THE EFFECTS OF COLD EXPOSURE AND ENERGY DEFICIT ON ENERGY BALANCE

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Thesis submitted to the University of Ottawa

in partial Fulfillment of the requirements for the

Doctorate in Philosophy Human Kinetics

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THESIS ABSTRACT

The main objective of this thesis was to examine the effects of cold exposure (CE) on energy intake (EI), energy expenditure (EE), hunger, and body weight in individuals living with obesity and determine its feasibility as a weight loss tool. The first article reviewed the current state of the literature, highlighted the gaps in knowledge surrounding individuals living with obesity and EI in CE research, and identified the research questions to be addressed. The second article examined the effects of acute CE on energy balance, hunger, and thermoregulatory outcomes. CE caused both EI and EE to increase, but the magnitude of both changes was quite small while having no impact of appetite. Skin temperature, peptides, thermal comfort and thermal sensation all demonstrated that the CE stimulus was potent, but EE only increased by an average of 20.3±20.6 kcal over 90 minutes, a mere 12% of what lean individuals undergoing the same stimulus would experience. The third article examined the effects of three 8-week weight loss interventions on energy balance, body weight and appetite: a dietary restriction of 30% (DIET), 28 cold sessions (CE), or both stimuli together (DIET+CE). CE alone did not result in weight loss or significantly improve weight loss outcomes when combined with a dietary restriction. EE increased for all three groups post intervention, thermal sensation improved post intervention for all groups, and subjective appetite changed between the three groups. EI and skin temperature did not change significantly post intervention. For the first time, CE was shown to not significantly improve weight loss outcomes alone or when used in combination with a dietary restriction. High levels of thermal discomfort, minimal

increases in heat production during CE, and high dropout rates highlight the challenges in feasibility of using CE as a weight loss adjunct.

ACKNOWLEDGEMENTS

This PhD has been a long journey. I could not have accomplished it on my own and there are so many people who have made this possible. Firstly, I want to thank my supervisor Dr. Éric Doucet for providing continual guidance and support throughout this process. You have helped me through the ups and downs, kept me motivated throughout it, and most importantly taught me the difference between being critical and cynical, at least when it comes to research. I would also like to thank my thesis committee and evaluators Dr. Imbeault, Dr. Haman, Dr. De Lisio, and Dr. Melanson for accepting the invitation, providing valuable feedback, and helping me complete this journey.

Thank you to all my peers from the BMRU and at Lees, both involved in the project and outside of it, especially Hannah St John, Noémie Beauregard, Alexanne Larocque, and Luzia Jaeger Hintze. You all helped make this possible, provided so much support, advice, and patience throughout this endeavor. Also, to Dr. Travis Saunders for helping me find my way to the University of Ottawa from PEI.

A big thank you to all the volunteers that took part in the study. The programs you all went through were tough and I will forever be grateful for the time and commitment donated to this study. Also thank you to all the graduate and undergraduate students who helped run this trial, it was a massive undertaking, and your time was invaluable.

Thank you to my friends and family here in Ottawa who provided me with support, care, love, and encouragement, particularly when things were difficult. I came to Ottawa for a new challenge and have found a second family here because of Jane and David making it

feel like home, and Katie and Katie providing space to be myself and grow. I also want to thank my parents, Mary and Roddy, who taught me to always be resilient, honest, and take pride in everything I do, as well as my sister, Nicole, who inspires me daily and taught me to push myself constantly and consistently, even if I felt outmatched.

Finally, I would like to thank my wife, Nikola. None of this could have been done without your unwavering support, from keeping me functional during comps to celebrating every achievement along the way, to letting me ramble my thoughts and practice my presentations. I love you and would not be at this stage without you.

PREFACE

The research conducted within this thesis was funded by the Canadian Institute of Health Research and was approved by the University of Ottawa Research Ethics Board (H-09-19-4928).

The work presented herein is my own, and I take full responsibility for its content. K.M. and E.D. were involved in the conception and writing of this thesis, as well as interpreting findings and collaboration to write the articles described within and the thesis as a whole. Contribution by article are as follows:

Article 1: K.M. and E.D. conceived and designed the review. K.M. completed data acquisition. K.M. and E.D. analyzed data. K.M., F.H., E.D. interpreted data. K.M. and E.D. created the original manuscript. K.M., F.H., E.D. edited and revised the final manuscript.

Article 2: E.D. and F.H. conceived the study. E.D., F.H. and K.M. designed the study. K.M., A.L., N.B. and L.J.H. completed data acquisition. K.M., N.B., L.J.H., G.F. and E.D. analyzed data. K.M., A.L., N.B., L.J.H., G.F., F.H. and E.D. interpreted data. K.M. and E.D. created the original manuscript. K.M., A.L., N.B., L.J.H., G.F., F.H. and E.D. edited and revised the manuscript.

Article 3: E.D. and F.H. conceived the study. E.D., F.H. and K.M. designed the study. K.M., A.L., N.B. and L.J.H. completed data acquisition. K.M., N.B., L.J.H. and E.D. analyzed data. K.M., A.L., N.B., L.J.H., F.H. and E.D. interpreted data. K.M. and E.D. created the original manuscript. K.M., A.L., N.B., L.J.H., F.H. and E.D. edited and revised the manuscript.

In addition to the thesis manuscripts, a list of non-thesis related published articles from my PhD duration can be found in the Appendix.

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LIST OF ABBREVIATIONS

η_p^2 – partial eta squared

AgRP/NPY – agouti-related protein/neuropeptide Y

AUC – area under the curve

BAT – brown adipose tissue

BMI – body mass index

CE – cold exposure

DEXA – dual-energy x-ray absorptiometry

DIET – dietary restriction

DIET+CE – dietary restriction and cold exposure

EE – energy expenditure

EI – energy intake

FFM – fat-free mass

FM – fat mass

GLP-1 – glucagon-like peptide-1

HFSA – high fat savoury

HFSW – high fat sweet

LCS – liquid conditioned suit

LFSA – low fat savoury

LFPQ – Leeds food preference questionnaire

LFSW – low fat sweet

NST – non-shivering thermogenesis

PFC – prospective food consumption

POMC – proopiomelanocortin

PYY – peptide YY

REE – resting energy expenditure

RG_{ox} – carbohydrate oxidation rate

RF_{ox} – lipid oxidation rate

RMR – resting metabolic rate

SD – standard deviation

SNS – sympathetic nervous system

ST – shivering thermogenesis

T_3 – triiodothyronine

T_{sk} – skin temperature

UCP1 – uncoupling protein 1

VAS – visual analog scale

CHAPTER 1. INTRODUCTION

The prevalence of obesity continues to climb globally, with more than 1 billion people worldwide having a body mass index (BMI) above 30 kg/m^2 [1]. As such, finding weight loss interventions and lifestyle modification tools that can help with body weight regulation have become increasingly important to scientists and policymakers. Traditionally, exercise and dietary interventions, namely caloric restriction, have been used to create a state of sustained negative energy balance, requiring the mobilization of one's energy stores to meet caloric demands, thus decreasing body weight. This can be done by manipulating either of the two factors in energy balance: energy intake (EI) and/or energy expenditure (EE). Caloric restriction works by having individuals decrease their EI for a prescribed period, ideally indefinitely, causing EI to be lower than EE, leading to weight loss. Exercise increases metabolic rate through purposeful physical activity, thus creating an energy deficit (if EI remains unchanged), and inducing weight loss. However, humans possess many compensatory mechanisms that mitigate attempts to maintain negative energy balance, such as the decrease of resting EE (REE) [2-4], the increased rewarding value of food [2, 5, 6], and upregulation of appetite hormones that produce an orexigenic state [2, 4, 7]. Although weight loss can be achieved with caloric restriction and exercise [2], these interventions often produce less weight loss than expected, as the duration of the intervention is positively correlated to the level of compensation occurring for both restrictive and exercise interventions [2]. The efficacy of these compensatory mechanisms can be clearly seen when looking at long term success rates, as individuals tend to regain 80-95% of weight lost within 5 years [8, 9]. The difficulty and strain placed on the individual,

combined with the low levels of success and increasing prevalence of obesity have opened the door to various new options for weight loss.

Bariatric surgeries, such as gastric banding or Roux-en-Y, have been shown to produce decreases in excess body weight of 45.9-74.1% [10]. Though this is impressive, bariatric surgery comes with several potential complications, such as surgical complications, nutritional deficiencies, gastric ulcers, and neuropathy [11], as well as being irreversible. Pharmacology has also been explored, as a variety of medications have been used over the years, with the current and most successful option being incretin receptor agonists. These molecules have been shown to be extremely effective in producing weight loss, up to 22kg with tirzepatide, with minimal side effects [12]. However, these medications provide substantial financial barriers and, like any other weight loss intervention, must be used for life to continue to see their effect, and long-term impacts in humans are currently unknown.

With the "rediscovery" of brown adipose tissue (BAT) in 2009 by a number of groups [13-16], cold exposure (CE) has garnered interest as a potential weight loss tool. Given the high thermogenic potential of BAT, it has been repeatedly proposed that CE could represent an efficient strategy to increase total EE and thus possibly facilitate weight control and induce weight loss as well [17-22]. Despite the multitude of human and animal studies demonstrating the thermogenic potential of CE [23-25], there are several important questions that need answers before these claims are substantiated. First, most studies have only included individuals who were normal weight. As such, the effects of CE in individuals with obesity, who represent the target population for these weight loss claims,

remains largely understudied. Second, although the thermogenic potential of CE is well demonstrated in individuals who are normal weight, we know almost nothing as to the effects of this intervention on appetite control and energy intake. Finally, the question as to whether individuals with or without obesity would be able to tolerate repeated CE sessions for a sufficient duration to produce appreciable weight loss also remains uncertain. Before concluding that CE could represent an efficient and feasible weight loss strategy, the above questions need to be correctly addressed.

1.1 Objectives and Hypotheses

The goal of this thesis is to answer the following objectives and determine the accuracy of the following hypotheses:

1. Determine the effects of a single dose of CE on EE, EI and hunger in individuals living with obesity.
    a. EE was hypothesized to increase following CE compared to a thermoneutral control.
    b. EI was hypothesized to increase following CE compared to a thermoneutral control, but to greater magnitude than EE.
    c. Hunger was hypothesized to increase following CE compared to a thermoneutral control.
2. Determine the effects of chronic CE on the regulation of body energy stores alone and in combination with a dietary restriction in individuals living with obesity.

    a. Weight loss was hypothesized to be lesser in the combined dietary restriction and CE group compared to the dietary restriction alone group following the 8-week intervention.

3. Determine the effects of chronic CE on EI and hunger alone and in combination with a dietary restriction in individuals living with obesity.
    a. EI was expected to decrease to a lesser extent in the combined group than in the dietary restriction or CE alone group following the 8-week intervention.
    b. Hunger was hypothesized to increase by a greater extent in the combined group than in the dietary restriction or CE groups following the 8-week intervention.

The thesis has been broken up into three original articles. The first original article is a review published in Obesity Reviews that provides an overview of the state of CE research pertaining to the measurement of EE and EI. The second original article included individuals living with obesity in a single cohort to determine the acute impacts of a single dose of CE on subjective appetite, EI, EE, skin temperature, thermal comfort and sensation, and food reward and determine if sex differences occur with these outcomes. The third article investigated the feasibility of CE as a weight loss tool. This was done by randomizing individuals into three weight loss group: diet alone (DIET), CE alone (CE), or CE and diet combined (DIET+CE). Body composition was measured before and after 8 weeks of weight loss, as well as EI, EE, skin temperature, and subjective appetite.

CHAPTER 2. LITERATURE REVIEW

For the purpose of this literature review, Section 2.1 includes the first original article entitled: “Humans in the Cold: Regulating Energy Balance”, which details the effects of CE on EE and EI in animals and humans. This manuscript clearly outlines the gaps in knowledge related to the use of CE as therapeutic weight loss intervention. In Section 2.2, an update of available literature since the publication of this manuscript is presented and discussed, as well as a more extensive discussion pertaining to the impacts of CE on energy balance specifically in individuals living with obesity.

Complete reference:

Kurt McInnis, François Haman, Éric Doucet. **Humans in the cold: Regulating energy balance. Obesity Reviews**. 2020Mar;21(3):e12978.

doi:10.1111/obr.12978. Epub 2019 Dec 21.

2.1 ARTICLE 1
Received: 7 October 2019 | Revised: 25 October 2019 | Accepted: 25 October 2019
DOI: 10.1111/obr.12978

PHYSIOLOGY

OBESITY Reviews WILEY

Humans in the cold: Regulating energy balance

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Funding information
Natural Sciences and Engineering Research Council of Canada (NSERC Canada), Grant/Award Number: RGPIN/2016-05291; Natural Sciences and Engineering Research Council of Canada (NSERC Canada) to F. H., Grant/Award Number: RGPIN/2016-05291

Summary

For humans to maintain a stable core temperature in cold environments, an increase in energy expenditure (EE) is required. However, little is known about how cold stimulus impacts energy balance as a whole, as energy intake (EI) has been largely overlooked. This review focuses on the current state of knowledge regarding how cold exposure (CE) impacts both EE and EI, while highlighting key gaps and shortcomings in the literature. Animal models clearly reveal that CE produces large increases in EE, while decreasing environmental temperatures results in a significant negative dose-response effect in EI (r=-.787, P<.001), meaning animals eat more as temperature decreases. In humans, multiple methods are used to administer cold stimuli, which result in consistent yet quantitatively small increases in EE. However, only two studies have measured *ad libitum* food intake in combination with acute CE in humans. Chronic CE (i.e., cold acclimation) studies have been shown to produce minimal changes in body weight, with an average compensation of ~126%. Although more studies are required to investigate how cold impacts EI in humans, results presented in this review warrant caution before presenting or considering CE as a potential adjunct to weight loss strategies.

KEYWORDS

appetite, cold exposure, compensation, energy balance

1 | INTRODUCTION

The effects of cold exposure (CE) on energy expenditure (EE) have been well documented in humans (see Table 1). This is achieved namely through the activation of both shivering (ST) and nonshivering thermogenesis (NST)[1,2] or increases in voluntary muscle contractions. Collectively, this has led to the discovery of numerous metabolic benefits of CE, such as increased GLUT4 translocation, amelioration of peripheral insulin sensitivity,[3] and increases in cold-induced muscular glucose uptake.[4] Repeated CE also leads to a subjective decrease in the discomfort normally experienced in cold environments.[5] Studies have mostly focused on heat production while paying little to no attention to the effects of CE on overall energy balance. A number of authors have suggested that the increased EE caused by CE could represent a possible way to induce weight loss and to also improve weight management.[5-10] However, these studies for the most part have not investigated or reported the effects of CE on energy intake (EI). Although there would seem to be an increase in EI with CE in humans,[11,12] the data are scarce and inconclusive at best. Furthermore, studies pertaining to seasonal variations in environmental temperature have shown that body weight increases in the winter,[13,14] although this is associated with increased EI and decreased physical activity. The purpose of this review is to provide a comprehensive overview of available literature on the effects of CE on EI and appetite from both animal and human studies, as well as compensation in response to chronic CE. The interactions between CE and the central nervous system and appetite-related hormones will also be discussed.

Abbreviations: CE, cold exposure; EE, energy expenditure; EI, energy intake; NST, non-shivering thermogenesis; LCS, liquid conditioned suit; RMR, resting metabolic rate; SNS, sympathetic nervous system; ST, shivering thermogenesis; T3, triiodothyronine

TABLE 1 Cold exposure studies in humans performed in respiration chambers

Reference	Study Group	Duration	Intervention (RC)	EE Baseline, MJ/day	EE CE, MJ/day	EE % Change	EI Baseline, MJ/day	EI CE, MJ/day
Wijers et al., 2007[43]	13 healthy males, BMI 22.96±0.90 kg/m^2	84 h	16°C *vs* 22°C	11.47±0.11	12.06±0.17	+5.1	Fed in energy balance (no values given)	
Warwick and Busby 1990[41]	4 females, 6 males, BMI 27.6±6.8 kg/m^2	24 h	20°C *vs* 28°C	8.77±0.93	9.17±0.67	+5.0±5.5		8.92±1.04
Dauncey 1981[38]	9 healthy females, BMI 21.2 kg/m^2	30 h	22°C *vs* 28°C	7.72±0.13	8.26±0.19	+7.0±1.1		10.46
Chen et al., 2013[39]	31 healthy individuals, BMI 21-27 kg/m^2	12 h	19°C *vs* 24°C	7.09±1.24	7.46±1.16	+5.3±5.9	Fed in energy balance (no values given)	
Wijers et al., 2010[44]	10 males, BMI 20-25 kg/m^2 10 males, BMI 28-41 kg/m^2	48 h	16°C *vs* 22°C	11.35±0.32 12.92±0.40	11.60±0.32 12.97±0.32	+2.2 +0.004	Fed in energy balance (no values given)	
Westerterp-Plantenga et al., 2002[42]	9 healthy males, BMI 22.7±2.1 kg/m^2	60 h	16°C *vs* 22°C EB 16°C *vs* 22°C AL	12.2±2.2 12.9±1.9	12.9±2.0 13.4±2.0	+5.7 +3.9	11.9±2.2 17.0±2.4	12.7±2.0 17.9±3.5
Langeveld et al., 2016[40]	10 individuals, BMI 20.8-24.9 kg/m^2	2.5 h	18°C *vs* 22°C,	NP	48±14 kJ increase	NP	2740±567 kJ	2878±492 kJ

Abbreviations: AL: ad libitum; BMI: body mass index; CE: cold exposure; EB: energy balance; EE: energy expenditure; EI: energy intake; NP: not provided; RC: respiration chamber.

2 | WHAT IS COLD

In the context of this review, CE will be considered a decrease in environmental temperature that leads to an increase in heat loss and, consequently, to an increase in EE or oxygen consumption, in an attempt to maintain stable core temperature. EE increases are caused though the activation of ST and NST. There are various modalities for producing CE, including cold air, liquid conditioned suits (LCS), or cold water. It is important to note that all CE is not equal, as different magnitudes of heat transfer occur. Water causes the greatest heat loss as both conductive and convective heat transfers are greater in water than air.[15] This is supported by the observation that the thermoneutral ambient temperature for humans is 26°C on average, whereas the thermoneutral temperature of water climbs to 36°C.[16] Due to this, the different CE modalities and temperatures will impact EE differently causing a wide range of responses,[17] similar to varying exercise modalities and intensities. Finally, CE, when done properly, is carried out in a manner that does not cause cold injuries such as frostbite or hypothermia.

2.1 | Shivering thermogenesis

ST is defined as involuntary muscular contractions caused by decreases in skin and core temperatures.[2] There are large interindividual differences that influence the contribution of ST to total EE, which can be partially explained by the quantity and subsequent activation of brown adipose tissue (BAT).[2,18] It has been reported that ST decreases and NST increases with chronic CE, or cold acclimation, without changing total EE.[18,19] This decreased shivering could make CE more tolerable at low intensities, where lower levels of heat production are needed, and ST levels are lower.

2.2 | Nonshivering thermogenesis

NST is the blanket term for any cold-induced increases in EE not associated with ST. Recently, NST research has focused on BAT. BAT was originally thought to increase daily EE by up to 20% based on animal models, however, but more recent estimates range from 20 kcal/day to a 3 to 5% increase in daily EE for humans based on extrapolations from animal models.[20] The primary contributors to NST are believed to be mitochondrial proton leak and sarcoplasmic reticulum calcium cycling.[21] Briefly, mitochondrial proton leak is the passive permeability of protons across the inner mitochondrial membrane resulting in heat waste from incomplete ATP synthesis.[22] This is the primary mechanism occurring in the mitochondria-rich BAT, where uncoupling protein 1 (UCP1) uncouples oxidative phosphorylation, generating excessive heat.[23,24] Sarcoplasmic reticulum calcium cycling is, briefly, the pumping of calcium ions from cytoplasm to the sarcoplasmic reticulum, generating heat as a by-product. For more detailed reviews, refer to Blondin and Haman,[21] and Gamu et al.[25]

3 | COLD EXPOSURE IN ANIMALS

3.1 | Energy balance in animal models

Available literature on long-term cold acclimation studies using rodents (Wistar, zucker, and F344 rats, Swiss and C57BL/6J mice, and Syrian and striped hamsters) shows decreases in body mass as the ambient temperature decreases, as demonstrated by our reanalysis of published evidence in Figure 1 (r=.457, P=.037). In addition, through our compilation of results from existing literature, we were able to identify a significant negative dose-response relationship between EI and ambient temperature through which EE can be inferred. This inference can be made due to the increases in heat loss as temperature decreases, requiring a matched increase in EE to maintain a stable core temperature.[2] Indeed, a progressive decrease in ambient temperature is associated with a progressive increase in EI for as long as sufficient food is available, as seen in Figure 2 (r=-.787, P<.001). In fact, significant decreases in body mass were noted for hamsters[32] and for Swiss mice[29] with decreases in room temperature from 23 to −8°C and from 15 to −8°C, respectively, despite the observation that animals had access to *ad libitum* food quantities. However, this increase in EI is not able to fully compensate for increases in EE as there are decreases in body mass. This could partially be explained by the fact that rodents are capable of reaching shivering intensities near, or at their maximal oxygen consumption,[32] thus generating EE that may exceed the animals capacity to increase caloric intake accordingly. It is important to note that in comparison, humans can reach a maximum shivering intensity at around 5 times their resting metabolic rate (RMR), which corresponds to approximately 40% of maximal oxygen consumption.[2,12] Two studies exposed cold-acclimated lean rats to ambient temperatures at 5[28] or 10°C[27] for a period of 7 to 9 weeks. EI increased significantly by up to 100% over that measured in their control counterparts living at room temperature. When the cold-acclimated lean rats were returned to the thermoneutral environment upon completion of CE, they displayed a sharp decrease in EI. However, they were still consuming more energy than their control counterparts. This discrepancy slowly diminished over the 2 weeks that followed cold acclimation.[27,28] The increased EI following CE consequently caused a positive energy balance in the lean rats, during which Hori et al found that both 3- and 21-month old rats increased their body mass following the cessation of sustained CE.[27] CE appears to be a potent enough stimulant of EI and appetite

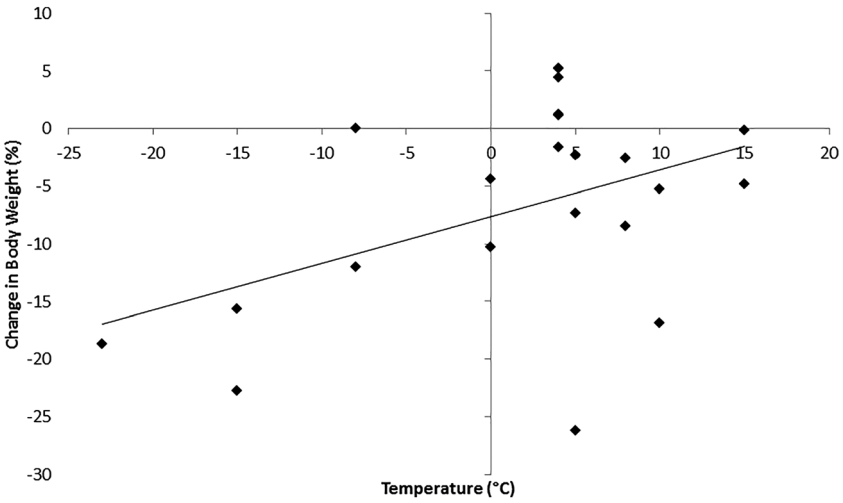

FIGURE 1 Correlation between relative changes in body weight (%) and decreasing ambient temperature in rodents (Wistar,[26] Zucker,[27] and F344 rats,[28] Swiss[29] and C57BL/6J mice,[30] Syrian[31] and Striped[32] hamsters). r=.457, P=.037

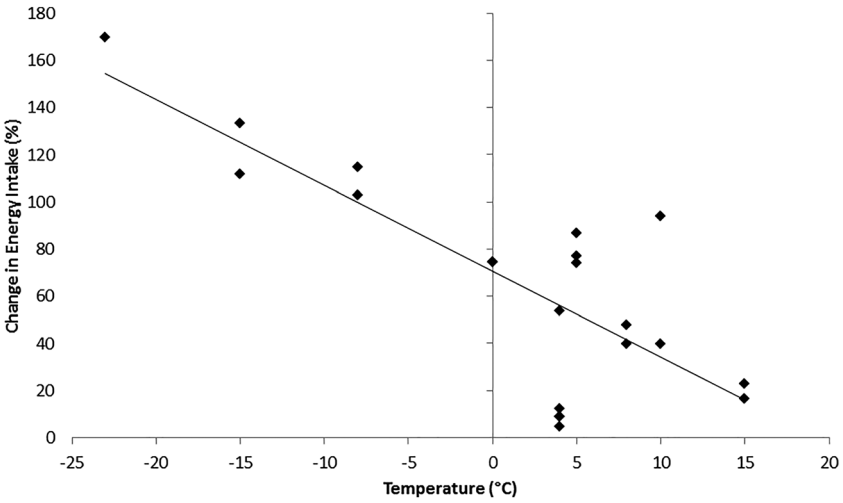

FIGURE 2 Correlation between relative changes in energy intake and decreasing ambient temperature in rodents (Wistar,[26] Zucker,[27] and F344 rats,[28] Swiss[29] and C57BL/6J mice,[30] Syrian[31] and Striped[32] hamsters). r=−.787, P<.001

that its impact is carried over after the cold acclimation is terminated. Interestingly, Hori et al[27] also used obese rats in the same study and reported that the obese and lean rats responded differently. The increased EI postacclimation was only seen in the lean rats, as the obese rats went from consuming ~8% more energy than their obese control counterparts during CE to ~13% less energy immediately after returning to a thermoneutral environment.[27] It is important to note that both lean and obese rats in this study increased body mass over time as per regular growth, and there were no significant differences in the body mass of the control lean and CE lean rats, as the increased EI compensated for increased EE. However, the growth of the CE obese rats was slowed compared with the obese control rats, demonstrating that the metabolic demands of CE can slow growth and may prevent increases in body mass under such conditions.[27] It is important to note that the control rodents were housed at room temperatures of approximately 23°C, which for some rodents can be outside their thermoneutral zone. This opens the possibility that the control rodents would also be undergoing a mild CE at room temperature and could be acclimatized to CE, impacting responsiveness to decreasing temperatures.[33]

3.2 | Metabolic demands of cold

Juvenile animals living in cold temperatures show significantly lower body mass and significantly higher EI as they age compared with their counterparts (often littermates) living in warm or thermoneutral temperatures.[28,34] Specifically, 3-month old F344 male rats had no changes in body weight over 9 weeks while living at 5°C, whereas their littermates living at 23°C gained an average of 46g over this time period. The EI of the cold exposed rats also increased by more than 100% of that observed in the control animals, again illustrating the metabolic demands of living in a cold environment.[28] Furthermore, Macari et al[34] reported a correlation of r=0.88 between EI and metabolic rate for piglets kept at 10°C when they were allowed to feed in *ad libitum* quantities. Both Swiss mice[29] and Syrian golden hamsters[31] decreased their physical activity time, quantified by the number of revolutions per night on a running wheel[31] or by video analysis,[29] in response to sustained CE. The Syrian golden hamsters completely stopped running on their wheels when exposed to 4°C for 14 days, while total movement for the Swiss mice decreased by 67% at −15°C compared with the baseline of 23°C. Feeding time also increased significantly for rodents in these two studies, as food was made available in *ad libitum* quantities; therefore, the rodents did not have to forage. A third study also found that intermittent (i.e., part day) CE resulted in increased EE during the exposures, but to a compensatory decrease in EE when returned to room temperature that resulted in no changes in body weight.[35] Exposing mice (C57BL/6J) to intermittent CE 3 days per week also stimulated EI and decreased leptin with exposure times of as little as 4 hours per day but resulted in no changes in body mass. These results again suggest that the EI increase is a compensatory mechanism to counteract the increase in EE during CE.[30] Furthermore, Yoo et al[36] found that using intermittent CE resulted in decreases in adipose tissue during initial CE sessions. However, compensation outside of the CE drove increases in adipose tissue, resulting in large daily fluctuations in adiposity. In the end, the study concluded with increased adiposity for these animals.[36] Given the large shivering capacity of rodents (i.e., to maximal oxygen uptake), other animal models need to be scrutinized before concluding that cold-stimulated increases in EE exert comparable effects on EI and compensation across species.

