THESIS

A HIGH ENERGY FLUX STATE ATTENUATES THE WEIGHT LOSS-INDUCED ENERGY GAP BY ACUTELY DECREASING HUNGER AND INCREASING SATIETY AND RESTING METABOLIC RATE

Submitted by

Rebecca Foright

Department of Food Science and Human Nutrition

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2014

Master's Committee:

Advisor: Chris Melby

Deana Davalos
Melissa Wdowik

ABSTRACT

A HIGH ENERGY FLUX STATE ATTENUATES THE WEIGHT LOSS-INDUCED ENERGY GAP BY ACUTELY DECREASING HUNGER AND INCREASING SATIETY AND RESTING METABOLIC RATE

Introduction: Maintaining weight loss is one of the greatest challenges facing obese dieters. Weight loss-induced, compensatory, biological adjustments increase hunger and decrease resting metabolic rate (RMR), resulting in a disconnect between desired and required calories. This phenomenon, termed the *energy gap*, results in strong biological pressures that promote weight regain. Previous research in athletes has shown that high levels of physical activity coupled with high energy intake may increase RMR and reduce hunger. It is possible that this high energy flux state characterized by high daily energy expenditure (resulting from increased physical activity) with matching high energy intake (high calorie throughput) may attenuate the weight loss-induced energy gap by reducing hunger and increasing RMR.

Methods: This proof-of-concept pilot study utilized a within-subjects cross-over experimental design. Six obese adults [age ($\bar{x}$ ± SD) =42+12 y; BMI=35.7±3.7 kg/m^2] underwent baseline measures of body weight, body composition, RMR via indirect calorimetry, fasting and post-prandial perceived hunger via visual analog scales, fasting and serial postprandial measures of glucose, insulin, and peptide YY (PYY, an anorexigenic hormone) and ad libitum energy intake from a mid-day food buffet. They

then underwent weight loss (7% of initial body weight achieved over several months) and were stabilized at this reduced weight for three weeks. Subjects were then placed in two different 4-day experimental conditions of energy balance in random order—Low Flux (**LF**): sedentary with energy intake (EI)=RMR x1.35; and **HF**: daily exercise net energy cost of ~500 kcal/d and EI= RMR x1.7. On each morning of the 4 days of the HF and LF conditions, RMR was measured and hunger and satiety monitored. On the day following the HF and the LF conditions, respectively, participants again underwent measures of RMR, fasting and post-prandial hunger and satiety, fasting and serial postprandial measures of glucose, insulin, and PYY, and ad libitum energy intake from the food buffet.

Results: Daily energy intake during HF (x±SD: 3,191±587 kcal/d) was significantly greater ($p<0.001$) than during LF (x±SD: 2,449±406 kcal/d), but in line with the experimental design, subjects were in energy balance and average weight did not differ between low flux (103±4.8 kg) and high flux (103.4±4.7 kg). Perceived hunger at the end of day was lower ($p=0.020$), fullness throughout the day was higher ($p=0.015$) and there was a trend for hunger throughout the day to be lower ($p=0.091$) in HF compared to LF conditions. Additionally, RMR was significantly higher in HF (1926±138 kcal/day) compared to LF (1847±126 kcal/day; $P = 0.05$). Fasting and post-prandial glucose concentrations did not significantly change with weight loss. Fasting and postprandial insulin concentrations were lower after weight loss the day following HF and LF compared to pre-weight loss baseline values, but did not differ by flux condition. Fasting PYY concentrations were not different among pre-weight loss, HF, and LF, but postprandial PYY was lower the day following HF compared to pre-weight loss baseline.

Ad libitum food intake and subjective feelings of hunger and satiety on the day following HF and LF did not differ between flux conditions or from pre-weight loss baseline values.

Conclusions: A 4-day high flux state resulted in decreased hunger, increased satiety and increased RMR in weight-reduced, weight-stable, obese individuals when compared to a low flux state. Our findings support the importance of a daily high energy flux state in attenuating the increase in hunger and the decrease in energy expenditure that accompany diet-induced weight loss. However, the greater satiety and lower hunger were not evident the day following the high flux state, suggesting that such benefits resulting from the acute state are not long-lasting.

DEDICATION

Dedicated to Dr. Chris Melby

How lucky I am to have found something that makes saying goodbye so hard.

-Winnie the Pooh

TABLE OF CONTENTS

INTRODUCTION

It is well known that obesity is a growing national issue. Sixty-eight percent of Americans are overweight or obese [1]. That is to say, healthy weight individuals are now the minority in America. Obesity is associated with a number of comorbidities which exacerbate the decline in health seen with the obese state. These include diseases such as type II diabetes, coronary heart disease, various cancers, and hypertension [2]. The high frequency in which individuals present with both diabetes and obesity has brought about the introduction of the term diabesity into the lexicon. These comorbidities contribute to the growing medical burden of obesity, which is estimated to cost Americans 190 billion dollars annually [3].

One of the greatest issues facing the obese population is the failure of weight loss programs to produce a sustainable, long-term, weight loss. Within one year of completing a weight loss program individuals gain back nearly half of the lost weight [4, 5]. At five years post weight loss, fifty percent of individuals have returned to their starting or higher weight [6]. Only about 16 percent are successful in maintaining the initial weight loss over the course of five years [6]. Many wrongly attribute this regression to a lack of willpower or inadequate diligence on a cognitive, behavioral or social level. With weight regain, however, it is increasingly recognized that not only are environmental pressures difficult to overcome, but in response to diet-induced weight loss, numerous biologic factors that regulate energy intake and expenditure defend

against further weight loss and promote weight regain. The term energy gap is used to describe the biological pressures of reduced energy expenditure and increased hunger following weight loss which drive weight regain [7].

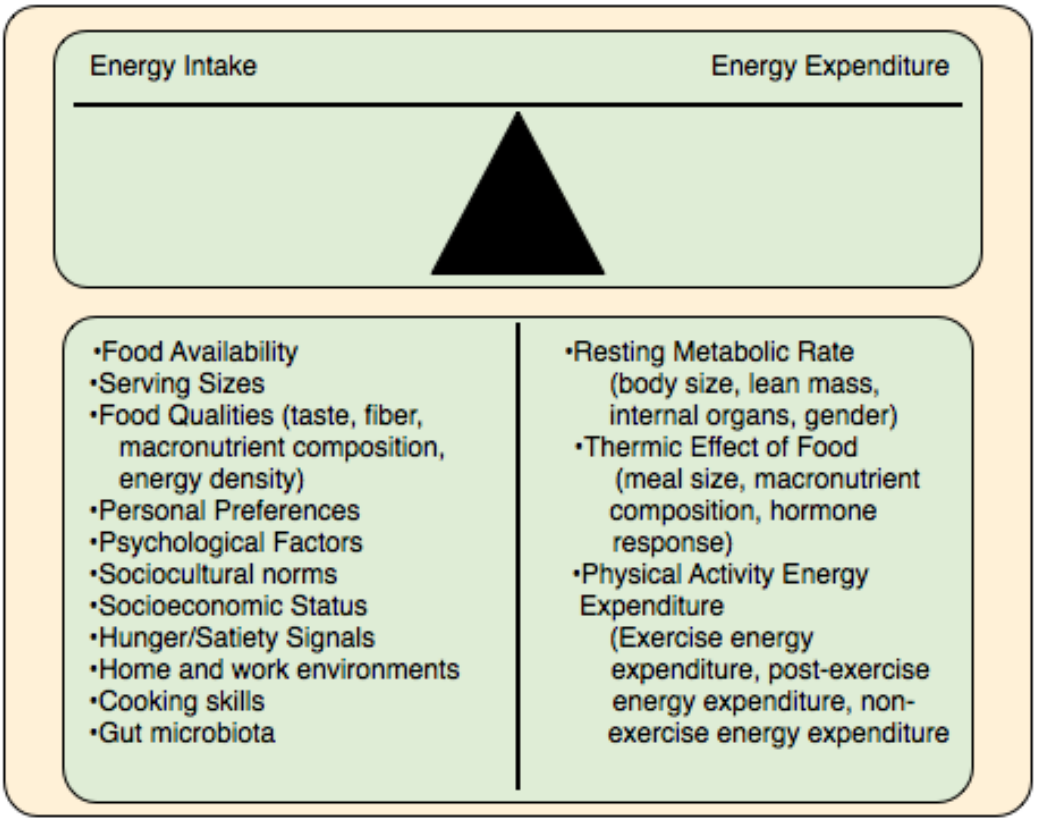

Figure 1: Energy balance

Factors influencing energy intake and energy expenditure [65].

Energy Gap

Weight loss initiates a series of compensatory biological changes aimed to defend against the further weight loss and promote weight regain. These include alterations in

hunger and satiety related hormones and peptides along with decreases in energy expenditure. The weight loss-induced compensatory changes result in a substantial elevation in hunger coupled with a decreased energy need. This mismatch between hunger and energy requirements leaves weight-reduced individuals susceptible to weight regain.

Body weight regulation

There are many elements that contribute to body weight regulation. The maintenance of body weight requires a balance be struck between energy intake and expenditure. The obese state is achieved through the additive effects of numerous episodic periods of energy intake greater than energy expenditure or chronic, sustained, daily, positive energy balance. Influencing intake and expenditure are countless interrelated factors. The components of energy intake and expenditure will be discussed separately along with several specific influencing factors.

Body weight regulation-energy intake

Energy intake is one side of the energy balance equation and can be defined as the amount of metabolizable kcalories consumed as food. This also includes the energy derived from resistant starch and dietary fibers resulting from microbial digestion. While simple to define, the regulation of energy intake is anything but simple.

Central Nervous System in the regulation of body weight

Appetite, food intake, and body weight are regulated by an array of hunger and satiety signals. These molecules signal to the central nervous system the acute and long-term energy status of peripheral tissues. Weight loss-induces changes in concentrations of these hunger and satiety signals, such that a state of energy depletion is communicated to the central nervous system, particularly the hypothalamus. The hypothalamus is the brain region best characterized as the center responsible for integrating these signals [8, 9]. Distinct neuronal circuits initiate the appropriate responses to changing hormone, nutrient and peptide concentrations and subsequently affect energy intake and expenditure to regulate body weight.

The arcuate nucleus (ARC) of the hypothalamus is a major circuit involved in the regulation of energy expenditure and intake. The ARC is composed of two neuronal populations with reciprocal functions. Neurons co-expressing neuropeptide Y (NPY) and agouti-related protein (AgRP) work in concert to increase food intake and decrease energy expenditure when stimulated [8, 9]. Weight loss results in the activation of these neurons to increase energy intake and decrease energy expenditure. Activating neurons that express pro-opiomelanocortin (POMC) leads to decreases in food intake and increases in energy intake [9]. Weight gain stimulates these neurons to decrease energy intake and increase in energy expenditure.

Adiposity-related signals

Insulin and leptin are two circulating hormones that have been identified as adiposity-related signals. These hormones regulate intake in the long-term. Concentrations of insulin and leptin are relatively stable and proportionate to adipose mass [11, 10]. As adiposity increases with weight gain, so do blood concentrations of insulin and leptin. Weight loss, in turn results in decrements to circulating leptin and insulin blood concentrations [13, 14, 17].

Leptin

Leptin is secreted by adipose tissue in amounts directly proportional to the magnitude of adipose mass, especially peripheral fat stores [11]. In healthy weight individuals, leptin indirectly activates the ARC in the hypothalamus [12]. The result is an inhibition of appetite and a promotion of energy expenditure to decrease fat stores. However, the majority of obese individuals present with hyperleptinemia [13]. The apparent inadequate or absent response to elevated blood leptin concentrations has led to the theory of leptin resistance among obese individuals, in which the hypothalamic neurons fail to elevate energy expenditure and reduce appetite in response to increased circulating leptin.

During the dynamic phase of weight loss, fasting leptin concentrations decrease [13, 14]—an expected response given the decrease in fat mass. However, the decrement in circulating leptin is disproportionately greater than the loss of fat mass. In one study, a loss of 10% body mass produced a 64% decrease in leptin concentrations [15]. This

discordant decrease in leptin concentrations in response to a modest weight loss is thought to be one factor contributing to the energy gap and the propensity for weight regain. Studies utilizing exogenous leptin supplementation in weight-reduced individuals as a means of countering hypothalamic leptin resistance have found beneficial effects on hypothalamic activity, satiety, and energy expenditure [15, 16].

During the static phase of weight maintenance following weight loss, leptin concentrations increase [17, 13]; however these concentrations remain significantly reduced even when adjusted for changes in fat mass after one [15, 17] and two years [18] of weight maintenance. Thus, a sustained leptin decrement following weight loss is likely a primary contributor to the weight loss-induced energy gap. However, it is possible that weight loss improves the hypothalamic neuronal response to leptin in a manner similar to weight loss-induced improvements in insulin sensitivity. If true, the lower leptin concentrations would be at least partially offset by increased leptin sensitivity, contributing less to the weight loss-induced energy gap than might be expected.

Insulin

Insulin is produced by beta-cells of the pancreas and 24-hour insulin concentrations are proportionate to adipose tissue mass, until such a time as the beta-cells wear out and insulin secretion diminishes. Insulin release is responsive to acute changes in nutrient intake [10]. It is secreted in response to acute meal ingestion in proportion to the rise in blood glucose concentrations. Insulin stimulates glucose uptake into muscle and

adipose tissue and indirectly activates glycogen synthase resulting in elevated rates of glycogen synthesis [46]. Additionally, insulin inhibits hepatic glucose production and lipolysis in adipose tissue [46]. Insulin is anorexigenic; central administration of insulin decreases intake and body weight [47]. It is believed these effects are derived from insulin binding to receptors in the ARC.

