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ADAPTIVE THERMOGENESIS AND METABOLIC CHANGES
FOLLOWING DIET- AND EXERCISE- INDUCED WEIGHT LOSS

by

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ADAPTIVE THERMOGENESIS AND METABOLIC CHANGES FOLLOWING DIET- AND EXERCISE- INDUCED WEIGHT LOSS

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University of Nebraska, 2017

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With more than two-thirds of adults in the United States considered as overweight or obese, a myriad of weight loss programs and products that promise quick results have infiltrated the market. While many individuals successfully lose weight over the short term, almost half of the initial weight lost is regained within one year, indicating a general predisposition for weight cycling and fat replenishment. As weight is lost, fat-free mass (FFM) also decreases, and resting metabolic rate (RMR) declines, decreasing energy expenditure. After accounting for decreases in FFM, there remains a greater-than-predicted decrease in RMR, referred to as adaptive thermogenesis (AT), which describes the suppression of non-vital functions to re-equilibrate energy status. The overall goal of this thesis is to quantify adaptive thermogenesis and determine the role of suppressed brown adipose tissue (BAT) activity in this adaptive response. An exploratory study in normal weight participants revealed a 5.4% increase in RMR following mild cold exposure as well as prominent increases in surface supraclavicular temperature, a region that indicate the presence of cold-induced thermogenesis (CIT) of BAT. Study 1 demonstrated that overweight/obese participants had a significantly greater RMR ($p=0.04$) and concentrations of leptin, insulin, and FGF-21 ($p=0.015$, $p=0.091$, $p=0.023$) than normal weight participants, but no significant difference in adaptive thermogenesis

(220 ± 40 kcal vs. 89 ± 15 kcal, $p=0.20$) or surface body temperatures were found. Study 2 revealed a reduction in RMR by 209 ± 1 kcal in normal-weight individuals undergoing severe calorie restriction, which was associated with reductions in insulin (-23.9 ± 14.8 pg/mL) and FGF-21 (-61.6 ± 28.7 pg/mL). In study 3, there were no significant changes in RMR, AT, or metabolic hormones over the course of a 3-month commercial weight loss intervention, which failed to produce significant weight loss. Indications of adaptive thermogenesis were present in both normal weight and obese/overweight participants, though AT was more pronounced in the latter. With the link between adaptive thermogenesis and modulation of BAT activity, cold exposure and brown fat activation may be an effective tool to increase energy expenditure during a period in which RMR is suppressed, thus preventing future weight regain.

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CHAPTER 1: INTRODUCTION

With staggering obesity statistics in the United States and around the world, the weight loss industry has become extremely lucrative, boasting nearly \$50 billion spent yearly on its efforts (Weis et al., 2002). Nearly half of all adults are attempting to control their weight and a significant portion is actively trying to lose it (~1/3 of all men and women). However, engaging in intentional weight loss may predispose individuals to future weight regain. A single bout of weight loss tripled the risk of becoming overweight in women and doubled it for men in comparison with individuals who do not attempt to lose weight (Pietilainen et al., 2012). This increased likelihood of weight regain can lead to the deleterious phenomenon of weight cycling and is intrinsically dependent upon fluctuations in adiposity and body composition.

Weight loss itself not only results in the loss of adipose tissue, but also in an undesired loss of fat-free mass. As fat free mass is an important predictor of resting metabolic rate (RMR), a decrease in fat free mass will result in a concomitant reduction in energy expenditure. In addition to the reduction in RMR due to decreased fat free mass, RMR is even further reduced during weight loss due to metabolic adaptations that occur in the energy-deficient state. The resultant suppression of RMR has been shown to result in attenuated weight loss and an increased propensity for fat mass accumulation once individuals return to their habitual diet. These metabolic factors work synergistically to promote weight regain and specifically fat overshooting following weight loss.

A key metabolic adaptation that occurs in energy deficiency involves regulation of energy loss via the reduction in heat production. This adaptation of thermogenic

capacity may be localized in Brown Adipose Tissue (BAT). Increased BAT activity results in sharp increases in energy expenditure, thus implicating its activation as a potential target to counteract reductions in RMR during and following weight loss, which may have tremendous implications for the prevention of weight regain, and in turn, decreased weight cycling bouts.

The overall purpose of this thesis was to evaluate the extent of adaptive thermogenesis in different populations and determine whether brown fat activity is modulated in response to adaptive thermogenesis, contributing to its overall effect on energy expenditure. Initially, an exploratory study was conducted to determine whether adaptive thermogenesis can be detected when individuals are exposed to a temperature slightly below thermoneutral, and whether modulations in body surface temperature in regions with BAT activity occur during the cold acclimation process. Study 1 assessed differences in metabolic markers of adaptive thermogenesis in overweight/obese individuals and normal weight individuals. The metabolic ramifications and fluctuations in selected markers of adaptive thermogenesis that occur during short-term, controlled semi-starvation were characterized in Study 2, and Study 3 examined metabolic adaptations in overweight/obese individuals over the course of a three-month weight loss program.

Objective 1:

The first objective of this thesis was to determine if adaptive thermogenesis could be measured when individuals are exposed to a temperature slightly below thermoneutral (*Exploratory Study*). We hypothesized that RMR measured at 21C would be greater than

RMR measured at 24C, indicating that increased heat production during the measurement at 21C could account for increased metabolic rate, and thus, increased energy expenditure. Additionally, the exploratory study sought to determine increases in heat production at body regions known for BAT activity (supraclavicular region) in response to mild cold exposure.

Objective 2:

The second objective of this thesis was to assess the relationship between adaptive thermogenesis, thermogenic activity at regions with high BAT activity, endocrine markers of BAT activity, and body weight status by comparing overweight and obese individuals to health controls (*Study 1*), by assessing change in response to short-term, controlled weight loss (*Study 2*), and in response to long-term weight loss in overweight and obese individuals (*Study 3*). It was our hypothesis that acute and chronic weight loss result in a suppressed metabolic state, characterized by a suppression of RMR along with a suppression of key metabolic hormones, such as leptin, insulin, adiponectin, and FGF-21. We further hypothesized that RMR suppression will be accompanied by a concomitant reduction in thermogenic activity of brown fat, as determined by non-shivering heat production through regional external temperature measurements, and a surrogate marker of BAT activity, FGF-21.

Results from these studies will inform the extent of adaptive thermogenesis in different populations in different states of energy balance and the contribution of brown fat thermogenesis to adaptive thermogenesis. If findings from these studies confirm the

decreased ability to produce heat as a major contributor to metabolic adaptations to weight loss, the activation of brown adipose tissue may be a viable strategy to improve the success of weight loss interventions and prevent future weight regain.

CHAPTER 2: LITERATURE REVIEW

Attenuating the Deleterious Consequences of Weight Cycling through Exercise

The United States currently boast a staggering prevalence of overweight and obesity, with more than two-thirds of adults over the age of 20 and one-third of young people between 2 and 19 years considered as overweight or obese (Ogden et al., 2014). With increasing societal and media pressure, as well as the desire to optimize performance by modulating fat and muscle mass in athletes, nearly half of all adults are actively trying to control or maintain their weight, while nearly 33% of men and 50% of women are attempting to lose weight, leading to over \$50 billion spent per year on weight loss assistance (Weis et al., 2002). Despite the staggering amount of individuals attempting to lose weight, dieting typically results in a weight-loss plateau and weight is often regained (Franz et al., 2007).

Participation in weight loss programs utilizing variations of diet and exercise prescriptions demonstrated that most overweight or obese individuals are able to lose on average 5-9% of their initial body weight within six months, although the amount of weight lost varies considerably upon the method of choice (Franz et al., 2007). However, almost half of the initial weight lost following diet and/or exercise is typically regained within one year (Curioni et al., 2005), and after four years, only 3-6% of the initial weight lost is maintained (Anastasiou et al., 2015; Franz et al., 2007). This propensity to regain weight following the cessation of weight-loss interventions predisposes dieters to begin weight cycling, which is defined as the repeated loss and gain of body weight (Bosy-

Westphal et al., 2015). Prolonged weight cycling further exacerbates the inability to maintain weight loss because of the preferential accumulation of fat mass over lean mass in the weight regain phase, manifesting into a vicious cycle that creates an increased desire to diet due to increased adiposity (Dulloo et al., 2015)

Initially, weight cycling and the consequential metabolic adaptations serve as a regulatory mechanism to prevent drastic metabolic consequences in periods of starvation, i.e., when food availability is scarce (Bosy-Westphal et al., 2015). While the phenomenon was beneficial to promote survival, it can be deleterious in an obesogenic environment where it promotes weight regain upon cessation of dieting. It has been recently postulated that the propensity to regain weight following weight loss occurs as a result of ‘fat overshooting’ as well as the individual’s initial partitioning characteristic, i.e., the ratio of fat mass to fat free mass reflected by body fat percentage (Friedl et al., 2000; Dulloo et al., 2015).

Fat overshooting refers to the disproportionate and accelerated replenishment of fat stores following weight loss, a rate that dramatically exceeds the replenishment of fat free tissue. Though the ultimate goal in weight loss is to eliminate fat mass while preserving fat free mass, the actual composition of weight loss is typically around 79% fat mass (FM) and 21% fat free mass (FFM), although the proportion of fat free mass losses may reach as much as 53% (Chaston et al., 2007; Goele et al., 2009). The loss of excessive FFM is undesirable, as it is the primary determinant of resting metabolic rate (RMR). Further, FFM also helps regulate core body temperature and confers benefits to skeletal integrity, so its loss should be minimized to mitigate large decreases in energy expenditure (Chaston et al., 2007).

After typical weight loss, however, an uneven redistribution in fat- and fat free mass occurs following chronic energy deficiency, which will result in a compensatory increase in energy intake. This leads to a state of hyperphagia, or increased appetite, which increases energy intake during a period where energy expenditure is suppressed (Keys et al., 1950). The depletion of both fat- and fat free mass compartments, however, affects the length of this hyperphagic response (Dulloo et al., 2015). Though fat mass will be accumulated at a much more rapid rate than fat free mass, the hyperphagic response persists until fat free mass stores have returned to their pre-weight loss levels, leading to fat regain beyond initial weight loss levels.

Suppression of the resting metabolic rate (RMR), the largest component of energy expenditure, will also continue until FFM stores have been replenished during weight regain. In the seminal Minnesota Starvation Experiment, twelve men underwent 24 weeks of semi-starvation, 12 weeks of restricted refeeding, and 8 weeks of ad libitum refeeding. Keys et al., found that RMR suppression can be reversed when an ad-libitum diet without regulation of food quantity and dietary intake is consumed upon cessation of weight loss. However, if feeding is controlled, RMR suppression will persist until FFM is fully recovered even when body fat stores have returned to their initial, pre-starvation state (Keys et al., 1950), predisposing individuals to 'fat overshooting' (Dulloo et al., 2015; Keys et al., 1950). Excessive fat overshooting has also been reported in US Army Rangers who completed an eight-week intensive training course while in an energy deficiency, followed by a recovery phase. The weight regain in the recovery period was characterized by an excessive amount of fat overshoot, as shown by an increase in body fat percentage by 40% from baseline values (Friedl et al., 2000).

The replenishment of fat and lean tissues is in part dictated by an individual's energy partitioning characteristic, which is ascertained by the initial body fat percentage and level of adiposity. A leaner individual with a lower initial fat content at the onset of weight loss will experience greater losses in FFM stores relative to FM (Dulloo et al., 2001), prolonging the amount of time necessary to replenish FFM stores in the weight recovery phase. This leads to a greater amount of time in the fat overshoot phase with concomitant, persistent hyperphagia as well as a greater, prolonged incidence of metabolic adaptations to counteract the energy imbalance (Dulloo et al., 2015). Thus, prolonged metabolic adaptations that occur during energy deficiency can persist for extended periods of time post weight loss, affecting future weight loss in an acute sense by decreasing resting metabolic rate and in a long-term sense by predisposing certain individuals with accelerated weight regain and fat recovery.

I. Alterations in Metabolic Rate in Periods of Weight Loss

In order to successfully lose weight, a state of energy deficiency must be reached, which can be induced either by decreasing energy intake, increasing energy expenditure, or a combination of both (Byrne et al., 2012). The prolonged state of energy deficiency can alter whole body metabolism. In a natural effort to return to an equilibrated energy status, metabolic adaptations occur that decrease energy expenditure in response to the decreased energy intake (Wade et al., 1996). The largest component of daily energy expenditure is resting metabolic rate (RMR), which comprises approximately 60-70% of total daily expenditure in the normal population. Theoretically, decreasing RMR would then enable the return to a balanced energy state (Steigler et al., 2006).

The primary determinant of resting metabolic rate is fat free mass, which is composed of highly metabolically active tissues, such as organs and muscle, and lower metabolically active tissues, such as bone and connective tissue. A loss in fat free mass will result in a concomitant decrease in resting metabolic rate, the effect of which is largely driven by the size and metabolic activity of the tissue that are lost during weight loss. The brain and other vital organs, which are typically conserved, have been found to consume more energy when compared to resting skeletal muscle, adipose tissue, and bone, and as such, the respective losses of each tissue will affect the reductions in metabolic rate differently (Steigler et al., 2006). Measuring the different tissue compartments helps elucidate whether RMR suppression following weight loss occurs merely as a function of the reduced size of energy-consuming organs or if it is a result of metabolic adaptations that occur at the tissue level (Byrne et al., 2012; Koehler et al., 2016).

After segregation of the metabolically active tissue components however, actual weight loss remained 67% lower than predicted (Byrne et al., 2012). This indicates that metabolic adaptations occur at the tissue level and account for the differences in predicted and observed weights. The adaptations suppress energy-demanding processes deemed inessential, such as reproduction, in order to maintain processes rendered critical for survival, such as thermoregulation and locomotion (Wade et al., 1996). The suppression of these non-vital processes and the resulting decreases in energy expenditure account for the greater-than-predicted reduction in metabolic rate that occurs independently of loss of lean tissue mass in the energy-deficient state, thus representing a

secondary factor that reduces RMR during weight loss or periods of chronic energy deficiency.

II. Reduced Metabolic Rates Indicate Suppression of Thermogenic Capacity and Presence of Adaptive Thermogenesis

Adaptive Thermogenesis (AT) has been referred to as the modulation of metabolic rate that occurs independently of changes in fat free mass or the mass of individual metabolically active components. Specifically, it represents the decrease in the metabolic activity of cells (Muller et al., 2013). AT either occurs when there are significant decreases in fat stores and/or during weight loss induced by chronic energy deprivation, and serves to defend non-adipose tissues from lipid accumulation (Kosmiski et al., 2011). This is accomplished by a rapid and disproportionate replenishment of fat over lean tissue, a reduction in sympathetic nervous system activity, and a concomitant decrease in circulating leptin and adiponectin, all acting to counteract weight loss (Dulloo et al., 1998, Goele et al., 2009).

In white adipose tissue (WAT), AT is dictated by a “fat stores memory,” which can either attenuate energy balance or accelerate tissue recovery by suppressing thermogenesis during both weight loss and weight recovery (Dulloo et al., 2015). Adaptive thermogenesis, however, does not solely occur in WAT. In fact, the previous definitions of AT actually represent metabolic adaptations that occur post weight loss. Adaptive thermogenesis, in the context of this thesis, is the response localized in brown adipose tissue, where it can represent an increased ability to produce heat after continuous exposure to cold, thus increasing energy expenditure by increasing non-

shivering thermogenesis (NST) (Hanssen et al., 2016). Furthermore, dietary compounds can induce brown fat activity to increase thermogenic capacity. Metabolic adaptations can affect all components of daily energy expenditure (DEE) (Redman et al., 2009), but the adaptive response to the resting component of EE occurs with contributions from brown adipose tissue thermogenesis, incorporating the effects of diet-induced thermogenesis, cold-induced thermogenesis, and NST. While AT can alter EE tremendously, there are a multitude of mechanisms through which EE can be reduced in the state of energy deficiency (Diagram 1).

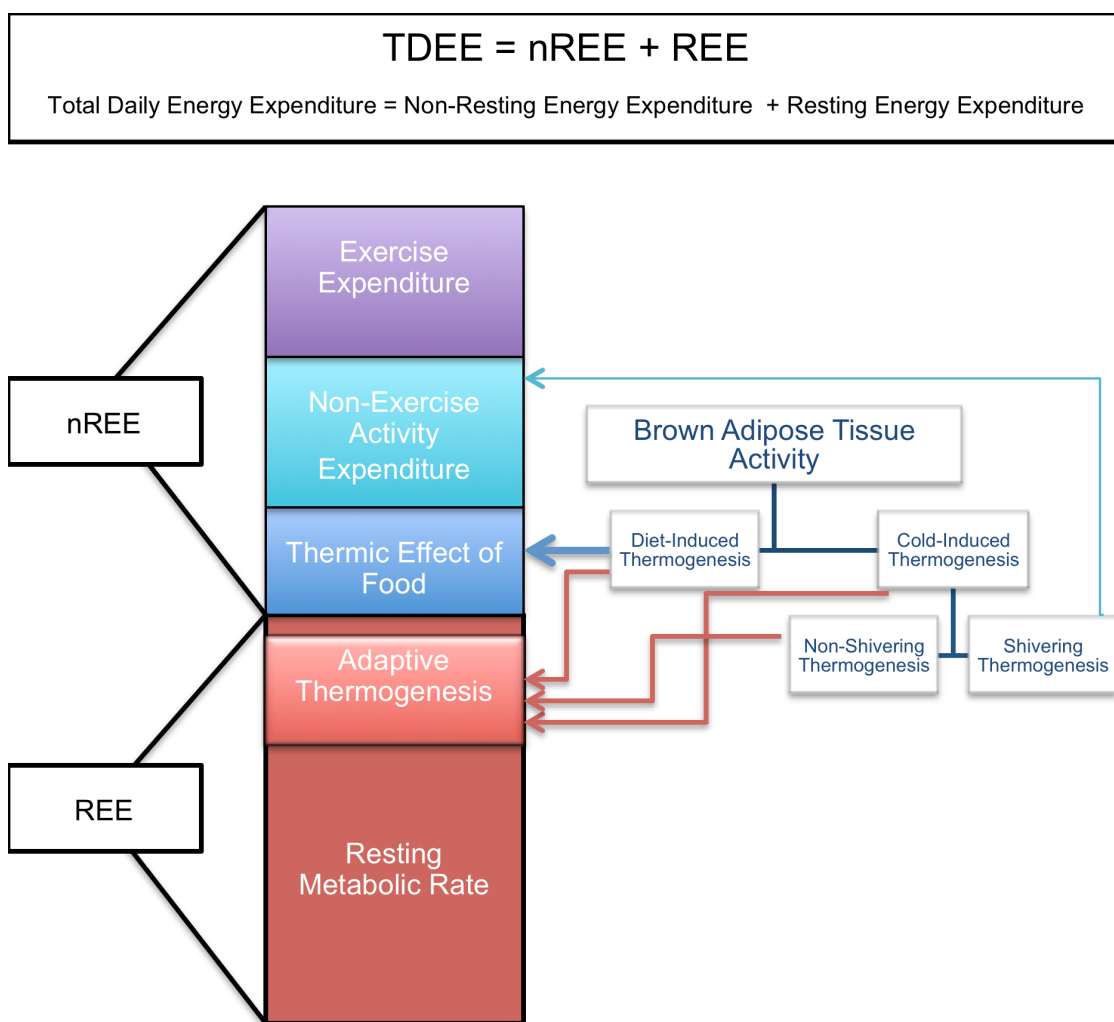


Diagram 1: Contributions of thermogenic components to Adaptive Thermogenesis (TDEE=Total Daily Energy Expenditure, nREE=non-Resting Energy Expenditure, REE=Resting Energy Expenditure)

Non-Exercise Activity Expenditure

Non-Exercise Activity Expenditure is a component of non-resting energy expenditure (nREE) defined by the energy produced by spontaneous physical activity, including the activities of daily living, postural maintenance, fidgeting, and spontaneous muscular contraction (Levine et al., 1999). When comparing age- and weight-matched subjects who lost 10% of their initial body weight within 5-8 weeks versus those who had maintained the weight loss for over a year, it was found that the differences between measured and predicted total energy expenditure (TEE) were more significant in the nREE component (Rosenbaum et al., 2008). Further, reductions in nREE appear to depend upon the amount of weight lost. In examining sedentary individuals with no prescribed physical activity, reductions in weight of 10% and 20% resulted in a corresponding decrease in nREE of 25% and 46% respectively, which can thus be attributed to a modulation of non-exercise activity (Weigle et al., 1988).

This was corroborated by Levine's study, where sixteen non-obese individuals were instructed to increase their daily intake by 1000 kcal a day for eight weeks. There was considerable variation in the amount of fat gained by the participants (0.36 kg to 4.23 kg) at the end of the eight weeks, but NEAT seemed to be the principal mediator in resisting excessive fat accumulation. Increases in NEAT activity accounted for nearly 2/3 of the increase in daily energy expenditure, while basal metabolic rate solely accounted for 8% and postprandial (diet-induced) thermogenesis was found to be an insignificant contributor (Levine et al., 1999). Overfeeding, which occurs with the hyperphagic response in the weight regain phase, was associated with substantial decreases in NEAT activity. Individuals that could effectively activate NEAT were able to dissipate excess

energy rather than promoting its long-term storage in adipose tissue, protecting the individual from excess fat gain (Levine et al., 1999, Anastasiou et al., 2015). Though it is difficult to quantify the decrease in NEAT activity with energy deficiency due to the concomitant reduction in the energy cost of physical activity with weight loss, successful weight maintenance may be aided by preventing large reductions in NEAT activity (Redman et al., 2009)

Diet-Induced Thermogenesis (DIT)

The predominant component of Diet-Induced Thermogenesis (DIT), the thermic effect of food (TEF), accounts for the increase in energy expenditure that occurs as a result of digestive processes, including absorption, processing, and storage of nutrients as well as the resultant sympathetic nervous system response (Byrne et al., 2012). DIT is thus modulated by perturbations in energy intake, but more specifically, by the energy content of the food. When food consumption is reduced, there is a decrease in postprandial peak metabolism as well as length of the thermic response. If an individual with an initially large habitual energy intake enters a state of chronic energy deficiency and caloric restriction, the significant decrease in energy intake can have ramifications in decreasing the thermic effect of food, and thus diet-induced thermogenesis (Byrne et al., 2012; Dulloo et al., 2015).