Macari et al[34] tested four groups of piglets, living at either 10 or 35°C and allocated either 300 or 600 g of food per day. They reported that piglets living at 10°C ate 533 g of food per day and maintained similar body mass to animals exposed to 35°C, which consumed 250 g of food per day. When allowed to feed in *ad libitum* quantities, the cold exposed piglets consumed twice as much energy as the heat exposed piglets. Interestingly, when all four groups were returned to 25°C, the cold exposed piglets maintained significantly higher EI for four days after the completion of CE (P<.05). The latter effect is similar to that noted in rat models discussed previously.[27,28] Collectively, studies employing animal models (rodents and pigs) report increases in EI to match the metabolic demands of CE, an increase that seems to be associated to the magnitude of the cold stimulus as suggested by our reanalyses of available studies presented in Figure 2, which resulted in strong inverse correlation (r=−.787, P<.001). In fact, this increase in EI ranged from 15% at 6°C to 170% at −23°C, which on average resulted in a 77% increase in EI. Whether or not CE results in comparable effects in humans, who present a much lower shivering response than smaller mammals, will be addressed in the following sections.

4 | COLD EXPOSURE AND ENERGY BALANCE IN HUMANS

4.1 | Acute cold in humans

To our knowledge, studies examining how humans respond to the cold began shortly after World War 2,[37] with a renewed interest occurring over the last decade with the re-discovery of BAT and its thermogenic capabilities in humans.[38] Many recent studies looking at the effects of CE on EE in humans have been done using respiratory chambers. Typically, these studies included relatively healthy participants staying in a respiratory chamber for 2.5 to 84 hours, following a set schedule of activities, wearing fixed clothing, and consuming predetermined meals at two different temperatures. These studies have focused primarily on measuring changes in EE while feeding participants in energy balance to attenuate the effects of diet-induced thermogenesis. One of the first of these studies was performed by Dauncey in 1981,[39] in which nine women were exposed to either 28 or 22°C in a respiratory chamber for 30 hours while being fed a predetermined number of calories. The average EE was 7% higher at 22°C compared with 28°C. Table 1 presents a series of studies in humans where EE and/or EI were assessed in a whole-room indirect calorimeter. Overall, studies reported increases in EE of 0 to 7%,[39-45] with an average

increase of 4.3% or 430 kJ/day. In addition, the aforementioned studies reported relatively large interindividual EE differences in response to CE. Although there is no singular theory to explain these interindividual differences, some explanations have been proposed. Blondin et al[46] found that burst shivering rates are partially related to muscle fibre composition (i.e., fibre typing), which varies between individuals. There is also a large variation in ST for the same amount of heat production due to motor unit recruitment and the aforementioned differences in burst rates.[2] However, neither of these can explain the difference in total heat production between individuals. There are also difficulties in comparing studies, as the temperatures and durations of the exposure vary, making the cold stimulus different between the studies. As can be seen in Table 1, the majority of studies also included participants who were for the most part normal weight,[39-41,43-45] which limits the generalizability to individuals living with increased adiposity. Although more studies are needed, it is tempting to speculate that the response to CE, as far as EE is concerned, could be influenced by variations in morphology. In support of this postulation, a study by Quaade et al[47] had participants lay between two blankets infused with a solution of cold water and alcohol at 4°C for 30 minutes while measuring heat production. Upon separating participants into underweight, normal weight, and overweight groups, it was observed that the underweight increased their oxygen consumption to a greater extent relative to their baseline, when compared with the normal and overweight groups. However, it should be noted that the overweight still had significant increases in thermogenesis under the conditions described in this work.

Numerous studies (see Table 2) have used a LCS, a garment that can be perfused with cold water. Being able to perfuse the suits with a variety of water temperatures allows for a range of responses, from mild shivering (1-2 xRMR) to high-intensity shivering (~3-4 xRMR), as can be seen in Table 2. The EE increase due to CE elicited by the LCS ranges from 20.6[48] to 250.6%,[49] averaging 105.6%, depending on the temperature of the perfused water. Clearly, the LCS can be used for a more potent, yet controlled, stimulus than ambient air, with an average relative increase of 836 kJ/session or 105.6%, compared with 430 kJ/day or 4.33%, respectively.

As previously mentioned, humans are only able to increase their heat production to 4 to 5 times their RMR through ST and NST.[2,12] As such, if a link does indeed exist between cold-induced EE and EI, the effects of CE on EI may not be as pronounced in humans as what is seen in smaller mammals.

4.2 | Chronic cold in humans

A number of cold acclimation studies have been done where body weight was reported both pre- and post-acclimation.[4,10,50-54] Across these studies, the weighted average weight change for participant was an increase of 0.04±0.47kg. There were no significant differences in body weight pre- vs post-cold acclimation. It is important to note that the modality of exposure used in these studies varied widely, using ambient temperature changes,[4,10,53] LCS,[50-52] and cold-water

TABLE 2 Liquid conditioned suit cold studies in humans

Reference	Study Group	Duration	Intervention	EE Baseline, kJ/min	EE CE, kJ/min	EE % Change
Blondin et al., 2015[87]	12 healthy males, BMI 25.5±0.8 kg/m^2	3 h	25°C ambient *vs* LCS with 18°C water	6.7±0.3	12.2±0.8	+82.1
Ouellet et al., 2012[18]	6 healthy males, BMI 23.7-31.0 kg/m^2	2.5 h	25°C ambient *vs* LCS with 18°C water	7.74±0.36	13.64±1.30	+76.2
Gosselin and Haman 2013[88]	8 healthy males, BF% <15	3 h	22.7°C ambient *vs* LCS with 15°C water	4.74±0.11	8.72±0.62	+84.0
Blondin et al., 2017[45]	8 health males, BMI 24.5 kg/m^2	3 h	22.5°C ambient *vs* LCS with 18°C water	4.9±N/A	8.2±N/A	+67.3
Blondin et al., 2015[89]	12 healthy young males, BMI 25.4 kg/m^2 7 healthy older males, BMI 26.3 kg/m^2 6 diabetic older males, BMI 28.6 kg/m^2	3 h	23-25°C ambient *vs* LCS with 18°C water	6.7±N/A 5.0±N/A 5.0±N/A	12.1±N/A 8.4±N/A 9.2±N/A	+80.6 +68.0 +84.0
u Din et al., 2016[47]	7 healthy individuals, BMI 25.5±3.3 kg/m^2	~4 h	22°C ambient *vs* cooling blanket with 6°C water	4.94±0.82	5.96±1.67	+20.6
Blondin et al., 2014[50]	6 healthy males, BMI 24.5±1.2 kg/m^2	3 h	25°C ambient *vs* LCS with 18°C water	5.9±0.4	11.3±0.8	+91.5
Blondin et al., 2017[51]	8 healthy males, BMI 23.1 kg/m^2	4 h	21°C ambient *vs* LCS with 18°C water	5.0±N/A	9.6±N/A	+92.0
Blondin et al., 2011[90]	6 healthy females, BF% 27.8±2.4 Luteal Phase Follicular Phase	2 h	24.5°C ambient *vs* LCS with 4°C water	4.7±0.1 4.8±0.4	11.9±0.7 11.3±0.6	+153.2 +135.4
Blondin et al., 2010[91]	6 healthy males, BF% 12.1±1.4	2 h	23-25°C ambient *vs* LCS with 4°C water	6.2±0.2	16.5±0.5	+166.1
Haman et al., 2005[48]	6 healthy males, BF% 13.3±1.98 8 healthy males, BF% 12.7±0.8	1.5 h	25.5°C ambient *vs* LCS with 10°C water 25.5°C ambient *vs* LCS with 5°C water	5.9±N/A 5.02±N/A	13.7±1.2 17.6±1.1	+132.2 +250.6

Abbreviations: BF: body fat; BMI: body mass index CE: cold exposure; EE: energy expenditure; EI: energy intake; LCS: liquid conditioned suit; NP: not provided.

baths.[54] The temperatures and durations achieved within each exposure modality also vary greatly between studies, accenting the lack of standardization within existing literature as far as the cold stress is concerned. For example, EE in the cold ranges from ~1.1 to 2.7 xRMR across these studies. Interestingly, a near significant relationship emerged between change in body weight and cold intensity, as seen in Figure 3 (r=−.718; P=.069), indicating that a stronger cold stimulus resulted in greater weight loss, even if the magnitude of weight change averaged an increase of 40g. Using these changes in body weight and the values provided for cold-stimulated increases in EE, we calculated compensation. Compensation is defined as the difference between predicted and actual changes in body energy stores.[55] A positive compensation is indicative of a lesser than predicted weight loss, while compensation values over 100% are associated with weight gain. The average compensation for the aforementioned studies was 335%. Removing one outlying study[4] where compensation was 1593% resulted in an average compensation of 126%, reflecting a slight increase in body weight in these studies. It could be established that these studies represent an accurate depiction of how humans compensate to chronic cold stimuli in a free-living situation. As per the details provided in these studies, no instructions were provided to individuals as far as dietary intake was concerned (other than Lee et al[53]), meaning that participants were free to self-select foods during these trials.[4,10,50-52,54] Lee et al[53] had participants undergo a 1-month weight maintenance diet prior to a month of ambient CE with *ad libitum* food consumption while living in their laboratory. Given that the weighted average revealed no changes in weight during the trials, it could be assumed that EI increased to match the cold-induced increase in thermogenesis. However, it is also possible that individuals decreased free-living EE, as seen in animals.[31]

5 | COLD EXPOSURE, *AD LIBITUM* ENERGY INTAKE, AND APPETITE

To date, only two studies have directly examined the effects of acute CE on *ad libitum* EI. In the first of these studies, by Westerterp-Plantenga et al,[43] participants were assessed while in a calorimeter at either 22°C or 16°C and while fed in energy balance or *ad libitum* quantities. There were no temperature effects on appetite, hunger, fullness, satiety, thirst, or desire to eat. As expected, EE increased significantly when participants were kept at 16°C during both conditions (ie, energy balance and *ad libitum* feeding) by 700 and 500 kJ/day, respectively, but EI did not increase significantly between temperatures. Interestingly, the percentage of overeating while at 16°C (although not significant) was inversely correlated to the decrease in rectal temperature (r^2=−.7; P<.01). In other words, this means that participants who displayed the greater decreases in rectal temperature were also the ones in whom a greater increase in food intake was more likely. Increased EE was also positively correlated with increased EI at 16°C (r=.8, P<.01), but not 22°C (r=.6, P=.09). In the second study to investigate EI and appetite in humans during CE,[41] participants were acclimated overnight to 24°C in a respiratory chamber. Then, they either remained in the same room or were moved to an identical room at 18°C for 2.5 hours. After exposure at 24 or 18°C for 2.5 hours, participants were served an *ad libitum* meal. As expected, EE was significantly higher during the 18°C trial, an increase of 48±14 kJ compared to that measured during the control condition over the 2.5 hour exposure time. Although it was not significant, a clear trend denoting an increase in hunger of 29% following CE was observed compared with the control condition (P=.064). There are, however, some shortfalls to this study, such as the use of thermal cameras to measure skin temperature and shivering, and not feeding breakfast to participants, possibly skewing the amount of energy consumption at lunch.[56,57]

It is important to note that during the study by Westerterp-Plantenga et al[43] although it was not statistically significant, EI increased by an average of 900 kJ when individuals were exposed to 16°C, compared with the average increase in EE of 500 kJ. Similarly, in the Langeveld et al[41] study, EI increased by a magnitude of 138 kJ (P=.69), compared with a 48 kJ increase in EE (P=.01). These results seem to suggest that acute CE increases EI by a much larger magnitude than EE. There is also a concern that cold-induced changes in EI may require longer collection periods to capture, similar to the

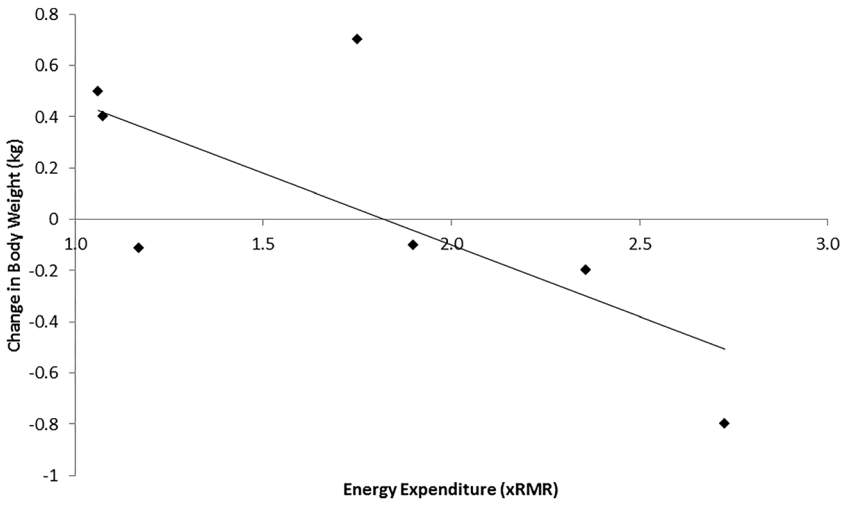

FIGURE 3 Correlation between changes in body weight (kg) and cold-induced thermogenesis. Energy expenditure is represented as a multiple of resting metabolic rate. Each point represents an individual study.[4,10,50-54] r=-.718, P=069

effects of exercise on appetite and EI.[12] Both these studies have looked at the acute effects of CE in humans, greatly reducing the window during which they can capture EI changes, as all intake measures occurred in the laboratory setting. These results suggest that CE is also orexigenic, although results are partial at best. Further studies are needed to clearly delineate whether chronic CE causes an increase in appetite and EI in humans, and the extent to which this alters energy balance and adiposity.

6 | COLD EXPOSURE, CIRCULATING APPETITE-RELATED PEPTIDES, AND THE CENTRAL NERVOUS SYSTEM

6.1 | Central nervous system

When exposed to decreased temperatures, cold-sensitive thermoreceptors activate the sympathetic nervous system (SNS), stimulating the release of norepinephrine, resulting in downstream lipolysis and release of free fatty acids. CE also results in decreases in triiodothyronine (T3) in the brain, further exciting the SNS and inhibiting the agouti-related peptide neurons, an effect linked to decreases in appetite and EI, which has been previously explained in more detail.[58] Conversely, peripheral T3 increases with CE,[58-60] resulting in increases in thermogenesis. Studies in a variety of rodent species[61-66] found significant increases in norepinephrine turnover,[63-65] plasma norepinephrine,[62,63] or urinary norepinephrine[61,66] following a CE stimulus between 3 and 6°C. Human exposures[44,67-69] have shown increases in norepinephrine ranging from 1.7[44] to 5.5[67] times their baseline value depending on the specific stress elicited by the CE. This sizable increase from the baseline state is due to norepinephrine being a primary stimulator of BAT.[1] Although most research regarding peptides and catecholamines is associative in nature, there has been considerable research using sympathomimetic compounds in both humans and animals to mimic increases in norepinephrine. Epinephrine and norepinephrine supplementation in rats have been shown to decrease subsequent EI by 35-55%.[70] Reuptake inhibitors also cause dose-dependent reductions in EI of up to 50%, accompanied by an increase in heart rate.[71] Various sympathomimetic compounds acting on the central catecholamine pathways have also been tested in humans, the majority of which have shown a decrease in body weight over time in humans[72,73] but have a variety of negative side effects, ranging from insomnia and irritability to worsening of dysrhythmia and angina.[73] The combined effects of norepinephrine and T3 changes open the possibility for short term negative energy balance, similar to exercise-induced[12] or adrenaline-induced anorexia.[70] The half-life of catecholamines, however, is quite short, meaning the anorexic state recedes quickly after removal of the stimulus. This is demonstrated, as mentioned above, by rodents rapidly decreasing body mass during intermittent CE and regaining it upon return to thermoneutral conditions,[35,36] creating a daily flux in body weight.

6.2 | Leptin

Leptin has been studied extensively in response to CE using a variety of rodent models,[26,74-81] as well as Suffolk rams.[82] Interestingly, all animals had significant decreases in serum leptin levels when exposed to the cold, ranging from −42 to −80% and averaging −59% of their thermoneutral control counterparts, with the exception of B6 mice[78,80,81] who had either no change or an increase in serum leptin levels. In humans, Chondronikola et al[83] found that mild CE in a LCS resulted in leptin values that were ~21% lower than during a thermoneutral control. This result has been seen in multiple other studies involving humans,[46,67,68,84,85] which could allow for decreased satiety and increased drive to eat, facilitating an increase in EI.[86] Zeyl et al[85] found that undergoing daily CE for 15 days using cold water immersion led to an increase in resting leptin levels on days 8 and 15 compared with day 1. During the CE leptin decreased on all 3 days, but the magnitude of the decrease was smaller on days 8 and 15, showing a possible adaptation effect.[85] Longer studies are needed to confirm this finding.

6.3 | Ghrelin

Ghrelin, a hormone primarily known for increasing hunger levels and desire to eat, has not been as extensively studied in CE as leptin. Korhonen et al[79] found that Siberian hamsters had an ~2.5-fold increase in plasma ghrelin levels after chronic exposure. Similarly, ghrelin has only been measured in humans under cold exposed conditions in three studies, where Tomasik et al[11] and Blondin et al[46] found 79 and 10% increases in ghrelin, respectively, whereas Chondronikola et al[83] reported a 27% decrease in ghrelin following acute CE. Collectively, the effect of CE on circulating peptides is relatively clear for norepinephrine and leptin, whereas controversy remains as far as ghrelin is concerned, but the wide variety of CE stimuli between studies complicates the comparison of results. What is more, establishing a link between the peptides and appetite and/or EI in the cold is simply not possible at this point in time, because neither appetite nor energy intake was measured in these studies. A homogeneous cold stimulus between studies as well as controlled measures of feeding would allow for conclusions to be more clearly drawn as to the effects CE on circulating peptides and EI.

7 | CONCLUSION

This review focused specifically on the effects of CE on both EE and EI, as well as various appetite-related peptides. Overall, the studies presented suggest that CE may have an orexigenic effect in humans, as it does in other animals. Decreased blood leptin levels brought on by CE create a potential orexigenic environment by stimulating appetite and, consequently EI,[85] although more work remains to fully understand how humans modulate EI in response to various cold stimuli. This could be achieved through studying EI for longer periods after acute CE, as well as examining chronic CE. Various other aspects have

been either neglected or ignored, such as the effects of acute vs chronic CE, the type of cold stimuli used, the relationship between the strength of the cold stimuli and degree of response, the standardization of the food environment, and the morphology of participants. Evidently, more studies are needed that address the entirety of energy balance and measure EI in an objective, verified manner to fully understand the impact of CE on energy balance in humans.

ACKNOWLEDGEMENTS

All authors have read and approved the final manuscript. François Haman is supported by the Natural Sciences and Engineering Research Council of Canada (NSERC Canada) to F. H. (RGPIN/2016-05291).

CONFLICT OF INTEREST

No conflict of interest was declared.

FUNDING

This work was supported in part by a grant from the Natural Sciences and Engineering Research Council of Canada (NSERC Canada) to F. H. (RGPIN/2016-05291).

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How to cite this article: McInnis K, Haman F, Doucet É. Humans in the cold: Regulating energy balance. *Obesity Reviews*. 2020;21:e12978. https://doi.org/10.1111/obr.12978

2.2 Cold Exposure, Obesity and Energy Balance

Cold induced thermogenesis responses do not appear to be the same between individuals who are lean and those living with obesity. Across a variety of modalities, temperatures, and durations, it has been shown that the increase in EE due to CE is smaller in individuals living with obesity [26-30], reported as being 20% [27], 32% [28] and 42% [26] of the increase seen in lean counterparts undergoing the same stimulus. This effect, however, is not seen when EE is corrected for fat free mass [15, 27]. The ambient temperature at which EE increases is also lower in individuals living with obesity compared to individuals who are lean [26], and EE also increases at a lower rate in cold water immersion for individuals living with obesity [29]. Skin and core temperature cooling rate is slower in individuals living with obesity [29, 30], and this population is able to maintain a higher core temperatures during exposure [29]. Collectively, it would appear that CE has a much lesser acute thermoregulatory impact on individuals living with obesity during compensable CE, needing either a stronger stimulus or longer duration to create the same increase in EE compared to lean individuals.

There are multiple possible explanations for why there are differences in CE response between people with differing levels of adiposity. Having an increased level of adiposity could decrease the amount of heat lost from the core to the environment due to the insulative properties of adipose tissue [31]. The thickness of subcutaneous adipose tissue has been shown to directly impact cooling rate with intramuscular measurement, with greater skinfold thickness increasing cooling time [32]. Interestingly, individuals with obesity have been shown to change skin temperature in a different manner than individuals

who are lean. Individuals living with obesity have a smaller decrease in distal skin temperature (-5.70 vs -4.48°C), but a larger decrease in proximal skin temperature (-.95 vs -1.48°C) [27]. This could be due to the thickness of subcutaneous tissue enabling a greater separation of their core from the external environment, making decreases in proximal skin temperature less impactful to core temperature compared to lean counterparts. REE is also typically higher in individuals living with obesity and, as such, has been suggested as a possibility for the difference in metabolic cold responses across varying levels of adiposity [27]. This is plausible for mild CE where lean individuals are only increasing their EE to the approximate value of the REE of individuals living with obesity, possibly due to the larger quantity of fat free mass of individuals living with obesity, and the small metabolic demand of the external stimulus. As mentioned previously, differences in EE due to mild CE can be seen when viewing EE in absolute values, but not when corrected for fat free mass [15, 27], meaning the higher basal metabolism of individuals living with obesity, who often posses greater fat free mass, could be sufficient for mild cold stimulus. The driving mechanisms behind differences in cold response between individuals who with or without obesity, as well as the best morphological predictors of cold response continue to be explored.

In the past five years several new studies have explored the impacts of CE on energy balance. Zakrzewski-Fruer *et al*. measured the impacts of acute ambient CE (10°C), finding minimal impacts on EI but a suppression of overall appetite across 5.5 hours when compared to a neutral control (20°C) in an environmental chamber [33], though no EE measurements were made for comparison. This is problematic, as without EE measurements it is impossible to determine the total effect on EB or compare the cold

response in different interventions. The study sample consisted of lean individuals, with body fat percentage ranging from 8.0-18.2%. Similarly, Coca *et al*. exposed individuals to a 24h cold, thermoneutral, or hot stimulus in an environmental chamber (16 vs 24 vs 32°C, respectively) with no change in EI (14.49±2.64, 14.06±2.85, and 14.96±2.99 MJ, respectively) or impacts on subjective appetite perception [34]. Once again, participants were lean as they had an average body fat percentage of 13.2±5.8%. Using the Leeds Food Preference Questionnaire (LFPQ), Coca *et al*. found that implicit wanting for high fat foods was slightly higher during CE compared to thermoneutral, suggesting a potential shift in food preferences brought on by the cold environment.

Unlu *et al*. [35] measured EI and EE across four 24h stays in a respiratory chamber, with participants being fed a prescribed eucaloric diet or in *ad libitum* quantities for both a cold and thermoneutral exposure, at 19.1±0.3 and 23.4±0.23°C, respectively. This study had a large range of body composition in their cohort, from 17.5-52.7 kg/m^2, though they averaged 32.4±8.6 kg/m^2. It was found that *ad libitum* EI was significantly higher during CE compared to control, an increase of 411±987 kcal over 24h. There were no changes to 24h EE during CE, likely due to the relatively mild 19°C ambient exposure, but respiratory exchange ratio significantly decreased during CE.

In combination with the studies of Westerterp-Plantenga *et al*. [36] and Langeveld *et al*. [37] there is a growing, though still small, body of research pertaining to the impact of resting, or passive, CE on energy balance. Together, these studies show through both trends and significance that EE increases by less than EI during CE, and show possible increases in hunger following acute exposure. However, the small sample sizes and mild

ambient cold stimuli create obvious limitations and areas for improvement. Also of concern is the lack of data focused upon individuals living with obesity and the absence of longitudinal studies testing the efficacy of CE to regulate body energy stores, as CE is often described as a potential weight loss tool [17-22, 38]. Currently, the only manner in which to infer the effects of chronic CE is by examining studies in individuals who are lean undergoing interventions not designed for weight loss as proxies, as previously discussed [25]. Recent studies have instead attempted to focus on the phenotyping of BAT activity in metabolically healthy or unhealthy individuals living with obesity [39].