Insulin resistance is a hallmark of obesity. In insulin resistance tissues inadequately respond to insulin. To compensate, the pancreas secretes additional insulin, often leading to hyperinsulinemia. As beta cell mass is reduced, the hyperinsulinemic state often leads to beta-cell decompensation, reduced insulin secretion and type 2 diabetes.

Weight loss results in a significant reduction in insulin concentrations [17]. This has been shown to persist at least one year following weight loss [17]. Decreased insulin concentrations signal to the hypothalamus a state of energy deficit. This results in adjustments to increase energy intake and decrease expenditure to return to initial body weight. Complicating these findings is a weight loss-induced increase in insulin sensitivity [48-50]. This stipulates that lower concentrations seen with weight loss are potentially able to produce an enhanced response compared to the obese-insulin-resistant state. This would indicate that the weight loss-induced decrease in insulin concentration may play less of a role than expected to the establishment of the energy gap.

Gastrointestinal signals

A host of hunger and satiety hormones and peptides are produced in the stomach and intestines. These gastrointestinal signals operate in the short-term on a meal-to-meal basis. They communicate the amount of food ingested in a single sitting to regulate total meal size. Weight loss results in compensatory adjustments in the production and circulating concentrations of these satiety/hunger signals. In many cases, these adverse alterations are disproportionate to the change in fat mass. These adjustments have been shown to persist up to a year following weight loss [17] and are likely instrumental in the establishment of the energy gap. Several of these signals including ghrelin, peptide YY, and cholecystokinin will be discussed in detail.

Ghrelin

Ghrelin is the only known orexigenic hormone and is produced primarily by oxyntic cells of the stomach [19]. This peptide is the endogenous ligand for the growth hormone secretagogue receptor type 1a (GHS-R1a) which is located throughout the body including the hypothalamus, pancreas, stomach and vagus nerve [19, 20]. Peripherally secreted ghrelin does not cross the blood-brain-barrier in high concentrations. Instead, vagal afferent neurons are required for ghrelin-mediated effects on appetite regulation [21]. These vagal afferents terminate on the nucleus tractus solitarius (NTS), which is connected to the ARC through noradrenergic neurons. There is also evidence of ghrelin-containing neurons in the hypothalamus, which when stimulated release ghrelin directly into the ARC [51]. Within the ARC, ghrelin binds to GSHR-1a which is co-localized with NPY secreting neurons [22]. Additionally, ghrelin indirectly inhibits POMC

secreting neurons leading to an attenuation of POMC release [22, 23]. Outside of the ARC, ghrelin has an additional effect in driving food intake. Ghrelin is known to stimulate reward centers of the brain. When activated these pathways are capable of overriding the homeostatic control mechanisms designed to maintain energy balance [24].

Ghrelin increases food intake and weight gain when administered peripherally to humans [25] and rodents [26] and does so in a dose-dependent manner when administered intracerebroventricularly in rodents [27]. Normally, ghrelin levels rise during fasting and drop rapidly with meal ingestion proportional to meal kcalorie content [28, 29]. However, obese individuals do not display the same suppression of ghrelin in response to kcalorie ingestion [28, 29]. It is speculated that the failure to adequately respond to kcalorie intake is one mechanism that perpetuates the obese state. It is also suggested that obese individuals are more susceptible to intravenous administration of ghrelin due to disproportionate increases in food intake in obese compared to lean individuals [26]. In one study, a low dose ghrelin infusion had no effect on food intake in the lean group but produced a 20% increase in intake in the obese group [26]. A high dose ghrelin infusion increased the intake in both groups with an average increase of 20% in the lean and 70% in the obese group [26]. Taken a step further, this research suggests that obese individuals are more sensitive to increases in circulating ghrelin concentrations with weight loss.

The majority of research has demonstrated that ghrelin concentrations rise initially with acute weight loss [14]. This increase in ghrelin is a biologic driver of increased food intake in weight-reduced individuals. However, there are mixed findings concerning the duration in which the elevated levels persist in the long-term [17, 30]. A study by Sumithran et al. in which 14% of initial body was lost over the course of 8 weeks, showed that ghrelin concentrations following weight loss decreased over the subsequent year. However, even one year later circulating ghrelin remained significantly higher compared to baseline ghrelin concentrations [17]. The ambiguity in the literature over the persistence of elevated ghrelin levels long-term may be explained by differences in the methods of measuring ghrelin concentrations, magnitude of weight lost and the means by which the weight loss is achieved.

Peptide YY

Peptide YY (PYY) is co-secreted with GLP-1 by L cells in the gastrointestinal tract in proportion to kcalories ingested. By way of binding to the Y family of G protein-coupled receptors, PYY acts to decreases hunger and increase satiety. Concentrations are lowest during fasting and peak approximately one hour after eating. Obese individuals seem to have a dampened PYY response to meal ingestion which may foster increased food intake [31]. Dynamic weight loss has been shown to decrease PYY concentrations [17]. Concentrations remain depressed for at least a year following weight loss [17]. This adaptation to weight loss contributes to the energy gap, promoting weight regain.

Cholecystokinin

Cholecystokinin (CCK) is secreted from I-cells of the intestines upon food ingestion. CCK secretion pays a role in digestion including stimulating enzyme release from the pancreas and bile from the gall bladder, slowing gastric emptying and enhancing intestinal motility. CCK is also an appetitive hormone, decreasing hunger and increasing satiety through interactions with the vagus nerve [52]. Additionally, circulating CCK can cross the blood-brain-barrier and directly affect the central nervous system. The use of CCK as an anti-obesity treatment has been invalidated. Peripheral administration reduces meal size while increasing meal frequency with no net effect on weight [53]. Like PYY, weight loss decreases circulating CCK concentrations which persist at least one year following initial weight loss [17].

Extra-hypothalamic regulation of energy intake

Body weight regulation in human beings, unlike laboratory animals, is subject to influences beyond biology. Varying environments and complex psychosocial pressures influence cognitive-driven eating behaviors. These behaviors recruit extra-hypothalamic brain regions that are capable of overriding the hypothalamic signals, at least to an extent.

Brain imaging techniques have allowed for the visualization of the complex brain functioning involved in hunger, satiety, and feeding. Vast brain circuits beyond the hypothalamus and brainstem are involved in the initiation of food intake and feeding behavior and respond to changes in weight. The application of brain imaging techniques

has demonstrated the profound effects food stimuli have on the human brain [32]. In addition, these techniques have demonstrated that the weight status of an individual influences brain responses to food images [33, 34].

Several studies have highlighted the importance of prefrontal cortex inhibitory control for successful weight loss [33, 34]. The results demonstrated that individuals, successfully maintaining weight loss, display greater activation in frontal and temporal regions compared to non-dieting individuals [33]. The conclusions drawn from this research suggest enhanced inhibitory control is associated with an inherent ability to maintain control over food intake through increased dietary restraint.

Insight into the brain's response to overfeeding has been provided by the work of Cornier and colleagues [35]. They demonstrated that thin individuals, resistant to weight gain, display a dampened response to food images in response to short term overfeeding [35]. Reduced weight overweight/obese individuals fail to produce this same attenuated response indicating an inability to properly sense the state of acute positive energy balance [35]. It is hypothesized that this inadequacy is partially responsible for the high rates of weight regain seen in overweight/obese individuals.

The prior discussion has outlined the role that the energy intake side of the energy balance equation plays in weight regain. Largely attributed to compensatory changes in circulating hunger and satiety signals, the weight-reduced individual is vulnerable to a marked elevation in hunger. Unfortunately, this increased hunger is compounded by

weight-loss induced decrements in energy expenditure. A thorough discussion of the energy expenditure contribution to the energy balance equation is not within the scope of this thesis. However, due to the interrelatedness of energy intake and expenditure a brief discussion follows.

Body weight regulation-energy expenditure

Energy expenditure can be divided into three components: resting metabolic rate, thermic effect of feeding, and thermic effect of physical activity [38]. Resting metabolic rate is the largest contributor to energy expenditure. It includes the cost of maintaining essential body systems, temperature and ion gradients while at rest. Fat free mass is the largest contributor to RMR. Weight loss often results in a decrease in RMR greater than would be expected for changes in total mass [38, 43, 44, 45]. A decreased RMR results in a decreased energy need. This directly contributes to the energy gap. The thermic effect of feeding is comprised of the energetic cost of digestion, absorption and storage of dietary nutrients and typically accounts for approximately 5-10% of kcalories ingested. Weight loss achieved through caloric restriction predictably reduces TEF. The thermic effect of physical activity includes both exercise thermogenesis and non-exercise activity thermogenesis, both of which are also often reduced in response to weight loss. With the increased biological and environmental pressures to increase energy intake and the reduced expenditure following weight loss, the success of future weight loss maintenance interventions is dependent upon finding effective methods to minimize this energy gap.

Energy flux

The term energy flux used in this thesis, describes the rate of turnover of kcalories entering and exiting the body i.e. the throughput of energy within the body. Both high and low flux states, respectively, can be achieved under conditions of energy balance. That is to say, energy intake is matched to either a high or low energy expenditure so that body weight is maintained. A high flux state would indicate high energy expenditure in combination with an increased intake to compensate for the expenditure. A low flux state implies a sedentary condition in which very low energy expenditure is matched by a low kcalorie intake. Both human and animal data provide some support for the potential role of a high flux state in attenuating the energy gap and thus aiding in the maintenance of body weight.

As far back as 1956, Mayer observed in West Bengal workers that as daily energy expenditure decreased as a function of their occupation, their energy intake proportionately decreased [36]. This, however, only occurred to a certain point. The low daily energy expenditure of sedentary shop keepers, supervisors and clerks was not matched by low energy intake. In other words, the sedentary workers failed to appropriately balance their energy intakes with their low rates of energy expenditure. Instead, they were found to exhibit energy intakes in line with those workers engaging in very heavy physical activity and high daily energy expenditure. This would promote weight gain in this sedentary group of individuals.

Further evidence that an increased activity level may promote weight maintenance has been shown by Jakicic et al [37]. Reduced-weight, over weight and obese women were prescribed an exercise regimen ranging in intensity and duration. After 24 months, it was found that those sustaining a weight loss of at least 10% of initial body weight reported participating in greater physical activity [37].

The National Weight Control Registry was established in 1994 by Rena Wing and James Hill to investigate the characteristics of individuals successfully maintaining a weight loss of at least 30 pounds [42]. Ninety percent of the members report exercising one hour per day in order to maintain weight loss [42].

Not only is there some evidence that energy intake is more accurately regulated to maintain energy balance in high flux compared to low flux state, but there is also evidence that the high flux states are characterized by higher resting metabolic rates. In several observational studies in young men [56], young women [57] and older adults [58], a state of high energy flux resulting from high levels of daily exercise was associated with higher resting metabolic rates. If this phenomenon were true following weight loss, a high flux state could help attenuate the energy gap so readily associated with weight regain.

Rigorous randomized control trials in animal models of obesity confirm the observational data seen in humans. Ad libitum fed, weight-reduced obese animals subjected to daily exercise bouts better match intake to energy expenditure [39, 40]. Exercise does not

completely prevent weight gain; however exercised animals are better able to defend against a lower body weight compared to non-exercised animals [39, 40]. This is significant because even small reductions (~5%) in body weight have beneficial effects on biomarkers of health in humans [41]. These animals do not demonstrate significant differences in circulating hunger and satiety signals compared to sedentary animals [39]. Instead it appears that exercise sensitizes the animals to existing concentrations through differing hypothalamic neuropeptide expression [39, 40].

STUDY HYPOTHESES AND AIMS

The above evidence supports the notion that a high flux state may attenuate the weight-loss-induced energy gap. Therefore, the purpose of this study was to investigate, after clinically relevant weight loss, differences in hunger, satiety, ad libitum food intake, and RMR during a four-day high flux condition compared to a low flux condition. We hypothesized that the high flux state would foster great satiety, suppress hunger, decrease ad libitum intake and increase RMR compared to a low flux state.

METHODS

Subjects

We recruited eleven obese study participants from the Colorado State University community and surrounding areas. Enrollment criteria included: BMI between 30-43 kg/m^2, age 18-55 years, weight stable over the prior 12 months, desire to lose weight, and ability to exercise as assessed by electrocardiogram (ECG), resting blood pressure and a normal incremental exercise test to exhaustion with simultaneous ECG. Exclusionary criteria included: pregnancy or breastfeeding, smoking, use of medication known to affect appetite or metabolism (including but not limited to antidepressants and statins), or prior surgery for weight loss. The study was approved by the Colorado State University Internal Review Board. Informed consent was obtained from each subject prior to participation in the study.

Experimental Design

The approach used in this study was a within-subjects cross-over experimental design to test the effect of high and low flux states following weight loss on resting metabolic rate and perceptions of hunger and satiety. The study protocol was divided into four distinct phases: 1.) baseline testing phase prior to weight loss; 2.) weight loss phase induced by a hypocaloric diet over the course of several months; 3.) weight maintenance phase in which subjects were maintained at the reduced weight for 3 weeks; and 4.) experimental phase in which measures were obtained of subjects' resting metabolic rates, fasting and post-prandial perceived hunger and satiety, fasting

and post-prandial circulating glucose, insulin, and PYY concentrations, and ad libitum food intake on the 5th day following low flux and high flux phase conditions, respectively, completed in random order with a three-day washout period in between (see Figure 2).

