporation, Cat. No. 5602-01, Austin, TX). The dilution factor used was 1:26 where 10 µl of urine was diluted in 250 µl of 0.9% sodium chloride (N° 04888, DIN 00037796). All assays were performed in duplicate.

3.5 Data Reduction and Analysis

Metabolic, thermal and blood saturation data were transferred to Igor Pro 6.3 (WaveMetrics Inc., Lake Oswego, Ore, USA) for further analyses. The area under the curve

(AUC) was calculated with the trapezoidal method over 30-min period throughout the 120-min experimental conditions.

3.5.1 EMG

Maximal activation for each muscle were calculated from MVC's, via the root mean squared (RMS) technique using a 50 ms moving window. All raw EMG data were filtered using a 20 Hz dual-pass, high-pass filter to remove any noise in the signal (De Luca, Gilmore, Kuznetsov, & Roy, 2010). Shivering activation throughout was measured in one-minute intervals, every five minutes throughout exposure. Shivering activity was then calculated using RMS and integrated EMG (iEMG) for the full one-minute duration. RMS values were calculated using a 50 ms moving window, and were amplitude normalized to the max activation from the MVC trials. iEMG was first normalized by taking the bias removed signal, and calculating the RMS using a 50 ms moving window. The normalized data were then integrated using the trapezoid rule, and the frame by frame iEMG was then summed over the entire one-minute duration to give a total iEMG value.

3.5.2 Skin Temperature

Mean skin temperature ($\overline{T}_{SK}$) was calculated using a modified Hardy Dubois 12-point system (Hardy & DuBois, 1938). Our protocol measured temperature from 11 body sites, excluding the foot, which is the 12th site in the Hardy & DuBois protocol. Using a similar technique as Ducharme and Brooks (1998) mean skin temperature was calculated using the following equation:

(eq.1) $\overline{T}_{SK} = (\overline{T}_1 \times 0.07 + \overline{T}_2 \times 0.088 + \overline{T}_3 \times 0.088 + \overline{T}_4 \times 0.14 + \overline{T}_5 \times 0.063 + \overline{T}_6 \times 0.065 + \overline{T}_7 \times 0.088 + \overline{T}_8 \times 0.088 + \overline{T}_9 \times 0.063 + \overline{T}_{10} \times 0.063\ \overline{T}_{11} \times 0.065)/0.88$

Table 3-1: Weighted Values for Skin Locations

Skin Location	**Weighted Value**
$\overline{T}_1$ = Forehead skin temperature	0.07
$\overline{T}_2$ = Chest skin temperature	0.088
$\overline{T}_3$ = Abdominal skin temperature	0.088
$\overline{T}_4$ = Forearm skin temperature	0.14
$\overline{T}_5$ = Anterior thigh skin temperature	0.063
$\overline{T}_6$ = Shin skin temperature	0.065
$\overline{T}_7$ = Upper back skin temperature	0.088
$\overline{T}_8$ = Lower back skin temperature	0.088
$\overline{T}_9$ = Buttocks skin temperature	0.063
$\overline{T}_{10}$ =Posterior thigh skin temperature	0.063
$\overline{T}_{11}$= Calf skin temperature	0.065

3.5.2 Metabolic Rate and Substrate Partitioning

Substrate oxidation and energy production were calculated from metabolic rate AUC outcomes post-data acquisition. Glucose (Gox) and lipid (Lox) oxidation rates were calculated using the following equations from Simonson and DeFronzo (1990) during each experimental condition. Protein oxidation rates were determined through urinary urea nitrogen analysis.

(eq.2) $G_{ox}\ (g \cdot min^{-1}) = 4.57\ \dot{V}CO_2 - 3.23\ \dot{V}O_2 - 2.60N$

(eq.3) $L_{ox}\ (g \cdot min^{-1}) = 1.69\ \dot{V}O_2 - 1.69\ \dot{V}CO_2 - 2.03N$

(eq.4) $Pro_{ox}\ (g \cdot min^{-1}) = 6.25N$

$\dot{V}O_2$ and $\dot{V}CO_2$ are expressed in both STPD and $L \cdot min^{-1}$ and were calculated via the flow rate of inspired (FR_i) and expired (FR_e) air using the following equations from Lighton (2008):

