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Autonomic Nervous System Response to Repeated Cold Exposure

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AUTONOMIC NERVOUS SYSTEM RESPONSE TO REPEATED COLD EXPOSURE

A Thesis

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Louisiana State University and
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TABLE OF CONTENTS

ABSTRACT

Recent work suggests repeated cold exposure raises energy expenditure, partly due to sympathetic nervous system stimulation, which indicates repeated cold exposure as a possible strategy to combat obesity without significant behavioral changes.

PURPOSE: (1) To examine the acute resting metabolic rate (RMR) response to mild cold exposure of 16° C and the acute autonomic nervous system (ANS) response to two cold exposures of 16° C and 4° C through heart rate variability (HRV) indices; and (2) to examine whether 4 weeks of daily cold exposure would result in adaptations in the metabolic and autonomic responses to acute cold exposure.

METHODS: RMR and HRV measurements were used to measure acute cold exposure response at 22° C and prolonged cold exposure response after 90 minutes at 16° C. HRV measurements were also used for acute exposure at 22° C and continuously during 20 minutes of cold exposure at 4° C. RMR and ANS responses to 16° C following 20 sessions (400 minutes) of repeated cold exposure were measured. RMR was measured using a metabolic cart and ANS activity was examined through the measurement of HRV.

RESULTS: A significant increase in RMR of 11% (p=0.03) and an increase in Total Power that approached significance (p=0.07) was measured after 90 minutes of exposure to 16° C. Upon exposure to 4° C, significant (p<0.05) increases in Total Power, High Frequency, and RMSSD indices of HRV were measured with Low Frequency approaching significance (p=0.07). No significant findings were measured after repeated cold exposure in comparison to baseline measurements.

CONCLUSION: Acute cold exposure of 16° C resulted in a significant increase in RMR of 11% with increased activation of the autonomic nervous system measured at both 16° C and 4° C. Twenty sessions of repeated cold exposure of 4° C did not significantly alter acute responses to cold exposure. There was no evidence of a change in autonomic balance toward a hyper-adrenergic state due to cold exposure. These findings suggest repeated cold exposure may be an effective tool for significantly increasing metabolic rate without changing autonomic balance toward a hyper-adrenergic state.

CHAPTER 1 - INTRODUCTION

Obesity rates in the U.S. continue to increase with 33% of adults and 17% of children and young adults (ages 2-19) classified as obese according to data from the NHANES (2010). Obesity has been linked to numerous health problems such as high blood pressure, cardiovascular disease, type 2 diabetes, cancer, osteoarthritis, gout, limited mobility, and early mortality. It is estimated that the health problems associated with overweight and obesity cost 2.1 billion dollars annually (Bahia et al., 2012; Squires, 2012). This is mainly due to the costs associated with the treatment of diseases caused by chronic obesity such as cardiovascular disease, high blood pressure, type 2 diabetes mellitus, and some cancers (Cornier et al., 2008; Samaranayake, Ong, Leung, & Cheung, 2012). Therefore, it is imperative that we make progress in combatting the rising obesity rates to ensure the health of our citizens and control the rising costs of healthcare.

Obesity is the result of a long-term imbalance between energy intake and energy expenditure (Samaranayake, Ong, Leung, & Cheung, 2012). Body weight and fat mass may be reduced as a result of creating a negative energy balance through decreased energy intake and/or increased energy expenditure. Energy intake can be reduced through interventions that promote dietary restrictions, use of pharmacologic agents that decrease appetite or alter nutrient absorption, or surgical procedures such as bariatric surgery. The three most common techniques used to stimulate thermogenesis and increase energy expenditure are: increasing energy expenditure through physical activity (Alahmadi, Hills, King, & Byrne, 2011; Borresen & Lambert, 2008), direct

stimulation of the sympathetic nervous system through beta-3 adrenoreceptor agonists (de Souza & Burkey, 2001), or exposure to cold temperatures (Haman et al., 2002).

A main concern regarding both energy intake (diet) and energy expenditure (exercise) is that the majority of strategies require an individual to make and maintain conscious lifestyle changes. While these strategies might have a significant effect on those individuals motivated to make necessary changes, they may not be sufficiently effective to alter the obesity epidemic in our society. Therefore, there is a need to develop additional strategies of creating a negative energy balance. Recent work suggests exposure to cold temperatures may be an important strategy to raise energy expenditure (Keith et al., 2006; Johnson et al.,2011; Ouellet et al., 2012). This has resulted in suggestions to decrease temperature in the work place, which could combat obesity in a significant number of individuals.

However, prior to moving toward implementing such a strategy it is critical to understand the mechanism(s) that are thought to contribute to an increase in energy expenditure due to cold exposure. The underlying concept is that cold exposure increases sympathetic drive (Jansky et al., 2006; Makinen et al., 2008). Arguably, inducing a chronic state of sympathetic excitation or hyper-adrenergic state may be ill-advised, as this is associated with poor health outcomes. In fact, most individuals who suffer from modern chronic diseases (obesity, cardiovascular disease, hypertension, and type 2 diabetes mellitus) are hyper-adrenergic. Unfortunately, there is very little published research that has examined the effects of acute and chronic cold exposure on energy expenditure, the autonomic nervous system, and clinical outcomes (Johnson et al., 2011).

Purpose and Specific Aims

The purpose of this study was to examine whether acute and chronic cold exposure increased resting metabolic rate (RMR), in part due to an increase in sympathetic nervous system activity. The study has two specific objectives: (1) To examine the acute resting metabolic rate response to mild cold exposure of 16° C and the acute autonomic response to two cold exposures of varying degree (i.e. 16° C and 4° C) through heart rate variability (HRV) indices; and (2) to examine whether 4 weeks of daily exposure to bouts of low temperature would result in adaptations in the metabolic and autonomic responses to acute cold exposure. In regards to the first objective it was hypothesized that acute cold exposure would increase RMR and alter indices of HRV in favor of a hyper-adrenergic state. In regards to the second objective, it was hypothesized that 4 weeks of daily exposure to low temperature would increase the metabolic response and decrease autonomic responses to acute cold exposure.

CHAPTER 2 – BACKGROUND

Energy Expenditure and Exercise

Increasing energy expenditure through exercise training is valuable. Exercise training can result in acute increases in metabolic rate and has been shown to increase non-exercise activity thermogenesis (NEAT) chronically after a significant duration and intensity of aerobic and/or resistance training. In addition to an increase in energy expenditure, normal physiological responses to exercise are improvements in cardiovascular performance, increased efficiency in substrate utilization, and in most cases, an increase in skeletal muscle mass. An improvement in substrate utilization efficiency is a response to stress that enables the body to perform activity for longer durations and at higher intensities through the increased use of fatty acids as a fuel source. Therefore, exercise is a common strategy adopted for reducing fat mass (McQueen, 2009). An increase in muscle mass can increase basal metabolic rate if performed for an extended period of time. The most effective method to increase muscle mass is through a resistance training routine. However, a less active individual will likely see an increase in muscle mass from an aerobic training routine as well. Increasing muscle mass can lead to improvements in insulin sensitivity and glucose uptake and increase RMR (Sundell, 2011) which will lead to a net decrease in fat mass. However, exercise alone is not the most sufficient strategy to lose weight, with less than 2 kg of weight loss due to aerobic exercise after one year of training (Thorogood et al., 2011).

Energy Expenditure and Daily Activity

Increasing energy expenditure can also be accomplished chronically through an increase in daily activity, which can increase both acute and chronic energy expenditure. In fact, one of the most effective ways to increase daily energy expenditure is through increasing NEAT (Levine, Eberhardt, & Jensen, 1999). NEAT is the amount of energy consumed through activity other than purposeful exercise. Increasing NEAT can be done by making small lifestyle changes such as using stairs instead of elevators, standing instead of sitting, and walking when possible. Since most of these changes are small, they can be done by almost any population. The goal of increasing NEAT is to make small increases in energy expenditure that will create a negative energy balance over time. Evidence suggests that mass can be decreased by 7 kg on average per year through diet and behavioral modifications that increase NEAT (Avenell et al., 2004). However, as with exercise training, the main issue with increasing NEAT is that it requires an individual to make and maintain conscious lifestyle changes and the effects of NEAT on weight loss are rather slow. (Alahmadi et al., 2011)

Energy Expenditure and Energy Intake

Without dietary restrictions in addition to exercise, there is a tendency for an individual to overcompensate the increase in exercise energy expenditure with increased food intake (McQueen, 2009). This can lead to a net increase in weight gain which is counterintuitive to the goals of an exercise intervention for weight loss. The main issue with exercise as an intervention is that it requires individuals to make a

dedicated lifestyle change many are not willing to do, or lack the practical knowledge to start and maintain an exercise routine on their own accord (Avenell et al., 2004).

Energy Expenditure and Pharmacotherapy

Despite extensive evidence of the importance of modifying diet and energy expenditure to decrease fat, a significant number of individuals struggle with this strategy. The value of pharmacotherapy to decrease fat mass continues to be problematic with risks often outweighing the benefits (Simonyi, Pados, Medvegy, & Bedros, 2012). Surgical interventions are expensive and are not applicable for the population at large due to the risks associated with surgical procedures. Therefore, there is a need to continue to explore other paths that may lead to successful fat loss in obese individuals. To this extent, recent work suggests exposure to cold temperatures may be an useful strategy for increasing energy expenditure (Jansky et al., 2006; Makinen et al., 2008; Ouellet et al., 2012; Johnson, Mavrogianni, Ucci, Vidal-Puig, & Wardle, 2011).

