Hunger and Satiety in Response to Positive Energy Balance at Two Levels of Energy Flux

by

Chad M. Cook

A dissertation submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

(Nutritional Sciences)

at the

UNIVERSITY OF WISCONSIN-MADISON

2012

Date of Final Oral Examination: March 6, 2012

The dissertation is approved by the following members of the Final Oral Committee:

Dale A. Schoeller, Professor, Nutritional Sciences
Denise M. Ney, Professor, Nutritional Sciences
Chi-Liang (Eric) Yen, Assistant Professor, Nutritional Sciences
James M. Ntambi, Professor, Biochemistry & Chair of Nutritional Sciences
Plamen D. Penev, Assistant Professor, University of Chicago Medicine-Endocrinology

Abstract

Background: Obesity is by definition a product of excessive mismatch between energy intake and energy expenditure. It is not clear, however, whether the energy balance system is differentially regulated at high and low fluxes of energy. Daily exercise during short-term positive energy balance may improve objective and subjective appetite regulation in obese individuals by modulating plasma levels of hormones involved in the homeostatic regulation of dietary behavior. To test this hypothesis, this study had two primary aims. We examined the effects of exercise and overfeeding on (1) leptin and self-selected food intake, and (2) perceived hunger and peripheral hormonal concentrations at two levels of physical activity.

Methods: Thirteen sedentary individuals aged 19–39 y were recruited by BMI status (healthy BMI, n=6; obese BMI, n=7). In a randomized cross-over design, subjects were seen as in-patients in a metabolic research ward for two different six-consecutive overnight visits separated by four weeks. During one of the visits, subjects remained sedentary (SED) at a daily physical activity level (PAL) of 1.4 x RMR. During the other visit, cycling exercise (EX) was performed twice daily to increase daily PAL to 1.8 x RMR. Each SED and EX visit consisted of two consecutive treatment periods lasting three days each. Subjects were in energy balance (EB) during the first three days of each visit followed by three days of overfeeding (OVER). Daily energy expenditure and energy intake were objectively measured. On the third day of each EB and OVER period, multiple blood samples and appetite questionnaires were administered over 24h. Plasma hormones measured included leptin, ghrelin, and peptide YY; perceived appetite responses measured included hunger and fullness. The morning after each EB and OVER period an *ad-libitum* breakfast was provided as an objective measure of self-selected food intake.

Results: Aim (1): We did not find an effect of OVER on the 24 h leptin mesor when subjects were grouped by BMI status. Therefore, subjects were grouped according to HOMA status: Normal HOMA (NH; range = 1.4 to 2.3; *n*=6) and high HOMA (HH, range = 3.0 to 8.5, *n*=7). The 24 h leptin mesor in the NH group was increased in response to OVER during the SED condition (15.1±13.8%, *p*=0.02) but was unchanged during EX (3.0±5.2%, n.s.). There was no effect of OVER on the 24 h leptin mesor during the SED or EX condition in the HH group. Both groups reduced self-selected breakfast intake in response to OVER during the SED visit (NH = -229 ± 112 kcals, $p<0.01$; HH = -165 ± 157 kcals, $p<0.05$) with a trend for a reduction during EX; however, these changes in *ad-libitum* breakfast intake were not explained by relationships with changes in 24h average leptin. Aim (2): All 13 participants from the previous aim were combined and analyzed together. Observed reductions in *ad-libitum* breakfast intake in response to OVER and EX were not explained by relationships with changes in meal related perceived hunger or PYY levels. Also, beneficial changes in 24h average perceived appetite (decreased hunger/increased fullness) were found in response to OVER and EX, but these changes were not explained by relationships with 24h leptin or PYY. Neither perceived appetite nor hormones measured explained more than 5% of the variance in *ad-libitum* breakfast energy intake following OVER.

Conclusions: The results of the present study are a testament to the complexity of the regulation of human appetite as we were unable to find any association between hormonal markers of hunger and satiety and either perceived appetite or objectively measured food intake. While we cannot rule out the risk of a type II error to explain our lack of significant relationships, power was sufficient to find relationships that explained 30% of the variance. Neither 24h average leptin nor PYY explained much of the average daily perceived hunger, fullness, or feeding at a

subsequent morning meal in habitually sedentary individuals; however, more work is required to further delineate the role of leptin in the regulation of appetite response to day-to-day perturbations in energy balance or energy flux (i.e. exercise). Additionally, our subjective appetite data may suggest that increasing habitual high daily physical activity may improve perceived responsiveness to hunger and satiety cues; however, the lack of an association with PYY or leptin suggests future research should measure additional hormones in conjunction with objectively monitored *ad-libitum* food intake over 24 hour periods (instead of a single meal).

ACKNOWLEDGMENTS

First and foremost I would like to express my deepest gratitude to my mentor, Dr. Dale Schoeller, for his never-ending support and guidance while allowing me the freedom to explore opportunities and conduct interesting research. I can honestly say that if I can be half the scientist (and person) that Dale is, I will be immensely successful in the future. It has truly been a great privilege working in his lab. I would also like to acknowledge and extend my heartfelt gratitude to my dissertation committee members: Dr. Plamen Penev, Dr. Denise Ney, Dr. Eric Yen, and Dr. James Ntabmi.

I would also like to thank the members of the Schoeller lab who have helped & supported me throughout my project. I am eternally grateful to Tim Shriver for "showing me the ropes" and for his expertise in fixing anything that broke down in the lab.

A special thank you goes out to the UW-Madison hospital CRU nursing staff and Lisa Davis, the registered dietician who made each subject's diet from scratch. Many hard hours were put in by these individuals and this study would not have been completed without them.

Last but not least I wish to thank my family, especially my wife Kristen, for undivided support and encouragement. I would not have been unable to complete this project without each of you.

TABLE OF CONTENTS:

ABSTRACT……i

ACKNOWLEDGMENTS……iv

LIST OF TABLES…….....vii

LIST OF FIGURES……..ix

CHAPTER 1: Literature Review…….....1

The obesity epidemic and the regulation of body weight……...2

Central nervous system (CNS) control of hunger and satiety…….5

Hypothalamus: "The appetite center"……5

Appetite suppressing neurons……7

Appetite stimulating neurons……..9

Peripheral control of hunger and satiety……11

Long term signals

Leptin……11

Insulin…….....16

Short term meal-to-meal signals

Ghrelin……...……18

Peptide YY (PYY)……19

Disturbed energy homeostasis……...……...21

Leptin resistance ……...22

Insulin resistance ……..23

References…….24

CHAPTER 2: Study background, hypotheses, and specific aims……..39

Background……....40

Hypotheses and Aims……....46

References……....47

CHAPTER 3: 24h leptin response to short-term positive energy balance and exercise in habitually sedentary humans……..51

Abstract…….....52

Introduction…….....53

Methods…….....55

Results…….....63

Discussion…….....66

Tables…….....72

Figures…….....73

References……80

CHAPTER 4: Relationships between perceived appetite, food intake, and appetite regulating hormones in sedentary humans in response to overfeeding and exercise……87

Abstract…….....88

Introduction…….....89

Methods…….....91

Results…….....95

Discussion…….....97

Figures…….....103

References……..107

CHAPTER 5: Summary and future directions……..110

Summary……..111

Research Proposal…..……...114

References…….……...126

APPENDIX

A. Visual Analog Scale (VAS) questionnaire…….……...130

B. Diet menu……..……..131

LIST OF TABLES

Chapter 3

Table 1: Subjects baseline characteristics as grouped by homeostasis model assessment (HOMA) estimate of insulin resistance…….72

LIST OF FIGURES

Chapter 1

Figure 1: Inter-individual variability in body weight change over time…….3

Figure 2: Diagram of energy balance…..……...4

Figure 3: Anatomical localization of the arcuate nucleus and the surrounding hypothalamic nuclei in a frontal plane view of the brain…..…….6

Figure 4: Structure of leptin……12

Figure 5: Schematic of the "leptin system" regulation of body fat mass through food intake……..13

Figure 6: Leptin receptor signaling in the hypothalamus……15

Figure 7: Insulin receptor signaling in the hypothalamus……17

Figure 8: Structure of acylghrelin……19

Chapter 2

Figure 1: Potential sites of leptin resistance……..……...40

Chapter 3

Figure 1: The Deltatrac (DTC) metabolic cart indirect calorimeter system in-use…….73

Figure 2: Study protocol……..74

Figure 3: Diagram of the whole room indirect calorimeter (i.e. metabolic chamber) at the UW-Madison CRU……75

Figure 4: Homeostasis model assessment estimated insulin resistance (HOMA) during each visit…….76

Figure 5: Percent change (±SD) of the overfeeding (OVER) leptin mesor from the energy balance (EB) leptin mesor during the sedentary (SED) and exercise (EX) visit……...77

Figure 6: *Ad-libitum* breakfast energy intake……..78

Figure 7: Relationship between change in mesor leptin levels in response to overfeeding and change in *ad-libitum* breakfast food intake following overfeeding during the sedentary (SED) and exercise (EX) visits…….79

Chapter 4

Figure 1: Relationship between change in PYY measured after the ad-libitum breakfast (post-breakfast = 08:00) and change in post-breakfast hunger in response to combined overfeeding and combined exercise conditions…….....**.....**103

Figure 2: Relationship between change in PYY measured after the ad-libitum breakfast (post-breakfast = 08:00) and change in self-selected breakfast food intake in response to combined overfeeding and combined exercise conditions……...104

Figure 3: Relationship between change in fasting hunger prior to the *ad-libitum* breakfast and change in self-selected breakfast food intake in response to combined overfeeding and combined exercise conditions……...105

Figure 4: Relationship between change in the 24h weighted average of plasma leptin and subjective appetite in response to overfeeding and exercise conditions……..106

Chapter 5

Figure 1: Change in body fat mass in 55 women following a 7 week exercise program……..115

Figure 2: Baseline Testing…….120

Figure 2: Main Study Design…….120

CHAPTER 1

Literature Review

The obesity epidemic and the regulation of body weight

Approximately two thirds of the United States adult population is classified as overweight or obese (defined as a body mass index ≥25kg/m^2) (1). In addition, adults and children in developing countries are experiencing an increase in overweight and obesity (2, 3). Carrying excess weight predominantly in the form of adipose tissue increases the risk of early death from chronic diseases such as cardiovascular disease, stroke, diabetes, and certain cancers, as well as decreasing overall quality of life (4). Using population survey data from the National Health and Nutrition Examination Survey (NHANES), Wang and colleagues (5) estimated in 2008 that if current trends continue, over 80% of U.S. adults will be considered overweight or obese (BMI ≥25kg/m2) by the year 2030.

While weight change over time is variable between individuals, it is estimated that the average U.S. adult gains approximately 0.5kg of BW annually (6, 7). The dramatic rate at which the prevalence of obesity has increased over the last few decades could be interpreted as evidence that body weight is not regulated; however, evidence does exist to suggest body weight, or body fat mass, is under some biological control.

In general, rapid weight change (loss or gain) is followed by a strong defense or drive to restore body weight to a generally stable value with a slight positive trend as approximated in Figure 1. For example, the majority of humans participating in weight loss intervention trials lasting 3 to 6 months regrettably return to or exceed baseline weight following cessation of the intervention (8). Similarly, many individuals in short-term overfeeding studies ultimately self-correct by losing weight following cessation of the overfeeding intervention, but tend to stabilize at a weight slightly higher than pre-study baseline body weight (9).

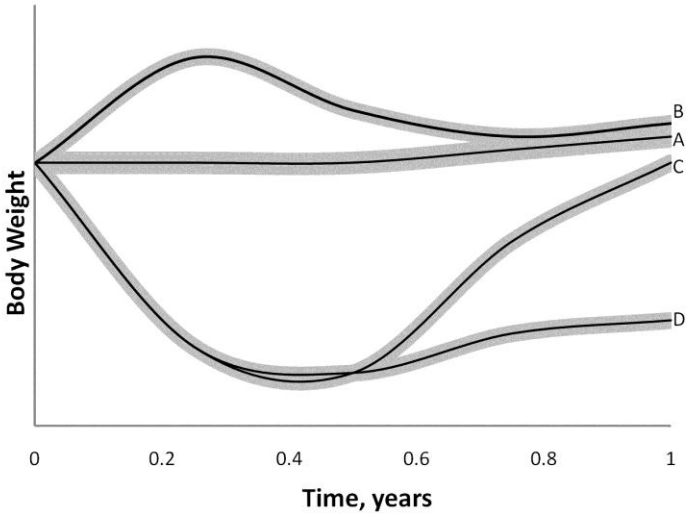

Figure 1: Inter-individual variability in body weight change over time. From Cook CM and Schoeller DA (10): (A) Hypothetical pattern of average annual body weight gain in U.S. adults inferred from population data (i.e. NHANES); (B) Hypothetical body weight gain during a short-term overfeeding intervention and subsequent weight loss during *ad-libitum* feeding resulting in body weight stabilizing at a slightly higher weight than pre-study baseline weight; (C & D) Typical variable body weight re-gain following a 3 to 6 month weight loss intervention. Black line = average body weight over time; Gray shading = day-to-day variability in body weight over time.

Indeed, human daily food intake can be highly variable and typically does not exactly match daily energy expenditure; however, when measured over several days, cumulative energy intake often closely matches total energy expenditure (11). In addition, the body weight of animals and humans in controlled over- or under-feeding trials has been shown to return to pre-intervention body weight once the stimulus (i.e. high fat diet or food restriction) is removed (12, 13). These observations, among others, have suggested a biological system exists to regulate food intake to maintain homeostatic control of body weight, albeit not at a pre-determined set point.

Body weight gain is a consequence of chronic positive energy balance, which in turn results from a combination of genetic, behavioral, and environmental factors interacting to bring about an energy imbalance via changes in both food consumption and energy expenditure (14). While a chronic positive energy balance is the thermodynamic cause of weight gain, this

physiological process may be described as a failure of homeostatic mechanisms working to match intake to expenditure and thus maintain energy balance (15). Longitudinal studies examining change in body weight while using objective measures of energy expenditure (i.e. doubly labeled water) suggest the energy intake side of the energy balance equation has a larger influence on change in body weight. Thus, understanding mechanisms impacting appetitive feelings of hunger and satiety that influence energy intake may hold importance in understanding the role of energy intake in chronic positive energy balance and weight gain (Figure 2).

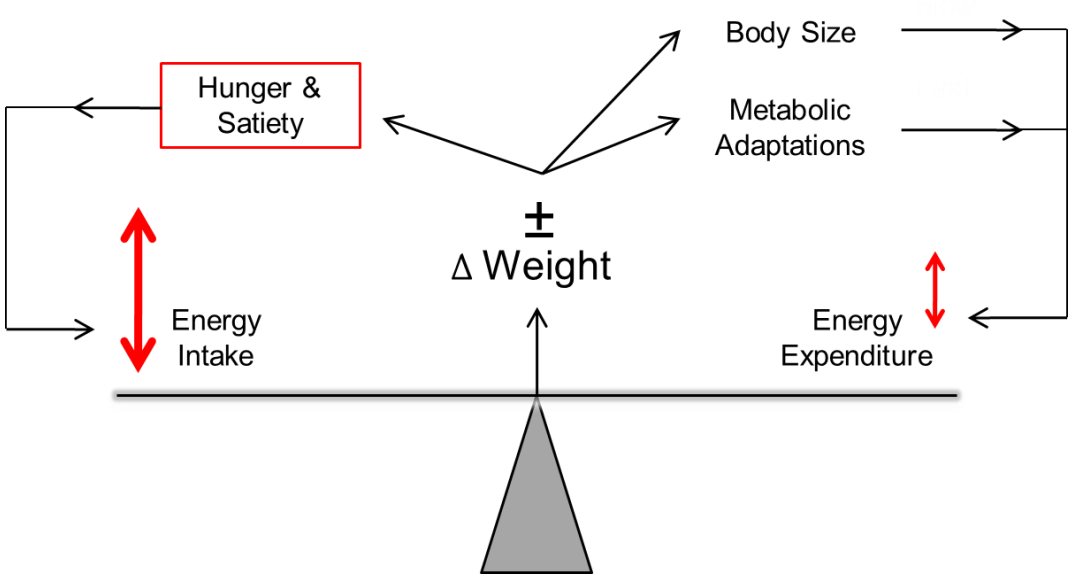

Figure 2: Diagram of energy balance. Adapted from Schoeller DA (16).

Research conducted primarily in monogenic rodent models of obesity, such as the leptin deficient *ob*/*ob* mouse (17) provided early insight into the complex neuro-hormonal processes that modulate energy homeostasis and body weight regulation. To-date, multiple gastrointestinal and adipose tissue derived hormones have been shown to act in the central nervous system in both the short and long-term regulation of food intake, energy balance, and body fat mass (18).

Central nervous system control of hunger and satiety

Hypothalamus: "The appetite center"

In humans, the hypothalamus is a roughly almond shaped collection of distinct neural cells grouped together approximately 3 to 4 millimeters thick, located on the anterior end of the third ventricle (fluid-filled cavity) of the brain connected just below the thalamus and above the pituitary gland (19). This specialized region of the brain is where multiple central and peripheral signals are integrated to maintain internal homeostasis, including the control of body temperature (20), blood pressure coupled with fluid and electrolyte balance (21), along with other functions. It is now well established that the hypothalamus also plays a critical role in the regulation of food intake.

Research conducted in the middle of the 20th century demonstrated that body weight can be altered by introducing damaging lesions or electrical stimulation to specific regions of the hypothalamus. For example, lesions in the ventromedial nucleus (VMN) were shown to cause increased food intake and excessive weight gain, while lesions in the lateral hypothalamic area (LHA) caused anorexia and weight loss (22, 23). In turn, electrical stimulation of intact VMN resulted in decreased feeding and weight loss, whereas stimulation of intact LHA increased feeding resulting in weight gain (22, 24). Taken together, these data suggested the hypothalamus was the "appetite center" of the brain, with the VMN serving as the "satiety center" and the LHA as the "feeding center" (25). Further investigations have since revealed the regulation of food intake in the hypothalamus and other brain regions involves complex neural circuits rather than specific individual hypothalamic nuclei; however, the hypothalamus is a critical first step as distinct hypothalamic neurons integrate central and peripheral signals that are linked to maintaining energy homeostasis. These neurons of the hypothalamus (Figure 3) include those of

the arcuate nucleus (ARC), which project and innervate other areas of the hypothalamus including the VMN, LHA, paraventricular nucleus (PVN), and dorsomedial nucleus (DMN) (26), which in turn project to further downstream regions of the brain to mediate feeding behavior.

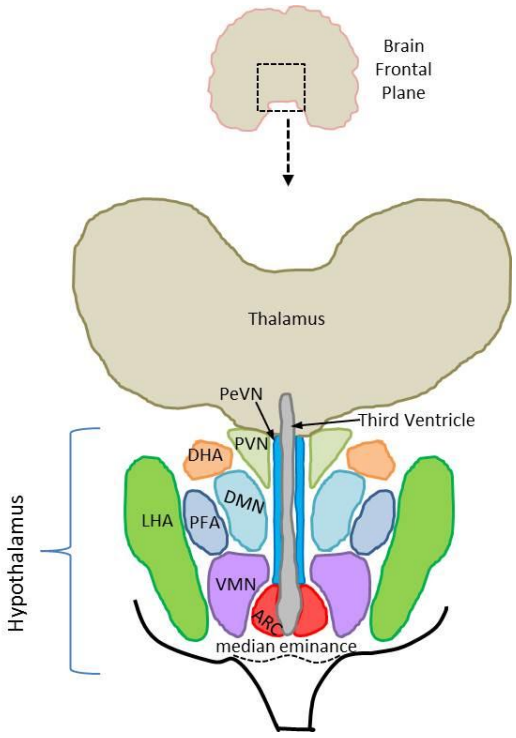

Figure 3: Anatomical location of the arcuate nucleus (ARC) and frontal section view of associated hypothalamic nuclei. DHA, dorsal hypothalamic area, DMN, dorsomedial nucleus, LHA, lateral hypothalamic area, PeVN, periventricular nucleus, PFA, parafornicular nucleus, PVN, paraventricular nucleus, VMN, ventromedial nucleus. Adapted from (27) and (28)

Located in the anterior mediobasal hypothalamus, the arcuate nucleus (ARC) is a particularly important region of the hypothalamus in the control of feeding behavior. The ARC lies immediately above the median eminence, where capillaries lack tight junctions offering a more permeable region of the blood brain barrier (29). This is important because neurons in the ARC are positioned to sense peripheral metabolic and hormonal signals (i.e. leptin, insulin, PYY,

glucose, etc…) that play a role in regulating food intake and relay this information to other brain regions to drive changes in energy intake or energy expenditure to maintain energy balance.

The ARC consists of two populations of neurons containing receptors for appetite regulating hormones, including gastrointestinal and adipose tissue derived hormones. One subset of neurons, when stimulated, express the orexigenic (stimulate food intake) neuropeptide Y (NPY) and agouti related peptide (AgRP) (30). The other group of neurons express the anorexigenic (suppress food intake) neuropeptides pro-opiomelanocortin (POMC) and cocaine and amphetamine regulated transcript (CART) (31). Thus, the ARC of the hypothalamus serves a critical function as the primary integrator of peripheral and central signals regulating food intake (32). Understanding how the hypothalamus coordinates peripheral peptide hormonal signals to control energy balance is important for understanding the development of obesity. The current view on hypothalamic control of feeding behavior, which will be reviewed in the following sections, is that food intake is regulated via a balance between orexigenic NPY/AgRP neurons and anorexigenic POMC/CART neurons, which in turn are activated or repressed by peripheral peptide hormones, such as leptin, insulin, and peptide YY (26, 33, 34).

Appetite suppressing neuropeptides

Pro-opiomelanocortin (POMC)

Evidence from gene and protein expression studies in rodents demonstrates that specific neurons within the ARC produce pro-opiomelanocortin (POMC) (35). POMC's role in regulating energy balance via food intake has been demonstrated in humans congenitally lacking *POMC* gene products (36) and in rodent models with disruption of both *POMC* alleles (37), as each display hyperphagia and become obese at an early age. In rodents, food restriction or

complete fasting reduces hypothalamic *POMC* mRNA expression (38), while re-feeding, overfeeding or exogenous leptin administration increases (39) hypothalamic *POMC* mRNA expression. POMC is technically a precursor polypeptide whose anorexigenic effects are actually mediated by smaller peptide fragments of POMC generated from extensive post-translational modification (40, 41). The resulting biologically active peptides that bring about a reduction in food intake include α-, β- and γ-melanocyte stimulating hormone (MSH), collectively known as the melanocortins.

Studies in rodents have demonstrated that α-MSH is one of the more important melanocortins in suppressing food intake. Intracerebroventricular (ICV) injection of α-MSH to *POMC* knockout (*POMC* -/-) mice markedly reduces food intake and body weight (42). The appetite suppressing effects of α-MSH are mediated via binding to specific melanocortin receptors (MCR). Five MCR's have been identified and are distributed throughout the body, but the most important receptors involved in regulating energy balance are MC3R and MC4R containing neurons within the ARC and PVN of the hypothalamus (43). Both receptors have been shown to regulate food intake, but more is currently known about MC4R. MC4R dysregulation has been studied in rodents (44) and humans (45) and is perhaps the most prevalent monogenic variant in the susceptibility of human obesity (46). A mutation in the MC4R gene, thereby rendering the receptor non-functional, or blocking α-MSH from binding functional MC4R, leads to extreme hyperphagia and obesity (44). Importantly, POMC neurons within the hypothalamus express receptors for peripheral peptide hormones like leptin, and when bound stimulates the expression of POMC with the result of inhibiting feeding. Satiety neurons of the ARC also express another peptide with anorexigenic properties, cocaine and amphetamine-regulated transcript (CART), in response to peripheral peptide hormones.

Cocaine and amphetamine regulated transcript (CART)

CART was first identified when screening rat brain areas for mRNA responsive to acute administration of cocaine or amphetamine (47). CART is particularly concentrated in regions of the hypothalamus, including the ARC, PVN, LHA, as well as in a few other regions of the brain (48, 49). CART's ability to inhibit feeding has been shown in rodent models. Chronic ICV administration of CART in lean and obese Zucker (*fa/fa*) rats has been shown to decrease food intake and body weight (50), while treatment with antibodies directed against CART reverses the CART-induced hypophagia (51). Furthermore, overexpression of CART in the rat PVN has been shown to result in significantly increased cumulative food intake and body weight gain over time in adult male rats compared with control animals regardless of being fed either a normal chow or high fat diet (52). Mice lacking the CART gene (*CARTPT* -/-) are typically hyperphagic and have a higher body weight than wild type mice (53) when fed either a regular diet or a high fat diet, although results from these genetic knockout models have been mixed (54). CART expression is regulated in-part by peripheral peptide hormones, including leptin, as leptin receptors have been found on CART peptide containing neurons in the ARC and other hypothalamic regions (55) and CART mRNA levels in the rat ARC are increased by administration of leptin (56).

Appetite stimulating neuropeptides

Neuropeptide Y (NPY)

NPY is a 36 amino acid peptide discovered in the early 1980's (57, 58) and has been shown to stimulate feeding. NPY belongs to the pancreatic polypeptide (PP) family containing a

characteristic hairpin tertiary structure called the PP-fold (59). NPY neurons are mostly restricted to the ARC of the hypothalamus (60, 61), with low levels of expression within the DMH of the hypothalamus and the brainstem (62). ARC NPY neurons have receptors for peripheral peptide hormones, including leptin, and have extensive efferent projections to numerous hypothalamic and other brain regions, which coordinately play an important role in the regulation of food intake and energy balance (63). ARC NPY mRNA, along with NPY protein released, are increased with fasting and negative energy balance and decrease after feeding (64). Similar to studies using direct ICV injection of POMC or CART peptides to suppress food intake, ICV injection of NPY into the PVN results in hyperphagia and obesity (65).

Changes in food intake are likely controlled by receptors for NPY, of which five subtypes have been identified: NPY1-5 (66). The Y1 and Y5 receptors are thought to be heavily involved in the regulation of food intake. Y1 receptor agonists stimulate hyperphagia in rats, whereas antagonists significantly reduce food intake in the presence of ICV administered NPY or food deprivation (67). Similarly, antagonists to Y5 receptors reduce food intake in rodents and Y5 receptor knockout mice become hyperphagic and obese (68, 69). Thus, it has been demonstrated that NPY is a potent stimulator of food intake and neurons of the ARC that express NPY are regulated, in-part, by peripheral peptide hormones like leptin.