(eq.5) $\dot{V}O_2 = FR_i(F_iO_2) - FR_e(F_eO_2)$

(eq.6) $\dot{V}CO_2 = FR_e(F_eCO_2) - FR_i(F_iCO_2)$

Energy production was calculated from individual contributions of each substrate to the fuel mixture (Simonson & DeFronzo, 1990) as follows:

(eq.7) $EP\ (kcal \cdot min^{-1}) = EP = 3.74\ \ Gox + 9.46\ Lox + 4.32\ Pox$

3.6 Statistical analysis

Based on results of previous cold and hypoxic exposure studies, a statistical power analysis was used to determine the minimum number of participants required to reach statistical significance. First, data were checked for normality, sphericity, and heteroscedasticity. Second, t-tests were performed to determine any statistical difference between the three BMR measurements of each experimental session to ensure accuracy and reliability of the measurements. Then, a 2-way repeated measure ANOVA [3 conditions X 5-time intervals] was run on metabolic rate, substrate partitioning, SpO_2, EMG, skin and core temperatures, to detect any differences between the three conditions (cold, hypoxia, and combined conditions). Statistical significance was set at $p < 0.05$. Statistical Package for the Social Sciences (SPSS) for Windows was used to evaluate the data.

Chapter 4: Results

4.1 Participants

Anthropometric data shown in the table below are based off information collected from the eight participants during the familiarization session.

Table 4-1: Participant Anthropometrics (*n*=8)

Age	Height (cm)	Weight (kg)	BMI	BSA
28.9 ± 11.6	175.9 ± 6.7	80.7 ± 11.5	26.0 ± 2.7	1.97±0.16

Mean ± SD

Based on previous research results on oxygen uptake (Johnston et al., 1996) a statistical power analysis was run using G*Power 3.1 to determine the minimum number of participants. Calculations were performed on a mean difference of 243 $mL \cdot min^{-1}$, (based on the above-mentioned study) with a total variance of 4.44 resulting in a sample size of 8.1, with an effect size, critical F, and p-value of 0.5, 0.96, and 0.05 respectively. Note that oxygen uptake represents the main variable for subsequent calculations of substrate partitioning.

4.2 Metabolic (Respirometry)

The statistical analysis revealed a significant interaction between conditions and time for CHO ($F_{(2,4)} = 3.489$; $p = 0.005$), Figure 4-1. *Post-hoc* test showed that as time elapsed CHO oxidation significantly increased during cold exposure from 90 to 120 min compared to CH and H. The mean difference between Cold and CH and between Cold and

H were 101.8 mg·min^{-1} (95% CI = 9.4 to 194.2) and 148.5 mg·min^{-1} (95% CI = 56.1 to 240.9) after 90 minuets of exposure and 135.7 mg·min^{-1} (95% CI = 43.3 to 228.1) and 178.5 mg·min^{-1} (95% CI = 86.1 to 270.9) after 120 minuets of exposure, respectively. As for lipid oxidation, the statistical analysis revealed no significant interaction but a significant main effect of conditions ($F_{(2,4)}$ = 14.450; p = 0.001) and of time ($F_{(2,4)}$ = 9.416; p = 0.001), Figure 4-2. *Post-hoc* test showed a greater lipid oxidation ($F_{(2,4)}$ = 9.416; p = 0.001) during CH compared to the two other conditions and a linear increase ($F_{(2,4)}$ = 9.416; p = 0.001) through time.

The statistical analysis revealed a significant interaction between conditions and time for EP ($F_{(2,4)}$ = 4.045; p = 0.003), Figure 4-3. *Post-hoc* test showed that as time elapsed EP significantly increased during cold and CH exposure from 60 to 120 min compared to H. The mean difference between Cold and H and between CH and H were 2.02 kcal·min^{-1} (95% CI = 0.49 to 3.55) and 1.88 kcal·min^{-1} (95% CI = 0.35 to 3.41) at 60 min marked and 2.88 kcal·min^{-1} (95% CI = 1.34 to 4.41) and 3.39 kcal·min^{-1} (95% CI = 1.86 to 4.92) at 90 min marked and 3.91 kcal·min^{-1} (95% CI = 2.37 to 5.44) and 3.91 kcal·min^{-1} (95% CI = 2.38 to 5.44) at 120 min marked, respectively.