2.3 Cold Exposure and Circulating Appetite-Related Peptides

Circulating peptides continue to be an avenue of interest for possible mechanistic impacts of CE on appetite regulation. The rationale for interest in these peptides, and peptides not covered in the first original article, are discussed below.

Leptin

Leptin was recently shown not to change following 24 hours of mild CE [40], however, chronic usage of whole body cryostimulation over 4 weeks resulted in a significant decrease in circulating leptin in individuals living with obesity [41]. This decrease in leptin is commonly seen across various types of CE [25, 35], an effect which can be viewed through multiple lenses. The primary physiological role of leptin is to serve as a marker of long-term energy stores [42], stimulating, among other things, proopiomelanocortin (POMC) - containing neurons in the arcuate nucleus, which cause downstream anorexigenic effects

by decreasing food reward and appetite while stimulating EE, and inhibiting agouti-related protein/neuropeptide Y (AgRP/NPY) containing neurons, which are orexigenic [43]. As such, CE lowering the levels of leptin would be expected to have orexigenic effects. Individuals living with obesity typically present with higher-than-normal levels of circulating leptin, often accompanied by central leptin resistance, where the efficacy of leptin is decreased [42, 44]. The impact of exogenous leptin administration is also minimal [45], though the exact mechanisms through which leptin resistance occurs are still unknown. The decrease in leptin associated with CE could be interpreted in two manners. First, the decrease in leptin is orexigenic. Second, it is possible that the decrease in leptin due to CE could be caused by a recovery of leptin sensitivity [46]. This could be indicative of an improvement of leptin function and possibly create an anorexigenic state.

Ghrelin

Ghrelin acts as both a short-term appetite stimulant and a lesser impactor of long-term body weight regulation. Ghrelin responses to CE in humans are less clear than that of leptin. Acute CE has been shown to increase ghrelin values, ranging from a marginal increases of 10% [34, 35, 47] to 45% above thermoneutral control [48]. Conversely, ghrelin has also been shown to not change during CE [33] or even decrease by 28%, although this was only a statistical trend [49]. The large variability in methodology and temperature combined with relatively small, typically lean sample sizes, and the small number of studies make it hard to draw conclusions about ghrelin's response to CE in humans. Currently, no studies measure the effect of chronic CE on ghrelin. This is of importance because ghrelin does not change acutely with exercise but long-term exercise

interventions capable of producing weight loss increase circulating ghrelin levels [50]. The suppression of ghrelin levels following food intake is also decreased for individuals living with obesity [51].

Ghrelin acts on a variety of central and peripheral areas of the central nervous system, but of key interest is that, acutely, ghrelin acts by stimulating AgRP/NPY neurons in the arcuate nucleus, increasing food intake and appetite, while inhibiting the activity of POMC neurons, suppressing anorexigenic signals [52, 53]. In a series of robust mouse model experiments, a potential orexigenic pathway for CE to influence appetite directly was discovered [40]. Using several knockout mouse models, hypothalamic AgRP neurons in the arcuate nucleus were found to be stimulated though CE. Cold-responsive neurons in the medial preoptic area that make excitatory synapses onto the AgRP neurons in the arcuate nucleus were discovered, with the inhibition of either neuron group mitigating the increase in EI typically seen in mice during CE, and the activation of the cold-responsive neurons increasing food intake. The mice were also injected with ghrelin at thermoneutral or cold conditions. Ghrelin infusion increased EI for both groups compared to control, but the combination of ghrelin and CE together had a greater effect than ghrelin alone, suggesting that CE may heighten ghrelin effectiveness.

Peptide YY

Peptide YY (PYY) is a peptide released from L cells in the intestines in response to food consumption, inhibiting gastric mobility and suppressing appetite [54]. PYY has been shown through meta analysis to have a smaller increase over time in response to food

consumption in individuals living with obesity compared to individuals with normal weight [55], leading to interest in increasing PYY for individuals living with obesity. CE has been found to have no impacts on PYY during ambient passive exposures at 10°C [33] and 16°C [34], but both studies have been conducted in individuals who are lean, leaving a gap pertaining to the effects of acute CE on PYY concentrations in individuals living with obesity.

Glucagon-like Peptide-1

Glucagon-like peptide-1 (GLP-1), a peptide hormone with a wide variety of functions, is a key regulator of appetite and energy homeostasis. It is produced in L cells in the intestine, α cells in the pancreas and the nucleus of the solitary tract [56]. As shown extensively with the use of medications binding to the GLP-1 receptor, increasing GLP-1 signaling pathways decreases EI, increases satiety, and decreases body weight [57]. CE has been shown to increase GLP-1 during passive ambient cold at 16°C when compared to 32°C, but not thermoneutral [34], or comparing thermoneutral to 10°C [33]. Similar to PYY, CE changes in individuals living with obesity have not been studied for GLP-1.

2.4 Summary

While CE has been repeatedly touted as a potential weight loss tool [17-22, 24] the evidence does not support this claim. Individuals living with obesity have a smaller increase in heat production due to CE compared to individuals of normal body weight [26-30], both individuals of normal body weight [25] and living with obesity [58] have been

shown to have minimal changes to body energy stores due to chronic CE that was not intended for weight loss, and CE has either minimal or orexigenic effects on EI [33-37]. Taken together, the acute impact of CE on EI in humans, especially individuals living with obesity, remains understudied and should be expanded to combat the misinterpretation that CE is a feasible weight loss tool, especially with direct comparison to EE measures to clearly show the full impact on energy balance. Further, no study has been conducted to determine the effects of chronic CE as a weight loss tool, alone or in combination with other weight loss methods. This creates a situation where CE can and is being recommended as a weight loss tool when it may have no impact or indeed be orexigenic and make weight loss more difficult. It is therefore imperative to conduct robust studies to explore the acute and chronic effects of CE on the entirety of energy balance, both EI and EE, as well as body energy stores to determine the feasibility and applicability of CE as a weight loss tool.

CHAPTER 3. METHODS

The methods used for data collection in this thesis are presented in the respective original research article to which they apply. This was deemed appropriate to avoid unnecessary repetition.

CHAPTER 4. ARTICLE 2

Energy Intake and Energy Expenditure are Minimally Impacted by Acute Cold Exposure in Individuals Living with Obesity

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Abstract:

Context: Cold exposure (CE) has been purported to be a possible weight loss strategy and to be anorexigenic, however, the impacts of CE on appetite and energy intake have not been fully explored, even less so in individuals living with obesity.

Objective: Determine the effects of a single dose of moderate CE on energy expenditure, intake, and appetite regulation in individuals living with obesity.

Design: Forty-seven individuals living with obesity (18 males) underwent two experimental sessions, one 90-min CE (10°C fluid using a liquid conditioned suit) and one control. Energy expenditure (indirect calorimetry), energy intake (food menu), subjective appetite (Visual analogue scales), food reward (Leeds Food Preference Questionnaire), appetite-related peptides (ELISA), skin temperature (Thermocouples), thermal comfort and thermal sensation (Likert scales) were assessed.

Results: CE produced a small (18%) but significant increase in energy expenditure over 90 minutes ($p<0.001$). Energy intake during lunch increased slightly (10%), yet significantly following CE ($p=0.008$) before decreasing for the remainder of the day ($p=0.049$). There were no differences in subjective appetite ($p>0.05$), but there was a decrease in the rewarding value of low-fat sweet foods ($p<0.05$). Leptin levels decreased significantly ($p=0.004$), whereas GLP-1 values were increased ($p=0.003$) in response to CE.

Conclusion: Although both energy expenditure and intake significantly changed, CE caused minimal impacts to energy balance in individuals living with obesity. When combined with the high levels of discomfort, these results question the efficiency of CE as

an intervention able to produce meaningful changes in body weight and composition in individuals living with obesity.

Introduction

Obesity is becoming increasingly prevalent Worldwide [59], with latest estimates of approximately 27% of Canadians having a BMI above 30 kg/m^2 [60]. Traditional weight loss interventions are challenging for individuals and have been shown to have high rates of regain following weight loss [2], creating an opportunity for novel interventions. With the rediscovery of brown adipose tissue in adult humans [13-16], cold exposure (CE) has gained traction as a possible avenue to increase energy expenditure (EE) and create the negative energy balance required for changes in body energy stores [17-22, 24]. Importantly, most studies documenting the effects of CE on EE have done so in relatively lean individuals [25, 26, 34, 37, 61, 62], whereas, individuals living with obesity typically have much smaller increases in EE during CE [26-30].

The energy intake (EI) and appetite regulation aspects of CE in obesity have been largely ignored, with all research occurring in individuals who are lean. A recent meta-analysis [63] highlighted a small increase in EI due to CE, however, only a handful of studies [33, 34, 36, 37] measured EI in a controlled, non-exercise environment, using ambient CE of 10-18°C. Available studies that assessed EI revealed trends toward increases in hunger level following mild ambient cold [37], with most reporting no statistically significant effects on EI [33, 34, 36, 37]. However, a common occurrence in these studies [34, 36] is prolonged stays in a metabolic chamber under *ad libitum* conditions, leading to relatively high EI values at thermoneutral, up to 32% above 24h EE, which could conceal any possible orexigenic effect of the cold. The EI increase in response to CE leads to increases in the thermic effect of feeding that could thereby reduce the reliance on shivering and

nonshivering thermogenesis to increase heat production. The latter is supported by an inverse correlation reported between decreases in rectal temperature and percentage of overeating (r^2=0.7, $p<0.01$) [36]. More importantly, none of these studies focused recruitment on individuals living with obesity. Indeed, only one study has measured EI in individuals living with obesity, where EI increased by 411 ± 987 kcal over 24h, but the CE stimulus was quite minimal using 19°C vs 23.5°C ambient exposures, creating no change in EE [35]. It thus seems relevant and important to investigate the possible orexigenic effects of CE in individuals living with obesity with a more potent cold stimulus, given that they represent the population most often included in weight loss intervention.

Therefore, the objective of this study was to determine the effects of a single dose of moderate CE on EE, EI, and appetite regulation in individuals living with obesity. We hypothesized that CE would increase EE, EI, and hunger levels in individuals living with obesity. We also hypothesized that CE would drive an acute state of positive energy balance, with EI increases being greater than increases in EE.

Methods

Eighteen males and 29 females living with obesity (33.2±3.9 and 33.7±4.4 kg/m^2, respectively) underwent two experimental sessions in a randomized order separated by a minimum 2-week washout. These participants were part of an 8-week trial investigating the impact of chronic CE on appetite and body weight regulation, ClinicalTrials.gov ID: NCT05076357. Participants were eligible for the study if they had a BMI between 27-40

kg/m^2, aged 18-55, and were sedentary (<2 time/week of 30 mins of continuous exercise). Participants were excluded for changes in body weight exceeding ±4 kg in the 6 months prior to enrollment, if they were peri- or post-menopausal (minimum 2 menses in previous 3 months), currently or planning to become pregnant, actively dieting or smoking, had a heart condition, diabetes, an axis 1 psychiatric disorder, thyroid disease, Raynaud's syndrome, excessive alcohol intake, use of opiates or stimulants, or were taking medications that could impact appetite regulation, including but not limited to selective serotonin reuptake inhibitors or thyroid medications. Sessions were completed within 8 days of the beginning of follicular phase for females to ensure consistent hormone levels and ease of phase identification. Females were not excluded for the use of oral contraceptives. This study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the University of Ottawa Research Ethics Board (H-09-19-4928). Written informed consent was obtained from all participants.

Experimental Protocol

Participants arrived at the laboratory after a 12 hour overnight fast, as shown in **Figure 1**. Height and weight (HR-100 Height Rod and HR-100, BWB-800AS, respectively; Tanita Corporation of America, Inc.) measurements, and a dual-energy X-ray absorptiometry scan (Lunar Prodigy; General Electric) were performed at 7h00. Resting energy expenditure (REE) was measured over 30 minutes following a 20-minute rest period beginning at 7h15. Subjective appetite visual analog scales (VAS) were completed before and after a standardized breakfast at 8h30, every 40 minutes into the postprandial period for 2 hours, as well as immediately before and after an *ad libitum* lunch at 12h30, as described

previously [64]. An indwelling catheter was inserted into the antecubital vein, with blood draws occurring fasted, immediately before, at the midpoint, and immediately following exposure, and before lunch.

Participants were then fitted with thermocouples to measure average skin temperature (T_{sk}) at 9h00. For the CE condition, each participant was then fitted with a three-piece liquid conditioned suit. Beginning at 9h45, a 30-minute postprandial EE measurement was collected before a 90-minute exposure to either ambient control or 10°C diluted antifreeze perfused in the liquid conditioned suit. Thermal comfort and sensation were collected at baseline and every 10 minutes during exposure with Likert scales, with EE measurements every other 15 minutes as previously described [65]. Following exposure, at 11h45 individuals completed a smell test, taste test, and the Leeds Food Preference Questionnaire (LFPQ), then consumed an *ad libitum* lunch at 12h30, as previously described [66]. At 13h00 they completed the LFPQ again and left the session with food boxes for the remainder of the day and following day. The entire sequence of measurements for both experimental sessions is presented in **Figure 1.**

Measurements

Cold Exposure

CE was achieved using a three-piece liquid conditioned suit (three-piece high density; Allen-Vanguard, Inc.) lined with tubing that was perfused with diluted antifreeze chilled to

10°C using a circulating bath (Neslab Refrigerated RTE-7 Bath Circulator, Thermo Scientific), as described previously [67].

Energy Expenditure

EE was calculated from indirect calorimetry measurements (O_2/CO_2) obtained with a metabolic cart (Vmax Encore 29N metabolic cart; Sensor Medics Corporation) and a ventilated hood. REE measurements were performed in a fasted state for 30 minutes following a 20-minute rest period, and then for 15 minutes following a 15-minute rest period for postprandial and during each exposure. Ethanol burning tests were performed to control the quality of measurements, as previously described [68, 69]. Our most recent analyses revealed a 4.3% and 0.39% difference for VCO_2 and VO_2 measures, respectively.

Carbohydrate (RG_{ox}) and lipid (RF_{ox}) oxidation rates were calculated using the following formulas, as previously described [70, 71], while assuming that protein oxidation contributed to 10% of the total EE measured:

$$RG_{ox}\ (g/min) = 4.59\ VCO_2\ (L/min) – 3.23\ VO_2\ (L/min)$$

$$RF_{ox}\ (g/min) = -1.70\ VCO_2\ (L/min) + 1.70\ VO_2\ (L/min)$$

Skin Temperature

Twelve thermocouples (DS1921H High-Resolution Thermochron iButton Devices, Maxim Integrated) were fitted to the participants at the following sites: forehead, upper back, lower back, abdomen, quadriceps, hamstring, front calf, back calf, chest, biceps, forearm,

and hand, as previously described by Gosselin and Haman [62]. A weighted average formula was used to determine average T_{sk} across the 12 sites [72].

Thermal Comfort and Sensation

Thermal comfort was measured using a 6-point Likert scale ranging from “very uncomfortable”, -3, to “very comfortable”, +3. Thermal sensation was measured using an 11-point scale ranging from “coldest I’ve ever felt”, -5, to “hottest I’ve ever felt”, +5. These scales are a modification of the scale used by Passias *et al* [73].

Standardized Breakfast

The standardized breakfast was comprised of whole-wheat toast (*D’Italiano*, 2 slices), peanut butter (*Kraft Smooth*, 20g), strawberry jam (*Smuckers*, 20g) and orange juice (*Tropicana,* 250g), equating to 446 kcal with a macronutrient breakdown of 63% carbohydrates, 25% fat and 12% protein.

Energy Intake

EI in and outside of the laboratory setting was measured using a validated [66] food menu containing a variety of foods that can be consumed within and outside of the laboratory. Participants chose items from the curated list and were served in *ad libitum* quantities, with food being weighed before and after consumption to determine the net food consumed using an electronic scale (Scout Pro SP2001, Ohaus Corporation). The consumed quantity was then analyzed with food processing software (Food Processor SQL, version 9.6.2, ESHA Research). Outside the laboratory setting, food and beverage

items were packed into plastic containers and bottles, then stored in two portable coolers for transport, one for the remainder of day 1 and one for day 2. Participants were asked to only consume food and beverages provided for the 2-day period.

Peptides assays

Venous blood samples were collected to measure total peptide YY (PYY), total glucagon-like peptide-1 (GLP-1) and leptin levels. An intravenous catheter was inserted into an antecubital vein of the non-dominant arm in a subsample of 15 participants (9 males) due to logistical restraints. All samples were placed into tubes containing EDTA. Blood samples were then centrifuged at 3500 rpm at 4˚C and stored at –80˚C until assayed. Leptin, GLP-1, and total PYY (includes both PYY 1-36 and 3-36) were assayed with commercially available ELISA kits (Human Leptin ELISA kit-EZHL-80SK, Multi Species GLP-1 total ELISA kit-EZGLP1T-36 K, and Human PYY (Total) ELISA kit-EZHPYYT-66K, Millipore Corporation, Billerica, MA). In our hands, intra-kit coefficient of variation for leptin, GLP-1 and PYY were 2.1%, 5.7% and 4.8%, respectively. All samples for every participant were assayed using the same kit.

Subjective Appetite

Subjective appetite was measured 8 times throughout the sessions, as highlighted above. Desire to eat, fullness, prospective food consumption (PFC), and hunger were derived from the following 4 questions: 1. “How strong is your desire to eat?” (Very weak – Very strong); 2. “How full do you feel?” (Not full at all – Very full); 3. “How much food do you think you could eat?” (Nothing at all – A large amount); 4. “How hungry do you feel?” (Not hungry at

all – As hungry as I have ever felt), respectively. Participants were instructed to place a vertical line along a 100 mm scale for each question, giving a score out of 100 for each aspect of appetite, as described previously [64]. VAS have been previously validated in single meal studies [74].

Leeds Food Preference Questionnaire

Participants completed the LFPQ, a validated computer-based test that measures food reward, immediately before and after *ad libitum* lunch, as described previously in greater detail [75]. The test compares 16 foods of the participants choice that are broken down into four categories (high-fat sweet [HFSW], low fat-sweet [LFSW], high-fat savoury [HFSA], low-fat savoury [LFSA]). It uses a forced choice task that compared each food option to every other option, where participants chose "which food they wanted more right now," determining the implicit wanting of foods. Participants were also shown each image accompanied by a 100-mm VAS asking how much they "liked" or "wanted" each option.

Olfaction

Olfactory performance was measured using Sniffin' Sticks (Burghart Instruments, Wedel, Germany) [76]. It consists of three tests: odor detection threshold, odor discrimination, and odor identification. Each test used scented markers in their respective tests. Threshold detection involved a stepwise repeated smelling of markers that contain varying concentrations of odor until the threshold is found. Discrimination involved having sets of three markers, two with identical scents and one with a different scent that must be correctly identified. Identification involved the presentation of 16 different scents that must

be identified by choosing from a multiple-choice list. The sum of the scores from the three tasks gave the olfaction score.

Gustation

Gustatory performance was measured using Taste Strips (Burghart Instruments, Wedel, Germany) [77]. This test used filter paper strips infused with a tastant (sweet, sour, salty, or bitter) of varying concentrations. The strip was placed upon the tongue, at which point the participant determined which of the categories they were tasting. The score received was the total number of correct identifications from each category, as well as the final value of all correct identifications allowing for a total and flavor specific objective measurement of gustatory changes.

Statistical Analysis

Changes in EE, substrate oxidation rates, subjective appetite, skin temperature, peptides, thermal comfort and thermal sensation, LFPQ, gustation, olfaction, EI and macronutrient consumption were analyzed using repeated measures ANOVA, with condition as a within-subjects variable (control and CE) and across time where applicable (varying per outcome) with sex as a between-subject factor. Effect size is also reported as partial eta squared (η_p^2), whereby values above 0.2, 0.5, and 0.8 were considered small, medium, and large effects, respectively. Data are presented as mean ± standard deviation (SD) unless otherwise specified. Statistical analyses were performed using Statistical Product and Service Solutions software, version 29.0 (Chicago, IL) with an alpha set at 0.05 to establish

statistical significance. Reported p-values were adjusted for multiplicity with the Bonferroni correction where necessary.

Results

Energy Expenditure

REE and thermic effect of feeding at time postprandial were not significantly different between conditions as seen in **Figure 2**. However, CE caused a significant increase in EE (condition by time $p<0.001$, $\eta_p^2=0.470$) from time 30 onward. EE was 18.0% higher during CE compared to control, as participants expended, on average, an additional 20.3±20.6 kcal over 90 minutes of CE. This increase in EE is mostly due to an increase in glucose oxidation during CE (condition by time, $p<0.001$, $\eta_p^2=0.178$), as there was no significant change in lipid oxidation between conditions (condition by time, $p=0.076$). There were significant sex by time ($p=0.024$) and sex by condition ($p<0.001$) interactions, indicating females increased EE by more than males during the CE condition.

Skin Temperature

Average T_{sk} was 33.0±0.7 vs 33.0±0.7°C for control and CE at time 0, respectively. Over 90 minutes of exposure, control T_{sk} increased slightly to 33.9±0.8°C, whereas CE T_{sk} decreased significantly to 24.5±1.1°C ($p<0.001$, $\eta_p^2=0.977$), as seen in **Figure 3A**.

Thermal Comfort and Sensation

Thermal comfort was comparable between conditions at baseline, with control and CE reporting an average of 2.4±0.7 vs 2.2±0.8 respectively, or approximately “comfortable”. Throughout experimental conditions comfort level significantly changed (condition by time $p<0.001$, $\eta_p^2=0.427$), as during control individuals maintained “comfortable”, averaging 2.0±1.1 after 90 minutes, whereas 90 minutes of CE decreased individuals to -1.2±1.5, or “just uncomfortable”, as seen in **Figure 3B**. There were no differences in thermal comfort between sexes.

Similarly, thermal sensation was comparable at baseline, with control and CE reporting an average of 0.7±1.0 vs 1.0±1.3, respectively, or “slightly warm.” Throughout exposure thermal sensation significantly changed (condition by time $p<0.001$, $\eta_p^2=0.548$), as control maintained “slightly warm”, averaging 0.8±1.1after 90 minutes, whereas 90 minutes of CE significantly decreased sensation to -2.9±1.5 or “cold”, as seen in **Figure 3C**. There were no differences in thermal sensation between sexes.

Energy Intake

Ad libitum EI at lunch was significantly different between CE and control sessions ($p=0.008$, $\eta_p^2=0.147$) as seen in **Figure 4**, with individuals consuming 957.3±361.8 vs 870.3±330.7 kcal, respectively (mean difference of 87.0±223.8 kcal more following CE). There were no differences in fat ($p=0.338$) consumption between conditions at lunch, however, carbohydrate and protein consumption were significantly higher following CE compared to control ($p=0.007$, $\eta_p^2=0.153$, $p=0.014$, $\eta_p^2=0.127$, respectively).

Ad libitum EI (p=0.049, η_p^2=0.085), protein (p=0.016, η_p^2=0.125) and carbohydrate (p=0.046, η_p^2=0.088), but not fat (p=0.183), consumption were lower for the remainder of Day 1 following CE compared to thermoneutral control, as seen in **Figure 4**. There were no significant differences in EI or macronutrient consumption during Day 2.

Sex was a significant between-subjects factor for total EI (p<0.001, η_p^2=0.217), protein (p<0.001, η_p^2=0.284) and carbohydrate (p<0.001, η_p^2=0.249) consumption at lunch, driven by females consuming fewer calories for all variables as there was not a statistical difference in proportions of macronutrient consumption.

Interindividual variability in T_{sk}, EE, EI are shown in **Figure 5**. Of note, there were no significant correlations between CE induced changes in EI and those in EE or T_{sk}. There were also no significant correlations between body morphology and heat production during CE.

Peptide Responses

There was a significant condition by time interaction for GLP-1 (p=0.003, η_p^2=0.263) and leptin (p=0.004, η_p^2=0.482) but not for PYY (p>0.05) plasma levels, as seen in **Figure 6**. GLP-1 levels were elevated during and following CE compared to control, while the leptin decrease over time was greater following CE. Sex was a significant between-subjects effect for both GLP-1 (p=0.002, η_p^2=0.546) and leptin (p<0.001, η_p^2=0.624), with females displaying lower GLP-1 concentrations and higher levels for leptin, but there was only a trend toward significance for a condition by time by sex interaction for leptin (p=0.057).

Subjective Appetite

There were no significant differences in hunger (p=0.527), desire to eat (p=0.100), fullness (p=0.826), or PFC (p=0.784) between cold and control conditions over time, as seen in **Figure 7**. A sex by time interaction was noted for PFC (p<0.001) indicating that females had a lower PFC score than males, irrespective of condition. No other sex differences were found.

Leeds Food Preference Questionnaire

There was as significant condition effect for the implicit wanting (p=0.014, η_p^2=0.156), explicit wanting (p=0.027, η_p^2=0.128), and explicit liking (p=0.030, η_p^2=0.124) for LFSW food types, with CE preference being lower for all 3 measures. A trend towards significance for condition was also seen for LFSA implicit wanting (p=0.068, η_p^2=0.090), with CE being higher than control. There was also a significant time effect (p<0.05) for implicit wanting, explicit wanting, and explicit liking for HFSW, LFSW, HSFA and LFSA measures. There were no condition by time interactions or sex differences.

Olfaction

There were no significant condition or sex effects for threshold, identification, discrimination, or total olfaction scores (p>0.05). Data are not presented.

Gustation

There were no significant condition or sex effects for sweet, salty, sour, bitter, or total gustation scores (p>0.05). Data are not presented.

Discussion

The objective of this study was to determine the acute effects of a single dose of CE on EI, EE and appetite control in individuals living with obesity. Although no increase in hunger was found, our remaining hypotheses were supported, as it was found that both EE and EI significantly increased, however, the magnitudes are the important aspect. EE increased on average by 20.3±20.6 kcal over 90 minutes of CE, whereas EI during the *ad libitum* lunch increased by 87.0±223.8 kcal, creating a mean net positive state of energy balance of 66.7 kcal after 90 minutes of CE for individuals living with obesity. However, EI was lower for the remainder of the day following CE, likely resulting in a marginal effect on energy balance over the 48h period.

