THESIS

WHAT GOES DOWN NEED NOT GO BACK UP: DECREASING THE BIOLOGICAL DRIVE TOWARD WEIGHT REGAIN BY INCREASING ENERGY FLUX

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ABSTRACT

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DECREASING THE BIOLOGICAL DRIVE TOWARD WEIGHT REGAIN BY INCREASING ENERGY FLUX

INTRODUCTION: Weight regain after weight loss is the experience of most obese dieters. Metabolic adjustments characterized by decreased resting metabolic rate (RMR) and increased hunger can prevent long-term success. Possibly this energy gap could be attenuated by a high flux (HF) state (higher expenditure coupled with higher intake).

METHODS: 6 obese adults [age (mean±SE) = 42±12 y; body mass index (BMI)=35.7±3.7 kg/m^2] underwent 7% diet-induced weight loss and were stabilized at this weight for 3 weeks. RMR via indirect calorimetry, and hunger via visual analog scale were then examined during two 4-day 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.

RESULTS: Average 5-day weight did not differ between HF (103.4±4.7 kg) and LF (103±4.8 kg) (P>0.10). Average daily RMR was higher during HF (1926±138 kcal/day) compared to LF (1847±126 kcal/day; P = 0.05). Resting fat oxidation was also higher during HF (0.073$\pm$0.010 g/min) compared to LF (0.059$\pm$0.012 g/min; P<0.05). Average daily, perceived end-of-day hunger was lower during HF compared to LF (P<0.05).

CONCLUSION: These preliminary data suggest that compared to a sedentary LF state of energy balance, a HF energy balance state is associated with a greater RMR, resting fat

oxidation, and less hunger - all of which may attenuate the energy gap and protect against weight regain.

ACKNOWLEDGEMENTS

Batter my heart, three-person'd God, for you

As yet but knock, breathe, shine, and seek to mend;

That I may rise and stand, o'erthrow me, and bend

Your force to break, blow, burn, and make me new.

I, like an usurp'd town to another due,

Labor to admit you, but oh, to no end;

Reason, your viceroy in me, me should defend,

But is captiv'd, and proves weak or untrue.

Yet dearly I love you, and would be lov'd fain,

But am betroth'd unto your enemy;

Divorce me, untie or break that knot again,

Take me to you, imprison me, for I,

Except you enthrall me, never shall be free,

Nor ever chaste, except you ravish me.

-John Donne

TABLE OF CONTENTS

CHAPTER I

LITERATURE REVIEW

Examined globally, an estimated 400 million adults are obese (body mass index, or BMI, ≥ 30 kg/m^2), and 1.6 billion are overweight (BMI ≥ 25 kg/m^2) [1]. Nearly 150 million adults in the United States are categorized as overweight or obese [2,3], and more than two thirds of U.S. adults are reportedly attempting to lose or maintain weight - spending $33 billion annually for products and services designed to reduce weight [4,5]. Overweight and obesity are characterized by excessive adiposity resulting from a long-term imbalance between energy expenditure (EE) and energy intake (EI) [6,7]. This imbalance elevates the risk for diseases such as hypertension, type 2 diabetes, cancer, and cardiovascular disease [8-12]. Because of this, chronic disequilibrium between expenditure and intake exists as a major health concern in the United States. Conversely, the *reduction* of weight is accompanied by *decreases* in disease risk. Weight loss of even modest proportions (≈5-10% of body weight) in overweight/obese individuals improves glycemic control, reduces blood pressure, and lowers cholesterol concentrations [13]. This same modest reduction in weight has been associated with improvements in cardiovascular disease risk factors, and decreased reliance on medications [14,15]. However, despite the public awareness and money invested in combating obesity, the common experience among most individuals who diet to lose weight is ultimately, weight regain [16].

Environmental contributors to obesity

The environment of the United States is one that facilitates energy consumption, but does not lend itself as readily to physical activity [17,18]. Over the past two decades, energy-dense foods, such as sugars and fats, have become relatively cheaper than other foodstuffs; and while the price of these items has risen, this elevation has been less than that of the consumer price index. However, the price of less energy-dense foods, such as fruits and vegetables, has increased at a rate above that of the consumer price index [19] making the purchase of energy-dense food more economically sensible in the short-term. The monetary practicality, as well as the accompanying ease of availability [20], are two of many environmental factors attributed to weight (re)gain. The inverse relationship between energy density and energy cost appears to be a driving motivational and behavioral contributor to the rise in overweight and obesity [21], but this is not the only contributing influence. Other commonly listed behavioral and environmental correlates to weight gain include: education, motorization, suburbanization, increased portion sizes, elevations in sedentary activity, reductions in physical activity, and the dwindling number of jobs involving physical labor [19,20, 22-24].