4.3 EMG

Recall that no EMG was recorded during H condition owing to the fact that no shivering was expected in a thermoneutral condition. Therefore, the EMG analyse compare C to CH. No significant difference was observed in shivering activity in individual muscles. However, *t*-test ($t(3)$ = 1.484, p = 0.086) showed that during CH, pooled muscle shivering

activity was slightly higher (4.1±1.4%MVC; 95%CI = 1.9 to 6.2) compared to C condition (3.5±1.2%MVC; 95%CI = 1.6 to 5.4).

4.4 Core Body Temperature

Core body temperature remained constant through time in all experimental conditions (from 37.1±0.3°C to 36.8±0.4°C, from 37.3±0.3°C to 37.1±0.3°C, and from 37.0±0.5 to 37.0±0.5°C for CH, C, and H, respectively). The mean difference between cold and CH was -0.16°C (95%CI = -0.37°C to 0.06°C); it was 0.05°C (95%CI = -0.16°C to 0.27°C) between cold and H, and 0.21°C (95%CI = -0.003°C to 0.42°C) between CH and H.

4.5 Skin Temperature

The statistical analysis revealed a significant interaction between conditions and time for Skin temperature ($F_{(2,4)} = 27.89$; $p = 0.001$), Figure 4-6. *Post-hoc* test showed that as time elapsed skin temperature significantly decreased during cold and CH exposure from 30 to 120 min compared to H. The mean difference between Cold and H and between CH and H were -4.7°C (95% CI = -5.7 to -3.7) and -4.0°C (95% CI = -4.9 to -3.0) after 30 min of exposure, -6.0°C (95% CI = -6.9 to -5.0) and -5.5°C (95% CI = -6.5 to -4.5) after 60 min of exposure, -6.6°C (95% CI = -7.6 to -5.6) and -6.0°C (95% CI = -6.9 to -5.0) after 90 min of exposure, and -6.8°C (95% CI = -7.8 to -5.8) and -6.7°C (95% CI = -7.7 to -5.7) after 120 min of exposure respectively, as shown in Figure 4-4

4.6 Heart Rate

The statistical analysis revealed a significant main effect of condition on heart rate ($F_{(2,4)} = 5.518$; $p = 0.005$), Figure 4-5. In fact, *post-hoc* test showed that the mean difference between cold and CH was -6.3 beats·min^{-1} (95%CI = 10 to -2). As well, there was a significant main effect of time ($F_{(2,4)} = 3.313$; $p = 0.053$). Indeed, as time elapsed heart rate went from 63±4 beats·min^{-1} to 72±5 beats·min^{-1}. Although statistically significant, these outcomes have no clinical relevance.

4.7 SpO_2

The statistical analysis revealed a significant interaction between conditions and time for SpO_2 ($F_{(2,4)} = 10.13$; $p = 0.001$). *Post-hoc* test showed that as time elapsed SpO_2 significantly decreased during CH and H exposure from 30 to 120 min compared to cold. The mean difference between CH and cold and between H and cold were 8.9% (95% CI = 6.2 to 11.6) and 11.5% (95% CI = 8.7 to 14.2) after 30 min of exposure and 8.3% (95% CI = 5.6 to 11.0) and 11.6% (95% CI = 8.9 to 14.3) after 60 min of exposure, and 7.7% (95% CI 4.9 to 10.4) and 10.8% (95% CI = 8.1 to 13.5) after 90 min of exposure and 8.1% (95% CI = 5.4 to 10.8) and 10.3% (95% CI = 7.6 to 13.1) after 120 min of exposure, respectively, as shown in Figure 4-6.