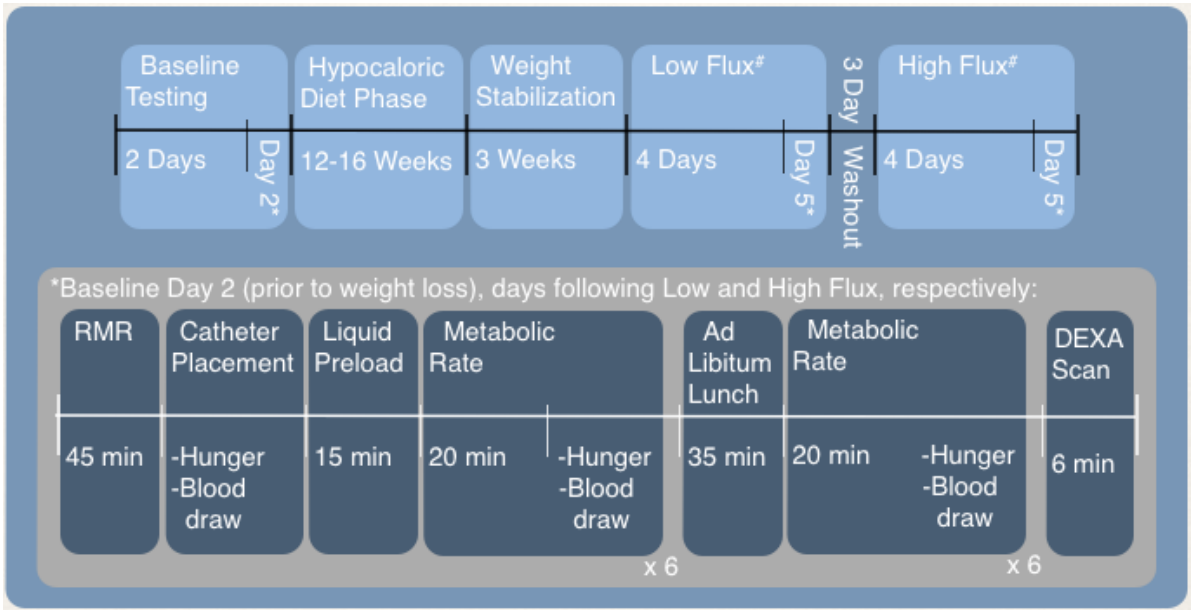

Figure 2: Experimental Timeline

#Order of Low Flux and High Flux were randomly assigned

During the low flux condition subjects remained sedentary for four consecutive days. All food was provided so that energy intakes were adjusted to maintain energy balance. During the high flux condition subjects exercised on four consecutive days (approximately 500 net exercise kcal expenditure at 60% $V0_2$ max) and were fed additional food necessary to maintain energy balance. Most of the study procedures were performed in the Human Performance and Clinical Research Laboratory (HPCRL) at Colorado State University. The dietary counseling for weight loss took place in the

Kendall Anderson Nutrition Center (KANC) located on the Colorado State University campus. The specific details of each of these flux conditions are provided later in this section.

Baseline Pre-Weight Loss Testing

Baseline testing prior to subjects losing weight was scheduled for two sessions. The first included initial screening, completion of a health history questionnaire, measurement of blood pressure and electrocardiogram during both rest and an incremental exercise-to-exhaustion test. The second was used to assess resting metabolic rate (RMR), thermic effect of feeding (TEF), subjective ratings of hunger and satiety, collection of blood samples for analysis of various metabolic hormone concentrations and body composition assessed by dual-energy x-ray absorptiometry (DEXA). Subjects reported to the HPCRL between 05:00 and 08:00 after a minimum ten-hour fast and underwent an initial 45-minute test to determine resting metabolic rate (RMR) by indirect calorimetry prior to venous catheter insertion. A baseline blood sample, subjective hunger rating and blood pressure were taken followed by a liquid breakfast preload with an energy content of 20 percent of RMR. The subsequent three hours were comprised of identical 30 minute cycles in which 20 consecutive minutes were dedicated to collecting metabolic rate and data on subjective hunger/satiety ratings and a blood sample were obtained at the end of each 30 minute period. Three hours after breakfast, participants were provided with a pre-weighed ad libitum buffet, which was consumed during a 30 minute lunch period. After lunch, the 30 minute cycles

continued for an additional three hours. The day was completed with an assessment of body composition by DEXA scan.

Weight Loss Phase

Weekly individual nutritional counseling appointments were provided to the subjects by trained graduate students from the Department of Food Science and Human Nutrition at Colorado State University. Together, the assigned counselor and subject developed an individualized plan to achieve a weight loss goal of 7% of initial body weight over the course of an estimated 12-16 weeks. Weight loss was focused on achieving a hypocaloric state solely by way of a reduced kcalorie intake, not by increased physical activity. Participants were weighed weekly using a Tanita bioimpedance scale (Tokyo, Japan) to assess weight loss progress.

Weight Stabilization Phase

To minimize the acute effects attributable to the dynamic phase of weight loss on metabolic rate and on hunger and circulating appetitive hormone concentrations, subjects were maintained at the seven percent lower body weight for a three-week period prior to the start of the low and high flux conditions. During these three weeks subjects reported to the KANC every three days to monitor weight and minimize weight fluctuations. Subjects were instructed to consume a slightly increased kcalorie intake compared to the weight loss phase to maintain weight.

Low Flux Experimental Condition

Study participants were randomly assigned to either low flux or high flux as their first experimental phase except for one subject, who for work-related reasons, needed to perform high flux during the first week of the experimental conditions. A three-day washout period occurred prior to the start of the second flux phase. The low flux phase occurred over the course of five days. Subjects reported to the HPCRL on the morning of days 1-4 for a RMR measurement. RMR was then used to calculate the subject's kcalorie intake required for daily energy balance based on the formula RMR x 1.3, reflective of little daily physical activity energy expenditure. Prepared meals were weighed and provided the morning of each flux day to the subject to eat throughout the day. Subjects were expected to remain extremely sedentary and were instructed to keep daily steps below 3,000 each day. They were supplied a pedometer to track the number of steps taken. On day 5, the same measures taken at baseline prior to weight loss were again obtained. These included RMR, fasting and postprandial blood sampling for measures of appetitive hormones and glucose, ad libitum food consumption, and subjective measures of hunger and satiety. Following the completion of day 5 testing, subjects completed a hunger/satiety questionnaire used to assess general feelings of hunger/satiety over the prior four days of the low flux condition.

High Flux Experimental Condition

Similar to the low flux condition, the high flux phase occurred over the course of five days. Subjects reported to the HPCRL on the morning of days 1-4 for a RMR measurement. RMR was then used to calculate the subject's kcalorie requirement for

the day at RMR x 1.7. To achieve energy balance, subjects' energy expenditure was elevated through an increased step count and supervised exercise bout. Prepared meals were weighed and provided to the subject to eat throughout the day. Subjects were supplied a pedometer (NL-1000 pedometer, NEW-LIFESTYLES, Inc., Lees Summit, MO) to track their step count and instructed to achieve a minimum step count of 7,500. The daily supervised exercise bout was performed on a cycle ergometer or motorized treadmill depending on subject preference. Exercise was performed at 60 percent of VO_{2peak} to produce a net exercise energy expenditure of about 500 kcalories. On day 5, the same testing protocol was performed as that of day 5 for the low flux condition including fasting, post-breakfast and post buffet measures. Following the completion of these measurements on day 5 subjects completed a hunger/satiety questionnaire used to assess general feelings of hunger/satiety over the prior four days of high flux.

Specific Procedures

Resting Metabolic Rate

Subjects arrived at the HPCRL after a minimum ten hour fast prior to participating in physical activity. Calibration of the instruments with known gas concentrations (Mass Spectrometer (Perkin Elmer MGA 1100, MA Tech Services, Inc., St. Louis, MO) or Parvo (Parvo TrueOne 2400 Metabolic Measurement System, Parvo Medics, Sandy, UT)) took place prior to subject arrival. VO_2 and VCO_2 values were obtained using

indirect calorimetry to estimate RMR while subjects lay quietly for 45 minutes in a dim lit room. The Weir equation was used to convert respiratory gas exchange measures to kcal [54].

Diet Standardization

Subjects were provided meals during baseline testing Day 2 and during all four days of low and high flux, respectively. All meals and snacks were provided to subjects on days 1-4 of low and high flux. Meals were designed to achieve a daily macronutrient composition of 50/35/15 (carbohydrate/fat/protein). Total energy expenditure was calculated at RMR multiplied by an activity factor. The low flux phase activity factor was 1.3 and high flux 1.7. Meals were prepared and weighed ahead of time. Estimations of the required kcalorie intake for each of the four days of low and high flux were made based on the measured RMR values determined the morning of the same day.

Experimental Days: Baseline Day 2, Low/High Flux Day 5

Three identical experimental days were used to examine possible differences in perceptions of hunger and satiety, blood glucose, insulin, and PYY in response to breakfast preload, and ad libitum intake from a meal buffet. One experimental day was a baseline day prior to weight loss and the other two experimental days were the day following the high and low flux conditions, respectively. On each of these days participants reported to the HPCRL between 0600 and 0800. Following measurement of RMR, a venous catheter was placed in an antecubetal vein and connected to a saline IV drip for catheter patency. A fasting blood sample was obtained, which was then

followed by subjects consuming a liquid breakfast preload (Ensure, Ross Laboratories, Abbott Park, IL; 64% CHO, 22% fat, 14% protein, chocolate, strawberry or vanilla) with a caloric value of 20 percent of measured RMR. Subjects were given 15 minutes to complete the liquid meal and then blood samples were collected at 30 minute intervals for the next 3 hours. For lunch, an ad libitum buffet of pre-weighed food was provided. Food items included, a pre-made sandwich cut into small sections with the subject choosing the types and amounts of meat, greens and other vegetables, tomatoes, cheese, and dressing. They were also provided with a variety of other foods including chips, yogurt, apples, bananas, various candy bars, cookies, water, apple juice and milk. Subjects ate in a quiet room, were allowed to bring their cellphones or reading material, and were given 30 minutes to complete their meal. Remaining food was reweighed to determine the total amount of ingested food for each food item for later conversion to total kcalorie ingestion using diet analysis software (Nutritionist Pro Axxya Systems). Blood samples were collected from the indwelling catheter at 30 minute intervals for the next 3 hours, and participants rated their perceptions of hunger and satiety at the same time points.

Measures of Hunger and Satiety

Hunger and satiety were analyzed using a 100mm visual analog scale questionnaire at each 30 minute interval during baseline day 2 and low/high flux day 5. The questionnaire consisted of four questions (1) how full do you feel? (2) how much could you eat right now? (3) how strong is your desire to eat? (4) how hungry are you?
At the completion of the fifth day of each low and high flux condition subjects were

asked to complete a short questionnaire designed to address their overall feeling of hunger and satiety when reflecting on the prior four days. Subjects answered the following questions by marking a vertical line on a 100mm visual analog scale. (1) “On average, how hungry did you feel at the end of these 4 days?” with anchors with 0- Not hungry, plenty of food and 100- Very hungry, not enough food. (2) “On average, how hungry did you feel throughout each of these 4 days?” with the anchors 0- Not hungry, plenty of food and 100- Very hungry, not enough food. (3) “On average, how full did you feel in the evening just prior to bedtime during these 4 days?” with the anchors 0- Never full, wanted more to eat and 100- Full, could not eat anymore.

Exercise Standardization

Subjects performed a supervised exercise bout on days 1-4 of the high flux phase. Subjects chose between completing the exercise bout on the cycle ergometer, motorized treadmill or a combination of the two. Exercise was performed at 60 percent of VO_{2peak} to produce a net energy expenditure of approximately 500 kcalories. The duration of the exercise bout varied among participants based on individual fitness levels, i.e. VO_{2peak} values, ranging from 60-100 minutes. Respiratory gas exchange measures were taken at regular intervals and any adjustments in intensity were made to maintain the workload at 60% VO_{2peak}. Heart rate (beats/min) was measured using a heart rate monitor (FT1, Polar Electro Inc., Lake Success, NY). Ratings of perceived exertion were measured using the Borg scale [55].

Plasma Assays and Analysis

As indicated earlier, on the baseline day 2 and on days 5 of the low and high flux conditions, respectively, a fasting blood sample was obtained as well as samples obtained at 30 minute intervals following the pre-load breakfast. On each of these occasions, two ml of blood were drawn for immediate analysis of glucose concentrations using the glucose oxidase method on an automated glucose analyzer (YSI 2300 Stat Plus, YSI Inc., Yellow Springs, Ohio). An additional blood sample was collected in EDTA tubes and kept on ice. Tubes were then centrifuged (3200 rpm x 10min at 4 degrees C). Blood serum was collected and transferred to plastic Eppendorf tubes and stored at -70 degrees Celsius for later analysis.