Dieting seems to precipitate its own set of environmental and behavioral pressures that encourage a return to a positive energy state. Those going through weight loss may be faced with feelings of depravity – that they are missing out on desired and enjoyable foods [25], and rapidly diminishing feelings that the benefits of weight loss are worth the relative effort [26]. Regainers may associate failures in other aspects of their lives with their attempts at weight reduction, place excessive importance on weight as a self-evaluating tool, and may be pressured to respond to life events with elevations in caloric intake [27]. With all the potential contributing factors and areas of pressure, little wonder can be had at the diverse approaches to weight loss or the emphasis placed on behavioral and environmental modification. However, even with all the

listed potential contributing influences, these pressures may not be telling the entire story in regard to obesity. Though behavioral and environmental factors are commonly discussed as reasons for the obesity epidemic, biological/metabolic factors may be playing a larger role than previously supposed. While interventions and weight loss programs attempt to consider the external pressures, and address EI and EE at a level of behavior - oftentimes advocating will-power and moderate portion sizes - increasing emphasis is being given to the biological and metabolic regulators of energy balance: those internal mechanisms that can influence EI and EE, and which contribute to the external behavior.

Metabolic/genetic contributors to obesity

The contributions of biological and metabolic factors to the etiology of obesity are becoming more widely recognized. As a part of this, genetic factors are being subject to greater scrutiny as a potential influence on body weight regulation. One study found that nearly 40% of individual differences in adjusted resting metabolic rate (RMR, the primary contributor to daily energy expenditure for most individuals) were genotype-dependent [28]. Current findings support the idea that obesity develops, not independently of genetics, but as an interaction between genetic predisposition and environmental influences [29]. In support of this are studies that examine physical and metabolic characteristics of the U.S. and Mexican Pima Indians. Whereas genetics links the regionally distinct groups, Mexican Pimas have a lower degree of obesity, which may be explained, in part, by differences in diet and physical activity - thus displaying the role of genetics *and* environment [30,31]. Genetics influence total daily energy expenditure (TDEE), but not to an extent that can be considered independent of, or higher than, a person's individual and behavioral contributions. Therefore, being mindful of what factors

determine body weight, and how these contributors are modified by specific behaviors, remains pertinent.

Weight regain among dieters

The majority of individuals who successfully reduce their weight, commonly regain this lost weight, and by so doing, lose the health benefits they worked to achieve [32-35]. Individuals who diet to lose weight generally regain one-third of lost weight in the year after dieting [36]. By five years, over 50% of dieters have returned to their starting weight [37]. For some, dieting may even have the opposite effect of what is intended, and can lead to long-term weight gain, or act as a predictor for future elevation in body weight [38,39]. While dieting methods may differ, the result is often the same: recidivism back into positive energy balance and subsequent weight regain. [33,35,40].

Weight regain can come at a cost to health. Just as weight gain has been associated with an elevated risk of diabetes [41,42], colon cancer [43], and breast cancer [44], weight *re*gain also has accompanying comorbidities. Whereas weight loss is associated with improvements in cardiometabolic risk factors [11], weight regain has been seen to elevate low-density lipoprotein, result in insulin resistance, and raise total cholesterol concentrations *beyond* original, pre-weight loss values. [45]. Additionally, weight regain results in a preferential and disproportionate replacement of fat mass compared to lean mass [46,47]. Specifically, one study found that for every 1 kg of fat lost, 0.26 kg of lean tissue was lost. However, every 1 kg of fat regained was accompanied with a return of only 0.12 kg of lean tissue [47]. Whereas reduced body fat is associated with improved blood pressure, reductions in inflammatory proteins, and improved mental and social function, these advances regress towards pre-weight loss values on the regaining of lost weight [48-50]. Despite the well-known benefits of maintaining lost weight,

most dieters fail to sustain long-term reductions in body mass. This phenomenon of weight regain is often blamed on volitional behavior and pressures from an obesogenic environment [32]. However, these traditional explanations only account for a portion of the weight regain observed among most individuals who experience diet-induced weight loss [28]. The high propensity for recidivism leads one to consider the existence of additional pressures that must act in the opposing direction – driving even the most self-controlled individual towards a positive energy balance.