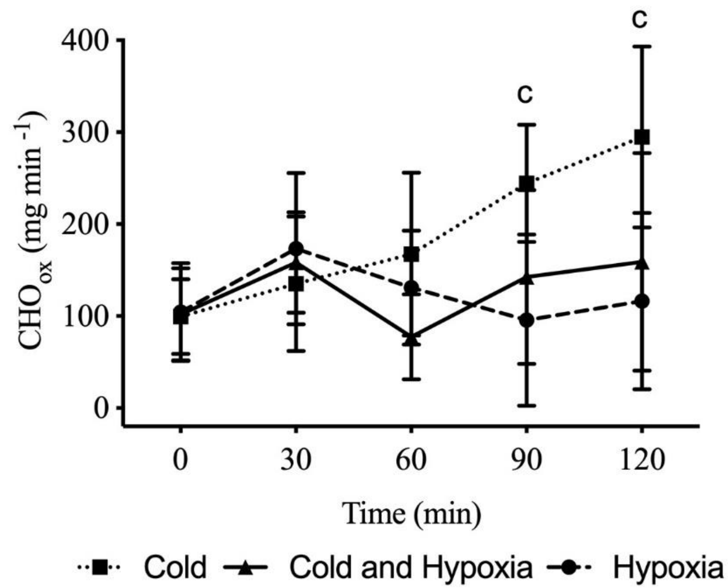

Figure 4-1: CHO utilization over time during experimental conditions.
[c] Indicates a significant interaction between conditions and time. CHO_{ox} increased during Cold from 90 min to 120 min with no change in Cold Hypoxia and Hypoxia ($p<0.005$).

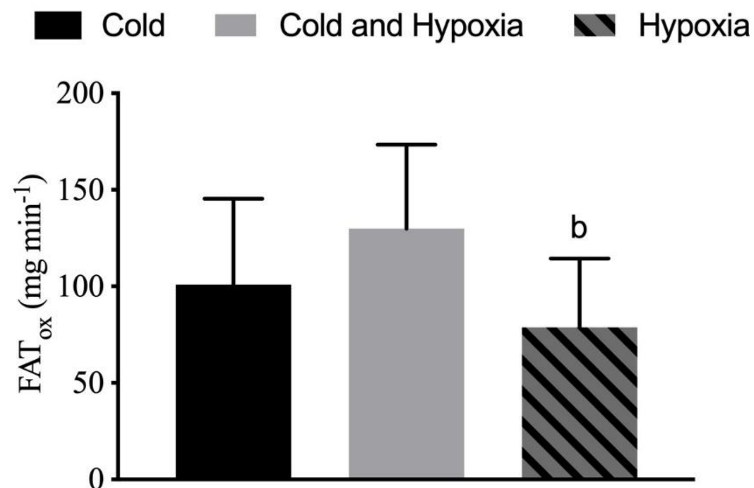

Figure 4-2: Lipid utilization during experimental conditions.
[b] Indicates a significant effect of conditions ($p<0.01$). FAT_{ox} was lower in Hypoxia compared the two other conditions.

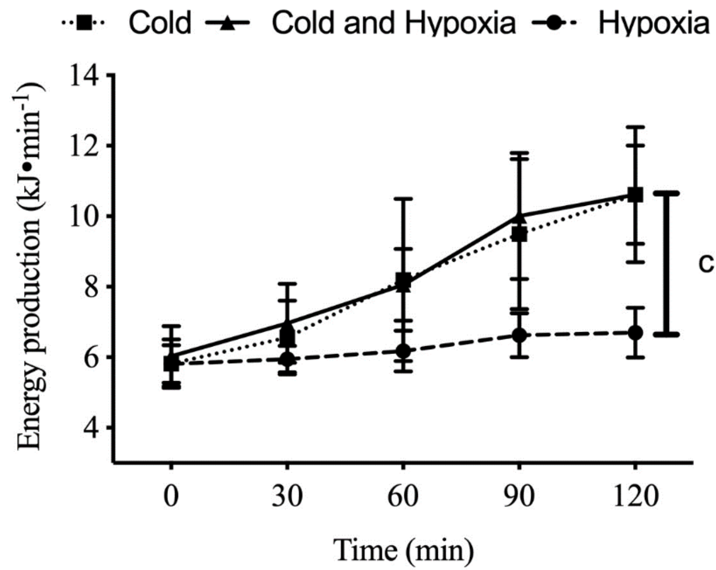

Figure 4-3: Energy Production during experimental conditions over time.
[c]Indicates a significant interaction between conditions and time (p<0.03). Cold Hypoxia and Cold significantly increased EP over time with no change in Hypoxia.