A commercially available MILLIPLEX MAP Human Metabolic Hormone Magnetic Bead Panel (Millipore, Billerica, MA, USA) was used to measure plasma insulin, acetylated ghrelin, and peptide YY (PYY). The technology utilized by this method allows for the simultaneous quantification of the targeted analytes. The protocol provided by Millipore was followed as is briefly described. Two hundred micro-liters of assay buffer were added to each well and left to shake at room temperature for 10 minutes. The assay buffer was removed and 25 µL of control or standards were added to the appropriate wells. Twenty-five micro-liters of assay buffer were added to sample wells followed by 25 µL of sample. Twenty-five micro-liters of matrix solution were then added to control and standard wells. Finally, 25 µL of beads were added to all wells and left to shake at 4℃ overnight. The following morning, well contents were removed and the plate was washed three times. Fifty micro-liters of detection antibody were added to each well and

incubated for 30 minutes at room temperature with agitation. After incubation, 50 µL of streptavidin-phycoerythrin were added to all wells and again incubated for 30 minutes at room temperature with agitation. Well contents were removed and the plate was washed 3 times. One hundred micro-liters of sheath fluid were added to each well. After re-suspending the beads for five minutes the median fluorescent intensity was read using a weighted 4-parameter logistic fitting model to calculate the analyte concentrations. Samples were run in duplicate and a minimum of 50 beads of each targeted analyte were collected on a luminex (Luminex 11/200 System, Luminex, Austin, TX).

Statistical Analysis of Data

Data analyses were performed using SPSS version 22. Comparisons among baseline, high flux, and low flux condition variables (e.g. blood analytes) were made using repeated measures analysis of variance (ANOVA). Least Significant Differences Tests were used for post-hoc comparisons among the three conditions. For those variables examined between low and high flux conditions only, data were analyzed using paired t-tests and repeated measures ANOVA. Statistical significance was set at $p<0.05$.

RESULTS

Participant Characteristics

Twelve subjects initially enrolled in the study. Six subjects were unable to complete all aspects on the study due to various reasons (three failed to lose 7% of body weight within the timeframe expected; two subjects experienced work conflicts; and one participant was found to be using a medication that prevented him from further participation following baseline screening). The baseline subject characteristics of the 6 participants who completed the study are shown in Table 1. All were obese based on both BMI and body composition measures and exhibited RMR values within the range expected for sex and fat-free mass.

Table 1: Subject Baseline Characteristics (n=6, 4-males, 2-females)	
Variable	Mean ± SE
Age (yr)	42 ± 5
Height (cm)	176 ± 4
Weight (kg)	110.7 ± 4
BMI (kg/m^2)	35.7 ± 1.5
Fat Mass (kg)	45.0 ± 2.2
Lean Mass (kg)	61.1 ± 4.1
BF (%)	42 ± 2
RMR (Kcal/day)	1933.0 ± 117.7
RER (VCO_2/VO_2)	0.86 ± 0.04
BMI, Body Mass Index; BF, Body Fat; RMR, Resting Metabolic Rate; RER, Respiratory Exchange Ratio	

In accord with our study design, the six obese subjects succeeded in losing 6.9% of their body weight (Figure 3.) Weight loss was maintain during the scheduled three weeks of weight maintenance and was monitored by visits to the KANC two times per week.

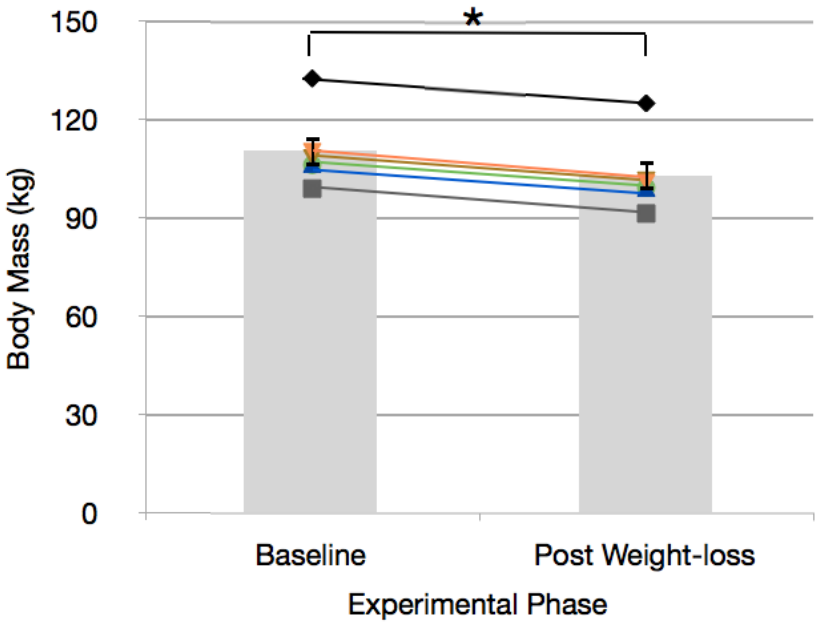

Figure 3: Change in weight following weight-loss phase

Individual and average participant weights at baseline and post weight-loss phases, * $p<0.001$ (n=6).

As designed, the energy intake for high flux (x±SD: 3,191±587 kcal/d) was significantly greater ($p<0.001$) than for low flux (x±SD: 2,449±406 kcal/d) (Figure 4A). In accord with the study design, there was no difference in macronutrient composition between the two conditions (Figure 4B).

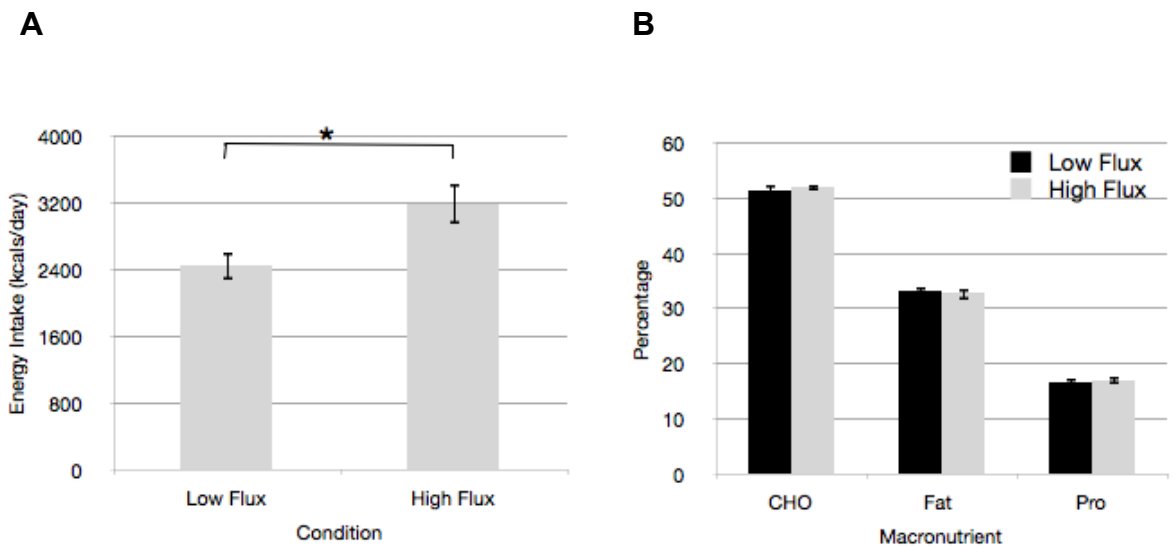

Figure 4: Average energy intake and macronutrient composition during days 1-4 of both low and high flux.

(**A**) Average daily kcalorie intake of prepared meals, snacks and food modules during days 1-4 of low (activity factor 1.34) and high (activity factor 1.67) flux, p=0.001 (n=6). (**B**) Average percentage of energy consumed as carbohydrate, fat and protein on days 1-4 of low and high flux, no statistically significant differences in macronutrient composition were found between conditions (n=6). * indicates $p<0.05$.

Subjects were kept weight stable (Figure 5) during and across the four days of low and high flux conditions, respectively, despite the large difference in daily energy intake. This approximate energy balance was achieved by the low total daily energy expenditure under low flux and the high daily energy expenditure during high flux, the latter a function of the exercise bout and the increased number of daily steps taken.

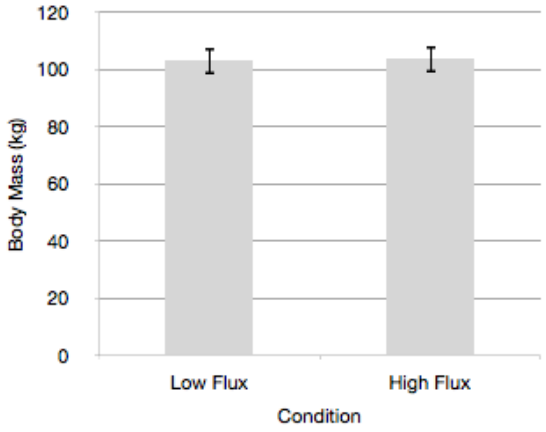

Figure 5: Average body mass across five days of low/high flux for the 6 study participants.

Average weight (kg) of participants across five days of each low and high flux. There were no statistically significant differences were found between conditions.

On the day following each of the low and high flux conditions, subjects were asked to rate their perceptions of overall hunger and satiety during the respective four day periods (Figure 6). Despite energy intakes adequate to maintain energy balance and body weight in both conditions, the participant responses indicated that they were significantly more hungry ($p=0.020$) at the end of each of the days during low flux. They were significantly more full at the end of each of the days during high flux ($p=0.015$). There was a strong trend for the subjects to exhibit greater hunger throughout the day during low compared to high flux ($p=0.09$).

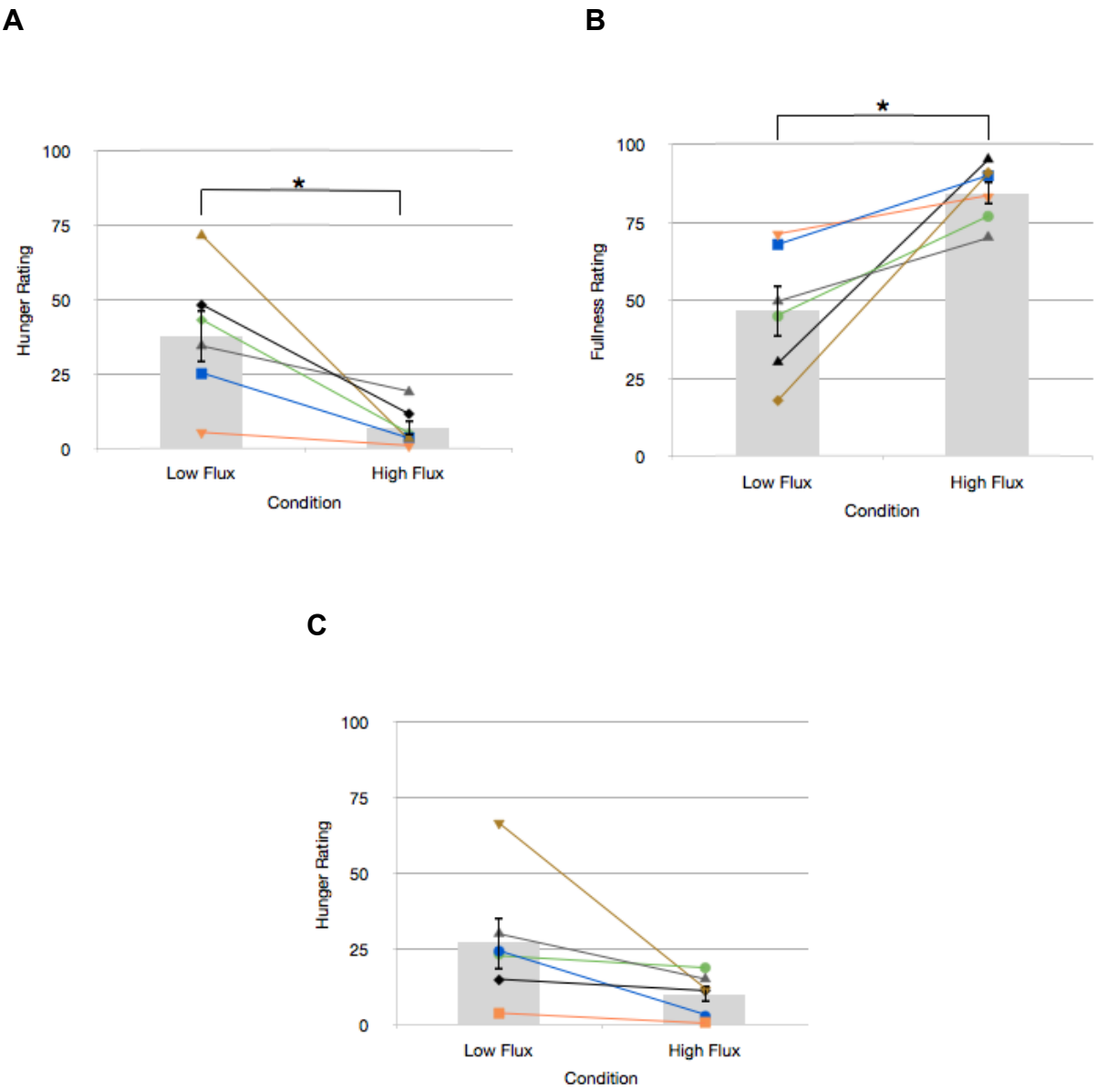

Figure 6: Results of the questionnaire addressing overall perceived hunger/fullness during low/high flux phases completed at the end of each low and high flux (n=6).