Energy Expenditure and Thermoregulatory Outcomes

While EE increased significantly during CE, the magnitude of the response was quite small. In comparison, a similar CE experiment was previously conducted using the liquid conditioned suit perfused with 10°C diluted antifreeze for 120 minutes where participants had an average body fat percentage of 13.3±1.9 [67]. The EE of the lean participants increased by 160% after 90 minutes, compared to 18% in our study population. This supports the historical view of individuals living with obesity having lesser increases in EE during CE [27-30]. Furthermore, T_{sk} decreased from 34.0±0.02°C to 27.2±0.02°C in lean individuals [15, 27], whereas T_{sk} decreased from 32.9±0.2°C to 24.5±0.1°C in the current cohort, denoting a larger decrease in skin temperature amongst individuals living with obesity. Data presented here also corroborate individuals living with obesity having a

slightly lower skin temperature at thermoneutral [27]. The thermal comfort and sensation reported in the current study are also like lean individuals with skin temperature clamped to 27°C in the liquid conditioned suit [61]. Taken together, this shows that individuals living with obesity have a much smaller increase in EE in response to a similar CE stimulus, while also having a large decrease in skin temperature and possibly changes similar thermal comfort and sensation throughout the exposure. What is more, our results also highlight the interindividual variability in CE induced changes in EE, as previously reported [61]. Nonetheless, it is important to put this variability into perspective, as the strongest response was still ~80 kcal over 90 minutes including the thermic effect of breakfast as seen in **Figure 5**.

Sex Differences in Thermoregulatory Outcomes

Sex differences in thermoregulatory responses to CE display a wide array of results, likely due in part to the different methods employed to produce the CE stimulus. Here, we show that during CE females with obesity increased their EE to a greater extent than males, a result also seen in lean females [78]. Conversely, studies with lean individuals have found no difference in EE between sexes [61, 78, 79]. Sex differences in thermal comfort and sensation have mixed results, as it is often reported that females have greater thermal discomfort and report a fixed temperature as being colder than that reported by males [80, 81] and that males and females respond similarly [61]. In a cohort of individuals living with obesity we found no significant differences in thermal comfort and sensation between sexes.

Energy Intake

EI was found to significantly increase at lunch immediately following CE, but this was compensated by a lower EI for the remainder of the day. In corroboration with previous literature in lean individuals where no change in EI has been observed in varying durations [33, 34, 36, 37] and the singular study in individuals living with obesity [35]. Overall, CE had no impact on EI over 48 hours. The increase in EI seen at lunch predominantly came from an increase in carbohydrate consumption, which could possibly be due to carbohydrates being the main fuel source used for shivering during CE [82], even if we did not see a correlation between CE induced changes in carbohydrate intake and oxidation (results not shown).

Subjective Appetite

Contrary to our hypotheses, no differences in subjective appetite measured with VAS were found. Of note, available literature is more divided on appetite responses to CE. Coca *et al* found that hunger, PFC and overall appetite were lower pre-lunch during CE, but there was no difference between conditions when including values post-lunch [34]. Similarly, a trending increase in hunger during CE was found by Langeveld *et al* [37]. Conversely, no change in appetite has also been reported following ambient CE exposure [33, 36].

Food Reward

CE caused a significant decrease in the implicit wanting, explicit wanting, and explicit liking of LFSW foods. Interestingly, CE has also been shown to increase the implicit wanting of high fat foods [34], shifting preferences towards foods that are higher in fat and typically

more calorically dense. Intriguingly, the significant increase of implicit wanting for high-fat foods did not translate into increases of these foods at lunch, as we observed an increase in the carbohydrates and protein instead. This is in line with other results that have reported that changes in preference do not always translate into concomitant changes in intake [83].

Peptide Responses

Results from our sub-sample analysis revealed that plasma leptin concentrations decreased significantly more over time due to CE relative to control (-12.5% vs -30.0%, respectively), an outcome that reinforces the potency of the stimulus used in the current study as this depression in leptin has been repeatedly reported during and following CE [49, 84, 85]. This difference could also contribute to the acute increase in EI seen during the *ad libitum* lunch, although this was a short-lived effect as no differences in 48-hour EI measures were noted between conditions. Conversely, we report that plasma GLP-1 levels, which increases satiety [86], increased during CE. This gut peptide has been minimally studied during non-exercise CE, where it has been found to be unaltered compared to a thermoneutral control [33, 34], but elevated compared to a hot environment [34]. The exact mechanism through which CE could increase GLP-1 is unclear. However, GLP-1 secretion has been shown to increase through the activation of α1, α2 and β-adrenergic receptors in enteroendocrine L cell line GLUTag cells [87]. These receptors can be activated by norepinephrine, which has been repeatedly shown to increase in response to CE [88]. Together, it could be speculated that CE could be causing an orexigenic effect, decreasing leptin, and an anorexigenic effect, increasing GLP-1. More data are needed, especially

pertaining to GLP-1, to conclusively state the effects of CE on circulating peptides relating to appetite and body weight regulation.

Limitations and strengths

The cold-induced EE measurements here need to be interpreted in light of the fact that participants were in a postprandial state during the measurement period, as these EE measurements are most often done after an overnight fast [26, 37, 61]. Due to this, the thermic effect of feeding may have masked the true magnitude of EE increases in response CE in our population of individuals with obesity. The singular dose of CE used here is novel for understanding the acute impacts of CE on energy balance in individuals living with obesity, however, it is only a singular dose of CE and cannot be used to ascertain the impacts of repeated exposure on body weight regulation. The strengths of the study are in its large sample size, target population of individuals living with obesity, inclusion of females as participants, focus on cold alone (not in combination with exercise), and the standardization of the cold stimulus (ambient cold vs liquid conditioned suit), which can be seen by comparing EE and skin temperature of the previously mentioned lean individuals [67] to the other studies measuring EI and EE [33, 34, 36, 37]. Together, this study design has a greater generalizability of results pertaining to the acute impacts of CE on energy balance.

Conclusion

CE has been shown to have various benefits for individuals living with obesity or at risk of type 2 diabetes, including reductions in glycemia [89] and improvements in peripheral insulin sensitivity [90]. In this study, we show that a well standardized CE for 90 minutes decidedly impacted objective markers such as T_{sk}, cold comfort and circulating leptin in individuals with obesity. Despite these, EE was minimally increased by ~20 kcal over 90 minutes, a mere 12% of what has been observed in individuals with no obesity. The negligeable impact on EE as well as the overall absence of effects on EI, seem to make CE an unlikely approach for improved body weight control in individuals with obesity. This is even more likely considering the level of discomfort CE caused in the present cohort. Nonetheless, the chronic effects of CE in individuals with obesity remain to be fully elucidated.

Acknowledgements

This research was supported through a grant by the Canadian Institute of Health Research (CIHR) for E.D. and F.H.

Competing Interests

E.D. is supported by Bausch Health Canada.

Authorship

This work is original and has not been published elsewhere. E.D. and F.H. convinced the study. E.D., F.H. and K.M. designed the study. K.M., A.L., N.B. and L.J.H. completed data acquisition. K.M., N.B., L.J.H., G.F. and E.D. analyzed data. K.M., A.L., N.B., L.J.H., G.F., F.H. and E.D. interpreted data. K.M. and E.D. created the original manuscript. K.M., A.L., N.B., L.J.H., G.F., F.H. and E.D. edited and revised the manuscript. All authors approved the final manuscript version.

Data Availability

The collected and analysed datasets of the current study are not publicly available. De-identified and processed data can be requested from the corresponding author for academic purposes after completing a signed data access form. Only de-identified data can be provided to protect participant privacy.

Table1. Participant Characteristics.

	Females (n=29)	Males (n=18)	*P value*
Age (years)	27.6±9.4	31.5±9.0	0.148
Height (m)	1.67±0.08	1.76±0.06	**<0.001**
Body Weight (kg)	94.7±16.7	102.9±13.7	0.081
Fat Mass (kg)	43.8±11.0	37.9±8.8	0.055
Fat Free Mass (kg)	48.2±6.5	61.9±8.0	**<0.001**
Body Fat %	46.6±3.9	37.7±5.1	**<0.001**
BMI (kg/m^2)	33.7±4.4	33.2±3.9	0.713

Results are means +/- SD. Boldface indicates signficant *P* values. BMI = body mass index.

Figure legends

Figure 1. Study protocol.  = blood draw;  = VAS.

Figure 2. Energy expenditure over time for females (A) and males (B). RMR = resting metabolic rate; PP = postprandial. ✱ denotes $p<0.05$. There was a significant condition by time effect ($p<0.001$) for both females and males, as well as a sex by time ($p=0.024$) and sex by condition ($p<0.001$) but not sex by condition by time ($p=0.442$).

Figure 3. T_{sk} (A), thermal comfort (B) and thermal sensation (C) changes following 90 mins of CE. ✱ denotes $p<0.05$. There was a significant condition by time effect for T_{sk} ($p<0.001$, $\eta_p^2=0.977$), thermal comfort ($p<0.001$ $\eta_p^2=0.427$), and thermal sensation ($p<0.001$, $\eta_p^2=0.548$).

Figure 4. Energy intake over time. Con = control; CE = cold exposure. ✱ denotes $p<0.05$. *Ad libitum* EI, protein, and carbohydrate consumption were significantly higher during lunch ($p=0.008$, $\eta_p^2=0.147$, $p=0.007$, $\eta_p^2=0.153$, $p=0.014$, $\eta_p^2=0.127$, respectively). *Ad libitum* EI, protein, and carbohydrate consumption were significantly different for day 1 ($p=0.049$, $\eta_p^2=0.085$, $p=0.016$, $\eta_p^2=0.125$, $p=0.046$, $\eta_p^2=0.127$, respectively). Lunch – in lab lunch; Day 1 = lunch bag for remainder of day 1; Day 2 = lunch bag for entire day 2.

Figure 5. Individual T_{sk} (A), EE (B), and EI (C) changes due to 90 minutes of CE sorted by bodyweight. T_{sk} and ad libitum lunch EI are presented as the difference between CE and control sessions, with EE presented as the total number of calories expended above REE. Each bar represents a unique individual, sorted from the smallest body mass to the largest

(range of 62.9 kg to 140.0 kg). All individuals are represented in the same order across the three graphs. Grey bars denote females, black bars denote males. T_{sk} = skin temperature; EE = energy expenditure; EI = energy intake.

Figure 6. Plasma levels of GLP-1 (A), PYY (B), and Leptin (C) changes over time following 90 mins of CE or control. GLP-1 = glucagon-like peptide-1; PYY = peptide YY. ✱ denotes $p<0.05$. There was a significant condition by time interaction for GLP-1 ($p=0.003$, $\eta_p^2=0.263$) and leptin ($p=0.004$, $\eta_p^2=0.482$) but not for PYY ($p>0.05$). Sex was a significant between-subjects effect for both GLP-1 ($p=0.002$, $\eta_p^2=0.546$) and leptin ($p<0.001$, $\eta_p^2=0.624$).

Figure 7. Hunger (A), desire to eat (B), fullness (C), and PFC (D) changes over time following 90 mins of CE or control. PFC = prospective food consumption. There were significant time effects for hunger, desire to eat, fullness, and PFC ($p<0.05$).

Figure 1.

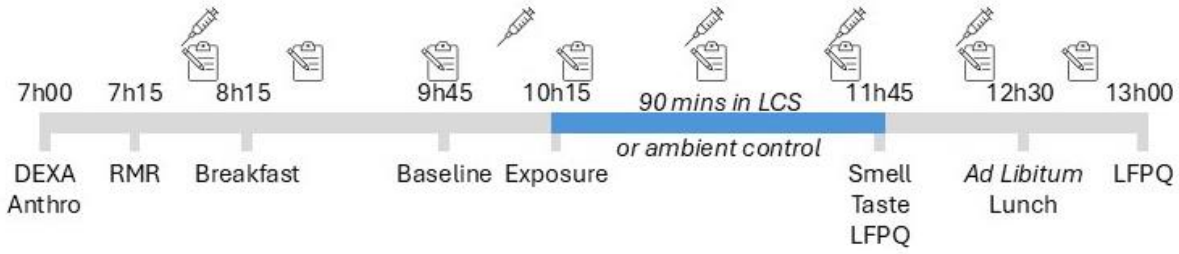

7h00
7h15
8h15
9h45
10h15
90 mins in LCS
or ambient control
11h45
12h30
13h00
DEXA
Anthro
RMR
Breakfast
Baseline
Exposure
Smell
Taste
LFPQ
Ad Libitum
Lunch
LFPQ

Figure 2.

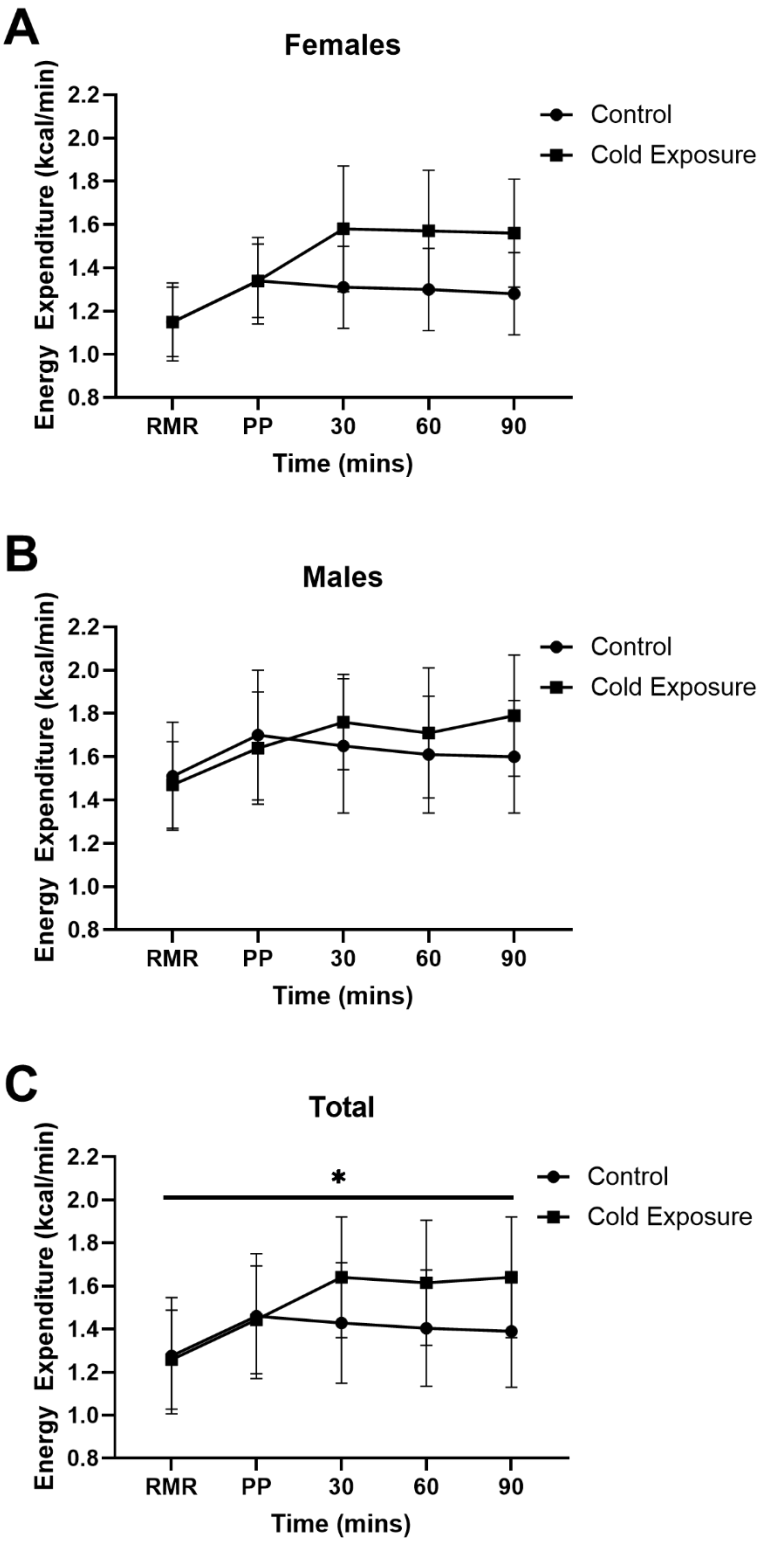

A
Females
Energy Expenditure (kcal/min)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
RMR
PP
30
60
90
Time (mins)
Control
Cold Exposure
B
Males
Energy Expenditure (kcal/min)
Time (mins)
Control
Cold Exposure
C
Total
*
Energy Expenditure (kcal/min)
Time (mins)
Control
Cold Exposure

Figure 3.

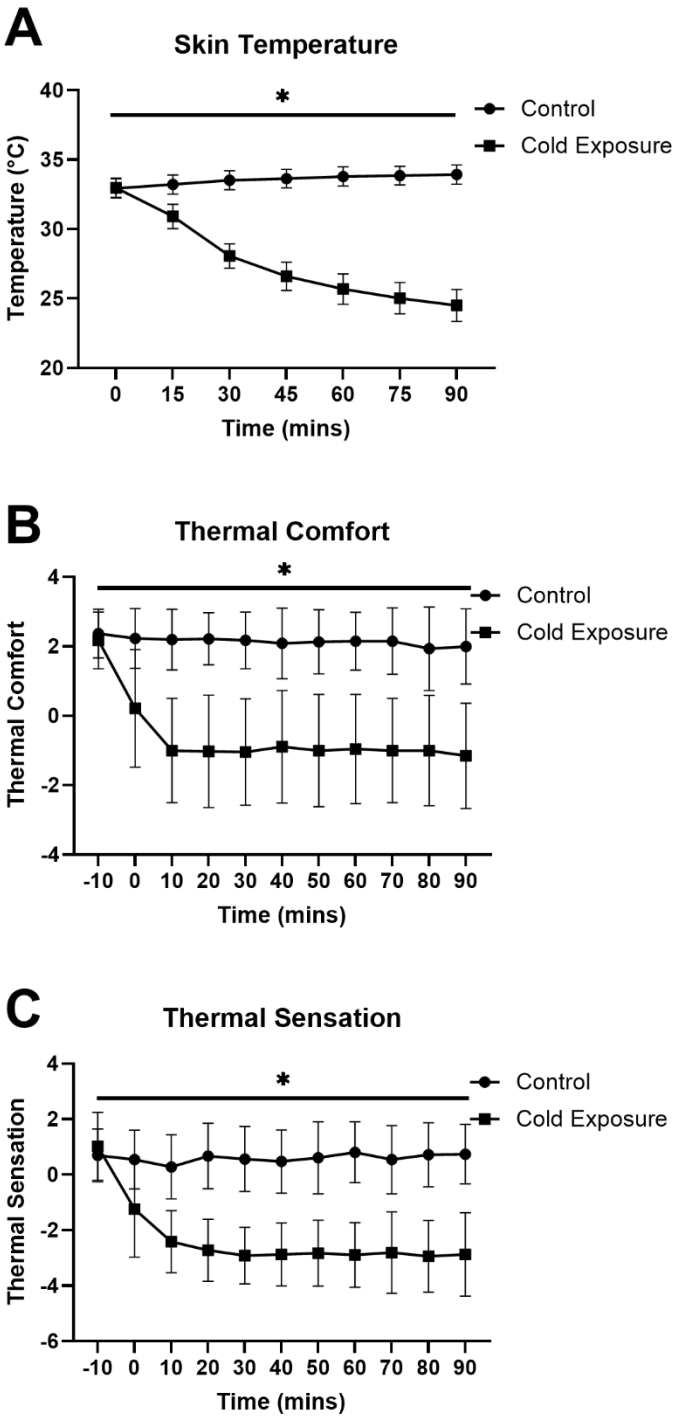

A
Skin Temperature
Temperature (°C)
40
35
30
25
20
*
Control
Cold Exposure
0 15 30 45 60 75 90
Time (mins)
B
Thermal Comfort
Thermal Comfort
4
2
0
-2
-4
*
Control
Cold Exposure
-10 0 10 20 30 40 50 60 70 80 90
Time (mins)
C
Thermal Sensation
Thermal Sensation
4
2
0
-2
-4
-6
*
Control
Cold Exposure
-10 0 10 20 30 40 50 60 70 80 90
Time (mins)

Figure 4.

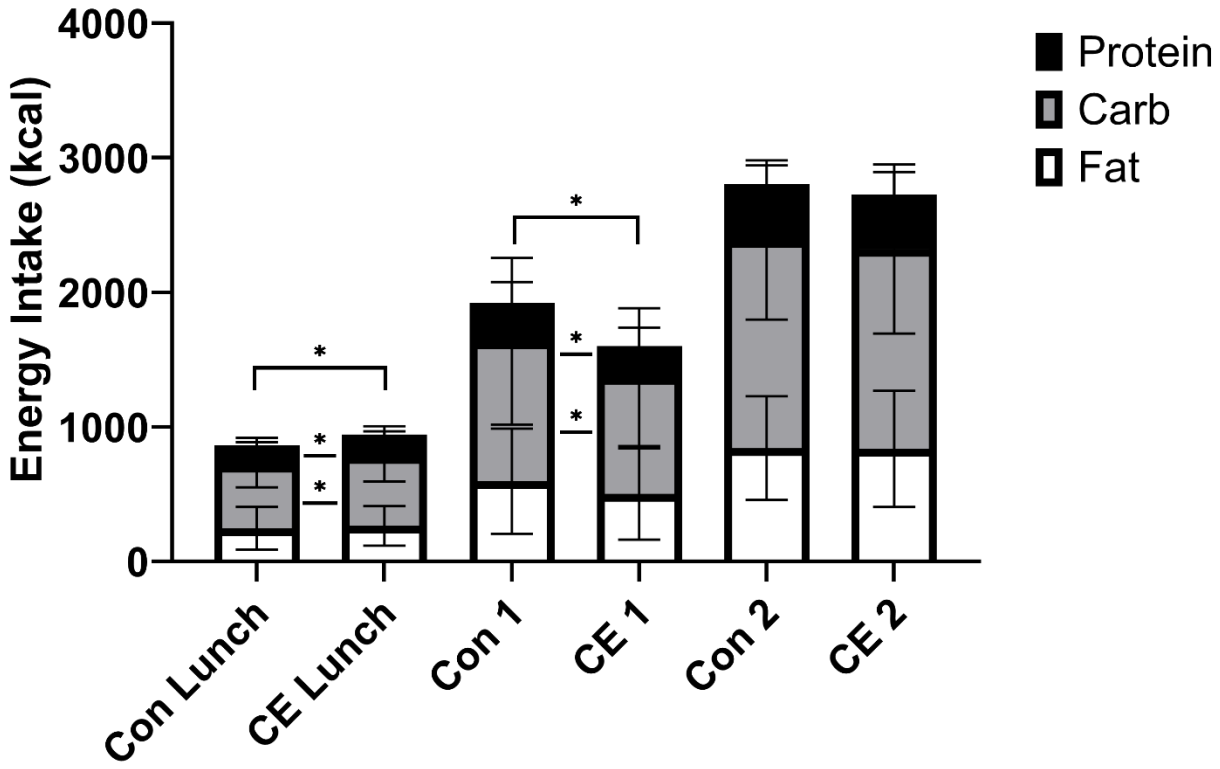

Energy Intake (kcal)
4000
3000
2000
1000
0
Protein
Carb
Fat
*
*
*
*
*
Con Lunch
CE Lunch
Con 1
CE 1
Con 2
CE 2

Figure 5.

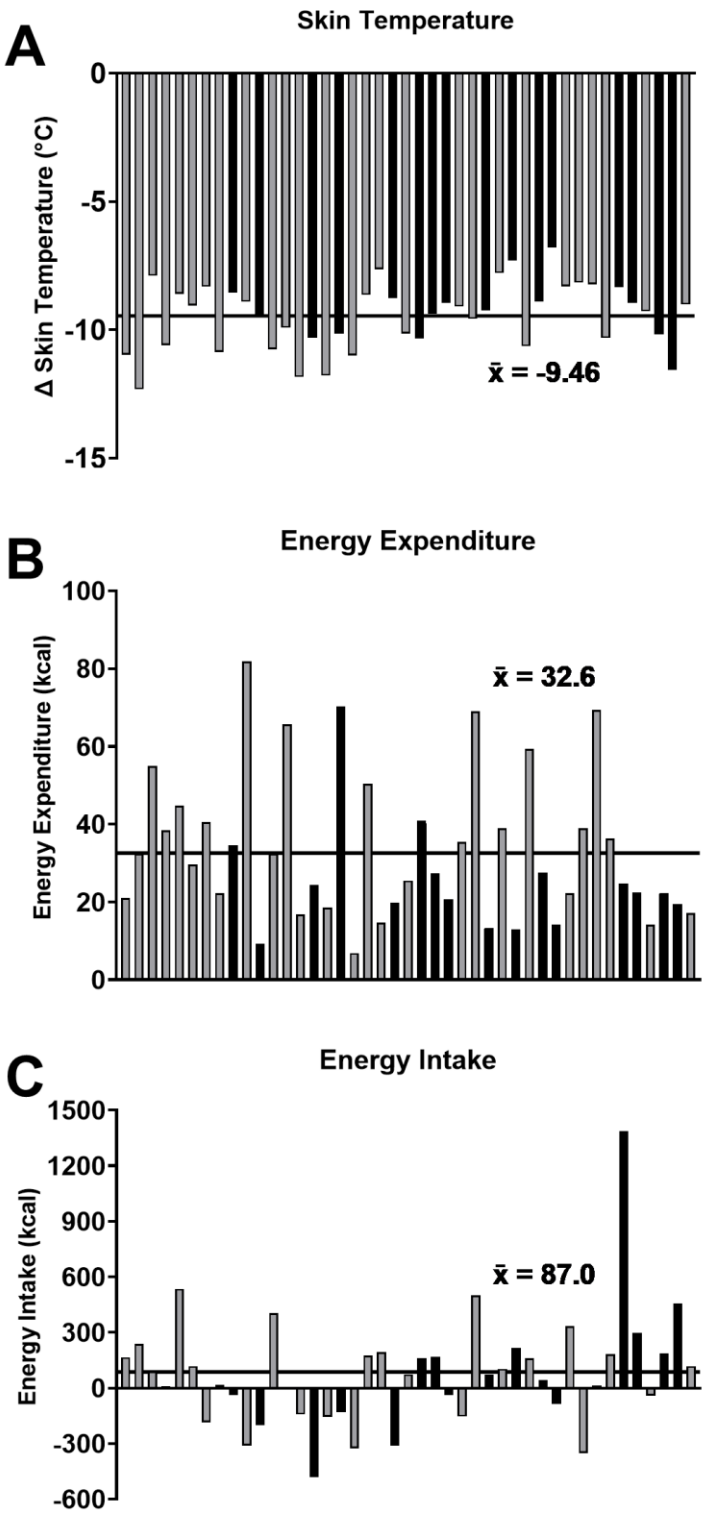

A
Skin Temperature
Δ Skin Temperature (°C)
0
-5
-10
-15
x̄ = -9.46
B
Energy Expenditure
Energy Expenditure (kcal)
100
80
60
40
20
0
x̄ = 32.6
C
Energy Intake
Energy Intake (kcal)
1500
1200
900
600
300
0
-300
-600
x̄ = 87.0

Figure 6.