Metabolic regulators of weight regain: energy intake

During even initiation of body weight reduction, specifically from a calorie-restricted diet, compensatory metabolic adjustments occur that disrupt further weight reduction, and even push towards a positive energy state [51]. In order to continue, or maintain weight loss, these compensatory responses must be addressed. One of the resultant biological adjustments to a calorie-restricted diet is an elevated appetite [52]. Whereas chronic appetite is generally down-regulated in the overfed [53], those attempting to lose or maintain lost weight are confronted with the opposite biological response: energy restricted weight loss results in an elevated appetite such that energy intake may even exceed that of pre-weight loss measures [54-64]. Alteration of energy intake is the behavioral response to changes in hunger and satiety [65], and although hunger and satiety are influenced by the media and social stimuli, they are also attributable to measurable, biological processes. When weight loss is achieved via caloric restriction, compensatory mechanisms respond such that a strong internal desire to eat pushes behavior towards an elevated EI. The hypothalamus acts as one of the key players in this compensatory response, and functions in accord with hunger and satiety hormones that provide feedback as to the current energetic state of the biological system as a whole. Because the

primary emphasis of this paper revolves around the influence of energy flux on expenditure, a comprehensive discussion on the hormonal and neurological regulators of intake will not be undertaken. Due to the integrated nature of energy balance however, mention of some of the foremost hormonal constituents is relevant. These hormones are summarized in the table that follows:

Table 1. The influence of weight loss on selected hunger and satiety signals in the overweight and obese. ↑, increase; ↓, decrease; NPY, neuropeptide Y; AgRP, agouti-related peptide; POMC, proopiomelancortin; CCK, cholecystokinin; PYY, peptide YY [51,52,61,64,66].

APPETITE-RELATED SIGNAL	**FASTING CONCENTRATION POST WEIGHT LOSS**	**INFLUENCE ON HUNGER/SATIETY**
NEUROPEPTIDE SIGNALS		
NPY	↑	Orexigenic signal. Administration leads to hyperphagia, obesity, lowered energy expenditure, and reduced SNS activity.
AgRP	↑	Orexigenic signal. Reductions in AgRP result in reduced body weight.
POMC	↓	Anorexigenic signal. Loss of POMC elevates risk for obesity.
ADIPOSE SIGNALS		
Insulin	↓	Anorexigenic. Produced by pancreas and responsive to changes in adiposity. NPY and POMC may be downstream targets.
Leptin	↓	Anorexigenic. Administration results in reduced energy intake. Mutations of the *ob* gene, which controls leptin production, results in hyperphagia.

GASTROINTESTINAL SIGNALS		
CCK	↓	Anorexigenic. Released from gut in response to nutrients. Treatment results in reduced intake and smaller meal sizes.
PYY	↓	Anorexigenic. Administration delays gastric emptying and reduces intake. Effects are inhibited by NPY.
Ghrelin	↑	Orexigenic. Concentrations are elevated during fasting. Treatment raises caloric intake and lowers fat oxidation.

All of the aforementioned hormones work in concert to establish an individual's appetite and thus influence caloric consumption. While these biological regulators can be useful in terms of maintaining homeostasis in light of over-nutrition, more often the case in regards to weight loss and maintenance is their role in elevating the drive to eat in the face of a diet-induced caloric deficit. However, caloric intake is only one of two key factors contributing to overall energy balance and body weight regulation. The other factor, energy expenditure, is also under voluntary and involuntary control, as described below.

Metabolic regulators of weight regain: energy expenditure

Caloric intake alone is not always what separates and determines the obese from the lean. In some instances, obese individuals may even consume less than those in the lean category [67,68]. Energy balance therefore, is not solely explicable in terms of caloric consumption, and must also be discussed with consideration of energy expenditure. Just as weight loss activates compensatory mechanisms designed to maintain energy homeostasis, resulting in a drive for

caloric consumption, so too does weight loss result in metabolic counterbalances characterized by reduced EE [69,70].