(**A**) Individual and average ratings of perceived hunger based on the question “On average, how hungry did you feel at the end of these 4 days?” (p=0.020). (**B**) Individual and average ratings of perceived hunger based on the question “On average, how hungry did you feel throughout each of these 4 days?” (p=0.091). (**C**) Individual and average ratings of perceived fullness based on the question “On average, how full did you feel in the evening just prior to bedtime during these 4 days?” (p=0.015). * indicates $p<0.05$.

On the three different experimental days (baseline day 2 and day 5 of low and high flux), participants were provided a liquid breakfast preload. The energy content of the breakfast was not significantly different between the three conditions (x±SE: baseline 387±24 kcalories, low flux 374±31 kcalories, high flux 381±23 kcalories). Additionally, participants were provided an ad libitum lunch buffet. Average kcalories consumed did not differ between experimental conditions (x±SE: baseline 898±57 kcalories, low flux 1047±108 kcalories, high flux 1046±80 kcalories) although there was significant individual variability (Figure 7).

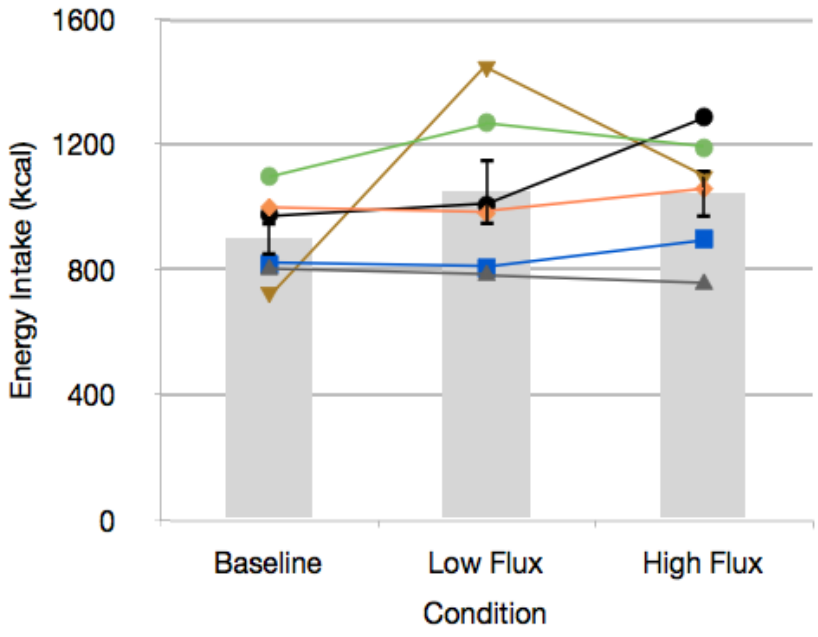

Figure 7: Individual and average kcalorie intake during the 30 minute ad libitum lunch buffet provided on the 5th day of each low and high flux (n=6). There were no statistically significant differences among baseline, low flux and high flux conditions.

The fasting, post-breakfast and post ad libitum buffet blood glucose and plasma insulin and PYY determinations are shown in Figure 8. There were no significant changes in fasting blood glucose with weight loss. As expected, fasting insulin decreased following weight loss and was significantly lower on the LF (8.3±1.1 μU/ml) and HF (6.4±0.8 μU/ml) experimental days compared to the pre-weight loss baseline (11.8±0.6 μU/ml). These data reflect an increase in insulin sensitivity. During the post-prandial periods following breakfast and lunch, there were significant main effects of time and condition for plasma insulin, with the insulin response being significantly lower for the low and high flux states compared to pre-weight loss. This again reflects an increase in insulin sensitivity. There were no differences in fasting PYY concentrations among pre-weight loss, low and high flux conditions respectively. There was a significant time effect such that PYY increased across all conditions in response to breakfast followed by a rapid drop and increase again in response to ingestion from the buffet. However, for PYY there was not a significant main effect of condition, nor was there a condition by time interaction. However, note the lower PYY from 180-360 minutes in the high flux condition compared to the baseline (pre-weight loss) and low flux. There was a significant condition effect such that average PYY from 180-360 minutes was significantly lower for the high flux that the same time points at baseline prior to any weight loss.

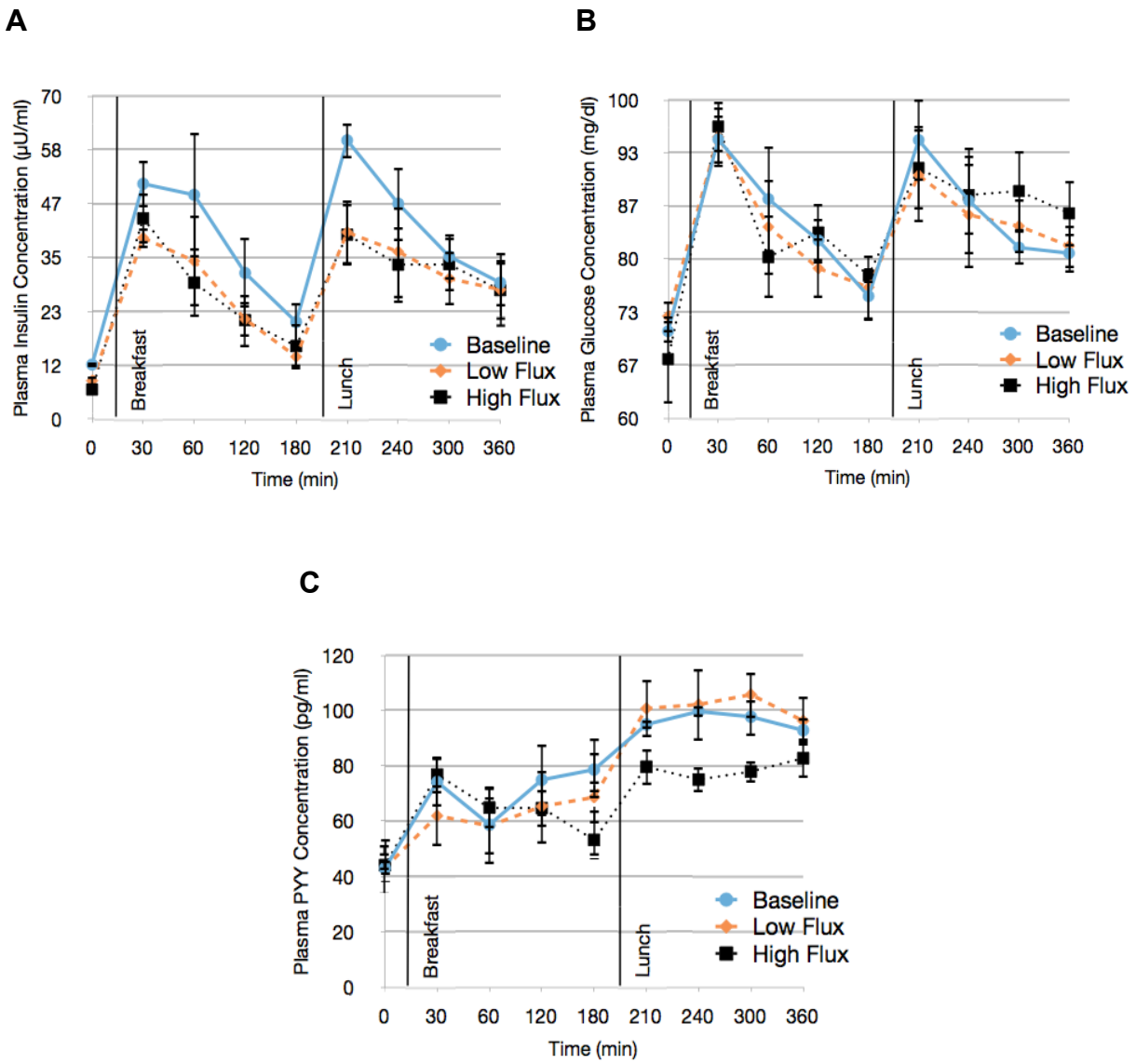

Figure 8: Average, fasting and postprandial plasma analyte concentrations at selected time points taken while fasting and during 3-h periods following breakfast and lunch during baseline (pre-weight loss), low flux day 5 and high flux day 5 testing. (**A**) Group mean plasma insulin concentrations (n=5). There was a significant condition effect, with plasma insulin concentrations significantly lower for the two flux conditions compared to pre-weight loss baseline values. (**B**) Group mean plasma glucose concentrations (n=6). (**C**) Group mean plasma PYY concentrations (n=5). PYY was significantly lower for HF compared to baseline from 180-300 min.

The hunger and satiety data for the three respective experimental days are shown in Figure 9. There was an obvious and expected significant main effect of time for these subjective feelings relative to before and after meal consumption. However, there were no significant condition effects or condition by time interactions. Thus, despite some differences in insulin and PYY concentrations across conditions over the course of the 6 hours on the experimental days, these were not associated with differences in subjective hunger and satiety or in differences in ad libitum energy intake.

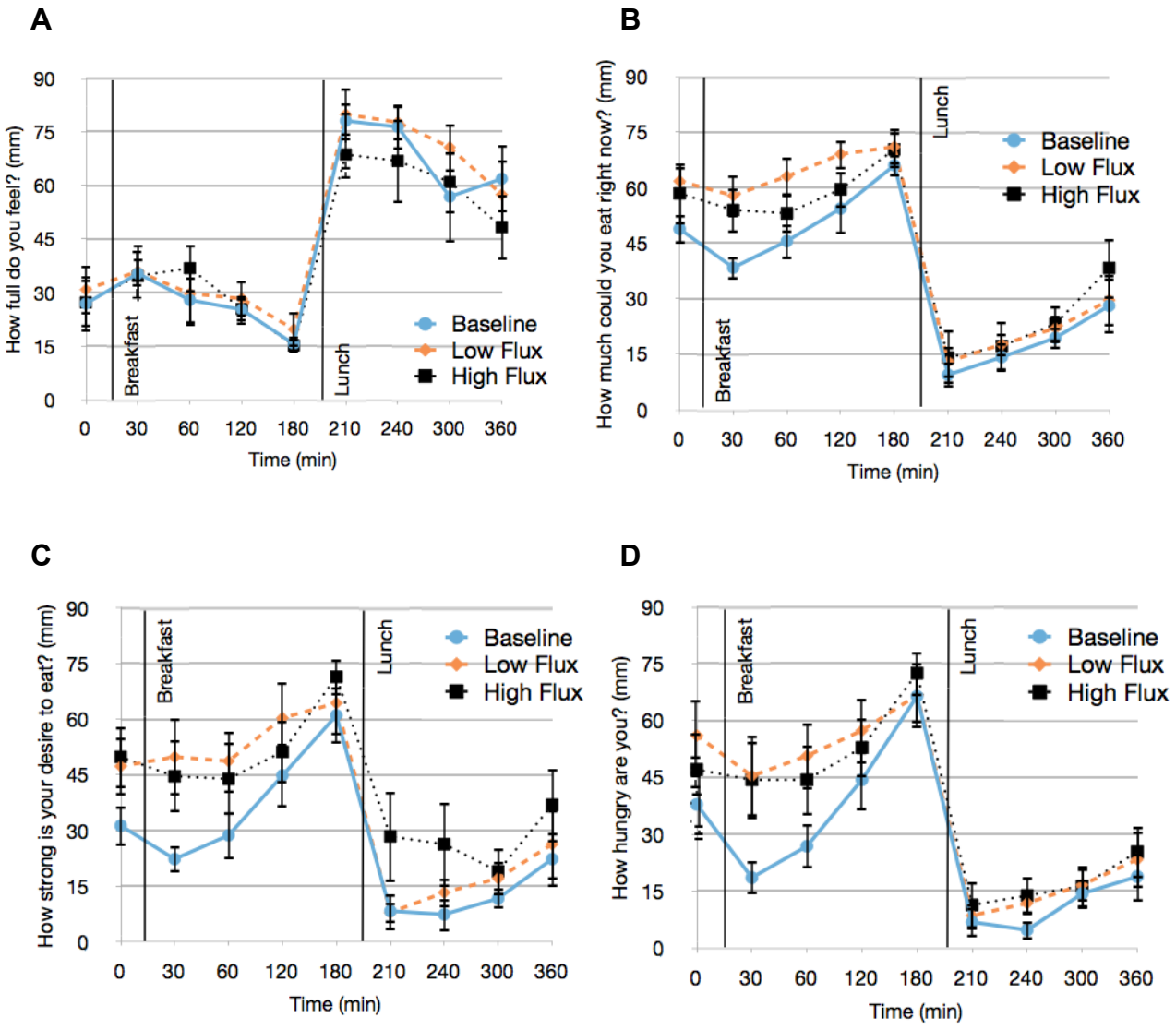

Figure 9: Average participant responses to VAS questionnaire addressing hunger and satiety. Questionnaire was completed at regular time points during baseline, low flux day 5 and high flux day 5 testing.(n=6)

(**A**) Average participant responses to the question how full do you feel? (**B**) Average participant responses to the question how much could you eat right now? (**C**) Average participant responses to the question how strong is your desire to eat? (**D**) Average participant responses to the question how hungry are you?

There were significant time effects for all 4 variables, but condition effects and time by condition interactions were not significant. Timing of breakfast preload and lunch are indicated.