Additionally, the thermic effect of food is also dependent upon the energy cost of converting nutrients to metabolic fuels, the macronutrient composition of which dictates the energy cost (Levine et al., 1999). The various ATP requirements for nutrients depending upon the initial steps of metabolism has resulted in divergent contributions to

DIT: fat accounts for 0-3% of the DIT response, carbohydrates account for 5-10%, protein accounts for 20-30%, and alcohol accounts for 10-30% (Johnston et al., 2002). Individuals consuming a mixed diet will then have a DIT response that represents nearly 10% of total daily energy expenditure (Westerterp et al., 2004). Changes in DIT activity, however, are dependent upon changes in food consumption, rather than by fat depletion. When comparing high-protein/low-fat diets with high-carbohydrate/low-fat diets, it was found that DIT activity increased by 100% with the high-protein diet. Further, the increase in body temperature led to increased feelings of satiety, possibly implicating a high-protein diet as a protective measure against decreases in diet-induced thermogenesis, and thus metabolic rate, in periods of energy deficiency (Johnston et al., 2002).

While the term “thermic effect of food” has often been used interchangeably with diet-induced thermogenesis, it only represents the component of diet-induced thermogenesis that is involved with intestinal absorption, metabolic processing, and nutrient storage, i.e., digestion in general (Tappy et al., 1995). Of recent interest, however, is the idea that dietary compounds and/or factors may be able to promote brown fat activity or brown-like (“beige”) cell formation as an additional component of diet-induced thermogenesis (Okla et al., 2017). Depending upon the source of the thermogenic stimuli, there can be an increase in BAT activity in previously existing brown fat cells, the induction of “browning” in white adipocytes, or an increase in the brown-like cells from progenitor cells (Okla et al., 2017). Capsaicin and capsinoids, compounds found in peppers, have been studied extensively due to their ability to increase energy expenditure, enhance fat oxidation in rodents and humans, and stimulate

brown adipose tissue activity via β -adrenergic signaling (Ludy et al., 2012). Green tea catechins are abundant in polyphenols, which have antihypertensive and hypocholesteremic properties, among other health benefits (Hursel et al., 2011). Between the catechins in green tea, which stimulate thermogenesis via β -adrenergic signaling, and caffeine, which inhibits cAMP degradation, green tea has become an interesting target to fight obesity (Diepvens et al., 2007). These individual dietary factors, which have been predominately studied in animals, could represent an additional component of diet-induced thermogenesis that affects brown fat activity and in turn increases energy expenditure and metabolic rate.

Cold-Induced Thermogenesis (CIT)

Cold-Induced Thermogenesis (CIT) pertains to modulations in energy expenditure that occur to maintain a consistent core body temperature in response to fluctuations in environmental temperatures (Brychta et al., 2017). Specifically, as the environmental temperature decreases, a concomitant increase in energy expenditure occurs to prevent heat loss (Swift et al., 1932). CIT is comprised of non-shivering thermogenesis and shivering thermogenesis. Non-shivering thermogenesis is primarily mediated through brown adipose tissue, a thermogenic tissue that produces heat via uncoupling protein 1, and has been shown to increase energy expenditure and heat production by approximately 11% during cold exposure in comparison with thermoneutral conditions (Lee et al., 2013; Swift et al., 1932).

Non-Shivering Thermogenesis (NST) refers to an increase in energy expenditure without the induction of shivering in response to decreased ambient temperature (van der

Lans et al., 2013). When the ambient temperature decreases beyond that of an individual's thermoneutral zone, thus requiring heat generation and increased energy expenditure to maintain core temperature, internal mechanisms to prevent heat loss are first utilized, such as cutaneous vasoconstriction (Schmidt-Nielsen et al., 1990).

Vasoconstriction serves to both decrease skin temperature and elevate circulatory catecholamines from sympathetic nervous system activation. This will in turn lead to increased lipolysis in adipocytes, the result of which increases plasma triglycerides that likely fuel the non-shivering thermogenic response (Din et al., 2016).

While all mammals initially shiver when exposed to cold, generating heat via involuntary muscle contractions that hydrolyze ATP, prolonged cold exposure leads to the cessation of shivering with the simultaneous retention of the elevated metabolic rate, allowing for more comfort in cold environments and increased heat production through non-shivering thermogenesis (Griggio et al., 1982; Cottle and Carlson, 1956). This heat production occurs in brown adipose tissue, the recruitment of which has been inconsistently shown to parallel enhanced NST (Giralt et al., 2016; Hanssen et al., 2016). Though several studies (Hanssen et al., 2015; Yoneshiro et al., 2013; Chen et al., 2016) have shown effective recruitment of BAT through cold acclimation which paralleled enhanced non-shivering thermogenesis, the extent to which BAT activity directly contributes to measured oxidative metabolism in humans remains unclear, and thus, an increase in BAT activity is not definitively accompanied by an increase in non-shivering thermogenesis (Hanssen et al., 2016).

Despite this unclear association, enhancing the non-shivering thermogenic response via cold acclimation remains intriguing as a potentially effective weight loss

tool. When exposing seventeen individuals for six hours each day to an environmental temperature of 15-16 C for 10 days, the increase in NST was 11% before and 18% after cold acclimation. This occurred in conjunction with a 37% increase in detectable BAT volume, thus indicating that brown adipose tissue contributed to NST and its enhancement during cold acclimation (van der Lans et al., 2013). An inability or impairment of BAT to produce heat may then cause acceleration of muscles to shiver, and an impaired NST response (Bakker et al., 2014). Enhancing non-shivering thermogenesis through brown fat recruitment and subsequent activation could increase energy expenditure and metabolic rate, potentially opposing the decrease in metabolic rate that occurs post weight loss.

When heat demand exceeds the heat production ability of non-shivering thermogenesis, shivering thermogenesis is recruited. Shivering is a common defense mechanism to prevent heat loss by increasing skeletal muscle contraction, thus affecting skeletal muscle as opposed to brown adipose tissue (Haman et al., 2006). The induction of shivering itself results in steep increases in resting energy expenditure and is associated with systemic metabolism increases that may range 2-3 fold above the expected expenditure (Badjatia et al., 2008). While shivering can drastically increase energy expenditure, and thus metabolic rate, it is transient and unsustainable.

Continued and prolonged exposure to colder temperatures leads to increased cold acclimatization, which minimizes shivering and maximizes heat production through non-shivering thermogenesis, increasing the overall CIT response (van der Lans et al., 2013; Hanssen et al., 2015). Cold exposure at 17C for two hours every day for a total of 6 weeks resulted in increased brown adipose tissue activity and cold-induced

thermogenesis, with a simultaneous decrease in body fat in non-obese participants with initially low brown fat activity (Yoneshiro et al., 2013). Despite the exciting potential of cold exposure for weight loss, it is still difficult to delineate the contributions of both affected organs, skeletal muscle and brown adipose tissue, in cold-induced thermogenesis because of difficulties in measuring the organs' separate metabolic activity in vivo (Dulloo et al., 2013).

Brown Adipose Tissue (BAT) Activity

Brown adipose tissue thermogenesis refers to the utilization of uncoupling protein 1 (UCP1), which uncouples energy production from ATP synthesis via mitochondrial oxidative phosphorylation towards energy dissipation in the form of heat production (Lowell et al., 2000). Located throughout areas where circulatory temperature regulation is critical for blood supply to vital organs such as the cervical, supraclavicular, axillary, paravertebral, mediastinal, and upper abdominal regions, brown fat can be a significant contributor to energy expenditure and whole-body temperature regulation (Saely et al., 2012; Saito et al., 2009). BAT thermogenesis is stimulated by β -adrenergic-mediated activation of lipolysis, which increases the amount of circulating fatty acids (Lowell et al., 2000). The increased fatty acids in circulation remove the inhibitory effective of cytosolic nucleotides on UCP1, allowing for heat production (Cannon et al., 2004).

Brown adipose tissue thermogenesis also encompasses cold-induced thermogenesis and diet-induced thermogenesis (Saito et al., 2009), as both components occur fully or partially in brown adipose tissue. As aforementioned, when BAT is activated by increased circulatory catecholamines, specifically norepinephrine, it acts

primarily via β -adrenergic signaling. This sympathetic nervous response also activates cold- and diet-induced thermogenesis in brown adipose tissue, as both can also be activated via β -adrenergic signaling (Cannon et al., 2004; Peronnet et al., 1981).

Continued sympathetic nervous system activity can activate several different thermogenic responses, which exacerbates the amount of producible heat from brown adipose tissue. Variations in BAT thermogenic activity may then explain the considerable individual variations in metabolic rate and diet-induced thermogenesis, even after using predictive models accounting for age, sex, and body composition (Saito et al. 2009).

III. White Adipose Tissue Metabolism is a Dynamic Interplay Between Lipolytic Modulation and Hormonal Circulation

When food is consumed, the gastrointestinal tract transmits satiety signals to the brain via the vagal nerve, resulting in a release of satiety hormones into circulation (Harrold et al., 2012). Plasma glucose, which increases tremendously upon eating, is cleared from the circulation by an increased secretion of insulin, which promotes its uptake into skeletal and cardiac muscle for energy utilization. This in turn shifts the predominant fuel source of energy from fatty acids to glucose (Elia et al., 1988). After a meal, plasma triglycerides levels also increase, which must then be transported into white adipose tissue for storage. When the glucose and triglycerides have normalized in the post-absorptive states, free fatty acids become the primary fuel source once again. Through the coordinated activity of lipoprotein lipase (LPL) and hormone-sensitive lipase, fatty acids are released through increased lipolysis, allowing for increased levels of non-esterified fatty acids in circulation (Frayn et al., 1999). Other tissues are then able to oxidize the

fatty acids obtained from circulating VLDL particles and chylomicrons, implicating white adipose tissue as a controller of energy flux throughout the body.

Similar to the increased release of NEFA as an energy source in the post-absorptive state, certain substrates released into blood, such as catecholamines, can replicate this response. Catecholamines initiate lipolysis by binding to beta-adrenergic receptors on adipocytes, interacting with guanosine triphosphate-binding regulatory proteins and activating adenylate cyclase (AC). When ATP is then converted to cyclicAMP (cAMP), hormone-sensitive lipase is phosphorylated and inactivated (Horowitz et al., 2005). Similarly, catecholamines can also act through alpha-adrenergic stimulation, which in turn works via a G_i or inhibitory pathway, decreasing lipolysis (Saely et al., 2012; Horowitz et al., 2005). This enables catecholamines to control the lipolytic rate based off their concentrations and receptor affinity. Increasing catecholamine release, which can occur through cold exposure, exercise, or exposure to stressful environments, also increases sympathetic nervous system activity and beta-adrenergic activity, mimicking a fasted state that aims to release more NEFAs into circulation (Coyle et al., 1995). The effect will further increase vascular conductance and blood flow, allowing for increased systemic circulation of hormones and adipokines released by adipose tissue (Horowitz et al., 2005).

Leptin

The adipokine leptin suppresses food intake and stimulates energy expenditure, making it a key regulator of energy homeostasis (Dubuc et al., 1998, Anastasiou et al., 2015). When bound to its receptor ObR in the hypothalamus, it induces the STAT3

signaling pathway, modulating melanocortin production and altering energy balance, allowing for increased insulin sensitivity and reduced intracellular lipid accumulation (Bates et al., 2003, Rosen et al., 2006). In parallel, the activation of extracellular signal-related kinase (ERK) and PI3K kinase by leptin may regulate neuropeptide Y, which in turn regulates the hypothalamic-pituitary-thyroid axis (Bates et al., 2003, Fekete et al., 2002), implicating the indirect regulation of leptin on thyroid hormones. Upon leptin repletion, thyroid hormone levels are decreased and there is a combined increase in food responsiveness with decreased energy expenditure (Anastasiou et al., 2015). Therefore, when leptin is suppressed during diet-induced weight loss, it cannot inhibit orexigenic neurons in the hypothalamus, thus stimulating hunger and increasing food intake (Munzberg et al., 2015).

Though leptin expression and its circulating levels are positively correlated with adiposity, it drops considerably upon energy restriction, even within a few days (Bryson et al., 1999, Weigle et al., 1997). Because its gene expression occurs proportionately to adipocyte volume, a weight loss-induced depletion of lipid content in adipocytes can lead to an influx of glucose into adipocytes, which is followed by a subsequent preferential reaccumulation of fat (Zhang et al., 2002, Bosy-Westphal et al., 2015). Though decreased levels of leptin after weight loss can persist during weight maintenance, leptin repletion may be able to reverse concomitant weight loss-induced reductions in energy expenditure upon repletion to pre-weight loss levels (Sumithran et al., 2011, Rosenbaum et al., 2002). This makes the replenishment of leptin levels a potential mechanism to help prevent weight regain by decreasing hunger and energy intake after weight loss.

Thyroid Hormone

Triiodothyronine (T_3) is also an important regulator of energy balance. Plasma T_3 concentrations fall during weight loss and may be continuously reduced past baseline values for years after the weight loss intervention (Muller et al., 2013, Fothergill et al., 2016). Serum levels of T_3 and leptin have been shown to correlate significantly with the ratio of measured to predicted metabolic rate in energy deficient individuals, implicating T_3 and leptin as endocrine markers that indicate the presence of metabolic adaptations (Koehler et al., 2016, Goele et al., 2009, Rosenbaum et al., 2002). Because hyperthyroidism has been associated with marked elevations in resting energy expenditure, it follows that its plasma levels will significantly decrease as a metabolic adaptation to decrease energy expenditure in times of energy deficiency (Kosmiski et al., 2011). The decrease in thyroid hormones is due to alterations in the hypothalamic-pituitary-thyroid axis as well as reduced peripheral uptake of thyroid hormones (Muller et al., 2013).

Thyroid hormones also activate sympathetic activity via regulation of AMP kinase, thus allowing for indirect control of Brown Adipose Tissue (BAT) thermogenic activity (Giralt et al., 2016). Further, thyroid hormones stimulate Uncoupling protein 1 (UCP1) activity in brown adipose tissue, which serves to uncouple mitochondrial ATP production and shift it towards heat production, thereby increasing energy expenditure (Muller et al., 2013). The decrease of thyroid hormone concentrations that occur as a result of weight loss can then decrease energy expenditure by suppressing thermogenic activities and other processes that upregulate metabolism and increase metabolic rate.

Adiponectin

Adiponectin became an adipokine of interest for its insulin-sensitizing effects. When isolated, adiponectin plasma concentrations were significantly lower in obese subjects compared with non-obese subjects, a notion that seemed inherently paradoxical in relation to other adipokines, the concentrations of which increase in obese conditions (Arita et al., 1999). It was further found that adiponectin concentration is negatively regulated by visceral fat accumulation and the state of hypoadiponectinemia is associated with the pathogenesis of type 2 diabetes, coronary artery disease, hypertension, and left ventricular hypertrophy, making adiponectin an important molecule involved in limiting the pathogenesis of obesity-linked disorders (Lopez-Jaramillo et al., 2016, Goldstein et al., 2009).

Adiponectin is mainly synthesized by adipocytes, but it can also be expressed by skeletal muscle cells, cardiac myocytes, and endothelial cells (Kadowaki et al., 2015). It serves as an insulin-sensitizing hormone by curtailing the rise of plasma free fatty acids after a high-fat meal as well as by activating AMPK and inhibiting TNF- α . (Yamauchi et al., 2001). Upregulation of adiponectin can enhance its insulin-sensitizing effects by enhancing lipid catabolism, reducing triglyceride contents and lowering hepatic-glucose production (Combs et al., 2004). It has also been shown to enhance nitric oxide (NO) production as well as inhibit inflammatory signaling (Cai et al., 2015). Because low levels are associated with metabolic syndrome, its decrease during weight loss or energy deficiency could lead to serious health consequences (Saely et al., 2012).

IV. The Effect of Exercise on White Adipose Tissue

When examining the efficacy of weight-loss programs incorporating exercise interventions, the amount of weight maintained was directly related to the amount of exercise performed, whether it is duration or a function of energy expenditure (Jakicic et al., 2008). This is largely due to the effect of exercise on white adipose tissue metabolism, and more specifically, the resultant increase in lipolysis that is accompanied by a simultaneous decrease in de novo lipogenesis (Askew et al., 1975, Habitante et al., 2010). During exercise, insulin concentrations are decreased, as increased substrates are required in circulation for energy utilization. The suppression of insulin is thus one mechanism in which lipolysis is increased during exercise (Hodgetts et al., 1991). Exercise further increases lipolysis through the release of many circulatory substrates, including catecholamines, that activate the beta-adrenergic signaling, the stimulatory G_s pathway that promotes release of non-esterified fatty acids into circulation for subsequent oxidation (Baptista et al., 2008).

In order to be used as an energy source, triglycerides need to be initially be hydrolyzed into glycerol and fatty acids, the latter of which must travel from adipose tissue to the tissues where oxidation will occur. This process is furthered by the increased adipose tissue and skeletal muscle blood flow that occurs during exercise, allowing for increased delivery of NEFAs (Horowitz et al., 2005). Even a single bout of exercise can decrease fatty acid synthesis, or lipogenesis, prevent an increase in FA esterification enzymes, and increase lipolysis instead, thus decreasing fat storage and increasing fat utilization (Horowitz et al., 2000).

While exercise enables mobilization of fatty acids through modulations of white adipose tissue metabolism, it also affects energy output and appetite regulation.

Prolonged and habitual exercise is associated with rapid gastric emptying, allowing for more feelings of satiety through production of anorexigenic digestive hormones (Horner et al., 2011, Anastasiou et al., 2015). This increase in satiety effectiveness overcomes the increased urge to eat following exercise, counteracting its effects (King et al., 2009). An additional counter-regulatory mechanism surrounds the effect of exercise on browning of white adipose tissue. Through promotion of thermogenic genes, the browning of white adipose tissue - turning white adipose tissue or progenitor stem cells into brown adipose tissue - could ultimately increase total daily energy expenditure (Sapa-Kishi et al., 2016).

V. Brown Adipose Tissue Thermogenic Activity May Indicate Metabolic Adaptation Presence

Reducing the loss of energy via heat production is a key metabolic adaptation that occurs during weight loss. The main location for this adaptation is likely Brown Adipose Tissue (BAT), a thermogenic tissue that produces heat by uncoupling mitochondrial processing from ATP synthesis towards heat production (Hanssen et al., 2015, Giralt et al., 2016). Stimulation of BAT thermogenic activity has been correlated with the induction of non-shivering thermogenesis, in that both increase as individuals acclimate to cold exposure (van der Lans et al., 2013, Giralt et al., 2016). Furthermore, BAT has been shown to secrete adipokines that affect whole body metabolism in addition to its ability to attract circulating metabolites, allowing for significant clearance of circulating fatty acids, triglycerides, and glucose molecules (Giralt et al., 2016; Gifford et al., 2016; Din et al., 2016).

Though the link between brown fat thermogenic activity and weight loss has yet to be elucidated, its activity may be reduced during RMR suppression, which could indicate that its activity may also reflect adaptations that occur during weight loss. If modulations in BAT thermogenic activity are determined to be a significant metabolic adaptation that occur during weight loss, maintaining or increasing the thermogenic activity during prolonged periods of energy deficiency may be an intriguing anti-obesity target.

VI. Brown Fat Activation Confers Long-Term Metabolic Benefits

The activation of brown adipose tissue is controlled primarily by the sympathetic nervous system, which in turn triggers BAT to produce heat (Din et al., 2016). Sympathetic nerves release norepinephrine, which activates G protein-coupled receptors, thereby inducing cyclic AMP (cAMP) production. Production of cAMP results in the subsequent activation of protein kinase A and ultimately lipolysis, which helps serve as a fuel for non-shivering thermogenesis (Chen et al., 2016; Din et al., 2016). When sympathetic activity adrenergically acts upon brown adipocytes, two other processes occur in addition to the induction of lipolysis. These include an upregulation of lipoprotein lipase (LPL) activity, as LPL can promote uptake of fatty acids from plasma triglycerides, and induction of uncoupling protein-1 (UCP-1) gene expression (Giralt et al., 2016). UCP-1, a biomarker of brown adipose tissue presence, enables the uncoupling of mitochondrial ATP production towards heat generation and is stimulated by the fatty acids produced from lipolysis (Chen et al., 2016, Din et al., 2016).

Though UCP-1 is only present in brown adipose tissue, continuous activation of BAT can lead to the production of brown adipocyte-like cells in areas that are designated for fat storage, i.e., in white adipose tissue depots. This process is referred to as “browning,” with the brown adipocyte-like cells being referred to as beige or ‘brite’ cells (Giralt et al., 2016). These cells contain UCP1 and can thus produce heat, which serves to help ameliorate the effects of cold exposure and return the body to a thermoneutral, or more metabolically favorable, state (Lee et al., 2015). Cold exposure, without the induction of shivering, has been shown to be an efficacious way of upregulating UCP-1 expression and brite-cell production, thus activating brown fat via sympathetic nervous system stimulation (Chen et al., 2016; Din et al., 2016; Lee et al., 2015).

Activation of brown fat enables increased heat generation, which allows for increases in energy expenditure and metabolic rate, ultimately alleviating metabolic stress and improving overall metabolic functionality. Because BAT is intrinsically involved in clearance of plasma triglycerides and glucose, its activation, in combination with dietary restrictions, drastically improves insulin sensitivity, thus promoting uptake of glucose into tissues and increasing lipolysis, a necessary process to provide the fuel (FFA) for continued brown fat activation (Bakker et al., 2014; Bosy-Westphal et al., 2015). When BAT was activated via cold exposure, it was found that GLUT4, which was previously distributed all across the myocytes, was translocated to the sarcolemma, allowing for increased facilitation of glucose uptake. The localization of GLUT4 at the sarcolemma of the myocytes paralleled an increased glucose uptake, thus improving whole-body insulin sensitivity by reducing circulating glucose levels (Hanssen et al., 2015).