Total daily energy expenditure is comprised of three facets: RMR, the thermic effect of feeding (TEF), and the thermic effect of physical activity (TEA) [71,72]. Each of the contributors to EE is biologically malleable, capable of responding to environmental fluctuations, and while RMR accounts for the largest portion of EE (60-75%), in most individuals/under most circumstances, TEA (~20%) and TEF (~10%) also contribute considerably towards daily energy output [73]. Because overall energy balance can be summarized by the equation: $E_{balance} = E_{in} - E_{out}$ (or that balance is achieved when $E_{in}=E_{out}$, as depicted in the figure below), each of the components of E_{out}, especially because of their plasticity in the face of non-homeostatic pressures, is important in overall weight stability. Reductions in energy expenditure due to weight loss can be attributed to alterations in each of the three components of EE [69,74].

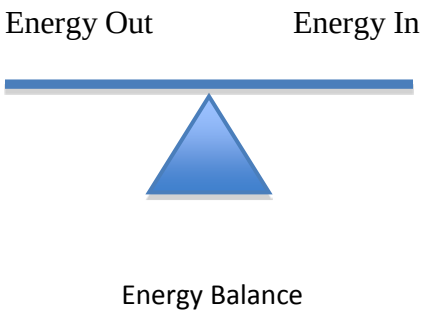


Figure 1. Scale of energy balance: A stable body weight results from equilibrium between energy intake and energy expenditure.

The thermic effect of feeding contributes to approximately 10% of overall energy expenditure and constitutes the energy spent above basal levels in the hours following a meal.

Absorption, transportation, digestion, and storage comprise one component of thermogenesis, termed the obligatory TEF. Absorption and transportation of foods account for a relatively small proportion of TEF compared with the synthesis of storage forms of glycogen, protein, and fat [73,75,76]. The term facultative TEF makes up the other component of TEF, and refers to heat production beyond that represented by obligatory demands [77]. Facultative TEF is not always apparent or understood, but seems to represent metabolic processes of limited efficiency, many of which result in heat production, but are not clearly linked to the type of substrate mobilization seen in the obligatory component. Examples of facultative TEF might be heat production due to the futile cycling of substrates in anabolic and catabolic pathways, and/or because of an uncoupling of oxidation to phosphorylation resulting from alterations in the sympathetic nervous system (SNS), [72,73]. The thermic effect of feeding is variable from person to person, and is influenced by genetics, meal size, and meal composition. While isocaloric protein meals raise TEF beyond that induced by either lipid or carbohydrate intake, the change in TEF from consumption of foods with mixed degrees of macronutrients is due more to the positive correlation between TEF and meal size than to meal composition alone [78,79].

The contribution of TEF to TDEE is well described, but the link between obesity and TEF is not as lucid. Though some studies report a relationship between obesity and a reduction in TEF [80-84], this relationship has lacked consistency [78,85,86]. If overweight and obese individuals do have a blunted TEF, this could provide one possible area of intervention for maintaining energy balance. When this relationship *is* observed, and obesity is coupled with a reduced TEF, whether a lowered TEF contributes to obesity, or is the result of obesity is still uncertain - though a reduced TEF observed in formerly obese individuals supports the former [87]. A potential connection between a blunted TEF in response to obesity can be made, and

points largely towards the SNS [81,88]. Fat depots, and the associated adiposity signals, are positively correlated with SNS activity, which places the overweight and obese in a state of chronic SNS stimulation. Persistently elevated SNS activity may result in Beta Adrenergic Receptor (β-AR) desensitization and reductions in tissue responsiveness [89]. Because the β-AR pathway accounts for 30-40% of TEF [90], a desensitized pathway would lead to a reduced TEF. The degree of insulin resistance may also play a role in the relationship between obesity, sympathetic activity, and TEF [81]. Whereas an overactive SNS results in long-term vasoconstriction, limits glucose uptake, and could result in insulin resistance, insulin resistance itself may blunt the postprandial, sympathetic response, therefore limiting the degree of TEF [88,91]. Interventions that can address this insulin resistance then, may prove valuable in raising postprandial thermogenesis and ultimately, TDEE.

The thermic effect of activity makes up the second largest component of TDEE, and is the most easily manipulated of the three. TEA is the energy expended for muscular activity above basal levels, both during and after physical work [73]. Though values for TEA are generally 20% of TDEE, the actual contribution of TEA to total daily expenditure is highly variable. The percentage of total expenditure due to TEA can be increased by elevating the intensity, duration, and frequency of exercise, and increasing the energy expended in activities of daily living [71,72]. TEA is often subdivided into two additional components. These subdivisions will be addressed more fully elsewhere in the review.