Agouti Related Peptide (AgRP)

Previously, it was shown that the melanocortins (specifically α-MSH), are potent endogenous ligands for melanocortin receptors functioning to reduce food intake and/or increase energy expenditure to regulate energy balance. Conversely, agouti-related protein (AgRP), which is expressed in the medial part of the ARC (70), acts as the main natural endogenous

peptide directly antagonizing the action of a-MSH by competing for binding on MC3R and MC4R thereby inhibiting receptor activation (34). In mice, overexpression of AgRP leads to hyperphagia and obesity (71), while targeted destruction of AgRP neurons leads to lowered body weight and anorexia. The appetite stimulating effects of AgRP are inhibited by the hormone leptin and activated by the hormone ghrelin.

Summary of the CNS role in regulating food intake

The identification of neuropeptides that control food intake and possibly energy expenditure in animals suggests a biological system exists that regulates homeostatic control of body weight. Indeed, metabolic signals that monitor energetic state, including metabolites and hormones like leptin and insulin, target the ARC and other regions of the hypothalamus to modulate the activity of NPY/AgRP and POMC/CART neurons, whose downstream effects stimulate feelings of hunger or fullness to modulate food intake.

Peripheral control of hunger and satiety

Long term (e.g. day to day) appetite regulation: Leptin and Insulin

Leptin

The existence of a signal derived from adipose tissue involved in energy homeostasis was first supported by parabiosis experiments in mice (surgically creating conjoined twins to share a common blood supply) conducted in the 1970's. These studies demonstrated that genetically obese (*ob/ob)* mice lacked a circulating factor that inhibited feeding, whereas obese mice with a different gene mutation (*db/db*) were resistant to this factor (72, 73), eventually identified as leptin in 1994 (17). The discovery of leptin was at the forefront of a new research paradigm

focusing on adipose tissue as an active endocrine organ that has a role in the development of obesity. Within this paradigm leptin acts as a "lipostat;" an afferent signal traveling to the brain communicating the size of fat stores in the periphery.

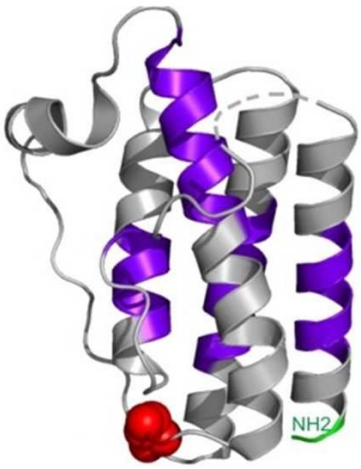

Figure 4: Structure of Leptin (74)

Leptin (figure 4) is a 167 amino acid protein product of the *ob* gene (17). Production has been shown to be stimulated by both insulin and glucocorticoids and inhibited by fasting/caloric restriction (75). Leptin circulates in the plasma in a circadian rhythm at concentrations closely related to body fat mass (76), although females have up to twice as much plasma leptin as males given the same amount of body fat (77). Plasma leptin is entrained to meal timing (78) as concentrations reach a nadir (lowest level) in the morning between 08:00 and 12:00 hours and rise throughout the afternoon and evening to hit a zenith (highest level) between 24:00 and 04:00 hours. Leptin is secreted primarily by adipocytes of white adipose tissue, but has also been identified in other tissues including the stomach (79), hypothalamus and pituitary gland (80), and reproductive tissues (81).

While leptin is involved in many physiological processes, including effects on reproduction and immunity, the most well described function is the regulation of food intake.

Importantly, leptin receptors are expressed by key hypothalamic neurons involved in food intake and energy homeostasis. Lack of leptin production (i.e. *ob/ob* mice) or lack of the CNS leptin receptor (i.e. *db/db* mice) in rodents or humans, while rare, causes extreme hyperphagia and obesity. Peripheral administration of leptin in the *ob/ob* condition normalizes food intake and body weight. Leptin transport across the blood-brain barrier occurs by a saturable receptor-mediated process [12] and leptin concentrations in human cerebrospinal fluid directly correlate with plasma concentrations [161]. Taken together, this information led to a general hypothesis that the 'leptin system' exists to maintain body fat within a narrow range in healthy humans and other animals. In general, an increase in body fat or acute overfeeding (i.e. 24-72 hours) in healthy individuals is associated with an increase in plasma leptin levels and subsequent reduction in food intake (Figure 5). In contrast, decreasing body fat or acute fasting/caloric restriction is associated with a decrease in plasma leptin concentrations, which in-turn stimulates food intake.

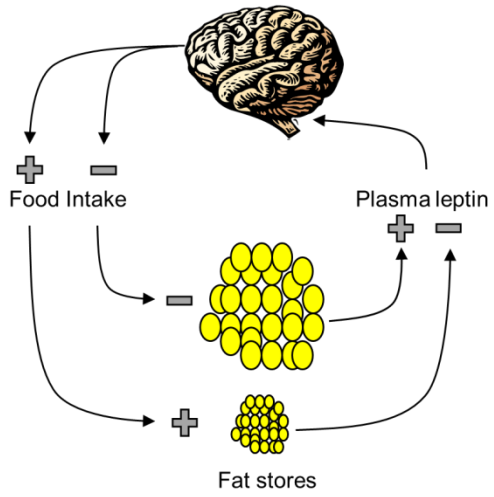

Figure 5: Schematic of the "leptin system" regulation of body fat mass through food intake in healthy humans. Leptin circulates in proportion to body fat stores and short-term changes in energy balance, and ultimately has effects on important hypothalamic neuronal pathways that mediate corresponding changes in food intake and ultimately changes in body fat mass. In the proposed leptin system model, energy restriction or weight loss decrease plasma leptin concentrations and increase food intake, whereas overfeeding or weight gain increase plasma leptin and decrease subsequent food intake.

There are five different isoforms of the leptin receptor (OB-R) and all share identical extracellular ligand-binding domains but differ at the C terminus due to alternative mRNA splicing (82, 83). These isoforms are classified into three groups; short, long, and secreted. While leptin receptors have been identified in the CNS and in many peripheral tissues, studies in genetically modified mice have demonstrated that leptin action in the brain mediated by the long form (OB-Rb) of the receptor is sufficient to regulate body weight, feeding, energy expenditure, and glucose metabolism (84-86). OB-Rb is the specific receptor responsible for leptin action on energy homeostasis, as demonstrated in *db/db* mice that lack functional OB-Rb and exhibit extreme hyperphagia, obesity, and diabetes (87). In addition, the Zucker fatty rat (*fa/fa*) has a mutation in OB-Rb that disrupts normal leptin mediated signal transduction pathways (88) resulting in hyperphagia and obesity.

Ob-Rb is present in the ARC, VMH, DMN, and LHA nuclei (89). Of the five leptin receptors, only Ob-Rb (a type 1 cytokine receptor) encodes all protein motifs capable of activating the Janus Kinase/Signal Transduction and Activator of Transcription (JAK-STAT) pathway. In this pathway (figure 6), STAT3 molecules are activated upon leptin binding, which leads to the transcription of genes associated with the regulation of energy homeostasis (90). In addition to STAT3 dependent effects of leptin, at least some of the hypothalamic effects of leptin appear to be mediated by phosphoinositide-3 kinase (PI3K) signaling, as the PI3K pathway is involved in the regulation of gene transcription and may induce rapid non-genomic events affecting neuronal activity and neuropeptide release (91).

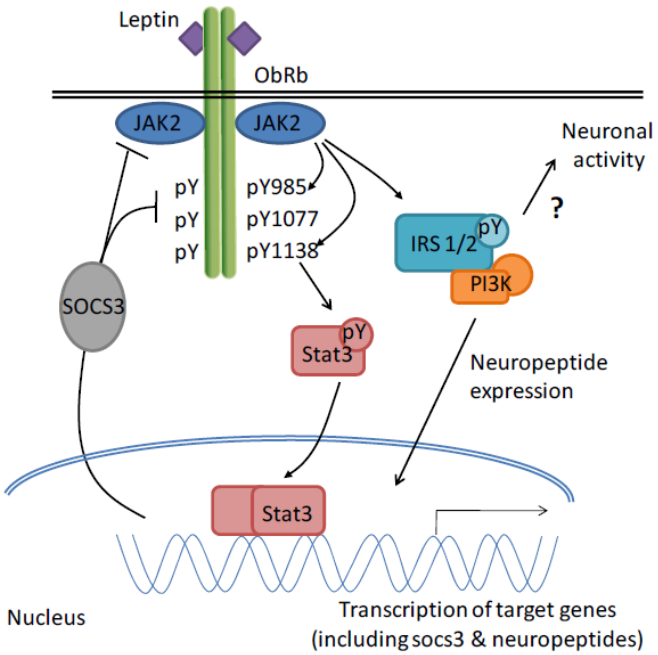

Figure 6: Leptin receptor signaling in the hypothalamus. Leptin binding to the long form of the leptin receptor (Ob-Rb) activates the intracellular tyrosine kinase Jak2, resulting in Signal Transducer and Activator of Transcription-3 (STAT3) phosphorylation. Once phosphorylated, STAT3 molecules induce transcription of several target genes encoding neuropeptides and Suppressor of Cytokine Signaling (SOCS-3). SOCS-3 expression leads to feedback inhibition of the leptin receptor signaling pathway. Phosphatidylinositol-3 Kinase (PI3K) pathway is also involved in the regulation of gene transcription and may potentially induce non-genomic events affecting neuronal activity and neuropeptide release. Adapted from Bjorbaek *et al.* (91).

In the ARC, both the appetite stimulating NPY/AgRP neurons and appetite suppressing CART/POMC neurons express OB-Rb (92). As such, leptin binding stimulates the aforementioned JAK/STAT signaling pathway, which results in a downregulation in expression of the orexigenic neuropeptides NPY/AgRP, thereby suppressing food intake behavior. Leptin signaling through Ob-Rb in the hypothalamus activates the appetite suppressing CART/POMC neurons leading to an increase in POMC synthesis and neuropeptide release [48, 182]. POMC neurons, which ultimately produce the anorectic α-MSH as a product of POMC protein cleavage, are believed to be key mediators of leptin appetite suppressing action (32). In the absence of leptin, NPY/AgRP neurons inhibit catabolic POMC neurons via synapses with these neurons in

the ARC. Thus, leptin binding NPY/AgRP neurons (i.e. inhibition) results in a disinhibition of POMC neurons to further mediate reduced food intake. Leptin has also been shown to impact energy expenditure in rodents, with an increase or decrease in energy expenditure mediated by an increase or decrease in sympathetic nervous system activity (93). Taken together, the leptin response in target hypothalamic neurons appears to be coordinately regulated to maintain energy homeostasis via regulation of neuropeptide gene expression as well as neuronal activity and neuropeptide release.

Insulin

Insulin is produced and secreted from pancreatic β-cells within the islets of Langerhans. Apart from the well-known function in glucose metabolism and the stimulation/suppression of anabolic/catabolic pathways in insulin sensitive tissues (liver, adipose, skeletal muscle, etc...), insulin has a similar role to leptin as an adiposity signal in the brain. Consistent with insulin's role as an adiposity signal, plasma levels are positively correlated with body fat mass; insulin is transported across the BBB (94) and binds receptors distributed in the brain, particularly in the ARC of the hypothalamus (95), and; insulin or insulin mimetics (insulin-like regulation in the absence of insulin) administered ICV into the third ventricle of rodent brains leads to decreased food intake and body weight in a dose dependent manner (96, 97). The anorectic effect of insulin is mediated through insulin receptor substrate-2 (IRS-2) mediate insulin signaling, as demonstrated in IRS-2 knockout mice (98). Intracellular insulin signaling has been studied in classical insulin target tissues, such as skeletal muscle and liver, and because insulin signaling is similar in neuronal cells it is briefly reviewed in figure 7.

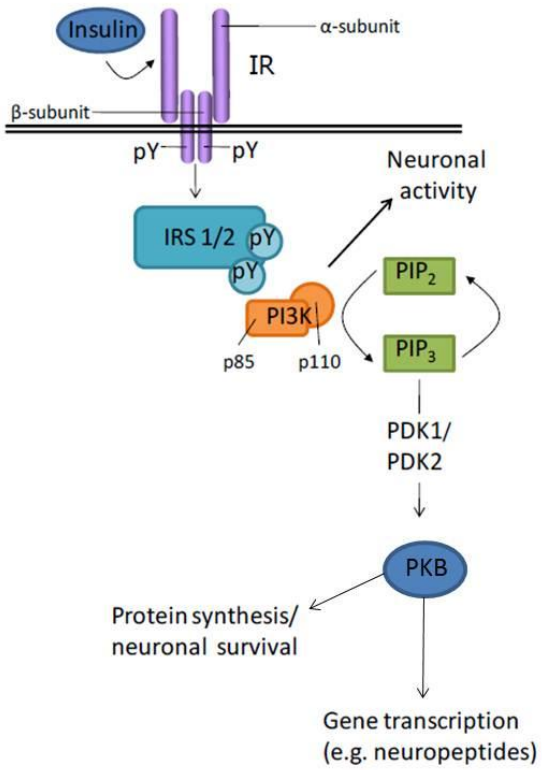

Figure 7: Insulin receptor signaling in the hypothalamus. Insulin binding to the insulin receptor (IR) results in receptor auto-phosphorylation and subsequent phosphorylation of intracellular insulin receptor substrate (IRS) proteins ultimately initiating downstream signaling via serine/threonine kinases (i.e. PDK and PKB). These signals result in the diverse biological effects of insulin signaling in the brain including regulation of gene transcription. Like leptin, insulin activation of PI3K signaling pathways in hypothalamic neurons appears to alter neuronal activity and neuropeptide release. Figure adapted from Plum *et al.* (99).

As with leptin, the energy homeostatic effects of insulin in the CNS are mediated by intracellular insulin signaling pathways that facilitate neuropeptide gene expression as well as neuronal activity and neuropeptide release. In some rodent models of obesity, NPY/AgRP neurons are inhibited by insulin and this inhibition is thought to drive some of the anorectic actions of insulin (100). For example, insulin signaling via PI3K has been shown to reduce the firing rate and corresponding release of NPY in NPY/AgRP neurons (101). In addition, central administration of insulin reduces the expression of the orexigenic NPY gene in the ARC (100). Conversely, POMC neurons are activated by insulin resulting in an increase in POMC gene

expression and the anorexigenic peptide α-MSH (102). Up-regulation of α-MSH appears to mediate at least some of the anorexigenic effects of insulin since administration of a melanocortin antagonist prevents insulin-induced reduction in food intake in these studies (102). Interestingly, central insulin administration reduces weight to a greater extent than can be accounted for by reduced caloric intake alone, suggesting a role for insulin in the regulation of energy expenditure, at least in rodent models. Insulin, like leptin, induces a spectrum of responses to regulate energy homeostasis and adipose tissue mass.

Short term meal-to-meal signals: Ghrelin and Peptide YY

Even though there are numerous short-term signals having a role in regulating food intake, including circulating metabolites like glucose and several hormones secreted by the gastrointestinal tract, this portion of the literature review will focus on two well described gut peptides known to be involved in the regulation of hunger and satiety: Ghrelin and Peptide YY (PYY).

Ghrelin

Ghrelin is a 28 amino acid peptide (Figure 8) secreted by enteroendocrine cells in the fundus region of the stomach, and is the only known endogenous hormone that stimulates food intake (103). Initial evidence for ghrelin's role in control of appetite came from the observation that mice lacking either ghrelin or its receptor (GSHR) are protected from diet-induced obesity. Plasma levels are closely related to meal patterns, rising before meals and falling within one hour after eating, suggesting involvement in pre-meal hunger sensations and meal initiation (104). Blood ghrelin levels are typically highest in the fasting state and fasting ghrelin levels have been shown to be decreased in human obesity (105), which may reflect an adaptive response to weight

gain. Acute ghrelin injections potently stimulate appetite and food intake in multiple species, including humans, and chronic administration increases body weight by stimulating food intake but also by decreasing energy expenditure and fat catabolism, at least in rodents (103, 106). These observations suggest that ghrelin may serve as a signal to the brain of nutrient excess or depletion.

Acylghrelin, the biologically active form, has a medium chain fatty acid on Ser-3 (octanoylation) through the action of a membrane bound enzyme called ghrelin O-acyltransferase (GOAT) (107). Octanoylation is required for binding to the ghrelin receptor (GHSR) and stimulating food intake. Rather than being transported to the hypothalamus across the blood brain barrier like leptin and insulin, ghrelin produced in the stomach may modulate appetite behavior through interaction with GHSR present on afferent neurons of the vagus nerve. This in turn results in the local release of ghrelin in the ARC of the hypothalamus and stimulates appetite by increasing the activity of the orexigenic neurons NPY/AgRP neuron (108).

Figure 7: Structure of acylghrelin. Adapted from (108).

Peptide YY (PYY)

PYY is a 36 amino acid peptide secreted from L cells of the ileum (distal part of the small intestine) and in the colon (large intestine) in response to nutrients and plays a role in reducing energy intake by inducing feelings of satiety. The major secreted form contains 36 amino acids (PYY_{1-36}), but there also is a truncated form (PYY_{3-36}) produced by the cleavage of two amino

acids at the NH2 terminus that is thought to be the active form functioning as a satiety factor in the hypothalamus (109). Both forms have been shown to decrease food intake when infused peripherally; however, PYY_{3-36} seems to be more potent. PYY modulates a reduction in food intake by two mechanisms: 1) Decreasing motility and secretory activity of the stomach and small intestine ("ileal brake"), and 2) a central effect on appetite within the hypothalamus via the inhibition of NPY. Importantly, PYY acts directly in the ARC hypothalamic circuits that regulate energy homeostasis, but additional work has implicated brainstem and vagal inputs (110).

PYY plasma concentrations are lowest in the fasting state and increase in response to food intake. The post-prandial increase in PYY is significantly associated with increased feelings of satiety (111). Peripheral administration of PYY_{3-36} to rodents, primates, and humans acutely decreases food intake (112, 113), while chronic administration reduces adiposity in rodents (113). PYY knockout mice are hyperphagic and develop marked obesity but are sensitive to exogenous PYY and chronic treatment reverses the obese phenotype. It has been reported that obese adults have lower baseline and post-meal peptide YY concentrations and exhibit lower satiety compared with lean controls (114), raising the possibility that decreased peptide YY concentrations could play a role in the development and/or maintenance of obesity. Results from a recent randomized crossover study showed that acute exercise significantly increased postprandial levels of PYY (as well as glucagon-like peptide 1 and pancreatic polypeptide) in normal weight adults (115). This suggests that acute exercise can trigger physiological changes in concentrations of hormones secreted from the gastrointestinal tract which may help explain the short-term suppression of hunger or, "exercise induced anorexia,"

observed after exercise. These findings provide compelling evidence that PYY is a physiologically relevant regulator of food intake and body weight.

Disrupted energy homeostasis: Cause or consequence of obesity?

Despite the evidence supporting a role for the hypothalamus in the regulation of energy homeostasis, the prevalence of obesity in the United States is increasing at an alarming rate. While the mechanisms involved in the development of obesity remain to be fully elucidated, the obese state has been well studied. Obese individuals have markedly increased plasma insulin and leptin concentrations, reflecting an increase in total body fat mass, yet food intake and energy expenditure are not appropriately regulated as predicted from a homeostatic feedback loop. This suggests that the homeostatic effects of insulin and leptin are impaired at one or more levels.

Leptin Resistance

The idea that obese humans may be resistant to the appetite suppressing and weight reducing effects of leptin was first indicated by the finding of elevated, rather than reduced, plasma leptin concentrations in obese humans compared to normal weight peers (116). Leptin resistance may occur at multiple levels. Obese individuals have higher plasma-to-cerebrospinal fluid (CSF) ratios of leptin in comparison to healthy weight controls, suggesting a potential source of leptin resistance is due to impaired leptin transport across the blood brain barrier (117). A rodent model exits suggesting impaired leptin transport into the CNS; the New Zealand Obese (NZO) mouse does not respond to peripheral leptin injections but is responsive to central leptin administered ICV (118); however, resistance to the food consumption lowering effects of

centrally administered leptin is observed in other genetically obese rodent models as well as in high fat diet-induced obese rodents (119) suggesting another possible site of leptin resistance may occur as a result of reduced leptin signaling in the brain. Specifically, the ability of leptin to activate the downstream signaling molecule, STAT3, in hypothalamic neurons is reduced in mice with diet induced obesity (120) suggesting a defect in central leptin signaling. A final possibility is that leptin resistance may reflect that it is a relatively weak satiety system, as some investigators (reference) have suggested the role of leptin is to prevent starvation rather than to avoid obesity. For example, mice that are put on a high-fat diet to promote diet induced obesity begin with a normal functioning leptin system. The diet, rather than alterations of the leptin system *per se*, trigger the increased energy intake, as the rewarding properties of highly palatable foods may override negative feedback signals to positive energy balance; however, the developing feedback inhibition/cellular leptin resistance likely exacerbates and stabilizes the ensuing increase in body weight (121).

Insulin Resistance

Like leptin, most obese mammals have elevated plasma concentrations of insulin yet exhibit inappropriate levels of food intake and energy expenditure for the level of insulin. As with leptin, similar mechanisms may underlie insulin resistance in obesity. CNS uptake of insulin from the plasma appears to be reduced in obese Zucker rats (122) and high-fat fed dogs (123), suggesting that insulin transport is a site of resistance. Previous work has demonstrated resistance to the food consumption lowering effects of insulin when administered ICV into the brain of rats fed a high-fat diet (124). Therefore, inadequate transport does not fully explain the CNS insulin resistance seen in obesity. Inactivation of insulin signaling via serine

phosphorylation of IRS proteins is a common feature of peripheral insulin resistance (125), which may also be implicated in the hypothalamus of high-fat diet-induced obese rats. In addition, activation of PI3K signaling in hypothalamic neurons has been shown to be required for the ability of centrally administered insulin to reduce food intake and hyperpolarize NPY/AgRP neurons (126). Thus, impaired signal transduction is a potential mechanism of central insulin resistance in diet-induced obesity.

Obesity and physical inactivity are associated with peripheral insulin resistance, and insulin is known to affect plasma leptin levels. Studies have shown that insulin increases both leptin gene expression (127) and circulating leptin concentrations (128), while in other experiments insulin appeared to increase leptin secretion from both human and rodent isolated adipocytes (129, 130). Indeed, insulin-stimulated glucose metabolism in adipocytes may be a key factor linking leptin secretion to body fat mass (131). It should be noted that contradictory results have also been reported, as some studies found increased leptin secretion only after prolonged insulin treatment of isolated human or rat adipocytes or cultured 3T3-L1 adipocytes (75); however, it is possible that impaired insulin-induced leptin production in obese insulin-resistant individuals may contribute to the development or worsening of obesity (128).

References

1. Flegal KM, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999-2008. JAMA : the journal of the American Medical Association 2010;303(3):235-41.
2. Cattaneo A, Monasta L, Stamatakis E, et al. Overweight and obesity in infants and pre-school children in the European Union: a review of existing data. Obesity reviews : an official journal of the International Association for the Study of Obesity 2010;11(5):389-98.
3. Ng SW, Zaghloul S, Ali HI, Harrison G, Popkin BM. The prevalence and trends of overweight, obesity and nutrition-related non-communicable diseases in the Arabian Gulf States. Obesity reviews : an official journal of the International Association for the Study of Obesity 2011;12(1):1-13.
4. Malnick SD, Knobler H. The medical complications of obesity. QJM : monthly journal of the Association of Physicians 2006;99(9):565-79.
5. Wang Y, Beydoun MA, Liang L, Caballero B, Kumanyika SK. Will all Americans become overweight or obese? estimating the progression and cost of the US obesity epidemic. Obesity (Silver Spring) 2008;16(10):2323-30.
6. Hill JO, Wyatt HR, Reed GW, Peters JC. Obesity and the environment: where do we go from here? Science 2003;299(5608):853-5.
7. Flegal KM, Troiano RP. Changes in the distribution of body mass index of adults and children in the US population. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 2000;24(7):807-18.

8. Jakicic JM, Marcus BH, Lang W, Janney C. Effect of exercise on 24-month weight loss maintenance in overweight women. Arch Intern Med 2008;168(14):1550-9; discussion 9-60.

9. Levitsky DA, Obarzanek E, Mrdjenovic G, Strupp BJ. Imprecise control of energy intake: absence of a reduction in food intake following overfeeding in young adults. Physiology & behavior 2005;84(5):669-75.

10. Cook CM, Schoeller DA. Physical activity and weight control: conflicting findings. Current opinion in clinical nutrition and metabolic care 2011;14(5):419-24.

11. Edholm OG. Energy balance in man studies carried out by the Division of Human Physiology, National Institute for Medical Research. Journal of human nutrition 1977;31(6):413-31.

12. Pasquet P, Apfelbaum M. Recovery of initial body weight and composition after long-term massive overfeeding in men. The American journal of clinical nutrition 1994;60(6):861-3.

13. Heyman MB, Young VR, Fuss P, Tsay R, Joseph L, Roberts SB. Underfeeding and body weight regulation in normal-weight young men. Am J Physiol 1992;263(2 Pt 2):R250-7.

14. Schoeller DA. Insights into energy balance from doubly labeled water. Int J Obes (Lond) 2008;32 Suppl 7:S72-5.

15. Flatt JP. How NOT to approach the obesity problem. Obes Res 1997;5(6):632-3.

16. Schoeller DA. The energy balance equation: looking back and looking forward are two very different views. Nutrition reviews 2009;67(5):249-54.

17. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994;372(6505):425-32.

18. Coll AP, Farooqi IS, O'Rahilly S. The hormonal control of food intake. Cell 2007;129(2):251-62.

19. Delcomyn F. Foundations of Neurobiology. New York: W. H. Freeman and Company, 1998.

20. Hammel HT, Jackson DC, Stolwijk JA, Hardy JD, Stromme SB. Temperature Regulation by Hypothalamic Proportional Control with an Adjustable Set Point. J Appl Physiol 1963;18:1146-54.

21. Denton DA, McKinley MJ, Weisinger RS. Hypothalamic integration of body fluid regulation. Proceedings of the National Academy of Sciences of the United States of America 1996;93(14):7397-404.

22. Kennedy GC. The hypothalamic control of food intake in rats. Proc R Soc Lond B Biol Sci 1950;137(889):535-49.

23. Hetherington AW, Ranson SW. The spontaneous activity and food intake of rats with hypothalamic lesions. Am J Physiol 1942;136:609-17.

24. Valenstein ES, Cox VC, Kakolewski JW. Modification of motivated behavior elicited by electrical stimulation of the hypothalamus. Science 1968;159(819):1119-21.

25. Elmquist JK, Elias CF, Saper CB. From lesions to leptin: hypothalamic control of food intake and body weight. Neuron 1999;22(2):221-32.

26. Mercer RE, Chee MJ, Colmers WF. The role of NPY in hypothalamic mediated food intake. Frontiers in neuroendocrinology 2011;32(4):398-415.