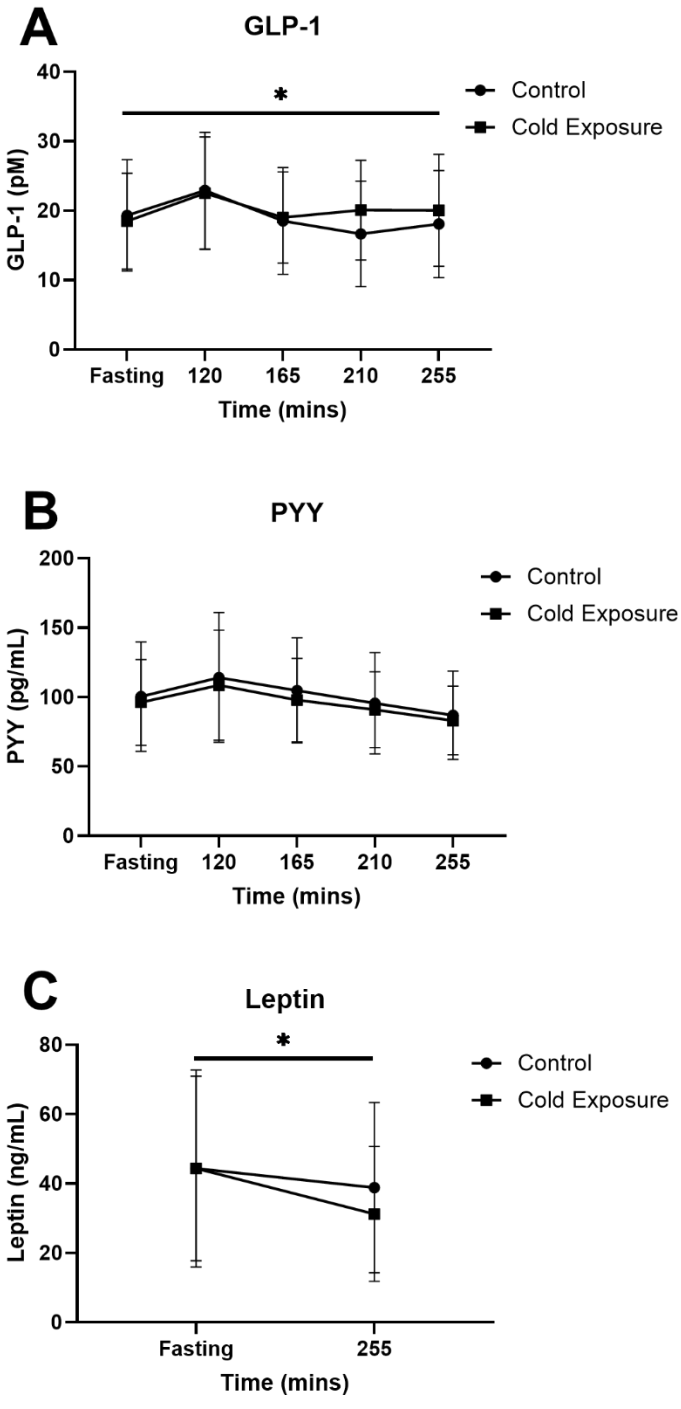
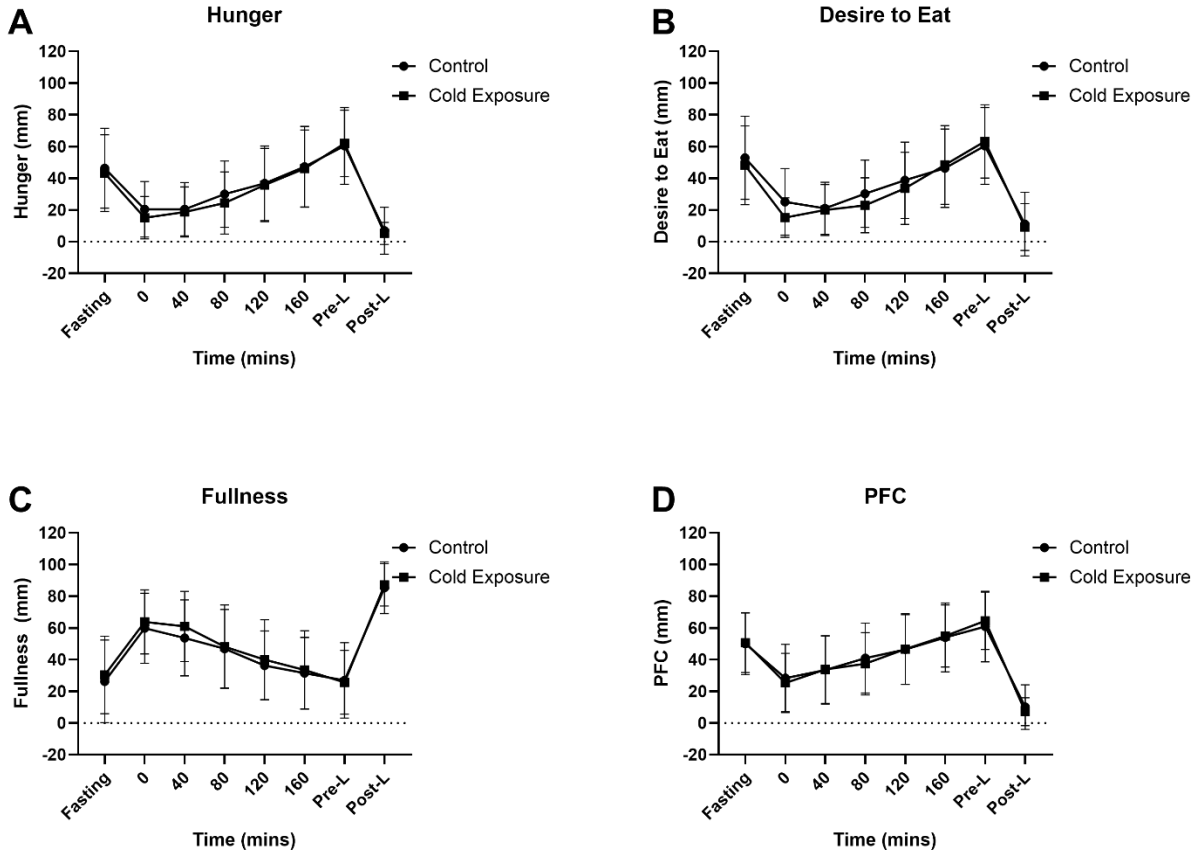

A
GLP-1
*
Control
Cold Exposure
GLP-1 (pM)
40
30
20
10
0
Fasting
120
165
210
255
Time (mins)
B
PYY
Control
Cold Exposure
PYY (pg/mL)
200
150
100
50
0
Fasting
120
165
210
255
Time (mins)
C
Leptin
*
Control
Cold Exposure
Leptin (ng/mL)
80
60
40
20
0
Fasting
255
Time (mins)

Figure 7.


A
Hunger
Hunger (mm)
Control
Cold Exposure
120
100
80
60
40
20
0
-20
Fasting
0
40
80
120
160
Pre-L
Post-L
Time (mins)
B
Desire to Eat
Desire to Eat (mm)
Control
Cold Exposure
Time (mins)
C
Fullness
Fullness (mm)
Control
Cold Exposure
Time (mins)
D
PFC
PFC (mm)
Control
Cold Exposure
Time (mins)

CHAPTER 5. ARTICLE 3

Chronic Cold Exposure Does Not Improve Weight Loss in Individuals Living with Obesity

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Abstract

Introduction: Cold exposure (CE) has been repeatedly touted as a possible weight loss adjunct, but no chronic CE studies have been conducted in individuals living with obesity with the goal of assessing weight loss outcomes.

Methods: Thirty-two individuals (21 females) underwent one of three 8-week weight loss interventions: 30% dietary restriction (n=14), 28 CE sessions (n=10), or both simultaneously (n=8). Body weight and composition (dual-energy x-ray absorptiometry), energy expenditure (indirect calorimetry), energy intake (food menu), subjective appetite (visual analog scales), skin temperature (iButtons), thermal comfort and sensation (Likert scales) were assessed before and after the interventions under control and CE conditions.

Results: Weight loss was not significantly different between groups. Energy expenditure in the cold increased for all groups post-intervention. All groups had a significant improvement in thermal sensation post intervention. Skin temperature and energy intake were not significantly different post intervention. All aspects of subjective appetite changed significantly between groups post intervention.

Discussion: For the first time, CE has been shown to not significantly improve weight loss outcomes alone or in combination with a dietary restriction. High levels of thermal discomfort, minimal increases in heat production during CE and high dropout rates of 45-55% highlight the challenges in feasibility of CE as a weight loss adjunct.

Introduction

Rates of obesity continue to rise globally [1], furthering the demand for novel, viable weight loss and maintenance strategies. Traditional weight loss interventions, like dietary restrictions or exercise, have been shown to have low rates of success for most individuals living with obesity [91-93]. In alignment with this, the re-discovery of brown adipose tissue has led to speculations that chronic cold exposure (CE) could represent an effective adjunct to weight loss therapies [17-22, 24], although this has never been formally tested in individuals with obesity.

However, there are three main issues with this expectation. First, individuals living with obesity have repeatedly been shown to have a much smaller increase in energy expenditure (EE) than their lean counterparts when exposed to cold [26-30](McInnis *et al.,* submitted). Second, the impact of CE on energy intake (EI) and appetite regulation have been minimally explored, with only two studies in individuals living with obesity. In the few studies where it has been explored in lean individuals, EI has not changed [33, 34, 36, 37], and the increase in EE has also been minimal, averaging 11.5±3.3 kcal over 180 mins [37] and ~167 kcal over 24h, [36], but EE is not always measured [33, 34]. In individuals living with obesity, EI has been shown to increase significantly during acute *ad libitum* conditions in a metabolic chamber at 19°C [35] and following CE in a liquid conditioned suit (McInnis *et al.,* submitted). Third, the only lens through which to currently view chronic CE on body weight regulation is by evaluating changes in body energy stores during cold acclimation studies in individuals who are lean. As previously reviewed [25], chronic CE does not create meaningful changes in body energy stores in lean individuals in free living conditions with

no instructions to modify their EI or lifestyle. When compounded with the discomfort often reported during CE [94], the feasibility of CE being a weight loss tool is questionable. However, there has been no study to directly test the use of chronic CE as a weight loss tool.

Therefore, the objective of this study was three-fold. The first goal was to determine the effects of chronic CE on body weight in individuals living with obesity. We compared the effects of chronic CE alone (CE), in combination with a dietary restriction (DIET+CE), and a dietary restriction alone (DIET). We hypothesized that CE would not create a significant reduction in body weight alone, and that when implemented in combination with a dietary restriction would produce less weight loss than a dietary restriction alone. The second goal was to determine the effects of chronic CE interventions on EI and resting EE (REE). We hypothesized that the DIET+CE would see greater increases in EI and a smaller decrease in REE than the DIET following the interventions. The third goal was to determine the effects of chronic CE and weight loss on heat production in the cold. We hypothesized that the DIET+CE and DIET groups would see an increase in cold-induced EE, but the CE group would remain unchanged.

Methods

Two-hundred and forty-nine individuals living with obesity were screened, of which 52 individuals (33 females) were randomized for an 8-week weight loss intervention with two experimental sessions both before and after the intervention, with 32 (21 females) that

completed the study, as seen in **Figure 1**. Participants were randomized in a 1:1:1 ratio stratified by sex to one of three weight loss interventions: 1) CE; 2) DIET; 3) DIET+CE. Participants were eligible for the study if they had a BMI between 27-40 kg/m^2, aged 18-55, and were sedentary (<2 time/week of 30 mins of continuous exercise). Participants were excluded for variations in body weight exceeding ±4 kg in the 6 months prior to the study, if they were peri- or post-menopausal (minimum 2 menses in previous 3 months), currently or planning to become pregnant, actively dieting or smoking, had a heart condition, diabetes, an axis 1 psychiatric disorder, thyroid disease, Raynaud's syndrome, excessive alcohol intake, use of opiates or stimulants, or were taking medications that could impact appetite regulation, including but not limited to selective serotonin reuptake inhibitors or thyroid medications. Sessions were completed within 8 days of the beginning of follicular phase for females to ensure consistent hormone levels and ease of phase identification. Females were not excluded for the use of oral contraceptives. This study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the University of Ottawa Research Ethics Board (H-09-19-4928). Written informed consent was obtained from all participants. This trial is registered with ClinicalTrials.gov ID: NCT05076357.

Weight Loss Interventions

Participants in the DIET group underwent a dietary restriction of 30% of total energy expenditure, calculated by combining REE, as described below, and physical activity data measured via an Actical accelerometer (Actical-Mini, Mitter Co. Inc.), with a 10% allowance for the thermic effect of feeding, as previously described [95], with a floor value

of 1200 kcal/day. The DIET was implemented using the food exchange system from Diabetes Canada [96], which allows participants to select foods they prefer but at restricted quantities, with a macronutrient composition of 50% carbohydrates, 30% lipids and 20% protein.

The CE group underwent chronic CE with a 90-min cold session every other day for the 8-weeks, resulting in a total of 28 CE sessions using the liquid conditioned suit and circulating bath as described below. During the first 7 sessions the liquid conditioned suit was perfused with 18°C diluted antifreeze before decreasing to 14°C for sessions 8-14 and finally 10°C for sessions 15-28 to allow for a training effect. The DIET+CE group underwent both the CE and DIET interventions.

Experimental Protocol

For the full protocol of the baseline and follow-up sessions see **Figure 2**. Participants underwent two experimental sessions in a randomized order both before and after an 8-week weight loss intervention. Briefly, participants arrived at the lab fasted for anthropometrics, a dual-energy X-ray absorptiometry scan (Lunar Prodigy; General Electric), then underwent REE measurement over 30 minutes following a 20-minute rest period. Subjective appetite visual analog scales (VAS) were completed before and after a standardized breakfast, every 40 minutes into the postprandial period for 2 hours, as well as immediately before and after an *ad libitum* lunch at 12h30, as described previously [64]. The standardized breakfast was consumed after REE and consisted of whole-wheat toast (D'Italiano, 2 slices), peanut butter (Kraft Smooth, 20g), strawberry jam (Smuckers, 20g)

and orange juice (Tropicana, 250g), equating to 446 kcal with a macronutrient breakdown of 63% carbohydrates, 25% fat and 12% protein. Participants were then fitted with thermocouples to measure average skin temperature (T_{sk}). For the CE condition, each participant was then fitted with a three-piece liquid conditioned suit. A 30-minute postprandial EE measurement was collected before a 90-minute exposure to either ambient control or 10°C diluted antifreeze perfused in the liquid conditioned suit. Thermal comfort and sensation were collected at baseline and every 10 minutes during exposure with Likert scales, with EE measurements every other 15 minutes as previously described [65]. Following exposure participants consumed an *ad libitum* lunch, as previously described [66].

Measurements

Cold Exposure

CE was achieved using a three-piece liquid conditioned suit (three-piece high density; Allen-Vanguard, Inc.) lined with tubing that was perfused with diluted antifreeze chilled to 10°C using a circulating bath (Neslab Refrigerated RTE-7 Bath Circulator, Thermo Scientific), as described previously [67].

Energy Expenditure

EE was calculated from indirect calorimetry measurements (O_2/CO_2) obtained with a metabolic cart (Vmax Encore 29N metabolic cart; Sensor Medics Corporation) and a

ventilated hood. REE measurements were performed in a fasted state for 30 minutes following a 20-minute rest period, and then for 15 minutes following a 15-minute rest period for postprandial. Three experimental measures were then taken, again for 15 minutes following a 15-minute rest. Ethanol burning tests were performed to control the quality of measurements, as previously described [68, 69]. Our most recent analyses revealed a 4.3% and 0.39% difference for VCO_2 and VO_2 measures, respectively.

Carbohydrate (RG_{ox}) and lipid (RF_{ox}) oxidation rates were calculated using the following formulas, as previously described [70, 71], while assuming that protein oxidation contributed to 10% of the total EE measured:

$$RG_{ox}\ (g/min) = 4.59\ VCO_2\ (L/min) - 3.23\ VO_2\ (L/min)$$

$$RF_{ox}\ (g/min) = -1.70\ VCO_2\ (L/min) + 1.70\ VO_2\ (L/min)$$

Thermal Comfort and Sensation

Thermal comfort was measured using a 6-point Likert scale ranging from “very uncomfortable”, -3, to “very comfortable”, +3. Thermal sensation was measured using an 11-point scale ranging from “coldest I’ve ever felt”, -5, to “hottest I’ve ever felt”, +5. These scales are a modification of the scale used by Passias *et al* [73].

Skin Temperature

Twelve thermocouples (DS1921H High-Resolution Thermochron iButton Devices, Maxim Integrated) were fitted to the participants at the following sites: forehead, upper back, lower back, abdomen, quadriceps, hamstring, front calf, back calf, chest, biceps, forearm,

and hand, as previously described by Gosselin and Haman [62]. A weighted average formula was used to determine average T_{sk} across the 12 sites [72].

Standardized Breakfast

The standardized breakfast was comprised of whole-wheat toast (D'Italiano, 2 slices), peanut butter (Kraft Smooth, 20g), strawberry jam (Smuckers, 20g) and orange juice (Tropicana, 250g), equating to 446 kcal with a macronutrient breakdown of 63% carbohydrates, 25% fat and 12% protein.

Energy Intake

EI in and outside of the laboratory setting was measured using a validated [66] food menu containing a variety of foods that can be consumed within and outside of the laboratory. Participants chose items from the curated list and were served in ad libitum quantities, with food being weighed before and after consumption to determine the net food consumed using an electronic scale (Scout Pro SP2001, Ohaus Corporation). The consumed quantity was then analyzed with food processing software (Food Processor SQL, version 9.6.2, ESHA Research). Outside the laboratory setting, food and beverage items were packed into plastic containers and bottles, then stored in two portable coolers for transport, one for the remainder of day 1 and one for day 2. Participants were asked to only consume food and beverages provided for the 2-day period.

Subjective Appetite

Subjective appetite was measured 8 times throughout the sessions, as highlighted above. Desire to eat, fullness, prospective food consumption (PFC), and hunger were derived from the following 4 questions: 1. “How strong is your desire to eat?” (Very weak – Very strong); 2. “How full do you feel?” (Not full at all – Very full); 3. “How much food do you think you could eat?” (Nothing at all – A large amount); 4. “How hungry do you feel?” (Not hungry at all – As hungry as I have ever felt), respectively. Participants were instructed to place a vertical line along a 100 mm scale for each question, giving a score out of 100 for each aspect of appetite, as described previously [64]. VAS have been previously validated in single meal studies [74].

Statistical Analysis

Sample size was calculated using means and standard deviations derived from Langeveld *et al* [37], with an $\alpha<0.05$ and a 1-β of 80% for a three-group intervention, giving a sample size of 21 subjects per group. Data were analyzed in two manners. First, changes in AUC EE, AUC subjective appetite, AUC skin temperature, AUC thermal comfort and AUC thermal sensation, EI and body composition were analyzed using repeated measures ANOVA, with condition as a within-subjects variable (pre control and post control) and group as a between-subjects factor. Second, the same variables were analyzed comparing the pre CE to post CE session, to determine the effects of the interventions on thermoregulatory outcomes and appetite responses in the cold. Area under the curve (AUC) was calculated using the trapezoid method for subjective appetite, excluding the fasting values, as previously described [97]. AUC for thermal comfort, thermal sensation

and skin temperature were also calculated using the trapezoid method. AUC EE was calculated using the trapezoid method while subtracting REE.

Effect size is reported as partial eta squared (η_p^2), whereby values above 0.2, 0.5, and 0.8 were considered small, medium, and large effects, respectively. Data are presented as mean ± standard deviation (SD) unless otherwise specified. Statistical analyses were performed using Statistical Product and Service Solutions software, version 29.0 (Chicago, IL) with an alpha set at 0.05 to establish statistical significance. Reported p-values were adjusted for multiplicity with the Bonferroni correction where necessary.

Results

Compliance and Drop Out

As shown in **Figure 1**, of the 249 potential participants screened, 52 participants were randomized into the three groups. Ten completed the CE intervention with 8 dropouts, 14 completed the DIET with 3 dropouts, and 8 completed the DIET+CE with 9 dropouts. As far as compliance to the CE protocol, participants in the CE and DIET+CE groups completed an average of 25.1±3.3 (~90%) and 22.6±4.7 (~81%) of the 28 CE sessions prescribed for their corresponding weight loss interventions, respectively (p=0.211), however the missing sessions were not evenly distributed, as 95.8±6.7, 94.1±8.8, and 80.6±18.9% of sessions were completed at 18, 14, and 10°C, respectively (p=0.015, η_p^2=0.450). There were no group by temperature interactions for number of sessions completed amongst completers (p=0.514, η_p^2=0.091).

Body Composition

Body weight (p=0.677), body fat percentage (p=0.496), fat mass (p=0.906) and lean mass (p=0.425) were not significantly different between groups at baseline. There were no significant differences between groups for body weight (p>0.05), however, there was a significant time effect (p=0.006, η_p^2=0.236) and a trend towards significance for a group by time interaction (p=0.057, η_p^2=0.180). The CE group experienced an average increase in body weight of 0.3 kg over 8 weeks, the DIET group and DIET+CE group experienced decreases in body weight of 3.8 and 3.0 kg, respectively, as seen in **Figure 3**. Fat mass had a significant time effect (p=0.004, η_p^2=0.256) but no group by time interaction (p=0.129) and there were no significant time or group by time interactions for lean mass (p=0.675 and 0.142, respectively).

Energy Expenditure – Control

REE was not significantly different between groups at baseline (p>0.05) and there were no time (p=0.545) or group by time interaction (p=0.211) effects. There were no significant time (p=0.402) or group by time interaction (p=0.839) effects for EE during control exposure comparing the pre and post control sessions.

Energy Expenditure – Cold

Cold induced thermogenesis, defined as the number of calories expended due to CE above REE, was not different between groups at baseline (p=0.345). There was a significant time effect (p=0.009, η_p^2=0.217) but no group by time interaction (p=0.644) when comparing pre and post CE sessions, as seen in **Figure 4**.

During the CE sessions, there was no significant time effects (p=0.487), but there was a trend towards significance for a group by time interaction (p=0.058, η_p^2=0.184) for the total grams of carbohydrates oxidized above resting when comparing the pre and post CE sessions, as both the DIET group oxidized more carbohydrates and the DIET+CE group oxidized less following their respective interventions (data not shown)**.** There were no group (p=0.640) or group by time interaction (p=0.106) effects for total grams of lipids oxidized above REE when comparing pre and post CE sessions.

Thermal Comfort

There were no significant time (p=0.531) or group by time interaction (p=0.234) effects when comparing pre and post control sessions for AUC thermal comfort. There were also no significant time (p=0.272) or group by time interaction (p=0.441) effects when comparing pre and post CE sessions.

Thermal Sensation

There were no significant time (p=0.155) or group by time interaction (p=0.781) effects when comparing pre and post control sessions for AUC thermal sensation. There was a significant time effect (p<0.001, η_p^2=0.334) for AUC thermal sensation comparing pre and post CE sessions, but not a group by time interaction (p=0.387), as seen in **Figure 5**.

Skin Temperature – Control

There were no significant time (p=0.909) or group by time (p=0.688) effects for T_{sk} AUC when comparing the pre and post control sessions. Average T_{sk} for the final 15 minutes of baseline and final 15 minutes of exposure are shown in **Figure 6.**

Skin Temperature – Cold

There were no significant time (p=0.285) or group by time (p=0.178) effects for skin temperature AUC when comparing pre and post CE sessions. Average T_{sk} for the final 15 minutes of baseline and final 15 minutes of exposure are shown in **Figure 6.**

Energy Intake – Control

There was no significant time effect (p=0.707), but *ad libitum* lunch EI in control sessions had a trend towards significance for a group by time interaction (p=0.085, η_p^2=0.156), as seen in **Figure 7**, with the DIET+CE group consuming fewer kcal post intervention. There were no significant time (p=0.915) or group by time interaction (p=0.494) effects for 48h food intake (data not shown).

Energy Intake – Cold

There were no significant time (p=0.101) or group by time interaction (p=0.882) effects for *ad libitum* EI during cold sessions lunch. There were also no significant time (p=0.219) or group by time interaction (p=0.364) effects for 48-hour food intake (data not shown).

Visual Analog Scales – Control

Fasting desire to eat (p=0.247), hunger (p=0.921), fullness (p=0.896), and PFC (p=0.922) levels did not change following weight loss for any of the groups. When comparing pre and

post control sessions there was no time effect for AUC desire (p=0.791), hunger (p=0.971), fullness (p=0.153), but there was a trend for PFC (p=0.060, η_p^2=0.121). There were significant group by time interactions for desire to eat (p=0.022, η_p^2=0.238), hunger (p=0.048, η_p^2=0.195), fullness (p=0.007, η_p^2=0.301), and PFC (p=0.016, η_p^2=0.255), as depicted in **Table 2**.

Visual Analog Scales – Cold

When comparing pre and post CE session there were was a significant time effect for desire (p=0.012, η_p^2=0.203), hunger (p=0.008, η_p^2=0.229), and PFC (p=0.020, η_p^2=0.177) but not fullness (p=0.192). There were no significant group by time interactions effects for desire (p=0.703), hunger (p=0.742), fullness (p=0.570), or PFC (p=0.444), as seen in **Table 3**.