The largest contributor to TDEE on most days is RMR. Resting metabolic rate accounts for up to 75% of TDEE, and includes the energetic cost of sustaining the integrated systems of the body while in a rested post-absorptive state - usually 10-12 hours fasted and 10-12 hours following any strenuous physical activity [72,72]. Some of these costs include maintaining

homeothermic temperature, ion gradients, activity of the central nervous and cardiopulmonary systems, and the continuation of other chemical reactions required by the body in the resting state [92]. Fat free mass (FFM) accounts for the bulk of resting metabolism, as it is comprised of highly metabolically active muscle and organs, as well as bone and connective tissue of a lesser metabolic magnitude [93-97]. While not an exhaustive list, additional contributors to the energy expended in a resting, fasting state, include genetics, age, emotions, and disease, with the magnitude and direction of these factors at times differing due to intervariable interaction, i.e. infection in an elderly male may have different effects on metabolism vs. an infection in a younger female [98,99]. Hormones such a thyroxine, triiodothyronine, leptin, and insulin, also contribute to RMR, many through an association with the SNS, and can lead to variation amongst individuals even of similar body characteristics [100-105]. In addition to its contribution towards TDEE, RMR may also assist in the regulation of EI. Ongoing research suggests an association between RMR, hunger, and meal size [106-107]. Characteristics such as sex and chronic exercise also influence RMR, but not to the extent of FFM. Because of its large contribution to TDEE, as well as its potential role in regulating energy intake, alterations in RMR can have a major influence on energy balance.

Although the role of a reduced RMR in the etiology of obesity is not fully agreed upon, a low RMR may be a predictor of future weight gain [94,95,108,109]. One of the potential, compensatory mechanisms the body employs to maintain homeostasis resides in its ability to alter RMR. Whereas RMR is often elevated in conjunction with an increase in BMI, thus potentially raising TDEE, a reduction in body weight can lead to the opposite effect. In one study, maintenance of a body weight at least 10% below initial weight resulted in a reduction of TDEE of roughly 8 kcal per kilogram of FFM per day in obese participants [69]. This was

confirmed in one meta-analysis which found that formerly obese individuals typically have a 3-5% lower RMR, adjusted for FFM and FM, relative to those without a history of obesity [110]. Some attribute this reduction in RMR post weight loss to elevated energy efficiency in tissue with high metabolic activity [111], whereas other potential variants include changes in thyroid hormones [29] and/or sympathetic activity [112]. In reality, a combination of adaptations is likely at work. In those instances of RMR reductions with weight loss, the act of weight reduction may, paradoxically, predict future weight gain because of suppressed EE due largely to the compensatory actions observed in RMR.

When an individual loses weight, which is often a combination of a reduction in water weight, FM, and FFM, a reduction in RMR accompanies this loss. Because RMR is largely determined by metabolically active tissue, reductions in tissue mass coincide with reductions in RMR. Additionally, TEA and TEF are altered by diet-induced body weight fluctuations as well [113,114]. These alterations are, in part, predictable because of the known relationships between mass, composition of that mass, and RMR, TEF, and TEA. While the direction of these alterations may be predictable, the magnitude of these changes is not. In one study, a diet-induced body weight reduction of 10% resulted in a TEF 15% below that which could be explained by body mass and composition alterations [69]. In another study, previously obese women had an RMR of 72 kcal/day below what was predicted based on FFM [115]. Not only does a discrepancy exist in EE, but EI appears to be disproportionately affected as well. Individuals with high energy stores should, in theory, experience high levels of satiety and lower levels of hunger than those of lesser energy stores. But the opposite tends to be observed [116,117]. When dieting is undertaken, as described previously, these values become exacerbated. A dissociation occurs then, upon weight reduction, not only between EE and that

which can be explained by alteration in body composition, but also between EI and that which can be explained by energy requirements. While energy expenditure lessens, the drive for energy intake actually elevates. This disconnect between intake and expenditure is often described as the energy gap.

Energy gap

The term energy gap was first used to characterize the change in EE and EI needed to arrest weight gain and achieve energy balance [20]. The premise was based on the idea that a discrepancy exists between the calories ingested versus the calories burned, and that after weight loss, biological pressures to regain lost weight emerge on both sides of the energy balance scale. The energy gap following weight loss is characterized by an elevated desire to eat, beyond an individual's energy needs and expenditure [51]. Following weight loss, on one side of the scale, the biological drive for EI is elevated to promote weight regain, i.e. the drive to eat increases. One theory behind the method of regulating intake and inducing weight gain pertains to appetite hormones. Hypoleptinemia and reduced levels of insulin have been observed in connection with weight reduction [61,118,119]. These adipokines normally relay information to the brain regarding the energy status of the system. During weight loss, the concentrations and effectiveness of these hormones are compromised, which diminishes neural-mediated satiety regulation and thus can raise EI [120-122].