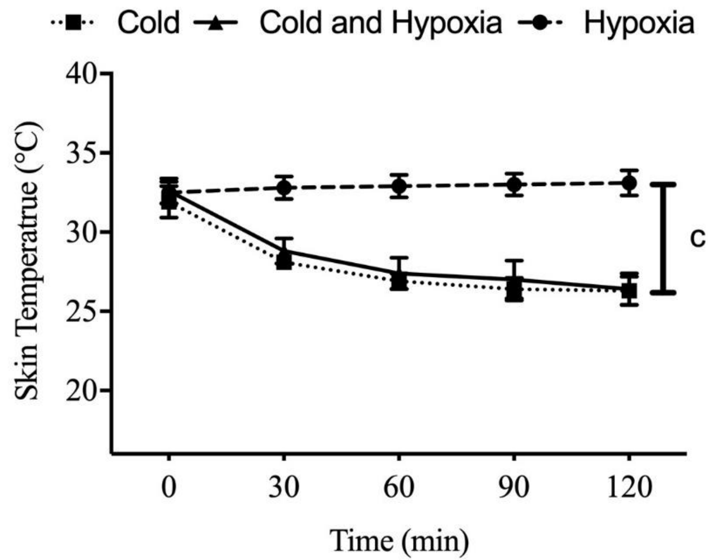

Figure 4-4: Change in skin temperature (°C) during experimental conditons over time.
[c]Indicates a significant interaction between conditions and time (p<0.05). Cold and Cold Hypoxia significantly decreased over time with no change for Hypoxia.

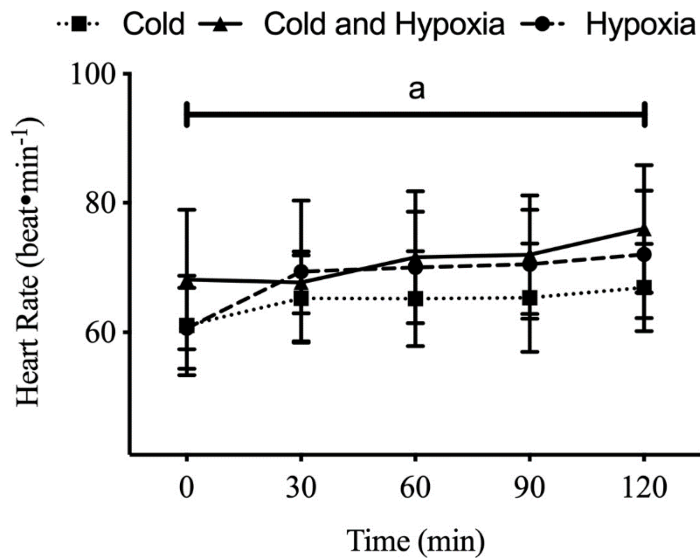

Figure 4-5: Change in heart rate (beats·min^{-1}) during experimental conditons over time. [a]Indicates a significant time effect (p<0.005). As time elapsed, HR increased in all conditions.

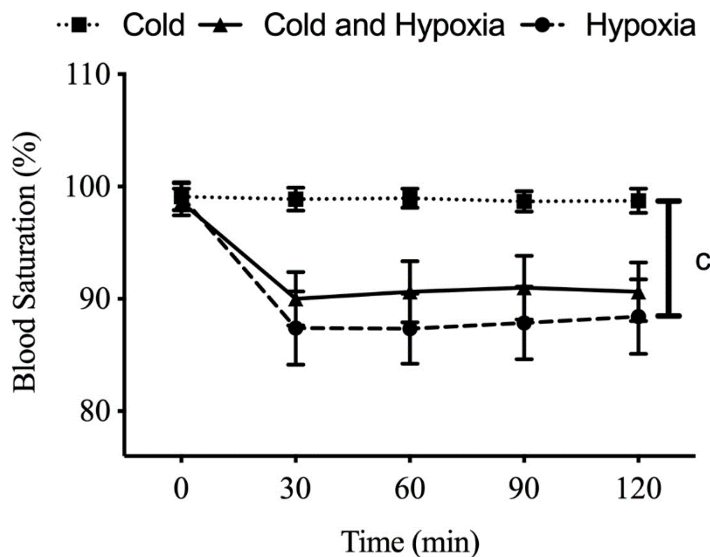

Figure 4-6: Change in hemoglobin oxygen saturation (%) during experimental conditons over time.