The increased uptake of glucose allows for decreased secretions of insulin, and because increased concentrations of insulin inhibit lipolysis and promote lipid storage, it could help prevent the accumulation of fat that occurs as a result of fat-overshooting and reduce hyperinsulinemic and hypertriglyceridemic dispositions (Giralt et al., 2016; Gifford et al., 2016). Circulating levels of glucose can also be decreased by the insulin-mediated suppression of gluconeogenesis, an energy-consuming process that produces glucose from non-carbohydrate substrates, allowing for increased fat oxidation to be used as energy substrates (Bosy-Westphal et al., 2015). In order to further reduce insulin resistance, these physiological mechanisms can be enhanced by dietary restriction of glucose. The increase in insulin sensitivity that occurs as a function of BAT activation will be reversed upon weight regain, but can be maintained upon a post-weight loss diet that limits glucose, i.e., a low glycemic load (Hanssen et al., 2015, Bosy-Westphal et al., 2015).

VII. Linking Weight Loss, Metabolic Adaptations, and Brown Adipose Tissue

Thermogenic Activity

The ability of brown adipose tissue to attract fatty acids, triglycerides, and glucose from circulation provides a mechanism by which continued BAT activation and recruitment can occur, thus increasing energy expenditure and maintaining metabolism. As the substrates are cleared from circulation, insulin sensitivity is improved, allowing for decreased insulin secretion, and thus increased lipolysis, the products of which allow for continuous BAT activation and recruitment. Furthermore, the presence of insulin will hinder new glucose production via gluconeogenesis, allowing for fat oxidation to occur

instead of glucose oxidation. The ability of brown fat activation and recruitment could potentially negate the long-term ramifications of the metabolic adaptations that occur in periods of chronic energy deficiency, perhaps making the individual less prone to regain weight and remain in a hyperphagic state for prolonged periods of time by altering energy partitioning.

Although the metabolic principles underlying the reduction of RMR during weight loss remain to be fully elucidated, the potential of BAT activation as an obesity target has been recognized. While BAT activation has been experimentally linked to improved metabolic functionality, the link between metabolic suppression from weight loss and reduced BAT thermogenic activity has not yet been divulged. This leads to the question of whether a decrease in BAT activity can explain the suppression of metabolic rate that occurs independently of fat free mass loss during weight loss. If it represents the metabolic adaptations that occur during weight loss, or a large proportion of metabolic rate suppression, counteracting its effects with activation could be an extremely promising venue in decreasing the propensity to regain weight.

VIII. The Effect of Exercise on Brown Adipose Tissue

While activation of brown fat is intriguing as a weight loss mechanism, engaging in exercise may enhance its induction as well as brite cell formation. During exercise, the expression of proliferator-activated receptor-gamma coactivator-1 α (PGC-1 α) is upregulated, which in turn increases mitochondrial biogenesis and fat oxidation (Lin et al., 2002). Its action further upregulates the expression of thermogenic genes, including UCP-1, allowing for increased substrate availability for brown fat activity (Roberts et al.,

2013). Several compounds have been postulated to increase brown fat activity through PGC-1 α mediation, including natriuretic peptides, FGF-21, and irisin, among several others.

The secretion of atrial- and brain natriuretic peptides (ANP and BNP, respectively) increases during acute exercise due to an increase in heart rate and cardiomyocyte stretching (Hansen et al., 2012). As heart rate and cardiomyocyte stretching increases rapidly at the onset of exercise, the release of ANP and BNP also increases rapidly, and can account for increased lipolysis, UCP-1 expression, mitochondrial biogenesis, and increased respiration (Bordicchia et al., 2012). The actions of ANP and BNP contribute to the substrate sink utilized by brown fat activity via lipolysis in addition to an increase in brown fat metabolism. Its effects are similar to that of β -aminoisobutyric acid (BAIBA), an amino acid generated by the breakdown of valine, which increases hepatic fatty acid oxidation while simultaneously decreasing lipogenesis through a PPAR α -mediated mechanism (Oh et al., 2006).

Fibroblast Growth Factor-21 (FGF-21) is also associated with brown fat activity, especially during acute cold exposure. Cold acclimation that caused increased brown fat activity was accompanied by a concomitant increase in FGF-21 levels (Hanssen et al., 2015). FGF-21 levels were also increased after a one-leg cycling exercise bout of one hour in duration, yet no increase was visualized in the non-exercising leg (Catoire et al., 2014). Though there is a lack of research connecting FGF-21 levels with acute and chronic exercise, FGF-21 activates PGC-1 α and UCP1 expression, thereby indicating that it may also play a role in providing substrates for brown fat activation in addition to activating brown fat through UCP1 gene expression (Collins et al., 2001).

While exercise itself, and the release of the aforementioned substrates, stimulate brown fat activity through β -adrenergic signaling and the production of NEFAs, the myokine irisin has been implicated recently as a substance released from exercise that can actually induce brite-cell formation (Giralt et al., 2016). Released by skeletal muscle, irisin activates the p38-MAP kinase pathway, which mediates brown adipose tissue activation (Bostrom et al., 2012). Irisin is also secreted in response to cold exposure, with its induction paralleling shivering intensity, suggesting that it may have evolved from shivering-induced muscle contractions (Lee et al., 2013). Further, cleaved from Fibronectin type III domain-containing protein 5 (FNDC5), irisin also binds to white adipocytes, upregulating UCP1 and inducing the transformation from white to brown adipocytes, allowing for increases in energy expenditure and improvements in glucose tolerance (Timmons et al., 2012).

While these results are exciting, it is unclear whether irisin levels are upregulated after exercise in humans. Norheim et al.'s detected reduced irisin levels in the exercising group (Norheim et al., 2013) while Bostrom and his colleagues found increased levels of irisin after endurance training (Bostrom et al., 2012). Despite these uncertainties, enhanced expression of PGC-1 α occurs through exercise and is associated with providing increased amounts of usable fat and the transition into an oxidative skeletal muscular type (Lin et al., 2002). Though the literature is inconsistent, irisin may be an effective tool to increase brown fat activity through exercise, thereby increasing metabolic rate via energy expenditure.

IX. Conclusion

Though weight loss induces a state of energy deficiency, which in turn suppresses metabolic rate in order to reduce energy expenditure, there are variable responses in each of the resting- and non-resting energy expenditure components (Diagram 1). Metabolic adaptations, and the components that contribute to its response (including adaptive thermogenesis) can hinder the ability to maintain weight loss by affecting white and brown adipose tissue metabolism, as well as altering responses of skeletal muscle and the digestive system. If metabolic suppression can be reversed or hindered through mechanisms such as brown fat recruitment or exercise, effective anti-obesity tools and programs can be created to help ensure successful weight maintenance and the cessation of weight cycling.

CHAPTER 3: APPROACH

The overall purpose of this thesis, which is to quantify changes in thermogenic activity of brown adipose tissue and metabolic adaptations occurring during acute and chronic energy deprivation or weight loss, will be addressed in four studies. An exploratory study was conducted to determine the extent of adaptive thermogenesis in cold acclimation and to determine the thermogenic capacity of various body regions (supraclavicular region, forehead, trunk, and forearm). To measure metabolic markers of adaptive thermogenesis and changes in thermogenic activity between overweight/obese participants and normal weight, habitually exercising men, an observational study was conducted for both populations before meaningful weight loss had been initiated in the overweight/obese group (Study 1). To determine whether changes in adaptive thermogenesis occur in a short-term induced starvation period, we conducted a repeated measures three-way crossover design in habitually exercising men (Study 2). To assess the changes in metabolic markers of adaptive thermogenesis over participation in a three-month weight loss program in overweight/obese participants, we conducted an observational study with measurements before participation in the program and upon completion (Study 3). We will further compare measures of thermogenic activity and metabolic markers among the four study populations.

Exploratory Pilot Study

The exploratory pilot study assessed internal and external temperature measurements (CorTemp Data Recorder, HQInc., Palmetto FL) during RMR tests, further analyzing whether adaptive thermogenesis and cold-induced thermogenesis were

visible and whether the extent of which would correlate with the other. Both males and females were included to participate in pilot testing, with ages ranging from 21 to 36. Participants were instructed to swallow the CorTemp Core Body Temperature Sensor, which was encased in a large pill at least 4-6 hours before accurate temperature assessment. Internal temperature was measured with the corresponding data recorder every minute during the resting metabolic rate test and was accompanied by a concomitant measurement of external temperature via ear thermometer. External temperature was also quantified with thermoimaging analysis. Thermoimages were taken of the supraclavicular region, forehead, trunk, and forearms with an IR camera (A655sc Infrared Camera, FLIR Systems, Inc., Wilsonville, OR) and temperature measurements at these regions were subsequently analyzed. The analysis in this pilot study included the assessment of body composition, resting metabolic rate, thermogenic activity, and internal versus external temperature measurements.

Study 1

Participants

The overweight and obese participants were recruited from a weight loss program conducted by the Campus Recreation Center of the University of Nebraska-Lincoln (UNL). Recruitment occurred at informational meetings and through fliers around UNL and the community. All individuals who were overweight or obese, as indicated by an initial body mass index (BMI) $> 25 \text{ kg/m}^2$, were eligible for participation in the research study. Women who were currently pregnant or individuals taking medications that interfere with the study outcomes, including medication affecting the metabolism of sex

hormones (androgens, anabolic steroids), thyroid medication (e.g. thyroxin, thyroid stimulating agents, antithyroid agents), insulin and antidiabetic drugs, growth hormone, and immunosuppressants, were excluded. Furthermore, individuals determined to be at a high cardiovascular risk as identified by ACSM Risk Stratification (ACSM's Guidelines for Exercise Testing and Prescription, Ninth Edition) were excluded as well. Inclusion and exclusion criteria were verified through the administration of surveys to determine health status, pre-existing conditions, and use of medication. Participants provided written informed consent prior to study enrollment.

The normal weight, healthy exercising men were recruited online, i.e., through listserv announcements and the lab website, as well as through fliers on UNL campus and in recreational and athletic settings in the surrounding Lincoln community. The men were between the ages of 19 and 40 who habitually exercise at least four hours a week, with a primary mode of exercise (either aerobic or resistance). In order to be eligible for participation, the individual needed to have a BMI between 18.5 - 30 kg/m², a body fat percentage less than or equal to 29%, and/or a sum of seven skinfolds of less than or equal to 201 mm, representing the 95th percentiles for body fat percentage and skinfolds in a male athletic population (Santos et al., 2014). Participants were excluded from the study if they smoke, have a history of metabolic abnormalities (high fasting glucose, dyslipidemia, or hypertension), or if they have a history of a disease or chronic illness that would interfere with measurement outcomes, including medications that would alter results (anti-hypertensive medications, lipid-lowering medications, thyroid medications, etc.). Inclusion and exclusion criteria were verified through surveys administered at the

beginning of participation in the study. Participants provided written informed consent prior to study enrollment.

Study Design

Upon consenting to participate in the study, baseline anthropometric measurements were collected. Height and weight were measured on a digital scale in the laboratory, collected to the nearest millimeter and 0.1 kg, respectively (Digital Column Scale with Body-Mass-Index Function, Seca, Hamburg, Germany). Body fat percentage and body density were estimated by measuring skinfold thickness at 7 sites utilizing skin calipermetry, including the abdomen, triceps, chest, midaxillary, subscapular, suprailiac, and thigh regions in normal weight participants (ACSM's Guidelines for Exercise Testing and Prescription, Ninth Edition).

Participants in both populations (overweight/obese and normal weight) were assessed on body composition, resting metabolic rate, circulating concentrations of metabolic hormones and surrogate markers of brown adipose tissue, and thermogenic activity. Dietary energy intake was assessed through 7-day (normal weight) or 3-day (overweight/obese) diet logs, while exercise energy expenditure was obtained using exercise logs. Non-exercise energy expenditure was measured via accelerometry (wGT3X-BT, Actigraph, Pensacola, FL, USA). The measurements were all completed within 2-3 weeks in 3-5 visits, depending upon the availability of the participant.

Study 2

Participants

Participants in Study 2 were recruited from both campuses of the University of Nebraska-Lincoln. The study was limited to men between the ages of 18 and 30 who habitually engage in aerobic exercise at least 4 hours a week. The individuals must have had a body mass index between 19-25 kg/m², no more than 15% body fat, and had been weight stable (\pm 2.5 kg) during the past six months. Participants were excluded if they smoke or if they had a past or present diagnosis of a clinical eating disorder, an infectious disease within the 4 weeks prior to participation, or cardiovascular disease or orthopedic issues that impair long bouts of moderate- to vigorous-exercise. They were also excluded if they take medications for any pre-existing conditions that could interfere with study outcomes or if they have diabetes mellitus. Inclusion and exclusion criteria were verified through surveys administered at the beginning of participation in the study and participants provided written informed consent prior to study enrollment.

Study Design

Study 2 was a repeated-measures 3-way crossover design. Participants completed two low energy availability (EA) conditions, calculated at 15 kcal/kg FFM/day, and one condition with a normal energy availability, calculated at 40 kcal/kg FFM/day. During the low EA conditions, participants either consumed a high intake of protein at 1.7 g/kg body weight (denoted as CR+HP) or a normal protein intake at 0.8 g/kg body weight (denoted as CR+LP). In the normal energy availability condition, participants will consume a high intake of protein at 1.6 g/kg body weight (denoted as CON for control condition). Though three conditions were assessed in this study, only the control

condition and high protein/low energy availability conditions were included in the analysis.

Dietary intake was manipulated to attain the prescribed energy availability levels such that the target EA for the low EA groups would be 15 kcal/kg FFM/day and 40 kcal/kg FFM/day for the control condition after accounting for exercise energy expenditure. The amount of prescribed exercise was calculated by a desired equivalent of 15 kcal/kg FFM/day, which was accomplished by participants cycling at 60% of their individual $\text{VO}_{2\text{max}}$ until the exercise expenditure prescription was attained. Each condition lasted five days with the order of conditions randomized. Between each condition, participants completed washout periods with ad libitum diet and exercise for at least 14 days to allow protein balance to re-equilibrate. This analysis includes assessment of body composition, resting metabolic rate, and circulating concentrations of metabolic hormones.

Study 3

Participants

The overweight/obese participants recruited in Study 1 were the same individuals assessed in study 3, a study which examined how the metabolic parameters changed over the course of a three-month weight loss intervention. Participants were recruited from a weight loss program (Fit +Fueled Program) conducted by the Campus Recreation Center of the University of Nebraska-Lincoln (UNL). Recruitment occurred at informational meetings and through fliers around UNL and the community. All individuals who were overweight or obese, as indicated by an initial body mass index (BMI) $> 25 \text{ kg/m}^2$, were

eligible for participation in the research study. Women who were currently pregnant or individuals taking medications that interfere with the study outcomes, including medication affecting the metabolism of sex hormones (androgens, anabolic steroids), thyroid medication (e.g. thyroxine, thyroid stimulating agents, antithyroid agents), insulin and antidiabetic drugs, growth hormone, and immunosuppressants, were excluded. Furthermore, individuals determined to be at a high cardiovascular risk as identified by ACSM Risk Stratification (ACSM's Guidelines for Exercise Testing and Prescription, Ninth Edition) were excluded as well. Inclusion and exclusion criteria were verified through the administration of surveys to determine health status, pre-existing conditions, and use of medication. Participants provided written informed consent prior to study enrollment.

Study Design

Before entering the weight loss program, participants underwent testing to assess body composition, resting metabolic rate, circulating concentrations of metabolic hormones and surrogate markers of BAT, and thermogenic activity. During the weight loss program, weekly body weights, dietary energy intake, and exercise energy expenditure were obtained either from the weight loss program staff or through diet-, exercise-, and body weight logs completed by the participants. Non-exercise energy expenditure was measured via accelerometry (wGT3X-BT, Actigraph, Pensacola, FL, USA). Upon completion of the weight loss program (4-12 weeks), participants were tested to assess body composition, resting metabolic rate, circulating concentrations of metabolic hormones and surrogate markers of BAT, and non-shivering heat production.

To monitor weight regain following the completion of the study, body composition will be assessed every 3 months for one year.

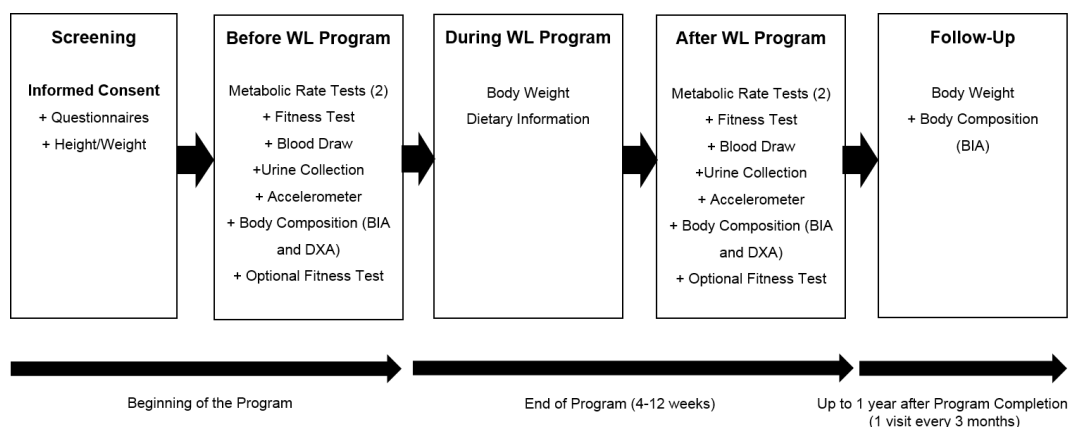


Diagram 2: Illustration of the Study Design of Study 3

Detailed Description of Methods

Body Composition. Body composition was measured by skin calipermetry and bioimpedence analysis. The participant's weight was measured to the nearest 0.1 kg on a digital scale (Digital Column Scale with Body-Mass-Index Function, Seca, Hamburg, Germany) as well as their height to the nearest millimeter using a stadiometer (Digital Column Scale with Body-Mass-Index Function, Seca, Hamburg, Germany) in a standard outfit of t-shirt and gym shorts. A skinfold caliper was then used to determine the participant's body fat percentage and body density by taking measurements of skinfold thickness at 7 sites: abdomen, triceps, chest, midaxillary, subscapular, suprailiac, and thigh (ACSM's Guidelines for Exercise Testing and Prescription, Ninth Edition).

Body composition was further measured by a bioimpedance analyzer (Quadscan 4000, Bodystat, Isle of Man, British Isles) before each RMR and at each follow-up visit. Four electrodes were placed on the participant's wrist, hand, ankle, and foot on the right side of the body while lying in a supine position. Upon connecting the electrodes to the analyzer and beginning the test, an electrical current runs through the body to estimate body composition. Bioimpedance analysis provided fat mass, fat free mass, and dry lean weight in kilograms as well as total body water in liters.

Resting Metabolic Rate. Resting Metabolic Rate was measured following an overnight, 12-hour fast with no food or beverage, excluding water. Participants were also required to avoid caffeine and alcohol and to refrain from exercise for 24 hours prior to the test. Before the test, participants completed a 15-30 minute rest period in a supine position to achieve a steady state at 24°C. Thereafter, a ventilated hood was placed above the participants' head and oxygen consumption and carbon dioxide production were measured (Quark CPET Metabolic Testing System, COSMED, Rome, Italy) for the next 30-45 minutes. RMR was calculated from oxygen uptake and carbon dioxide production using the Weir equation (Weir, 1949). To quantify RMR suppression, measured RMR was compared to predicted RMR (Gibbs et al., 2011). RMR was predicted using the Harris-Benedict equation (Harris-Benedict, 1918), denoted as RMR_{HB} , and from the proportion of lean mass, denoted as RMR_{LM} , using the Cunningham equation (Cunningham 1991).

Metabolic Hormones and Surrogate Markers of BAT. Fasting blood samples were collected from the forearm vein by a certified phlebotomist. Participants were fasted, with no food or drink except water, for 12-hours prior to the beginning of the blood draw and had abstained from caffeine, alcohol, and exercise 24-hours prior as well. A portion of the blood sample was used for a complete blood count (hemoglobin, hematocrit, red blood cell count, white blood cell count). The remaining blood was allowed to clot for 30 minutes at room temperature and subsequently centrifuged at 1000-1200 g for 10 minutes. The serum was then aliquoted and stored at -80C until further analysis.

Metabolic hormones of interest (leptin, insulin, adiponectin) and surrogate markers of brown adipose activity (FGF-21) were analyzed using commercially available-enzyme-linked immunoabsorbent assay (ELISA) kits (Quantikine ELISA, R&D Systems, Minneapolis, Minnesota). The working ranges (lowest and highest standard concentrations) were 15.6-1000 pg/mL (leptin), 3.9-250 ng/mL (adiponectin), 15.6-500 pg/mL (insulin), and 31.3-2000 pg/mL (FGF-21). Upon completion of the assay procedures, optical densities were measured using a microplate reader (Synergy H1 Hybrid Microplate Reader, BioTek Instruments, Winooski, VT) and concentrations were determined using the accompanying software (Gen5, BioTek Instruments, Winooski, VT). Concentrations outside of the assay working range were manually corrected to the lower and upper limit.

Thermogenic Activity. Thermogenic activity of brown adipose tissue was quantified by determination of non-shivering heat production, or cold-induced thermogenesis, which was measured during an additional resting metabolic rate (RMR) test conducted at 21C, a

temperature slightly below thermoneutral that induces cold acclimation and an adaptive thermogenic response. Oxygen consumption and carbon dioxide production were measured with a ventilated hood system (Quark CPET Metabolic Testing System, COSMED, Rome, Italy). Adaptive thermogenesis was quantified as the increase in RMR between 24°C and 21°C and cold-induced thermogenesis was quantified as the increase in regional external temperatures between 24°C and 21°C (Image 1). To minimize dietary influences on RMR, a 24-hour dietary recall was performed (Nutrition Data System for Research, Nutrition Coordinating Center, Minneapolis, Minnesota, USA) for the day before the first RMR test. The participant was then asked to replicate this diet for the day before the second RMR. Both RMR tests were conducted in randomized order (24°C or 21°C) and within seven days or less.