Energy Expenditure and Cold Exposure

The underlying mechanism for the rise in energy expenditure using cold exposure is beta-3 adrenoreceptor stimulation. Exposure to temperatures below 19° C stimulates the sympathetic nervous system and/or alters the parasympathetic nervous system through beta-3 adrenoreceptord, thus leading to an increase in energy expenditure (Bateman, Boychuk, Philbin, & Mendelowitz, 2012). Research indicates that the increase in energy expenditure ranges from 6-22% (Celi et al., 2010; Yoneshiro et al., 2011), and the effect is dependent on the dose (time and temperature) of the cold

exposure. Exposure to cold temperatures has been shown to cause an increase in muscle glycogen depletion, lipid metabolism, and heart rate (HR) (Haman et al., 2002). Exposure to temperature changes are becoming less common in habitual environments due to indoor heating and air conditioning, with increases in comfortable ambient temperature correlating to a decrease in daily energy expenditure (Johnson et al., 2011). Therefore, it may be possible to decrease temperature in the work place or home in order to raise energy expenditure and provide and additional means to treat obesity in a significant number of individuals. However, prior to moving toward implementing cold exposure as a therapeutic agent for obesity, it is critical to understand the mechanism(s) that could contribute to increased energy expenditure as well as risks and benefits of this method. If indeed cold exposure increases sympathetic drive, this may lead to a chronic hyper-adrenergic state, which is known to be associated with poor health outcomes. In fact, most individuals who suffer from modern chronic diseases (such as obesity, cardiovascular disease, hypertension, and type 2 diabetes mellitus) are hyper-adrenergic. There is very little research that has examined the effects of acute and chronic cold exposure on the autonomic nervous system and clinical outcomes in humans (Johnson et al., 2011).

Mechanisms of Increasing Energy Expenditure through Cold Exposure

The main effects of increasing adrenergic receptor stimulation concerning weight loss are an increase in HR and stimulation of lipolysis, due to mediation of norepinephrine and epinephrine. There are three types of beta adrenoreceptors that have different functions but are not stimulated exclusively. Stimulation of beta-1 adrenoreceptor normally causes an increase in HR, decreased blood pressure through

renin secretion, and increased hunger through increasing ghrelin secretion. Beta-2 adrenoreceptors main actions are smooth muscle relaxation and lipolysis. Beta-3 adrenoreceptors are responsible for regulation of thermogenesis and lipolysis (Krcmar et al., 2006). Beta-1 and beta-2 adrenoreceptors are normally stimulated pharmacologically to treat asthma, chronic pulmonic disease, and bradycardia. Due to the possible side effects of chronic stimulation of beta-1 and beta- 2 adrenoreceptors, beta-3 adrenoreceptor stimulation is believed to be the best target for weight loss.

Direct stimulation of the sympathetic nervous system through beta-3 adrenoreceptor agonists is currently under investigation as an intervention to increase thermogenesis in humans to stimulate weight loss. In animal models, beta-3 adrenoreceptor agonists have been shown to increase lipolysis of white adipose tissue and increase thermogenesis in brown adipose tissue (Krcmar et al., 2006). When norepinephrine is released, it will primarily stimulate beta-3 adrenoreceptors, which are associated with autonomic control of the heart, specifically the vagus nerve. The action of beta-3 adrenoreceptor agonists on the vagus nerve will lead to a decrease in parasympathetic activity and increase thermogenesis (Bateman et al., 2012). However, the effectiveness of beta-3 adrenoreceptor agonists have not been fully supported in humans as a weight loss intervention and the long-term side effects are not known (Redman et al., 2007). There is still much to study regarding the role of adrenoreceptor agonists in increasing thermogenesis before they can be used as an effective treatment of obesity.

Due to the difficulty of manipulating energy intake and energy expenditure to control body weight, novel and efficacious strategies should be tested to be used alone

or in combination with energy intake and energy expenditure interventions. Cold

exposure has been indicated as a possible strategy due to the effect of cold exposure

on increasing thermogenesis through stimulation of beta-3 adrenoreceptors. The role of

the sympathetic and parasympathetic nervous system during acute and chronic cold

exposure has not been fully tested, as well as the effectiveness of repeated cold

exposure to enhance the thermogenic response.

CHAPTER 3 - METHODS

Study Subjects

Male subjects between the ages of 18-35 years were sought for this study.

Eligible subjects were required to have a body mass index (BMI) between 18.5 and 25.0

kg/m^2 and weight stable, which is less than a 2 kg weight fluctuation in the past 3

months. Individuals were excluded if they were smokers, involved in intensive exercise

routines, such as marathon training or long distance jogging or biking, taking

medications or recreational drugs, or had evidence of overt signs of disease determined

by medical history. Subjects were recruited through the Pennington Biomedical

Research Center (Baton Rouge, LA) website, e-mail distribution lists and word of

mouth. All subjects completed a phone screening where they were asked yes or no

questions to determine eligibility. Once deemed eligible, subjects were scheduled for a

clinical screening to ensure adequate health status to participate in the trial. Subject

enrollment took place from May 2011 to May 2012.

Study Design

This study was a prospective/non-randomized trial consisting of a screening visit

and 2 distinct phases of testing. The screening visit included information about the risks,

benefits and procedures of the study, followed by signing of the informed consent.

Subsequently, anthropomorphic measures and a blood draw were obtained to

determine study eligibility. Phase 1 aimed to measure the effects of acute cold exposure

on energy expenditure and indices of autonomic function. Phase 2 aimed to examine

the effects of 20 sessions of cold exposure (over a 4 week period) on energy

expenditure and indices of autonomic function.
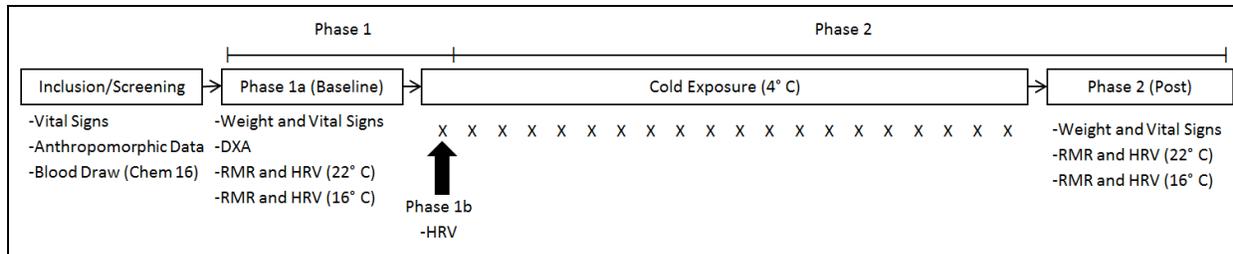
Figure 1: Main study experimental design from screening to post-testing.

Experimental Procedures

Screening visit: The screening visit occurred after a 10 hour fast and the subject

abstaining from alcohol, caffeine, and strenuous exercise for 24 hours prior to the visit.

A written informed consent, approved by the institutional review board at Pennington

Biomedical Research Center, was reviewed and signed prior to any study procedures.

Eligibility testing included blood pressure, pulse rate, and temperature measurements

as well as a blood draw with chemistry panel to evaluate fasting glucose, cholesterol,

triglycerides, and creatnine levels. The blood draw results, vital sign measurements,

and results from a medical health screening form were reviewed and approved by the

medical investigator prior to participation of Phase 1 procedures.

Phase 1: Examination of energy expenditure and indices of autonomic function

before and during acute mild and moderate cold exposure.

Baseline testing included 2 study visits to the inpatient and outpatient clinic at

Pennington Biomedical Research Center. Subjects reported for visits each morning

after fasting for 10 hours. Subjects were asked to refrain from caffeine, alcohol, and strenuous activity for 24 hours prior to each study visit.

Study Visit 1 (Phase 1a): During Study Visit 1 the metabolic and autonomic responses to mild cold (16-17° C) were measured. Subjects arrived at Pennington Biomedical Research Center at 7:00 AM. The subject's vital signs (blood pressure, pulse, and temperature) and weight were assessed after voiding their bladder. Body composition was measured by dual-energy X-ray absorptiometry (DXA) while wearing a hospital gown. After the DXA, subjects wore shorts and a T-shirt for the reminder of testing. RMR and HRV were then measured under thermo-neutral conditions (22-23° C) in a supine position after a 30-minute period of uninterrupted rest. HRV data were acquired continuously for 8 minutes using the POLAR heart rate monitor, after which RMR was obtained using a canopy hood system. The participant was then moved by wheelchair to a room set to mild cold temperature (16-17° C), where they rested supine for 80 minutes. After 82 minutes of resting, HRV was measured for 8 minutes followed by another 30 minute RMR measurement. The subject was then provided lunch and dismissed.