[c]Indicates a significant interaction between conditions and time (p<0.001). As time elapsed, SpO_2 decreased in Hypoxia and Cold Hypoxia with no change in Cold.

Chapter 5: Discussion

This study was designed to examine the combined effect of cold and hypoxic exposure on various metabolic parameters of male subjects at rest. Using multiple experimental conditions, this study compared the effects on subjects exposed to cold and hypoxia combined to their results when exposed both cold and hypoxic conditions individually. Results from all experimental trials indicate that exposure to cold and hypoxia simultaneously will increase the rate of lipid oxidation, which would indicate a greater reliance on lipids as a source of energy when compared to the other two conditions.

5.1 Metabolic & Thermoregulatory Response

As was expected, a significant drop in skin temperature over time was shown for C and CH conditions, with no change reported during hypoxic exposure alone. These results are supported by Blatteis and Lutherer (1976) who reported that skin temperature fell rapidly during initial exposure to cold and hypoxia, then stabilized. Measurements of skin temperature during our study were able to show that skin temperature will drop quickly during the first 30 minutes of exposure, and then began to taper off for the remainder of the experimental session. Cipriano and Goldman (1975) concluded that skin temperature will not decrease as much during cold exposure at altitude when compared to cold exposure at sea level. These findings did not relate to those of our study, which showed very similar decrease in skin temperate during C and CH. However, we must consider Cipriano and Goldman (1975) utilized HH during exposure, while our study used NH.

Although a decrease in skin temperature was observed in C and CH, no changes in core temperature were observed. According to the definition of Haman and Blondin (2017), the heat produced by increased shivering was sufficient to maintain core temperature. This would lead us to conclude that the cold condition we elicited was compensable. These results, however, are contrary to the findings of Blatteis and Lutherer (1976) that core temperature decreased with increasing altitude. However, as our study utilized a hypoxic and cold exposure at a controlled constant level, there is potential that we would see changes in core temperature had these levels decreased to mimic conditions at higher altitudes. An increase in core cooling was also reported by Reading et al., (1996) despite an increase in shivering activity. Johnston et al., (1996) proposed that acute NH exposure will lower thermoregulatory responses, which will lead to accelerated core cooling, which again was not an outcome supported by our study. Similar NH conditions to our study were used by the research team (12% compared to 14% O_2). However, as there was exercise involved, results from this study do not correlate directly to our study conducted with participants at rest.

Measures of energy production showed a significant increase in EP during C and CH, while a slight non-significant (seated) position effect was observed in H. An increase in EP is the likely result of increased energy requirements during C and CH conditions. Robinson and Haymes (1990) hypothesized an increase in energy requirements during combined exposure to cold and hypoxia was the result of increased cardiac output, combined with increased peripheral resistance from vasoconstriction response to cold exposure. This explanation would support our findings of increased EP. Workman and

Basset (2012) also found an increase in energy expenditure during acute hypoxic exposure, however this was shown in overweight sedentary subjects, only during hypoxic exposure, which may explain the difference in results. As there were only significant increases in energy production during both C and CH and not H alone, it is likely that the resulting increase in EP during our study can be attributed to the body's thermoregulatory response.

Interestingly, results for substrate contribution towards energy production significantly differ between the conditions over time. Glucose oxidation was greater during C compared to CH and H, while lipid oxidation was higher during CH compared to C and H. A shift in substrate utilization towards lipid sources was show by Workman and Basset (2012) which supports our findings from the CH condition, but not H alone. This results in the rejection of our hypothesis, as it was thought that exposure to the CH condition would result in an increase in glucose oxidation. From these results, we can conclude that during CH, a shift in substrate utilization towards lipid sources will occur, which may indicate the body's preferred response to combat effects of cold exposure rather than decreased oxygen levels.