Thermogenic activity was also visualized through the usage of thermoimaging (A655sc Infrared Camera, FLIR Systems, Inc., Wilsonville, OR). A thermoimage was taken before and after each RMR measurement. The image focused on the cervical, supraclavicular, axillary, paravertebral, mediastinal, and upper abdominal regions, as they are the regions brown fat is predominately distributed in (Saely et al., 2012). The image was taken with the participant standing with their shirt removed from a standardized distance (six feet). Thermogenic activity, assessed through cold-induced thermogenesis, was quantified as differences in heat production in thermogenically active body regions (e.g. thoracic region) between visits. The regions specifically analyzed were the supraclavicular region, forehead, trunk, and forearms. Temperature of the skin overlying these regions was measured using infrared thermography, and the temperature representative of the 95th percentile temperature in that region was utilized. Trunk and

forearm values included both right- and left-side images, but were averaged during analysis. Further, these regions were all compared to the supraclavicular temperature, where BAT activity was most prominent.

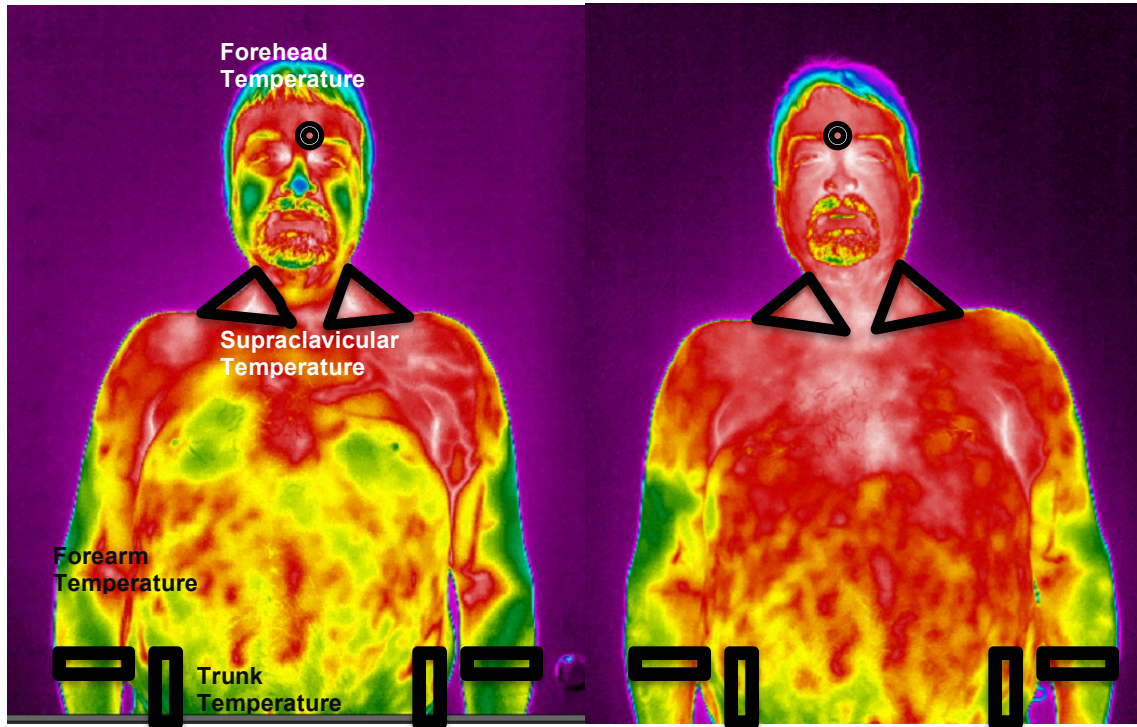


Image 1: Thermoimages collected at room temperatures of 21C (left) and 24C (right)

Cold-induced thermogenesis was calculated by averaging left and right temperature measurements in each region where applicable, and subtracting the regional temperature average at 24C from the regional temperature average at 21C. CIT was assessed in the following regions: supraclavicular temperature, forehead temperature, forearm temperature, and trunk temperature, in addition to supraclavicular temperature in relation to room temperature and all of the aforementioned regions, including a five-region average of the regions not associated with BAT Activity (Forehead, Forearm left and right, Trunk left and right).

Energy Balance. Dietary information and energy intake were determined using a 7-day diet log, which was analyzed by trained lab personnel upon its completion (Nutrition Data System for Research, Nutrition Coordinating Center, Minneapolis, Minnesota, USA). Exercise data was collected using 7-day exercise logs and was translated into exercise expenditure, expressed in kcal, using standardized literature MET values (Ainsworth et al., 2011). Non-exercise activity was measured through accelerometry for seven consecutive days (wGT3X-BT, Actigraph, Pensacola, FL, USA).

Internal Temperature. Internal temperature was measured in the exploratory study using a CorTemp Data Recorder in conjunction with a HQI Temperature Sensor (CorTemp Data Recorder, HQInc., Palmetto FL). Participants obtained an ingestible, wireless sensor at least four hours before accurate core body temperature measurements (within 0.1C). The sensor transmitted a magnetic signal to the data recorder when in close proximity.

Statistical Analysis

Statistical analyses were performed with Microsoft Excel for Mac 2011. If not stated otherwise, data are reported as mean \pm standard error of the mean (SEM). Student's T-test was used to compare difference between groups (unpaired) as well as within-subject differences between conditions and within-subject changes over time (both paired). Pearson's correlation coefficients were determined to assess correlations between measures of adaptive thermogenesis, cold-induced thermogenesis, and endocrine markers.

CHAPTER 4: RESULTS

Exploratory Study - Observation of adaptive thermogenesis and regional thermogenic capacity

Description of Participants

Two men and one woman participated in this exploratory study. The participants were 25.5 ± 1.8 years of age with an average body weight of 78.2 ± 4.0 kg, a BMI of 24.4 ± 0.76 , and a body fat percentage of 21.9 ± 1.4 (Table 1).

Table 1: Anthropometric data and body composition in normal weight, healthy individuals (Exploratory Study)

Exploratory Study (n=4)	
Age (y)	25.5 ± 1.8
Sex	2 M, 2 F
Body Weight (kg)	78.2 ± 4.0
BMI	24.4 ± 0.8
Body Fat (%)	21.9 ± 1.4
Fat Mass (kg)	16.7 ± 1.1
Fat-Free Mass (kg)	61.5 ± 3.8
Dry Lean Weight (kg)	19.8 ± 1.4
Total Body Water (L)	41.7 ± 2.5

Resting Metabolic Rate

RMR measured at 24C amounted to 1569 ± 62 kcal/d and increased by 168 kcal when measured at 21C (1727 ± 76 kcal) (Figure 1). The respiratory quotient also increased from 0.84 at 24C to 0.86 at 21C.

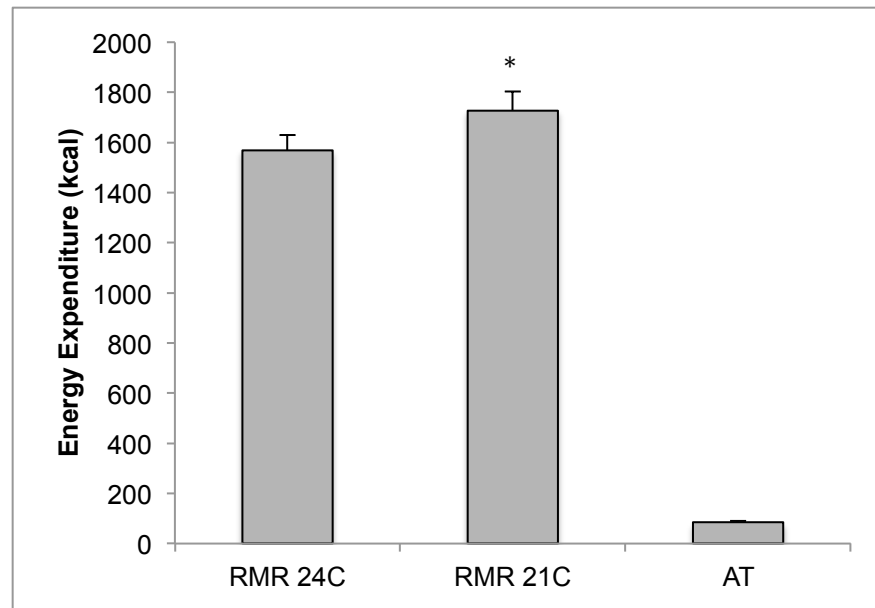


Figure 1: Comparison of RMR measurements at 24C and 21C with resultant adaptive thermogenesis value ($AT = RMR_{21} - RMR_{24}$) in normal weight, healthy individuals

Adaptive Thermogenesis

RMR measured at 21C was significantly higher than RMR measured at 24C, indicating a significant presence of adaptive thermogenesis ($p=0.011$). Adaptive thermogenesis, quantified as the difference in metabolic rates measured at 21C and 24C ($RMR_{21} - RMR_{24}$), was measured at 84 ± 7 kcal.

Thermoimaging Analysis

At 24C, there were no significant differences in body region temperatures (Figure 2) or body temperatures relative to supraclavicular temperature (Figure 3) between before and after acclimation images. At 21C, the five-region of interest temperature average (forehead, trunk left and right, forearm left and right) increased by 0.72C when acclimated to room temperature ($p=0.015$) (Figure 4). There were no significant

differences between body temperatures relative to supraclavicular temperature between before and acclimation images (Figure 5, Table 2).

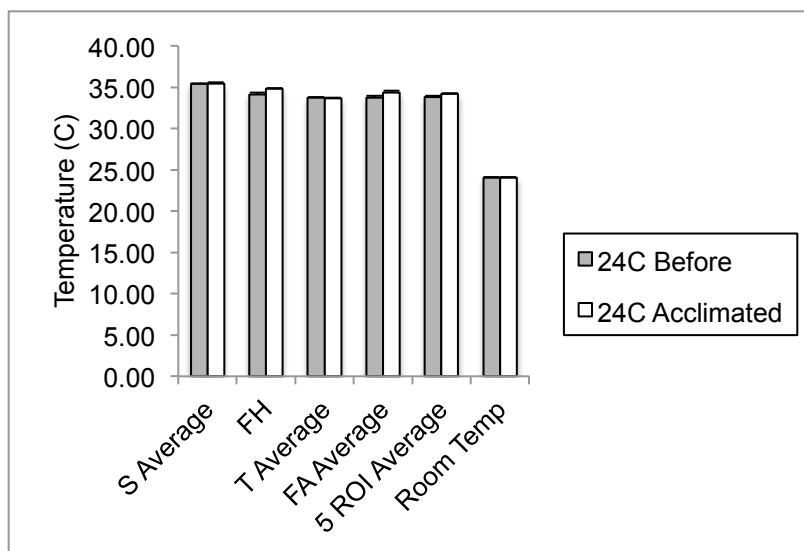


Figure 2: Comparison of external regional body temperatures at 24C in normal weight individuals (S=Supraclavicular, FH=Forehead, T=Trunk, FA=Forearm, ROI=Region of Interest)

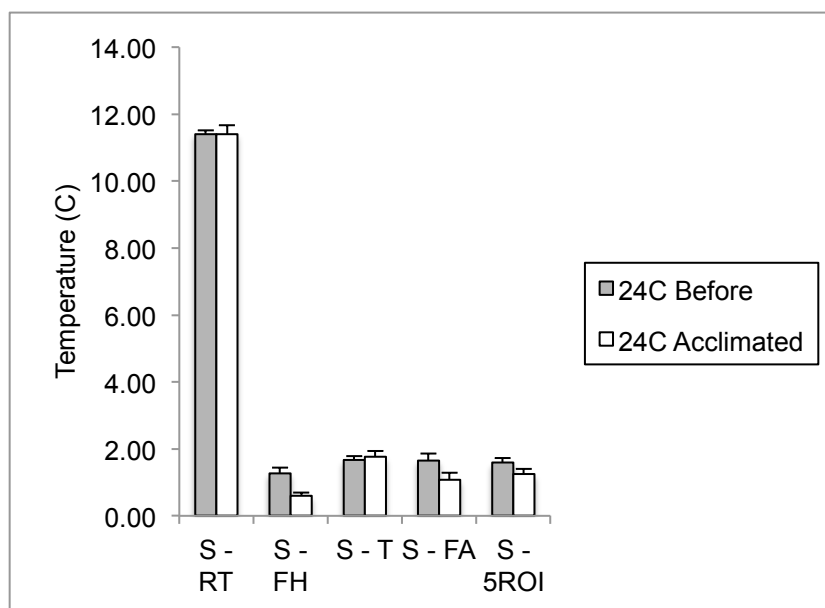


Figure 3: Comparison of body temperatures at different sites relative to the supraclavicular temperature at 24C in normal weight individuals (S=Supraclavicular, FH=Forehead, T=Trunk, FA=Forearm, ROI=Region of Interest)

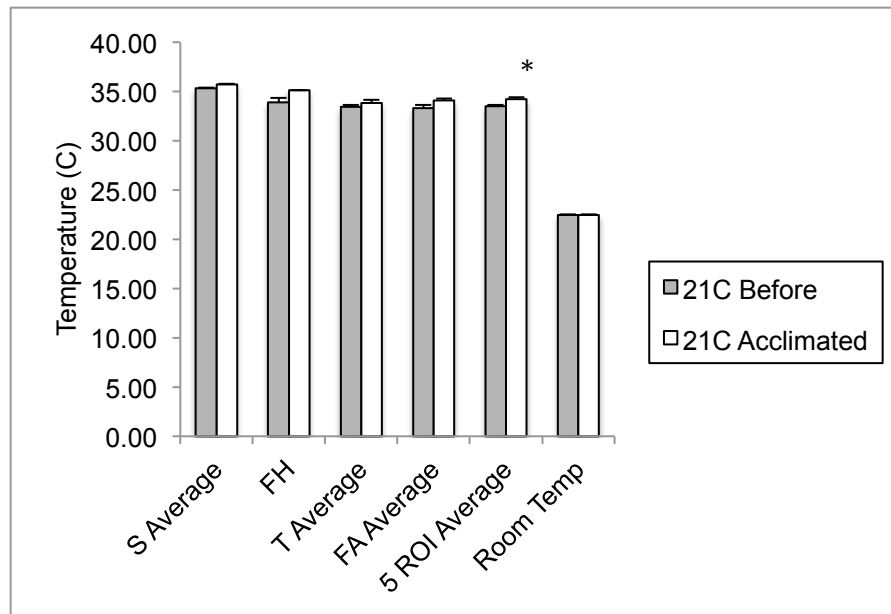


Figure 4: Comparison of external regional body temperatures at 21 C in normal weight individuals (S=Supraclavicular, FH=Forehead, T=Trunk, FA=Forearm, ROI=Region of Interest)

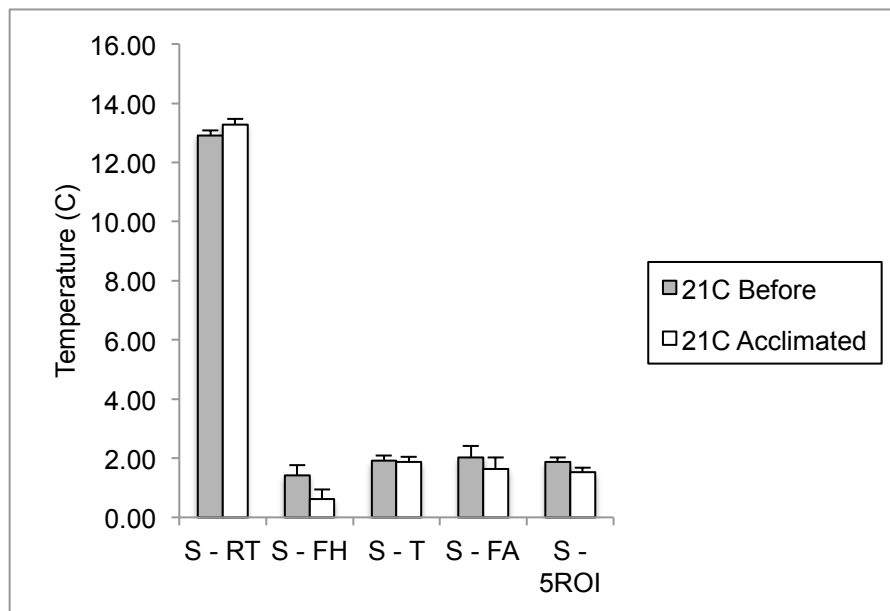


Figure 5: Comparison of body temperatures at different sites relative to the supraclavicular temperature at 21C in normal weight individuals (S=Supraclavicular, FH=Forehead, T=Trunk, FA=Forearm, ROI=Region of Interest)

Table 2: Comparison of body region temperatures before and after room temperature acclimation (Exploratory Study)

	21C			24C		
	Before	Acclimated	p-value	Before	Acclimated	p-value
Supraclavicular Temperature (S)	35.35 ± 0.07	35.73 ± 0.06	0.105	35.41 ± 0.05	35.42 ± 0.20	0.491
Forehead Temperature (FH)	33.92 ± 0.40	35.11 ± 0.03	0.113	34.13 ± 0.19	34.82 ± 0.11	0.089
Trunk Temperature (T)	33.43 ± 0.22	33.86 ± 0.32	0.202	33.74 ± 0.11	33.65 ± 0.08	0.344
Forearm Temperature (FA)	33.32 ± 0.33	34.08 ± 0.19	0.095	33.76 ± 0.23	34.34 ± 0.24	0.166
5 Regions of Interest Temperature (ROI)	33.48 ± 0.16	34.20 ± 0.21	0.015	33.82 ± 0.16	34.16 ± 0.13	0.164
S - RT	12.91 ± 0.18	13.29 ± 0.14	0.105	11.40 ± 0.12	11.41 ± 0.26	0.491
S - FH	1.43 ± 0.33	0.62 ± 0.09	0.123	1.28 ± 0.16	0.59 ± 0.09	0.114
S - T	1.92 ± 0.17	1.87 ± 0.38	0.454	1.67 ± 0.11	1.76 ± 0.18	0.293
S - FA	2.03 ± 0.39	1.64 ± 0.24	0.283	1.65 ± 0.22	1.07 ± 0.20	0.122
S - 5ROI	1.87 ± 0.16	1.53 ± 0.26	0.122	1.58 ± 0.15	1.25 ± 0.14	0.116
Room Temperature	22.44 ± 0.12	22.44 ± 0.12	1	24.01 ± 0.08	24.01 ± 0.08	1

Cold-Induced Thermogenesis

Cold-Induced Thermogenesis (CIT), defined as the difference in regional temperatures and temperatures in relation to supraclavicular temperature at 24C and 21C ($\text{Temp}_{21 \text{ acclimated}} - \text{Temp}_{24 \text{ acclimated}}$), increased in all regions and regions relative to supraclavicular temperature (Figure 6). Moreover, CIT of the supraclavicular area ($r = -0.909$) and forehead ($r = -0.956$) were negatively correlated to adaptive thermogenesis (Table 3).

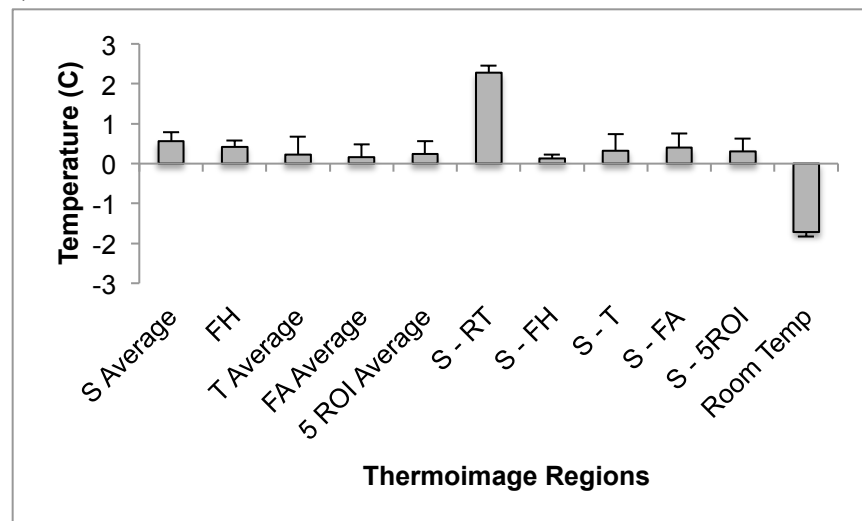


Figure 6: Cold-Induced Thermogenesis calculated by $\text{Temp}_{21} - \text{Temp}_{24}$ in normal weight, healthy individuals (S=Supraclavicular, RT=Room Temperature FH=Forehead, T=Trunk, FA=Forearm, ROI=Region of Interest)

Table 3: Correlation between AT and CIT in normal-weight individuals

AT and S	-0.91
AT and FH	-0.96
AT and S-RT	-0.64
AT and S-FH	-0.79
AT and S-T	0.24
AT and S-FA	-0.04
AT and S-5ROI	0.06

(S=Supraclavicular, FH=Forehead, T=Trunk, FA=Forearm, ROI=Region of Interest)

Study 1 - Comparison of metabolic markers of adaptive thermogenesis in overweight/obese and normal-weight individuals

Description of Participants

Six overweight/obese participants, five men and one woman, were compared to eight normal weight men. Due to missing data, four normal weight men are included in thermoimaging analysis and five overweight/obese participants are included in assay analysis. There was no difference in age between the two groups ($p=0.51$). Body weight values of the overweight/obese group exceeded that of the normal weight group by 68% ($p<0.001$). Further, body mass index and body fat percentage were 63% and 108% greater in the overweight/obese individuals ($p<0.001$).