Study Visit 2 (Phase 1b): During Study Visit 2 the response of the autonomic nervous system to moderate cold (4° C) was measured. The acute autonomic nervous system response to cold was assessed using the same tools and pre-test instructions as described above. After a 5 minute seated rest, HRV was measured in a seated position for 5 minutes in thermo-neutral condition at 22° C and at 5-minute intervals during the 20 minutes of cold exposure at 4° C in a climate controlled chamber.

Phase 2: Examination of energy expenditure and indices of autonomic function before and after 20 sessions of cold exposure over a 4-week period.

Phase 2 consisted of a single study visit to the inpatient and outpatient clinics at Pennington Biomedical Research Center. Subjects reported for the visit in the morning after fasting for 10 hours. Subjects were asked to refrain from caffeine, alcohol, and strenuous activity for 24 hours prior to each study visit. The tests described for Phase 1 study visit 1 were replicated, with the exception of a DXA scan.

Daily cold exposure: Subjects reported to Pennington Biomedical Research Center 5 days a week for 4 weeks for 20 minutes of cold exposure in a climate controlled chamber with a temperature of 4° C. The participants wore shorts, a T-Shirt, and light shoes for each cold visit. Each subject completed at least 400 minutes of cold exposure over the 4-week period.

Study Procedures

Assessment of autonomic nervous system activity: The autonomic nervous system is comprised of two systems: sympathetic (SNS) and parasympathetic (PNS). Both the sympathetic and parasympathetic nervous systems use hormones to control physiological processes such as HR, breathing rate, blood pressure and glucose metabolism (Figure 2a-b). The sympathetic nervous system is described to have a "fight-or-flight" response to stress, such as pain, fear, or cold exposure, through the use of the hormones epinephrine and norepinephrine. During a sympathetic nervous system response, HR, respiration rate, blood pressure, and muscle glycogen usage increases, whereas digestion decreases (Figure 2b). The parasympathetic nervous system is

responsible for the resting state in which food is digested, and HR, respirations, and

blood pressure are decreased through the neurotransmitter Acetylcholine (Figure 2a).

Acetylcholine is removed from the action site at a faster rate than norepinephrine and

epinephrine, thus the stimulation rate of the parasympathetic nervous system must be

higher than the sympathetic nervous system for the equivalent control effect on the

same organ (Marino, Sockler, & Fry, 1998). Therefore, if we measure the rate of

stimulation of the cardiac system, we can measure the activity of the sympathetic

nervous system and parasympathetic nervous system. The SNS and PNS work

together to control responses to stressors and resting states of the body throughout

daily activity, therefore both systems are active at different capacities.
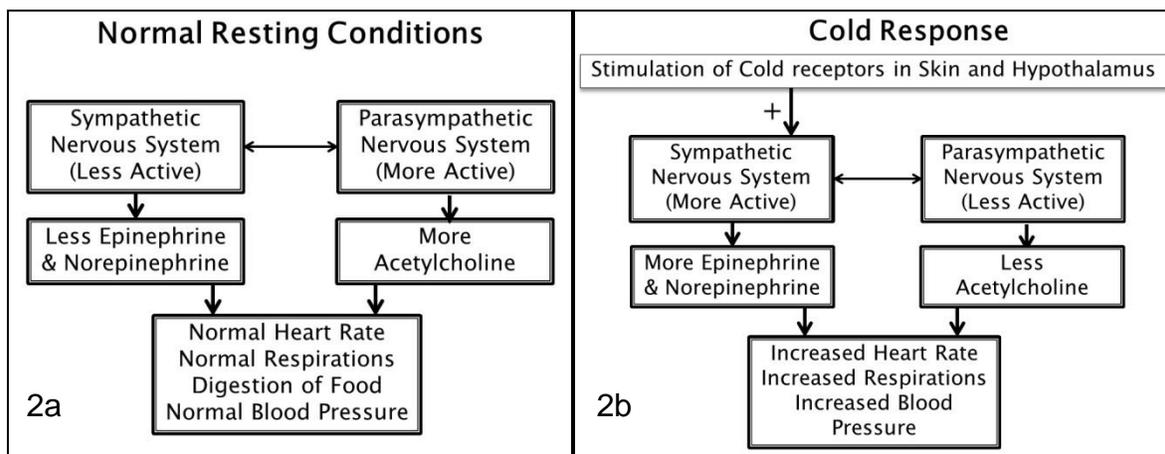
Figure 2a-b: Autonomic nervous system activity under normal and cold conditions.

To determine a systematic response of cold exposure on the subject, we will

measure the response of the autonomic nervous system. Direct measures of autonomic

nervous system activity can be obtained by examining the hormonal markers

norepinephrine and epinephrine through a blood draw. Because of cost and availability,

autonomic balance in this study was measured using an end-organ response, in the

form of heart rate variability (HRV) (Sinski et al., 2006). HRV is a valid and reproducible

measurement, sensitive to acute and chronic changes in the autonomic nervous system through environmental and exercise stimuli, and allows differentiation of physiological states such as age, fitness level, and certain disease states (Pomeranz et al., 1985).

HRV measurements are derived from the analysis of R-R interval data measured by an Electrocardiogram (ECG). R-values are the large peaks in a normal ECG, which correlate to ventricular depolarization of the heart. HRV variables are divided into two categories; time domain variables and variables derived through spectral analysis. The time domain variables were calculated from the measurement of R-R intervals during the time course of the measurement. The most common time domain variables used to measure autonomic nervous system response are HR, mean time between R-R intervals (mean RR), and root mean square of normal R-R intervals (RMSSD). Spectral analysis variables were determined through the calculation of R-R frequency oscillations using a fast fourier transformation of the R-R intervals during a measured time period. The frequencies derived from the fourier transformation are then divided into three categories: high frequency (0.15-0.4 Hz),which is associated with parasympathetic nervous system activity; low frequency (0.04-0.15 Hz), which is associated with sympathetic nervous system activity; and very low frequency (0-0.04 Hz) (Akselrod et al., 1981). These associations were derived from studies during which HRV was monitored during the removal of sympathetic nervous system and parasympathetic nervous system activation through pharmacological means (Bateman et al., 2012). Very low frequency measurements have yet to be correlated to autonomic nervous system activity but are sometimes reported to represent sympathetic nervous system activity when combined with Low Frequency values. We use the analysis of R-R intervals to

determine sympathetic nervous system and parasympathetic nervous system response to acute cold exposure and adaptation of the autonomic nervous system after repeated cold exposure. HRV can be affected acutely through alteration of breathing rate, stress, or pharmaceuticals that enhance heart rate, and has been indicated to be affected chronically by changes in cardiorespiratory fitness and age (Borresen & Lambert, 2008). Therefore it is important to control for medications and breathing rate, as well as limit environmental stimulation to ensure valid HRV measurements.

For the purpose of this study a holter style electrode HR monitor (POLAR Model RS 800, Lake Success, NY) was fitted to the participant. Similar POLAR models have been shown to produce valid and reliable measurements of HRV (r^2=0.85-0.99) comparable to HRV measurements obtained using a 12-lead ECG (Nunan et al., 2009). After a 20 minute supine rest in a thermo neutral room (22-23° C), the subject was asked to begin breathing in sync with an audible metronome set to 15 beats per minute to control breathing rate during HRV measurement.

Dependent variables of HRV were reported in both time and frequency domains. Each of these variables were derived from 5-minute continuous measurements of HRV and expressed as mean values unless otherwise stated. The time domain values reported are mean HR (bpm), mean RR, and RMSSD. The frequency domain variables of High Frequency, Low Frequency, and Very Low Frequency were calculated through R-R spectral analysis using a Fourier transformation algorithm. The frequency domain variables are reported as power of the frequency band in ms^2. To determine the spectral power in the frequency domain, the areas under the curves were quantified for the following frequency bands: Very Low Frequency (VLF; 0.007 to 0.035 Hz), Low

Frequency (LF; 0.035 to 0.15 Hz), High Frequency or vagal component (HF; 0.15 to 0.5 Hz), and Total Power (0.007 to 0.5 Hz).

RMR in thermo neutral and in mild-cold conditions: RMR in thermo neutral and cold conditions was measured in a climate-controlled room, in which ambient temperature was tightly controlled. The subjects wore light clothing. After 30 minutes of bed rest in a supine position, at an ambient temperature of 22-24° C, RMR was measured for 30 minutes with a Max II metabolic cart (AEI Technologies, Naperville, Il) equipped with a canopy hood system. The subject was then moved by wheelchair to a cold room set to 15-17° C. Subjects remained in the closed room alone but under supervision for 2 hours at this temperature, still wearing light clothing. Attention was given to ensure the subject was not shivering during the cold exposure. After 90 minutes, energy expenditure induced by cold-induced thermogenesis was measured by indirect calorimetry for 30 minutes, with the subjects lying quietly in a supine position. A full software and hardware calibration was performed on the metabolic cart with standard calibration gasses prior to each test. All values from the metabolic cart are expressed as Standard Temperature and Pressure, Dry (STPD) through the use of a sample drying system and adjusted to environmental measurements taken from a National Institute of Standards and Technology traceable weather station. The accuracy and precision of the metabolic carts are assessed once a month with ethanol combustion tests.