27. Peyron C, Faraco J, Rogers W, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nature medicine 2000;6(9):991-7.

28. Schwartz MW, Woods SC, Porte D, Jr., Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature 2000;404(6778):661-71.

29. Norsted E, Gomuc B, Meister B. Protein components of the blood-brain barrier (BBB) in the mediobasal hypothalamus. Journal of chemical neuroanatomy 2008;36(2):107-21.

30. Broberger C, Johansen J, Johansson C, Schalling M, Hokfelt T. The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. Proceedings of the National Academy of Sciences of the United States of America 1998;95(25):15043-8.

31. Kristensen P, Judge ME, Thim L, et al. Hypothalamic CART is a new anorectic peptide regulated by leptin. Nature 1998;393(6680):72-6.

32. Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. Nature 2006;443(7109):289-95.

33. Meister B. Neurotransmitters in key neurons of the hypothalamus that regulate feeding behavior and body weight. Physiology & behavior 2007;92(1-2):263-71.

34. De Jonghe BC, Hayes MR, Bence KK. Melanocortin control of energy balance: evidence from rodent models. Cellular and molecular life sciences : CMLS 2011;68(15):2569-88.

35. Dhillo WS, Small CJ, Stanley SA, et al. Hypothalamic interactions between neuropeptide Y, agouti-related protein, cocaine- and amphetamine-regulated transcript and alpha-melanocyte-stimulating hormone in vitro in male rats. Journal of neuroendocrinology 2002;14(9):725-30.

36. Krude H, Biebermann H, Luck W, Horn R, Brabant G, Gruters A. Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. Nature genetics 1998;19(2):155-7.

37. Yaswen L, Diehl N, Brennan MB, Hochgeschwender U. Obesity in the mouse model of pro-opiomelanocortin deficiency responds to peripheral melanocortin. Nature medicine 1999;5(9):1066-70.

38. Mizuno TM, Kleopoulos SP, Bergen HT, Roberts JL, Priest CA, Mobbs CV. Hypothalamic pro-opiomelanocortin mRNA is reduced by fasting and [corrected] in ob/ob and db/db mice, but is stimulated by leptin. Diabetes 1998;47(2):294-7.

39. Hagan MM, Rushing PA, Schwartz MW, et al. Role of the CNS melanocortin system in the response to overfeeding. The Journal of neuroscience : the official journal of the Society for Neuroscience 1999;19(6):2362-7.

40. Mountjoy KG. Functions for pro-opiomelanocortin-derived peptides in obesity and diabetes. The Biochemical journal 2010;428(3):305-24.

41. Bicknell AB. The tissue-specific processing of pro-opiomelanocortin. Journal of neuroendocrinology 2008;20(6):692-9.

42. Tung YC, Piper SJ, Yeung D, O'Rahilly S, Coll AP. A comparative study of the central effects of specific proopiomelancortin (POMC)-derived melanocortin peptides on food intake and body weight in pomc null mice. Endocrinology 2006;147(12):5940-7.

43. Lee M, Wardlaw SL. The central melanocortin system and the regulation of energy balance. Frontiers in bioscience : a journal and virtual library 2007;12:3994-4010.

44. Huszar D, Lynch CA, Fairchild-Huntress V, et al. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell 1997;88(1):131-41.

45. Santini F, Maffei M, Pelosini C, Salvetti G, Scartabelli G, Pinchera A. Melanocortin-4 receptor mutations in obesity. Advances in clinical chemistry 2009;48:95-109.

46. Farooqi IS, O'Rahilly S. Monogenic obesity in humans. Annual review of medicine 2005;56:443-58.

47. Douglass J, McKinzie AA, Couceyro P. PCR differential display identifies a rat brain mRNA that is transcriptionally regulated by cocaine and amphetamine. The Journal of neuroscience : the official journal of the Society for Neuroscience 1995;15(3 Pt 2):2471-81.

48. Keller PA, Compan V, Bockaert J, et al. Characterization and localization of cocaine- and amphetamine-regulated transcript (CART) binding sites. Peptides 2006;27(6):1328-34.

49. Koylu EO, Couceyro PR, Lambert PD, Kuhar MJ. Cocaine- and amphetamine-regulated transcript peptide immunohistochemical localization in the rat brain. The Journal of comparative neurology 1998;391(1):115-32.

50. Larsen PJ, Vrang N, Petersen PC, Kristensen P. Chronic intracerebroventricular administration of recombinant CART(42-89) peptide inhibits and causes weight loss in lean and obese Zucker (fa/fa) rats. Obes Res 2000;8(8):590-6.

51. Lambert PD, Couceyro PR, McGirr KM, Dall Vechia SE, Smith Y, Kuhar MJ. CART peptides in the central control of feeding and interactions with neuropeptide Y. Synapse 1998;29(4):293-8.

52. Smith KL, Gardiner JV, Ward HL, et al. Overexpression of CART in the PVN increases food intake and weight gain in rats. Obesity (Silver Spring) 2008;16(10):2239-44.

53. Asnicar MA, Smith DP, Yang DD, et al. Absence of cocaine- and amphetamine-regulated transcript results in obesity in mice fed a high caloric diet. Endocrinology 2001;142(10):4394-400.

54. Steiner RC, Hsiung HM, Picciotto MR. Cocaine self-administration and locomotor sensitization are not altered in CART knockout mice. Behavioural brain research 2006;171(1):56-62.

55. Elias CF, Lee CE, Kelly JF, et al. Characterization of CART neurons in the rat and human hypothalamus. The Journal of comparative neurology 2001;432(1):1-19.

56. Wang ZW, Zhou YT, Kakuma T, et al. Comparing the hypothalamic and extrahypothalamic actions of endogenous hyperleptinemia. Proceedings of the National Academy of Sciences of the United States of America 1999;96(18):10373-8.

57. Tatemoto K. Neuropeptide Y: complete amino acid sequence of the brain peptide. Proceedings of the National Academy of Sciences of the United States of America 1982;79(18):5485-9.

58. Tatemoto K, Carlquist M, Mutt V. Neuropeptide Y--a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature 1982;296(5858):659-60.

59. Sheikh SP, Hakanson R, Schwartz TW. Y1 and Y2 receptors for neuropeptide Y. FEBS letters 1989;245(1-2):209-14.

60. Chronwall BM. Anatomy and physiology of the neuroendocrine arcuate nucleus. Peptides 1985;6 Suppl 2:1-11.

61. White JD, Kershaw M. Increased hypothalamic neuropeptide Y expression following food deprivation. Molecular and cellular neurosciences 1990;1(1):41-8.

62. Li C, Chen P, Smith MS. Neuropeptide Y (NPY) neurons in the arcuate nucleus (ARH) and dorsomedial nucleus (DMH), areas activated during lactation, project to the paraventricular nucleus of the hypothalamus (PVH). Regulatory peptides 1998;75-76:93-100.

63. Bai FL, Yamano M, Shiotani Y, et al. An arcuato-paraventricular and -dorsomedial hypothalamic neuropeptide Y-containing system which lacks noradrenaline in the rat. Brain research 1985;331(1):172-5.

64. Sanacora G, Kershaw M, Finkelstein JA, White JD. Increased hypothalamic content of preproneuropeptide Y messenger ribonucleic acid in genetically obese Zucker rats and its regulation by food deprivation. Endocrinology 1990;127(2):730-7.

65. Stanley BG, Kyrkouli SE, Lampert S, Leibowitz SF. Neuropeptide Y chronically injected into the hypothalamus: a powerful neurochemical inducer of hyperphagia and obesity. Peptides 1986;7(6):1189-92.

66. Parker RM, Herzog H. Regional distribution of Y-receptor subtype mRNAs in rat brain. The European journal of neuroscience 1999;11(4):1431-48.

67. O'Shea D, Morgan DG, Meeran K, et al. Neuropeptide Y induced feeding in the rat is mediated by a novel receptor. Endocrinology 1997;138(1):196-202.

68. Schaffhauser AO, Stricker-Krongrad A, Brunner L, et al. Inhibition of food intake by neuropeptide Y Y5 receptor antisense oligodeoxynucleotides. Diabetes 1997;46(11):1792-8.

69. Marsh DJ, Hollopeter G, Kafer KE, Palmiter RD. Role of the Y5 neuropeptide Y receptor in feeding and obesity. Nature medicine 1998;4(6):718-21.

70. Shutter JR, Graham M, Kinsey AC, Scully S, Luthy R, Stark KL. Hypothalamic expression of ART, a novel gene related to agouti, is up-regulated in obese and diabetic mutant mice. Genes & development 1997;11(5):593-602.

71. Graham M, Shutter JR, Sarmiento U, Sarosi I, Stark KL. Overexpression of Agrt leads to obesity in transgenic mice. Nature genetics 1997;17(3):273-4.

72. Coleman DL. Effects of parabiosis of obese with diabetes and normal mice. Diabetologia 1973;9(4):294-8.

73. Coleman DL. Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. Diabetologia 1978;14(3):141-8.

74. Zhang F, Basinski MB, Beals JM, et al. Crystal structure of the obese protein leptin-E100. Nature 1997;387(6629):206-9.

75. MacDougald OA, Hwang CS, Fan H, Lane MD. Regulated expression of the obese gene product (leptin) in white adipose tissue and 3T3-L1 adipocytes. Proceedings of the National Academy of Sciences of the United States of America 1995;92(20):9034-7.

76. Maffei M, Halaas J, Ravussin E, et al. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nature medicine 1995;1(11):1155-61.

77. Couillard C, Mauriege P, Imbeault P, et al. Hyperleptinemia is more closely associated with adipose cell hypertrophy than with adipose tissue hyperplasia. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 2000;24(6):782-8.

78. Schoeller DA, Cella LK, Sinha MK, Caro JF. Entrainment of the diurnal rhythm of plasma leptin to meal timing. The Journal of clinical investigation 1997;100(7):1882-7.

79. Bado A, Levasseur S, Attoub S, et al. The stomach is a source of leptin. Nature 1998;394(6695):790-3.

80. Morash B, Li A, Murphy PR, Wilkinson M, Ur E. Leptin gene expression in the brain and pituitary gland. Endocrinology 1999;140(12):5995-8.

81. Masuzaki H, Ogawa Y, Sagawa N, et al. Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. Nature medicine 1997;3(9):1029-33.

82. Wang MY, Zhou YT, Newgard CB, Unger RH. A novel leptin receptor isoform in rat. FEBS letters 1996;392(2):87-90.

83. Lee GH, Proenca R, Montez JM, et al. Abnormal splicing of the leptin receptor in diabetic mice. Nature 1996;379(6566):632-5.

84. de Luca C, Kowalski TJ, Zhang Y, et al. Complete rescue of obesity, diabetes, and infertility in db/db mice by neuron-specific LEPR-B transgenes. The Journal of clinical investigation 2005;115(12):3484-93.

85. Cohen P, Zhao C, Cai X, et al. Selective deletion of leptin receptor in neurons leads to obesity. The Journal of clinical investigation 2001;108(8):1113-21.

86. Morton GJ, Niswender KD, Rhodes CJ, et al. Arcuate nucleus-specific leptin receptor gene therapy attenuates the obesity phenotype of Koletsky (fa(k)/fa(k)) rats. Endocrinology 2003;144(5):2016-24.

87. Chua SC, Jr., Chung WK, Wu-Peng XS, et al. Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. Science 1996;271(5251):994-6.

88. da Silva BA, Bjorbaek C, Uotani S, Flier JS. Functional properties of leptin receptor isoforms containing the gln-->pro extracellular domain mutation of the fatty rat. Endocrinology 1998;139(9):3681-90.

89. Fei H, Okano HJ, Li C, et al. Anatomic localization of alternatively spliced leptin receptors (Ob-R) in mouse brain and other tissues. Proceedings of the National Academy of Sciences of the United States of America 1997;94(13):7001-5.

90. Baumann H, Morella KK, White DW, et al. The full-length leptin receptor has signaling capabilities of interleukin 6-type cytokine receptors. Proceedings of the National Academy of Sciences of the United States of America 1996;93(16):8374-8.

91. Bjorbaek C, Kahn BB. Leptin signaling in the central nervous system and the periphery. Recent progress in hormone research 2004;59:305-31.

92. Hakansson ML, Hulting AL, Meister B. Expression of leptin receptor mRNA in the hypothalamic arcuate nucleus--relationship with NPY neurones. Neuroreport 1996;7(18):3087-92.

93. Haynes WG, Sivitz WI, Morgan DA, Walsh SA, Mark AL. Sympathetic and cardiorenal actions of leptin. Hypertension 1997;30(3 Pt 2):619-23.

94. Baura GD, Foster DM, Porte D, Jr., et al. Saturable transport of insulin from plasma into the central nervous system of dogs in vivo. A mechanism for regulated insulin delivery to the brain. The Journal of clinical investigation 1993;92(4):1824-30.

95. Marks JL, Porte D, Jr., Stahl WL, Baskin DG. Localization of insulin receptor mRNA in rat brain by in situ hybridization. Endocrinology 1990;127(6):3234-6.

96. Woods SC, Lotter EC, McKay LD, Porte D, Jr. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. Nature 1979;282(5738):503-5.

97. Air EL, Strowski MZ, Benoit SC, et al. Small molecule insulin mimetics reduce food intake and body weight and prevent development of obesity. Nature medicine 2002;8(2):179-83.

98. Burks DJ, Font de Mora J, Schubert M, et al. IRS-2 pathways integrate female reproduction and energy homeostasis. Nature 2000;407(6802):377-82.

99. Plum L, Schubert M, Bruning JC. The role of insulin receptor signaling in the brain. Trends in endocrinology and metabolism: TEM 2005;16(2):59-65.

100. Schwartz MW, Marks JL, Sipols AJ, et al. Central insulin administration reduces neuropeptide Y mRNA expression in the arcuate nucleus of food-deprived lean (Fa/Fa) but not obese (fa/fa) Zucker rats. Endocrinology 1991;128(5):2645-7.

101. Spanswick D, Smith MA, Mirshamsi S, Routh VH, Ashford ML. Insulin activates ATP-sensitive K+ channels in hypothalamic neurons of lean, but not obese rats. Nature neuroscience 2000;3(8):757-8.

102. Benoit SC, Air EL, Coolen LM, et al. The catabolic action of insulin in the brain is mediated by melanocortins. The Journal of neuroscience : the official journal of the Society for Neuroscience 2002;22(20):9048-52.

103. Wren AM, Seal LJ, Cohen MA, et al. Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab 2001;86(12):5992.

104. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 2001;50(8):1714-9.

105. Otto B, Cuntz U, Fruehauf E, et al. Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa. European journal of endocrinology / European Federation of Endocrine Societies 2001;145(5):669-73.

106. Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature 2000;407(6806):908-13.

107. Gardiner J, Bloom S. Ghrelin gets its GOAT. Cell metabolism 2008;7(3):193-4.

108. Kojima M, Kangawa K. Ghrelin: structure and function. Physiological reviews 2005;85(2):495-522.

109. Onaga T, Zabielski R, Kato S. Multiple regulation of peptide YY secretion in the digestive tract. Peptides 2002;23(2):279-90.

110. Abbott CR, Monteiro M, Small CJ, et al. The inhibitory effects of peripheral administration of peptide YY(3-36) and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal-brainstem-hypothalamic pathway. Brain research 2005;1044(1):127-31.

111. Karra E, Chandarana K, Batterham RL. The role of peptide YY in appetite regulation and obesity. The Journal of physiology 2009;587(Pt 1):19-25.

112. Batterham RL, Cowley MA, Small CJ, et al. Gut hormone PYY(3-36) physiologically inhibits food intake. Nature 2002;418(6898):650-4.

113. Chelikani PK, Haver AC, Reeve JR, Jr., Keire DA, Reidelberger RD. Daily, intermittent intravenous infusion of peptide YY(3-36) reduces daily food intake and adiposity in rats. American journal of physiology Regulatory, integrative and comparative physiology 2006;290(2):R298-305.

114. le Roux CW, Batterham RL, Aylwin SJ, et al. Attenuated peptide YY release in obese subjects is associated with reduced satiety. Endocrinology 2006;147(1):3-8.

115. Martins C, Morgan LM, Bloom SR, Robertson MD. Effects of exercise on gut peptides, energy intake and appetite. The Journal of endocrinology 2007;193(2):251-8.

116. Considine RV, Sinha MK, Heiman ML, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. The New England journal of medicine 1996;334(5):292-5.

117. Schwartz MW, Peskind E, Raskind M, Boyko EJ, Porte D, Jr. Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. Nature medicine 1996;2(5):589-93.

118. Van Heek M, Compton DS, France CF, et al. Diet-induced obese mice develop peripheral, but not central, resistance to leptin. The Journal of clinical investigation 1997;99(3):385-90.

119. Lin S, Thomas TC, Storlien LH, Huang XF. Development of high fat diet-induced obesity and leptin resistance in C57Bl/6J mice. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 2000;24(5):639-46.

120. El-Haschimi K, Pierroz DD, Hileman SM, Bjorbaek C, Flier JS. Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. The Journal of clinical investigation 2000;105(12):1827-32.

121. Myers MG, Cowley MA, Munzberg H. Mechanisms of leptin action and leptin resistance. Annual review of physiology 2008;70:537-56.

122. Stein LJ, Dorsa DM, Baskin DG, Figlewicz DP, Porte D, Jr., Woods SC. Reduced effect of experimental peripheral hyperinsulinemia to elevate cerebrospinal fluid insulin concentrations of obese Zucker rats. Endocrinology 1987;121(5):1611-5.

123. Kaiyala KJ, Prigeon RL, Kahn SE, Woods SC, Schwartz MW. Obesity induced by a high-fat diet is associated with reduced brain insulin transport in dogs. Diabetes 2000;49(9):1525-33.

124. Posey KA, Clegg DJ, Printz RL, et al. Hypothalamic proinflammatory lipid accumulation, inflammation, and insulin resistance in rats fed a high-fat diet. American journal of physiology Endocrinology and metabolism 2009;296(5):E1003-12.

125. Zick Y. Insulin resistance: a phosphorylation-based uncoupling of insulin signaling. Trends in cell biology 2001;11(11):437-41.

126. Niswender KD, Morrison CD, Clegg DJ, et al. Insulin activation of phosphatidylinositol 3-kinase in the hypothalamic arcuate nucleus: a key mediator of insulin-induced anorexia. Diabetes 2003;52(2):227-31.

127. Saladin R, De Vos P, Guerre-Millo M, et al. Transient increase in obese gene expression after food intake or insulin administration. Nature 1995;377(6549):527-9.

128. Saad MF, Khan A, Sharma A, et al. Physiological insulinemia acutely modulates plasma leptin. Diabetes 1998;47(4):544-9.

129. Gettys TW, Harkness PJ, Watson PM. The beta 3-adrenergic receptor inhibits insulin-stimulated leptin secretion from isolated rat adipocytes. Endocrinology 1996;137(9):4054-7.

130. Wabitsch M, Jensen PB, Blum WF, et al. Insulin and cortisol promote leptin production in cultured human fat cells. Diabetes 1996;45(10):1435-8.

131. Mueller WM, Gregoire FM, Stanhope KL, et al. Evidence that glucose metabolism regulates leptin secretion from cultured rat adipocytes. Endocrinology 1998;139(2):551-8.

Chapter 2

Hypothesis and Specific Aims

Background

Humans and other animals with genetic mutations that result in no function leptin production (1) or pathways in the brain that respond to leptin (2) display extreme hyperphagia (increased hunger and food consumption) and gain body fat rapidly. Administration of leptin in the former condition reverses the phenotype. These conditions clearly demonstrate that leptin has a direct role in energy balance by modulating food intake and, to a smaller degree, energy expenditure; however, these genetic mutations are very rare in the human population. Most obese individuals actually have plasma leptin concentrations that are much higher compared to their healthy weight counterparts. In theory, this should lead to decreased energy intake and weight loss back to a pre-obese state, but this is not supported by the increasing overweight and obesity prevalence in the United States (3). It is thought that part of the failure to reduce energy intake may be the development of resistance to leptin, which may occur at one or multiple levels (Figure 9) including; 1) impaired leptin production/secretion from adipocytes; 2) impaired plasma transport and/or uptake across the blood brain barrier into the CNS; and 3) impaired leptin signaling within target tissues (e.g. the hypothalamus).

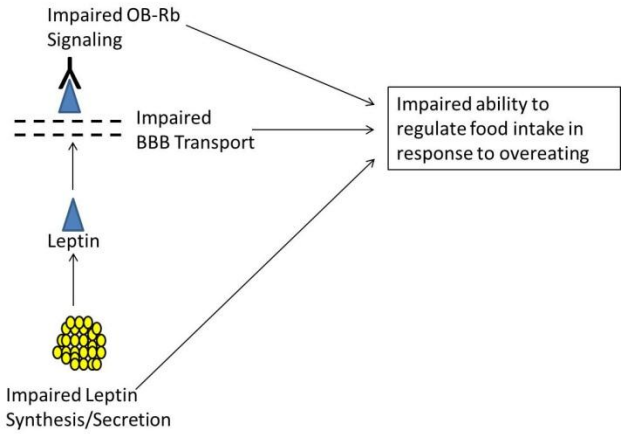

Figure 1: Potential sites of leptin resistance. Adapted from Houseknecht KL, *et al.* (4).

In humans, there is little information available regarding day-to-day changes in 24h plasma leptin levels; however, some data does suggest a defect in the short-term leptin response to overfeeding that may explain why some individuals gain more weight than others. Previous research has shown three days of overfeeding (130% of daily caloric needs) in non-obese men increases average 24h leptin concentrations while three days of underfeeding (70% of daily caloric needs) significantly decreases average 24h leptin concentrations when compared to eucaloric feeding (5). These changes in the 24h leptin response to acute change in energy balance were also significantly correlated with changes in self-selected energy intake of a breakfast meal following each treatment. These results are in agreement with other studies in healthy weight individuals for both overfeeding (6) and fasting (7) conditions, in which the change in plasma leptin concentrations are driven by short term changes in energy balance that are essentially independent of significant changes in adipose tissue mass. On the other hand, it has been shown that obese males do not respond to overfeeding by increasing 24h plasma leptin levels (8), but 24h leptin levels decrease as expected in response to underfeeding. In essence, the homeostatic leptin signal detects short-term under-eating and induces hunger in the obese, but fails to detect short-term overfeeding and does not produce a satiety response, potentially reinforcing continued slow weight gain.

In a series of unrelated studies, it has also been noted that sedentary behavior may reduce the sensitivity of appetite control. In one of the classic papers leading to the modern hypothesis that physical activity protects against excess weight gain, Mayer et al. (9) reported associations suggesting a disruption in the accuracy by which energy intake is matched with energy expenditure at low levels of physical activity. Support for the idea of better coupling of energy intake to expenditure in habitually high active individuals has been demonstrated using the pre-

load/test meal paradigm. When the caloric density of a meal (usually liquid) is covertly manipulated and an *ad libitum* meal is served shortly thereafter, habitually active non-elderly individuals are better able to distinguish between high and low energy preloads compared to sedentary individuals by adequately adjusting energy intake at a subsequent *ad-libitum* meal (10, 11). In a longitudinal study, Martins and colleagues studied the effects of a six week aerobic exercise training intervention (4 days/wk, 30-45min/day @ 65-75% max heart rate) on energy intake compensation in response to covertly manipulated preloads in 25 normal weight sedentary men and women (12). The authors observed an improved appetite control after the six week exercise intervention, as participants displayed a more sensitive eating behavior on average by decreasing 24h cumulative food intake following a high energy breakfast pre-load compared with a low-energy breakfast pre-load.

The strongest evidence supporting the role of physical activity in regulating body weight at healthy levels comes from studies of individuals who have lost significant weight and maintained a reduced body weight. For example, Jakicic and colleagues (13) studied overweight women randomly assigned to 1 of 4 groups based on physical activity energy expenditure (1000 versus 2000 kcal/week) and intensity (moderate versus vigorous) with a concomitant decrease in daily energy intake (−1200 to −1500 kcal/day) over 24 months. Despite no difference in average weight loss at 6 and 24 months between the groups, post hoc analyses showed that individuals sustaining a loss of 10% or more of initial body weight at 24 months reported performing more physical activity (1835 kcal/week or 275 min/week) compared to those sustaining a weight loss of less than 10% of initial body weight. In addition, Schoeller et al, (14) have demonstrated that weight reduced women with high levels of measured total energy

expenditure (physical activity level, PAL >1.75 or ~80 min/day of moderate-intensity physical activity) maintained most of their reduced weight after 12 months of follow-up.

A potential mediator of the reduced peripheral leptin response to overfeeding is insulin resistance. In the above described study of Cooper et al (8), it was noted that plasma insulin levels in the obese men did not increase in response to overfeeding. This may be important because studies have shown insulin increases both leptin gene expression (15) and circulating leptin concentrations (16). Thus, inadequate insulin-induced leptin production or secretion as a result of peripheral insulin resistance at the level of the adipocyte may contribute to the development or worsening of obesity. It is known that regular exercise or sustained physical activity, even after one bout of sufficient volume and duration, improves whole body insulin sensitivity in sedentary overweight/obese individuals (17). As insulin sensitivity is improved with regular exercise, it is reasonable to suggest that improved insulin signaling within the adipocyte may better regulate leptin synthesis or secretion, and this component of "leptin resistance" may be improved in obese subjects during short-term overfeeding.

Most research in humans studying the effects of exercise on leptin are limited, as many have analyzed only a single fasting sample and drawn conclusions from this 'snapshot' of leptin's 24h diurnal plasma secretion rhythm (18-20). Few studies have examined the 24h plasma leptin response to exercise and short-term changes in energy balance (21). van Aggel-Leijssen and colleagues (21) studied 8 healthy weight (14.1% fat) sedentary males under four conditions: (I) no exercise + energy balance; (II) exercise + energy balance (2 h at 50%VO2max, equivalent to ~800 kcal); (III) exercise + negative energy balance (energy deficit of ~800 kcal); and (IV) exercise + positive energy balance (energy surplus of ~800 kcal). Interestingly, the exercise + energy balance condition resulted in a 20% reduction in average 24h leptin, while the

exercise + negative energy balance condition did not significantly alter 24h leptin levels. Exercise + positive energy balance increased the amplitude of the 24h leptin curve by 2-fold compared with the exercise + energy balance condition, while average 24h leptin concentration showed a tendency to increase as well. The exercise + positive energy balance data from this study suggest that moderate intensity aerobic exercise of sufficient duration during periods of positive energy balance may increase the leptin response to overfeeding in sedentary obese humans. Unfortunately, objective measures of self-selected food intake following each treatment, or measures of subjective appetite (i.e. "how hungry are you"), were not examined in this previous study. Based on the available literature, the 24h leptin response to exercise and sedentary conditions during energy balance or overfeeding has not been examined in sedentary obese men and women.