Body Composition

Fat mass in the overweight/obese group exceeded normal weight individuals by 261% ($p<0.001$). Fat free mass and total body water also exceeded normal weight individuals by 21% ($p=0.032$) and 26% ($p=0.011$), respectively (Table 4). Dry lean weight was not significantly different between the two groups ($p=0.29$).

Table 4: Comparison of anthropometric data and body composition between overweight and obese participants and normal weight participants (Study 1)

	Overweight/ Obese (n=6)	Normal Weight (n=8)	p-value
Age (y)	35.7 ± 1.6	32.7 ± 0.8	0.51
Sex	5 M, 1 F	8 M	-
Body Weight (kg)	144.7 ± 4.6	86.1 ± 1.5	<0.001
BMI (kg/m ²)	41.7 ± 1.3	25.6 ± 0.2	<0.001
Body Fat (%)	40.8 ± 1.9	19.6 ± 0.4	<0.001
Fat Mass (kg)	60.9 ± 4.1	16.9 ± 0.4	<0.001
Fat-Free Mass (kg)	83.8 ± 2.2	69.2 ± 1.2	0.032
Dry Lean Weight (kg)	24.2 ± 0.7	21.7 ± 0.5	0.29
Total Body Water (L)	59.6 ± 1.6	47.4 ± 0.7	0.011

Resting Metabolic Rate

RMR measured at 24C in the overweight/obese participants was 319 kcal greater than RMR measured at 24C in the normal weight participants (p=0.04) (Figure 7) and 450 kcal greater in overweight/obese participants than normal weight participants at RMR measured at 21C (p=0.013). Predicted RMR according to Cunningham's equation, which factors in lean mass, was a more accurate predictor of measured RMR than Harris-Benedict, and the ratio of measured vs. Cunningham-predicted RMR did not differ significantly between groups (p=0.64) (Cunningham et al., 1980). Predicted RMR according to the Harris-Benedict equation overestimated RMR by 20% and 7% in overweight/obese and normal weight individuals, respectively, with a greater ability to predict RMR more accurately in normal weight individuals (p=0.016). The respiratory quotients did not differ significantly between groups (p=0.56) (Table 5).

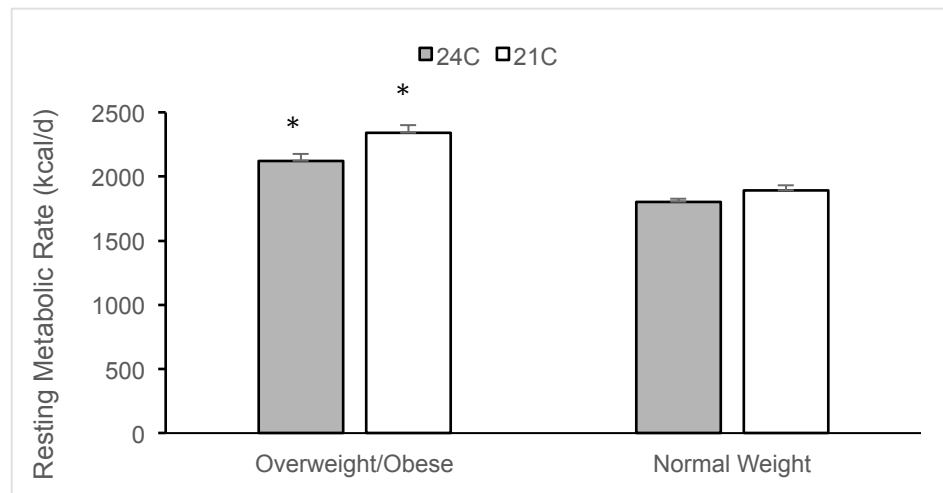


Figure 7: Changes in RMR during measurements at 24C and 21 C in overweight/obese and normal weight participants

Table 5: Study 1 Resting Metabolic Rate (RMR) measured at 24C

	Overweight/ Obese (n=6)	Normal Weight (n=8)	p-value
RMR (kcal/d)	2124 ± 53	1805 ± 25	0.04
Respiratory Quotient	0.89 ± 0.02	0.94 ± 0.02	0.56
measured vs. predicted ¹ RMR	0.80 ± 0.01	0.93 ± 0.01	0.016
measured vs. predicted ² RMR	1.01 ± 0.02	0.98 ± 0.01	0.64

¹predicted according to Harris-Benedict (1919); ²predicted according to Cunningham (1991)

Adaptive Thermogenesis

Adaptive thermogenesis was 220 kcal on average in the obese/overweight population and 89 kcal on average in the normal weight men (Figure 8). Though adaptive thermogenesis in the overweight/obese population exceeded that of the normal weight population by 131 kcal, the difference did not reach statistical significance (p=0.20).

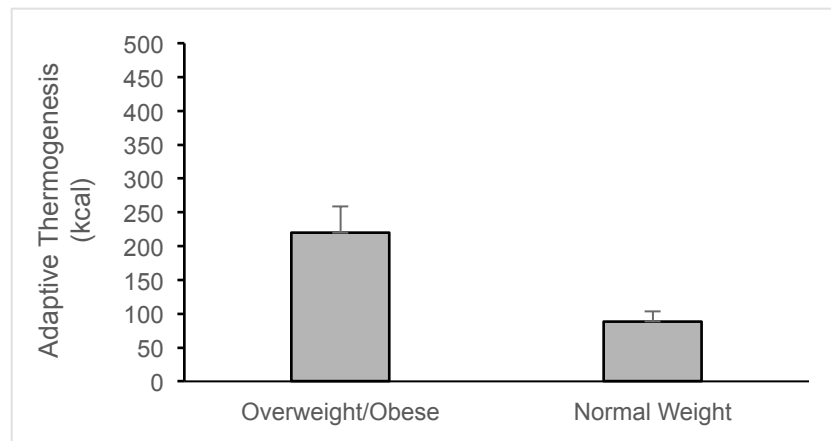


Figure 8: Adaptive thermogenesis ($RMR_{21} - RMR_{24}$) calculated by RMR measurements at 24C and 21C in overweight/obese and normal weight participants

Thermoimaging Analysis

In the overweight/obese participants, there were no significant differences in external regional body temperatures between measurements at 24C and 21C (Figure 9). In normal weight participants, there were also no significant differences in external regional body temperatures between measurements at 24C and 21C (Figure 10). At 21C, the regional forearm temperature was 0.76C greater in normal weight participants when compared to overweight/obese ($p=0.018$), but there were no other significant differences in regional temperature. At 24C, supraclavicular temperature ($p=0.019$), trunk temperature ($p=0.017$), forearm temperature ($p=0.012$), and the five averaged region temperature ($p=0.0037$) were all greater in normal weight participants when compared to overweight/obese participants.

When relating supraclavicular temperature to other regions and room temperature, there was a greater difference in supraclavicular temperature relative to room temperature ($p<0.001$) and trunk temperature ($p=0.046$) at 21C in overweight/obese participants when compared to measurements at 24C (Figure 11). There were no significant differences in supraclavicular temperature to other regions and room temperature in normal weight

participants (Figure 12). At 21C, there was a greater difference (0.69C) in supraclavicular temperature relative to forearm temperature in overweight/obese participants in comparison with normal weight participants ($p=0.015$). At 24C, there were no significant differences in supraclavicular temperature to other regions and room temperature in both groups.

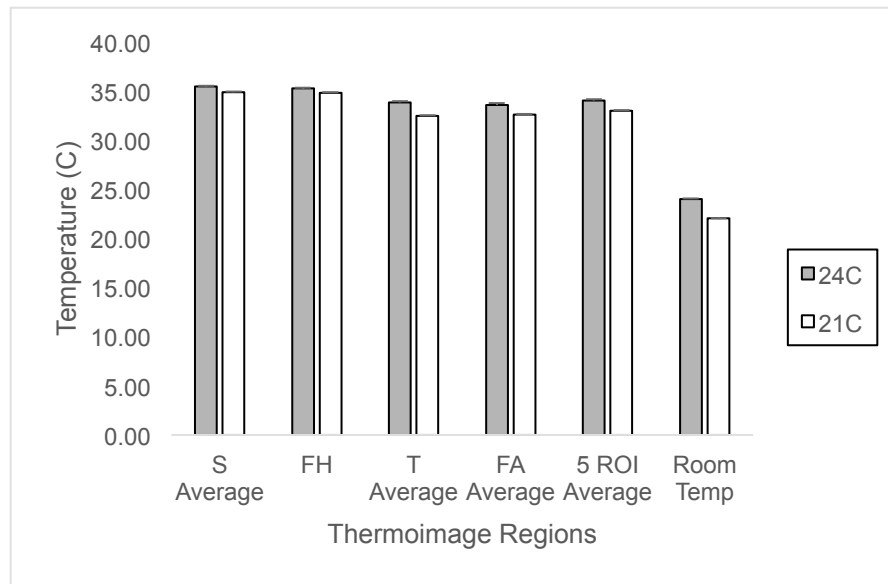


Figure 9: Comparison of external body temperatures at different anatomical locations during RMR measurements at 24C and 21C in overweight/obese participants (S=Supraclavicular, FH=Forehead, T=Trunk, FA=Forearm, ROI=Region of Interest)

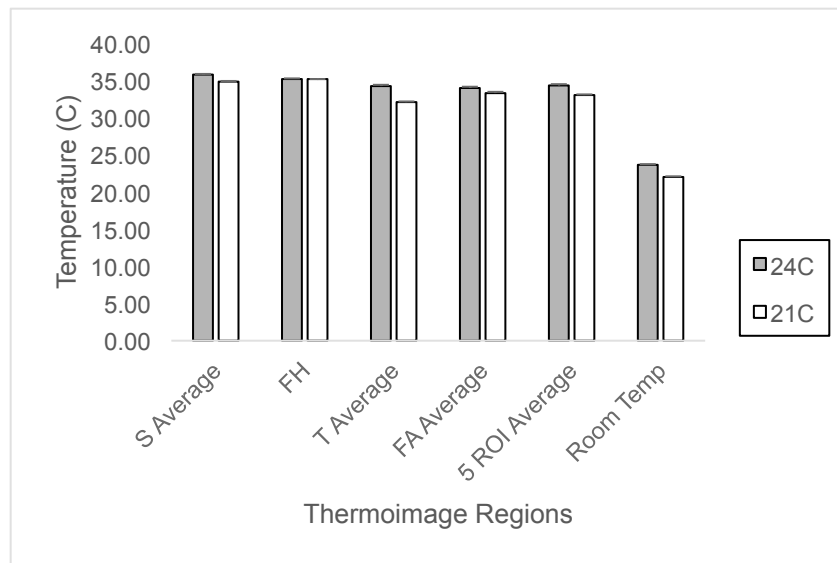


Figure 10: Comparison of external body temperatures at different anatomical locations during RMR measurements at 24C and 21C in normal weight participants (S=Supraclavicular, FH=Forehead, T=Trunk, FA=Forearm, ROI=Region of Interest)

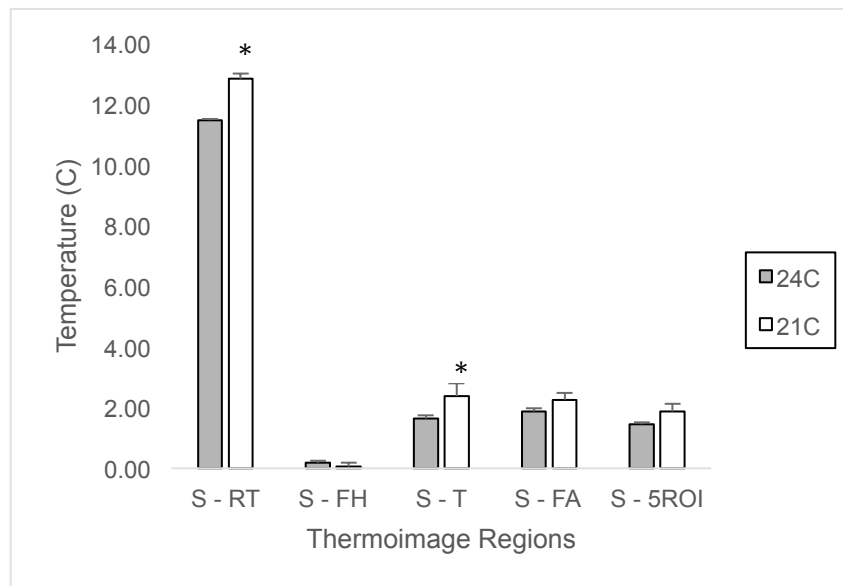


Figure 11: Comparison of body temperatures at different sites relative to the supraclavicular temperature during RMR measurements at 24C and 21C in overweight/obese participants (S=Supraclavicular, RT=Room Temperature, FH=Forehead, T=Trunk, FA=Forearm, ROI=Region of Interest)

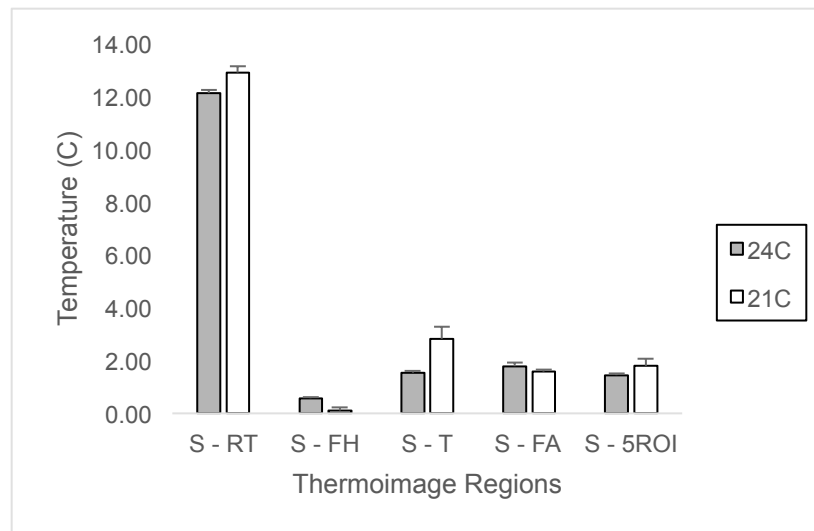


Figure 12: Comparison of body temperatures at different sites relative to the supraclavicular temperature during RMR measurements at 24C and 21C in normal weight participants (S=Supraclavicular, RT=Room Temperature, FH=Forehead, T=Trunk, FA=Forearm, ROI=Region of Interest)

Cold-Induced Thermogenesis

Cold-Induced Thermogenesis (CIT) did not significantly differ between overweight/obese and normal weight participants (Figure 13).

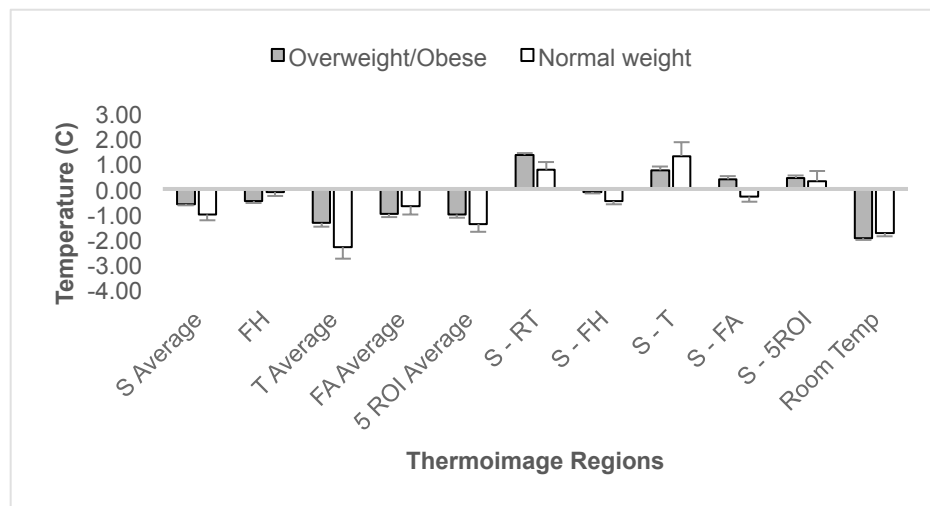


Figure 13: Cold-Induced Thermogenesis calculated by $TempAverage_{21} - TempAverage_{24}$ in overweight/obese and normal weight individuals (S=Supraclavicular, RT=Room Temperature FH=Forehead, T=Trunk, FA=Forearm, ROI=Region of Interest)

Hormones

Overweight/obese participants had significantly greater concentrations of leptin ($p=0.015$), insulin ($p=0.091$), and FGF-21 ($p=0.023$) than normal weight participants (Table 6). Adiponectin concentration did not differ significantly between the two groups ($p=0.42$). There were no correlations between adaptive thermogenesis and adiponectin, leptin, insulin, or FGF-21 in obese/overweight and normal weight participants (Figure 14). Cold-induced thermogenesis was assessed by supraclavicular temperature and the relation of supraclavicular temperature to room temperature between measurements at 24C and 21C. Supraclavicular temperature was strongly correlated with adiponectin ($r = -0.74$), leptin ($r=0.86$), insulin ($r=0.78$), and FGF-21 ($r=0.54$) in normal weight participants and strongly correlated with leptin ($r=0.59$) and FGF-21 ($r=0.68$) in overweight/obese participants (Figure 15). Supraclavicular temperature relative to room temperature was significantly correlated with adiponectin ($r = -0.75$), leptin ($r=0.97$), and insulin ($r=0.95$) in normal weight participants, but there were no strong correlations in overweight/obese participants (Figure 16).

Table 6: Comparison of hormone levels in overweight/obese and normal weight participants (Study 1)

	Overweight/ Obese (n=6)	Normal Weight (n=8)	p-value
Adiponectin (ng/mL)	29.6 ± 5.0	26.9 ± 2.7	0.42
Leptin (pg/mL)	237.5 ± 48.0	27.7 ± 4.0	0.015
Insulin (pg/mL)	414.6 ± 30.9	268.3 ± 26.7	0.091
FGF-21 (pg/mL)	451.1 ± 87.1	107.9 ± 8.8	0.023

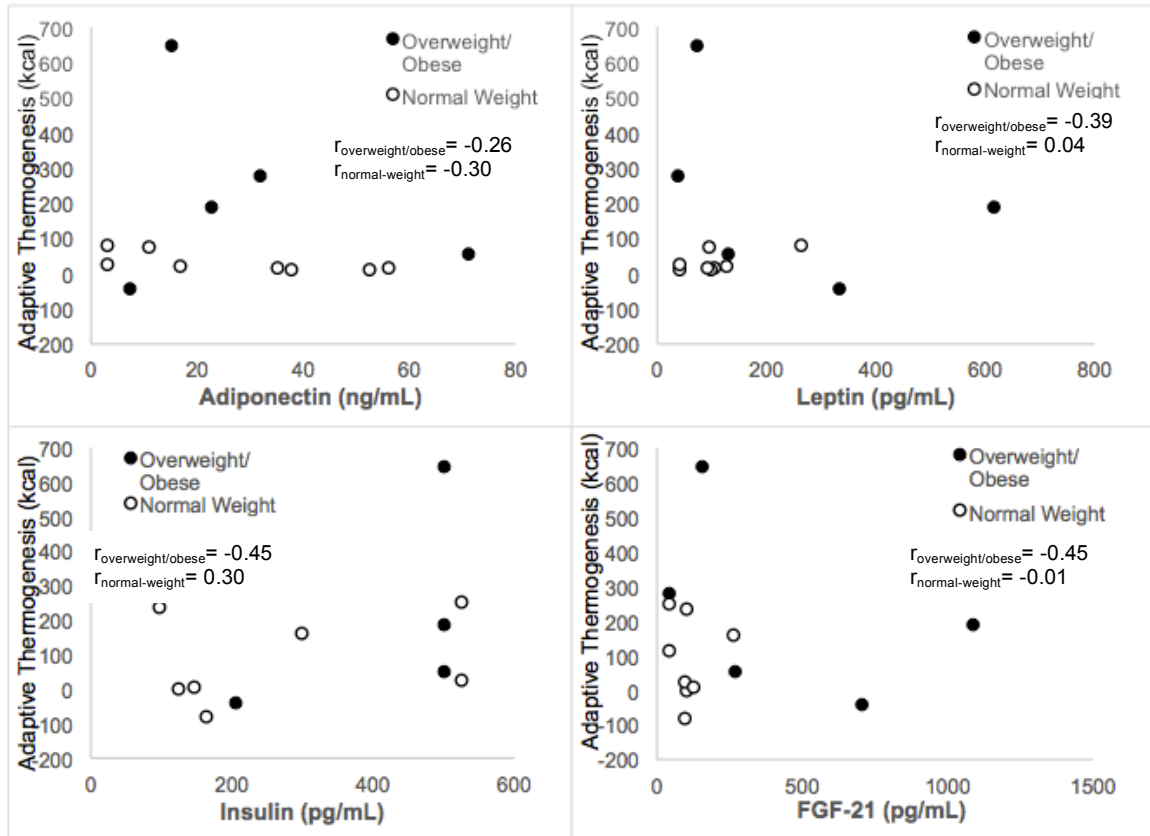


Figure 14: Correlations between adaptive thermogenesis and metabolic hormones/surrogate markers of BAT activity (Adiponectin, Leptin, Insulin, and FGF-21) in overweight/obese and normal weight participants