RMR measurements are reported as mean RMR extrapolated to 24 hours. RMR was calculated using the Weir equation (Mansell & Macdonald, 1990) using measurement of VO_2 (ml/min) and VCO_2 (ml/min). Weight factored RMR was calculated

by dividing RMR by the subject's measured weight on the day of the RMR measurements, and was reported as RMR per day per kg of body weight (kcal/d/kg). The respiratory quotient (RQ) was calculated as VCO_2/VO_2 from the minute average of VO_2 (ml/min) and VCO_2 (ml/min). The first 10 minutes of each 30-minute measurement were discarded to allow for acclimation to the hood and flow adjustments. Any minutes with RQ <0.7 and >1.0 (outside of normal physiological range) were not included in calculations (Compher, et al., 2006). The mean of the minute averages for the remaining 20 minutes of VO_2 (ml/min) and VCO_2 (ml/min) were used to calculate RMR and RQ.

Anthropomorphic measurements: Height and weight were evaluated according to standard Pennington Biomedical Research Center procedures to determine inclusionary criteria at screening. Height was measured in centimeters on a calibrated wall-mounted stadiometer without shoes. Weight was measured in a hospital gown immediately after voiding the bladder under a fasting state. The gown weight was measured and subtracted from total weight to determine metabolic weight. Metabolic weight is reported in kilograms.

Dietary intake: Dietary intake was determined through self-reported diet logs recorded by subjects for a 24 hour period during the week of testing. Dietary intake was calculated as total calories consumed during a 24 hour period (kcal/d). Calculations of the percentage of intake of carbohydrates, fat, and protein during this period were also performed and reported as percent of total consumption.

DXA scan: Dual X-Ray absorptiometry (DXA) scans were performed using the GE iDXA (GE Healthcare, Madison, Wi) whole-body scanner. The protocol requires that participants lie on a table wearing a hospital gown and no metal containing objects, while the scanner emits low energy X-rays and a detector passes along the body. The scan takes 10 minutes and the radiation dose is less than 1 mrem, equal to about 12 hours of background radiation. Two distinct energies are used to determine body mineral and soft tissue content. An attenuation ratio was determined from known tissue content. Variations of the attenuation ratio determine the fat content of the tissue at each pixel thereby calculating the percentage body fat (Encore v13.4 software). Fat mass was calculated from weight on a scale and % body fat in Kg and fat-free mass by subtracting fat mass from metabolic weight (kg).

Vital signs: Vital signs were measured with the subject seated for 5 minutes. Body temperature (sublingual) was measured using an electronic thermometer (Sure Temp 679, Welch Allyn) and blood pressure using a manometer (Baumanometer. W.A. Baum Co., Inc. USA). HR was measured by radial arterial pulse for 30 seconds. Vital signs were performed at screening to determine inclusion and prior to daily testing to monitor subject health for participation in study procedures.

Blood draw: The screening blood draw during the screening visit consisted of a 15ml venous sample obtained from the arm using sterile technique. The blood was analyzed by the Clinical Research Laboratory at Pennington Biomedical Research Center for blood glucose, cholesterol, triglycerides, and creatnine levels using a Beckman DXC 600 (Beckman coulter, Inc, USA). Results were reviewed by the medical investigator to determine eligibility.

Statistical analysis: All data is reported as mean and standard deviation and analyzed using SPSS statistical software (IBM SPSS Stats version 20). A repeated measures analysis of variance was performed to determine effect of acute cold exposure on HRV for the phase 1 portion of the trial. Significance of changes in RMR and weight factored RMR changes were determined through the use of a paired t-test. For phase 2 of the trial, we used a 2 by 2 analysis of variance to determine change in HRV and RMR from thermo neutral to cold conditions, and compare HRV and RMR responses in both pre and post-repeated cold exposure testing periods. A paired t-test was used to determine significance of weight change, and a regression correlation was used to determine if changes in weight correlated to changes in RMR or HRV indices either at baseline or during the cold response. A P value of <0.05 was considered to be statistically significant.

CHAPTER 4 - RESULTS

Anthropometric Data

Nine subjects (all male) participated in all facets of the study. None exhibited overt signs of disease. Mean age, body weight, BMI, and body fat percentage were 23 ±2.5 years (range: 20-27 years), 71.4 ±6.4 kg (range: 61-82.6 kg), 22.9 ±1.8 kg/m^2 (range 20.2-25 kg/m^2), and 23.1 ±5.9 % (range: 14.9-30.7), respectively. To the best of our knowledge all subjects maintained their regular exercise routines, and did not alter other behaviors (i.e. smoking status, use of medications or recreational drugs). However, regular diet was not kept constant according to self-reported dietary intake logs.

Effects of Acute Cold Exposure on Resting Metabolic Rate and Heart Rate Variability (Phase 1a): Exposure to Mild-Cold (16° C) Conditions for 90 Minutes

Resting Metabolic Rate: RMR measured during exposure to 16° C ambient temperature for 90 minutes, resulted in an 11% increase in the average RMR from 1676.2 ±138.9 kcal/d at 22° C to 1860.6 ±190184 ±272 kcal/d (p=0.03). Closer examination of the individual responses revealed 6 of the 9 participants tested increased and 3 decreased RMR. A 12 % increase in weight factored RMR was also measured significant with 23.5 ±1.3 kcal/d/kg measured at 22° C and 26.3 ±3.9 kcal/d/kg measured during 16° C ambient temperature (p=0.04). In addition to the increase in RMR, RQ also increased from 0.78 ±0.06 to 0.83 ±0.1 after 90 minutes of 16° C cold exposure, but did not reach statistical significance (p=0.21).

Heart rate and heart rate variability: Table 1 presents the HR and HRV data before and after 90 minutes of exposure to 16° C ambient temperatures. Mean HR was not significantly affected after 90 minutes of exposure to 16° C ambient temperatures. Responses in mean RR, RMSS, low frequency, and high frequency were not statistically different between pre and post 90 minutes of exposure. In contrast, there was a significant difference in total power (Low Frequency + High Frequency) which increased ~76% (p=0.07) between pre and post 90 minutes of exposure.

Table 1. Energy Expenditure and heart rate variability for Phase 1 Study Visit 1 (n=9)

	21-23° C	**16° C**	**P value**
Resting Metabolic Rate (kcal/d)	1676.2 ±138.9	1860.6 ±190	p=0.03*
Respiratory Quotient	0.78 ±0.06	0.83 ±0.1	p=0.21
Mean HR (bpm)	60 ±11	60 ±10	p=0.93
Mean RR (ms)	1045 ±185	1043 ±198	p=0.98
RMSSD (ms)	76 ±21	86 ±32	p=0.47
Low Frequency HRV (ms^2)	2660 ±2217	3121 ±2758	p=0.70
High Frequency HRV (ms²)	1843 ±1269	3021 ±2990	p=0.29
Total Power HRV (ms²)	8160 ±2544	14367 ±9186	p=0.07

* Denotes significant change from normal room temperature (p<0.05)

Effects of Acute Cold Exposure on Resting Metabolic Rate and Heart Rate Variability (Phase 1b): Time Course of Exposure to Moderate-Cold (4° C) Conditions

Heart rate and heart rate variability: Table 2 and Figures 3a-e present the time course of the HR and HRV responses before and during 20 minutes of exposure to 4^0 C ambient temperatures.

No statistical significant changes were noted for mean HR and mean RR at any time point compared to baseline. In contrast, significant increases in all measurements of HRV were noted from baseline to the first 5 minutes after exposure to 4° C, except for

22

Low Frequency, which approached significance (p=0.07). RMSSD remained

significantly elevated at all exposure time points compared to pre-exposure. Total

Power also remained elevated and approached significance at all time points compared

to baseline. High Frequency and Low Frequency remained elevated compared to

baseline for 10 and 15 minutes, respectively, but not thereafter.

Table 2. Heart Rate Variability for Phase 1 Study Visit 2 (n=9)

	22-23° C			Continuous Cold Exposure at 4° C						
	Baseline	**0-5 minutes**		**5-10 minutes**		**10-15 minutes**		**15-20 minutes**	
Mean HR (bpm)	69.6 ±5.5	71.1 ±4	p=0.62	70.2 ±4.4	p=0.90	70.6 ±4.3	p=0.80	69.9 ±3.2	p=0.91
Mean RR (ms)	909 ±203	897 ±145	p=0.73	594 ±157	p=0.78	884 ±141	p=0.59	887 ±115	p=0.56
RMSSD (ms)	50 ±25	77 ±16	p=0.002*	67 ±26	p=0.05*	66 ±24	p=0.03*	66 ±21	p=0.004*
Low Frequency HRV (ms²)	2074 ±1464	4361 ±3510	p=0.07	3821 ±3238	p=0.07	3608 ±3072	p=0.07	3260 ±3012	p=0.21
High Frequency HRV (ms²)	1391 ±1596	2350 ±1391	p=0.002*	2116 ±1966	p=0.12	1726 ±1498	p=0.52	18890 ±1193	p=0.16
Total Power HRV (ms²)	6961 ±4595	26891 ±20474	p=0.01*	10697 ±8405	p=0.15	9860 ±6761	p=0.11	10678 ±7162	p=0.07

Significant increases (p<0.05) measured in RMSSD for all minutes of cold exposure at 4° C compared to normal room temperature. High Frequency and Total Power measured significant increases during the first 5 minutes of cold exposure at 4° C.