Taken together, these data suggest that being physically active on a regular basis is required for optimal functioning of the appetite regulatory system and that the 24h leptin response may be involved. It should be noted, however, that the physiological system responsible for appetite regulation is extremely complex, involving several hormones in addition to leptin. Measures of these hormones should be included in physiologic studies of leptin and appetite.

In addition to leptin, two hormones secreted principally from the gastrointestinal tract, ghrelin and peptide YY (PYY), are also involved in the regulation of hunger and satiety and thus involved in the regulation of food intake and energy balance. Ghrelin is the only known blood hormone that stimulates food intake (22), while PYY is a satiety factor (23). It has been reported that obese adults may have lower baseline (24) and post-prandial (25) PYY plasma levels while also exhibiting lower subjective satiety ratings ("how full are you?") compared with healthy

weight controls, although not all studies are in agreement (26). If true, this raises the possibility that decreased peptide YY plasma concentrations could play a role in the development and/or maintenance of obesity. Fasting ghrelin levels have actually been shown to be lower in obese humans relative to healthy weight peers (27), but obese individuals may have a diminished ability to reduce ghrelin secretion after initiating a meal (28), which may work in concert with the lower postprandial peptide YY levels and aforementioned leptin dysregulation to reinforce obesity.

Several limitations are apparent in the literature regarding the interaction between exercise and ghrelin/PYY. As with leptin, many studies have only measured the ghrelin and/or PYY hormone response to exercise in the fasted state or for relatively short time periods (i.e. 30 minutes post-exercise). Additionally, most study participants have been almost exclusively healthy weight or exercise trained individuals. Also, little is known about the influence of exercise on the active forms of ghrelin (acylghrelin) and PYY (PYY_{3-36}), as most studies have examined total ghrelin and total PYY. Few studies have assessed post-exercise gut hormone responses to changes in energy balance over a prolonged period (i.e. 24 h) or attempted to correlate these responses with changes in subjective appetite sensations (feelings of hunger and fullness). Fewer still have attempted to answer these research questions in sedentary healthy weight and obese individuals together.

Assessing 24 h plasma levels of leptin, insulin, ghrelin, and PYY during short-term changes in energy balance (overfeeding) and energy use (exercise) in relation to body adiposity will help determine the impact of these hormones on objective and subjective measures of appetite. Differences in plasma levels of these hormones between healthy weight and obese individuals may help explain why some individuals defend against weight gain despite being

exposed to an obesogenic environment (29). If positive effects are revealed, this will reinforce the critical role of exercise in body weight management. Therefore, the purpose of this study is to examine the effects of short-term positive energy balance and exercise on 24h plasma levels of insulin, leptin, acylghrelin, and PYY_{3-36} in sedentary healthy weight and obese men and women.

Overarching hypothesis: Daily exercise during short-term positive energy balance will improve objective and subjective appetite in obese individuals by modulating plasma levels of hormones involved in appetite regulation.

Specific Aim 1: The first aim of this study is to demonstrate that moderate intensity aerobic exercise will increase 24h plasma leptin response in obese individuals following overfeeding compared to sedentary treatments. We hypothesize that plasma levels will fail to increase in response to overfeeding during sedentary conditions in the obese, but healthy weight controls will demonstrate an appropriate increase in 24h plasma leptin levels in response to overfeeding regardless of activity level (sedentary or exercise).

Additionally, we aim to determine if changes in 24h plasma leptin response correlate with changes in self-selected energy intake of an *ad-libitum* breakfast meal. We hypothesize that increased 24h plasma leptin levels will be inversely correlated with self-selected breakfast energy intake in obese individuals only after the overfeeding + exercise treatment.

Specific Aim 2: A secondary aim of this study is to characterize the 24 h plasma levels of acylated ghrelin and peptide YY_{3-36} in habitually sedentary healthy weight and obese individuals. We hypothesize that exercise will improve responsiveness of these hormones to short-term overfeeding and will correlate with changes in subjective appetite ratings (e.g. decreased hunger/increased fullness) and changes in self-selected energy intake of an *ad-libitum* breakfast meal.

References

1. Farooqi IS, Jebb SA, Langmack G, et al. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. The New England journal of medicine 1999;341(12):879-84.
2. Clement K, Vaisse C, Lahlou N, et al. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature 1998;392(6674):398-401.
3. Flegal KM, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999-2008. JAMA : the journal of the American Medical Association 2010;303(3):235-41.
4. Houseknecht KL, Baile CA, Matteri RL, Spurlock ME. The biology of leptin: a review. Journal of animal science 1998;76(5):1405-20.
5. Chin-Chance C, Polonsky KS, Schoeller DA. Twenty-four-hour leptin levels respond to cumulative short-term energy imbalance and predict subsequent intake. The Journal of clinical endocrinology and metabolism 2000;85(8):2685-91.
6. Kolaczynski JW, Ohannesian JP, Considine RV, Marco CC, Caro JF. Response of leptin to short-term and prolonged overfeeding in humans. The Journal of clinical endocrinology and metabolism 1996;81(11):4162-5.
7. Boden G, Chen X, Mozzoli M, Ryan I. Effect of fasting on serum leptin in normal human subjects. The Journal of clinical endocrinology and metabolism 1996;81(9):3419-23.
8. Cooper JA, Polonsky KS, Schoeller DA. Serum leptin levels in obese males during over- and underfeeding. Obesity (Silver Spring) 2009;17(12):2149-54.

9. Mayer J, Roy P, Mitra KP. Relation between caloric intake, body weight, and physical work: studies in an industrial male population in West Bengal. Am J Clin Nutr 1956;4(2):169-75.

10. Long SJ, Hart K, Morgan LM. The ability of habitual exercise to influence appetite and food intake in response to high- and low-energy preloads in man. Br J Nutr 2002;87(5):517-23.

11. Van Walleghen EL, Orr JS, Gentile CL, Davy KP, Davy BM. Habitual physical activity differentially affects acute and short-term energy intake regulation in young and older adults. Int J Obes (Lond) 2007;31(8):1277-85.

12. Martins C, Truby H, Morgan LM. Short-term appetite control in response to a 6-week exercise programme in sedentary volunteers. Br J Nutr 2007;98(4):834-42.

13. Jakicic JM, Marcus BH, Lang W, Janney C. Effect of exercise on 24-month weight loss maintenance in overweight women. Arch Intern Med 2008;168(14):1550-9; discussion 9-60.

14. Schoeller DA, Shay K, Kushner RF. How much physical activity is needed to minimize weight gain in previously obese women? Am J Clin Nutr 1997;66(3):551-6.

15. Saladin R, De Vos P, Guerre-Millo M, et al. Transient increase in obese gene expression after food intake or insulin administration. Nature 1995;377(6549):527-9.

16. Saad MF, Khan A, Sharma A, et al. Physiological insulinemia acutely modulates plasma leptin. Diabetes 1998;47(4):544-9.

17. Hawley JA, Lessard SJ. Exercise training-induced improvements in insulin action. Acta Physiol (Oxf) 2008;192(1):127-35.

18. Hickey MS, Considine RV, Israel RG, et al. Leptin is related to body fat content in male distance runners. The American journal of physiology 1996;271(5 Pt 1):E938-40.

19. Perusse L, Collier G, Gagnon J, et al. Acute and chronic effects of exercise on leptin levels in humans. J Appl Physiol 1997;83(1):5-10.

20. Racette SB, Coppack SW, Landt M, Klein S. Leptin production during moderate-intensity aerobic exercise. The Journal of clinical endocrinology and metabolism 1997;82(7):2275-7.

21. van Aggel-Leijssen DP, van Baak MA, Tenenbaum R, Campfield LA, Saris WH. Regulation of average 24h human plasma leptin level; the influence of exercise and physiological changes in energy balance. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 1999;23(2):151-8.

22. Wren AM, Seal LJ, Cohen MA, et al. Ghrelin enhances appetite and increases food intake in humans. The Journal of clinical endocrinology and metabolism 2001;86(12):5992.

23. Batterham RL, Bloom SR. The gut hormone peptide YY regulates appetite. Annals of the New York Academy of Sciences 2003;994:162-8.

24. Batterham RL, Cohen MA, Ellis SM, et al. Inhibition of food intake in obese subjects by peptide YY3-36. The New England journal of medicine 2003;349(10):941-8.

25. le Roux CW, Batterham RL, Aylwin SJ, et al. Attenuated peptide YY release in obese subjects is associated with reduced satiety. Endocrinology 2006;147(1):3-8.

26. Pfluger PT, Kampe J, Castaneda TR, et al. Effect of human body weight changes on circulating levels of peptide YY and peptide YY3-36. The Journal of clinical endocrinology and metabolism 2007;92(2):583-8.

27. Tschop M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. Diabetes 2001;50(4):707-9.

28. le Roux CW, Patterson M, Vincent RP, Hunt C, Ghatei MA, Bloom SR. Postprandial plasma ghrelin is suppressed proportional to meal calorie content in normal-weight but not obese subjects. The Journal of clinical endocrinology and metabolism 2005;90(2):1068-71.

29. Jebb SA, Siervo M, Fruhbeck G, Goldberg GR, Murgatroyd PR, Prentice AM. Variability of appetite control mechanisms in response to 9 weeks of progressive overfeeding in humans. Int J Obes (Lond) 2006;30(7):1160-2.

Chapter 3

24h leptin response to short-term positive energy balance and exercise in habitually sedentary humans

Chad M. Cook, MS, Britt Erikkson, PhD, Alexandra Adams, MD, PhD, Dale A. Schoeller, PhD

Abstract

Introduction: Previous research has shown three days of overfeeding (OVER) increases leptin levels in healthy weight, but not obese, men. Daily exercise (EX) during positive energy balance (EB) may improve appetite regulation in the obese by modulating plasma leptin levels. **Objective:** To examine the effects of EX and OVER on 24h leptin and self-selected food intake at two levels of physical activity. **Methods:** In a randomized cross-over design, 13 habitually sedentary individuals (19-39y) were in-patients in a metabolic research ward for two different six-consecutive overnight visits. During one visit, subjects remained sedentary (SED) and during the other performed cycling EX at 50% VO_{2max} twice daily. Subjects were in EB on days 1-3 and OVER on days 4-6 of each visit. Multiple blood samples were taken over 24h on days 3 & 6 for leptin and insulin. The morning after EB and OVER, subjects were fed an *ad-libitum* breakfast. **Results:** No effect of OVER on 24h leptin was found when stratified by BMI status; however, when stratified by HOMA status (normal HOMA = NH, n=6 vs. high HOMA, n=7) the 24 h leptin mesor in the NH group increased in response to OVER during SED (15.1±13.8%, p=0.02) but was unchanged during EX (3.0±5.2%). There was no effect of OVER on 24h leptin during SED or EX in the HH group. Both groups reduced *ad-libitum* breakfast intake in response to OVER during SED (NH = -229±112 kcals, p<0.01; HH = -165±157 kcals, p<0.05) with a trend during EX. **Conclusions:** The results of the present study are a testament to the complexity of human appetite regulation as changes in *ad-libitum* breakfast intake were not explained by relationships with changes in 24h leptin responses.

Introduction

The biology responsible for the regulation of energy balance (EB) depends, at least in-part, on the integration of central and peripheral neuro-hormonal signals acting in a homeostatic fashion to adjust food intake and energy expenditure to maintain a stable body weight. These include adiposity signals such as leptin and insulin; short-term meal-to-meal episodic signals like macronutrients and gastrointestinal peptide hormones; and several other neural and hormonal signaling candidates that all likely interact with different regions of the central nervous system to exert effects on food intake, metabolism, and EB.

Animal studies using forced overfeeding as a way to study positive EB and body weight regulation have shown that rodents forced to overeat will significantly reduce voluntary food intake and display changes in metabolism that favor weight loss following the overfeeding intervention (1, 2). In addition, parabiosis experiments have shown that overfeeding one rodent reduces food intake and body weight/adiposity in the conjoined parabiotic partner (3). Overfeeding studies in humans have also demonstrated significant decreases in voluntary food consumption following prescribed overfeeding (4). These studies provide evidence for an adaptive resistance to weight gain primarily through subsequent reductions in food intake. This led to the hypothesis that there was a 'leptostat' that informed the brain regarding an animal's EB for periods of days to weeks (5). A variety of hormones are now known to contribute to this resistive response to overfeeding, but leptin is currently the prime candidate for signaling related to body fat stores and thus EB over periods of days to weeks.

Previous research has shown three days of overfeeding at 130% of EB needs in healthy weight human males increases 24 h average leptin concentrations (relative to eucaloric feeding), and this increase in leptin strongly correlates with a reduction in food intake at a self-selected

breakfast the morning after the overfeeding period (6). In contrast, data from a follow-up study utilizing the same design demonstrated that 24 h leptin does not increase (relative to eucaloric feeding) in response to three days of overfeeding in obese males and there is no significant relationship between the 24 h leptin response to overfeeding and self-selected food intake (7). Interestingly, 24 h leptin concentrations were significantly decreased in response to acute underfeeding in both groups of men. In essence, the purported homeostatic leptin signal detects short-term underfeeding and induces hunger in both healthy weight and obese individuals, but fails to respond to short-term overfeeding in the obese by not producing a leptin signal nor satiety response. These data, albeit comprised of a limited sample size, suggest that the leptin system response to short-term changes in EB appears dependent on adiposity and the observed pattern of the 24 h leptin response to over- and underfeeding may set an obese person up for continued slow weight gain.

Because obesity is often accompanied by insulin resistance (8) and because insulin is involved in regulating leptin gene expression (9) and circulating leptin concentrations (10-12), we hypothesized that the lack of leptin response to overfeeding in the obese might be related to insulin resistance. Moreover, because insulin resistance is reduced following exercise and because high habitual daily physical activity or structured exercise is important in the maintenance of weight loss in humans (13), we hypothesized that exercise would restore the leptin response to overfeeding in the obese. The combination of exercise and energy surplus on plasma concentrations of leptin has rarely been studied, although it has relevance from both a basic science perspective (regulation of energy balance and body weight) and from a practical perspective (to minimize weight gain during short periods of energy excess, such as during weekends and holidays). Thus, the purpose of this study was to determine if increasing a

sedentary individual's daily physical activity level with structured aerobic exercise during overfeeding will improve short-term appetite control by increasing the 24 h plasma leptin response.

Methods

Study Subjects

Six individuals (five females and one male) with a healthy BMI (20<25 kg/ m^2) and seven individuals (six females, one male) with an obese BMI (30<35 kg/m^2), aged 19–39 y participated in this study. Each participant had normal thyroid function as determined by plasma thyroid-stimulating hormone concentrations (0.4–3.6 IU/l). Due to the large volume of blood drawn, subjects were required to have normal hemoglobin (12.0 - 16.0 g/dL for females and 13.5 - 17.5 g/dL for males) and hematocrit (36 – 46% for females and 41 – 53% for males). In addition, all subjects had a normal fasting lipid profile, were not hypertensive, and were identified as non-restrained eaters using the Three Factor Eating Questionnaire (14).

Exclusion criteria included current tobacco use, a history of metabolic or cardiovascular disease, history of claustrophobia (which might preclude use of a whole-room indirect calorimeter in this study), special dietary habits (e.g. vegetarian, low-carbohydrate), use of prescription medications (other than oral hormonal birth control), use of over-the-counter substances that could potentially alter energy metabolism, and females who were pregnant, lactating, or experienced irregular menstrual cycles. All subjects passed a complete medical history and physical exam by the study physician. Individuals who exercised regularly or did not eat three meals a day (including breakfast or an early morning meal) at least five days/week were also excluded. Subjects were recruited through advertisement in local newspapers, information

posted on the University of Wisconsin Department of Nutritional Sciences website, mass campus e-mail, and through flyers. The study was approved by the University of Wisconsin-Madison IRB and all subjects provided written informed consent. The study was performed at the Clinical Research Unit (CRU) at the University of Wisconsin-Madison Hospital.

Baseline Testing

One to two weeks prior to the first main study visit, subjects arrived at the University of Wisconsin Hospital's Clinical Research Unit (CRU) in the morning between 07:00 and 08:00 to complete the following procedures: Deuterium oxide (^2H_2O) for total body water (TBW) measurement to estimate body composition; resting metabolic rate (RMR); fasting blood sample (TSH, hemoglobin, hematocrit, lipid panel); complete medical history and physical exam; and an exercise test. Subjects fasted and avoided caffeine for 8-12 hours and avoided strenuous exercise and alcohol for 24 hours prior to baseline testing.

Body Composition

Body composition was estimated using a two-compartment model; fat free mass (FFM) and fat mass (FM) determined by calculating TBW using the ^2H_2O dilution technique (15). At the beginning of the baseline testing visit, saliva samples were collected before and 3 h after administration of 0.05g ^2H_2O/kg body weight. Saliva samples were centrifuged at 3,000rpm for 20 minutes and the resultant water was decolorized and cleaned with carbon black, filtered, and transferred into vials for measurement of isotopic enrichment by isotope-ratio mass spectrometry. FFM was assuming a hydration constant of 0.732 such that FFM = TBW/0.732. FM = body weight - FFM.

Resting Metabolic Rate (RMR)

Following $^{2}H_2O$ administrations, subjects were instructed to lie supine and awake in a quiet room free of bright light and at a comfortable temperature (20-24^{o}C) for at least 10 minutes prior to a 30 minute respiratory gas collection period using the Deltatrac (DTC) Metabolic Monitor (VIASYS Healthcare, Inc, SensorMedics, Yorba Linda, CA). The DTC has been established as a valid and reliable portable metabolic cart indirect calorimeter system (16). Before each RMR measurement, the DTC was calibrated to a reference gas of known O_2 and CO_2 concentrations. Gravametric methanol burns were performed periodically and calibrations adjusted accordingly. During testing, subjects were instructed to remain motionless without sleeping while respiratory gases were drawn from a clear hood canopy placed over the participant's entire head, as shown in figure 1. The final 20 minutes were used to calculate respiratory exchange ratio (RER; $VCO_2 \div VO_2$), as well as RMR (kcals/day) using the modified Weir equation (17): $[3.941 \times VO_2] + [1.106 * VCO_2]$

Exercise Test

Each participant performed an exercise test on a cycle ergometer to estimate maximal oxygen uptake (VO_{2max}) from a protocol developed by Storer et al (18). Briefly, subjects cycled for four minutes at 0 watts (W). Thereafter, work rate increased in 15 $W \cdot min^{-1}$ increments at a pedal rate of 60rpm until the participant reached his or her limit of tolerance and could no longer maintain a constant pedal rate of 60rpm as confirmed by a digital pedal revolution counter. Subjects were verbally encouraged by the test administrator to provide a maximal effort. The

final work rate (W), along with the participant's body weight (kg) and age (y) were used in the following equations to estimate VO_{2max}:

1) Males VO_{2max} ($ml \cdot min^{-1}$) = 10.51 (W) + 6.35 (kg) – 10.49 (y) + 519.3 $ml \cdot min^{-1}$
2) Females VO_{2max} ($ml \cdot min^{-1}$) = 9.39 (W) + 7.7 (kg) – 5.88 (y) + 136.7 $ml \cdot min^{-1}$

This estimated VO_{2max} was used as the criterion measure of cardiorespiratory fitness and used to calculate each individual's submaximal workload (~50% of VO_{2max}) for the exercise portion of the study. Heart rate and blood pressure were monitored during the exercise test and subjects showing a hypertensive response according to the American College of Sports Medicine guidelines (19) were not included in the study.

Protocol

In a randomized cross-over design, subjects were seen as in-patients for two different six-consecutive overnight visits separated by four weeks (Figure 2). During one of the visits, subjects remained sedentary (SED) at a daily physical activity level (PAL) of 1.4 x RMR. During the other visit, cycling exercise (EX) was performed twice daily to increase daily PAL to 1.8 x RMR. Each SED and EX visit consisted of two consecutive treatment periods lasting three days each. Subjects were kept in energy balance (EB) during the first three days of each visit followed by three days of overfeeding (OVER). During the SED visit, subjects were fed a diet to maintain EB at 1.4 x RMR for the first three days, followed by a 130% increase in energy intake during the following three days of overfeeding. During the EX visit, subjects were fed a diet to maintain EB at 1.8 x RMR for the first three days, followed by a 130% increase in energy intake during the following three days of overfeeding. All female subjects completed each visit during the early part of the follicular phase of their menstrual cycle to control for potential variations in

sex hormone levels. The PAL of 1.4 x RMR during the SED visit was determined from previous observational experience of subjects from multiple cohorts under sedentary conditions after entire days in the room calorimeter (described below). The PAL of 1.8 x RMR for exercise visits was based on higher fat utilization reported by Smith et al, (20), and data indicating that a PAL of approximately 1.8 x RMR is the minimum threshold to prevent weight regain following significant weight loss in intervention trials (21).

Whole room indirect calorimeter

A whole room indirect calorimeter (i.e. metabolic chamber; figure 3) was used to measure total energy expenditure (TEE) during the first two days of both the EB and OVER periods during the SED and EX study visits to verify subjects were fed the appropriate amount of food to match the study design. Design of the metabolic chamber at the UW-Madison CRU is similar to the metabolic chamber in the Department of Human Biology at Maastricht University in Maastricht, The Netherlands (22) and the specifications and diagnostics have been previously described (23). Additionally, information on chamber temperature, humidity, airflow, pressure, and data collection instrumentation has been described elsewhere (24). Briefly, the composition of air is measured with CO_2 (Hartman and Braun Uras-4) and O_2 (Magnos-6) gas analyzers (Applied Automation, Bartlesville, OK). The chamber was calibrated against gravametric methanol burns prior to each study visit. Average recoveries for O_2 and CO_2 = 102.8±5.1% and 95.2±2.8%, respectively. The percentage recoveries were used to develop correction factors for the corresponding chamber data for each study visit.

Blood Draws

On days three and six of each visit, subjects were moved to a single bed patient room in the CRU for 24 h blood sampling and were instructed to not leave the room, thereby mimicking conditions of the metabolic chamber. Subjects were relocated because the metabolic chamber is not equipped for blood sampling. An indwelling catheter was placed in a forearm vein for blood draws and the line was kept patent with saline infusions following each blood draw. Blood draws took place every hour from 08:00 to 23:00, every 30 minutes after each meal (08:30, 13:30, 18:30, 21:30), and every two hours after midnight to minimize potential sleep disruption until 08:00 the following morning. Blood was drawn into chilled pink-top K_2 EDTA tubes (BD Vacutainer) and immediately spun at 1800 x g for 15 min at 4ºC. Plasma was aliquoted into low-protein binding micro tubes (Sarstedt, Inc.) and stored at -80ºC until assayed.

Revised protocol for final three subjects

During the final months of the study, the UW-Madison CRU underwent complete renovations that forced us to shut down the metabolic chamber. Therefore, the study protocol was revised to utilize a different method of measuring daily energy expenditure for the final three subjects. All study activities continued at the UW-hospital in a temporary location. In place of the metabolic chamber, the final three subjects resided in a single bed patient room and were not allowed to leave in an effort to mimic the environment of the metabolic chamber. The SenseWear Pro_3 Armband (SW) accelerometer (BodyMedia Inc., Pittsburgh, PA), along with doubly labeled water (25), were used in place of the metabolic chamber to measure daily energy expenditure. The SW armband was worn every day except during the morning shower. Wear time and energy expenditure was calculated every minute using proprietary equations included in version 5.12 of the accompanying Innerview Research software. Daily energy expenditure

measured using the SW armband was compared against average daily energy expenditure measured using doubly labeled water.

Exercise

During the EX visit, subjects rode a stationary bike at 50% of predicted VO_{2max} twice each day (10:00–11:00 and 21:00–22:00). The goal for the exercise visit was to raise 24 h TEE to a PAL of 1.8 x RMR. To calculate duration of exercise, the energy cost of cycling at 50% of predicted VO_{2max} was estimated from the individual linear equation between VO_2 and work (in watts) during each participant's exercise test, which was used in combination with RMR to estimate the minutes of cycling needed to raise 24 h TEE from 1.4 x RMR (SED) to 1.8 x RMR (EX). The total number of minutes was then split into the morning and evening sessions of exercise.

Diet

All meals were prepared by the research dietician at the hospital CRU. Daily energy intake was divided over three meals and one snack as follows: Breakfast 20% (except during *ad-libitum* breakfast described below); lunch 30%; dinner 35%, and snack 15% and each meal comprised 50% carbohydrates, 35% fat, and 15% protein. Breakfast was served at 8:00 am, lunch at 1:00 pm, dinner at 6:00 pm, and the snack at 9:00 pm. Subjects were required to consume all of the food provided to them, except for an *ad-libitum* breakfast (described below).

The first meal after each three day EB and OVER period (morning of day four and seven) was an *ad-libitum* breakfast provided in a family style format. Subjects were given 30 minutes and instructed to "eat until comfortably full." Foods were pre-weighed and post-weighed to

calculate energy intake. Each participant received the same food at every *ad-libitum* breakfast, which included a large bowl of yogurt and extra-large muffins, each made by the CRU dietician to include the same relative proportion of fat, carbohydrate, and protein as the other study meals. The energy contents of the remaining meals on day four were the amounts that would normally be given during days five and six of each visit. Any adjustments needed to match total daily energy intake to the treatment period based on how much was consumed at the *ad-libitum* breakfast were made using the evening snack.

Hormone measurements

Leptin was measured in plasma in duplicate by radio-immunoassay (RIA) (kit# HL-81K; Linco Research, Inc., St. Charles, MO) with a lowest limit of detection of 0.5ng/mL and average intra- and inter-assay coefficients of variation of 5.7% and 9.2%, respectively. Insulin was also measured in plasma in duplicate by RIA (kit# HI-14K; Linco Research, Inc., St. Charles, MO) with a lowest limit of detection of 2μU/mL and average intra- and inter-assay coefficients of variation of 4.6% and 8.2%, respectively. All samples for each subject were analyzed in a single assay kit.

Statistical Analysis

SAS version 8.2 (SAS Institute Inc, Cary, NC) was used for all data analysis. The 24 h weighted average of leptin was calculated from all blood draw time points on the third day of each treatment period and is referred to as the leptin mesor; however, there is a slight mathematical difference as the mesor is calculated from the cosinor fit of the plasma leptin curve, but the mesor and 24 h weighted average are roughly equivalent. The leptin amplitude

(fraction above the mesor) is calculated as the peak of the 24 h leptin curve ÷ the mesor. Not all diet X activity interactions were of interest (diet = EB vs. OVER; activity = SED vs. EX). Therefore, the following two planned comparisons were analyzed using an ANOVA with repeated measures: 1) the leptin response to OVER vs. EB when the subjects were stratified by BMI status (healthy BMI vs. obese BMI) compared to when the subjects were stratified according to degree of insulin resistance defined by the homeostasis model assessment (HOMA) method (normal HOMA vs. high HOMA); 2) leptin response during EX vs. SED conditions. Pearson correlations were used to examine linear relationships between plasma leptin and insulin, as well as leptin and *ad-libitum* breakfast food intake. Data = mean ± SD unless otherwise indicated and statistical significance was set as $p \leq 0.05$.