5.2 Muscle Activity

Previous research of cold and hypoxic exposure had yielded mixed results with regards to shivering activity. Kotteke et al., (1947) reported that humans will have inhibited shivering levels when exposed to cold and hypoxia. In contrast, Robinson and Haymes (1990) observed an increased reliance on shivering at rest, which they believed was a result of the inhibition of non-shivering thermogenesis. Reading et al., (1996) also reported an

increase in shivering levels despite the reduction in inspired O_2. No significant difference in shivering intensity was found between the C and CH conditions. This result is supported by Lim and Luft (1962) who reported an insignificant effect of hypoxia on shivering thermogenesis, as they did not observe any changes in O_2 uptake or heat production. It is however important to note that their measure of shivering intensity did not use EMG analysis and was instead inferred by unchanged O_2 consumption and heat production. Without a direct measure of muscle activity, it is difficult to compare these results to our findings, as it is possible that low level shivering did occur, and the muscle activity was not captured. Blatteis and Lutherer (1976) studied the metabolic effects of subjects at altitudes of 150m, 3350m, and 4360m. They found that shivering was present during cold exposure at all altitudes and seemed to progress to more vigorous levels with greater altitudes. However, 4360m would represent a lower (F_IO_2) than what was used in our study, and this result cannot be used in comparison with ours.

5.3 Methodological considerations

There are several methodological issues to consider in this study. First and foremost, the difficulty of creating a truly hypoxic environment, without contamination from outside air, was discovered by the research group. In the initial project design, it was intended for the subjects to be in a supine position on a bed during exposure, breathing hypoxia air via a face mask attached to supply of hypoxic air. The purpose for the supine position was to ensure the participants were in a resting state, an expending as little additional energy as possible. However, during pilot testing, it was quickly noted that the face mask could not be sealed effectively to the participant's face, which led to

contamination from outside air during exposure. Because of the leaks in the face mask, we were unable to create a truly hypoxic environment. To solve this issue, the research team was able to re-purpose a plethysmograph into an air-tight chamber, which was able to create a hypoxic environment with no outside contamination. While the chamber proved to be an excellent solution to creating and maintaining a hypoxic environment, there were some minor issues that must be noted.

One issue with the use of the chamber is that while the chamber was very good at maintaining a hypoxic environment while sealed, there was no way to allow participants to enter the chamber without opening the door and exposing the chamber to room air. Because of this issue, during trials with hypoxic exposure, participants would be required sit in the chamber for ~20 minutes while waiting for the oxygen level to decrease to ~14%. This meant the participants were required to sit in the chamber for ~20 minutes longer than during the cold trials, extending these trials longer than was anticipated.

One other issue with the chamber is that based on the size and orientation, participants were required to be seated in the chamber, instead of the initial supine position. A non-significant increase in energy production during the hypoxic condition indicated that the seated position did impact the participant's energy level. One hypothesis for this occurrence was due to increased muscle activity during postural changes that were not captured during the 1-minute shivering measurements. While the increase was not found to be significant, it is important to note that had the participants been in a supine position in a similar chamber, this increase may have been absent.

5.4 Future Research

While this study has yielded some very interesting results with regards to the effects of cold and hypoxic exposure on metabolic rate, there is still potential for more work to be conducted on this subject in the future. One idea to expand on the research completed during this project would be to analyze the effects of substrate loading prior to exposure, by controlling participant's diet and supplementation. As shown by Haman et al. (2004b), when CHO stores are depleted, an increase in lipid oxidation will occur in order to fuel heat production. It could be very interesting to see if similar effects would be produced by the combined exposure of cold and hypoxia, or if alternate changes to metabolic response would occur. An alternative proposition would be to replicate the conditions of the study, but instead introducing physical activity as a parameter instead of participants at rest.

Chapter 6: Conclusion

The main purpose of this study was to examine the combined effects of cold and hypoxia on substrate metabolism in men at rest. In addition to substrate metabolism, the effects of various other metabolic responses, such as skin temperature, core temperature, heart rate, and EMG activity were also examined. The findings of this study, in response to the original hypotheses are as follows:

Hypothesis 1: Exposure to both cold and hypoxic conditions simultaneously will lead to an increase in the rate of core cooling, leading to earlier onset of shivering thermogenesis than during exposure to cold alone.

The hypothesis was based off findings from Johnston et al. (1996) which reported an increased rate in core cooling during exposure to cold and hypoxia. Based on our results, we must reject this hypothesis as there were no changes in core temperature observed during all three experimental conditions.

Hypothesis 2: As a consequence of early onset of shivering thermogenesis, substrate partitioning will shift towards CHO sources to maximize energy production during low partial pressure of oxygen, induced by hypoxic condition.