* Denotes significant change from baseline (p<0.05)
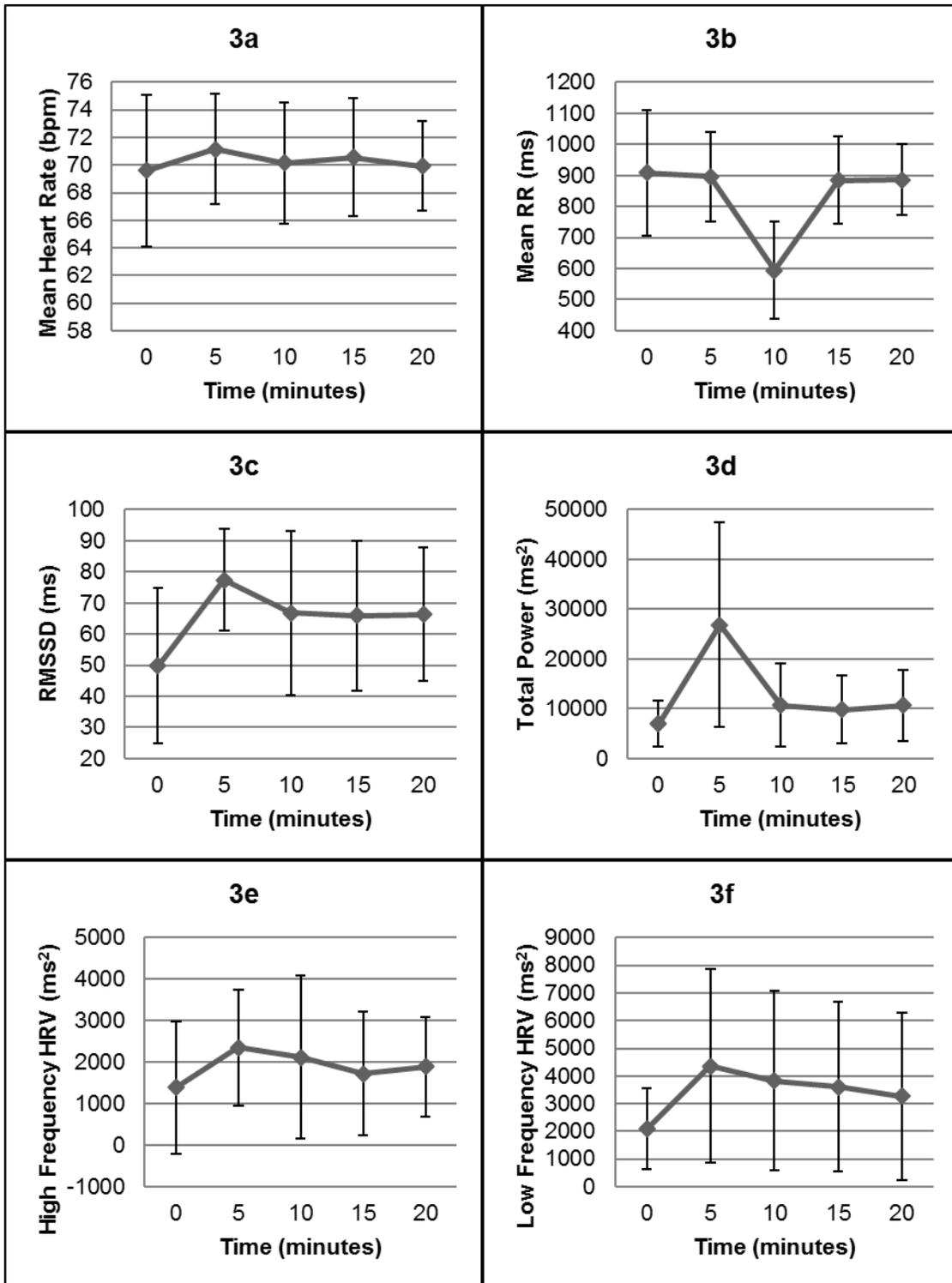
Figure 3a-f. Acute HRV Response to 4° C. Data represents the mean HR (bpm) and Mean RR (ms), mean RMSSD (ms), Total Power (ms^2), High Frequency (ms^2), and Low Frequency (ms^2) components of HRV for each 5 minute period measured during pre-cold exposure at minute 0 and during cold exposure of 4° C for 20 minutes.

Effects of 20 sessions of Cold Exposure on Resting Metabolic Rate and Heart Rate Variability (Phase 2)

Resting Metabolic Rate: Table 3 and Figures 4a-f present the RMR, HR, and HRV data during normal ambient temperature exposure of 22-23° C and after 90 minutes of exposure to 16° C ambient temperature collected prior to and after 20 sessions of cold exposure. After repeated cold exposure, RMR increased on average from 1621.3 ±184.9 kcal/d at 22° C to 1791.7 ±234.2 kcal/d upon exposure of 16° C ambient temperature. The increase in energy expenditure (11%) following 90 minutes was not statistically significant (p=0.24).
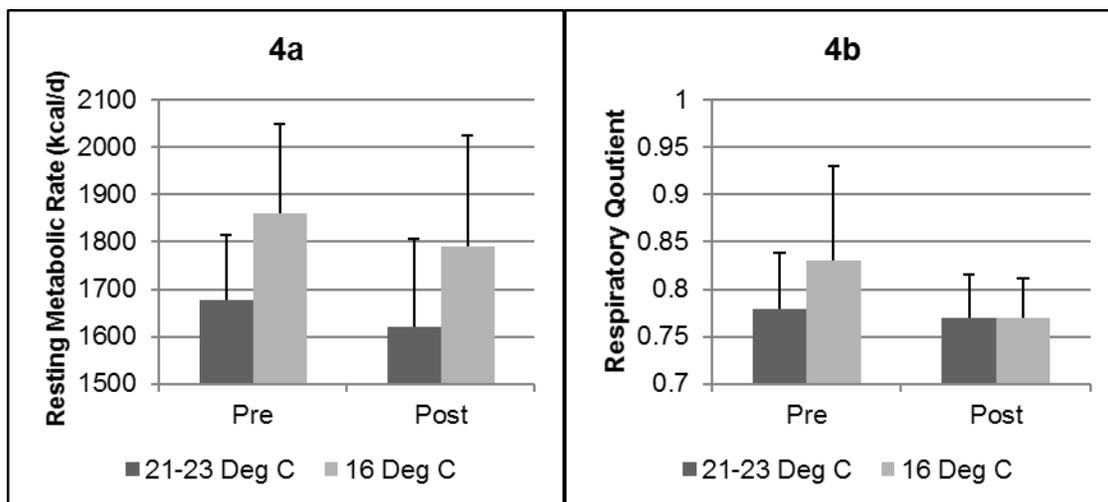
Figure 4a-b. Resting Metabolic Rate and Respiratory Quotient. Data represents RMR and RQ measured at normal temperature (21-23° C) and mild-cold exposure (16° C) both prior to (Pre) and after (Post) repeated cold exposure.

There were no statistically significant differences in RQ responses after 90 minutes of exposure to 16° C ambient temperature before and after the 20 sessions of cold exposure. Pre-intervention RQ responses increased by 6.5% from 0.78 ± 0.06 to 0.83 ± 0.1. After the 20 sessions of cold exposure, the RQ response to cold increased by 1.3% from 0.77 ± 0.05 to 0.78 ± 0.04.