The resting metabolic rate and thermic effect of feeding data were the primary dependent variables for another thesis and thus are not a focus for the present thesis. Nevertheless, for the sake of full disclosure involving both energy intake and expenditure sides of the energy gap, we found that the mean resting metabolic rate was significantly higher averaged over the 4 days of high flux when compared to low flux ($p=0.048$) (Figure 10).

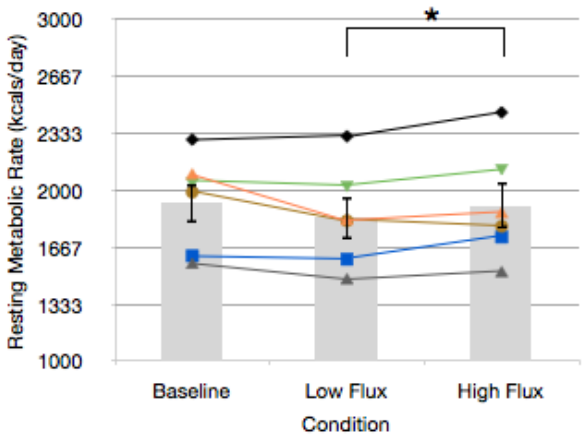

Figure 10: Average resting metabolic rate at baseline and across 5 days of low and high flux (n=6).

Statistically significant difference between low and high flux RMRs $p=0.048$. * indicates $p<0.05$.

DISCUSSION

Major findings

The present study was designed to investigate if a weight-stable, high flux state could minimize the energy gap by dampening the weight loss-induced effects on hunger, satiety and metabolic rate in reduced-weight, obese individuals. Additionally, we assessed the possibility of carryover from the high flux state during a sedentary day following four days of high flux. The main findings of this study include decreased subjective hunger, increased subjective satiety and increased RMR in the weight-reduced participants during four-days of a high flux state compared to a four-day low flux (sedentary) state. These data are consistent with previous work from this laboratory in which young male [56] and female [57] athletes were found to exhibit higher metabolic rates during high flux states compared to low flux states or sedentary controls.

The data regarding the ability of exercise to decrease hunger and/or increase satiety are not consistent. Exercise is generally prescribed to aid in weight loss; however, the magnitude of weight loss is often less than would be expected for the projected exercise-induced caloric deficit. This is thought to be due to energy intake compensation- that is individuals consciously or subconsciously increase energy intake to compensate for the increased energy expenditure. Evidence of the variable response to a 12 week supervised exercise program has been shown by King et al. [64]. The study enrolled 35 overweight and obese sedentary individuals into a supervised

exercise-induced weight loss program. The average weight loss of the participants was 3.7±3.6kg which was comparable to the predicted weight-loss based on the projected exercise induced energy expenditure. The average weight loss, however, does not tell the whole story. Body weight changes upon the completion of the exercise program ranged from a weight loss of 14.7kg to a weight gain of 1.7kg. The authors found that those who failed to lose as much weight as projected had increased their energy intake in response to exercise, while those who lost the most weight actually experienced a decrease in energy intake in response to exercise. Reasons for this response variability are not clear.

In support of the benefits of a high flux state, a study by Rosenkilde et al. [60] found that only participants in the high exercise group (60 min/day at 67% Vo_{2max}) experienced higher fasting and postprandial rating of fullness and elevated postprandial PYY_{3-36} concentrations when compared to the sedentary control. A recent meta-analysis concluded that an acute exercise bout results in increased concentrations of PYY over a 2-4 hour period following the exercise bout, all which may suppress food intake [62]. Along these lines, we found that during the 4-day high flux state, weight-reduced obese participants reported decreased subjective ratings of hunger and increased ratings of satiety compared to a 4-day low flux, sedentary state. Others [61, 63] have previously found that increases in satiety ratings coincided with increases PYY in weight-stable healthy populations following an acute exercise bout. However, we made no attempt to measure appetitive hormone concentrations during the 4-day low and high flux conditions, respectively, but rather to examine these the day after each of these

conditions. This decision was based on evidence that individuals who engage in acute strenuous exercise often do not compensate even during 24-48 hours following the exercise. However, our findings indicate any alterations in circulating hormone and peptide concentrations as a result of the four-day high flux condition do not carry over to the day following the last exercise bout. We found no differences in plasma PYY, insulin or glucose concentrations the day following the high and low flux conditions. These data are also concordant with results of VAS questions addressing hunger, satiety, desire to eat, prospective food consumption, and ad libitum food intake, none of which differed in response to a standardized breakfast and ad-libitum intake at lunch the day after the high and low flux conditions.

Strengths and Limitations

There are numerous strengths of this study. 1.) To our knowledge this is the first investigation designed to examine the possible role of a high flux state on key aspects of the energy gap following clinically relevant weight loss. 2.) While the study participants remained free-living, energy intake and expenditure were carefully controlled during the low and high flux states and there is every indication that energy balance was achieved during these conditions in the face of large differences in energy expenditure. All meals, snacks and food modules were provided to the participants based on measured rather than estimated RMR to minimize errors in estimating energy intake requirements. Daily physical activity was monitored by a pedometer and all exercise bouts performed during the high flux condition were supervised with measures of indirect calorimetry to achieve the net energy cost of 500 kcal per exercise bout. Thus, the higher resting metabolic rates, greater satiety and less hunger during high

compared to low flux are not likely attributable to positive energy balance during high flux and/or negative energy balance during low flux. Circulating insulin is a sensitive marker of energy balance and the lack of significant differences in fasting and postprandial insulin concentrations on the day following the high and low flux states suggest that energy balance did not differ between these conditions. 3.) Additionally, we controlled for the possible confounding effects of acute weight loss on various outcome variables by maintaining subjects' weight loss for three weeks prior to their entry into high and low flux states.

There are several noteworthy weaknesses in the present study. 1.) The weight loss phase was anticipated to take 12-16 weeks; however several participants took much longer than expected to achieve the loss of 7% of initial body weight. It is conceivable the gradual weight loss attenuated the magnitude of the compensatory homeostatic adjustments that are seen with rapid weight loss. 2.) The small sample size could obviously be viewed as a weakness. However, despite this potential limitation, statistically significant differences in key outcome variables were found between the low and high flux conditions. 3.) To minimize the length of the study and burden for the subjects, we did not control for the menstrual cycles of the two female participants. The extent to which this may have influenced our findings is unknown. 4.) In retrospect, a different approach was warranted to examine the potential role of orexigenic and anorexigenic peptides in explaining the difference in hunger and satiety between high and low flux days. As indicated earlier, we measured these peptides the day after the high and low flux conditions, rather than on a day during these respective conditions and thus we can provide no insights as to the possible role of appetitive hormones in

contributing to the reported greater satiety and less hunger during high compared to low flux. 5.) The duration of the low and high flux conditions were only 4 days each. Thus it is unclear if weeks and months of high flux would produce the same beneficial effects on resting metabolic and hunger and satiety as were seen in this short-term study. 6.) Finally, we only assessed homoeostatic regulators of food intake (i.e. insulin, glucose, PYY). We did not attempt to assess the other psychological factors that influence food intake.

In conclusion, we found a 4-day high flux state resulted in decreased hunger, increased satiety and increased RMR in weight-reduced, weight-stable, obese individuals when compared to a low flux state. Our findings support the importance of a daily high energy flux state in attenuating the increase in hunger and the decrease in energy expenditure that accompany diet-induced weight loss. However, the greater satiety and lower hunger were not evident the day following the high flux state, suggesting that such benefits resulting from the acute state are not long-lasting. Future research is needed to address the mechanistic reasons behind these acute beneficial effects of high flux and to determine the effects of long-term high flux on the energy gap and maintenance of lost weight.

REFERENCES

1. Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999–2010. Journal of the American Medical Association 2012;307(5):491–97.
2. Centers for Disease Control and Prevention. Overweight and Obesity: Health Consequences. Accessed: June 2014, http://www.cdc.gov/obesity/adult/causes/index.html.
3. Reugters. Factbox: Obesity's dollars and cents. Accessed: June 2014. http://www.reuters.com/article/2012/04/30/us-obesity-factbox-idUSBRE83T0CG20120430.
4. Kouvelioti R, Vagenas G, Langley-Evans S. The effects of exercise and diet on weight loss maintenance in overweight and obese adults: a systematic review. J Sports Med Phys Fitness 2014 [Epub ahead of print].
5. Curioni CC and Louenco PM. Long-term weight loss after diet and exercise: a systematic review. International Journal of Obesity 2005;29:1168-1174.
6. Lowe MR, Kral TV, Miller-Kovach K. Weight-loss maintenance 1, 2 and 5 years after successful completion of a weight-loss programme. Br J Nutr. 2008;99(4):925-30.
7. Hill JO, Peters JC, Wyatt HR. Using the energy gap to address obesity: a commentary. J Am Diet Assoc. 2009;109:1848-1853.
8. Broberger C, Landry M, Wong H, Walsh JN, Hokfelt T. Subtypes Y1 and Y2 of the neuropeptide Y receptor are respectively expressed in pro-opiomelanocortin- and neuropeptide-Y-containing neurons of the rat hypothalamic arcuate nucleus. Neuroendocrinology 1997;66(6):393-408.
9. Kalra SP, Dube MG, Pu S, et al. Interacting appetite-regulating pathways in the hypothalamic regulation of bodyweight. Endocr. Rev. 20(1), 68-100 (1999).
10. Polonsky KS, Given E, Carter V. Twenty-four-hour profiles and pulsatile patterns of insulin secretion in normal and obese subjects. J Clin Invest 1988;81:442–448.
11. Ugrinska A, Miladinova D, Trajkovska M, Zdravkovska M, Kuzmanovska S, Tripunoski T, Vaskova O. Correlations of serum leptin with anthropometric parameters and abdominal fat depots determined by ultrasonography in overweight and obese women. Prilozi 2013;34(1): 115-119.
12. Oury F, Karsenty G. Towards a serotonin-dependent leptin roadmap in the brain. Trends Endocrinol Metab 2011;22(9):382-387.
13. Considine RV, et al. Serum immunoreatve-leptin concentrations in normal-weight and obese humans. New England Journal of Medicine 1996;334(5):292-295.
14. Wang TN, Chang WT, Chiu YW, Lee CY, Lin KD, Cheng YY, Su YJ, Chung HF, Huang MC. Relationships between changes in leptin and insulin resistance levels in obese individuals following weight loss. Kaohsiung Journal of Medical Sciences 2013;29:436-443.
15. Kissileff HR, Thorton JC, Torres MI, Pavlovich K, Mayer LS, Kalari V, Leibel RL, Rosenbaum M. Leptin reverses declines in satiation in weight-reduced obese humans. Am J Clin Nutr 2012;95:309-317.
16. Hinkle W, Cordell M, Leibel R, Rosenbaum M, Hirsch J. Effects of reduced weight

maintenance and leptin repletion on functional connectivity of the hypothalamus in obese humans. PLoS ONE 2013;8(3):e59114 doi:10.1371/journal.pone.0059114.
17. Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, Proietto J. Long-term adaptations to weight loss. N Engl J Med 2011;365(17):1597-1604.
18. Adam TCM, Jocken J, Westerterp-Plantenga MS. Decreased glucagon-like peptide 1 release after weight loss in overweight/obese subjects. Obesity Research 2005;13(4):710-716.
19. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999; 402:656-660.
20. Camina JP. Cell biology of the ghrelin receptor. Journal of Neuroendocrinology 2006;18:65-76.
21. Le Roux CW, Neary NM, Halsey TJ, Small CJ, Martinez-Isla AM, Ghatei MA, Theodorou NA, Bloom SR. Ghrelin does not stimulate food intake in patients with surgical procedures involving vagotomy. The Journal of Clinical Endocrinology and Metabolism 2005;90(8):4521-4524.
22. Cowley MA, et al. The distribution and Mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulation energy intake. Neuron 2003;37:649-661.
23. Riediger T, Traebert M. Schmid HA, Scheel C, Lutz TA, Scharrer E. Site-specific effects of ghrelin on the neuronal activity in the hypothalamic arcuate nucleus. Neuroscience Letters 2003;2(1):151-155.
24. Malik S, McGlone F, Bedrossian D, Dagher A. Ghrelin modulates brain activity in areas that control appetitive behavior. Cell Metab. 2008;7:400–409.
25. Druce MR, Wren AM, Park AJ, Milton JE, Patterson M, Frost G, Ghatei MA, Small C, Bloom SR. Ghrelin increases food intake in obese as well as lean subjects. International Journal of Obesity 2005;29:1130-1136.
26. Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DGA, Ghatei MA, Bloom SA. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology 2000;141(11):4325-4328.
27. Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature 2000;407(19):908-913.
28. English PJ, Ghatei MA, Malik IA, Bloom SR, Wilding JPH. Food fails to suppress ghrelin levels in obese humans. The Journal of Clinical Endocrinology and Metabolism 2002;87(6):2984-2957.
29. 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):1068-1071.
30. Garcia JM, Iyer D, Poston WSC, Marcelli M, Reeves R, Foreyt J, Balasubramanyam A. Rise of plasma ghrelin with weight loss is not sustained during weight maintenance. Obesity 2006;14(10):1716-1723.
31. Suzuki K, Jayasena CN, Bloom SR. The gut hormones in appetite regulation. Journal of Obesity 2011 doi:10.1155/2011/528401.