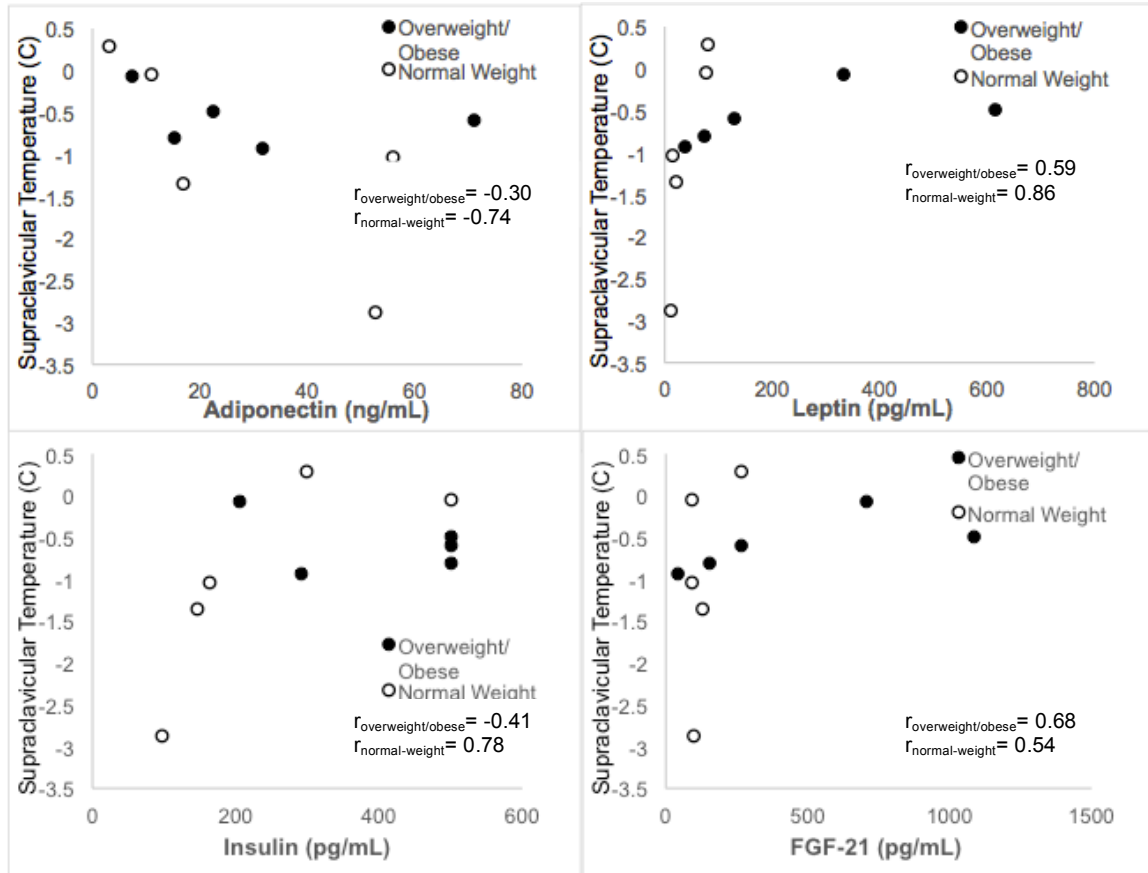


Figure 15: Correlations between cold-induced thermogenesis measured at the supraclavicular region and metabolic hormones/surrogate markers of BAT activity (Adiponectin, Leptin, Insulin, and FGF-21) in overweight/obese and normal weight participants

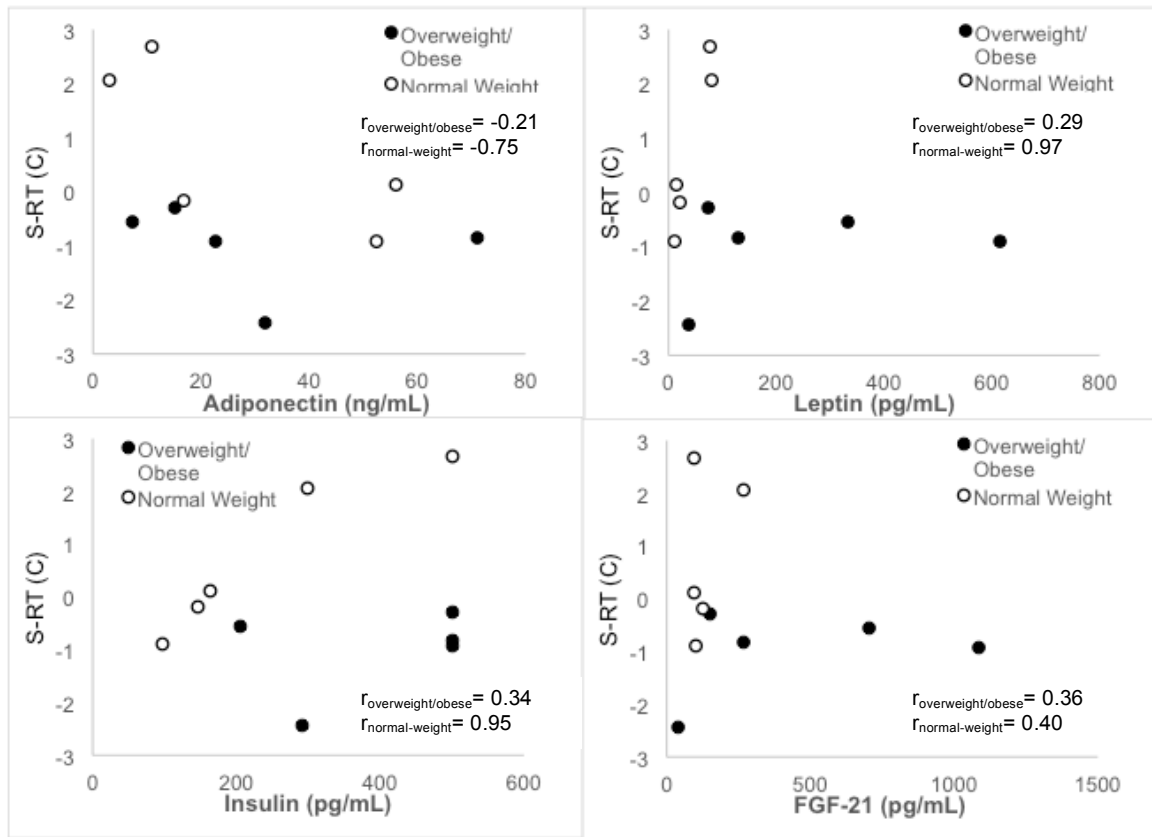


Figure 16: Correlations between cold-induced thermogenesis determined by the supraclavicular temperature (S) in relation to room temperature (RT) and metabolic hormones/surrogate markers of BAT activity (Adiponectin, Leptin, Insulin, and FGF-21) in overweight/obese and normal weight participants

Study 2 - Examination of the impact of induced short-term starvation on selected markers of adaptive thermogenesis in healthy, exercising men

Description of Participants

Of thirteen participants who enrolled in the study, two participants completed each condition, three participants discontinued after one condition, and one participant withdrew due to illness. The two participants included in this analysis completed one condition in energy balance and one condition in calorie restriction with matched levels of protein intake. The participants were 19.0 years of age with a body weight of 84.6 ± 0.6 kg. Initial BMI was 26.7 ± 0.7 and initial body fat percentage was 15.0 ± 0.1 % (Table 7).

Table 7: Baseline anthropometrics and body composition of healthy exercising men (Study 2)

Age (y)	19 ± 0
Body Weight (kg)	84.6 ± 0.6
BMI	26.7 ± 0.7
Body Fat (%)	15.0 ± 0.1
Fat Mass (kg)	12.7 ± 0.2
Fat-Free Mass (kg)	71.9 ± 0.4
Dry Lean Weight (kg)	23.1 ± 0.6
Total Body Water (L)	50.2 ± 1.2

Body Composition

In the energy balance condition, body weight decreased by 0.6 kg and fat free mass decreased by 2.0 kg throughout the five-day condition. Total body water increased by 0.6 L, fat mass increased by 1.4 kg, and dry lean weight increased by 0.2 kg. There

were no significant differences between body composition values at the beginning and end of the condition.

In the calorie restriction condition, body weight decreased by 3.5 kg, fat mass decreased by 3.6 kg, and dry lean weight decreased by 0.9 kg. Fat free mass and total body water increased by 0.1 kg and 1.0 L respectively. Again, there were no significant differences between body composition values at the beginning and end of the condition (Figure 17).

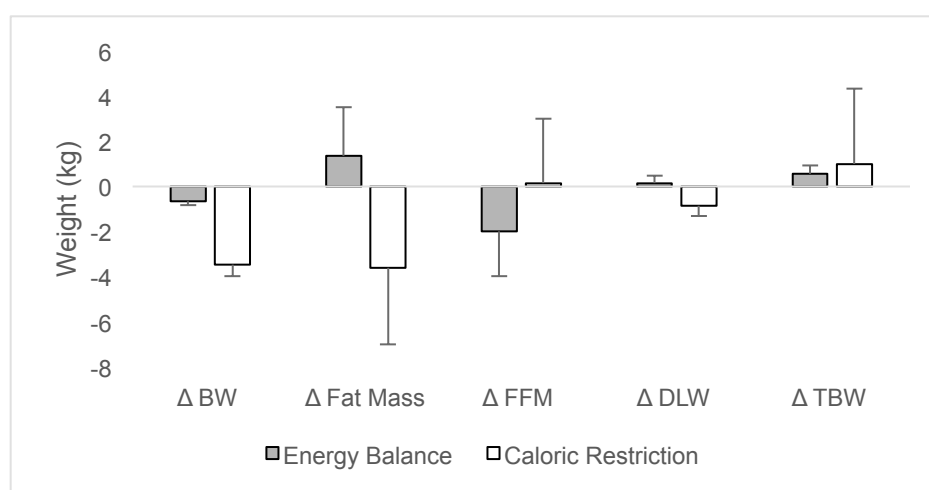


Figure 17: Changes in body composition over the course of each 5-day intervention, consisting of either caloric restriction (CR: 15 kcal/kg/FFM) or energy balance (CON: normal energy availability kcal/kg FFM) (BW=Body weight, FFM=Fat free mass, DLW=Dry lean weight, TBW=Total Body Water)

Resting Metabolic Rate

In the energy balance condition, RMR increased slightly from 1658 ± 33 kcal/d before the condition to 1694 ± 86 kcal/d after the condition (Table 8, Figure 18), while the respiratory quotient decreased from 0.95 ± 0.09 to 0.92 ± 0.01 . RMR and RQ at the end of the condition did not differ significantly from the beginning of the condition. In

the calorie restriction condition, RMR decreased by 209 kcal/d ($p=0.002$) and RQ decreased by 0.1 ($p=0.049$). There was no significant difference between the changes in RMR that occurred between conditions ($p=0.096$).

Table 8: Study 2 Resting Metabolic Rate (RMR) measured at 24C (n=2)

	Energy Balance		
	<i>Pre</i>	<i>Post</i>	Δ
RMR (kcal/d)	1658 \pm 33	1694 \pm 86	36 \pm 53
Respiratory Quotient	0.95 \pm 0.09	0.92 \pm 0.01	-0.03 \pm 0.07
measured vs. predicted ¹ RMR	0.83 \pm 0.01	0.85 \pm 0.04	0.02 \pm 0.03
measured vs. predicted ² RMR	0.88 \pm 0.02	0.92 \pm 0.03	0.04 \pm 0.01
	Calorie Restriction		
	<i>Pre</i>	<i>Post</i>	Δ
RMR (kcal/d)	1704 \pm 187	1495 \pm 186	-210 \pm 1.1
Respiratory Quotient	1.05 \pm 0.08	0.94 \pm 0.06	-0.11 \pm 0.01
measured vs. predicted ¹ RMR	0.84 \pm 0.09	0.75 \pm 0.09	-0.09 \pm 0.00
measured vs. predicted ² RMR	0.90 \pm 0.08	0.79 \pm 0.11	-0.11 \pm 0.03

¹predicted according to Harris-Benedict (1919); ²predicted according to Cunningham (1991)

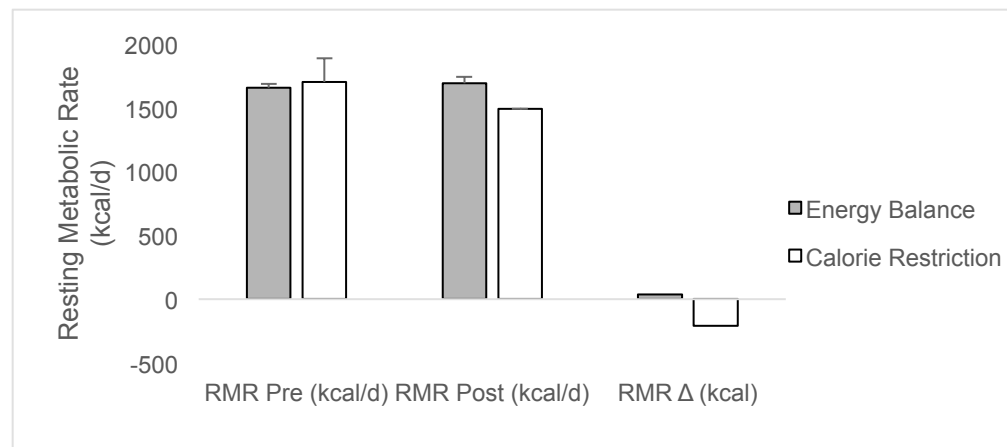


Figure 18: Changes in RMR over the course of each 5-day intervention, consisting of either caloric restriction (CR: 15 kcal/kg/FFM) or energy balance (CON: normal energy availability kcal/kg FFM)

Hormones

In the energy balance and calorie restriction conditions, there were no significant differences in pre- and post- levels of adiponectin, leptin, insulin, or FGF-21 (Table 9). In each condition, adiponectin and leptin increased by similar amounts, ~ 7.0 ng/mL and 0.4 pg/mL respectively. Insulin increased by 7.8 pg/mL throughout the energy balance condition, but decreased by 23.9 pg/mL in the calorie restriction condition. Similarly, FGF-21 increased by 4.5 pg/mL in the energy balance condition, but decreased by 61.6 pg/mL in calorie restriction.

Table 9: Hormone levels in healthy, exercising men (Study 2)

	Energy Balance		
	<i>Pre</i>	<i>Post</i>	Δ
Adiponectin (ng/mL)	25.6 ± 3.8	32.9 ± 1.2	7.3 ± 2.6
Leptin (pg/mL)	16.0 ± 0.3	16.3 ± 0.2	0.4 ± 0.5
Insulin (pg/mL)	166.6 ± 51.4	174.4 ± 20.2	7.8 ± 31.2
FGF-21 (pg/mL)	34.7 ± 9.3	39.2 ± 7.1	4.5 ± 2.2
	Calorie Restriction		
	<i>Pre</i>	<i>Post</i>	Δ
Adiponectin (ng/mL)	27.6 ± 9.6	34.6 ± 11.7	7.0 ± 2.1
Leptin (pg/mL)	21.1 ± 10.6	21.5 ± 3.1	0.4 ± 13.7
Insulin (pg/mL)	130.6 ± 16.8	106.8 ± 31.6	-23.9 ± 14.8
FGF-21 (pg/mL)	75.5 ± 38.4	13.8 ± 9.7	-61.6 ± 28.7

Study 3 - Comparison of metabolic markers of adaptive thermogenesis in overweight/obese individuals before and after participation in a weight loss program for 3 months

Description of Participants

Eight individuals initially signed informed consents to participate in the study. Two participants dropped out during pre-testing due to time constraints and illness, and one participant completed pre-testing, but relocated and did not return for post testing. As such, four men and one woman were assessed prior to and upon completing three months of a weight loss program. The participants were 37.8 ± 1.8 years of age at the beginning of the study.

Changes in Weight and Body Composition

Despite participation in a weight loss program, there were no significant changes in body weight ($p=0.14$), BMI ($p=0.18$), and body fat percentage ($p=0.22$) (Table 10). There were also no significant changes in fat mass ($p=0.20$), fat free mass ($p=0.29$), dry lean weight ($p=0.31$), or total body water ($p=0.32$).

Table 10: Comparison of anthropometric data and body composition between overweight and obese participants before and after WL (Study 1) (n=5)

	Pre	Post	p-value
Age (y)	37.8 ± 1.8	37.8 ± 1.8	-
Sex	4 M, 1 F	4 M, 1 F	-
Body Weight (kg)	137.3 ± 4.6	135.8 ± 5.0	0.14
BMI	40.0 ± 1.5	39.6 ± 1.6	0.18
Body Fat (%)	40.0 ± 2.5	39.5 ± 2.5	0.22
Fat Mass (kg)	56.7 ± 5.0	55.6 ± 5.2	0.20
Fat-Free Mass (kg)	80.6 ± 2.3	80.2 ± 2.3	0.29
Dry Lean Weight (kg)	23.8 ± 0.9	23.7 ± 1.0	0.31
Total Body Water (L)	56.9 ± 1.6	56.5 ± 1.6	0.32

Resting Metabolic Rate

RMR decreased from pre- to post- testing by 73 kcal/d, but the difference was not significant ($p=0.55$) (Table 11). Respiratory quotient increased by 0.01 in post testing, but was also not statistically significant ($p=0.45$) (Figure 19). Using the Harris-Benedict equation, RMR was overestimated more during post-testing than during pre-testing ($p=0.040$), but the Cunningham determination of RMR was again a more accurate predictor of measured RMR with no differences in measured vs. predicted RMR between pre- and post-testing ($p=0.19$).

Table 11: Study 3 Resting Metabolic Rate (RMR) measured at 24C (n=5)

	Pre	Post	p-value
RMR (kcal/d)	2004 ± 27	1931 ± 44	0.27
Respiratory Quotient	0.91 ± 0.03	0.92 ± 0.01	0.45
measured vs. predicted ¹ RMR	0.80 ± 0.02	0.78 ± 0.01	0.040
measured vs. predicted ² RMR	0.99 ± 0.03	0.96 ± 0.02	0.19

¹predicted according to Harris-Benedict (1919); ²predicted according to Cunningham (1991)

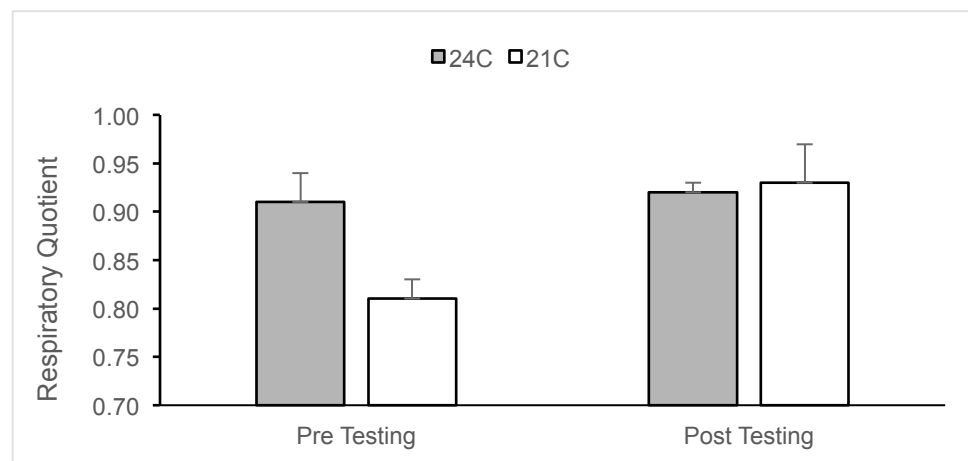


Figure 19: Changes in respiratory quotient during measurements at 24C and 21C before and after completing 3 months of a weight loss program in overweight/obese participants

Adaptive Thermogenesis

Adaptive thermogenesis decreased to 84 kcal over the course of the study, yet the decline was not statistically significant between pre- and post-testing ($p=0.28$) (Figure 20).

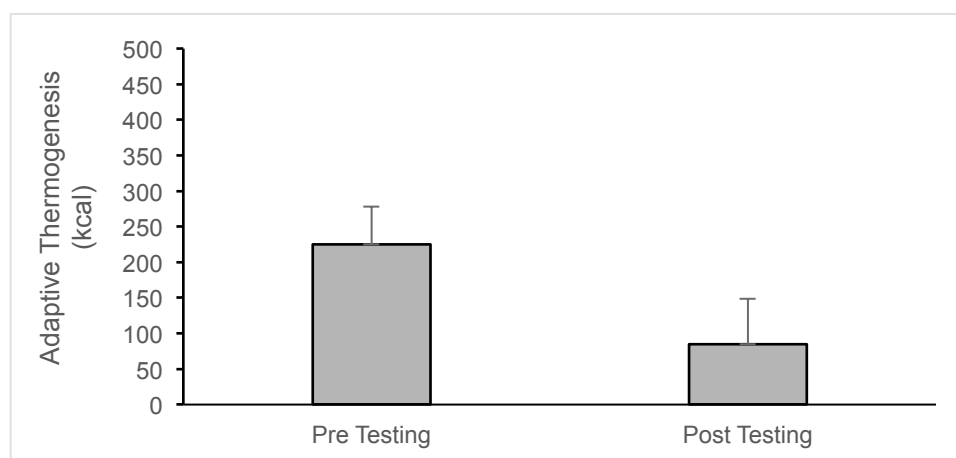


Figure 20: Adaptive thermogenesis ($RMR_{21} - RMR_{24}$) calculated by RMR measurements at 24C and 21C before and after completing 3 months of a weight loss program in overweight/obese participants

Thermoimaging Analysis

During the pre-testing RMR at 24C, supraclavicular temperature ($p=0.009$), forearm temperature ($p=0.004$), and trunk temperature ($p=0.024$) were all higher than corresponding temperatures during RMR at 21C (Figure 21). In post-testing, there were no significant differences in external regional temperatures at RMR measurements of 24C and 21C (Figure 22). There was a greater difference in supraclavicular temperature relative to room temperature ($p<0.001$) and trunk temperature ($p=0.042$) in pre-testing measurements at 21C (Figure 23). During post-testing, there was a greater difference in

supraclavicular temperature relative to room temperature ($p=0.001$) at 21C as well (Figure 24).

Between pre- and post-testing at 24C, supraclavicular temperature decreased ($p=0.047$), yet no other changes were significant. At 21C, supraclavicular temperature relative to trunk temperature ($p=0.021$) and the five-region of interest average temperature ($p=0.046$) decreased significantly over the course of the weight loss program.