Changes in body weight: Average body weight of the participants decreased slightly from 71.3 ±6.5 kg to 70.7±6.7 kg after 20 sessions of cold exposure. The weight decreased by ≥1 kg in five participants, did not change (<1kg fluctuation) in three participants, and increased in one participant by 1.3 kg. These changes were not found to be statistically significant (p=0.14) and changes in body weight did not statistically correlate to changes in RMR response to cold (p=0.16) or indices of HRV.
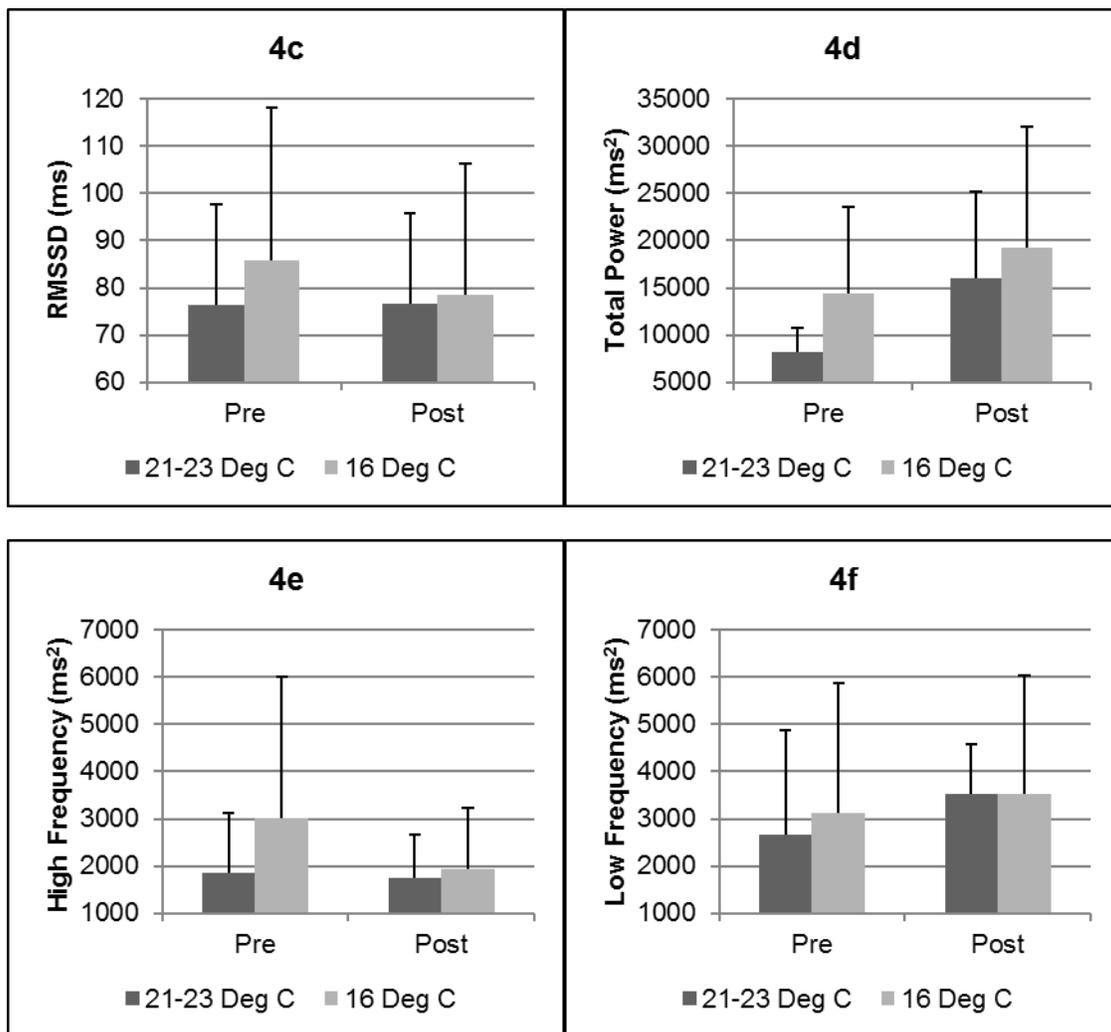
Figure 4c-f. HRV Responses. Data represent indices of HRV measured at normal temperature (21-23° C) and mild-cold exposure (16° C) both prior to (Pre) and after (Post) repeated cold exposure.

Table 3. Energy Expenditure and Heart Rate Variability for Phase 2, Post-Repeated Cold Exposure compared to Pre-Repeated Cold Exposure (n=9)

	Pre-Repeated Cold Exposure			Post Repeated Cold Exposure			
	21-23 ^{0}C	16 ^{0}C	Response	21-23 ^{0}C	16 ^{0}C	Response	P value
Resting Metabolic Rate (kcal/d)	1676.2 ±138.9	1860.7 ±190	184.5 ±235.3	1621.3 ±184.9	1791.7 ±234.2	170.3 ±298.4	p=0.24
Respiratory Quotient	0.78 ±0.06	0.83 ±0.1	0.05 ±0.1	0.77 ±0.05	0.78 ±0.04	0.01 ±0.06	p=0.15
Mean HR	59.7 ±10.9	60.1 ±9.7	0.46 ±14.5	56.5 ±8.3	60 ±9.2	3.5 ±12.3	p=0.39
Mean RR	1045 ±185	1043 ±198	-3 ±271	1097 ±1595	1042 ±153	-55.±220	p=0.45
RMSSD	76 ±21	86 ±32	10 ±39	77 ±19	79 ±28	2 ±34	p=0.40
Low frequency HRV (ms^2)	2660 ±2217	3121 ±2758	461 ±3539	3539 ±1039	3533 ±2495	-6 ±2703	p=0.97
High frequency HRV (ms²)	1843 ±1269	3021 ±2990	1178 ±3248	1747 ±922	1932 ±1290	185 ±1586	p=0.15
Total Power HRV (ms²)	8160 ±2544	14367 ±9186	6206 ±9532	16019 ±9232	19260 ±12798	3242 ±15780	p=0.37

Comparisons of pre intervention to post intervention responses upon cold exposure to 16° C from normal temperatures at 21-23° C indicated no significance (p>0.05).

Heart rate and heart rate variability: Mean HR was not significantly affected after 90 minutes of exposure to 16^0 C ambient temperatures. Responses in mean RR, RMSSD, Low Frequency, High Frequency, and Total Power (Low Frequency + High Frequency), as shown in figures 4c-f, were not statistically different between normal temperature and after 90 minutes of cold exposure.

Dietary Intake: Group average dietary intake increased 28% from 1984 ±652 kcal/d during pre-cold exposure to 2422 ±627 kcal/d post-cold exposure (p=0.23). Group average carbohydrate intake remained at 55 ±9%during both testing periods (p=0.99).   Average lipid intake decreased from 22 ±4% to 18 ±4% (p=0.03) while average protein intake increased from 23 ±11% to 27 ±7% (p=0.43) from pre to post cold exposure respectively.

CHAPTER 5 - DISCUSSION

The primary goal of this study was twofold: (1) To examine the acute resting metabolic rate response to mild cold exposure of 16° C and the acute autonomic response to two cold exposures of varying degree (i.e. 16° C and 4° C) through heart rate variability (HRV) indices; and (2) to examine whether 4 weeks of daily exposure to bouts of low temperature of 4° C would result in adaptations in the metabolic and autonomic responses to acute cold exposure. The data indicate that acute exposure to 16° C resulted in autonomic activation and increased RMR. Four weeks of daily exposure to cold temperature did not significantly alter the acute responses to cold exposure. Importantly there was no evidence of a change in autonomic balance toward a hyper-adrenergic state, following four weeks of daily exposure.

Participants

Participants tested were similar to those measured in other studies of cold exposure (Marino, Sockler, & Fry, 1998; van Marken Lichtenbelt et al., 2009). All subjects were deemed healthy, with normal bodyweights, but between the 10th and 20th percentile rank for body fat percentage for 20 to 29 year old males (NHANES III). Due to the nature of the study design (i.e. proof of concept, pilot study), overweight subjects were avoided.

Effects of Acute Cold Exposure on Resting Metabolic Rate and Heart Rate Variability (Phase 1a): Exposure to Mild-Cold (16° C) Conditions for 90 Minutes

The effects of cold exposure to 16° C for 90 minutes revealed a significant increase of 11% in the group average for RMR. Day to day variability in RMR of 26 ± 93 kcal/d has been measured in similar populations (Blond et al., 2010). The results in this study are similar to responses measured in previous cold exposure studies (van Marken Lichtenbelt et al., 2009; Yoneshiro et al., 2011; Marino, Sockler, & Fry, 1998). Most studies have reported increases in RMR ranging from 2-10 %, with increases depending on the extremity (Wijers, Saris, & van Marken Lichtenbelt, 2010) and duration (Johnson, et al., 2011) of the cold exposure. In regards to temperature, Celi et al. report a 6% increase in energy expenditure measured in a metabolic chamber with exposure to 19° C ambient temperature (Celi et al., 2010), compared to a 10% increase in RMR with exposure to 16° C ambient temperature for two hours (van Marken et al., 2009). Importantly the measure of energy expenditure in Celi (2010) and van Marken Lichtenbelt (2009) were obtained in a metabolic chamber without control of activity (Celi et al., 2010; van Marken Lichtenbelt et al., 2009). In contrast, energy expenditure was measured in the present study in a very controlled environment.

The underlying belief is that the increase in energy expenditure with acute cold exposure may be due to increased activation of the sympathetic nervous system, brown adipose tissue activation, or both. Activation of the sympathetic nervous system stimulates beta- 3 adrenoreceptors which results in an increase in thermogenesis and lipolysis (Krcmar et al., 2006). Activation of brown adipose tissue is also secondary to beta-3 adrenoreceptor stimulation. Activation of brown adipose tissue may further

increase thermogenesis and lipolysis (Cannon & Nedergaard, 2004). It is important to understand that the role of brown adipose tissue in human thermogenesis is in the early stages of investigation and not without criticism. In fact, some believe brown adipose tissue disappears shortly after the perinatal period in humans, whereas others report that PET imaging using the glucose analog (18)F-FDG has shown unequivocally the existence of functional brown adipose tissue in adult humans (Yoneshiro et al., 2011; Muzik et al., 2013). Given this area of research is still in its infancy it is not entirely understood what mechanisms are at play that stimulate brown adipose tissue and results in increasing thermogenesis. What is theorized is that brown adipose tissue is the main contributor of non-shivering thermogenesis, secondary to localized stimulation (through norepinephrine), causing a breakdown of triglycerides. The free fatty acids from the breakdown of the triglycerides stimulate uncoupling protein-1 (UCP1). UCP1 is normally inhibited by the presence of ATP in the cell. However, due to the free fatty acid stimulation, UCP1 is activated which then leads to cellular respiration without production of ATP. Instead, cellular respiration is used to produce heat instead of fuel (Cannon & Nedergaard, 2011). Clearly further research is needed to determine how much of the increase in energy expenditure in response to acute cold exposure is a consequence of brown adipose tissue stimulation and/or what other mechanisms are at play.