Discussion

The main objective of this study was to determine the effects of chronic CE on body weight and composition. Here we found for the first time that chronic CE did not improve weight loss outcomes for individuals living with obesity, supporting our hypothesis, as the DIET+CE group did not have greater weight loss than the DIET group, and the CE group had no change in body weight following 8 weeks of exposures, also supporting our hypotheses. Our secondary objectives included an investigation of the effects of chronic CE on EE, EI and appetite as well as on thermoregulatory outcomes. We also found that there were no changes in EI or REE due to the interventions, which did not support our hypotheses, but

there were decreases in subjective appetite for the DIET+CE group and increases for the DIET group. Finally, we found an improvement in thermal sensation in all groups post interventions. Taken together, chronic CE did not improve weight loss outcomes alone or in combination with a dietary restriction and did not work as a weight loss tool or adjunct.

Weight Loss and Body Composition

Chronic CE did not improve weight loss outcomes for individuals living with obesity. CE alone resulted in only a 0.3 kg decrease in body weight after 28 exposures. Chronic CE combined with a dietary restriction led to an average decrease in body weight that was smaller than dieting alone (3.0 vs 3.8 kg, respectively), even with cold as an additional source of caloric expenditure, despite the common claim that CE can cause weight loss [17-22, 24]. This is likely due in part to the relatively small increases in heat production in individuals living with obesity [27-30, 35] and repeated CE sessions over the 8 week intervention causing compensation through either increasing EI [35], causing participants to be less compliant with the prescribed dietary restriction, or modifying activity levels and decreasing total EE.

Energy Expenditure

REE did not change post intervention for any of the weight loss groups, but of greater interest are the changes in cold-induced heat production depicted in **Figure 4.** Typically, there can be a shift from shivering to non-shivering thermogenesis, but minimal changes to total heat production [98, 99], though data is scarcer in individuals living with obesity [100]. Recently it was reported that 10 days of cold acclimation resulted in a slight decrease in

the rate of heat production in individuals living with obesity, however the protocol had differences in the duration of CE before and after acclimation that could impact generalizability [101]. Here, all three groups had an increase in heat production following their respective weight loss interventions, but the magnitude is most important. Even with a significant increase in heat production, caloric expenditure was minimally impacted, as EE only increased by an average of 32.3±18.9 kcal over 90 minutes of exposure. This small increase can help to explain why the addition of CE to a dietary restriction did not improve weight loss outcomes. A longer or more intense cold stimuli would likely have a greater increase in heat production, but with the already high dropout rates associated with chronic CE of ~50% across both groups, it would likely further exacerbate attrition.

Energy Intake

There have been only a handful of studies that have measured EI changes due to passive CE, with only two focused upon individuals living with obesity [35](McInnis *et al,* submitted), where EI has been found to increase during CE, though the magnitudes varied widely. When comparing control conditions to determine the effects of weight loss on thermoneutral food intake, the DIET+CE group trended towards consuming approximately 200 kcal less during the *ad libitum* lunch, while the DIET and CE groups were relatively unchanged. This is supported by the changes in appetite but stands in direct opposition to the fact that the DIET+CE group had a smaller weight loss than the DIET group. A possible explanation for the lack of change in cold-induced EI and small change in EI seen acutely (McInnis *et al,* submitted) is the small increase in heat production in individuals living with obesity. Similarly, previous studies with individuals who are lean also reported relatively

small increases in EE (averaging 11.5±3.3 kcal over 180 mins [37] and ~167 kcal over 24h, [36]), though the stimuli was much milder, and no significant changes in EI. This could in part be due to the relatively small mean difference in EI (~33 kcal [37] or ~215 kcal [36]) being obscured or lost by the magnitude of total consumption (~670 kcal [37] or ~4000kcal [36]), which would also draw attention to the difference between the clinical and statistical significance of the values being discussed. However, an increase in EI of ~411 kcal was found in individuals living with obesity in a cold chamber (19°C) for 24h but had no change in EE. Further data are needed to elucidate the effects of CE on EI.

Subjective Appetite

Weight loss achieved through dietary restrictions often are accompanied by increases in desire to eat and hunger [102-104], as observed in the DIET group of this study. Interestingly, in support of the EI values obtained from the *ad libitum* lunch, individuals who completed the DIET+CE intervention had a general improvement in appetite between control sessions, decreasing their desire to eat and hunger while having a higher level of fullness, as seen in **Table 2.** As this is the first study to examine appetite outcomes after chronic CE, there is no real comparison point to extrapolate this finding to. It is possible that the lesser weight loss in the DIET+CE group resulted in a smaller impact on appetite regulation, but appetite not only shifted away from being orexigenic, but towards anorexigenic. Acute CE has been reported to decrease pre-lunch hunger [33], but this is not corroborated in other studies [34-37]. Further, all three groups in the present study experienced an increase in hunger, desire to eat, and PFC when comparing cold sessions before and after interventions. Acutely, it was found that CE increases GLP-1 concentration

in individuals living with obesity (McInnis *et al.,* submitted), which could contribute to decreased levels of appetite, as shown with the use of GLP-1 receptor agonist medications [105]. However, this stands in contrast to the changes in body energy stores for the respective groups. Although subjective appetite improved during this measurement period, the DIET+CE group did not experience a greater weight loss than the DIET alone.

Compliance and Dropout

Compliance for individuals who completed the CE and DIET+CE interventions was good on average for those who completed the study, with ~85% of assigned sessions being completed, though some participants had quite low compliance rates, particularly in the DIET+CE group. Participant dropout was also of concern, with the CE and DIET+CE groups having 8 (~44%) and 9 (~53%) dropouts, respectively, either during or before the start of the intervention. Comparatively, the DIET group only experienced 3 (~18%) dropouts, which is more in line with the 21% expected dropout rate for behavioural change interventions [106]. This difference is likely due to the higher time commitment and the high level of discomfort reported during CE acting as a possible deterrent to frequent exposures. The high level of dropout and ranging compliance values highlight the difficulty of repeated CE for participants who were involved in this study.

Limitations

A potential constraint of the study was the difference in time commitment required for the different interventions as the DIET group was required to visit the lab 1-2 times per week, while the CE and DIET+CE required 3-4 visits, however, the lack of exit interviews for

participants also makes the exact rationale for dropping out of the study unclear. The strengths of the study were the potency and reproducibility of the cold stimulus, as previously discussed (McInnis *et al.*, submitted), the study population being individuals living with obesity, and the generalizability of the CE interventions, particularly by combining the CE with a dietary intervention, as it is common for people to use multiple weight loss modalities at the same time, and focus on weight loss for individuals living with obesity as the primary outcome. The study was also designed to create ecological validity for the appetite-related outcomes by feeding participants breakfast to maintain habitual consumption patterns for participants, and that the thermic effect of feeding was considered as an increase in non-shivering thermogenesis when accounting for changes in heat production. The study also focused on EI and appetite related outcomes, which have not been reported to our knowledge, after a cold acclimation study in individuals who are lean or living with obesity.

Conclusion

Although the addition of chronic CE to a dietary restriction resulted in appetite improvements following weight loss, and a trend towards lower EI, it is shown here for the first time in individuals living with obesity that the administration of chronic CE did not improve weight loss outcomes when used alone or in combination with a dietary restriction. When scrutinizing the high levels of thermal discomfort, minimal increases in heat production, and high dropout rates, the feasibility of using CE for the management of

body energy stores alone or as an adjunct to a dietary restriction faces serious challenges in its implementation.

Table 1. Participant Characteristics.

	CE (n=10)	Diet (n=14)	DIET+CE (n=8)	*P* value
Age (years)	33.4±10.5	29.6±9.6	28.8±7.9	0.522
Height (m)	1.70±0.10	1.70±0.10	1.66±0.09	0.582
Body Weight (kg)	100.4±18.1	96.9±18.6	93.0±13.9	0.677
Fat Mass (kg)	42.4±11.0	40.6±9.7	40.9±7.9	0.906
Fat Free Mass (kg)	55.0±11.7	56.0±11.7	49.5±9.2	0.425
Body Fat %	42.7±5.8	42.0±6.6	45.2±5.3	0.496
BMI (kg/m^2)	34.5±4.6	33.4±3.5	33.7±2.9	0.766

Results are presented as mean +/- SD. CE = cold exposure; DIET+CE = diet and cold exposure; BMI = body mass index.

Table 2. Subjective Appetite Changes – Control

	CE (n=10)		Diet (n=14)		DIET+CE (n=7)		*P* value	
	Pre	Post	Pre	Post	Pre	Post	Time	Group by time
Fast Desire to Eat	60.1 ± 21.6	46.0 ± 26.8	45.1 ± 30.7	51.1 ± 23.0	46.6 ± 25.1	46.5 ± 25.3	0.585	0.220
AUC Desire to Eat	7544 ± 3999	7299 ± 3370	6940 ± 4036	8855 ± 4150	7749 ± 3188	5622 ± 2107	0.791	**0.022**
Fasting Hunger	46.4 ± 24.3	44.0 ± 27.2	40.9 ± 28.8	46.0 ± 25.2	42.0 ± 22.6	46.1 ± 22.3	0.666	0.807
AUC Hunger	7679 ± 2050	7619 ± 3444	7021 ± 3844	8661 ± 3683	7209 ± 2890	5572 ±1717	0.971	**0.048**
Fasting Fullness	23.7 ± 20.6	27.9 ± 19.7	28.2 ± 27.9	24.1 ± 17.1	21.3 ± 26.1	22.9 ± 24.1	0.877	0.607
AUC Fullness	9439 ± 3840	11149 ± 2895	10196 ± 4789	8757 ± 4674	8456 ± 2447	10284 ± 3464	0.153	**0.007**
Fasting PFC	48.2 ± 13.5	50.9 ± 26.1	50.1 ± 21.6	51.2 ± 19.9	50.4 ± 27.5	50.1 ± 21.4	0.774	0.963
AUC PFC	9133 ± 3474	8446 ± 3416	8934 ± 4264	9754 ± 3806	9708 ± 4751	6433 ± 2441	0.060	**0.016**

Results are presented as mean +/- SD. CE = cold exposure; DIET+CE = diet and cold exposure; PFC = prospective food consumption; AUC = area under the curve.

Table 3. Subjective Appetite Changes – Cold

	CE (n=10)		Diet (n=14)		DIET+CE (n=7)		*P* value	
	Pre	Post	Pre	Post	Pre	Post	Time	Group by time
Fast Desire to Eat	40.3 ± 24.2	52.4 ± 24.1	44.9 ± 23.4	51.5 ± 25.4	56.0 ± 25.0	48.4 ± 18.1	0.476	0.331
AUC Desire to Eat	4809 ± 3483	7208 ± 3478	4881 ± 2367	7294 ± 3884	4937 ± 1742	5936 ± 2859	**0.012**	0.703
Fasting Hunger	44.1 ± 25.8	50.6 ± 21.7	41.9 ± 24.2	49.9 ± 21.3	50.1 ± 21.7	46.5 ± 16.0	0.437	0.569
AUC Hunger	5092 ± 3930	7484 ± 3308	4687 ± 2532	7170 ± 3989	4710 ± 1944	5906 ± 2758	**0.008**	0.742
Fasting Fullness	26.0 ± 22.4	17.2 ± 14.6	29.2 ± 17.6	24.9 ± 21.2	22.0 ± 32.7	18.1 ± 18.8	0.173	0.862
AUC Fullness	9169 ± 3992	9684 ± 3703	9255 ± 3956	9551 ± 4682	8123 ± 2892	10377 ± 4071	0.192	0.570
Fasting PFC	51.0 ± 22.9	55.0 ± 24.5	49.1 ± 15.3	54.7 ± 20.9	57.9 ± 23.5	47.3 ± 18.7	0.918	0.164
AUC PFC	6262 ± 3346	8736 ± 3665	6919 ± 2856	8344 ± 3757	6418 ± 2077	6893 ± 3090	**0.020**	0.444

Results are presented as mean +/- SD. CE = cold exposure; DIET+CE = diet and cold exposure; PFC = prospective food consumption; AUC = area under the curve.

Figure Legends

Figure 1. Screening for study sample.

Figure 2. Timeline for (A) experimental sessions and (B) study protocol. Wks = weeks; DEXA = dual X-ray absorptiometry; REE = resting energy expenditure; Anthro = anthropometrics; LCS = liquid conditioned suit; Exp session = experimental session;  = visual analog scale.

Figure 3. Changes in (A) body weight, (B) fat free mass, and (C) fat mass before and after 8-week weight loss interventions. ✱ = significant time effect ($p<0.05$). CE = Chronic Cold Exposure; DIET = Dietary Restriction; DIET+CE = Chronic Cold Exposure and Dietary Restriction.

Figure 4. Changes in AUC energy expenditure for (A) control and (B) cold exposure sessions above resting energy expenditure before and after 8-week weight loss interventions. ✱ = significant time effect ($p<0.05$). CE = Chronic Cold Exposure; DIET = Dietary Restriction; DIET+CE = Chronic Cold Exposure and Dietary Restriction.

Figure 5. Changes in AUC thermal comfort and thermal sensation during (A,C, respectively) control and (B,D, respectively) cold sessions before and after 8-week weight loss interventions. ✱ = significant time effect ($p<0.05$). CE = Chronic Cold Exposure; DIET = Dietary Restriction; DIET+CE = Chronic Cold Exposure and Dietary Restriction.

Figure 6. Changes in skin temperature for (A) CE, (B) DIET, and (C) DIET+CE groups during control and cold sessions pre and post intervention. CE = Chronic Cold Exposure; DIET = Dietary Restriction; DIET+CE = Chronic Cold Exposure and Dietary Restriction.

Figure 7. Changes in *ad libitum* lunch EI during (A) control and (B) cold sessions before and after 8-week weight loss interventions. CE = Chronic Cold Exposure; DIET = Dietary Restriction; DIET+CE = Chronic Cold Exposure and Dietary Restriction.

Figure 1.

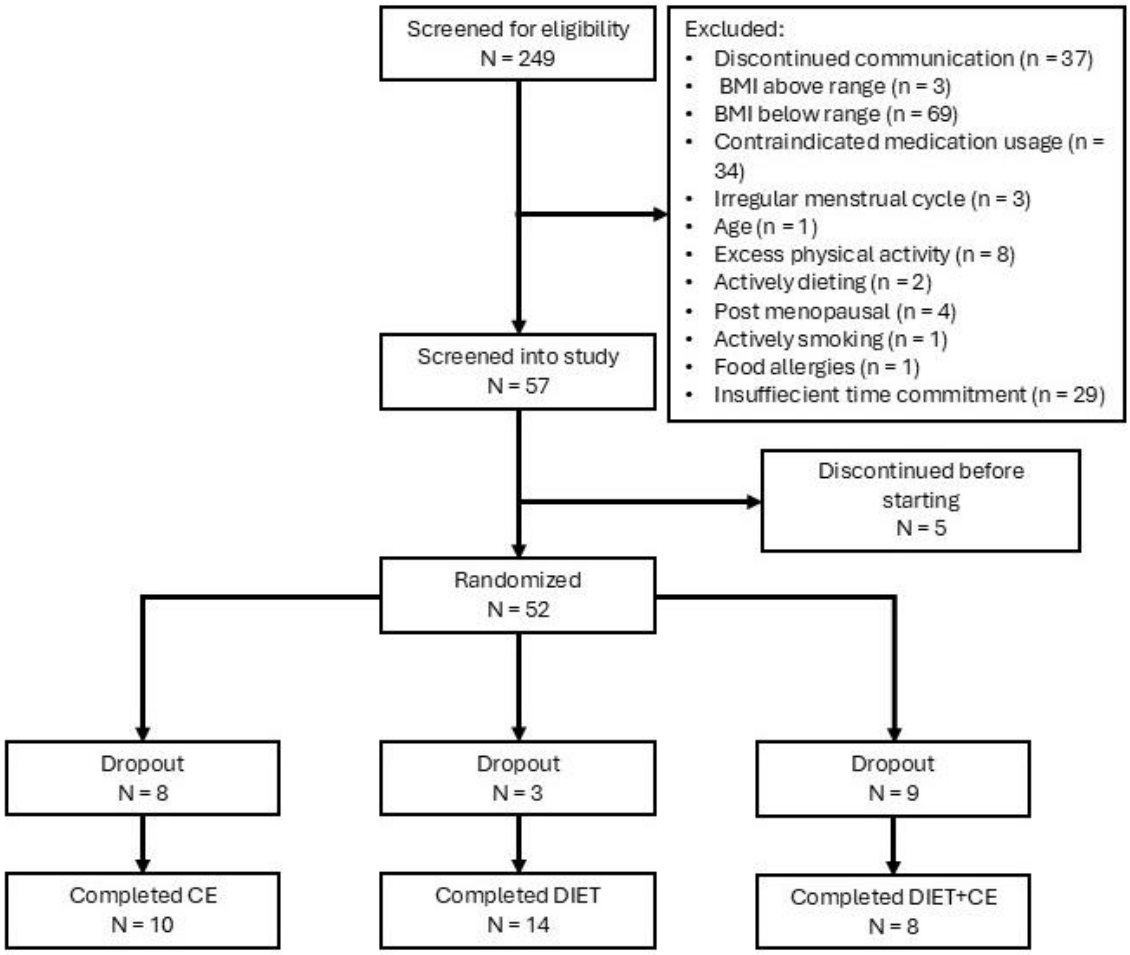

Screened for eligibility
N = 249
Excluded:
• Discontinued communication (n = 37)
• BMI above range (n = 3)
• BMI below range (n = 69)
• Contraindicated medication usage (n = 34)
• Irregular menstrual cycle (n = 3)
• Age (n = 1)
• Excess physical activity (n = 8)
• Actively dieting (n = 2)
• Post menopausal (n = 4)
• Actively smoking (n = 1)
• Food allergies (n = 1)
• Insuffiecient time commitment (n = 29)
Screened into study
N = 57
Discontinued before starting
N = 5
Randomized
N = 52
Dropout
N = 8
Dropout
N = 3
Dropout
N = 9
Completed CE
N = 10
Completed DIET
N = 14
Completed DIET+CE
N = 8

Figure 2.

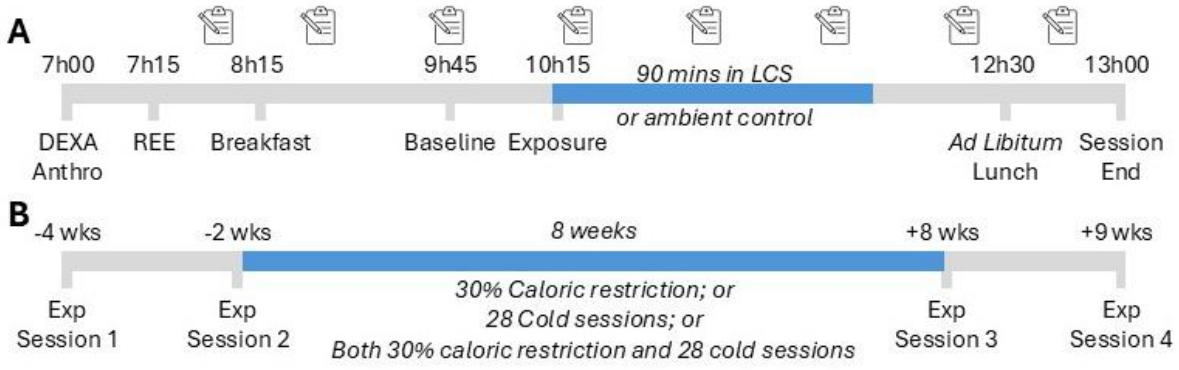

A
7h00
7h15
8h15
9h45
10h15
90 mins in LCS
or ambient control
12h30
13h00
DEXA Anthro
REE
Breakfast
Baseline
Exposure
Ad Libitum Lunch
Session End
B
-4 wks
-2 wks
8 weeks
+8 wks
+9 wks
Exp Session 1
Exp Session 2
30% Caloric restriction; or
28 Cold sessions; or
Both 30% caloric restriction and 28 cold sessions
Exp Session 3
Exp Session 4

Figure 3.

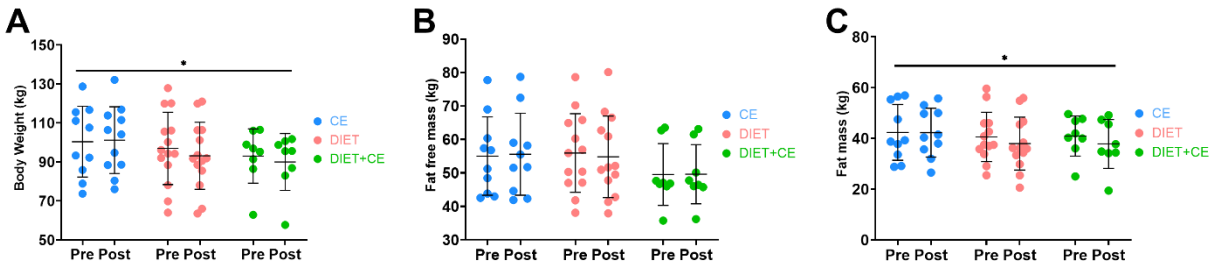

A
Body Weight (kg)
150
130
110
90
70
50
*
Pre Post
Pre Post
Pre Post
CE
DIET
DIET+CE
B
Fat free mass (kg)
90
80
70
60
50
40
30
Pre Post
Pre Post
Pre Post
CE
DIET
DIET+CE
C
Fat mass (kg)
80
60
40
20
0
*
Pre Post
Pre Post
Pre Post
CE
DIET
DIET+CE

Figure 4.

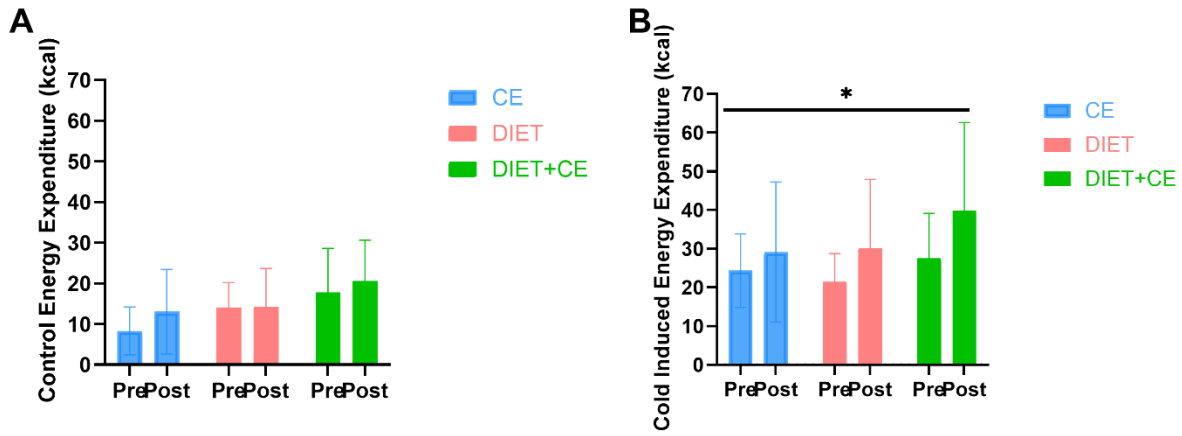

A
Control Energy Expenditure (kcal)
70
60
50
40
30
20
10
0
Pre
Post
Pre
Post
Pre
Post
CE
DIET
DIET+CE
B
Cold Induced Energy Expenditure (kcal)
*
70
60
50
40
30
20
10
0
Pre
Post
Pre
Post
Pre
Post
CE
DIET
DIET+CE

Figure 5.

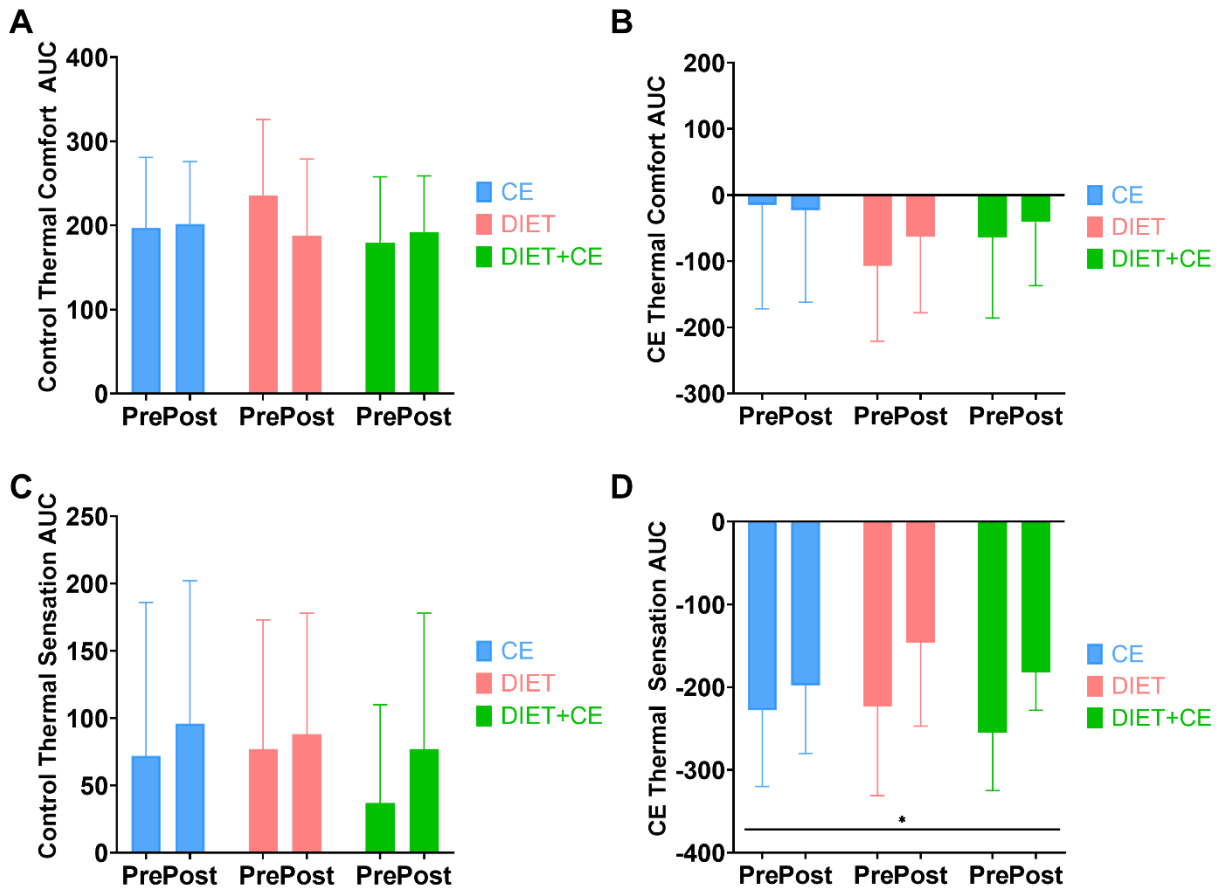

A
Control Thermal Comfort AUC
400
300
200
100
0
PrePost PrePost PrePost
CE
DIET
DIET+CE
B
CE Thermal Comfort AUC
200
100
0
-100
-200
-300
PrePost PrePost PrePost
CE
DIET
DIET+CE
C
Control Thermal Sensation AUC
250
200
150
100
50
0
PrePost PrePost PrePost
CE
DIET
DIET+CE
D
CE Thermal Sensation AUC
0
-100
-200
-300
-400
*
PrePost PrePost PrePost
CE
DIET
DIET+CE

Figure 6.