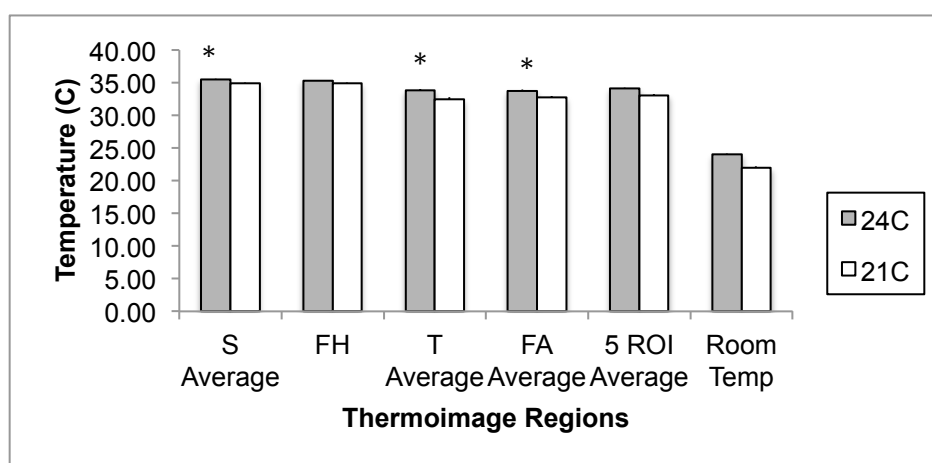


Figure 21: Comparison of external body temperatures at different anatomical locations during RMR measurements at 24C and 21C in overweight/obese participants before participating in a WL program (S=Supraclavicular, FH=Forehead, T=Trunk, FA=Forearm, ROI=Region of Interest)

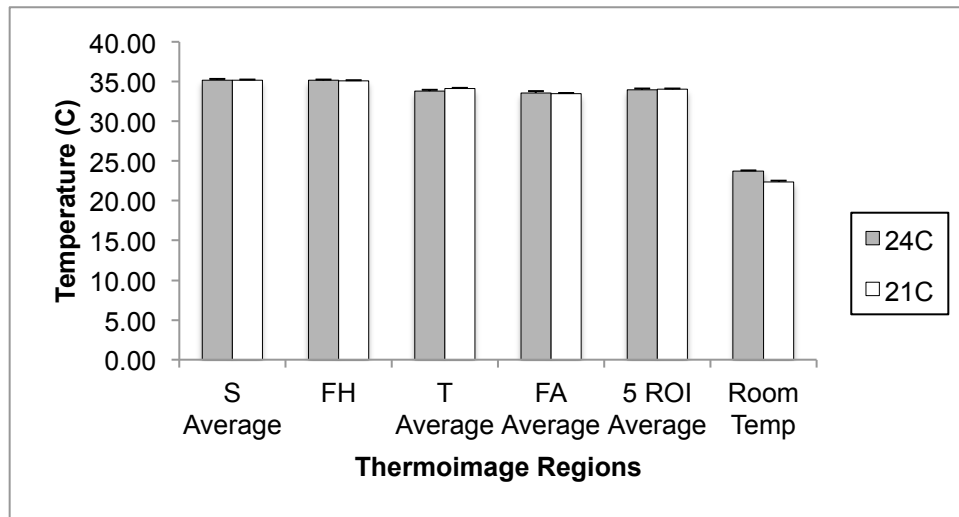


Figure 22: Comparison of external body temperatures at different anatomical locations during RMR measurements at 24C and 21C in overweight/obese participants after participating in a WL program (S=Supraclavicular, FH=Forehead, T=Trunk, FA=Forearm, ROI=Region of Interest)

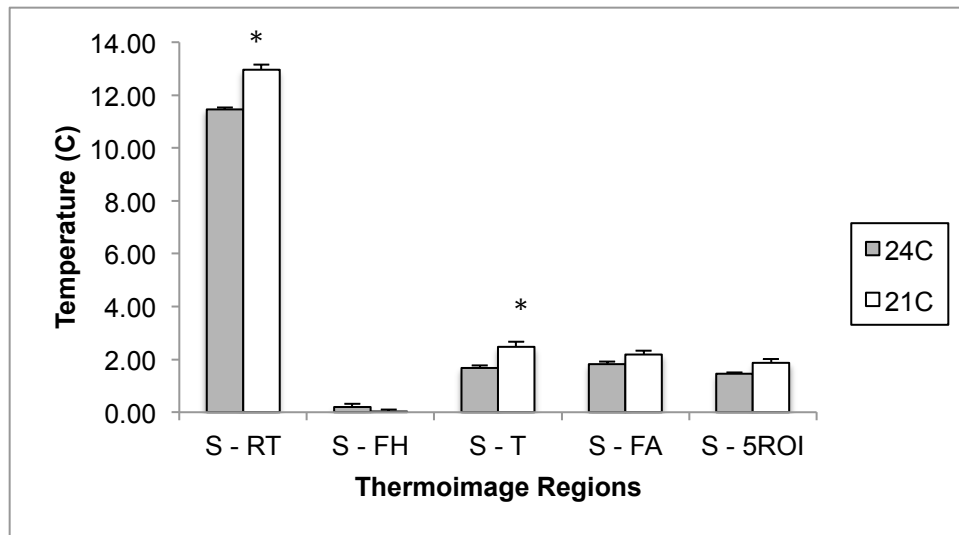


Figure 23: Comparison of body temperatures at different sites relative to the supraclavicular temperature during RMR measurements at 24C and 21C in overweight/obese participants before participating in a WL program (S=Supraclavicular, RT=Room Temperature, FH=Forehead, T=Trunk, FA=Forearm, ROI=Region of Interest)

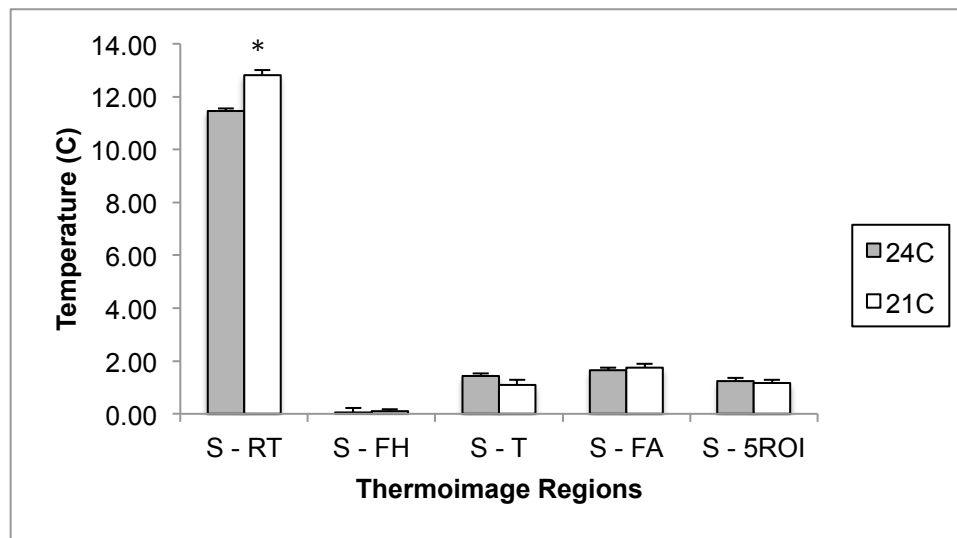


Figure 24: Comparison of body temperatures at different sites relative to the supraclavicular temperature during RMR measurements at 24C and 21C in overweight/obese participants after participating in a WL program (S=Supraclavicular, RT=Room Temperature, FH=Forehead, T=Trunk, FA=Forearm, ROI=Region of Interest)

Cold-Induced Thermogenesis

CIT increased significantly in the supraclavicular region ($p=0.022$), trunk region ($p=0.030$), and the average of 5-regions ($p=0.048$) over the course of the study (Figure 25). Supraclavicular temperature relative to trunk temperature decreased throughout participation in the weight loss program ($p=0.049$).

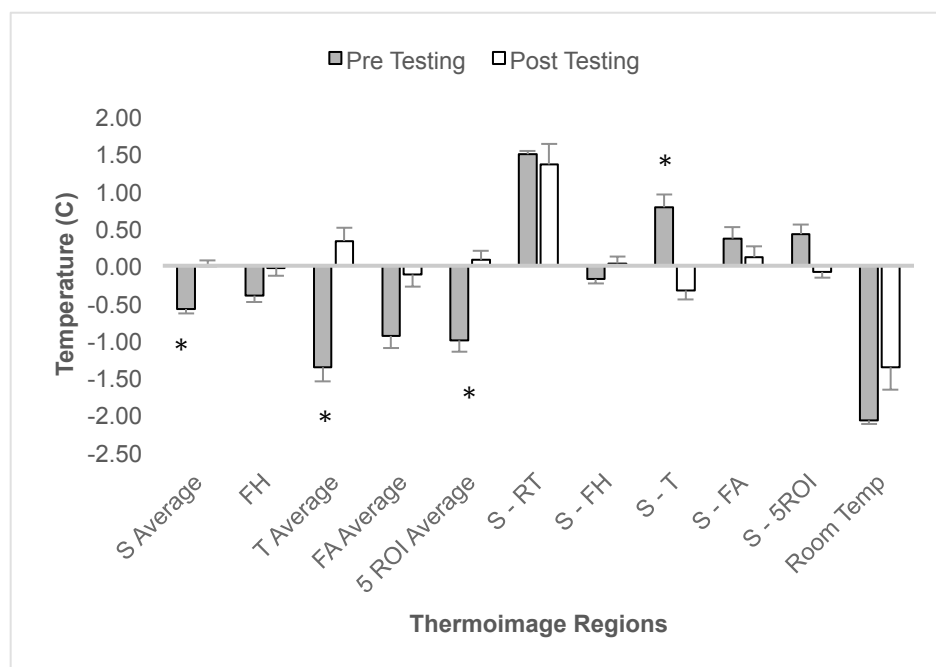


Figure 25: Cold-Induced Thermogenesis calculated by RMR tests measured at 21C and 24C in overweight/obese participants before and after weight loss (S=Supraclavicular, FH=Forehead, T=Trunk, FA=Forearm, ROI=Region of Interest)

Hormones

Throughout the three-month weight loss program, adiponectin concentration decreased by 9.5 ng/mL ($p=0.16$), leptin concentration decreased by 188.5 pg/mL ($p=0.12$), insulin concentration decreased by 128.3 pg/mL ($p=0.10$), and FGF-21 concentration decreased by 22.8 pg/mL ($p=0.46$) (Table 12). There were no significant changes between pre- and post- hormone levels. There were no correlations between adaptive thermogenesis and adiponectin, leptin, insulin, or FGF-21 in obese/overweight participants during pre-testing, but there were strong correlations in post-testing between adaptive thermogenesis and insulin ($r=-0.91$) and FGF-21 ($r=0.59$) (Figure 26). Cold-induced thermogenesis was assessed by supraclavicular temperature and the relation of

supraclavicular temperature to room temperature between measurements at 24C and 21C. During pre-testing, supraclavicular temperature correlated strongly with leptin ($r=0.52$) and FGF-21 ($r=0.63$). During post-testing, supraclavicular temperature correlated strongly with adiponectin ($r= -0.75$) and insulin ($r= -0.67$) (Figure 27). Supraclavicular temperature relative to room temperature was strongly correlated with adiponectin in pre-testing ($r=0.52$), but there were no other significant correlations between supraclavicular temperature relative to room temperature and leptin, insulin, or FGF-21 (Figure 28). In post-testing, there were no strong correlations between supraclavicular temperature relative to room temperature and adiponectin, leptin, insulin, and FGF-21.

Table 12: Comparison of hormone levels in overweight/obese participants before and after WL (Study 3) (n=5)

	Pre	Post	p-value
Adiponectin (ng/mL)	33.2 \pm 6.8	23.7 \pm 6.7	0.16
Leptin (pg/mL)	278.6 \pm 64.0	90.1 \pm 5.1	0.12
Insulin (pg/mL)	387.0 \pm 40.8	258.7 \pm 16.2	0.10
FGF-21 (pg/mL)	525.5 \pm 116.1	502.7 \pm 102.7	0.46

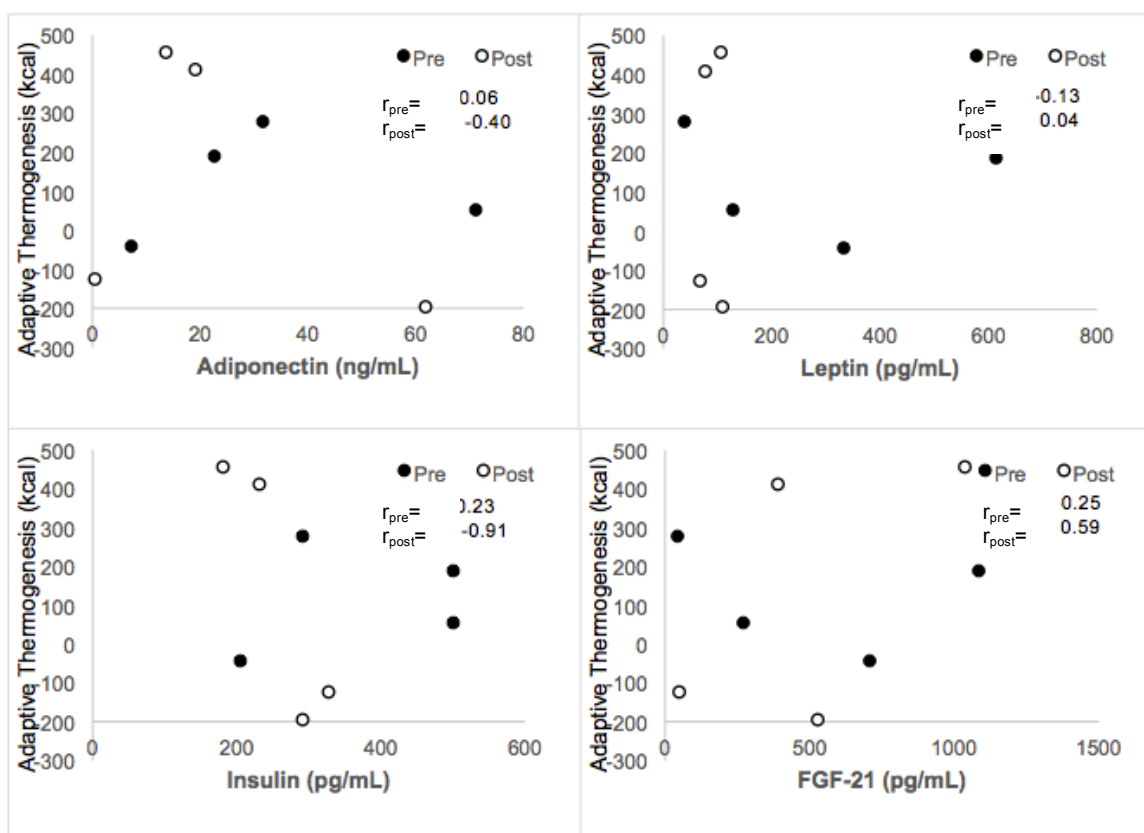


Figure 26: Correlations between adaptive thermogenesis and metabolic hormones/surrogate markers of BAT activity (Adiponectin, Leptin, Insulin, and FGF-21) in overweight/obese participants before and after participation in a three-month weight loss program

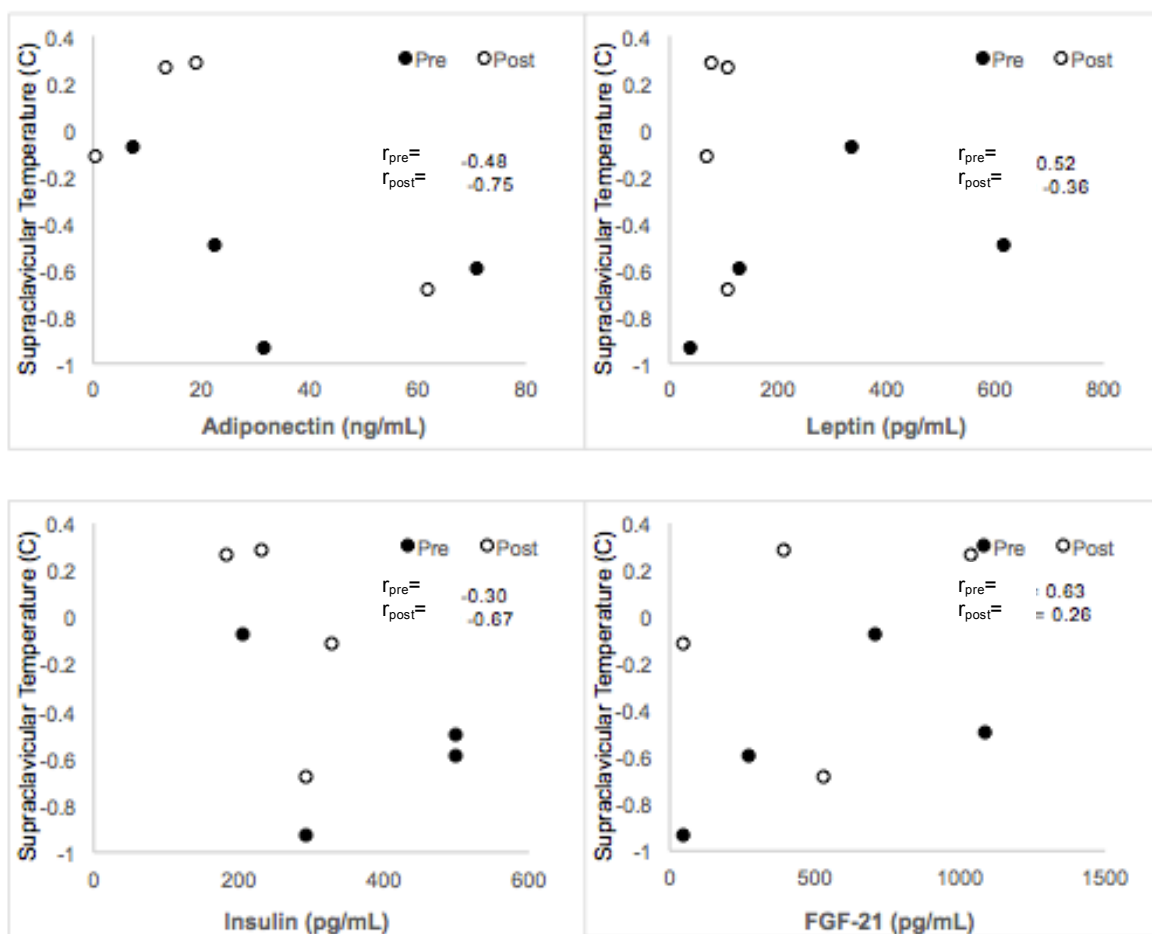


Figure 27: Correlations between cold-induced thermogenesis measured at the supraclavicular region and metabolic hormones/surrogate markers of BAT activity (Adiponectin, Leptin, Insulin, and FGF-21) in overweight/obese participants before and after participation in a three-month weight loss program

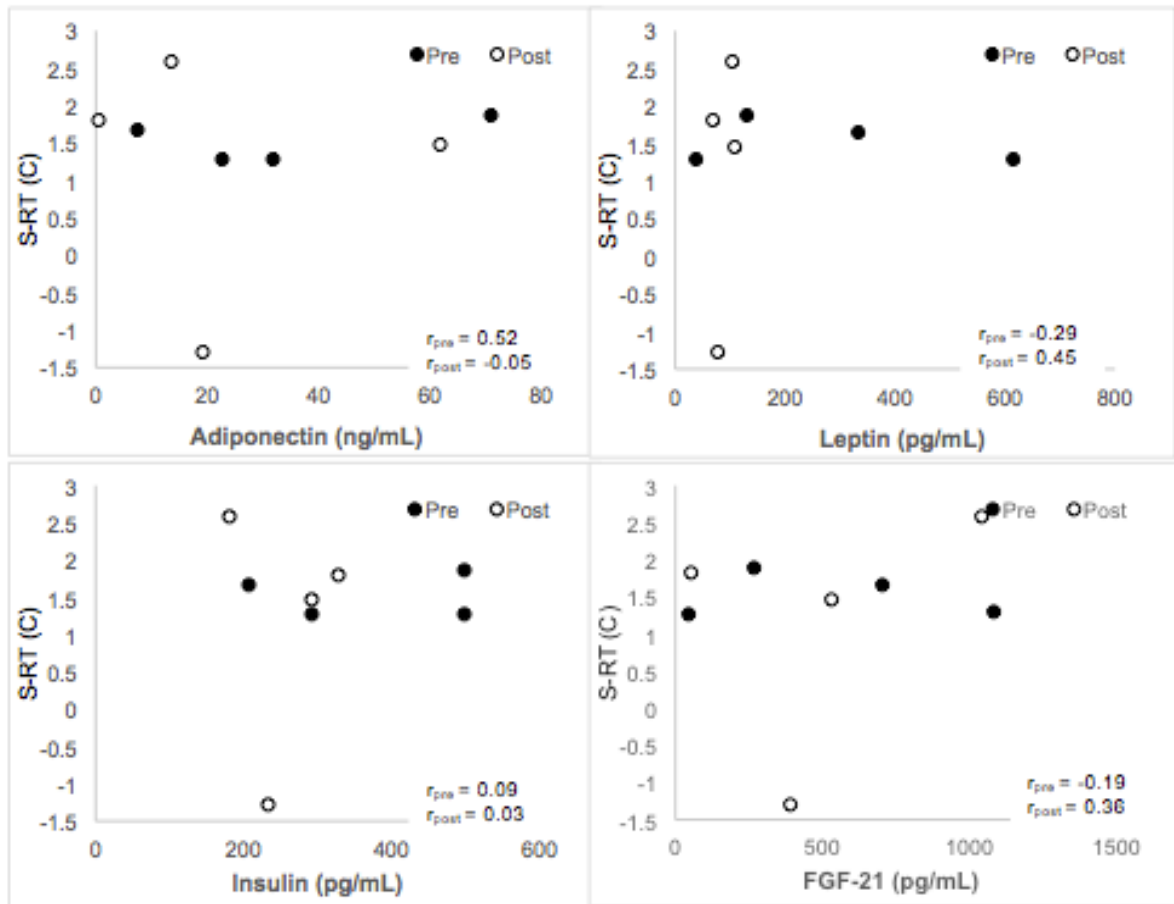


Figure 28: Correlations between cold-induced thermogenesis determined by the supraclavicular temperature (S) in relation to room temperature (RT) and metabolic hormones/surrogate markers of BAT activity (Adiponectin, Leptin, Insulin, and FGF-21) in overweight/obese participants before and after participation in a three-month weight loss program.