Results

Energy expenditure

Physical activity level (PAL) during the SED visit (days one, two, four, and five) averaged 1.34±0.04 in the NH group and 1.38±0.05 in the HH group. During the EX visit, PAL averaged 1.75±0.05 in the NH group and 1.74±0.04 in the HH group. For the three subjects on the revised protocol, PAL during the SED visit averaged 1.33 ± 0.06 and 1.80 ± 0.05 during the EX visit. Additionally, the average difference in total energy expenditure measured using the SW armband was -32±67 kcals/d compared with DLW during the SED visit, while SW tended to overestimate TEE by an average of 143±84 kcals/d compared to DLW during the EX visit in the three subjects on the revised protocol.

The average time and intensity spent exercising during each morning and afternoon exercise session was 55±5 minutes at 50±3% of predicted VO_2 max. Daily cycling exercise

energy expenditure averaged 564±36 kcals/d during the entire EX visit for all 13 subjects combined.

Leptin

In healthy weight individuals, we hypothesized that the 24 h average leptin would increase in response to OVER during SED or EX conditions, while the 24 h leptin response to OVER in the obese would be minimal in the SED condition and would normalize (increase) in response to EX. In our study, six subjects had a healthy BMI (19 to 25kg/m^2 and seven had an obese BMI (30 to 35kg/m^2). Contrary to our hypothesis, we did not find an effect of OVER on the 24 h leptin mesor during the SED or EX condition when subjects were grouped by BMI status (figure 1A; healthy BMI vs. obese BMI); however, two subjects with healthy BMIs (19.7 and 22.2 kg/m^2) and a percent body fat (BF%) of 25.6% and 26.6% had elevated HOMA values (5.4 and 7.5, respectively). Therefore, subjects were grouped according to HOMA status (table 1), defined as: normal HOMA (NH; range = 1.4 to 2.3; n=6) and high HOMA (HH, range = 3.0 to 8.5, n=7).

The 24 h leptin mesor in the NH group was increased in response to OVER during the SED condition (15.1±13.8%, p=0.02) but was unchanged during EX (3.0±5.2%, n.s.). There was no effect of OVER on the 24 h leptin mesor during the SED or EX condition in the HH group. HOMA values associated with diet (OVER vs. EB) and activity (SED vs. EX) treatments are shown in figure 3 and demonstrate a significant increase in response to OVER in the NH group ($p<0.05$), along with a significant decrease in response to EX in both the NH ($p<0.01$) and HH groups ($p<0.01$) suggesting improvements in insulin sensitivity in response to EX.

Repeated measures ANOVA revealed significant effects of EX on 24 h plasma leptin levels relative to SED conditions. 24 h average leptin expressed as percent change from the SED_EB mesor was significantly reduced in response to EX in the NH group (-28±27%, $p<0.05$) and in the HH group (-14±12%, $p<0.05$).

We next investigated the hypothesis that leptin response to OVER was related to insulin response. The leptin mesor for all 13 subjects was significantly correlated with the corresponding insulin mesor within each treatment: SED_EB ($R^2 = 0.34$, $p=0.04$); SED_OVER ($R^2 = 0.41$, $p=0.02$); EX_EB ($R^2 = 0.43$, $p=0.02$); EX_OVER ($R^2 = 0.55$, $p<0.01$). When we investigated the two groups categorized by HOMA, repeated measures ANOVA revealed that 24 h insulin was significantly increased in response to OVER in both the NH group (40±32%, $p<0.01$) and the HH group (35±19%, $p<0.01$) but there was no effect of EX on 24 h insulin levels relative to SED conditions.

Similar analyses of leptin response categorized by HOMA were done on the amplitude (fraction above the mesor) of the 24 h leptin curves in response to OVER during SED and EX conditions grouped by HOMA. There was no significant increase in the amplitude of the 24 h leptin curve in response to OVER during SED conditions in the NH group (EB = 17±2% vs. OVER = 16±3%) or in the HH group (EB = 19±5% vs. OVER = 14±3). Similarly, there was no change in the amplitude of the 24 h leptin curve in response to OVER during the EX visit in the NH group (EB = 20±3% vs. OVER = 18±3) or in the HH group (EB = 16±2% vs. OVER = 17±4).

Ad-libitum breakfast

Ad-libitum breakfast energy intake is presented in figure 6. In response to OVER, the NH group reduced self-selected energy intake during the SED condition (-229 ± 112 kcals, $p<0.01$), and demonstrated a trend for a reduction in self-selected energy intake during the EX condition (-214 ± 249 kcals, $p=0.09$). The HH group also reduced self-selected energy intake during the SED condition in response to OVER (-165 ± 157 kcals, $p<0.05$), but not during EX (-40 ± 242 kcals, n.s.). Similar results were found when energy intake was expressed as the percentage of each subject's average energy expenditure during each treatment.

The relationship between change in leptin mesor in response to overfeeding during each visit ($SED_{OVER-EB}$ and $EX_{OVER-EB}$) and change in *ad-libitum* breakfast food intake is shown in figure 7. Within the NH group, change in leptin mesor in response to OVER was not associated with a corresponding change in *ad-libitum* breakfast food intake in either the SED visit (r = 0.51; 95% C.I. = -0.46 to 0.91) or the EX visit (r = 0.35; 95% C.I. = -0.58 to 0.86). Similarly, within the HH group change in leptin mesor in response to OVER was not associated with a corresponding change in *ad-libitum* breakfast food intake in either the SED visit (r = 0.04; 95% C.I. = -0.69 to 0.72) or the EX visit (r = -0.02; 95% C.I. = -0.71 to 0.70).

Discussion

In a previous study, we reported that 24 h leptin levels in obese males did not respond to three days of moderate overfeeding, suggesting that the peripheral leptin system did not detect excess energy intake (7). These findings contrasted results in individuals with a healthy BMI who displayed an increased 24 h leptin response to the same level of overfeeding (6). In the current study we did not confirm these results when grouping subjects by BMI status, but did display a similar divergence of responses in sedentary overfeeding conditions when categorized

by insulin resistance as estimated by HOMA. Because insulin is involved in regulating plasma leptin levels (10-12), we suggest that categorization by insulin sensitivity is the more robust method to detect changes in circulating leptin in response to changes in energy balance. Although there are no comparable studies of leptin response to overfeeding with which to examine these two methods of categorizing subjects, as HOMA was not measured in the previous studies, elevated HOMA is associated with obesity although this correlation is not perfect (26-30).

To the best of our knowledge, only one study has previously examined the influence of exercise and positive energy balance on 24 h leptin levels. Similar to our results, van Aggel-Leijssen *et al* (31) showed that when energy balance was maintained through increased energy intake to match the greater energy expenditure on a day of relatively high physical activity, the 24 h leptin mesor decreased relative to the non-exercise day. Aggel-Leijssen *et al* (31) also reported that in the exercise treatment the leptin mesor increased during over-feeding. Their subjects were lean males and thus probably are comparable to our NH group. HOMA was not reported in their study nor are we aware of other studies that report both changes in leptin mesor during over-feeding and HOMA which might confirm the differences in leptin responses we report in our NH and HH subjects.

The leptin results from the present study are also similar to work from our lab where Cooper *et al* (32) studied the influence of exercise on appetite hormones during high fat energy balance diets. Our results contrast slightly with the findings of Hilton and Loucks (33) who found in healthy sedentary and exercising women that low energy availability, and not the stress of exercise, suppressed the 24 h mean and amplitude of the leptin curve and the authors concluded that changes in the 24 h rhythm of leptin depended on energy or carbohydrate

availability. A majority of the other previous studies examining the effect of exercise on plasma leptin levels only examined a single time point (e.g. fasting or immediately post-exercise) with little or no effect of acute exercise on these single time-point plasma leptin concentrations (34-37), and exercise training studies are often confounded by interventions designed to induce negative energy balance and corresponding body fat loss (38-40). Our findings and those of others highlight the importance of studying leptin over a 24 h period, as leptin secretion over 24 h has been shown to be pulsatile in nature, with ~32 pulses per 24 h and a pulse duration of ~30 minutes (41).

Although we did not study multiple mediators of leptin response, we can speculate on this. Leptin protein synthesis in adipocytes has been shown to be down-regulated by catecholamine induced β adrenergic stimulation (i.e. exercise) as demonstrated in studies using β-adrenergic agonists (42) and is similar to mechanisms responsible for decreasing leptin protein production in response to fasting (43). In addition, exercise induced improvements in whole-body insulin sensitivity and insulin-independent glucose uptake in skeletal muscle often reduces the relative amount of insulin required from the pancreas to metabolize a given load of carbohydrate. Taken together, exercise induced reductions in leptin production at the level of the adipocyte along with exercise induced decreases in 24 h average insulin levels (even during short-term periods of positive energy balance) may in effect remove the driving force (elevated insulin) behind increased in leptin protein production commonly associated with hyperinsulinemia.

The lack of an association between change in leptin mesor and change in *ad-libitum* breakfast food intake observed in our study does not support the previous findings of Chin-Chance *et al* (6), which suggested leptin was a principal regulator of energy intake following

three days of perturbations in EB. While we cannot rule out the possibility that the lack of a relationship between 24 h leptin levels and self-selected breakfast intake in the present study is a type II error, our data does suggest that the physiological regulation of appetite behavior is more complex than that of the simple leptin theory upon which we speculated based on the previous study in obese men. Indeed, numerous peptide hormones secreted from the gastrointestinal tract, such as ghrelin, glucagon like peptide-1, peptide YY, cholecystokinin, and many others, are known to by physiological regulators of food intake by influencing neuropeptide expression and activity in the brain (44).

Our study has limitations. First, although insulin resistance was not directly measured in the present study, HOMA has been shown to be highly correlated with the euglycemic-hyperinsulinemic clamp technique (26-30) and represents a surrogate marker of insulin resistance. In the two previous studies (6, 7), HOMA was not measured but we speculate that BMI and HOMA may have been correlated.

Second, while both men and women participated in this study, the predominantly female sample may not be representative of the general adult population. Plasma leptin concentrations in women can range approximately two to four times higher compared to men of similar BMI because women have a higher body fat mass at similar BMIs (45). Also, plasma leptin is roughly two to four times higher in obese individuals compared with healthy weight peers of the same sex on account of the higher body fat mass in the obese. Although the between-subject SD for the leptin mesor from all treatments was quite high in each group (NH ~10ng/mL; HH ~16ng/mL), the within-subject SD was smaller (NH ~1ng/mL; HH ~3ng/mL).

Third, in the previous studies in healthy weight (6) and obese men (7), the *ad-libitum* breakfast was a buffet consisting of a wide selection of common U.S. breakfast foods. In the

present study, each subject received the same food (large bowl of yogurt and large muffins) in a family style format at every *ad-libitum* breakfast. While the ad lib breakfast in the present study differed from the previous studies, this change was made to reduce potential confounding by dietary variety associated with a buffet.

Lastly, the use of a metabolic research ward has the potential to decrease an individual's activity level below their usual PAL, even if that person is classified as sedentary in free-living conditions. The average PAL of U.S. adults is between 1.60 and 1.65 (46), while the average inpatient sedentary PAL of our subjects was 1.35. Never-the-less, the greater precision and accuracy of controlling the imposed changes in energy balance compared to other 24 h leptin studies is a strength of our study.

In summary, the results of the present study are a testament to the complexity of the regulation of human appetite. Common human obesity is characterized by hyperleptinemia, which actually suggests an appropriate adaptive response to obesity and represents a state of positive energy balance where energy intake exceeds energy expenditure and the effects of adiposity signals fail to appropriately reduce food intake and adipose stores. Additionally, obese humans are only weakly responsive (or not at all) to exogenously administered leptin (47). It is possible that a lack of an association between change in leptin in response to overfeeding and change in subsequent food intake is not due to a pathological defect in leptin bioavailability, either at the level of the adipocyte or transport across the blood brainer barrier (48), but may simply reflect the limitations of the leptin "system" to regulate food intake and body fat stores as some investigators (49) have suggested the role of leptin is to prevent starvation rather than to avoid obesity. Many rodent models of obesity are characterized by hypothalamic resistance to leptin and insulin, and exercise has been shown to improve central actions of these hormones

(50-53). These data suggest future studies should focus on the central rather than peripheral action of leptin in the regulation of food intake.

Data = mean ± SD	NH (n=1M, 5F)	HH (n=1M, 6F)	*p*-value
HOMA	2.0 ± 0.4	6.5 ± 2.2	<0.001
FPI (μU/mL)	9.4 ± 1.9	27.0 ± 7.5	<0.001
FPG (mg/dL)	86.1 ± 5.5	95.0 ± 8.0	0.04
Age (y)	29 ± 4	25 ± 5	0.12
BW (kg)	66.4 ± 11.5	82.3 ± 15.1	0.06
BMI (kg/m^2)	25.0 ± 5.1	29.6 ± 7.0	0.21
Fat (%)	32.4 ± 7.2	36.1 ± 10.2	0.47
Fat (kg)	21.8 ± 7.4	30.4 ± 12.3	0.16
FFM (kg)	44.6 ± 6.7	51.9 ± 9.6	0.15
VO_2max (ml/kg/min)	25.7 ± 4.3	23.2 ± 3.1	0.26
RMR (kcals/d)	1330 ± 120	1510 ± 190	0.08

Table 1: Subjects baseline characteristics as grouped by homeostasis model assessment (HOMA) estimate of insulin resistance. Normal HOMA (NH; range = 1.4 to 2.3; *n*=6) and high HOMA (HH, range = 3.0 to 8.5, *n*=7); FPI = fasting plasma insulin; FPG = fasting plasma glucose; BW = body weight; BMI = body mass index; FFM = fat free mass; VO_{2max} = maximal aerobic fitness; RMR = resting metabolic rate. Data = mean ± SD.

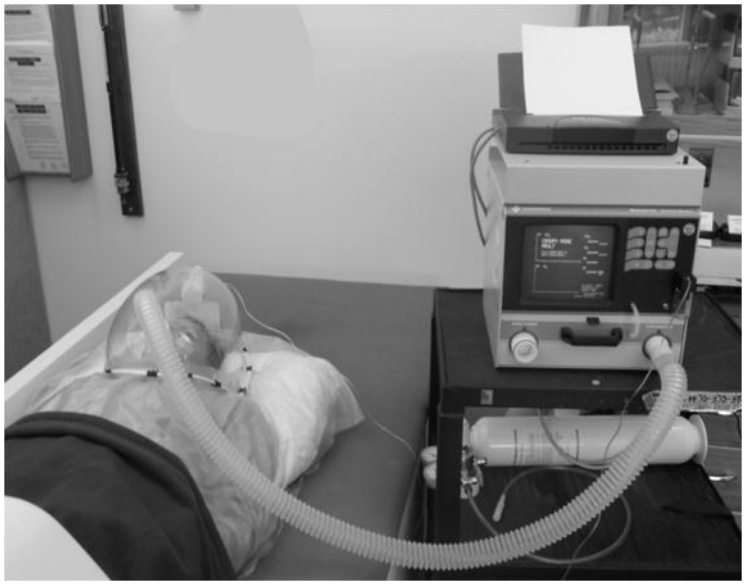

Figure 1: The Deltatrac (DTC) metabolic cart indirect calorimeter system in-use. During testing, subjects were instructed to remain motionless without sleeping while respiratory gases were drawn from a clear hood canopy placed over the participant's entire head, as shown. The final 20 minutes were used to calculate respiratory exchange ratio (RER; $VCO_2 \div VO_2$), as well as RMR (kcals/day) using the modified Weir equation (17): $[3.941 \times VO_2] + [1.106 * VCO_2]$

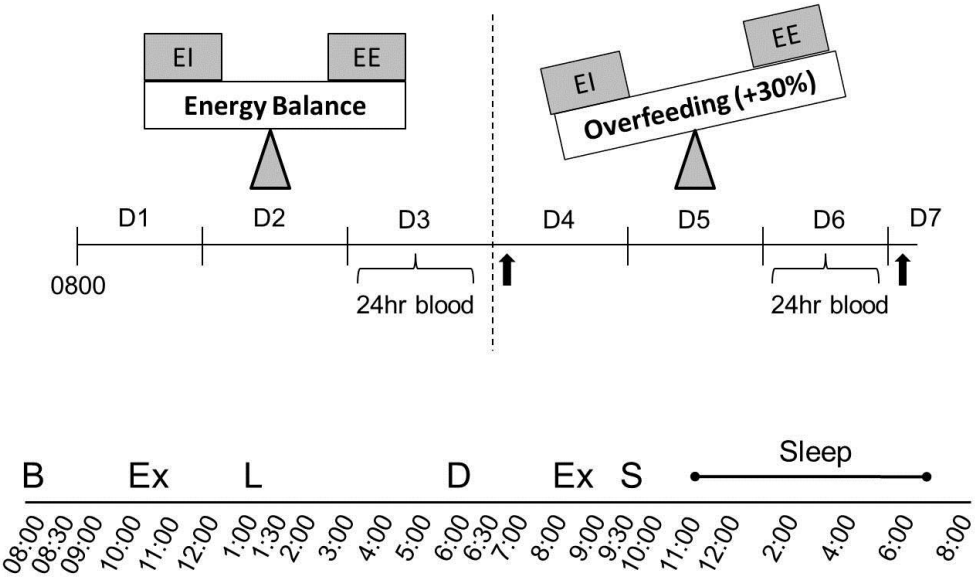

Figure 2: Study Protocol. In a randomized cross-over design, each participant took part in two separate one-week long interventions separated by a minimum of four weeks. Subjects were kept in energy balance during the first three days, and then overfed by 30% of daily energy balance requirements during the last 3 days. During one visit, subjects remained sedentary (kcals/d ≈ 1.4 x resting metabolic rate) and during the other visit exercised twice each day (kcals/d ≈ 1.8 x resting metabolic rate). EI = energy intake; EE = energy expenditure. The timeline on the bottom represents the blood draw, eating, and exercise schedule on days 3 and 6 of each treatment. B, L, D, S = breakfast, lunch, dinner, snack; Ex = during the exercise visit, subjects cycled on an indoor bicycle twice daily for ≈60 minutes @ ≈50% VO_{2max}; black arrow = *Ad-libitum* breakfast.

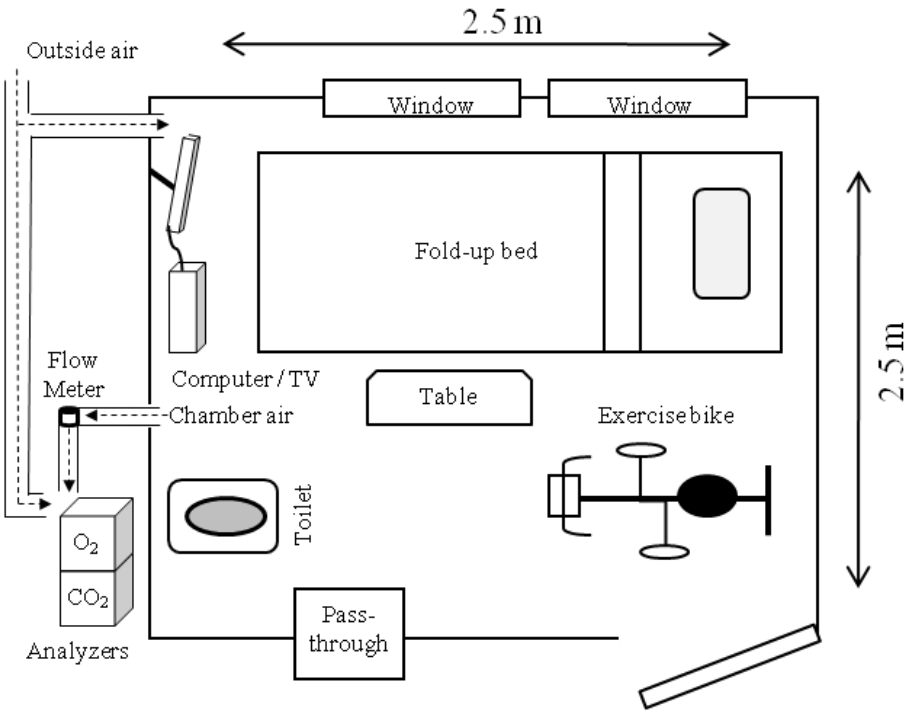

Figure 3: Diagram of the whole room indirect calorimeter (i.e. metabolic chamber) at the UW-Madison CRU. The design is similar to the metabolic chamber in the Department of Human Biology at Maastricht University in Maastricht, The Netherlands (22) and the specifications and diagnostics have been previously described (23). Additionally, information on chamber temperature, humidity, airflow, pressure, and data collection instrumentation has been described elsewhere (24). Briefly, the composition of air is measured with CO_2 (Hartman and Braun Uras-4) and O_2 (Magnos-6) gas analyzers (Applied Automation, Bartlesville, OK). The chamber was calibrated against gravametric methanol burns prior to each study visit. The percentage recoveries were used to develop correction factors for the corresponding chamber data for each study visit. The precision of the corrections averaged 5.4% and 3.5% for O_2 and CO_2, respectively.

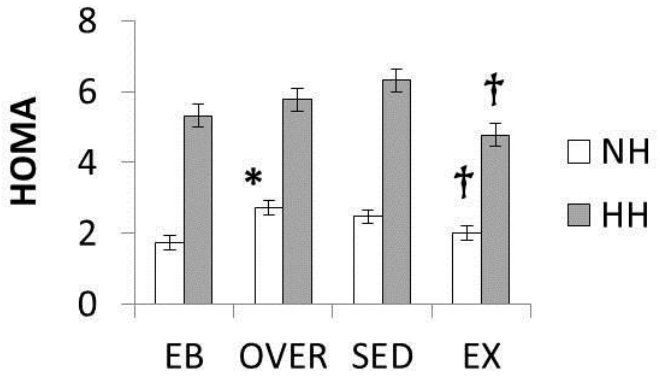

Figure 4: Homeostasis model assessment estimated insulin resistance (HOMA) during each visit in subjects with a normal HOMA (NR, n=6) and high HOMA (HR, n=7). EB = energy balance, OVER = overfeeding, SED = sedentary, EX = exercise. Data presented as mean ± SE. * = OVER significantly different from EB within NH group ($p < 0.05$). † = EX significantly different from SED within group ($p < 0.05$). Data = mean ± SEM

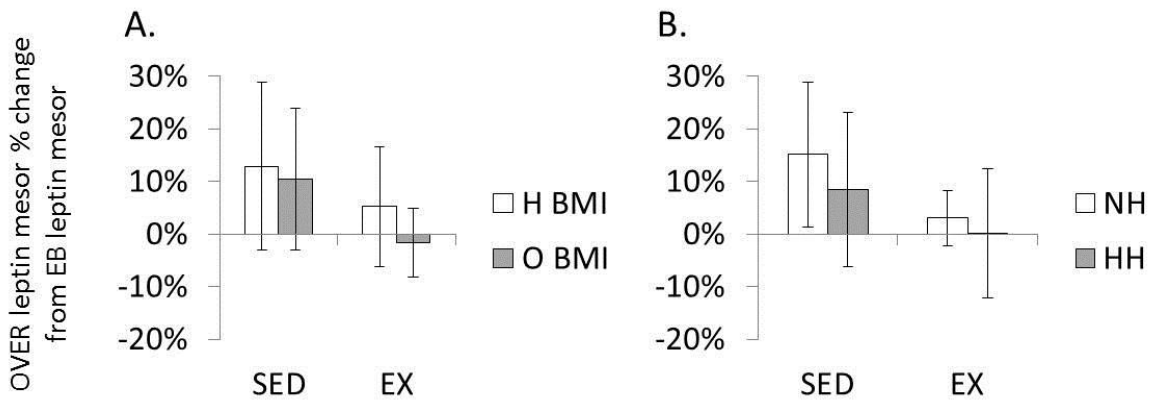

Figure 5: Percent change (±SD) of the overfeeding (OVER) leptin mesor from the energy balance (EB) leptin mesor during the sedentary (SED) and exercise (EX) visit. Graph on the left = subjects grouped according to BMI status; healthy BMI (H BMI, n=6) vs. obese BMI (O BMI, n=7). Graph on the right = the same subjects re-grouped by HOMA status; normal HOMA (NH, n=6) vs. high HOMA (HH, n=7). * = The only significant increase in the leptin mesor in response to OVER occurred in the NH group during the SED visit (p=0.02). Exercise did not normalize (increase) the 24 h leptin response to OVER in the obese or high HOMA groups. Data = mean ± SD

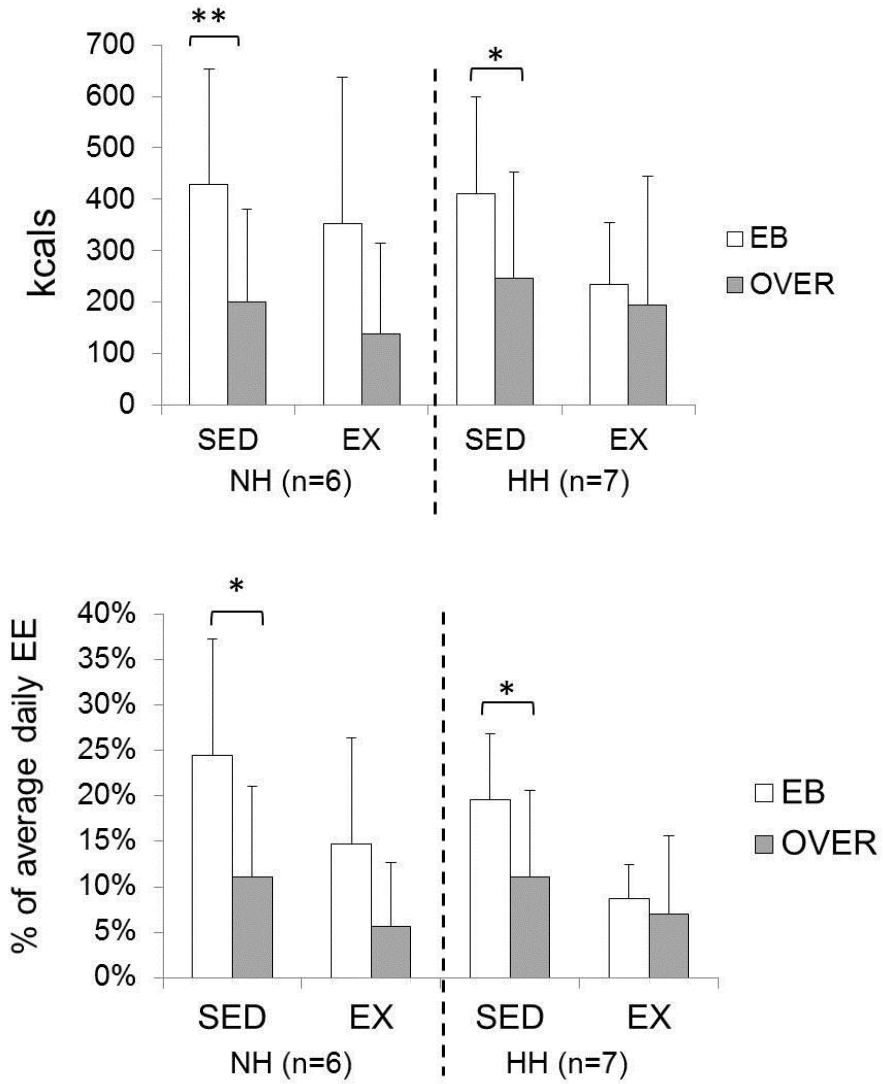

Figure 6: *Ad-libitum* breakfast energy intake. SED = sedentary; EX = exercise; EB = energy balance; OVER = overfeeding. Self-selected energy intake was significantly reduced in response to OVER during the SED condition in the NH group (-229±112 kcals, ** = $p<0.01$) and in the HH group (-165±157 kcals, * = $p<0.05$). There was a trend for a reduction in energy intake in response to OVER during the EX condition in the NH group (-214±249 kcals, $p=0.09$). Data = mean ± SD