The other side of the energy balance scale does little to mitigate the drive to regain lost weight in response to energy restriction. Though not found across all studies, the results from numerous investigations would suggest metabolic rate post weight loss is reduced beyond that which can be explained by the loss of body mass [69,74,111,123-126]. This enhanced

conservation of energy has been described as an increase in metabolic efficiency [127,128]. Following weight loss, metabolically active tissue responds with increased efficiency, thereby sparing calories that would have been spent in the pre-weight loss state, and lowering TDEE. This enhanced metabolic efficiency and suppressed metabolic rate seem also to be positively related to time spent in weight maintenance. As weight maintenance continues, not only does the energy gap remain, the rate of eventual weight regain seems actually to increase [129]. The energy gap then, although originally coined to describe the necessary change in intake and expenditure in order to achieve a more balanced body weight, can also be discussed as the *dissociation between the energy required, and the energy desired* – a disconnect that reveals, especially in the state of post-diet weight maintenance and regain, that the metabolic stimuli for appetite leads to energy intake that exceeds the overall energetic requirement of the individual [51].

The energy gap results from homeostatic adjustments on both sides of the energy balance equation in response to weight loss. On one side is the drive towards intake, especially observed in the up-regulation of certain orexigenic peptides and down-regulation of other anorexigenic peptides. On the other side is the conservation of energy expenditure, seen in the reduction of TDEE. Taken together, these metabolic adjustments promote a state of positive energy balance, and eventual regain. Therefore, developing an approach that attenuates the biological drive to regain lost weight is central to promoting the sustainability of weight loss and its health-related benefits.

Energy flux

Since 1956, EI and EE were seen to exist, not autonomously, but coupled and interrelated. At this time, Mayer *et al* [130] showed an association between EI and the EE required to perform daily, occupational demands. Mayer showed that an elevated EI was proportional to an elevated EE. However, this relationship only held true for those engaged in moderate-to-high activity. On average, the relationship between EE and EI was disrupted in more sedentary individuals, such that a lower EE was associated with a higher EI. This resulted in a positive energy state and weight gain. Energy intake, even then, was seen to be related to EE, but only once a certain level of activity was achieved. Once physical activity dropped, EI seemed to almost *replace* rather than *reflect* EE – i.e. that instead of burning calories, individuals were consuming these calories. Given the obesity epidemic, the importance of achieving a healthy body weight for obese individuals, the poor long-term success in maintaining lost weight owing to increased appetite and reduced energy expenditure, approaches to narrow the energy gap must be developed.

One possible approach to counter the tendency towards weight regain by attenuating the energy gap is by influencing energy flux. The concept of energy flux used in this thesis pertains to the total throughput of calories under conditions of energy balance. If energy flux is high, then caloric intake and expenditure are both elevated, therefore increasing the total throughput of calories, though energy balance is preserved. If flux is low, balance is maintained in the face of both diminished energy intake and expenditure. Both high flux (HF) and low flux (LF) states involve weight maintenance, but differ in how the stable body weight is achieved. Weight maintenance following diet-induced weight loss is often approached from a LF perspective, and is characterized by elevated hunger in the face of a required reduction in caloric consumption.

This results in difficulty maintaining lost weight. Alternatively, approaching weight maintenance through a HF status potentially offsets this drive towards weight regain, not only by influencing energy expenditure, but through alterations in appetite and EI as well.

The addition of exercise into a weight maintenance program may influence both sides of the energy balance equation. Exercise results caloric expenditure, but also has the potential to assist in appetite regulation as well. While a commonly held position is that exercise plays only a minor role in contributing towards weight loss due to a compensatory increase in EI [131,132], research shows a loose coupling between EE and EI, such that, although EI does increase upon the addition of exercise, this increase is, at least initially, only a fraction of the energy expended in activity [132,133]. Additionally, an acute post-exercise anorexia (loss of appetite) contributes to at least a short-term energy deficit [134]. Although long-term interventions examining the cross talk between physical activity and appetite control are lacking, appetite appears to have a dual response to exercise, displaying an increased hunger in the fasted state, but an elevated satiety efficiency in the postprandial state. Thus, exercise results in a heightened sensitivity to circulating markers of the body's energy status, such as insulin and leptin [135].