CHAPTER 5: DISCUSSION

The overall goal of the thesis was to evaluate the extent of adaptive thermogenesis in various states of energy balance in both overweight/obese and normal weight populations and to determine whether brown fat activity is a metabolic adaptation that occurs in response to adaptive thermogenesis. If reduction in BAT activity represented a considerable portion of adaptive thermogenesis and the suppression of metabolic rate after weight loss, strategies revolving around BAT recruitment and activation may be effective in preventing weight regain.

The *exploratory study* showed that mild cold exposure, induced by reducing the room temperature from thermoneutral (24) to slightly below thermoneutral (21C), was sufficient to cause a measurable increase in energy expenditure. The adaptive thermogenic response amounted on average to 84 kcal (a 5.4% increase when compared to RMR at 24C), illustrating an increased thermogenic response in temperatures slightly below the thermoneutral zone. This increase in resting energy expenditure associated with cold exposure has been attributed to the recruitment of brown adipose tissue (Hanssen et al., 2016).

The second purpose of the exploratory study was to determine whether the increase in RMR in response to acute mild cold exposure was associated with differences in body region surface temperatures. At 24C, there were no significant differences between before and after acclimation temperatures in all body regions and body region temperatures relative to supraclavicular temperature. At 21C, the five region of interest average was greater when acclimated than before acclimation ($p=0.015$), yet no other regions were significantly different between the non-acclimated and acclimated state. The

fact that body surface temperatures, especially in the supraclavicular region, which is the primary location of brown fat activity, did not change in response to acclimation was informative for future thermoimaging studies, in that thermoimages taken after room temperature acclimation reflect the external body temperature throughout the duration of the test.

Study 1 examined differences in markers of adaptive thermogenesis between obese/overweight individuals and normal-weight, healthy individuals. The overweight/obese participants exhibited a greater adaptive thermogenic effect, which was 220 ± 40 kcal (10.4% increase when compared to RMR at 24C) as opposed to the normal weight individuals at 89 ± 15 kcal (4.9% increase). Due to the small sample size, this difference failed to reach significance ($p=0.20$).

At 24C, supraclavicular temperature was greater in normal weight participants when compared to overweight/obese participants ($p=0.019$), but at 21C, there was a greater difference in supraclavicular temperature relative to room temperature ($p<0.001$) in overweight/obese participants when compared to normal weight participants, indicating that overweight/obese participants had greater thermogenic activity in the supraclavicular region. Overall, however, there was no statistical difference between cold-induced thermogenesis in normal weight and overweight/obese individuals.

Overweight/obese participants had significantly greater concentrations of leptin ($p=0.015$), insulin ($p=0.091$), and FGF-21 ($p=0.023$) than normal weight participants, yet adiponectin concentration did not differ significantly between the two groups ($p=0.42$). In normal weight participants, supraclavicular temperature was strongly correlated with adiponectin ($r= -0.74$), leptin ($r=0.86$), insulin ($r=0.78$), and to a lesser degree with FGF-

21 ($r=0.54$). Likewise, supraclavicular temperature relative to room temperature was significantly correlated with adiponectin ($r=-0.75$), leptin ($r=0.97$), and insulin ($r=0.95$) in normal weight participants. In overweight/obese participants, supraclavicular temperature was correlated with leptin ($r=0.59$) and FGF-21 ($r=0.68$), but there were no strong correlations in supraclavicular temperature relative to room temperature and hormonal concentrations.

Study 2 assessed the impact of short-term starvation on selected markers of adaptive thermogenesis in a small sample of healthy, young men. When calorie intake was restricted by about 60% for 5 days, body composition did not change significantly, but RMR decreased by 209 kcal/d ($p=0.002$) and RQ decreased by 0.1 ($p=0.049$). These metabolic changes were accompanied by a decrease in insulin (-23.9 pg/mL) and FGF-21 (-61.6 pg/mL), indicating that brown fat activity may decrease during a short-duration induced starvation period.

Study 3 examined the impact of diet- and exercise-induced weight loss on markers of adaptive thermogenesis in overweight/obese participants. However, it should be noted that there were no significant differences in any body composition measures from before participation in the weight loss program and after 3 months of participating. Without any significant loss of weight, we failed to see meaningful changes in metabolic markers of adaptive thermogenesis. Adaptive thermogenesis itself amounted to 225 kcal during pre-testing, but decreased to 84 kcal during post testing, but this difference was not significant ($p=0.47$). Nevertheless, the decreased ability to expend energy through heat generation coincided with a slight reduction in RMR following the weight loss program (2004 ± 27 kcal/d pre vs. 1931 ± 44 kcal/d post). This modest decrease in RMR may

reflect the persistence of metabolic adaptations post weight loss and upon beginning weight cycling.

At baseline, there was a greater difference in supraclavicular temperature relative to room temperature ($p < 0.001$) and trunk temperature ($p = 0.042$) at 21°C in overweight/obese participants. Upon completion of the weight loss program, there was also a greater difference in supraclavicular temperature relative to room temperature ($p = 0.001$) at 21°C. Supraclavicular temperature was greater at baseline at 24°C than upon completion of the weight loss program ($p = 0.047$), yet no other regions demonstrated significant differences in pre- and post- values measured at 24°C. Cold-induced thermogenesis increased substantially in the supraclavicular region ($p = 0.022$) over the course of the study, indicating that brown fat activity and recruitment may have been maintained over the course of the weight loss program, though no discernable weight was lost to contradict this finding.

Throughout the three-month weight loss program, there were no significant changes in hormone levels, nor were there any correlations between adaptive thermogenesis and adiponectin, leptin, insulin, or FGF-21 in obese/overweight participants at baseline. After completion of the weight loss program, however, adaptive thermogenesis was strongly correlated with insulin ($p = -0.91$) and FGF-21 concentrations ($p = 0.59$).

Adaptive Thermogenesis – Implications for RMR

The presence of adaptive thermogenesis can be assessed through changes in metabolic rate upon cold acclimation, changes in body surface temperatures in regions of prominent BAT activity, i.e., the supraclavicular region and its temperature in relation to room temperature, and changes in metabolic hormones and endocrine markers of BAT activity. In each of these studies, adaptive thermogenesis is present and manifested by modulations of metabolic rate in response to cold and energy deprivation in normal weight individuals, and by cold exposure in overweight/obese individuals.

In normal weight individuals in *Study 2*, the calorie restriction condition experienced a 0.15 kg increase in FFM despite a low energy availability, suggesting that high protein intake has a protective effect on lean mass in energy deficiency. Consuming large amounts of protein, defined as 1.07-1.60 g/kg BW, can prevent losses of FFM and large perturbations in body weight (Wycherley et al., 2012). Because FFM was fully preserved, the decrease in RMR throughout the condition of 210 kcal (12.3%) is fully attributable to the presence of metabolic adaptations that seek to re-equilibrate energy status by lowering energy expenditure to match the decreased energy intake, or adaptive thermogenesis.

The reduction in RMR was further accompanied by an increased reliance on fat oxidation, as demonstrated by a decrease in the RQ by 0.11. Similar metabolic adaptations were described by Van Proeyen et al in young men who participated in a 6-week endurance training program, where half trained whilst fasted and the others were provided with carbohydrate before and during the training sessions. She noted that individuals training in the fasted state were more effective at increasing muscular oxidative capacity by increasing type IIa fibers, though there was not a significant

difference between the fasted and fed groups ($p=0.07$). Further, the depletion of glycogen stores led to increased mobilization of fat stores, increased fat oxidation, and enhanced intramyocellular lipid breakdown (Van Proeyen et al., 2011). This was accompanied by a significant decrease in RQ from pre-testing to post-testing ($p<0.01$), which coincides with the observed decrease of 0.11 in the calorie restriction condition, indicating increased and improved fat oxidation as a metabolic adaptation of short-term starvation in healthy, exercising individuals.

Higher rates of fat oxidation were also seen in overweight/obese individuals as indicated by a lower respiratory quotient was (0.05) than normal weight individuals. In Schutz et al's examination of the association between body fat mass and fat oxidation, 106 weight-stable obese women and 24 moderately obese women who lost 12.7 kg of body weight and 9.8 kg of fat both displayed a significant negative association between fat mass and RQ ($r = -0.35$, $p<0.001$). The increased fat oxidation characteristic of obese individuals is partially reflected in lower RQs, indicating an increased contribution of fat as substrates for energy expenditure (Schutz et al., 1992). The observed difference in RQ between overweight/obese and normal weight participants in study 1 corroborates this evidence, attributing the lower RQ, and thus increased utilization of fat substrates in obese patients, to their 70% increase in FM relative to normal weight participants.

Though the overweight/obese participants did not lose a significant amount of weight, there was a minor decrease in RMR throughout the weight loss program (2004 ± 27 at baseline vs. 1931 ± 44 after completion, $p=0.55$). This slight decrease in metabolic rate may indicate that weight had been regained. In Fothergill et al's evaluation on the efficacy of successful weight maintenance in "The Biggest Loser" competitors, she found

that 41.0 of the 58.3 kg lost were regained and all but one regained some weight lost during the competition. From baseline to the end of the competition, RMR had decreased by 610 ± 483 kcal/d. Though a significant portion of the weight was regained, RMR six years later was still 704 ± 427 kcal/d lower than its baseline numbers, indicating that RMR suppression can persist long after weight loss has been discontinued (Fothergill et al., 2016).

The Contribution of Cold-Induced Thermogenesis to Adaptive Thermogenesis

Adaptive thermogenesis was also visualized in changes in body surface temperatures in regions of prominent BAT activity. In the exploratory study, the adaptive thermogenic response (84 kcal) was strongly correlated with indicators of cold-induced thermogenesis, including supraclavicular temperature ($r = -0.909$) and supraclavicular temperature relative to room temperature ($r = -0.644$), among other regions, in normal weight individuals. When examining the overweight/obese participants over a weight loss program, despite no substantial weight lost, there were greater differences in supraclavicular temperature relative to room temperature ($p < 0.001$) in both pre-and post-testing at 21C, which may indicate that heat production was still prominent in BAT regions, enabling increases in energy expenditure upon cold acclimation.

The increase in energy expenditure associated with cold acclimation seen in both populations has been attributed to the recruitment of brown adipose tissue. Hanssen et al measured energy expenditure in ten overweight/obese healthy men before and after a 10-day cold acclimation period. Acute mild cold exposure, as compared to thermoneutral exposure, increased energy expenditure by 10.2% ($p < 0.01$) before acclimation and by

14.0% ($p < 0.01$) after the acclimation period. Further, in the participants identified as BAT-positive, mean BAT activity increased by 26.1% ($p < 0.01$) and maximal upper-body BAT activity increased by 114.8% with cold exposure, showing that as cold exposure progressed, BAT recruitment and activity increased, increasing energy expenditure through its activation (Hanssen et al., 2016). Our observation of increased RMR with acute mild cold exposure concurred with Hanssen et al.'s finding, implicating increased BAT recruitment and/or activity as the adaptive response seen between thermoneutrality and cold exposure.

The greater adaptive thermogenic effect seen in overweight/obese participants in comparison with normal participants (Study 1), however, is in contrast to the work of Orava et al., who studied 36 obese individuals (20 individuals completed a 5-month weight loss program while 16 did not) and 27 lean subjects, comparing mean responses in BAT glucose uptake rate and blood flow in BAT in response to cold stimulation. BAT glucose uptake from cold stimulation was significantly greater and nearly double in the lean population than that of the obese population. BAT mass (24 ± 24 vs. 14 ± 29 g, $p = 0.009$) was significantly greater in lean individuals in both warm and cold environments, but BAT blood flow was only significantly greater in lean individuals when exposed to cold (Orava et al., 2013). While we observed the opposite effect, that is obese individuals with greater adaptive thermogenesis and surrogate markers of BAT activity, Orava's study indicates that the difference between RMRs should be greater at 21°C - cold activation - than at 24°C. The difference in RMR for overweight/obese participants and normal weight participants at 21°C was 449 kcal, while it was only 318 kcal at 24°C. While we measured surrogate markers of BAT activity, instead of BAT

activity itself, there is agreement on the contribution of cold-stimulation to the overall adaptive thermogenesis response.

Metabolic Markers of Adaptive Thermogenesis

Additional indicators of adaptive thermogenesis include changes in metabolic hormones, namely adiponectin, leptin, and insulin - as well as changes in endocrine markers of BAT activity, such as FGF-21. At baseline, the overweight/obese participants had significantly higher concentrations of leptin ($p=0.015$), insulin ($p=0.091$), and FGF-21 ($p=0.023$) when compared to normal weight participants. Upon completion of the weight loss program, and despite non-significant changes in body weight, leptin levels decreased by 68% over the three-month intervention in overweight/obese individuals ($p=0.12$).

The large decrease in leptin in overweight/obese participants, though statistically insignificant, indicates that weight loss may have occurred during the program, yet the participants underwent post-testing whilst in a weight regain phase. In Fothergill et al's evaluation of "The Biggest Loser" competitors, she found that leptin levels decreased by 94% of baseline values after the end of the competition. Even upon regaining most of the weight six years later (70% weight regain), leptin levels were still only 67% of its initial baseline value. This indicates that leptin repletion can last beyond weight loss and continue into weight regain (Fothergill et al., 2016).

When assessing leptin depletion in an acute fasting period in normal weight individuals, Chan et al found that 72-hour fasting suppressed leptin levels beyond that of

what is expected from change in fat mass. When leptin is fully re-administered, it does not significantly affect metabolic adaptations or changes in energy expenditure, but it decreases food intake, potentially counteracting the hyperphagic response that occurs post weight loss (Chan et al., 2017). The decrease in leptin from short-term induced starvation did not occur in Study 2, where leptin concentrations remained within 0.4 pg/mL before and after the calorie restriction condition. Despite these contradictory findings, the acute energy deficiency in Chan's study led to a 75% decrease in carbohydrate utilization in addition to a 45% increase in fat utilization. Between the beginning and end of the calorie restriction period, RQ decreased by 0.1 ($p=0.049$), indicating an increased utilization of fat, thus concurring with Chan's findings. Further, increased fat utilization and decreased leptin was accompanied by a 70% decrease in serum insulin levels in Chan's study, while Study 2 observed an 18% decrease in insulin (Chan et al., 2017). Though a substantial decrease in leptin was not seen in the short-term induced starvation period, other indices of metabolic adaptations to starvation were present, indicating adaptive thermogenesis.

FGF-21 concentrations, an endocrine marker of BAT activity, were also modulated in energy deficiency. Though insignificant due to the small sample size, FGF-21 decreased by 61.6 pg/mL after the five-day short-term induced starvation period in healthy exercising men. The large decrease in FGF-21 concentrations may be attributed to decreased brown fat activity, indicating suppressed BAT thermogenesis as a contributor of adaptive thermogenesis. In response to cold temperature, or more generally catecholamine release and sympathetic nervous system activation, FGF-21 gene transcription is induced and released into BAT. Increased concentrations of FGF-21

promote lipolysis via activation of hormone-sensitive lipase (HSL) (Hondares et al., 2011). If brown fat activity is suppressed during energy deprivation as a function and contributor of adaptive thermogenesis, consequential decreased concentrations of FGF-21 will result in decreased oxygen consumption, respiratory uncoupling, and "browning," leading to an overall decreased thermogenic capacity of brown adipose tissue (Lee et al., 2014).

In overweight/obese participants, FGF-21 concentrations actually exceeded those of normal-weight participants (Study 1). Further, there were no strong correlations between adaptive thermogenesis and FGF-21 concentrations in lean participants, but in overweight/obese participants after participation in a three-month weight loss program. Cold-induced thermogenesis, a measure of brown fat heat production assessed through supraclavicular temperature and its relation to room temperature, was strongly correlated with FGF-21 ($r=0.54$) in normal weight subjects, but was more strongly correlated ($r=0.68$) in overweight/obese subjects. These results are in contrast to previous findings, where exposure to cold was found to increase plasma FGF21 levels, resulting in significant heat production in brown fat regions in healthy normal-weight individuals, the extent of which dependent upon browning capacity of adipocytes and recruitability (Lee et al., 2014). Because BAT activity is negatively related to body fat percentage and fat mass, effective recruitment of brown adipose tissue should be more prominent in normal-weight individuals (Hanssen et al., 2016). Despite these conflicting results, increased FGF-21 levels were not the first indicator of an increased adaptive thermogenic response in the overweight/obese participants in comparison with normal-weight participants.

Limitations

Though the presence of adaptive thermogenesis was indicated through changes in metabolic rate, surface temperatures, and hormones, there were some limitations to the studies. The most notable limitation was that none of the overweight/obese participants who completed both pre- and post-testing surrounding a three-month weight loss intervention lost meaningful weight. As such, we were unable to see the effect of weight loss on metabolic markers of adaptive thermogenesis. Because we utilized a third-party weight loss program (Fit + Fueled – Campus Recreation University of Nebraska-Lincoln), we did not control diet and exercise prescriptions, nor did we monitor compliance to the weight loss intervention itself. In order to achieve desired weight loss future weight loss interventions should be administered through the lab or there should be more communication throughout the weight loss program between participant and research team to monitor progress more effectively and determine whether weight cycling may affect the results.

Although we were able to demonstrate an increase in RMR in response to mild cold exposure paradigm, there are several limitations with this specific procedure. In each of the studies, multiple RMRs were conducted on the participants. In studies 1 and 3, RMR were calculated at two different temperatures, 21C - slightly below thermoneutral - and 24C. In order to control for room temperature, participants were required come in on two different test dates. Though it was specified to consume as similar of a diet as possible on the days before each of the tests, macronutrient intake inevitably varied, and as such, measurements regarding RMR and RQ may have been skewed as a result. Further, room temperature was maintained through the usage of a

Space Heater (Ozeri OZH1 Dual Zone Ceramic Heater and Tower Fan with Adjustable Thermostat), so temperature may fluctuate around the desired value rather than remaining constant. In order to mitigate these limitations in future studies, Law et al's thermoimaging protocol with cold-water stimulation may reflect more sensitive changes in body temperature regions, control the temperature modulation more specifically, and reduce the visit and ability to quantify adaptive thermogenesis and BAT activity in one visit, eliminating the issue of confounding dietary variables. Law's method takes thermoimages continuously throughout the 25-minute study duration at 6 minute intervals. After the participant has acclimatized to the set room temperature for ten minutes, the hand is submerged in cold water (18C) for ten additional minutes. When the hand is removed, images will be taken for an additional eight minutes. Regions of interest in the supraclavicular area are isolated and analyzed with moving averages to reduce random fluctuation errors (Law et al., 2014).

Additionally, there were limitations due to small sample sizes. Because of the rigorous nature and time commitment in Study 2, only two individuals completed the two conditions to date. Likewise, several participants in Study 3 discontinued their participation due to time constraints and one participant completed all pre-testing, but moved out of state before completing post testing, drastically affecting our sample size. Furthermore, there were issues with compliance, in that participants in Study 3 would often reschedule visits, increasing the time between RMR measurements and leading to increased variability in body and meal composition between visits. For those who completed the study (n=5), we were unable to retrieve blood samples from two participants at post-testing. Though we utilize certified phlebotomists for blood

collection, there was extreme difficulty in locating the vein and obtaining any blood. To mitigate this limitation, finger-pricks as an alternative blood collection method may ensure that there is even a limited amount of blood to run assays and tests.

Due to the small sample size of each study, statistical analyses were limited by missing data, especially in the normal-weight participants. Because of errors in thermoimaging, i.e., missing regions of interest, participants were omitted from analysis. Further, participants were omitted from analysis if they did not have reliable RMR data with corresponding thermoimages at both temperature conditions. In order to mitigate this issue, there will be a stricter protocol and more comprehensive training regarding thermoimaging. Utilizing the alternative procedure outlined by Law et al could reduce error and improve the quality of images.

Conclusion:

In normal-weight and overweight/obese individuals, adaptive thermogenesis can be assessed reliably through analyzing RMR differences between measurements at 24C and 21C, examining differences in body surface temperatures in regions with heavy BAT activity, and through observing changes in metabolic hormones and surrogate markers of BAT activity. Though previous research indicates that normal weight individuals should have increased brown fat activity, assessed as a component of the adaptive thermogenesis response, overweight/obese participants displayed greater thermogenic capacity when exposed to temperatures slightly below thermoneutral than their normal weight counterparts. When both overweight/obese and normal weight participants were exposed to energy deficiency, adaptive thermogenesis was more pronounced in overweight/obese individuals. RMR increased at 21C, accompanied by a decrease in RQ, representing an increased mobilization of fats as substrates for brown fat activity and increased fat oxidation. Though we were unable to assess metabolic markers of adaptive thermogenesis in overweight and obese individuals who participated in a weight loss intervention, acute cold exposure increased metabolic rate, energy expenditure, and supraclavicular temperature – representing cold-induced thermogenesis. Serum concentrations of leptin, adiponectin, insulin, and FGF-21 were higher in overweight/obese participants in comparison with normal weight participants as well, though concentrations did not change significantly over the course of a weight loss intervention. More research is needed to confirm the relationship between adaptive thermogenesis and cold-induced thermogenesis, or the modulation of BAT activity, in larger populations. This will help elucidate the contribution of fluctuations in brown fat

activity to metabolic adaptations that occur post weight loss, allowing for recruitment and activation of BAT to potentially prevent future weight regain.

CHAPTER 6: REFERENCES

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