The HR response to cold exposure we observed is somewhat inconsistent in the literature with some studies reporting an increase with cold water immersion exposure (Jansky et al., 2006) and others a decrease (Makinen et al., 2008; Celi et al., 2010) upon exposure to cold ambient temperatures. In those previous reports, average change in HR was typically dependent on the severity and duration of cold exposure

(Launay & Savourey, 2009). In most studies, HR initially increases when a person is acutely exposed to a cold environment. However, as the cold exposure persists HR tends to return to normal. For example, Marino et al. (1998) reported an elevation of HR after 30 minutes of cold exposures with values returning to pre-exposure levels after 60 minutes (Marino, Sockler, & Fry, 1998). The present study did not find significant changes in HR following 90 minutes of exposure. This is consistent with Okamoto-Mizuno et al. (2009) and Makinen et al. (2008), suggesting the overall exposure did not serve as a significant challenge to the heart (Okamoto-Mizuno et al., 2009; Makinen et al., 2008).

Despite no significant changes in HR responses to acute cold exposure, the data present evidence to support activation of the autonomic nervous system. Specifically, both the low frequency (17%) and high frequency (64%) components of HRV increased, though not statistically significant, in response to cold exposure. The observed responses were similar to those found in other studies with low frequency increasing 16-29% and high frequency increasing 25-45% (Castellani et al., 2007; Celi et al., 2010). Thus it appears the autonomic nervous system was triggered by the cold exposure. Importantly, both branches of the autonomic nervous system were activated. Interestingly, in most cases of acute stress, e.g. exercise, the parasympathetic nervous system contribution of HR control is immediately removed followed by a slower increase in sympathetic nervous system activity (Paton et at., 2005). However, in this trial, both arms of the autonomic nervous system were activated in response to acute cold exposure as evidenced by an increase in total power. The mechanisms by which acute cold exposure activates the autonomic nervous system is hypothesized as follows: (1) a

decrease in skin temperature brought on by cold exposure, results in; (2) autonomic

nervous system activation, which in part triggers peripheral vasoconstriction (Launay &

Savourey, 2009); which leads to (3) shunting of blood to central tissues to protect the

core temperature; (4) contributing to an increase in central blood pressure; followed by

(5) activation of the baroreceptor reflex; and (6) normalization of HR and blood pressure

(Lanfranchi & Somers, 2002). Arguably, both arms of the autonomic nervous system will

be activated in this hypothesis, resulting in an increase in total power, as reported in this

study. Paton et al. (2005) suggest that the heart receives input from both sympathetic

nervous system and parasympathetic nervous system to varying degrees at all times

which allows for more efficient control of cardiac function. This infers that autonomic

nervous system control is more a balance of sympathetic nervous system to

parasympathetic nervous system activity to achieve the optimum reaction to external

stimuli and return to resting state. This is described as the "yin and yang of cardiac

autonomic control" (Paton et at., 2005).

Effects of Acute Cold Exposure on Resting Metabolic Rate and Heart Rate Variability (Phase 1b): Time Course of Exposure to Moderate-Cold (4° C) Conditions

Twenty minutes of cold exposure to 4° C did not significantly alter mean HR.

Consistent with exposure to 16° C, significant increases in most measures of HRV (High

Frequency, Total Power, and RMSSD) were observed. The most significant increase

was observed within the first 5 minutes of the cold exposure, followed by a gradual

return toward baseline values over the remaining 15 minutes. The initial responses, in

part, can be attributed to a change in position (i.e. participants were moved into the

chamber in an upright position). However, the fact, most of the components of HRV

(Low Frequency, High Frequency, Total Power, and RMSSD) remained elevated throughout the 20 minutes suggests the change in temperature also played a significant role. It is known that a change in posture induces a significant increase of HR with an increase of the low-frequency/high-frequency ratio of HRV (Jauregui-Renaud et al., 2001). However, the effect of postural changes is short-lived with evidence of a return to baseline within 5 minutes after a person is returned to the original position (Seiler, Haugen, & Kuffel, 2007). Given the HRV measures remained elevated for the remainder of the 20 minutes of cold exposure argues in favor of a temperature-induced effect as well.

Collectively, acute exposure to 16° C and 4° C confirms there is activation of the autonomic nervous system. Such activation could contribute to an increase in energy expenditure, more than likely secondary to stimulation of the beta-3 adrenoreceptor stimulation (Bateman et al., 2012). Interestingly, the present data do not indicate that acute cold exposure triggers a hyper-adrenergic state (evidenced by an increase in total power), which could be associated with deleterious consequences (de Morree et at., 2013). Therefore, results measured during the acute cold exposure testing support the hypothesis that acute cold exposure would increase RMR. However, although increases in HRV were measured, the indices of HRV did not indicate the inception of a hyper-adrenergic state due to acute cold exposure

Effects of Acute Cold Exposure on Resting Metabolic Rate and Heart Rate Variability Before and After 20 Sessions of Cold Exposure (Phase 2)

Following 20 sessions of cold exposure there were no statistically significant differences for either metabolic or autonomic nervous system responses to an acute mild-cold (16° C) exposure compared to normal room temperature (22° C). Similar to pre-repeated cold exposure, RMR also increased ~11% in response to acute cold, following the 20 session cold exposure intervention. This suggests the same mechanisms that stimulated the increase in RMR at baseline were still effective after the 20 sessions of cold exposure. However, the autonomic nervous system response to acute cold exposure following the intervention appears to have been slightly attenuated. Specifically, after repeated cold exposures, there was little noticeable low frequency activation, yet high frequency activation was still present during the acute cold exposure. The activation of the parasympathetic nervous system in absence of sympathetic nervous system stimulation has been noted elsewhere as well (Cannon & Nedergaard, 2011), and may be due to the increased sensitivity of measuring parasympathetic nervous system activity due to less interference caused by the sympathetic nervous system component (Taylor et al., 2001). The measurement of low frequency mainly corresponds to sympathetic nervous system activity. However, a portion of parasympathetic nervous system activity, normally attributed to high frequency, is also present in low frequency measurement (Akselrod et al., 1981). Therefore, it may be easier to detect changes in the parasympathetic nervous system due to reduced sympathetic nervous system activity.

The apparent reduction in sympathetic nervous system activation following the period of chronic exposure to cold, yet a consistent increase in metabolic rate does not exclude the possibility that brown adipose tissue stimulation may have significantly contributed to the increases in energy expenditure (Celi et al., 2010). An increase in metabolic rate in the absence of generalized sympathetic nervous system activity may imply a localized autonomic nervous system effect. Cannon and Nedergaard (2010) suggest that brown adipose tissue activation is due to localized sympathetic nervous system activation at the site of brown adipose tissue (Cannon & Nedergaard, 2011). It is important to recognize that HRV provides indirect information about autonomic nervous system activity through end organ responses. Subsequently, it does not allow inferences about localized effects at other organ sites. Thus the present results do not exclude the possibility that brown adipose tissue was significantly involved in the increased thermogenesis.

Overall, it does appear (although not statistically significant) activation of the autonomic nervous system was slightly less after the 20 sessions. For example, total power, or overall autonomic nervous system responsiveness to acute cold exposure, increased 20% after the 20 sessions, vs 76% prior to chronic exposure. The change in total power is mainly due to the decreased response of the high frequency component, which indicates parasympathetic nervous system activity, with very little change of the low frequency component, which indicates sympathetic nervous system activity. Specifically, the high frequency and low frequency responses to the acute cold exposure decreased from 64% to 11%, and 17% to -0.2%, after the 20 sessions of cold exposure, respectively. This could imply an adaptive process that altered activation of

the autonomic nervous system following repeated cold exposure. The current findings are in line with interventions such as exercise training which report a decrease in total power (Lee, Wood, & Welsch, 2003). However, the present findings stand in contrast with other studies of autonomic nervous system adaptation to cold exposure with reports of an increase of 50-54% measured in Total Power, High Frequency, and Low Frequency indices of HRV (Makinen et al., 2008) after 10 days of repeated cold exposure at 10° C for 2 hours. However, the temperature used in that study was much lower, which may have triggered shivering in which the sympathetic nervous system is known to play a greater role. The fact that the metabolic response to cold exposure after chronic exposure was similar to the increase seen at baseline, with lower autonomic nervous system responsiveness, suggests greater receptor sensitivity or number, or that other triggers involved in raising energy expenditure could be at play.

A potentially interesting finding was that the RQ response to acute cold exposure may be changing after the 20 sessions. For example, at baseline, acute cold exposure resulted in an increase in RQ from 0.78 to 0.83, suggesting a shift in substrate utilization away from lipid metabolism. After the 20 sessions, the RQ response to acute cold did not change (0.78 to 0.79). If these data are robust, this would imply substrate utilization in favor of fat metabolism (Haman et al, 2002). This could be potentially beneficial in populations where fat utilization is preferred. Unfortunately, the differential responses before and after the 20 sessions of cold exposure did not achieve significance, allowing mere speculation at this time.