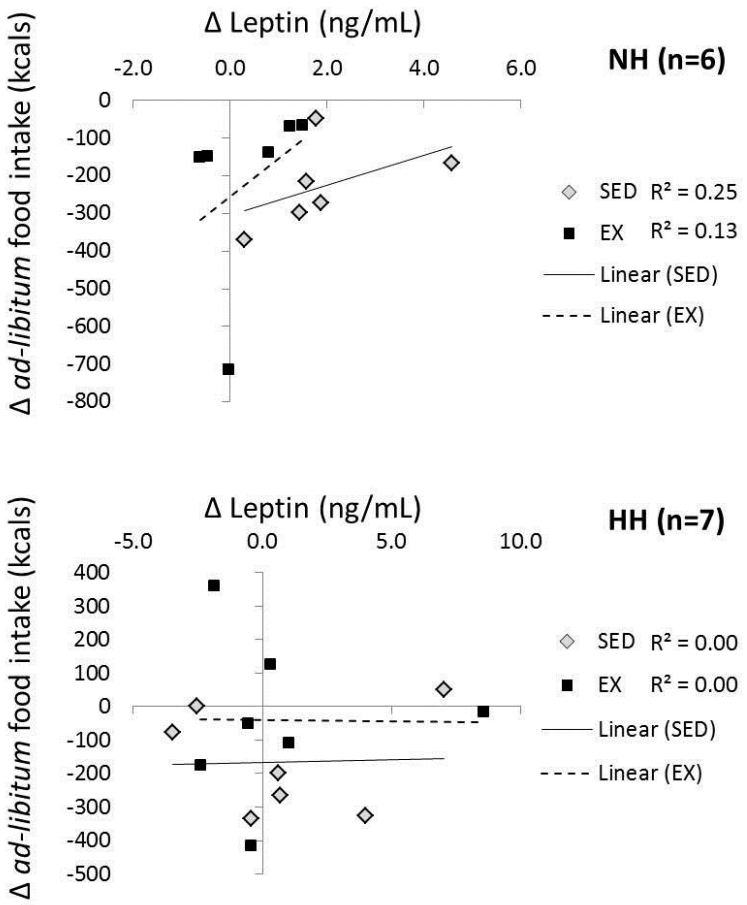

Figure 7: Relationship between change in mesor leptin levels in response to overfeeding and change in *ad-libitum* breakfast food intake following overfeeding during the sedentary (SED) and exercise (EX) visit for each group. No significant correlations were found between change in mesor leptin levels in response to overfeeding and change in *ad-libitum* breakfast food intake

References

1. Harris RB, Kasser TR, Martin RJ. Dynamics of recovery of body composition after overfeeding, food restriction or starvation of mature female rats. The Journal of nutrition 1986;116(12):2536-46.
2. Harris RB, Martin RJ. Changes in lipogenesis and lipolysis associated with recovery from reversible obesity in mature female rats. Proc Soc Exp Biol Med 1989;191(1):82-9.
3. Harris RB, Martin RJ. Metabolic response to a specific lipid-depleting factor in parabiotic rats. The American journal of physiology 1986;250(2 Pt 2):R276-86.
4. Roberts SB, Young VR, Fuss P, et al. Energy expenditure and subsequent nutrient intakes in overfed young men. The American journal of physiology 1990;259(3 Pt 2):R461-9.
5. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994;372(6505):425-32.
6. Chin-Chance C, Polonsky KS, Schoeller DA. Twenty-four-hour leptin levels respond to cumulative short-term energy imbalance and predict subsequent intake. The Journal of clinical endocrinology and metabolism 2000;85(8):2685-91.
7. Cooper JA, Polonsky KS, Schoeller DA. Serum leptin levels in obese males during over- and underfeeding. Obesity (Silver Spring) 2009;17(12):2149-54.
8. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 2001;414(6865):799-806.
9. Saladin R, De Vos P, Guerre-Millo M, et al. Transient increase in obese gene expression after food intake or insulin administration. Nature 1995;377(6549):527-9.
10. Saad MF, Khan A, Sharma A, et al. Physiological insulinemia acutely modulates plasma leptin. Diabetes 1998;47(4):544-9.

11. Lee MJ, Wang Y, Ricci MR, Sullivan S, Russell CD, Fried SK. Acute and chronic regulation of leptin synthesis, storage, and secretion by insulin and dexamethasone in human adipose tissue. American journal of physiology Endocrinology and metabolism 2007;292(3):E858-64.

12. Lee MJ, Yang RZ, Gong DW, Fried SK. Feeding and insulin increase leptin translation. Importance of the leptin mRNA untranslated regions. The Journal of biological chemistry 2007;282(1):72-80.

13. Donnelly JE, Blair SN, Jakicic JM, Manore MM, Rankin JW, Smith BK. American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. Medicine and science in sports and exercise 2009;41(2):459-71.

14. Stunkard AJ, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. Journal of psychosomatic research 1985;29(1):71-83.

15. Schoeller DA, Dietz W, van Santen E, Klein PD. Validation of saliva sampling for total body water determination by H2 18O dilution. The American journal of clinical nutrition 1982;35(3):591-4.

16. Cooper JA, Watras AC, O'Brien MJ, et al. Assessing validity and reliability of resting metabolic rate in six gas analysis systems. Journal of the American Dietetic Association 2009;109(1):128-32.

17. Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. The Journal of physiology 1949;109(1-2):1-9.

18. Storer TW, Davis JA, Caiozzo VJ. Accurate prediction of VO2max in cycle ergometry. Medicine and science in sports and exercise 1990;22(5):704-12.

19. Garber CE, Blissmer B, Deschenes MR, et al. American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. Medicine and science in sports and exercise 2011;43(7):1334-59.

20. Smith SR, de Jonge L, Zachwieja JJ, et al. Concurrent physical activity increases fat oxidation during the shift to a high-fat diet. The American journal of clinical nutrition 2000;72(1):131-8.

21. Schoeller DA, Shay K, Kushner RF. How much physical activity is needed to minimize weight gain in previously obese women? The American journal of clinical nutrition 1997;66(3):551-6.

22. Schoffelen PF, Westerterp KR, Saris WH, Ten Hoor F. A dual-respiration chamber system with automated calibration. Journal of applied physiology: respiratory, environmental and exercise physiology 1997;83(6):2064-72.

23. Votruba SB, Atkinson RL, Hirvonen MD, Schoeller DA. Prior exercise increases subsequent utilization of dietary fat. Medicine and science in sports and exercise 2002;34(11):1757-65.

24. Hansen KC, Zhang Z, Gomez T, Adams AK, Schoeller DA. Exercise increases the proportion of fat utilization during short-term consumption of a high-fat diet. The American journal of clinical nutrition 2007;85(1):109-16.

25. Schoeller DA, van Santen E. Measurement of energy expenditure in humans by doubly labeled water method. Journal of applied physiology: respiratory, environmental and exercise physiology 1982;53(4):955-9.

26. Colson A, Walker D, Besser GM, et al. Role of fasting plasma glucose, glycated haemoglobin and homeostatic model assessment in the detection of glucose intolerance in adult hypopituitary patients during growth hormone replacement therapy. Hormone research 2008;69(2):83-8.

27. Tresaco B, Bueno G, Pineda I, Moreno LA, Garagorri JM, Bueno M. Homeostatic model assessment (HOMA) index cut-off values to identify the metabolic syndrome in children. Journal of physiology and biochemistry 2005;61(2):381-8.

28. Dixon JB, Dixon AF, O'Brien PE. Improvements in insulin sensitivity and beta-cell function (HOMA) with weight loss in the severely obese. Homeostatic model assessment. Diabetic medicine : a journal of the British Diabetic Association 2003;20(2):127-34.

29. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7):412-9.

30. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes care 2004;27(6):1487-95.

31. van Aggel-Leijssen DP, van Baak MA, Tenenbaum R, Campfield LA, Saris WH. Regulation of average 24h human plasma leptin level; the influence of exercise and physiological changes in energy balance. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 1999;23(2):151-8.

32. Cooper JA, Watras AC, Paton CM, Wegner FH, Adams AK, Schoeller DA. Impact of exercise and dietary fatty acid composition from a high-fat diet on markers of hunger and satiety. Appetite 2011;56(1):171-8.

33. Hilton LK, Loucks AB. Low energy availability, not exercise stress, suppresses the diurnal rhythm of leptin in healthy young women. American journal of physiology Endocrinology and metabolism 2000;278(1):E43-9.

34. Hickey MS, Considine RV, Israel RG, et al. Leptin is related to body fat content in male distance runners. The American journal of physiology 1996;271(5 Pt 1):E938-40.

35. Houmard JA, Cox JH, MacLean PS, Barakat HA. Effect of short-term exercise training on leptin and insulin action. Metabolism: clinical and experimental 2000;49(7):858-61.

36. Perusse L, Collier G, Gagnon J, et al. Acute and chronic effects of exercise on leptin levels in humans. Journal of applied physiology: respiratory, environmental and exercise physiology 1997;83(1):5-10.

37. Racette SB, Coppack SW, Landt M, Klein S. Leptin production during moderate-intensity aerobic exercise. The Journal of clinical endocrinology and metabolism 1997;82(7):2275-7.

38. Kohrt WM, Landt M, Birge SJ, Jr. Serum leptin levels are reduced in response to exercise training, but not hormone replacement therapy, in older women. The Journal of clinical endocrinology and metabolism 1996;81(11):3980-5.

39. Kraemer RR, Kraemer GR, Acevedo EO, et al. Effects of aerobic exercise on serum leptin levels in obese women. European journal of applied physiology and occupational physiology 1999;80(2):154-8.

40. Okazaki T, Himeno E, Nanri H, Ogata H, Ikeda M. Effects of mild aerobic exercise and a mild hypocaloric diet on plasma leptin in sedentary women. Clinical and experimental pharmacology & physiology 1999;26(5-6):415-20.

41. Licinio J, Mantzoros C, Negrao AB, et al. Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. Nature medicine 1997;3(5):575-9.

42. Zhang F, Chen Y, Heiman M, Dimarchi R. Leptin: structure, function and biology. Vitamins and hormones 2005;71:345-72.

43. Lee MJ, Fried SK. Integration of hormonal and nutrient signals that regulate leptin synthesis and secretion. American journal of physiology Endocrinology and metabolism 2009;296(6):E1230-8.

44. Naslund E, Hellstrom PM. Appetite signaling: from gut peptides and enteric nerves to brain. Physiology & behavior 2007;92(1-2):256-62.

45. Hickey MS, Israel RG, Gardiner SN, et al. Gender differences in serum leptin levels in humans. Biochemical and molecular medicine 1996;59(1):1-6.

46. Institute of Medicine of the National Academies. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. The National Academies Press., 2005.

47. Heymsfield SB, Greenberg AS, Fujioka K, et al. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. JAMA : the journal of the American Medical Association 1999;282(16):1568-75.

48. Schwartz MW, Peskind E, Raskind M, Boyko EJ, Porte D, Jr. Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. Nature medicine 1996;2(5):589-93.

49. Flier JS. Clinical review 94: What's in a name? In search of leptin's physiologic role. The Journal of clinical endocrinology and metabolism 1998;83(5):1407-13.

50. Ropelle ER, Flores MB, Cintra DE, et al. IL-6 and IL-10 anti-inflammatory activity links exercise to hypothalamic insulin and leptin sensitivity through IKKbeta and ER stress inhibition. PLoS biology 2010;8(8).

51. Ropelle ER, Fernandes MF, Flores MB, et al. Central exercise action increases the AMPK and mTOR response to leptin. PloS one 2008;3(12):e3856.

52. Flores MB, Fernandes MF, Ropelle ER, et al. Exercise improves insulin and leptin sensitivity in hypothalamus of Wistar rats. Diabetes 2006;55(9):2554-61.

53. Zhao J, Tian Y, Xu J, Liu D, Wang X, Zhao B. Endurance exercise is a leptin signaling mimetic in hypothalamus of Wistar rats. Lipids in health and disease 2011;10:225.

Chapter 4

Relationships between perceived appetite, food intake, and appetite regulating hormones in sedentary humans in response to overfeeding and exercise

Chad M. Cook, MS, Britt Erikkson, PhD, Alexandra Adams, MD, PhD, Dale A. Schoeller, PhD

Abstract

Introduction: Daily exercise (EX) during short-term positive energy balance (EB) may improve objective and subjective appetite regulation in obese individuals by modulating plasma levels of hormones involved in the homeostatic regulation of dietary behavior. **Objective:** To examine perceived hunger and peripheral concentrations of leptin and peptide YY (PYY) at two levels of physical activity. **Methods:** In a randomized cross-over design, 13 habitually sedentary individuals (19-39y) were in-patients in a metabolic research ward for two different six-consecutive overnight visits. During one visit, subjects remained sedentary (SED) and during the other performed cycling EX at 50% VO_{2max} twice daily. Subjects were in EB on days 1-3 and overfed (OVER) on days 4-6 of each visit. Multiple blood samples and visual analog scale appetite questionnaires were taken over 24h on days 3 & 6 of each visit. The morning after EB and OVER, subjects were fed an *ad-libitum* breakfast. **Results:** All 13 participants were analyzed together. Significant reductions in average *ad-libitum* breakfast intake in response to OVER and EX were not explained by relationships with changes in meal related perceived hunger or appetite hormone levels. Also, beneficial changes in 24h average perceived appetite (decreased hunger/increased fullness) were found in response to OVER and EX, but these changes were not explained by relationships with 24h hormone values. Neither perceived appetite nor hormones measured explained more than 5% of the variance in *ad-libitum* breakfast energy intake following OVER. **Conclusions:** Subjective appetite and self-selected food intake are likely influenced by environmental and hedonic aspects of food that may override homeostatic biological mechanisms regulating appetite.

Introduction

The prevalence of obesity has reached epidemic proportions worldwide, with more than 1.5 billion adults considered overweight (BMI >25 kg/m2) and at least 500 million clinically obese (BMI >30 kg/m2) (1). It is generally accepted that the steady increase in obesity prevalence over the last several decades has paralleled an increase in the consumption of energy-dense foods along with an increasingly sedentary lifestyle; however, a better understanding of the causes of obesity lies in the study of the interactions between food intake and energy expenditure. To this end, the influence of exercise on appetite regulation has drawn renewed interest.

Cross-sectional studies have shown that food intake regulation in response to covertly manipulated breakfast pre-loads is more accurate in active vs. sedentary adults over the course of a day (2, 3). Martins *et al* (4) demonstrated significant improvements in the ability of previously sedentary individuals to self-regulate food intake following moderate-term (i.e. 6 weeks) exercise training. These data suggest maintaining a level of high habitual physical activity improves day-to-day appetite regulation by leading to a more sensitive eating behavior in response to previous energy intake, which may facilitate long-term energy balance. Improved appetite control associated with regular exercise may derive from alterations in subjective feelings of hunger and/or fullness mediated via changes in plasma levels of appetite-regulating hormones induced by the physiological stimulus of exercise. Of particular interest have been the hormones leptin and peptide YY (PYY).

Leptin is a 16-kDa circulating hormone produced primarily by adipose tissue in proportion to the amount of lipid stores and is best known for its role as a signal of chronic nutritional state and regulator of long-term energy balance (5). Previously, we could not confirm

results from a past study that demonstrated an association between the 24 h leptin response to overfeeding and subsequent food intake at an *ad-libitum* breakfast meal. Our most recent study included both three-day sedentary or exercise conditions in habitually sedentary individuals with low or high values for estimated whole body peripheral insulin resistance (chapter 3); however, we did find that average self-selected breakfast food intake was reduced in both groups of individuals following three-days of overfeeding and tended to be reduced during exercise compared to sedentary conditions. These findings suggest additional mechanisms are functioning in the physiological regulation of appetite during short-term changes in energy balance. Indeed, numerous peptide hormones have been found to modulate feelings of hunger and satiety and of these, PYY has received much attention.

It has been shown that PYY functions as an episodic signal of satiety (6), being released into circulation from endocrine L cells in the distal gastrointestinal tract partly in proportion to calories ingested such that plasma levels rise within 15 minutes, plateau at approximately 90 minutes, and fall back to pre-meal levels at a rate dependent on calories ingested (7). Aerobic physical activity of sufficient intensity and duration may alter plasma concentrations of PYY in a direction expected to suppress energy intake. Martins et al. (8) showed that PYY was elevated during and immediately after 60 minutes of moderate intensity aerobic exercise. Similarly, Broom et al. (9) found that aerobic exercise elevated PYY concentrations and suppressed hunger. These data lend physiological support to previous observational evidence that has shown acute exercise (at least aerobic exercise of moderate intensity) does not increase energy intake in the short term (10); however, further research is required to determine how long the exercise induced changes persist and whether the changes have any effect on subsequent subjective appetite or food intake.

In our previous study, we did not examine other peptide hormones that may impact changes in subjective appetite and food intake following overfeeding or exercise. We propose that the previously observed reductions in self-selected breakfast intake in response to overfeeding and exercise are related to changes in fasting and post-prandial plasma insulin and PYY levels. Thus, the aim of this study was to expand on our previous work by characterizing the relationship between changes in self-selected food intake, perceived appetite, and hormone (insulin, leptin, and PYY_{3-36}) responses to overfeeding and exercise in habitually sedentary individuals.

Methods

Study design

This was a sub-study to an investigation (chapter 3) of the 24 h plasma leptin response to exercise (EX) and overfeeding (OVER). In a randomized cross-over design, subjects were seen as in-patients for two different six-consecutive overnight visits separated by four weeks. During one of the visits subjects were sedentary (SED) and during the other visit exercised twice daily. All study visits took place at the University of Wisconsin-Madison Hospital's Clinical Research Unit (CRU). Each SED and EX visit consisted of two consecutive treatment periods lasting three days each. Subjects were kept in energy balance (EB) during the first three days of each visit followed by three days of OVER. During the SED visit, subjects were fed a diet to maintain EB at 1.4 x RMR for the first three days (SED_EB), followed by three days of OVER at 130% of SED_EB requirements (SED_OVER). During the EX visit, subjects were fed a diet to maintain EB at 1.8 x RMR for the first three days (EX_EB), followed by three days of OVER at 130% of

EX_EB requirements (EX_EB). All female subjects completed each visit during the early part of the follicular phase of their menstrual cycle.

Study Subjects

Thirteen healthy individuals (males n=2; females n=11) aged 19–39 y participated in this study. Individuals who exercised regularly or did not eat three meals a day (including breakfast or an early morning meal) at least five days/week were excluded. Additional exclusion criteria included current tobacco use, a history of metabolic or cardiovascular disease, history of claustrophobia (which might preclude use of a whole-room indirect calorimeter in this study), currently following a special diet (e.g. vegetarian, low-carbohydrate), use of prescription medications (other than oral hormonal birth control), use of over-the-counter substances that could potentially alter energy metabolism, and females who were pregnant, lactating, or experienced irregular menstrual cycles. The study was approved by the University of Wisconsin-Madison IRB and all subjects provided written informed consent. The study was performed at the Clinical Research Unit (CRU) at the University of Wisconsin-Madison Hospital.

Protocol

Treatment conditions are described in the study design section above. Prior to participation in study visits, subjects underwent a physical exam and screening where a fasting blood sample was taken to screen for abnormal blood lipids, glucose, insulin, thyroid stimulating hormone, hemoglobin, and hematocrit levels. Resting metabolic rate and estimated VO_{2max} were measured during screening via indirect calorimetry, as described previously (chapter 3). These

measures were used to establish energy intake, as well as exercise intensity and duration. For each SED and EX study visit, participants stayed in a metabolic chamber at the CRU and visits were separated by four weeks.

On the third and sixth day of each visit, an intravenous catheter was placed in the antecubital space of the subject at 0730 h and kept patent with saline. Blood draws took place every hour from 08:00 to 23:00, every 30 minutes after each meal (08:30, 13:30, 18:30, 21:30), and every two hours after midnight to minimize potential sleep disruption until 08:00 the following morning. Blood was drawn into chilled pink-top K_2 EDTA tubes (BD Vacutainer) and immediately spun at 1800 x g for 15 min at 4ºC. Aprotinin (0.6 TIU/mL, Phoenix Pharmaceuticals Inc., Burlingame, CA) and dipeptidyl peptidase IV inhibitor (10 μU/mL, item # DPP4; Linco Research, Inc., St. Charles, MO) was added to the plasma following centrifugation. Plasma was then aliquoted and stored at -80ºC until assayed.

Participants also completed 100mm visual analog scale (VAS) questionnaires to measure hunger, fullness, and prospective consumption (how much they thought they could eat), as previously developed (11). The questionnaire was given every hour from 0800 until 2300 h on days three and six of each visit, as well as before and after the ad-libitum breakfast (07:30 and 08:00) on days four and seven.

For the EX visits, cycling exercise was completed at 50% of VO2max for 2 h during each day, once in the morning from 10:00 to 11:00 and once in the evening from 20:00 to 21:00. A visual analog scale (VAS) was completed hourly between 0800 and 2300 h to assess subjective appetite. The morning after each three day treatment period, an *ad-libitum* breakfast was served to objectively measure self-selected food intake.

Diet

All meals were prepared by the research dietician at the hospital CRU. Daily energy intake was divided over three meals and one snack as follows: Breakfast 20% (except during *ad-libitum* breakfast described below); lunch 30%; dinner 35%, and snack 15% and each meal comprised 50% carbohydrates, 35% fat, and 15% protein. Breakfast was served at 8:00 am, lunch at 1:00 pm, dinner at 6:00 pm, and the snack at 9:00 pm. Subjects were required to consume all of the food provided to them, except for the *ad-libitum* breakfast (described below).

The first meal after each three day treatment period (morning of day four and seven) was an *ad-libitum* breakfast provided in a family style format. Subjects were given 30 minutes and instructed to "eat until comfortably full." Foods were pre-weighed and post-weighed to calculate energy intake. Each participant received the same food at every *ad-libitum* breakfast, which included a large bowl of yogurt and extra-large muffins, each made by the CRU dietician to include the same relative proportion of fat, carbohydrate, and protein as the other study meals. The energy contents of the remaining meals on day four were the amounts that would normally be given during days five and six of each visit. Any adjustments needed to match total daily energy intake to the treatment period based on how much was consumed at the *ad-libitum* breakfast were made using the evening snack.

Hormone measurements

Leptin was measured in plasma in duplicate by radio-immunoassay (RIA) (kit# HL-81K; Linco Research, Inc., St. Charles, MO) with a lowest limit of detection of 0.5ng/mL and average intra- and inter-assay coefficients of variation of 5.7% and 9.2%, respectively. PYY_{3-36} was also measured in plasma in duplicate by RIA (kit# PYY-67HK; Linco Research, Inc., St. Charles,

MO) with a lowest limit of detection of 20pg/mL and average intra-assay and inter-assay coefficient of variation (CV) of 10.9% and 13.7%, respectively. While high, these CV's are within published values supplied with the assay kit, as well as within the range of CV's reported by others (12). All samples for each subject were analyzed in a single assay kit.

Statistical analysis

SAS version 8.2 (SAS Institute Inc, Cary, NC) was used for all data analysis with subjects serving as their own controls due to the crossover design of the SED and EX visits. The 24 h weighted average of hunger, fullness, leptin, and PYY were each calculated from all available time points during each 24 h treatment period. Changes in subjective appetite and ad-libitum breakfast intake in response to OVER and EX were analyzed using ANOVA with repeated measures with all 13 subjects in one group. Pearson correlations were used to examine relationships between changes in meal related subjective appetite and hormones in response to OVER and EX with corresponding change in *ad-libitum* breakfast food intake in response to OVER and EX. To examine the effects of multiple variables on change in *ad-libitum* breakfast food intake in response to OVER and EX, a linear mixed effects model was used to examine the effect of treatment, age, sex, race, baseline BW, baseline HOMA status, change in meal related hunger (post breakfast – pre breakfast) in response to OVER and EX, change in 24h average leptin in response to OVER and EX, and changes in *ad-libitum* breakfast associated insulin and PYY_{3-36} (post breakfast – pre breakfast) responses to OVER and EX. The best model was found using backward elimination by eliminating insignificant variables in a stepwise manner. Results are presented as Mean ± SD unless otherwise stated. Statistical significance was set at $p \leq 0.05$.

Results

Participants

This study was an add-on to an investigation of the effects of exercise and overfeeding on leptin and *ad-libitum* food intake in sedentary individuals grouped according to degree of estimated insulin resistance as defined by the homeostasis model assessment (HOMA) method: Normal HOMA group (n= 1 male, 5 female) and high HOMA group (n=1 male, 6 female). For the present analysis, no significant correlations were found between any of the planned comparisons when subjects were analyzed by HOMA group within each treatment; therefore, all 13 participants were combined and analyzed together. Five participants were non-Hispanic Caucasian, three were Hispanic, and five were African-American with a group mean age of 26.6 ± 4.7 years and baseline body mass index (BMI) of 27.5 ± 6.4 kg/m^2. Per eligibility criteria, all subjects were sedentary (<3 h per week of low to moderate exercise and no vigorous exercise) and subjects were identified as non-restrained eaters using the Three Factor Eating Questionnaire (13).