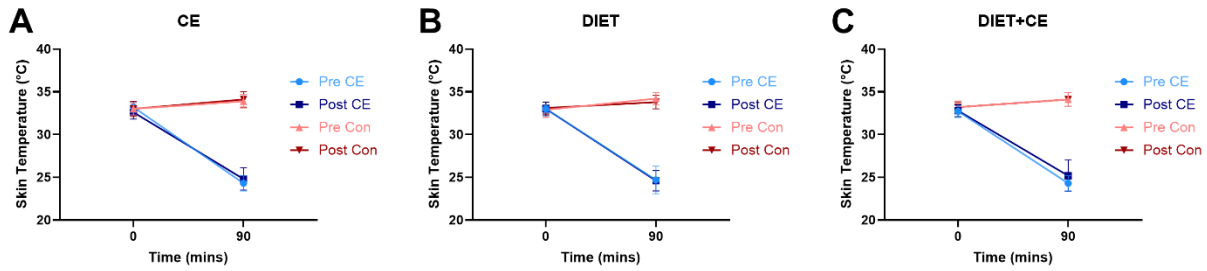
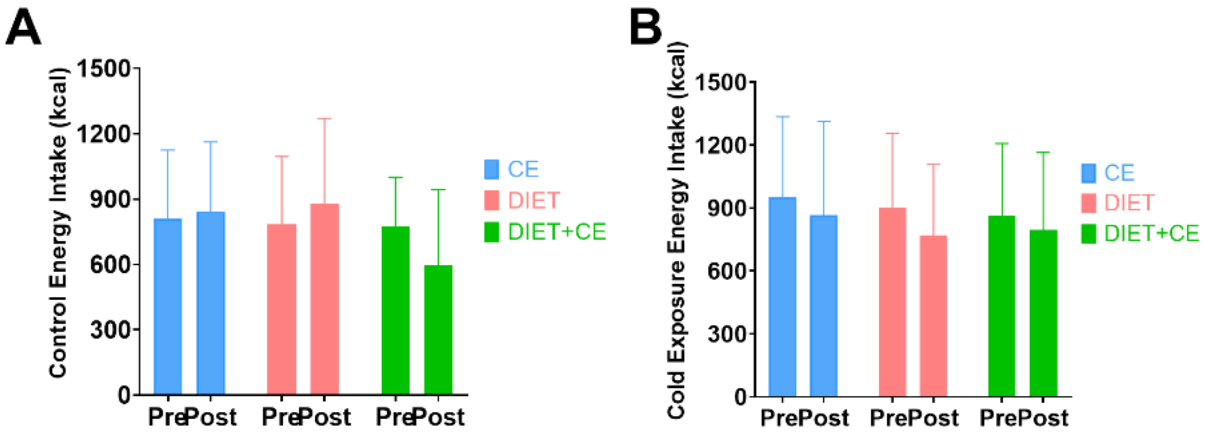

A
CE
B
DIET
C
DIET+CE
Skin Temperature (°C)
40
35
30
25
20
0
90
Time (mins)
Pre CE
Post CE
Pre Con
Post Con

Figure 7.


A
Control Energy Intake (kcal)
1500
1200
900
600
300
0
Pre
Post
Pre
Post
Pre
Post
CE
DIET
DIET+CE
B
Cold Exposure Energy Intake (kcal)
1500
1200
900
600
300
0
Pre
Post
Pre
Post
Pre
Post
CE
DIET
DIET+CE

CHAPTER 6. DISCUSSION

Summary

The three goals of this thesis were to: 1) determine the effects of a single dose of CE on EE, EI and hunger in individuals living with obesity; 2), determine the effects of chronic CE and chronic CE + diet on body energy stores; and 3) to determine the effects of chronic CE and chronic CE + diet on EI and hunger. The hypotheses that EE would increase more during CE than thermoneutral and that EI would increase more than EE during acute exposure were supported, but without significant changes in hunger. Weight loss was, when comparing means, lesser in the DIET+CE group compared to the DIET alone, supporting the hypothesis for the second goal. Finally, EI was not significantly different between groups and hunger decreased in the DIET+CE group, which does not support the hypotheses for the third goal of the thesis. Ultimately, CE did not improve weight loss outcomes, either by itself or in combination with a dietary restriction in individuals living with obesity.

Obesity and Thermoregulation

Multiple rationales have been discussed throughout the thesis that could explain the relatively small increase in EE due to CE seen in individuals living with obesity. They have a higher REE at baseline relative to individuals who are lean [27, 107] due, at least in part, to increased lean mass required to support the characteristic increased mass of adipose tissue found in individuals living with obesity. Therefore, absolute heat production at baseline is higher and can, if even only marginally, help compensate for increased metabolic demands that will be brought on by the cold environment. The increased level of

adiposity is also beneficial due to insulative properties of adipose tissue [108, 109] which result in a smaller amount of heat loss to the environment. This is likely due to the lower ratio of surface area to volume that is characteristic of obesity [110] and cutaneous heat loss being relatively proportional to skin surface area [111, 112], meaning that the amount of heat producing mass is increasing by more than the surface area over which heat loss can occur. Heat loss was not directly measured here throughout these studies, which could help with explaining the EE changes found. It is also possible that the increased level of adiposity creates a heat storage bank that can dissipate heat to the environment while minimizing the impact of heat loss to the core, because even though the specific heat capacity of adipose tissue is relatively low in humans (1.8-2.9 $kJ \cdot kg^{-1} \cdot °C^{-1}$) compared to muscle mass (2.6-3.8 $kJ \cdot kg^{-1} \cdot °C^{-1}$) [113] the sheer volume of adipose tissue in individuals living with obesity can store substantial amounts of heat. Together, this means that individuals living with obesity should theoretically, and as seen throughout the thesis, be more thermally stable when pertaining to passive heat exchange and exchange heat more slowly than individuals who are lean in cold environments [114].

Measurement of Food Intake

The accurate measurement of food intake is a hot debate that has raged for decades. Scientists argue over the accuracy and reliability of subjective (i.e. self-reported) vs objective (i.e. weighed and measured) methods, and the types of biases each can produce [115-117]. Subjective methods require participants to be honest with their reporting, which is often not the case, and even when they attempt to provide accurate information the reported values frequently do not match quantities consumed, with underreporting of

caloric intake of up to 38% [118, 119]. This can lead to vastly incorrect assumptions about food consumption habits for populations, misinform policy choices, and impede nutritional interventions. Objective analyses, such as vending machines and lunch boxes, can ensure that what is being consumed by the participant is accurately measured, but struggles with the ecological validity of its measures. Participants will often decrease food consumption or change eating habits if they know they are being monitored [118], possibly due to fear of judgement or an observer bias where they wish to change consumption patterns to appease researchers, creating an accurate but not generalizable assessment of caloric consumption. Doubly labeled water remains the most accurate assessment of EI, which ironically does not measure EI at all. By comparing EE assessed through doubly labeled water and assessing body energy stores, if participants are weight stable between measurements, then their EI is the total EE measured via doubly labeled water. In this thesis a validated food menu was used because, even if less generalizable, the repeated within-subjects design contains the bias within an individual participant, minimizing the impact of inherent biases.

Attrition

Attrition majorly impacted the study and could have influenced the results. A 20% attrition rate was expected, in line with typical weight loss interventions. The time required to complete the CE and DIET+CE intervention was substantial, with 3-4 lab visits per week, although this time commitment was modeled on a typical exercise intervention [3]. The high levels of discomfort reported by participants during CE sessions can partially explain the much higher levels of dropout seen in the cold interventions, ~44% for CE, ~53% for

DIET+CE and only ~18% for DIET. This high level of dropout likely created an attrition bias in the CE and DIET+CE groups, which could impact the generalizability of the results, though exactly how is unknown. It is possible that the participants willing and able to complete the 28 cold sessions were more tolerant of CE than those who dropped out, had differences in CE responses, or different cognitive, motivational, behavioural or mental health impacts from the repeated cold stimulus. The addition of psychological questionnaires, quality of life and mental health assessments, and qualitative interviews before and after intervention could elucidate what makes someone a completer or not with chronic CE. It is also possible that the high attrition rate could be influencing weight loss outcomes for the CE and DIET+CE groups, as those who experienced less weight loss, particularly in the DIET+CE group, may have less extrinsic motivation to continue the study and drop out, leaving those with greater weight loss to inflate the value, however, this is speculation [120]. Overall, the high attrition rates are a clear indicator of the feasibility problems of CE as a weight loss adjunct.

Blood Draws

Only a subset of participants were able to have blood draws completed due to a combination of scheduling problems, hemolysis of samples and clotting of the intravenous catheter used to collect samples. Due to this only a subsample of 15 participants had an adequate number of samples for analysis at baseline, and when combined with randomization into three groups and attrition rates, resulted in an inadequate number of participants with the necessary number of samples to run longitudinal analyses.

Modalities of Cold Exposure

The main modalities of CE used in research settings are ambient cold, LCS, and cold-water immersion. There are pros and cons to each method. Ambient CE is the easiest and safest method, as it is typically conducted in a respiratory chamber with a decreased ambient temperature and does not possess wind, which greatly impacts the risk of tissue damage and hypothermia in ambient CE due to the increase in convective heat transfer [121, 122]. The impact of ambient CE can be circumvented by some extent by the insulation level of clothing [123] and decreasing surface area exposed, such as by curling into the fetal position and placing hands in the armpits. Cold water immersion has a greater heat loss to the environment than ambient CE due to the greater thermal capacity of water, increasing the rate of conductive and convective heat loss. Due to this, cold water immersion can more easily change core temperature and has a higher risk of causing hypothermia if experiments are done incorrectly [124].

The LCS addresses some of these shortcomings by providing a cold stimulus that the participant cannot attempt to "hide" from, as it covers most of the skin surface area and any changes in body position would maintain the same amount of skin contact, if not increase it if hands are placed against the suit. It also allows for the administration of potent cold stress without the risk of triggering pre-existing respiratory complications that a low ambient temperature could, such as asthma or chronic obstructive pulmonary disease or increase the risk of respiratory infection due to bronchial inflammation [125]. The constant flow of the circulating bath also maintains the same thermal stress for the duration of exposure and limits the ability to warm the external environment and lessen the

impact of CE. The ability to set up multiple circulating baths and have a number of suits that can be cleaned easily and regularly between participants facilitates the scaling of the project for a smaller financial cost. It allowed more participants to be tested simultaneously and concurrently than a chamber, which would be limited to a single person, or a cold-water immersion bath, which needs to be drained, cleaned, refilled and cooled before another participant can be exposed. This was especially impactful for acclimation, where typically at least 5 participants were undergoing chronic CE at once, where after one participant completed a session, another participant was immediately able to begin a subsequent session.

Future Directions

Future chronic CE studies that attempt to further the understanding of body energy store changes should consider the implementation of more psychological measures to capture the subjective changes that occur throughout chronic exposure and determine what traits can predict success. The direct measurement of heat loss would also allow for a more holistic understanding of the thermoregulatory demands of individuals living with obesity and how they change with chronic exposure. The use of a colder temperature to increase heat production levels or a longer exposure to remove body heat stores could help to determine if a more potent cold stimulus is feasible for weight loss, but with even greater levels of discomfort, attrition would likely become too great. It would also be of interest to determine if there are predictors of CE success, including what traits both physiologically and psychologically influence contribute to the ability to complete cold acclimations.

CHAPTER 7. CONCLUSION

The main goal of this thesis was to determine the feasibility of using CE as a weight loss tool, while also measuring the impacts of CE on EI, EE and hunger in individuals living with obesity. Although it has been repeatedly touted that CE could be a potential tool in weight management and weight loss [17-22, 24], it has been shown within this thesis for the first time that repeated CE does not create meaningful changes in body energy stores alone or improve weight loss outcomes when used in combination with a dietary restriction for individuals living with obesity. The high levels of thermal discomfort, minimal changes in heat production and substantially greater drop out rates associated with chronic CE highlight the difficulties of its implementation as a weight loss adjunct. Together, these articles will help inform future studies pertaining to novel weight loss interventions and provide empirical evidence to those seeking to use CE as a therapeutic tool for mobilizing body energy stores.

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Office of Research Ethics and Integrity

CERTIFICAT D'APPROBATION ÉTHIQUE | CERTIFICATE OF ETHICS APPROVAL

Numéro du dossier / Ethics File Number	H-09-19-4928
Titre du projet / Project Title	Cold Exposure, Appetite Control and Weight Loss in Individuals Living with Obesity
Type de projet / Project Type	Recherche de professeur / Professor's research project
Statut du projet / Project Status	Renouvelé / Renewed
Date d'approbation (jj/mm/aaaa) / Approval Date (dd/mm/yyyy)	26/11/2019
Date d'expiration (jj/mm/aaaa) / Expiry Date (dd/mm/yyyy)	25/11/2025

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Conditions spéciales ou commentaires / Special conditions or comments

Université d'Ottawa
Bureau d'éthique et d'intégrité de la recherche

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Le Comité d'éthique de la recherche (CÉR) de l'Université d'Ottawa, opérant conformément à l'*Énoncé de politique des Trois conseils* (2014) et toutes autres lois et tous règlements applicables, a examiné et approuvé la demande d'éthique du projet de recherche ci-nommé.

L'approbation est valide pour la durée indiquée plus haut et est sujette aux conditions énumérées dans la section intitulée "Conditions Spéciales ou Commentaires". Le formulaire « Renouvellement ou Fermeture de Projet » doit être complété quatre semaines avant la date d'échéance indiquée ci-haut afin de demander un renouvellement de cette approbation éthique ou afin de fermer le dossier.

Toutes modifications apportées au projet doivent être approuvées par le CÉR avant leur mise en place, sauf si le participant doit être retiré en raison d'un danger immédiat ou s'il s'agit d'un changement ayant trait à des éléments administratifs ou logistiques du projet. Les chercheurs doivent aviser le CÉR dans les plus brefs délais de tout changement pouvant augmenter le niveau de risque aux participants ou pouvant affecter considérablement le déroulement du projet, rapporter tout évènement imprévu ou indésirable et soumettre toute nouvelle information pouvant nuire à la conduite du projet ou à la sécurité des participants.

The University of Ottawa Research Ethics Board, which operates in accordance with the *Tri-Council Policy Statement* (2014) and other applicable laws and regulations, has examined and approved the ethics application for the above-named research project.

Ethics approval is valid for the period indicated above and is subject to the conditions listed in the section entitled "Special Conditions or Comments". The "Renewal/Project Closure" form must be completedfour weeks before the above-referenced expiry date to request a renewal of this ethics approval or closure of the file.

Any changes made to the project must be approved by the REB before being implemented, except when necessary to remove participants from immediate endangerment or when the modification(s) only pertain to administrative or logistical components of the project. Investigators must also promptly alert the REB of any changes that increase the risk to participant(s), any changes that considerably affect the conduct of the project, all unanticipated and harmful events that occur, and new information that may negatively affect the conduct of the project or the safety of the participant(s).

Coordonateur / COORDINATOR
Coordonnateur de l'éthique / Ethics Coordinator
Pour/For **Daniel LAGAREC** Président(e) du/ Chair of the **Comité d'éthique de la recherche en sciences de la santé et sciences / Health Sciences and Sciences Research Ethics Board**

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CONSENT FORM

COLD EXPOSURE, APPETITE CONTROL, AND WEIGHT LOSS
IN INDIVIDUALS LIVING WITH OBESITY

Principal Investigator: Éric Doucet (Ph.D.)
Co-Investigator: François Haman (Ph.D)
Faculty of Health Sciences, University of Ottawa
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1. INVITATION TO PARTICIPATE: You are invited to participate in the above named research study funded by the Canadian Institute of Health Research.

2. PURPOSE OF THE STUDY: The primary purpose of this study is to determine the effects of weight loss induced by cold exposure and dieting on food intake and appetite. The present study will also measure blood hormone levels in order to look for relationships between changes in energy balance and cold exposure. It will also investigate the impact of chronic cold exposure on shivering.

3. BACKGROUND: When exposed to cold temperatures, humans have to increase their energy expenditure (i.e. calories burned) to maintain their core temperature. This has been shown repeatedly in both acute and chronic interventions, leading some researchers to believe that cold exposure could be used as a weight loss tool. However, the majority of these studies have been done in lean individuals, making results difficult to generalize to the entire population, as there is variation in how much energy expenditure increases between people. There has also been minimal research into how food intake and appetite are impacted by cold exposure, with no studies testing individuals living with obesity. Therefore, this is the first study is to investigate cold exposure as a weight loss tool. It will also measure how chronic cold exposure impacts appetite and food intake both before and after the weight loss intervention. This study will use cold exposure, alone and in combination with dieting, to determine the feasibility of cold exposure as a weight loss tool. Measurements during this study will include body composition, food intake, resting energy expenditure, appetite, olfaction (smell tests), hormone levels and accelerometry, all of which are explained below. Results from this study will enable us to better understand how the body compensates in response to chronic cold exposure and how it impacts food intake and appetite.

4. DESCRIPTION OF THE STUDY:

This study is an 8 week weight loss intervention with 3 randomly assigned groups: DIET, cold exposure (CE), and DIET+CE. You will undergo a screening session outlined below. There will be 4 experimental sessions, 2 before and 2 after the intervention you are assigned to. There will be one week between each experimental session, for a total of 13 weeks. All groups will undergo the same sessions, in a random order. There will be a control experimental session and cold exposure experimental session, as outlined below. The CE and DIET+CE groups will come to the lab every 2nd day for the 8 week intervention to undergo cold exposures as outlined below. The DIET and DIET+CE groups will have a weekly nutritional consultation at the lab as well as a weekly check-in via text, phone, or email. You will be

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assigned to one of the 3 groups after completing the screening session. A complete description of what you will need to do it provided below.

SCREENING

7h30 – Informed Consent and Screening. You will be asked to arrive at the lab after a 12h overnight fast. We will go over the informed consent form and verify inclusion criteria are met. Body weight and height will be measured to confirm body mass index (BMI).

8h00 – Resting energy expenditure. During this measurement, you will be lying still on your back with a plexiglass hood over your head in a quiet room. The expired air will be sampled to determine oxygen consumption and resting energy expenditure will be derived. This test requires that you lie quietly and relaxed for approximately 60 minutes. Breakfast will provided following this test. Afterwards, we will give you an accelerometer to measure your activity levels for 1 week. This data, in combination with your resting energy expenditure, will allow us to calculate your required energy intake, the number of calories you need to consume to maintain current body weight.

EXPERIMENTAL SESSIONS

There will be a total of 4 experimental sessions, one cold exposure (CE) and one ambient condition (AC) both before and after the intervention. They will be in a randomized order and separated by 1 week.

7h15 – Anthropometrics and DEXA. You will be asked to arrive after a 12h overnight fast and having refrained from vigorous activities for 48h. Body weight and height will be measured. A Dual Energy X-Ray Absorptiometry (DEXA) scan will be used to measure bone density, fat mass and lean body mass. You will have to lie on an examination table, fully clothed, while a low intensity x-ray will scan the entire body. The measurements take 15 minutes. The only risk is a minimal x-ray exposure of less than 0.5 millirem. This exposure is less than the natural background radiation from 1 day of exposure to sunlight.

7h30 – Insertion of catheter. A qualified nurse will place an intravenous catheter into the ante-cubital vein distal to the elbow to take blood draws at various points outlined below. A total of 8 collections will be done, with each collections being 10 mL for a total of 80 mL. The first will be taken immediately after insertion. This will be followed by a short rest period. Blood samples will be kept for 10 years after publication, and then will be destroyed through the university biohazard waste disposal process. Blood draws will occur at the following additional time points: **9h, 9h40, 10h20, 11h, 11h40, 12h20, 1h**.

8h00 – Resting energy expenditure. You will be asked to undergo a shortened version of the resting energy expenditure measurement outlined above, lasting approximately 30 minutes.

8h30 – Appetite measurement. You will be asked to fill out visual analog scales (VAS) at this point and various other points during the session. Briefly, desire to eat, hunger, fullness and prospective food consumption will be rated. Questions will be asked as follows: 1) “How strong is your desire to eat?” (Very weak- Very strong); 2) “How hungry do you feel?” (Not hungry at all- As hungry as I have ever felt); 3) “How full do you feel?” (Not full at all- Very full), and 4) “How much food do you think you could eat?” (Nothing at all- A large amount). VAS will be completed at the following additional time points: 9h, 9h40, 10h20, 11h, 11h40, 12h20, 1h.

8h35 – Standardized breakfast. You will be asked to choose food items from our validated food menu. You will be served the same breakfast for each session. You must consume the entirety of its contents within 20 minutes.
9h00 – Baseline measurements. You will be fitted with 4 surface electrodes on your sternocleidomastoid (neck), trapezius (shoulder), pectoralis major (chest), and rectus femoris (thigh) to measure muscular activity during shivering. You will do 3 maximal contractions of 5 seconds for each muscle to create a baseline measurement for comparison. You will then be fitted with 12 thermocouples at the following sites: forehead, upper back, lower back, abdominal area, quadriceps, hamstrings, front calf, back calf, chest, biceps, forearm, and hand. These discs will allow us to measure your skin temperature in various locations and get an overall mean skin temperature (T_{skin}). You will then be fitted with a 3 piece liquid conditioned suit that can be infused with either cold or ambient temperature water to manipulate skin temperature. We will begin with measuring your T_{skin} at rest while you lay down on a bed to determine your ambient T_{skin}. During this time the plexiglass hood will again be placed over your head to measure energy expenditure. You will also be asked to rate both your thermal comfort and thermal sensation on scales of – to +5 during the exposure.
10h00 – Exposure. You will then undergo either CE or AC. For CE, the suit will be perfused with cold water at 10°C for 2h. For AC, the suit will be perfused with thermoneutral water to maintain your T_{skin}. VAS and blood samples will be taken at **10h, 11h,** and **12h**. Once the exposure is completed, the suit, electrodes and thermocouples will all be removed.
13h00 – Olfaction and food reward. You will be required to complete 3 different odor tests. First, a set of 3 capsules will be subsequently presented to you and you must identify which of the 3 capsules presents an odor (the other 2 capsules are odorless). Second, a set of 3 capsules will be subsequently presented to you and you must identify which of the 3 capsules presents a different odor (the other 2 capsules will have the same odor). Finally, you will be required to identify the correct odor released by the capsule. Examples of some of the odors include rose, marker, banana, and grass. For the food reward task, you will do the Leeds Food Preference Questionnaire. This is a computerized task that uses 16 foods in 4 different categories: high-fat sweet, low-fat sweet, high-fat non-sweet, low-fat non-sweet. You will rank each of the 16 foods on a visual analog scale of 0-100. You will also undergo a choice task, where you will choose a preferred of 2 options on repeat, until all 16 foods have been matched with each other food.
13h30 – *Ad Libitum* lunch. You will be given a food menu, from which you can choose as many foods as you like. They will all be served to you in *ad libitum* quantities, where you can consume as much or as little of each food as you would like in the given time period.
14h15 – Olfaction and food reward. You will be asked to complete the tasks listed above again.
14h45 – Conclusion. At the end of the trial you will be asked to select foods to consume for the following two days from the validated food menu. They will be packed into labeled lunch boxes (coolers) and provided in *ad libitum* quantities, meaning you can consume as much or as little of each food as you would like, leaving any extra food, scraps, and wrappers in the cooler.

DIET Intervention

Your dietary energy prescription (i.e. how many calories you will eat) will be determined using your resting energy expenditure and accelerometer data to calculate your energy required to maintain current body weight. We will then

prescribe a caloric restriction of 30% from this value. Your diet will be personalized by a nutritionist, who will explain how the system works. Briefly, your diet will be prescribed using the Canadian Food Exchange System. This method will allow you to select foods you enjoy during the weight loss, however in smaller quantities. Exchange lists are groups of foods that contain a similar mix of carbohydrates, protein, fat, and calories. The Canadian exchange system there are seven exchange groups: Grains & Starches; Fruits; Milk & Alternatives; Vegetables; Meat & Alternatives; Fats; Unlimited consumption. Within any group, you can exchange one food serving for another. For example, in the Meats group, some sample foods that equal one lean meat exchange might be: 1 oz. of white meat chicken or turkey with no skin or 1 oz. of lean beef. The daily meal plan will contain foods from all seven Food Exchange lists in order to assure you a complete and a balanced nutrition. However, the number of portions from each group will be recommended based on your individual diet evaluated in the preliminary session. This session will also be used to clarify any potential questions that you have regarding the project and the intervention. You will also have weekly sessions with the nutritionist of roughly 30 minutes at the lab, as well as mid-week check-in via text, email, or phone call with our nutritionist.

COLD EXPOSURE Intervention

You will be asked to come to the laboratory every second day during the 8 week intervention for a 90 minute session in the liquid conditioned suit, where we will again perfuse the suit with 10°C water, similar to the experimental session. For these sessions you will be fitted with the liquid conditioned suit, but not the electrodes or thermocouples. During this time you will have access to Netflix or can bring your own entertainment. At the conclusion of the study you will be given a consultation session with the nutritionist to explain the Canadian Food Exchange System, as well as answer any dietary questions you may have.

DIET+CE Intervention

You will undergo both the dietary restriction of the diet group and the cold intervention of the CE group, described above.