Contrary to the original hypothesis, combined exposure to cold and hypoxia resulted in an increase in lipid oxidation. At this point, the exact mechanism as to why lipid sources were favoured is unknown. One hypothesis for this finding is that the body perceives prolonged cold exposure as a greater threat to survival than reduced O_2 availability. Because of this, the switch in metabolic processes towards lipid oxidation may

have occurred to fuel thermogenesis in order to combat heat loss. This would seem to show that the utilization of the CHO as the more oxygen efficient fuel source did not take priority, thus leading us to reject this hypothesis as well.

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Appendices

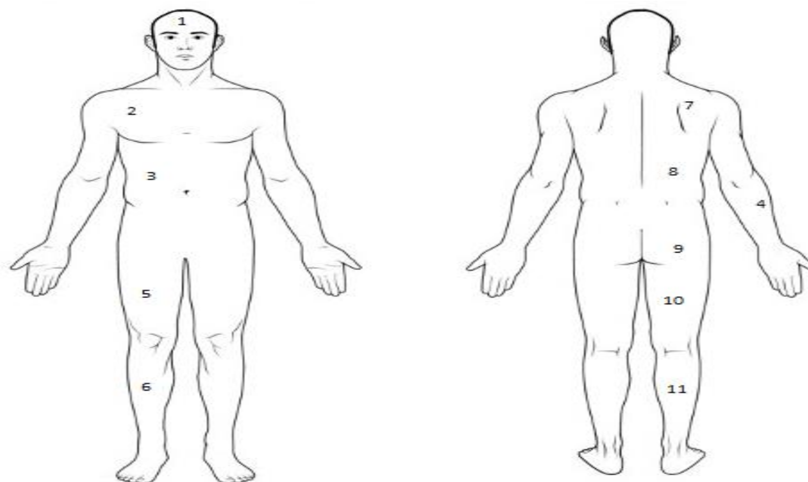
Appendix A: Thermochron Application Guide

Thermochron Application Guide

1. Participant will be asked to remove their shirt for thermochron application.
2. A total of 11 sites will be used for skin temperature measurements, as indicated in the below diagram
3. Each site will be shaved, cleaned with an alchool swap, and dried using guaze bandages
4. Once the sites have dried, on thermochorns will be applied to each site, and secured with transpore tape

Thermochron Sensor Locations:

1. Forehead
2. R Chest
3. R Abdomen
4. R Forearm
5. R Anterior Thigh
6. R Shin
7. R Upper Back
8. R Lower Back
9. R Buttock
10. R Posterior Thigh
11. R Posterior Calf



Appendix B: Lake Louise Mountain Sickness Score Questionnaire

Lake Louise Score (LLS) for the diagnosis of Acute Mountain Sickness (AMS)

A diagnosis of AMS is based on:
1. A rise in altitude within the last 4 days
2. Presence of a headache

PLUS

3. Presence of at least one other symptom
4. A total score of 3 or more from the questions below

SELF-REPORT QUESTIONNAIRE

Add together the individual scores for each symptom to get the **total score.**

Headache	No headache	**0**	
	Mild headache	**1**	
	Moderate headache	**2**	
	Severe headache, incapacitating	**3**	
Gastrointestinal symptoms	None	**0**	
	Poor appetite or nausea	**1**	
	Moderate nausea &/or vomiting	**2**	
	Severe nausea &/or vomiting	**3**	
Fatigue &/or weakness	Not tired or weak	**0**	
	Mild fatigue/ weakness	**1**	
	Moderate fatigue/ weakness	**2**	
	Severe fatigue/ weakness	**3**	
Dizziness/lightheadedness	Not dizzy	**0**	
	Mild dizziness	**1**	
	Moderate dizziness	**2**	
	Severe dizziness, incapacitating	**3**	
Difficulty sleeping	Slept as well as usual	**0**	
	Did not sleep as well as usual	**1**	
	Woke many times, poor sleep	**2**	
	Could not sleep at all	**3**	
	TOTAL SCORE:		

Total score of:

- 3 to 5 = mild AMS
- 6 or more = severe AMS

Note:

- Do not ascend with symptoms of AMS
- Descend if symptoms are not improving or getting worse
- Descend if symptoms of HACE or HAPE develop