32. Wang GJ, Volkow ND, Telang F, Jayne M, Ma J, Rao M, Zhu W, Wong CT, Pappas NR, Geliebter A, Fowler JS. Exposure to appetitive food stimuli markedly activates the human brain. NeuroImage 2004;21:1790-1797.
33. Mc Caffery JM, Haley AP, Sweet LH, Phelan S, Raynor HA, Del Parigi A, Cohen R, Wing RR. Differential functional magnetic resonance imaging response to food pictures in successful weight-loss maintainers relative to normal-weight and obese controls. Am J Clin Nutr 2009;90:928-34.
34. DelParigi A, Chen, K Sable AD, Hill JO, Wing RR, Reiman EM, Tataranni, PA. Successful dieters have increased neural activity in cortical areas involved in the control of behavior. International Journal of Obesity 2007;31:440-448.
35. Cornier MA, Salzburg AK, Endly DC, Bessesen DH, Rojas DC, Tregellas JR. The effects of overfeeding on the neuronal response to visual food cues in thin and reduced-obese individuals. PLoSone 2009:4(7):e6310.
36. Mayer J, Roy P, and 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.
37. 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.
38. Sims EA and Danforth E, Jr. Expenditure and storage of energy in man. J Clin Invest, 1987;79(4):1019-25.
39. MacLean PS, Higgins JA, Wyatt HR, Melanson EL, Johnson GC, Jackman MR, Giles ED, Brown IE, Hill JO. Regular exercise attenuates the metabolic drive to regain weight after long-term weight loss. Am J Physiol Regul Integr Comp Physiol 2009;297:R793–R802.
40. Levin BE, Dunn-Meynell AA. Chronic exercise lowers the defended body weight n and adiposity in diet-induced obese rats. Am J Physiol Regul Integr Comp Physiol 2004;286:R771–R778.
41. Wing RR, et al. Benefits of Modest Weight Loss in Improving Cardiovascular Risk Factors in Overweight and Obese Individuals With Type 2 Diabetes. Diabetes Care. 2011;34(7):1481-1486.
42. Klem ML, Wing RR, McGuire MT, Seagle HM, Hill JO. A descriptive study of individuals successful at long-term maintenance of substantial weight loss. Am J Clin Nutr 1997;66(2):239-46.
43. Leibel RL, Rosenbaum M and Hirsch J. Changes in energy expenditure resulting from altered body weight. N Engl J Med 1995;332(10):621-8.
44. Weigle DS, et al. Weight loss leads to a marked decrease in nonresting energy expenditure in ambulatory human subjects. Metabolism 1988;37(10):930-6.
45. Elliot DL, et al. Sustained depression of the resting metabolic rate after massive weight loss. Am J Clin Nutr 1989;49(1):93-6.
46. Dimitriadis G, Mitrou P, Lambadiari V, Maratou E, Raptis SA. Insulin effects in muscle and adipose tissue. Diabetes Research and Clinical Practice 2011;935:S52-59.
47. Air EL, Benoit SC, Blake Smith KA, Clegg DJ, Woods SC. Acute third ventricular administration of insulin decreases food intake in two paradigms. Pharmacol Biochem Behav 2002;72:423-429.
48. Ross R, Dagnone D, Jones PJ, Smith H, Paddags A, Hudson R, Janssen I.

Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in men. A randomized, controlled trial. Ann Intern Med 2000;133:92–103.
49. Friedman JE, Dohm GL, Leggett-Frazier N, Elton CW, Tapscott EB, Pories WP, et al. Restoration of insulin responsiveness in skeletal muscle of morbidly obese patients after weight loss. J Clin Invest 1992;89:701-5.
50. Niskanen L, Uusitupa M, Sarlund H, Siitonen O, Paljärvi L, Laakso M. The effects of weight loss on insulin sensitivity, skeletal muscle composition and capillary density in obese non-diabetic subjects. Int J Obes Relat Metab Disord 1996;20:154-60.
51. Hou Z, Miao Y, Gao L, Pan H, Zhu S. Ghrelin-containing neurons in cerebral cortex and hypothalamus linked with the DVC brainstem in rat. Regul Pept 2006;134(2-3):126-31.
52. Rogers RC, Herman GE. Mechanisms of action of CCK to activate central vagal afferent terminals. Peptides 2008;29(10):1716-1725.
53. West DB, Fey D, Woods SC. Cholecystokinin persistently suppresses meal size but not food intake in free-feeding rats. Am J Physiol. 1984;246(5 Pt 2):R776–87.
54. Weir J. New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol 1949;109:1-9.
55. Borg, GA. Psychophysical bases of perceived exertion.Med Sci Sports Exerc 1982;14:377-381.
56. Bullough, R.C., et al. Interaction of acute changes in exercise energy expenditure and energy intake on resting metabolic rate. Am J Clin Nutr 1995;61(3):473-81.
57. Burke CM, Bullough RC, Melby CL. Resting metabolic rate and postprandial thermogenesis by level of aerobic fitness in young women. Eur J Clin Nutr. 1993;47(8):575-85.
58. Bell, C., et al. High energy flux mediates the tonically augmented beta-adrenergic support of resting metabolic rate in habitually exercising older adults. J Clin Endocrinol Metab 2004;89(7):3573-8.
59. Martins C, Kulseng B, Rehfeld JF, King NA, Blundell JE. Effect of chronic exercise on appetite control in overweight and obese individuals. Medicine & Science in Sports & Exercise 2013;45(5):802-12.
60. Rosenkilde M, et al. Appetite regulation in overweight, sedentary men after different amounts of endurance exercise: a randomized controlled trial. J Appl Physiol 2012;115:1599-1609.
61. Deightin K, Karra E, Batterham RL, Stensel DJ. Appetite, energy intake, and PYY_{3-36} responses to energy-matched continuous exercise and submaximal high-intensity exercise. Appl Physiol Nutr Metab 2013;38:947-952.
62. Schubert MM, Sabapathy S, Leveritt M, Desbrow B. Acute exercise and hormones related to appetite regulation: a meta-analysis. Sports Med 2014;44:387-403.
63. Martins C, Morgan LM, Bloom SR, Robertson MD. Effects of exercise on gut peptides, energy intake and appetite. Journal of Endocrinology 2007;193:251-258.
64. King NA, Hopkins M, Caudwell P, Stubbs RJ and Blundell JE. Individual variability following 12 weeks of supervised exercise: identification and characterization of compensation for exercise-induced weight loss. International Journal of Obesity 2008;32:177-184.

65. Melby, C. (2012) Energy Balance. Sports Nutrition, ed. Rosenbloom CA, Academy of Nutrition and Dietetics, 5th edition, Chapter 10, 211-229.

APPENDIX

CONSENT FORM

Consent to Participate in a Research Study

Colorado State University

TITLE OF STUDY: Decreasing the Biologic Drive toward Weight Regain by Increasing Energy Flux

PRINCIPAL INVESTIGATOR: Chris Melby, Dr.P.H., Professor, *Dept. of Food Science and Human Nutrition,234 Gifford Building; chris.melby@colostate.edu;*
CO-PRINCIPAL INVESTIGATORS: Christopher Bell, Associate Professor, Department of Health and Exercise Science; christopher.bell@colostate.edu; Matthew Hickey, Professor, Department of Health and Exercise Science; matthew.hickey@colostate.edu

WHY AM I BEING INVITED TO TAKE PART IN THIS RESEARCH? **You are being invited to take part in this study because you are an adult between the ages of 18-55 years. You have a body mass index between 30 and 40 and want to lose weight.**

WHO IS DOING THE STUDY**? With support from the U.S. Department of Agriculture through the Colorado Agriculture Experiment Station, Professors and graduate students from the Department of Food Science and Human Nutrition and the Department of Health and Exercise Science are doing the study.**

WHAT IS THE PURPOSE OF THIS STUDY? **The purpose of this study is to help you lose weight. Then we want to find out whether or not increased exercise and energy intake after you lose weight can improve your metabolism to help you keep from gaining back the weight.**

WHERE IS THE STUDY GOING TO TAKE PLACE AND HOW LONG WILL IT LAST? **The study will take place on the Colorado State University campus in the Nutrition Center, Room 114 Gifford, and in the Nutrition and Fitness Laboratory, Room 216 Gifford Building. Some tests will also be performed in the Human Performance Clinical Research Laboratory in the Moby Complex. The entire study will last approximately 2 years, but your involvement will last 15-21 weeks.**

WHAT WILL I BE ASKED TO DO?

Screening Phase

Before beginning the weight loss program you will be asked to participate in several screening tests. These tests will help determine whether or not you qualify for the study.

Session 1, Screening tests: 1 hour and 15 minutes:

- Height- how tall you are without shoes will be determined using a height rod.
- Weight- how much you weigh while wearing light indoor clothing will be measured using a balance scale.
- Surveys- your current medical, health and family history will be measured using a health history questionnaire
- If you are woman, you will take a pregnancy test using a sample of your urine. If you are pregnant, you are not eligible to participate in this study.
- Your blood sugar level will be measured using a finger prick to determine your risk for diabetes. You are not eligible to participate if your blood sugar is too high.
- Your blood pressure will be measured using an automated cuff similar to what is used in a doctor's office. You are not eligible to participate if you have high blood pressure that needs medications to lower it.
- You will be given instructions as to how to keep track of everything you eat and drink for 3 days.
- You will be asked to complete 5 short questionnaires that measure your perceptions of your eating behaviors and relationship to food. It will only take about 15 minutes to complete all of these surveys.
- You will be asked to wear a pedometer to measure step counts over a 3 day period. You will bring the record of your food intake and your steps to your next session.

If you qualify to participate, you will then be expected to do the following:

Baseline Testing Phase

Session 2, Physical Measures: 2 hours: You will need to come to Moby Complex for the following measurements:

- **Body weight and height** measured with you wearing shorts and a t-shirt.
- **Blood pressure** using a standard automated cuff while you sit quietly.
- **Body composition**: We will measure how much fat you have in your body using a test called dual energy x-ray absorptiometry (DEXA). The DEXA test requires you to lie quietly on a padded table while a small probe gives off low-level x-rays and sends them over your entire body. This test gives very accurate measurements of your body fat and bone mineral density. This test lasts approximately 30 minutes.

- **Circumference measurements**: the distance around your waist, hips, thigh, and upper arm will be measured while you are wearing shorts and a t-shirt.
- **Blood sample**: a person trained in drawing blood will take a small amount of blood (approximately 3 teaspoons) from a vein in your arm. A tourniquet will be placed around your upper arm and a small needle will be inserted into the vein to obtain your blood sample. Your blood sample will be used to measure your levels of your blood sugar, insulin, and other hormones/molecules that can affect health and metabolism.
- **Exercise Stress Test**: This test will help determine if your heart is healthy. You will be asked to walk on a motorized treadmill or ride a stationary exercise cycle for approximately 10-12 minutes. The exercise will become more difficult every 2 minutes. While you are walking or riding we will measure your heart rate with an electrocardiogram (ECG) and your blood pressure with a cuff placed around your upper arm. A physician will supervise the test. If the physician does not think your heart is healthy you will be referred to your primary care physician for further testing. There is a chance that you may not be allowed to take part in our study. You will be asked to do this test once; it lasts roughly 1 hour.

Session 3: Breakfast/Lunch Test Day: 9 hours total.

- This will be a long day for you. You will report to the laboratory at approximately 7:00 in the morning. You should not have eaten since 7:00 the previous night. You will have your height, weight, and blood pressure measured as before.
- **Basal metabolic rate:** the amount of calories you burn at rest will be measured. You will lie quietly in a comfortable bed. A transparent plastic bubble will be placed over your head for approximately 45 minutes which allows us to measure how much air you breathe. This information will help us know how many calories you are burning.
- **Blood sampling**: A person trained in drawing blood will place a tourniquet around your upper arm. After disinfecting the skin, a small catheter (tiny hollow plastic tube) will be placed in a vein in your arm or hand. The catheter will remain in place for the next 7 hours, so that small amounts of blood can be obtained every 30-60 minutes. Your blood sample will be used to measure levels of your blood sugar, insulin, and other hormones that affect metabolism and appetite.
- **Breakfast:** You will be given a small breakfast to eat over a 20 minute period.
- **Calories Burned After Breakfast:** You will have your metabolic rate measured for 3 hours. You will be lying comfortably in bed with the transparent bubble over your head. You will be able to watch a DVD. You will also be able to get up and go to the bathroom when you need to. During this period of time you will be asked to rate your level of hunger and fullness.
- **Lunch:** 3 hours after breakfast you will be provided with lunch.
- **After Lunch Blood Sampling**: After lunch you will need to stay in the laboratory for another 3 hours. During this time we will obtain some blood samples from the catheter in your arm. A small amount of blood (approximately 1-2 teaspoons will be drawn from the tube at 30 minutes intervals for 3 hours after you have finished lunch. At these same time points you will be asked to rate your level of hunger

and fullness. After the final blood sample is taken, the catheter will be taken out. The amount of blood we draw will be much less than if you were to donate blood. During this 3-hour period after lunch you may read or watch DVDs. .

Diet to Lose Weight Phase:

Sessions 4-20: The number of sessions will depend on the time required to lose 7% of your weight.