Ad-libitum breakfast appetite responses

Within subject *ad-libitum* breakfast intake was significantly reduced in response to OVER compared to energy balance (EB) conditions (OVER - EB = -157±72kcals 95% CI, p<0.01) and in response to EX compared to sedentary conditions (EX - SED = -93±72kcals 95% CI, p<0.01; however, change in hunger during the *ad-libitum* breakfast (post breakfast – pre breakfast) was not associated with change in *ad-libitum* food intake in response to OVER (r = 0.16±0.35, 95% CI) or EX (r = -0.26±0.32, 95% CI). Also, changes in PYY during the *ad-libitum* breakfast (post breakfast PYY – pre breakfast PYY) in response to OVER or EX was not

inversely associated with corresponding change in *ad-libitum* breakfast food intake (Figure 1A = OVER; Figure 1B = EX). Although there were no significant correlations between changes in PYY and changes in *ad-libitum* breakfast intake, the relationship between changes in PYY and change in subjective appetite during the ad-libitum breakfast in response to OVER or EX was assessed to determine if change in PYY was inversely correlated with perceived hunger or positively correlated with perceived fullness. As shown in figure 3, change in PYY immediately after the ad-libitum breakfast PYY was not inversely associated with post-breakfast hunger in response to OVER (Figure 1A) or EX (Figure 1B). Similar non-significant correlations were found for each previously described analysis when feelings of fullness were used in place of hunger (data not shown) as change in hunger and change in fullness during the *ad-libitum* breakfast were significantly inversely correlated (r= -0.55±23, 95% CI; $p<0.01$).

A linear mixed effects model with backward elimination was used to examine the effects of multiple variables on change in *ad-libitum* breakfast food intake. Significant variables in the final model predicting change in *ad-libitum* breakfast food intake included the categorical variables sex ($p<0.01$) and race ($p<0.01$), along with the continuous variables baseline body weight ($p<0.05$) and change in breakfast insulin (post breakfast – pre breakfast) in response to OVER (figure 2; $p<0.001$). As shown in figure 2A, the change in *ad-libitum* breakfast insulin response (post breakfast – pre breakfast) to overfeeding was significantly positively correlated with change in food intake.

24 h appetite responses

Within subject 24h average hunger was significantly reduced in response to overfeeding compared to energy balance conditions (OVER - EB = -11±4mm 95% CI, $p<0.01$) and in

response to EX compared to sedentary conditions (EX - SED = -6±4mm 95% CI, $p<0.01$. Additionally, within subject 24h average fullness was significantly increased in response to OVER compared to energy balance conditions (OVER - EB = 7±4mm 95% CI, $p<0.01$) and in response to EX compared to sedentary conditions (EX - SED = 9±4mm 95% CI, $p<0.01$); however, these changes in subjective appetite were not explained by relationships with corresponding changes in 24h average leptin in response to OVER or EX, as shown in figure 3. These changes in subjective appetite were also not explained by relationships with corresponding changes in 24h average PYY (data not shown).

Discussion

While investigators have previously studied the effects of exercise on appetite, this study is one of the few to replace energy expended during exercise in an attempt to tease out the effects of exercise *per se* from negative energy balance on appetite responses, as well as to examine the effects of exercise on appetite during overfeeding. Other unique aspects of this study include the controlled research setting allowing precise and accurate measurements of daily energy expenditure and energy intake, along with subjective and objective measures of hunger and satiety over 24 h periods. Our findings suggest that habitually sedentary individuals are able to detect three-day increases in food intake relative to energy balance requirements over the course of an entire day, and aerobic exercise was associated with beneficial changes in 24 h measures of hunger and fullness compared to sedentary conditions; however, changes in subjective appetite responses to overfeeding or exercise were not explained by relationships with the putative appetite regulating hormones, leptin and PYY.

Previously, we reported a lack of an association between the 24 h leptin response to overfeeding and subsequent food intake at an *ad-libitum* breakfast meal during sedentary or exercise conditions in habitually sedentary individuals with low or high values for estimated whole body peripheral insulin resistance (chapter 3). In the present expanded data set, we also were unable to find a significant relationship between leptin and subjective appetite when subjects were grouped together. While we cannot rule out the risk of a type II error, the collective body of work suggests 24h average leptin does not reflect average daily hunger or fullness or influence feeding at a subsequent morning meal; however, more work is required to further delineate the role of leptin in the regulation of appetite response to short-term perturbations in energy balance or energy flux (i.e. exercise).

Most research examining the effects of exercise on appetite has focused on plasma levels of PYY, as this hormone is known to have acute episodic appetite suppressing effects (7). Interestingly, our data show no change in 24 h PYY or single meal changes in PYY associated with an ad-libitum breakfast following three-day overfeeding or exercise. This data is in disagreement with others, as it has been reported that mean plasma PYY concentration increase and hunger sensations temporarily decrease during and for a short while after resistance and aerobic exercise in both healthy weight (8, 10, 14) and obese (15) individuals. These effects are often short-lived, typically only detected during and immediately after cessation of the exercise bout. Thus, the within-day changes in PYY after exercise appear to be washed-out when data from the entire 24 h period is compared. We suggest that more frequent blood sampling may be needed to evaluate the extent of the within-day exercise effect and also to increase the precision of the 24 h mesor values which is limited under conditions of infrequent blood sampling because post-meal spikes are not optimally measured. Furthermore, it has recently been shown that

increased PYY levels in response to exercise depends on the intensity of exercise, with higher intensity exercise eliciting more pronounced changes in PYY (14) and our study employed low-to-moderate intensity exercise. Although these studies show that plasma PYY levels change during and after exercise, objective measures of food intake, subjective appetite, and PYY are often not measured over the course of a whole day and it does not necessarily follow that food intake will change. Further research is required to examine the influence of exercise training on changes in PYY and corresponding capacity to regulate satiety and influence body weight in habitually sedentary and obese individuals.

The 24 h subjective appetite response to exercise in the present study differs from previous work from our lab, in which eight healthy weight males fed eucaloric high fat diets differing in saturated and unsaturated fatty acids did not show a 24 h treatment effect for exercise vs. sedentary conditions (12). This previous study did, however, detect significant correlations between 24 h hunger and fullness and 24 h changes in leptin, insulin, and total ghrelin but not total PYY. A key difference between our study and the previous study is that we chose to measure the "active" form of PYY (PYY_{3-36}). At the time our study was initiated, few data were available on the effects of sample processing on measured $PYY_{3–36}$. Chandarana *et al* (16) recently studied how differences in sample processing affected measured gut hormone concentrations in human plasma. The authors point out that commercially available gut hormone assays recommend the addition of aprotinin, which inhibits several serine proteases, along with an inhibitor of the enzyme dipeptidyl peptidase IV (DPP4), which is thought to be responsible for proteolytic cleaveage of the first two amino acids from the full length $PYY_{1–36}$ to generate the selective Y2 receptor agonist $PYY_{3–36.}$ Chandarana and colleagues point out that there is no published evidence demonstrating aprotinin stabilizes active $PYY_{3–36}$ or total PYY. The authors

further demonstrated that addition of aprotinin with a DPP4 inhibitor had no effect on fasted, fed, or post-prandial area under the curve $PYY_{3–36}$ levels. These results suggest that one or more alternative enzymes may convert $PYY_{1–36}$ to $PYY_{3–36}$ (17) and that differences in sample processing could be responsible for discrepancies between our study and the results of others.

Additionally, the lack of an association between PYY_{3-36} and subjective appetite may be due to the inherent complexity of human appetite. In addition to environmental influences and hedonic characteristics of food, many peripheral hormones in addition to PYY signal satiation (cessation of eating) and satiety (the feeling of fullness that persists after eating) either via the vagus nerve (gut to brain communication) or via receptors in the hypothalamus. These include short-term or episodic hormones that occur in unison with episodes of eating, such as cholecystokinin, glucagon-like peptide 1, oxyntomodulin (secreted from the small and large intestines), pancreatic polypeptide, amylin, and insulin (all three secreted from the pancreas), and more long-term regulators of energy balance such as leptin.

The overfeeding induced changes in 24 h subjective appetite responses were likely driven by increased food volume, as satiation is initiated by neural input from the stomach to the brain signaling gastric distension after food consumption. The same foods were served at every meal during each treatment but portion size was increased by 30% during overfeeding, thus the percentage of total calories from carbohydrates, fats, and protein were held constant between treatments. A collective body of work from the laboratory of Barbara Rolls (18) has clearly demonstrated that sensory-specific satiety is influenced more by the volume of food consumed rather than the energy content for studies over periods of about one-day, while others have shown that this volume effect lessens over periods of days when the energy density of the meal is manipulated (19). Additional studies incorporating manipulation of energy density and food

volume during overfeeding in sedentary and exercise conditions could help parse out the different influences.

In addition to the previously discussed potential PYY sample processing issues, our study does have some limitations. Individuals may alter their food intake in a laboratory environment if they know they are being monitored and their appetites may be influenced by the foods they are offered rather than being allowed to self-select at each meal. That said, it should be pointed out that inferences drawn from out-patient or observational studies are often difficult given the limitations on the precision and accuracy of self-reported energy intake (20) and energy expenditure. Visual analogue scales (VAS) (11) are often employed to study the effect of a particular intervention (i.e. exercise) on appetite (hunger, fullness, desire for food, etc…) and have been shown to be reproducible and reliable for appetite research, particularly when used in a repeated measures design (21).

In summary, a sedentary lifestyle may predispose to a failure in appetite regulation, resulting in the inability to down-regulate food intake to match lower energy requirements of inactivity (22). Our subjective appetite data may suggest that increasing habitual high daily physical activity may improve responsiveness to hunger and satiety cues; however, the lack of an association between subjective and hormonal markers of satiety in our study suggest that additional studies of longer duration measuring additional hormones along with ad-libitum food intake over 24 hour periods (instead of a single meal) are needed, while also taking appropriate steps in blood sample processing. Further studies investigating plasma levels of PYY and similar gut hormones may require interventions that stimulate or block the peptide at critical times of the day to clarify their role in exercise-associated appetite responses. It is also important to emphasize that subjective appetite is likely influenced by environmental influences

and hedonic aspects of food that may override homeostatic biological mechanisms regulating appetite.

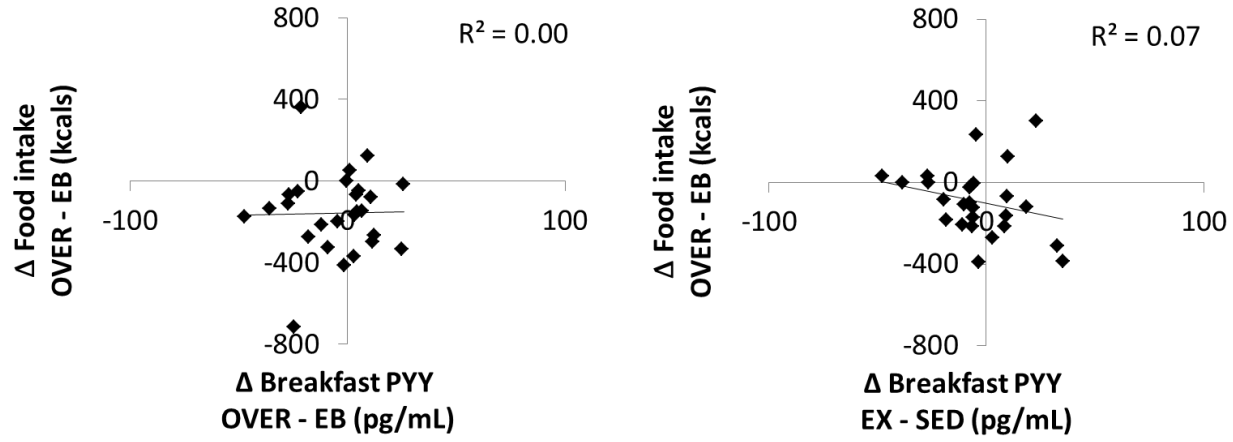

Figure 1: Relationship between the change in *ad-libitum* breakfast PYY (post breakfast – pre breakfast) response to overfeeding (A = OVER - SED) or exercise (B = EX – SED) and corresponding change in *ad-libitum* breakfast food intake in habitually sedentary individuals (n=13).

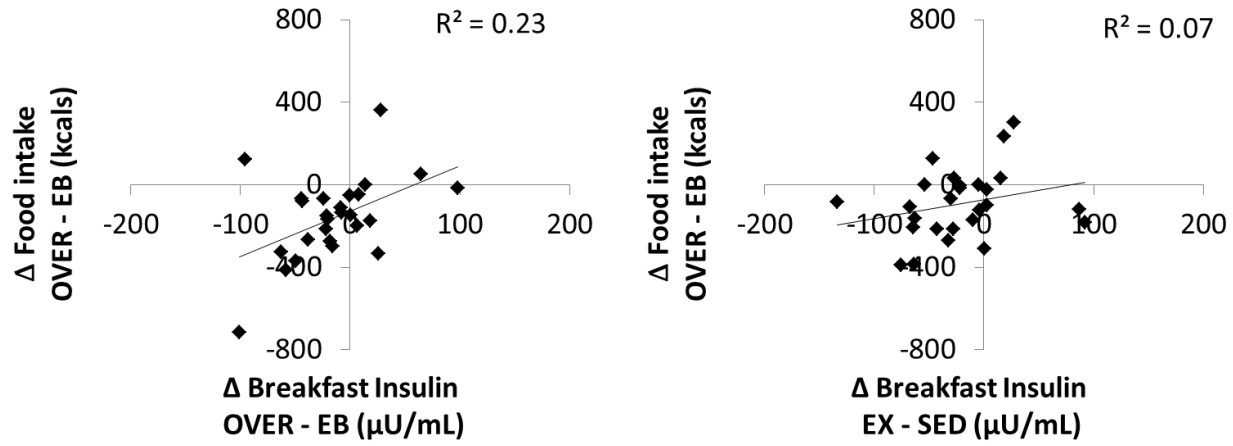

Figure 2: Relationship between the change in *ad-libitum* breakfast insulin (post breakfast – pre breakfast) response to overfeeding (A = OVER - SED) or exercise (B = EX – SED) and corresponding change in *ad-libitum* breakfast food intake in habitually sedentary individuals (n=13).

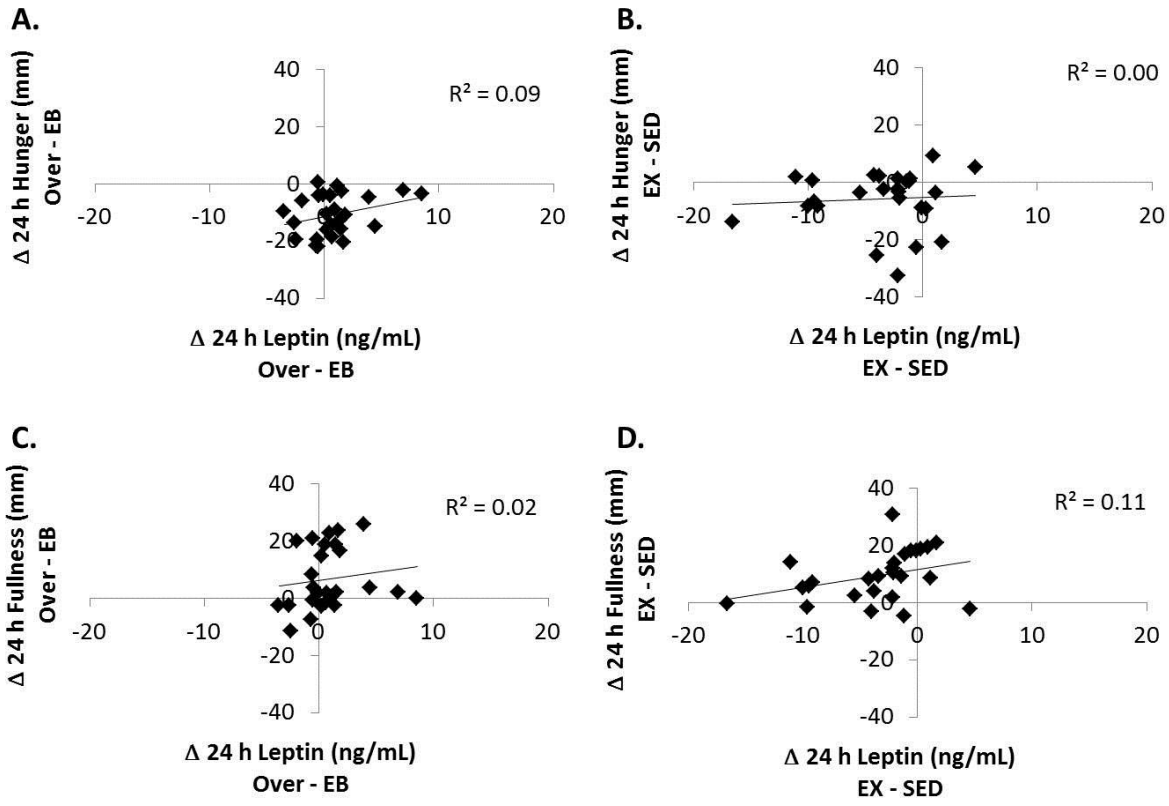

Figure 3: Relationship between change in the 24h weighted average of plasma leptin and subjective appetite in response to overfeeding (A = hunger; C = fullness) and exercise (B = hunger; D = fullness) in habitually sedentary individuals (n=13).

References

1. Organization WH. Obesity and Overweight Fact Sheet. http://www.who.int/mediacentre/factsheets/fs311/en/ accessed Feb 2012.
2. Van Walleghen EL, Orr JS, Gentile CL, Davy KP, Davy BM. Habitual physical activity differentially affects acute and short-term energy intake regulation in young and older adults. Int J Obes (Lond) 2007;31(8):1277-85.
3. Long SJ, Hart K, Morgan LM. The ability of habitual exercise to influence appetite and food intake in response to high- and low-energy preloads in man. The British journal of nutrition 2002;87(5):517-23.
4. Martins C, Truby H, Morgan LM. Short-term appetite control in response to a 6-week exercise programme in sedentary volunteers. The British journal of nutrition 2007;98(4):834-42.
5. Zigman JM, Elmquist JK. Minireview: From anorexia to obesity--the yin and yang of body weight control. Endocrinology 2003;144(9):3749-56.
6. le Roux CW, Bloom SR. Peptide YY, appetite and food intake. The Proceedings of the Nutrition Society 2005;64(2):213-6.
7. Batterham RL, Bloom SR. The gut hormone peptide YY regulates appetite. Annals of the New York Academy of Sciences 2003;994:162-8.
8. Martins C, Morgan LM, Bloom SR, Robertson MD. Effects of exercise on gut peptides, energy intake and appetite. The Journal of endocrinology 2007;193(2):251-8.
9. Broom DR, Batterham RL, King JA, Stensel DJ. Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy

males. American journal of physiology Regulatory, integrative and comparative physiology 2009;296(1):R29-35.

10. Broom DR, Stensel DJ, Bishop NC, Burns SF, Miyashita M. Exercise-induced suppression of acylated ghrelin in humans. J Appl Physiol 2007;102(6):2165-71.
11. Blundell J, de Graaf C, Hulshof T, et al. Appetite control: methodological aspects of the evaluation of foods. Obesity reviews : an official journal of the International Association for the Study of Obesity 2010;11(3):251-70.
12. Cooper JA, Watras AC, Paton CM, Wegner FH, Adams AK, Schoeller DA. Impact of exercise and dietary fatty acid composition from a high-fat diet on markers of hunger and satiety. Appetite 2011;56(1):171-8.
13. Stunkard AJ, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. Journal of psychosomatic research 1985;29(1):71-83.
14. Ueda SY, Yoshikawa T, Katsura Y, Usui T, Fujimoto S. Comparable effects of moderate intensity exercise on changes in anorectic gut hormone levels and energy intake to high intensity exercise. The Journal of endocrinology 2009;203(3):357-64.
15. Ueda SY, Yoshikawa T, Katsura Y, Usui T, Nakao H, Fujimoto S. Changes in gut hormone levels and negative energy balance during aerobic exercise in obese young males. The Journal of endocrinology 2009;201(1):151-9.
16. Chandarana K, Drew ME, Emmanuel J, et al. Subject standardization, acclimatization, and sample processing affect gut hormone levels and appetite in humans. Gastroenterology 2009;136(7):2115-26.

17. Unniappan S, McIntosh CH, Demuth HU, Heiser U, Wolf R, Kieffer TJ. Effects of dipeptidyl peptidase IV on the satiety actions of peptide YY. Diabetologia 2006;49(8):1915-23.

18. Rolls BJ. The relationship between dietary energy density and energy intake. Physiology & behavior 2009;97(5):609-15.

19. Poppitt SD, Prentice AM. Energy density and its role in the control of food intake: evidence from metabolic and community studies. Appetite 1996;26(2):153-74.

20. Trabulsi J, Schoeller DA. Evaluation of dietary assessment instruments against doubly labeled water, a biomarker of habitual energy intake. American journal of physiology Endocrinology and metabolism 2001;281(5):E891-9.

21. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 2000;24(1):38-48.

22. Murgatroyd PR, Goldberg GR, Leahy FE, Gilsenan MB, Prentice AM. Effects of inactivity and diet composition on human energy balance. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 1999;23(12):1269-75.

Chapter 5

Summary of findings and research proposal

Summary of findings

A review of the scientific literature presented in chapter one described biological mechanisms, albeit imperfect mechanisms, that function in a physiological homeostatic system to balance calories consumed (energy intake) with calories burned (energy expenditure) to maintain body weight; however, it was pointed out in chapter two that overconsumption and a sedentary lifestyle are both implicated in the development of the obesity epidemic. In light of these considerations, it was suggested that an improved understanding of how obesity occurs in the context of a homeostatic regulatory system is crucial to the development of effective therapies. A set of studies were described which suggested that the leptin system in healthy weight, but not obese, individuals was responsive to three days of overfeeding and this response was associated with reductions in *ad-libitum* breakfast food intake following overfeeding. Additional studies were described suggesting high levels of physical activity associated with successful maintenance of body weight may result in a better coupling between energy intake and energy expenditure, albeit in the presence of large inter-individual variability. In an extension of these findings, our overarching hypothesis was that high levels of physical activity during short-term positive energy balance may improve objective and subjective appetite regulation by modulating plasma levels of hormones involved in the homeostatic regulation of dietary behavior. To test this hypothesis we examined the effects of overfeeding on (1) leptin and self-selected food intake, and (2) perceived hunger and peripheral hormonal concentrations at two levels of physical activity in habitually sedentary individuals.

In chapter three, we report data for the first study aim. When stratified by degree of insulin resistance as estimated by the homeostasis model assessment (HOMA) method, both groups of subjects (normal HOMA and high HOMA) decreased self-selected breakfast intake in

response to overfeeding, with a trend for a reduction in response to exercise, but these changes in food intake were not explained by the 24 h leptin response to overfeeding or exercise. These findings suggest more work is required to further delineate the role of leptin in the regulation of appetite response to day-to-day perturbations in energy balance or energy flux. Instead of peripheral leptin response to changes in energy balance or energy flux recent work has focused on defects in leptin signaling within the central nervous system. Many rodent models of obesity have been shown to be characterized by hypothalamic resistance to leptin and insulin. Recent work (1-3) has shown that exercise improves both insulin and leptin signaling in an interleukin-6 dependent manner within the rat hypothalamus, a critical region in the brain for the integration of signals known to be involved in regulating energy homeostasis. Additional data shows that Ob-Rb mRNA from the hypothalamic arcuate nucleus of male Wistar rats is significantly increased following 12 weeks of endurance exercise training, along with increased activity (phosphorylation) of key molecules in the leptin signaling cascade even as circulating plasma leptin concentrations are significantly decreased with loss of fat mass (4). Based on this information, it is possible that exercise reinforces the central rather than peripheral action of leptin and further work is required to confirm. Additionally, while leptin resistance is a feature of already obese humans (5, 6), it is less clear that reduced leptin responsiveness actually pre-dates the onset of human obesity as it does in some obesity prone rodents. A key issue for future studies will be to elucidate the molecular mechanisms responsible for leptin resistance and whether this is a cause or consequence of obesity.

In chapter four, we report data for the second study aim. We were unable to find any association between hormonal markers of appetite and either perceived appetite or objectively measured food intake. These results are a testament to the complexity of the regulation of

human appetite. Neither 24h average leptin nor PYY explained much of the average daily perceived hunger, fullness, or feeding at a subsequent morning meal in habitually sedentary individuals. While our subjective appetite data may suggest that increasing habitual high daily physical activity may improve perceived responsiveness to hunger and satiety cues, the lack of an association with PYY or leptin suggests future research should measure additional hormones in conjunction with objectively monitored *ad-libitum* food intake over 24 hour periods (instead of a single meal).

Conclusions

It is important to keep in mind that there are multiple hormones that acutely suppress (or stimulate) food intake and that the integration of these signals influence overall energy balance in a manner that is not yet fully understood. Simply understanding the hormonal signaling system will not prevent obesity unless it is coupled with changes in behavior that alter energy balance. Decreased requirements for physical energy expenditure and an increased availability of palatable, high fat, high sugar, high salt, calorically dense foods may combine to act on neural reward mechanisms, similar to those involved in reinforcing drug seeking behavior (7), that generate a constant pressure on food intake that is not compensated by commensurate changes in intake or expenditure to alter energy balance enough to maintain a healthy weight for a long period in some individuals (8, 9).

Only during the past four to six decades has our species effectively engineered physical activity out of the daily lives of large portions of the US population. Physical inactivity has become so prevalent that it is common to refer to exercise as having health benefits, even though the exercise-trained state is likely the biological normal condition (10). It is reasonable to

hypothesize that modern influences on physical activity or physical inactivity are influencing, or even driving, the obesity epidemic. Thus, assessment of the feasibility of restructuring the human environment to reduce exposure to highly palatable and energy rich food and increase the opportunity for physical activity is warranted, particularly in the workplace where, for example, treadmill based walking workstations have been proposed as a strategy to increase daily activity levels (11). Additionally, characterization of the modes, volumes, and intensity of physical activity that would produce optimal diurnal between-meal appetite suppression may be helpful as well.

The need for additional knowledge in one particular area is suggested and a research proposal follows: Many studies have noted large inter-individual variability in change in body weight or body fat after sedentary individuals complete a structured exercise intervention (12-14). A likely possibility is that individuals who lose less weight than predicted (or even gain weight) may be compensating for the physical activity induced energy expenditure either via increases in energy intake or decreases in non-physical activity energy expenditure (or both). Thus, the purpose of this research proposal is to study the effects of a 24 week exercise intervention on behavioral and physiological mechanisms mediating change in body weight in overweight & obese women.