The effectiveness of cold exposure as a weight loss intervention may be questioned due to measured increases in intake during cold exposure in studies of

mice. However, in most instances this did not lead to an increase in body weight (Cannon & Nedergaard, 2009). Since no significant increase in body weight was measured, it is believed that most of the excess calories consumed are used to generate heat to counteract the cold and maintain body temperature. Therefore, the excess calories are used and not stored. This increase in thermogenesis has been shown most effective with a high fat diet and in animals with less insulating tissue (Cannon & Nedergaard, 2009). Although intake in relation to cold exposure has not been studied in humans, results from studies of cold exposure with mammals suggest that the same result would occur.

The data collected after 20 sessions of repeated cold exposure did not fully support or reject our second hypothesis due to failure to reach significance. However, although the metabolic response to cold was similar in comparison to the pre-repeated cold exposure measurement; there is indication of a decrease of autonomic nervous system activation due to cold exposure.

Limitations of the Study

Significant responses in RMR and HRV were measured during acute cold exposure. However, significant changes in RMR and HRV were not measured after 20 sessions of cold exposure in comparison to pre-repeated cold exposure measurements. The results indicated a trend towards decreased autonomic nervous system activation but were not significantly different from baseline; therefore there is a need to explore possible sources of variability.

This experiment was difficult to execute for a variety of reasons. In particular it was exceedingly difficult to recruit for this study, given the enormous amount of time commitment. Furthermore, since this was a pilot study that may provide initial data for future application, the subjects that participated were on average young, healthy, and arguably favorable body compositions. Clearly the impact of this experiment could be quite different in those whose body composition is less favorable (e.g. obese individuals). It is indicated that overweight individuals are less likely to increase thermogenesis due to cold exposure in comparison to lean individuals at the same temperature. This may be due to the insulating nature of fat, decreased activation of brown adipose tissue or both (Wijers, Saris, & van Marken Lichtenbelt, 2010). Therefore, future studies should explore the effectiveness and most effective dose of cold exposure to increase thermogenesis in obese individuals.

Dietary intake was not controlled throughout this study. Dietary intake has been shown to increase in cold exposure studies of mice yet the effect of cold exposure on energy intake in humans has not yet been fully realized (Cannon & Nedergaard, 2009). Therefore, the effect of cold exposure on intake during repeated cold exposure would have been of interest. There were significant changes in group average dietary intake that could have possibly impacted RMR, HRV, and/or weight loss results. However, the methods used to monitor dietary intake in this study was not ideal due to only one day of intake reported, and the time between the reporting day and measurement was not consistent across and within subjects. It should be recognized that for such trials a much larger budget is required to support the efforts by participants.

Limitations of HRV

HRV is a tool that measures the end-organ response to a stimulus with daily variations in HRV measured below 5% (Borresen & Lambert, 2008). In this case the heart. We cannot imply that the end-organ response to a stimulus is the same throughout the body. Thus it could be speculated that cold exposure triggers a localized autonomic nervous system response that is quite different from other locales.

There were other limitations that may have potentially influenced the experimental execution of this trial. These limitations have to do with positional shifts during moderate cold (4° C) exposure testing and from measuring RMR in two separate rooms. While the pre and post mild cold HRV measurement procedures were sound, the moderate cold exposure measurement could have been improved by decreasing participant movement when going from warm to cold environments and having participants remain in a supine position to limit any changes to the autonomic nervous system that may occur from postural change (Siebert et al., 2004). Future studies would also benefit from repeated measurement of HRV during the period of daily cold exposure to track changes in HRV over time. However, this would also require controlling for food intake in relation to measurement time. Since, feeding time may affect ANS response to cold due to role of the parasympathetic nervous system portion of the autonomic nervous system in the digestion of food (Himms-Hagen, 1972).

Another potential source of variability in our findings could be attributed to seasonal changes that can affect the basal as well as the responsiveness of the autonomic nervous system and metabolic systems to cold exposure. Although, the

climate where testing took place was controlled, seasonal changes were not controlled for in the current study. The measurements in this study took place from March 2011 to May 2012, with 4 participants tested between February and March. Metabolic responses to cold have been shown to be impacted by environmental conditions brought on by seasonal changes (De Lorenzo et al., 1999) and cold adapted individuals have been shown to have reduced sympathetic nervous system and metabolic response to cold (Makinen et al., 2008). In winter months, an individual normally resides in a thermo neutral, climate controlled environment. However, they are often exposed to temperatures similar to the intervention used in this study when they exit the climate controlled environment and are exposed to low temperatures in the external environment. Therefore, daily environmental cold exposure during winter months may possibly cause the same autonomic nervous system and metabolic changes as the intervention of this study. This may decrease the effectiveness of the intervention on individuals who have recently been acclimated to cold due to environmental exposure.

Relevance

Given the small number of individuals studied, the relevance of the present data must be presented with caution and remain largely speculative. It is imperative that all strategies are considered to combat obesity and protect and improve health and control the rising costs of healthcare. Many of the current strategies to reduce the burden of obesity call for individual behavioral changes (e.g. Increased physical activity and reduced caloric intake). Although these strategies have significant effects on those individuals motivated to make the necessary changes, they do not appear to slow down the on-going obesity epidemic. Consistent with previous work, the present data suggest

42

cold exposure may be an important strategy to raise energy expenditure (Ouellet et al., 2012; Johnson, Mavrogianni, Ucci, Vidal-Puig, & Wardle, 2011). Consequently cold exposure could be considered as a strategy that could combat obesity in a significant number of individuals, without requiring significant behavioral changes.

Although a significant decrease in average weight was not measured, increases in energy expenditure indicate cold exposure as a possible weight loss strategy. Not controlling for energy intake, along with the short duration of the study, could explain why more dramatic losses in weight were not seen.

The 11% increase in RMR as reported in this study would yield an additional 220 kcal expended for an individual with 2000 kcal/d energy requirement. Using the 3,500 calories per pound rule (Wishnofsky, 1952), if energy intake is maintained this would theoretically equate to a loss of 23 pounds over the course of a year. The impact of such an intervention would be invaluable to the fight against obesity. Perhaps of additional interest is the suggestion that repeated cold exposure may contribute to preferential utilization of fats as a fuel source. This could further support cold exposure as a strategy to fight obesity.

Further investigation is needed to determine the optimum temperature to simulate weight loss. For cold exposure to be a successful therapy for obesity; the subject must be willing to remain in decreased temperatures for an extended period of time. Evidence points to lower temperatures leading to greater increases in energy expenditure (Celi et al., 2010; Yoneshiro et al., 2011). However, if the temperature is

found to be uncomfortable, the subject may compensate by adding clothing, which would decrease the effectiveness of the intervention.

Importantly, the increased metabolic rate in the absence of a hyper-adrenergic state suggests cold exposure is potentially a safe method of increasing metabolic rate. However, further research is needed to confirm this speculation.

Although the current study does not allow for major inferences, the data does provide enough information to warrant the development of a larger trial aimed at determining the efficacy of repeated cold exposure, which could serve as a strategy to raise energy expenditure and contribute to weight loss.

CHAPTER 6 - CONCLUSION

Significant increases in RMR were measured during acute cold exposure at 16° C, which supports a portion of our first hypothesis. Acute cold exposure at 16° and 4° C resulted in activation of the autonomic nervous system (measured by HRV indices), but there was no evidence of a change in autonomic balance toward a hyper-adrenergic state. This refutes the portion of our first hypothesis regarding response of the autonomic nervous system to acute cold exposure. The autonomic nervous system response to cold exposure of 16° C after 20 sessions of repeated cold exposure at 4° C did not fully support or reject our second hypothesis due to a failure to reach significance. However, although the metabolic response to cold was similar in comparison to the pre-repeated cold exposure measurement; there was an indication of a decrease in autonomic nervous system activation due to cold exposure.

These findings suggest repeated cold exposure may be an effective tool for significantly increasing metabolic rate without changing autonomic balance toward a hyper-adrenergic state. Given the present findings future research should focus on determining the effectiveness of repeated cold exposure in more varied populations and over a longer term period. It would also be useful to measure responses to cold in relation to brown adipose tissue activation and generation to better understand this aspect of thermogenesis, of which little is known in relation to humans.

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VITA

Jonathan was born in 1984 in Baton Rouge, Louisiana. He graduated from Central High School in 2003 and attended Louisiana State University, majoring in Kinesiology. During college, he worked as a physical therapy technician gaining experience with patients and knowledge of the concepts of physical therapy.

After completing his degree in the spring of 2007, he worked full time as a physical therapy technician at an outpatient clinic and during which, chose not to pursue a career in the physical therapy field. In May of 2008, he took a position at Pennington Biomedical Research center as a Research Associate in the Energy Metabolism Core Lab, where he is currently employed. During his time as an employee of Pennington Biomedical Research Center, he has developed a better understanding of the principles of research, metabolism, and human physiology, as well as fully participated in the research process as a co-investigator.

In the fall of 2009, he enrolled as a graduate student in the college of Human Sciences and Education. Deciding on the thesis path to a Master of Science degree, he began to work with Dr. Michael Welsch as his advisor and mentor. With the assistance of Dr. Welsch, Jonathan began work on his thesis project in the spring of 2011. He defended his thesis in April of 2013 in order to be awarded a Master of Science in Kinesiology.

Jonathan plans to pursue a career as a high school biology instructor.