- **Meeting with Nutritionist/Dietitian**: 90 minutes. You will come to the Nutrition Center (114 Gifford) to receive counseling on weight loss. You will discuss the types of foods you like to eat. We will develop an individualized plan for you to reduce your calories. Your calorie intake will not be below 1200 calories per day. You will learn the types of foods that you should eat to obtain all of the essential nutrients you need. You will also have a goal of increasing the number of steps you walk each day by 2000. This is approximately one additional mile of walking. The goal will be for you to lose 7% of your body weight within a 10-16 week period. Example: 7% weight loss for a 200 pound person would be 14 pounds (200 pounds x 0.07 = 14 pounds).
- After this first visit to the Nutrition Center, you will return once a week for 15-30 minute appointments for dietary counseling and to monitor your weight loss progress.
- During this weight loss phase, every four weeks you will record your food and beverage intake for 3 days. During these 3-day periods, you will also use your pedometer to record the number of steps you take.
- After you have lost 7% of your initial weight, you will have your basal metabolic rate, body composition, and body circumferences measured again as before.

Maintain Your Weight Loss Phase:

Sessions 20-27:

After you have lost 7% of your body weight you will be asked to complete the 5 short questionnaires that measure your perceptions of your eating behaviors and relationship to food. You will then be instructed how to maintain your weight at this new level for 3 weeks. You should not gain or lose more than a pound during this time. You will meet with a nutritionist/dietitian twice per week for about 15 minutes per session. You will be weighed and receive advice to help you stay at the same weight. At the end of this period you will have your basal metabolic rate, body composition, and body circumferences measured again as before. You will also have an exercise test described below:

Exhausting Exercise Test (or VO2max test): 45 minutes

This test will tell us how fit you are and is very similar to the treadmill stress test. You will be asked to ride an exercise bike or run/walk on a treadmill, until you are too tired to continue. It will become more and more difficult to push the pedals or maintain your speed. While you are riding/walking/running we will measure your heart rate with an electrocardiogram (ECG). We will ask you to wear a nose clip (something that stops you breathing through your nose) and ask you to breathe through a mouthpiece. This will let

us measure the gases you breathe. This test lasts roughly 45 minutes. The results of this test will help us know how many calories you will burn during the four days of exercise.

After the three weeks when your weight has stayed the same, you will participate in the last phases of the study. In one of these 5-day periods you will exercise and the other 5-day period you will not. The order of these phases will be random. This means you may do the Exercise Phase first followed by the No Exercise Phase. Or the order might be the opposite. These last two phases will be separated by at least 2 days.

***Exercise Phase*:**
During this 5-day period you will exercise once per day and increase the number of steps you take. Because you will burn a lot of calories when you exercise on these days, you will be given more calories to eat. All of your food will be provided for you during the 5 days so your weight will not change. You will come to the Laboratory each of the first 4 mornings to be weighed. We will also measure your basal metabolic rate and you will pick up your day's supply of food. Your exercise on these days will be on a stationery cycle, a treadmill, or an elliptical machine. The exercise will be of moderate intensity for 45-70 minutes. This kind of exercise will raise your heart rate and cause you to sweat. You will be monitored by an exercise specialist. On the 4th day you will again be asked to complete the 6 short questionnaires that measure your perceptions of your eating behaviors and relationship to food. On the 5th day of this phase, you will repeat the same 9-hour Breakfast/Lunch Tests as you did during the baseline phase of the study.

***No Exercise Phase*:**
During this 5-day period you will not exercise at all. This means that you will maintain your new weight with a low level of physical activity. All of your food will be provided for you during the 5 days so your weight will not change. You will come to the Laboratory in the morning for each of first 4 days in order to be weighed. We will also measure your basal metabolic rate and you will pick up your day's supply of food. On the 4th day you will again be asked to complete the 6 short questionnaires that measure your perceptions of your eating behaviors and relationship to food. On the 5th day of this phase, you will repeat the same 9-hour Breakfast/Lunch Tests as you did during the baseline phase of the study.

ARE THERE REASONS WHY I SHOULD NOT TAKE PART IN THIS STUDY?

You will not be allowed to participate in these studies for any of the following reasons:
1) Your age is <u>not</u> between 18 and 55 years.
2) You are pregnant.
3) You are breast feeding.
4) You currently smoke.
5) Based on your medical history, your blood glucose, blood pressure, and ECG at rest and during incremental exercise, the research team has identified a physiological

characteristic/condition that may increase the likelihood of an unfavorable event during the study.
6.) You have had surgery for weight loss such as gastric bypass or gastric banding.
7) You are taking a medication that has the potential to affect your metabolism or appetite.
8.) You are not able to exercise.
9.) Your participation has not been approved by a physician, or by a senior member of the research team.

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?
It is not possible to identify all potential risks in research procedures, but the researchers have taken reasonable safeguards to minimize any known and potential, but unknown, risks. The Human Performance Clinical Research Laboratory keeps an automated defibrillator with built in transcutaneous pacing and a "crash-cart" stocked with oxygen and emergency medications. The investigators have a great deal of experience with all of the procedures. Some of the procedures you are being asked to volunteer for have several associated risks:

Exercise Stress Test, VO2 Max Test, and Moderate Exercise
There is a very small chance of an irregular heartbeat during exercise (< 1% of all subjects). Other rare risks of a stress test are heart attack (< 5 in 10,000) and death (<2 in 10,000). Exercise can make you tired and uncomfortable. Wearing a mouthpiece and nose-clip during the VO2 max test can sometimes cause dryness in the mouth and mild discomfort.

Body Composition
There is a small amount of radiation exposure (0.05 mRem) associated with the DEXA test that is less than 1/20 of a typical chest x-ray. The more radiation you receive over the course of your life, then the greater the risk of having cancerous tumors or of inducing changes in genes. The changes in genes possibly could cause abnormalities or disease in your offspring. The radiation in this study is not expected to greatly increase these risks, but the exact increase in such risks is unclear. Women who are or could be pregnant should receive no unnecessary radiation and should not participate in this study.

Blood Sampling
When the needle/catheter tube goes into a vein, it may hurt for a short period of time (a few seconds). Also there may be minor discomfort of having the needle/plastic tube taped to your arm. In about 1 in 10 cases, a small amount of bleeding will occur under the skin that will cause a bruise. The risk of forming a blood clot in the vein is about 1 in 100, and the risk of significant blood loss is 1 in 1,000. Other risks associated with blood drawing also include vein inflammation, slight risk of infection, local soreness, and fainting. These are all very minor risks and if present, are generally resolved in less than a day.

Basal Metabolic Rate

Some individuals may experience claustrophobia during the resting metabolic measurements. The canopy used for is measurement is a large, see-through plastic bubble with adequate space and breathing is unrestricted during this time. Should you begin to feel uncomfortable during this test, you will be free to remove the canopy.

ARE THERE ANY BENEFITS FROM TAKING PART IN THIS STUDY?
The major benefit for participants in this study is weight loss. From the results of your tests at start and end of the study of the study you will be told how much body fat you have lost. You will also learn your blood pressure, blood sugar, and bone density values, and your diet analysis results. Some of your results will be immediately available at the time of testing (body fat for example) while others will be provided later. It may take up to 6 months after the study for all of your information to be provided. Professor Melby (or a member of the research team) will provide your results to you in the manner you find most convenient, either regular mail or email. These results are not medical diagnoses and should only be used as general information.

DO I HAVE TO TAKE PART IN THE STUDY? Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to which you are otherwise entitled.
WHAT WILL IT COST ME TO PARTICIPATE? Cost of transportation to and from CSU for meetings and follow-up will be the responsibility of the participant.

WHO WILL SEE THE INFORMATION THAT I GIVE? We will keep private all research records that identify you, to the extent allowed by law. You will be identified by a code using a 3-digit combination. Only the study investigators will be able to link the code with your name.

Your information will be combined with information from other people taking part in the study. When we write about the study to share it with other researchers, we will write about the combined information we have gathered. You will not be identified in these written materials. We may publish the results of this study; however, we will keep your name and other identifying information private.

We will make every effort to prevent anyone who is not on the research team from knowing that you gave us information, or what that information is. For example, your name will be kept separate from your research records and these two things will be stored in different places under lock and key.

Your identity/record of receiving compensation (NOT your data) may be made available to CSU officials for financial audits.

CAN MY TAKING PART IN THE STUDY END EARLY?

We are aware that this study requires a significant time commitment from you as a volunteer. It is very important to the study that you not miss scheduled visits with study

personnel. In the event that something comes up that will make you miss a visit, please call and let us know. Please also note that we may call you if a visit is missed. We simply want to check and make sure that everything is OK. There are a number of reasons your participation could end early:
1.) if you become pregnant;
2.) if you do not follow the diet provided to you by the research team during those phases of the study where you must consume the foods we give you;
3.) If you miss more than 10% of your appointments; and
4.) If you are unable to lose 7% of your body weight within a 15 week period. If your participation ends early for any of the above reasons, we will contact you and let you know the reason why you will not be allowed to continue. We will make arrangements to send you the study results you have completed. Should our testing reveal information that suggests you need to be referred for medical care, we will refer you to your primary care physician. You will receive monetary remuneration only for those portions of the study that you complete.

WILL I RECEIVE ANY COMPENSATION FOR TAKING PART IN THIS STUDY? If you complete the entire study, along with your test results you will be given $350 to compensate you for your time. Should you choose to withdraw, or are removed from the study by investigators prior to completion, you will receive partial compensation as follows:
Successful completion of the *Baseline Testing Phase*: $50
Successful completion of *Diet to Lose Weight Phase and Maintain Your Weight Loss Phase*: $100
Completion of the 5-day Exercise Phase: $100
Completion of 5-day No Exercise Phase $100

WHAT HAPPENS IF I AM INJURED BECAUSE OF THE RESEARCH? The Colorado Governmental Immunity Act determines and may limit Colorado State University's legal responsibility if an injury happens because of this study. Claims against the University must be filed within 180 days of the injury.

WHAT IF I HAVE QUESTIONS?
Before you decide whether to accept this invitation to take part in the study, please ask any questions that might come to mind now. Later, if you have an emergency related to this study or questions about the study, you can contact the investigator, Chris Melby at 970-491-6736. If you have any questions about your rights as a volunteer in this research, contact Janell Barker, Human Research Administrator at 970-491-1655. We will give you a copy of this consent form to take with you.

This consent form was approved by the CSU Institutional Review Board for the protection of human subjects in research on December 13, 2012.

WHAT ELSE DO I NEED TO KNOW?

RETENTION OF BLOOD SAMPLES:

If there are any blood samples left over that are not used in the analysis of this study, we would like to keep them in a freezer in our lab. It is very possible that we will use all of the blood obtained in this study and will have none left, but in the event that we do, we would like your permission to keep the samples in the freezer so that they can be used for further research on hormones or other molecules that influence metabolism and body weight. We will use these samples in the future solely for the additional research on body weight and health. Your stored samples will be coded in such a way that your confidentiality will be maintained (you will be identified as a number rather than a name). Only the Principal Investigator (Professor Chris Melby) and members of the research team will have access to the coding system for your samples.

By checking 'the box below and signing the accompanying line, you are agreeing to allow the investigators to retain any leftover blood samples obtained during this study. You may prefer we contact you to obtain your permission to use the blood for other research purposes. If so, please provide a contact phone number below so we can do so. Please check only one of the following boxes adjacent to the statements and sign and date in the space by the statement you check.

1. ☐ The investigators may keep any of my leftover blood samples obtained during the course of this study for future research.

Signature and Date_____

2. ☐ The investigators may **NOT** keep any of my leftover blood samples obtained during the course of this study for future research.

Signature and Date_____

3. ☐ The investigators may keep any blood samples obtained during the course of this study for future research. However, I want them to contact me and explain the new research before I allow additional use of my tissue.

Signature and Date_____

Contact information:_____

Your signature below acknowledges that you have read the information stated and willingly sign this consent form. Your signature also acknowledges that you have received, on the date signed, a copy of this document containing 9 pages.

_____ _____

Signature of person agreeing to take part in the study Date

_____

Printed name of person agreeing to take part in the study

_____ _____

Name of person providing information to participant Date

_____

Signature of Research Staff

Participant ID_____
Date_____
Condition_____

Please answer the following questions based on your experience the **last four days in which your meals were provided**. Please place a single vertical mark on the line to indicate your response to each question.

1.On average, how hungry did you feel **throughout** each of these 4 days?

Not hungry, plenty of food _____ Very hungry, not enough food

2.On average, how hungry did you feel **at the end** of these 4 days?

Not hungry, plenty of food _____ Very hungry, not enough food

3.On average, how would you rate the food you were provided during these 4 days?

Terrible, not pleasant to eat _____ Outstanding, delicious

4.On average, how full did you feel in the evening just prior to bedtime during these 4 days?

Never full, wanted more to eat _____ Full, could not eat anymore

5.On average, how was your energy level throughout these 4 days?

No energy _____ Full of energy

6.On average, how tired did you feel throughout each of these 4 day?

Not at all tired _____ Extremely tired

If you exercised on each of the previous four days, please answer questions 7 and 8. If you did not exercise, leave questions 7 and 8 blank.

7.What are your feelings about the exercise you performed?

Extremely unpleasant _____ Extremely pleasant

8.How likely is it that you will regularly participate in this same type of exercise after the study?

Not at all likely _____ Very likely

9.Please indicate which days you ate the additional food module (circle applicable days)

Tuesday Wednesday Thursday Friday