Research Proposal Review of Literature:

A structured exercise program is often recommended as an intervention for reducing excess weight in overweight/obese populations (15); however, the evidence for the efficacy of this approach is not so clear. Large inter-individual variability in exercise-induced weight and fat loss has been documented, even when exercise is supervised and compliance is >90% (12, 13). For example, Barwell *et al.* (13) studied individual responsiveness to exercise-induced fat

loss in 55 women following a 7 week exercise program while continuing otherwise normal free-living conditions (**Figure 1).** When expressed as absolute change in fat mass (panel A), approximately 70% of the women lost body fat; however, when change in fat mass was adjusted to take into account exercise induced energy expenditure (panel B), the distribution of change in fat mass showed that roughly 50% of the women actually gained fat while approximately 50% lost body fat.

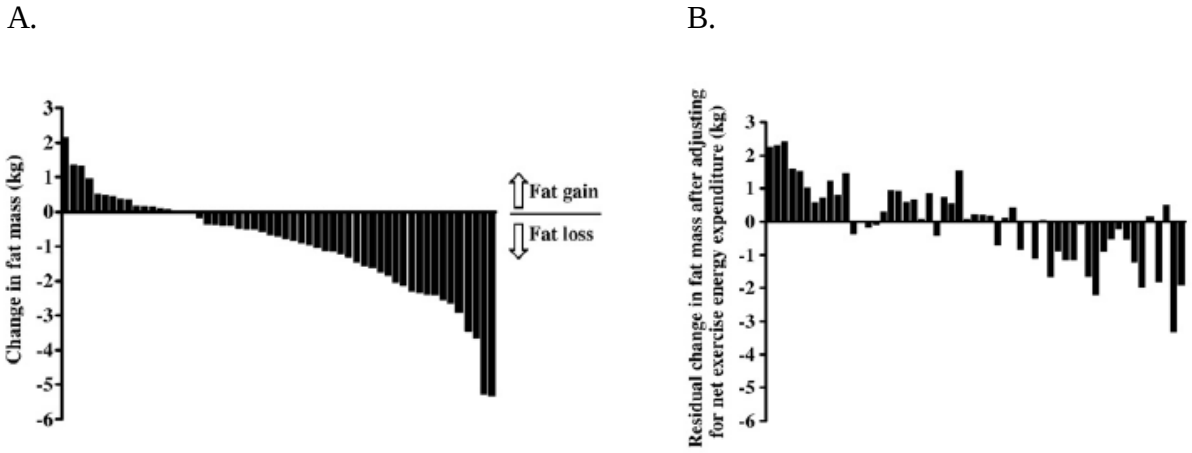

Figure 1: Change in body fat mass in 55 women following a 7 week exercise program. A = absolute change in fat mass; B = change in fat mass adjusted for net exercise energy expenditure. Adapted from Barwell *et al.* (13)

Individuals who lose less weight than predicted or even gain weight after undertaking an exercise program have been referred to as "compensators," while those who lose more than or equal to predicted weight loss have been referred to as "non-compensators" (12). Additional studies have reported similar results (14, 16) and it is also apparent that differences exist between men and women regarding the degree of compensation for exercise induced increases in energy expenditure (17, 18). The health benefits of exercise independent of weight loss (19) deserve recognition, but in the face of the current obesity epidemic it is extremely important to better understand the factors that drive compensation to exercise induced weight loss (20). It is apparent that behavioral and/or physiological mechanisms exist in compensators that result in the

resistance or susceptibility to exercise induced weight loss. Identifying these mechanisms is of fundamental importance, as this will lead to the design of more effective strategies for weight control. Data evaluating this phenomenon are limited, especially in women, as few studies have been designed to investigate compensatory mechanisms together using objective measures of energy expenditure, food intake, and appetite during supervised structured exercise.

Until fairly recently, the impact of structured exercise on daily PAEE has been difficult to quantify because of challenges in measuring both total daily PAEE and exercise energy expenditure objectively in a free-living environment. A limitation of many studies is that activity outside of planned exercise, as well as energy intake, are determined from self-report diaries. Self-report techniques have been common due to low cost and ease of administration, but tend to overestimate energy expenditure (21, 22) and underestimate energy intake (23, 24) on the individual level due to recall bias. On the other hand, the doubly labeled water technique (23) is relatively noninvasive and allows accurate and precise quantification of total energy expenditure; however, this method is expensive and cannot quantify subcomponents of PAEE, such as intensity and frequency. An alternative is the use of accelerometers and heart rate monitors, which are advantageous because duration and intensity of daily physical activity can be quantified. However, each has limitations when used alone. Therefore, the use of accelerometry and heart rate in combination has been used to improve the estimate of physical activity energy expenditure (25, 26). Turner and colleagues (17) used combined accelerometer + heart rate (Actiheart) to measure both daily exercise and non-exercise PAEE before, during, and after 6 months of structured exercise in sedentary, overweight middle aged men. Results indicated that non-exercise PAEE was actually maintained and not decreased, as hypothesized, in response to structured exercise. The authors speculated intuitively that the less-than-predicted

weight loss reflected compensatory increases in energy intake, although energy intake or factors influencing intake were not measured.

Knowledge concerning the impact of chronic exercise training on appetite and the regulatory control of energy balance and body weight is also limited. Emerging evidence suggests that longer term exercise may also trigger compensatory eating in susceptible individuals. King *et al.* (16) examined the effects of 12 weeks of supervised aerobic exercise on fasting and daily hunger in 58 overweight and obese individuals. Compensators who lost only 1% of their initial body weight exhibited increased fasting and daily hunger. In contrast, the non-compensators, who lost almost 6% of initial body weight, showed no increase in overall daily hunger despite an increase in fasting hunger. This study was unique because the investigators also utilized a technique called the "satiety quotient" (27) to measure the sensitivity of satiety signaling associated with a fixed energy breakfast meal. This allowed for direct comparisons in satiety signaling before and after the intervention in both compensators and non-compensators. The satiating effect of the fixed breakfast increased after the 12-wk exercise program in both the groups. A limitation of the study by King *et al.* is that blood hormones associated with appetite regulation were not measured and therefore a potential mechanistic explanation cannot be offered for the observed results. Novel gastrointestinal and adipose tissue derived hormones have been shown to have a role in the regulation of appetite and food intake, and ultimately in the regulation of energy balance and body weight/fat mass. It will be important for future research to examine changes in blood levels of these hormones in conjunction with psychological appetite ratings during both fixed and *ad-libitum* meals to link biology with behavior. For example, compensators may experience increased hunger and corresponding

increased energy intake in response to exercise due to elevated plasma levels of fasting acylated ghrelin, as recently demonstrated by Martins *et al*. (28).

The proposed study is important because it will determine if compensators decrease daily non-exercise (non-prescribed) PAEE in response to structured exercise and if compensators demonstrate changes in appetite that are associated with increased daily energy intake during the structured exercise program. A better understanding of the components of energy balance that may be susceptible to compensation in response to exercise in overweight/obese women will allow for targeting of weight loss intervention strategies and should result in improved weight loss outcomes.

Central hypothesis:

Overweight/obese women who experience a lower than predicted weight loss following structured exercise are compensating for the exercise-induced energy expenditure by reducing daily non-exercise physical activity energy expenditure (PAEE) and/or by increasing energy intake mediated by changes in appetite.

Specific Aim 1: Characterize the individual variability in change in body weight and body composition associated with a 24 week exercise intervention in overweight/obese women.

Specific Aim 2: Determine if compensators decrease daily non-exercise (non-prescribed) PAEE in response to structured exercise.

Specific Aim 3: Determine if compensators display changes in appetite that are associated with changes in objectively measured daily energy intake.

Specific Aim 4: Demonstrate beneficial changes in traditional biomarkers of health during a structured exercise program independent of changes in body weight.

Methods:

Population

Forty generally healthy, non-smoking women 20-39 years old with a body mass index (BMI) between $25 \leq 35$ kg/m^2 will be recruited. Participants must be sedentary (<60min of intentional moderate physical activity per week, including work related activity) and weight stable (±2kg over the previous 6 months). Personal and immediate family health histories will be obtained using criteria set forth by the American College of Sports Medicine (29) to exclude persons with known or diagnosed type I or II diabetes, cardiovascular disease, thyroid disorders, and/or orthopedic limitations. Participants who are pregnant, lactating, or taking medications or supplements that could affect physical performance or metabolism will also be excluded.

Study Design

Initial baseline testing is shown in figure 2. Prior to beginning the exercise intervention, participants will arrive at 0800 after a 10-12hr overnight fast for height, body weight (BW) and resting blood pressure (BP) and heart rate (HR) measurements. Resting metabolic rate (RMR) will be measured for 30 minutes via indirect calorimetry. Fasting blood samples will be drawn for baseline blood chemistries, including: glucose, insulin, thyroid stimulating hormone, thyroxine (free T4), and a basic lipid panel. Body composition will be measured by dual energy x-ray absorptiometry. Maximal oxygen uptake (VO_{2max}) will be determined on a treadmill using a progressive incline test similar to the modified Balke protocol (29). Baseline testing procedures will be repeated at the end of the exercise intervention (post-D1).

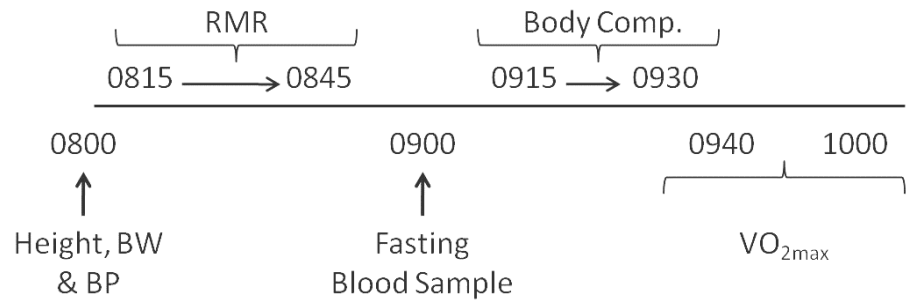

Figure 2: Baseline Testing

As shown in figure 3, participants will report one week before (week 0) and one week at the end of a 24 week exercise intervention to assess daily PAEE, appetite/energy intake, and body weight/composition. During the exercise intervention, participants will continue normal free-living conditions and consume an *ad-libitum* diet. All participants will be given a "cover story" to hide the true study purpose in an attempt to minimize the impact of knowledge of the study purpose on changes in normal behavior.

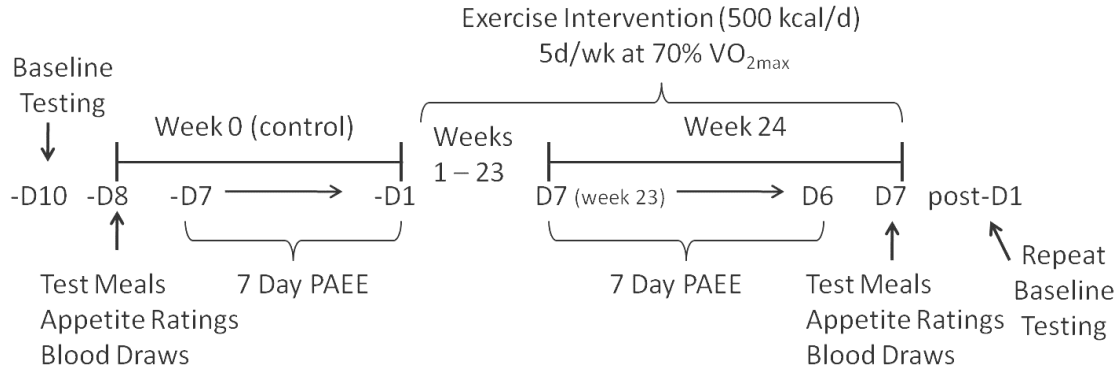

Figure 3: Study Design

Test Meals and Daily Energy Intake **(-D8 of week 0 and D7 of week 24):**

Personalized Breakfast Meal

48 hours following baseline testing (-D8), participants will arrive at 0800 after a 10-12hr overnight fast to be fed a breakfast meal. Participants will be asked to "eat until comfortably full" and the food will be weighed pre & post consumption to determine amount and energy intake (kcal). The amount of food consumed at this time will become a personalized fixed breakfast that will be consumed again at the end of the intervention (day 7 of week 24).

Lunch, Dinner, Snack

After the personalized fixed breakfast is consumed, total daily energy intake will be directly measured from *ad libitum* lunch and dinner test meals and a take-home evening snack. Thus, an objective measure of daily energy intake will be compiled from the energy consumed in the fixed breakfast and three subsequent eating episodes before starting the intervention (week 0) and at the end of the intervention (day 7 of week 24).

Appetite Ratings and Hormone Biomarkers of Appetite **(-D8 and D7 of week 24)***:*

Visual analog scale appetite rating scores (30) will be used before, during, and after all meals to assess degrees of: 1) hunger; 2) fullness; 3) desire to eat; and 4) prospective consumption. For the personalized fixed breakfast, appetite scores and blood samples to measure plasma levels of hormones known to be physiological regulators of appetite (i.e. insulin, ghrelin, PYY, and GLP-1) will occur before breakfast at minute -15, -10, -5, at the start of breakfast = minute 0, and after beginning breakfast at minutes 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, and 240. Because of the relatively short half-life of many of these peptide hormones (i.e. several minutes up to 30 minutes), frequent sampling times are required. Because the breakfast

meal time and energy content is the same before and after the exercise intervention, this should allow a direct examination of the effect of exercise on plasma levels of appetite hormones.

Appetite scores will also be measured immediately before consuming each *ad libitum* lunch and dinner (time = 0 minutes), as well as at 30, 60, 90, 120, 150, 180, and 240 minutes (4hrs in total) after beginning each meal. No blood samples will be taken with these meals.

To determine whether subjects engage in eating behaviors (i.e., weighing food, self-monitoring, self-weighing, etc.) consistent with weight control, the Eating Behavior Inventory (31) will be completed the morning of each test meal period. A higher score post-intervention is indicative of engagement in more eating behaviors recommended for weight control.

Average Daily PAEE **(7 days during week 0 and week 24)***:*

For seven full days and nights before (week 0) and at the end of the intervention (week 24), average daily PAEE will be assessed using combined accelerometry and heart rate (Actiheart; Cambridge Neurotechnology, Cambridge, United Kingdom) measurements. Briefly, Actiheart uses branched-equation modeling to estimate PAEE from synchronized accelerometry/heart rate data, providing estimates of energy expenditure in minute-by-minute epochs during a range of physical activities (25, 26, 32). Participants will be shown how to wear/use the Actiheart device following determination of their personalized fixed breakfast on –D8 (**figure 2**).

Exercise Intervention:

Aerobic exercise will be individually designed to expend 500kcal/session 5d/wk at approximately 70% VO_{2max}, following ACSM age-specific physical activity recommendations

(33). If feasible, all exercise sessions will be directly supervised. However, in the event this is not possible, participants will wear a heart rate monitor at every exercise session that will allow recording of exercise duration and heart rate. Participants will complete at least one of their weekly exercise sessions every week under direct supervision so that exercise intensity and heart rate response can be monitored and the exercise prescription altered accordingly. Indirect calorimetry will be performed once every 4 weeks during one of the supervised exercise sessions to assess energy expenditure of the prescribed exercise session. VO_{2max} will be recalculated after the first 4 weeks and after 12 weeks to account for changes in weight and fitness. In addition, a full practice exercise session will be carried out prior to beginning the intervention to familiarize each participant with their individual exercise prescription plan. The first two to three weeks of the intervention may require an individualized progressive build-up of intensity, frequency, and duration to achieve the full 5d/wk at 70% VO_{2max}, which may help minimize soreness and encourage continued participation.

Research Timeline:

This study is anticipated to last approximately three years. The first 12 months will be used to attain funding, equipment, IRB approval, and to set up arrangements to conduct the intervention. At the end of year one, twenty participants will begin the exercise intervention staggered over a five week period with two participants beginning the intervention each week to space out testing procedures. These participants will finish the intervention 24 weeks from their individual start date. At the end of year two, the same design will be followed for the remaining 20 participants, which should eliminate any seasonal differences (e.g. holidays) in energy intake or energy expenditure, while also allowing time for data analysis.

Statistics:

Compensators and non-compensators will be identified by comparing their predicted weight loss with actual weight loss. One kilogram of body weight (assuming 70:30 fat/lean tissue) has been estimated to be equivalent to 7700 kcal (34). Therefore, each individual's predicted weight loss will be calculated from the exercise energy expenditure (EE) using equation (1):

$$\text{Expected weight loss (kg)} = \text{Total Exercise EE (kcal)} \div 7{,}700 \text{ kcal/kg} \quad (1)$$

A participant will be classified as a compensator if actual weight loss is less than predicted and classified as a non-compensator if their actual weight loss is greater than or equal to predicted weight loss. Based on the previously described equation and knowing the exercise intervention is designed to expend 500kcal/session, 5d/wk for 24 weeks, the intervention should produce a weight loss of -7.8kg per individual, as calculated below:

$$60{,}000 \text{ kcal total exercise EE over 24 weeks} \div 7700 \text{ kcal/kg} = \mathbf{-7.8kg}$$

The primary outcome of this study is that the energy equivalent of the missing predicted weight loss in compensators is accounted for by increasing daily energy intake (mediated via changes in appetite) and/or decreasing daily non-exercise PAEE in response to structured exercise. Previously, King *et al.* (16) reported large variability in change in weight in 35 overweight and obese men and women (-14.7kg to +1.7kg) following 12 weeks of supervised exercise, with a difference in weight loss of 4.8kg between compensators and non-compensators. This difference represents an energy equivalent = 3,080 kcal/wk (4.8kg * 7,700 kcal ÷ 12wk) or a mean of 440 kcal/d in compensation. This is well within the analytical accuracy and precision of the Actiheart monitor for measuring daily energy expenditure. The estimated sample size based on a t-test to find an effect of 440 kcal/d in the face of an average SD of ±440kcal/d, $\alpha = 0.05$, and a

power of 80% would require a total of 32 participants. Allowing for attrition and in order to obtain more representative data, 40 participants will be recruited.

The immediate and delayed effects of the fixed breakfast will be assessed by calculating the satiety quotient (SQ), developed by Green et al (27). The SQ allows actual energy consumed to be related to the subsequent change in appetite ratings by using the following equation (2):

$$\textbf{(2)}\ SQ = \frac{\text{desire to eat (pre breakfast)} - \text{desire to eat (post breakfast)}}{\text{breakfast energy intake (kcals)}}$$

Area under the curve (AUC) values for appetite VAS scores and hormone biomarkers of appetite will be calculated using the trapezoidal rule. Within group (compensators or non-compensators) comparisons in weight, body composition, total daily energy intake, SQ appetite scores, average daily PAEE, and AUC values between week 0 and week 24 will be assessed with paired t-tests. Comparisons between groups will be assessed with an independent t-test. Pearson coefficients will also be calculated to examine relationships between appetite VAS scores and plasma appetite hormone levels, and between appetite VAS scores and change in weight/body composition.

References

1. Flores MB, Fernandes MF, Ropelle ER, et al. Exercise improves insulin and leptin sensitivity in hypothalamus of Wistar rats. Diabetes 2006;55(9):2554-61.
2. Ropelle ER, Fernandes MF, Flores MB, et al. Central exercise action increases the AMPK and mTOR response to leptin. PloS one 2008;3(12):e3856.
3. Ropelle ER, Flores MB, Cintra DE, et al. IL-6 and IL-10 anti-inflammatory activity links exercise to hypothalamic insulin and leptin sensitivity through IKKbeta and ER stress inhibition. PLoS biology 2010;8(8).
4. Zhao J, Tian Y, Xu J, Liu D, Wang X, Zhao B. Endurance exercise is a leptin signaling mimetic in hypothalamus of Wistar rats. Lipids in health and disease 2011;10:225.
5. Rosenbaum M, Goldsmith R, Bloomfield D, et al. Low-dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight. The Journal of clinical investigation 2005;115(12):3579-86.
6. Schwartz MW, Peskind E, Raskind M, Boyko EJ, Porte D, Jr. Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. Nature medicine 1996;2(5):589-93.
7. Berthoud HR. Multiple neural systems controlling food intake and body weight. Neuroscience and biobehavioral reviews 2002;26(4):393-428.
8. Borer KT. Nonhomeostatic control of human appetite and physical activity in regulation of energy balance. Exercise and sport sciences reviews 2010;38(3):114-21.
9. Berthoud HR. Homeostatic and non-homeostatic pathways involved in the control of food intake and energy balance. Obesity (Silver Spring) 2006;14 Suppl 5:197S-200S.

10. Booth FW, Lees SJ. Physically active subjects should be the control group. Med Sci Sports Exerc 2006;38(3):405-6.

11. Thompson WG, Foster RC, Eide DS, Levine JA. Feasibility of a walking workstation to increase daily walking. Br J Sports Med 2008;42(3):225-8; discussion 8.

12. King NA, Hopkins M, Caudwell P, Stubbs RJ, Blundell JE. Individual variability following 12 weeks of supervised exercise: identification and characterization of compensation for exercise-induced weight loss. Int J Obes (Lond) 2008;32(1):177-84.

13. Barwell ND, Malkova D, Leggate M, Gill JM. Individual responsiveness to exercise-induced fat loss is associated with change in resting substrate utilization. Metabolism 2009;58(9):1320-8.

14. Donnelly JE, Hill JO, Jacobsen DJ, et al. Effects of a 16-month randomized controlled exercise trial on body weight and composition in young, overweight men and women: the Midwest Exercise Trial. Arch Intern Med 2003;163(11):1343-50.

15. Donnelly JE, Blair SN, Jakicic JM, Manore MM, Rankin JW, Smith BK. American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. Med Sci Sports Exerc 2009;41(2):459-71.

16. King NA, Caudwell PP, Hopkins M, Stubbs JR, Naslund E, Blundell JE. Dual-process action of exercise on appetite control: increase in orexigenic drive but improvement in meal-induced satiety. Am J Clin Nutr 2009;90(4):921-7.

17. Turner JE, Markovitch D, Betts JA, Thompson D. Nonprescribed physical activity energy expenditure is maintained with structured exercise and implicates a compensatory increase in energy intake. Am J Clin Nutr 2010;92(5):1009-16.

18. Manthou E, Gill JM, Wright A, Malkova D. Behavioral compensatory adjustments to exercise training in overweight women. Med Sci Sports Exerc 2010;42(6):1121-8.

19. Blair SN, Morris JN. Healthy hearts--and the universal benefits of being physically active: physical activity and health. Ann Epidemiol 2009;19(4):253-6.

20. Martins C, Morgan L, Truby H. A review of the effects of exercise on appetite regulation: an obesity perspective. Int J Obes (Lond) 2008;32(9):1337-47.

21. Wareham NJ, Rennie KL. The assessment of physical activity in individuals and populations: why try to be more precise about how physical activity is assessed? Int J Obes Relat Metab Disord 1998;22 Suppl 2:S30-8.

22. Ainslie P, Reilly T, Westerterp K. Estimating human energy expenditure: a review of techniques with particular reference to doubly labelled water. Sports Med 2003;33(9):683-98.

23. Subar AF, Kipnis V, Troiano RP, et al. Using intake biomarkers to evaluate the extent of dietary misreporting in a large sample of adults: the OPEN study. Am J Epidemiol 2003;158(1):1-13.

24. Schoeller DA. Limitations in the assessment of dietary energy intake by self-report. Metabolism 1995;44(2 Suppl 2):18-22.

25. Brage S, Brage N, Franks PW, Ekelund U, Wareham NJ. Reliability and validity of the combined heart rate and movement sensor Actiheart. Eur J Clin Nutr 2005;59(4):561-70.

26. Crouter SE, Churilla JR, Bassett DR, Jr. Accuracy of the Actiheart for the assessment of energy expenditure in adults. Eur J Clin Nutr 2008;62(6):704-11.

27. Green SM, Delargy HJ, Joanes D, Blundell JE. A satiety quotient: a formulation to assess the satiating effect of food. Appetite 1997;29(3):291-304.

28. Martins C, Kulseng B, King NA, Holst JJ, Blundell JE. The effects of exercise-induced weight loss on appetite-related peptides and motivation to eat. J Clin Endocrinol Metab 2010;95(4):1609-16.

29. ACSM's Guidelines for Exercise Testing and Prescription. Philadelphia: Lippincott, Williams, &Wilkins, 2005.

30. Blundell J, de Graaf C, Hulshof T, et al. Appetite control: methodological aspects of the evaluation of foods. Obes Rev 2010;11(3):251-70.

31. O'Neil PM, Rieder S. Utility and validity of the eating behavior inventory in clinical obesity research: a review of the literature. Obes Rev 2005;6(3):209-16.

32. Thompson D, Batterham AM, Bock S, Robson C, Stokes K. Assessment of low-to-moderate intensity physical activity thermogenesis in young adults using synchronized heart rate and accelerometry with branched-equation modeling. J Nutr 2006;136(4):1037-42.

33. American College of Sports Medicine Position Stand. The recommended quantity and quality of exercise for developing and maintaining cardiorespiratory and muscular fitness, and flexibility in healthy adults. Med Sci Sports Exerc 1998;30(6):975-91.

34. Forbes GB, Kreipe RE, Lipinski B. Body composition and the energy cost of weight gain. Hum Nutr Clin Nutr 1982;36(6):485-7.

Appendix A: Sample Visual Analog Scale (VAS) Questionnaire

8:00am

Please make an up and down line across each horizontal line at the point on the line that best describes how you are feeling right now.

1) How hungry are you right now?

Not at all hungry Extremely hungry

_____

2) How full are you right now?

Not at all full Extremely full

_____

4) How much do you think you could eat right now?

Nothing at all A large amount

_____

Appendix B: Menu

Day 1 & 4 (except breakfast)	Day 2 & 5	Day 3 & 6
Breakfast Oatmeal Nuts (walnuts, almonds) Milk **Breakfast (morning of day 4& 7)** Citrus Yogurt Multigrain Muffins **Dinner** Tilapia w/ Dill Sauce Carrots w/ Butter White Rice w/ Butter Coleslaw Ambrosia Whole Wheat Bread **Evening Snack** Pudding/Cookie Bar	**Breakfast** Scrambled Egg Whole Wheat Bread Peanut Butter, Butter, Jelly Grape Juice Milk **Lunch** Chicken Salad Pita Bread Grapes Relish plate (carrots, broccoli, ranch dressing) Lemonade **Dinner** Spaghetti w/ Meat Sauce & Parmesan Cheese Broccoli w/ Butter Green Salad (lettuce, tomatoes, ranch dressing) Pears Whole Wheat Bread Vanilla Pudding Milk **Evening Snack** Pudding/Cookie Bar	**Breakfast** Kashi GoLean Crunch Fruit Yogurt Milk **Lunch** Tuna Salad Sandwich on Whole Wheat Bread Relish Plate: carrots, celery, ranch dressing Trail mix Ambrosia Milk **Dinner** Chicken w/ BBQ sauce Green Beans w/ Butter Citrus Rice Salad Whole Wheat Bread Citrus Tapioca Pudding Lemonade **Evening Snack** Pudding/Cookie Bar