5. POSSIBLE RISKS/DISCOMFORTS:

The risks associated with this project are low and minimal. The measure of body composition presents a low risk for you. However, it is important to underline that this apparatus will expose you to a minimal radiation (the equivalent of a day in the sun, at 0.02 – 0.05 millirem). The blood samples also present very few risks. However, a small local hematoma (a bruise at the venal puncture) could develop during the few days following the blood sampling. It is important to note that the risks of infection, of phlebitis (inflammation of the vein) and vaso-vagal shock (loss of consciousness) are very low, but still remain a possibility. There is also no risk associated with the resting energy expenditure measurement or the wearing of an accelerometer. The use of the liquid condition suit for cold exposure presents minimal risks, as the cold exposure will be compensable and not change core temperature, meaning you will be cold and minorly uncomfortable, but your body can produce enough heat to maintain the same core temperature. The physical practice of low intensity shivering can result in muscular and joint stiffness and/or cramps. In extreme cases, when exposure to cold is prolonged and severe, heart attacks and even death may occur, however this stimuli is mild. These risks depend largely on physical condition, age, and the level of physical fitness. Being healthy makes

it extremely unlikely for this to occur. Shivering can result in the same effects as low intensity exercise due to the increase in physical demands on the body. There is also the possibility of acute fatigue due to the prolonged shivering, meaning you may feel tired or sore after the cold exposure is completed. During the diet intervention, you might feel some physical discomfort (e.g. tiredness, weakness, dizziness, irritability and lethargy). However, we will provide a weekly consult throughout intervention, to address concerns you may have with your diet plan and to provide motivation to optimize compliance to your dietary plan.

6. BENEFITS:

This study will provide valuable information regarding the feasibility of cold exposure as a weight loss tool, how it impacts food intake and appetite, as well as the changes in shivering that occur with chronic cold exposure.

7. COMPENSATION:

Parking at the research center is free for participants, as are all scientific tests. You will be given information regarding your body composition and resting metabolic rate. You will receive nutritional counselling during either the DIET or DIET+CE interventions, or a consultation with the nutritionist upon the completion of the study if you are part of the CE group. Additionally, you will receive a compensation of $150.00 for your participation, with half being allocated after completing the baseline sessions pre-intervention, and half being allocated upon completing the study.

8. CONFIDENTIALITY AND ANONYMITY:

In order to guarantee the confidentiality and anonymity of participants, all precautions and necessary measures will be taken to ensure that results and personal information of participants is kept under the strictest of confidentiality.

-Only the following persons will have access to the material: Dr. Éric Doucet, Dr. François Haman, Dr. Luzia Hintze, Shawna Watt (study nurse) and Kurt McInnis (Ph.D student). Any other individuals involved in the study will not have access to participant's personal information and results.

-The names of participants will not appear on any reports. A number code will be used to identify participants on all research documents.

-All material and information which can be linked to participants will not be made public and will be kept under the strictest confidentiality.

-Participants will not be identified in any way in publications or reports.

-The data collected will be kept in a locked cabinet in the Behavioral and Metabolic Research Unit with restricted access where all participants' folders will be kept. In addition, the computer files will be protected by a password.

-Data will be destroyed five years after publication of study results.

-Your data will be destroyed if you choose to withdraw.

-Blood samples will be kept in a locked laboratory and will be destroyed after publication of study results.

9. VOLUNTARY PARTICIPATION

You are free to refuse to participate and if you choose to participate, you are free to withdraw from the study at any time for any reason. At any moment during this study, the best interests of participants will always prevail upon the objectives of the study. The participants will be made aware of new findings that might influence their decision to take part in the present study.

Any information about your rights as a research participant may be addressed to: Protocol officer for ethics in research, University of Ottawa, 550 Cumberland, Tabaret Hall, room 154, Ottawa, Ontario, K1N 6N5; Phone: (613) 562-5387, email: ethics@uottawa.ca.

If you have any questions about the conduct of the research project, you may contact Luzia Hintze at ljaeg051@uottawa.ca or 613-914-0634, or the Dr. Éric Doucet at edoucet@uottawa.ca or 613-562-5800 ext. 7364.

There are two copies of the consent form, one of which I may keep.

RESEARCH COORDINATOR
Luzia Hintze, Ph.D.: _____
Date: _____

PRINCIPAL INVESTIGATOR'S SIGNATURE

Eric Doucet, Ph.D: _____
Date: _____

PARTICIPANT'S SIGNATURE:
I agree to participate in this study,

_____ _____
Printed Name Signature

Date: _____

Should you choose to withdraw from the study, any partial data completed may be used with your permission.

Do you allow use to share your coordinates with Dr. Pascal Imbeault's research team for a partner study? This partner study entails adipose biopsies that will be performed before and at the end of the intervention. Briefly, a small incision will be performed at the level of the umbilicus and a small amount of subcutaneous adipose tissue will be taken from this incision site. If you should accept to share these coordinates, full details of this partner study would be provided by Dr. Imbeault's team. Please note that refusal to share your coordinates with Dr. Imbeault's research team will not affect in any way your eligibility for the study presented in this consent form.

I accept I refuse

uOttawa
Faculté des sciences de la santé
Faculty of Health Sciences

Université d'Ottawa
Faculté des sciences
de la santé

École des sciences de
l'activité physique

University of Ottawa
Faculty of Health Sciences

School of Human Kinetics

FORMULAIRE DE CONSENTEMENT

EXPOSITION AU FROID, CONTRÔLE DE L'APPÉTIT ET PERTE DE POIDS CHEZ DES INDIVIDUS VIVANT AVEC L'OBÉSITÉ

Chercheur principal : Éric Doucet (Ph.D.)
Co-chercheur : François Haman (Ph.D)
Faculté des sciences de la santé, Université d'Ottawa
École des sciences de l'activité physique

1. INVITATION À PARTICIPATER : Vous êtes invités à participer à l'étude mentionnée ci-dessus financée par l'Institut de recherche en santé au Canada.

2. OBJECTIF DE L'ÉTUDE : L'objectif principale de cette étude est de déterminer les effets de la perte de poids induit par l'exposition au froid et un régime sur la prise alimentaire et l'appétit. La présente étude mesurera également les niveaux d'hormones sanguines afin de déceler les relations entre les changements dans le bilan énergétique et l'exposition au froid. L'impact de l'exposition chronique au froid sur le frissonnement sera examiné.

3. CONTEXTE : Lorsque les humains sont exposés à de basses températures, ils ont besoin d'augmenter leur dépense énergétique (c.-à-d. les calories brulées) pour maintenir la température centrale. Ce phénomène a été démontré à plusieurs reprises lors d'interventions aigües et chroniques, amenant certains chercheurs à croire que l'exposition au froid pourrait être un outil pour la perte de poids. Toutefois, la majorité de ces études ont été réalisées avec des individus de poids santé, ce qui rend les résultats difficiles à généraliser à l'ensemble de la population, puisque la dépense énergétique varie entre individus. Il y a également peu de recherches sur l'impact de l'exposition au froid sur l'apport alimentaire et l'appétit. De plus, aucune étude n'a été menée sur des personnes vivant avec l'obésité. Par conséquent, il s'agit de la première étude utilisant l'exposition au froid comme intervention de perte de poids. L'impact de l'exposition au froid chronique sur l'appétit et la prise alimentaire avant et après l'intervention de perte de poids sera aussi évalué. Cette étude utilisera l'exposition au froid, seule ou en combinaison avec un régime, afin de déterminer la faisabilité de l'exposition au froid comme outil de perte de poids. Les mesures effectuées au cours de l'étude comprendront la composition corporelle, l'apport alimentaire, la dépense énergétique au repos, l'appétit, l'olfaction (tests olfactifs), les niveaux hormonaux et d'accélérométrie, qui sont tous expliqués ci-dessous. Les résultats obtenus permettront de mieux comprendre comment l'organisme compense à la suite de l'exposition chronique au froid et l'influence sur la prise alimentaire et l'appétit.

4. DESCRIPTION DE L'ÉTUDE :
Cette étude est une intervention de perte de poids de 8 semaines avec 3 groupes assignés au hasard : RÉGIME, exposition au froid (EF) et RÉGIME+EF. Vous participerez à une visite préliminaire décrite ci-dessous. Il y aura 4 séances expérimentales, 2 avant et 2 après l'intervention à laquelle vous êtes assignés. Il y aura une semaine entre chaque session expérimentale, pour un total de 13 semaines. Tous les groupes suivront les mêmes séances, dans un ordre aléatoire. Il y aura une session

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expérimentale de contrôle et une session expérimentale d'exposition au froid, tel que décrit ci-dessous. Les groupes EF et RÉGIME+EF se rendront au laboratoire tous les 2 jours pour l'intervention de 8 semaines afin de subir les expositions au froid décrites ci-dessous. Les groupes RÉGIME et RÉGIME+EF auront une consultation nutritionnelle hebdomadaire au laboratoire ainsi qu'une vérification hebdomadaire par SMS, téléphone ou courriel. Vous serez assignés à l'un des 3 groupes à la fin de la visite préliminaire. Une description complète de ce dont vous aurez besoin pour le faire est fournie ci-dessous.

VISITE PRÉLIMINAIRE

7h30 – Consentement éclairé et mesures de base.

Nous allons vous demander d'arriver au laboratoire après un jeûne nocturne de 12 heures. Nous allons passer à travers le consentement éclairé et vérifier à ce que les critères d'inclusions soient respectés. Votre poids corporel et grandeur seront mesurés pour confirmer l'indice de masse corporel (IMC).

8h00 – Dépense énergétique de repos.

Lors de cette procédure, vous serez couché sur le dos avec un bulle en Plexiglas au-dessus de votre tête dans une chambre calme. L'air expiré sera analysé pour déterminer votre consommation d'oxygène, ensuite dérivé pour donner une mesure de la dépense énergétique au repos. Ce test exige que vous vous allongiez en restant détendu pour une période d'environ 1 heure. Un petit-déjeuner vous sera servi après le test. Par la suite, nous vous remettrons un accéléromètre pour mesurer votre niveau d'activité pendant 1 semaine. Ces données, combinées à votre dépense énergétique au repos, nous permettront de calculer votre apport énergétique requis, le nombre de calories que vous devez consommer pour maintenir votre poids corporel actuel.

VISITES EXPÉRIMENTALES

Il va y avoir un total de 4 visites expérimentales, une exposition au froid (EF) et une condition ambiante (CA) avant et après l'intervention. Les visites seront randomisées et séparées par 1 semaine.

7h15 – Mesures anthropométriques et DEXA. Vous allez devoir arriver après un jeûne nocturne de 12 heures et vous abstenir de toute activité physique vigoureuse pendant les 48 heures précédant le test. Le poids corporel et la taille seront mesurés. Une mesure d'ostéodensitométrie (DEXA : *Dual Energy X-ray Absorptiometry*) sera utilisée pour mesurer la densité osseuse, la masse grasse et la masse maigre. Vous devrez vous allonger sur une table d'examen, entièrement vêtu, alors qu'une radiographie à faible intensité balayera tout le corps. Ces mesures prennent 15 minutes. Le seul risque est une exposition minimale aux rayons X inférieure à 0,5 millirem. Cette exposition est inférieure à l'exposition à la lumière du soleil lors d'une journée.

7h30 – Insertion du cathéter

Une infirmière qualifiée placera un cathéter intraveineux dans la veine cubitale distale du coude pour les prélèvements sanguins aux différents points décrits ci-dessous. Au total, 8 prélèvements seront effectués, chacun étant de 10 ml pour un total de 80 ml. Le premier sera effectué immédiatement après l'insertion du cathéter. Suivra une courte période de repos. Les échantillons de sang seront conservés pendant 10 ans après leur publication, puis seront détruits par le processus universitaire d'élimination des déchets biologiques dangereux. Les prises de sang auront lieu aux moments suivants: **9h, 9h40, 10h20, 11h, 11h40, 12h20, 13h**.

8h00 – Dépense énergétique de repos. On vous demandera de faire une version abrégée de la mesure de la dépense énergétique de repos décrite ci-dessus, d'une durée d'environ 30 minutes.

8h30 – Mesure de l'appétit. On vous demandera de remplir des échelles visuelles analogues (EVA) à ce stade et à divers autres moments lors de la visite. En somme, le désir de manger, la faim, la plénitude et la consommation alimentaire potentielle seront évalués. Les questions seront posées comme suit : 1) « À quelle intensité se situe votre envie de manger ?» (Très faible - très fort); 2) « À quel point avez-vous faim? » (Pas du tout faim - plus affamé que jamais); 3) "À quel point vous sentez-vous rassasié?" (Pas du tout - complètement), et 4) "Quelle quantité de nourriture pensez-vous pouvoir manger?" (Rien du tout - une grande quantité). L'EVA sera complétée aux moments suivants: 9h, 9h40, 10h20, 11h, 11h40, 12h20, 13h.

8h35 – Petit déjeuner standardisé. On vous demandera de choisir des aliments à partir de notre menu d'aliments validés. Le même petit-déjeuner vous sera servi à chaque séance. Vous devez consommer la totalité de son contenu dans un délai de 20 minutes.

9h00 – Mesures de références. Vous serez munis de 4 électrodes de surface sur votre muscle sterno-cléido-mastoïdien (cou), trapèze (épaule), grand pectoral (poitrine) et le droit fémoral (cuisse) pour mesurer l'activité musculaire pendant le frissonnement. Vous réaliserez 3 contractions maximales de 5 secondes pour chaque muscle afin d'avoir une mesure de référence à des fins de comparaison. Vous serez ensuite munis de 12 thermocouples aux endroits suivants : front, haut du dos, bas du dos, région abdominale, quadriceps, ischiojambiers, mollet avant, mollet arrière, poitrine, biceps, avant-bras et mains. Ces disques nous permettront de mesurer la température de votre peau à différents endroits et d'obtenir une température moyenne globale de la peau (T_{peau}). Vous serez ensuite munis d'une combinaison 3 pièces qui peut être infusées avec de l'eau froide ou à température ambiante pour manipuler la température de la peau. Nous commencerons par mesurer votre T_{peau} au repos pendant que vous vous allongez sur un lit pour déterminer votre T_{peau} ambiante. Pendant ce temps, la bulle en plexiglas sera à nouveau placée au-dessus de votre tête pour mesurer la dépense énergétique. On vous demandera également d'évaluer votre confort thermique et votre sensation thermique sur une échelle de - à + 5 pendant l'exposition.

10h00 – Exposition. Vous allez ensuite vous soumettre à EF ou CA. Pour l'EF, la combinaison sera perfusée avec de l'eau froide à 10°C pendant 2h. Pour CA, la combinaison sera perfusée avec de l'eau à température neutre pour maintenir votre T_{peau}. L'EVA et les prélèvements sanguins seront effectués à **10h, 11h** et **12h**. Une fois l'exposition terminée, la combinaison, les électrodes et les thermocouples vous seront retirés.

13h00 – Olfaction et récompense alimentaire. Vous devrez compléter 3 différents tests d'olfaction. Tout d'abord, un ensemble de 3 capsules vous sera présenté et vous devrez identifier laquelle des 3 capsules présente une odeur (les 2 autres capsules sont inodores). Ensuite, un ensemble de 3 capsules vous sera présenté et vous devrez identifier laquelle des 3 capsules présente une odeur différente (les 2 autres capsules auront la même odeur). Enfin, vous devrez identifier la bonne odeur dégagée par la capsule. Voici quelques exemples d'odeurs : rose, marqueur, banane et herbe. Pour la tâche de récompense alimentaire, vous devrez remplir un questionnaire (Leeds Food Preference Questionnaire). Il s'agit d'une tâche informatisée qui utilise 16 aliments de 4 catégories différentes : riche en gras et sucre, faible en gras et riche sucre, riche en gras et faible en sucre, faible en gras et non

sucré. Vous classerez chacun des 16 aliments sur une échelle visuelle analogique de 0 à 100. Vous serez également soumis à une tâche de choix, où vous choisirez une des deux options préférées, jusqu'à ce que les 16 aliments aient été jumelés avec les autres aliments.

13h30 – Diner *Ad Libitum*. On vous remettra un menu d'aliments dans lequel vous pourrez choisir autant d'aliments que vous le souhaitez. Ils vous seront tous servis en quantités ad libitum, où vous pourrez consommer autant ou aussi peu de chaque aliment que vous le souhaitez dans le temps alloué.

14h15 – Olfaction et récompense alimentaire. On vous demandera d'accomplir à nouveau les tâches énumérées ci-dessus.

14h45 – Conclusion. À la fin de la visite, on vous demandera de choisir les aliments à consommer pour les deux jours suivants du menu d'aliments validés. Ils seront emballés dans des boîtes à lunch (glacières) étiquetées et fournis en quantités ad libitum, ce qui signifie que vous pouvez consommer autant ou aussi peu de chaque aliment que vous le souhaitez, laissant tout aliment supplémentaire, les restants et emballages dans la glacière.

Intervention RÉGIME

Votre prescription d'énergie alimentaire (c.-à-d. le nombre de calories que vous mangerez) sera déterminée en fonction de votre dépense énergétique de repos et des données de l'accéléromètre pour calculer votre énergie requise pour maintenir votre poids corporel actuel. Nous prescrivons alors une restriction calorique de 30% de cette valeur. Votre régime sera personnalisé par un/une nutritionniste qui vous expliquera le fonctionnement du système. En bref, votre régime alimentaire vous sera prescrit par le système d'échange alimentaire canadien. Cette méthode vous permettra de choisir des aliments que vous aimez pendant la perte de poids, mais en plus petites quantités. Les listes d'échange sont des groupes d'aliments qui contiennent un mélange similaire de glucides, de protéines, de lipides et de calories. Le système d'échange alimentaire canadien compte sept groupes alimentaires : Céréales et amidons ; Fruits ; lait et substituts ; Légumes ; Viandes et substituts ; graisses ; autres aliments. Au sein d'un groupe, vous pouvez échanger une portion de nourriture contre une autre. Par exemple, dans le groupe Viandes, certains échantillons d'aliments qui équivalent à un échange de viande maigre pourraient être : 1 oz. de viande blanche de poulet ou de dinde sans peau ou 1 oz. de bœuf maigre. Le plan de repas quotidien contiendra des aliments provenant des sept listes du système d'échange afin de vous assurer une alimentation complète et équilibrée. Cependant, le nombre de portions de chaque groupe sera recommandé en fonction de votre alimentation individuelle évaluée lors de la séance préliminaire. Cette session sera également utilisée pour clarifier toutes les questions que vous pourriez avoir concernant le projet et l'intervention. Vous aurez également des séances hebdomadaires d'environ 30 minutes avec le/la nutritionniste au laboratoire, ainsi qu'une vérification en milieu de semaine par texto, courriel ou appel téléphonique avec notre nutritionniste.

Intervention EXPOSITION AU FROID

On vous demandera de venir au laboratoire tous les deux jours pendant l'intervention de 8 semaines pour une session de 90 minutes dans la combinaison conditionnée liquide, où nous perfuserons à nouveau la combinaison avec 10°C d'eau, comme pour la session expérimentale. Pour ces séances, vous serez équipés de la combinaison à conditionnement liquide, mais pas d'électrodes ou de thermocouples. Pendant ce temps, vous aurez accès à Netflix ou vous pourrez apporter votre propre divertissement. À

la fin de l'étude, vous aurez une séance de consultation avec le/la nutritionniste pour expliquer le système d'échange alimentaire canadien et répondre à toute question diététique que vous pourriez avoir.

Intervention RÉGIME+EF

Vous subirez à la fois les restrictions alimentaires du groupe diététique et l'intervention à froid du groupe EF, décrites ci-dessus.

5. RISQUES POSSIBLES/INCONFORTS:

Les risques associés à ce projet sont faibles et minimes. La mesure de la composition corporelle présente un faible risque pour vous. Cependant, il est important de souligner que cet appareil vous exposera à un rayonnement minimal (l'équivalent d'une journée au soleil, de 0,02 à 0,05 millirem). Les échantillons sanguins présentent également très peu de risques. Cependant, un petit hématome local (une ecchymose à la ponction veineuse) pourrait se développer au cours des quelques jours suivants le prélèvement sanguin. Il est important de noter que les risques d'infection, de phlébite (inflammation de la veine) et de choc vasovagal (perte de conscience) sont très faibles, mais demeurent néanmoins une possibilité. Il n'y a pas non plus de risque associé à la mesure de la dépense énergétique au repos ou au port d'un accéléromètre. L'utilisation de la combinaison pour l'exposition au froid présente des risques minimes, car l'exposition au froid sera compensée par votre corps et ne changera pas votre température centrale, ce qui signifie que vous aurez froid et serez légèrement inconfortable, mais que votre corps pourra produire suffisamment de chaleur pour maintenir la température centrale. Le frissonnement de faible intensité peut entraîner des raideurs musculaires et articulaires et/ou des crampes. Dans les cas extrêmes, lorsque l'exposition au froid est prolongée et sévère, des crises cardiaques et même la mort peuvent survenir, mais ce stimulus est léger. Ces risques dépendent en grande partie de la condition physique, de l'âge et du niveau de forme physique. Le fait d'être en bonne santé rend cela extrêmement improbable. Le frissonnement peut avoir les mêmes effets que les exercices de faible intensité en raison de l'augmentation des exigences physiques pour le corps. Il y a aussi la possibilité d'une fatigue aiguë due aux frissons prolongés, ce qui signifie que vous pouvez vous sentir fatigué ou endolori une fois l'exposition au froid terminée. Au cours de l'intervention diététique, vous pourriez ressentir un certain inconfort physique (p. ex. fatigue, faiblesse, étourdissements, irritabilité et léthargie). Cependant, nous vous fournirons une consultation hebdomadaire tout au long de l'intervention, afin de répondre à vos préoccupations concernant votre plan diététique et de vous motiver à optimiser la conformité à votre plan diététique.

6. AVANTAGES:

Cette étude fournira de précieux renseignements sur la faisabilité de l'exposition au froid en tant qu'outil de perte de poids, sur son incidence sur l'apport alimentaire et l'appétit, ainsi que sur les changements dans les frissons qui surviennent lors d'une exposition chronique au froid.

7. COMPENSATION:

Le stationnement au centre de recherche est gratuit pour les participants, de même que tous les tests scientifiques. Vous obtiendrez des informations sur la composition de votre corps et votre métabolisme au repos. Vous recevrez des conseils nutritionnels pendant les interventions RÉGIME ou

RÉGIME+EF, ou une consultation avec le/la nutritionniste à la fin de l'étude si vous faites partie du groupe EF. De plus, vous recevrez une compensation de 150,00 $ pour votre participation, dont la moitié vous sera allouée après avoir complété les visites pré intervention, et l'autre moitié après avoir complété l'étude.

8. CONFIDENTIALITÉ ET ANONYMAT:

Afin de garantir la confidentialité et l'anonymat des participants, toutes les précautions et mesures nécessaires seront prises pour s'assurer que les résultats et les renseignements personnels des participants sont gardés dans la plus stricte confidentialité.

-Seules les personnes suivantes auront accès au matériel : Dr Éric Doucet, Dr François Haman, Dre Luzia Hintze, Ann Beninato (infirmière de l'étude) et Kurt McInnis (étudiant au doctorat). Les autres personnes participant à l'étude n'auront pas accès aux renseignements personnels et aux résultats des participants.

-Les noms des participants n'apparaîtront sur aucun rapport. Un code numérique sera utilisé pour identifier les participants dans tous les documents de recherche.

-Tous les documents et informations pouvant être liés aux participants ne seront pas rendus publics et seront conservés dans la plus stricte confidentialité.

-Les participants ne seront pas identifiés de quelque façon que ce soit dans les publications ou les rapports.

-Les données recueillies seront conservées dans un classeur verrouillé de l'Unité de recherche sur le comportement et le métabolisme à accès restreint où seront conservés tous les dossiers des participants. De plus, les fichiers informatiques seront protégés par un mot de passe.

-Les données seront détruites cinq ans après la publication des résultats de l'étude.

-Vos données seront détruites si vous choisissez de vous retirer.

-Les échantillons de sang seront conservés dans un laboratoire fermé à clé et seront détruits après la publication des résultats de l'étude.

9. PARTICIPATION VOLONTAIRE

Vous êtes libre de refuser de participer et si vous choisissez de participer, vous êtes libre de vous retirer de l'étude à tout moment pour quelque raison que ce soit. À tout moment au cours de cette étude, l'intérêt supérieur des participants l'emportera toujours sur les objectifs de l'étude. Les participants seront informés des nouvelles découvertes qui pourraient influencer leur décision de participer à la présente étude.

Toute information sur vos droits en tant que participant à la recherche peut être adressée à : Agent du protocole pour l'éthique de la recherche, Université d'Ottawa, 550 Cumberland, Tabaret Hall, salle 154, Ottawa, Ontario, K1N 6N5; téléphone : (613) 562-5387, courriel: ethics@uottawa.ca.

Si vous avez des questions sur le déroulement du projet de recherche, vous pouvez communiquer avec Luzia Hintze à ljaeg051@uottawa.ca ou 613-914-0634, ou le Dr Éric Doucet à edoucet@uottawa.ca ou 613-562-5800 ext. 7364.

Il y a deux copies du formulaire de consentement, dont une que je peux conserver.

COORDINATEUR DE RECHERCHE
Luzia Hintze, Ph.D.: _____
Date: _____

SIGNATURE DU CHERCHEUR PRINCIPAL

Eric Doucet, Ph.D: _____
Date: _____

SIGNATURE DU PARTICIPANT:
J'accepte de participer à cette étude,

_____ _____
Nom (en caractère d'imprimerie) Signature

Date: _____

Si vous décidez de vous retirer de l'étude, toutes données déjà obtenues pourront être utilisées avec votre permission

Est-ce vous nous permettez de partager vos coordonnées avec l'équipe du Professeur Pascal Imbeault pour une étude de partnerariat? Cette étude de partenariat comporte des biopsies du tissus adipeux qui seront prélevées avant et après l'intervention décrite dans ce formulaire de consentement. En bref, une petite incision sera faite près du nombril afin de prélevé une petite quantité de tissus adipeux sous-cutané à ce site d'incision. Dans l'éventualité où vous accepteriez de partager vos coordonnées, tous les détails de cette étude de partenariat vous seraient expliqués en détails par l'équipe du Professeur Imbeault. Veuillez noter que le refus de partager vos coordonnées avec l'équipe du Professeur Imbeault n'affectera en rien votre éligibité à participer à l'étude présentée dans le présent formulaire de consentement.

J'accepte Je refuse

APPENDIX C. LIST OF PUBLISHED NON-THESIS ARTICLES DURING PHD TENURE

Beauregard, N., **McInnis, K.,** Goldfield, G. S., & Doucet, E. (2024). Energy balance and obesity: the emerging role of glucagon like peptide-1 receptor agonists. *Curr Opin Clin Nutr Metab Care, 27*(6), 472-478. doi:10.1097/MCO.0000000000001064

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