The role of increased physical activity in elevating caloric expenditure is a well-recognized benefit. What might not be as apparent is which components of EE are targeted by increased exercise. Research has shown an elevated TEF in obese individuals during post exercise meal consumption, but the generalizability of these findings is still debated [136]. Because HF includes elevated EE, with matching elevations in EI in order to maintain energy balance, the absolute TEF, due to a greater caloric consumption, is necessarily elevated in the HF state.

The thermic effect of activity can be divided into exercise energy expenditure (ExEE) and non-exercise activity thermogenesis (NEAT). High energy flux potentially affects both of these contributors to TEA. Energy expended *during* the exercise bout is well recognized. Dependent on duration, intensity, and mode of exercise, energy expended *following* exercise, commonly referred to as excess post-exercise oxygen consumption, or EPOC, also contributes to ExEE [137]. A HF state therefore raises EE not only through the calories spent in the act of exercising, but also results in residual caloric expenditure through both rapid (approximately one hour post exercise) and prolonged (potentially several hours post exercise) EPOC.

NEAT is the energy expended in daily activities aside from exercise, and has been described as the main contributor to activity thermogenesis [138]. NEAT is extremely variable between individuals, ranging from 15% of TDEE, to 50% [139-141], and therefore exists as potentially a major player in energy balance. Some studies suggest that ExEE and NEAT are counterbalanced – an elevation in ExEE for example, would result in decreased NEAT. This then would have serious implications in terms of TDEE. One study [142] found that a walking intervention designed to increase TDEE by 215 kcal/day actually resulted in no appreciable increase in TDEE due, in part, to a reduction in NEAT by 175 kcal/day. Whereas some have suggested this reduction in NEAT is due to altered work efficiency [138], an alternative explanation is that, either knowingly or subconsciously, individuals lessen NEAT because of misconceptions about, and reliance on, ExEE alone – i.e. “Because I exercised this morning, I can drive to work instead of riding my bicycle as I normally do.” The lowering of NEAT is potentially volitional then, and can be avoided through conscious decision making. If individuals are encouraged to maintain normal levels of activity, the reduction in NEAT could be offset.

Weight reduction results in decreased RMR, which drives towards weight regain. Because RMR accounts for the bulk of TDEE, any intervention that might elevate RMR in the face of a weight reduced state could have far reaching effects on abrogating body weight recidivism resulting from positive energy balance. Certain modes and intensities of exercise may result in an elevated RMR, even 15 hours post physical activity [143]. RMR is influenced by physical activity, caloric consumption, and the interaction of the two, such that HF may be more responsible for an elevated RMR than exercise or intake alone [144]. One mechanism by which HF contributes to RMR is via sympathetic tone. Maintaining HF through regular exercise contributes to enhanced β-AR support of metabolism (resulting in an elevated RMR) [103]. Additionally, the exercise promoted by HF maintenance may salvage, or mitigate the loss of FFM – the major determinant of RMR [145]. With respiring mass intact, reductions in RMR would be hindered. Further mechanisms by which HF could raise RMR include a prolonged influence of EPOC on RMR [146,147], an increase in protein turnover [148], and a higher incidence of uncoupling in the electron transport chain [149-150].

Statement of the problem

Taken together, the current findings and potential mechanisms of a HF state may attenuate a portion of the homeostatic regulation seen to drive weight regain. Whereas weight reduction and subsequent attempts at maintenance via LF are characterized by an energy gap in which appetite is greater than actual energy requirements, a HF state may mitigate this gap and better regulate EI due to intentional alterations on both sides of the energy balance equation – higher EE owing to daily physical activity, and caloric intake designed to counterbalance the elevations in EE. Therefore, HF would directly address the two antagonists to weight loss and

maintenance – an elevated appetite and reduced EE. Maintaining HF post weight loss is contrary to the norm, and while the idea that a person in a weight reduced state can better maintain energy balance with a higher caloric intake may seem counterintuitive, perhaps another trend it can counter is the one that is pushing the masses towards positive energy balance. Examining the potential impact HF may have on maintaining reduced weight is pertinent in light of the current, obesogenic environment. However, this state has not been studied experimentally in human participants. Therefore, the purpose of this study is to examine the differences in RMR, TEF, and ratings of hunger and satiety following a five day HF condition compared to a five day LF condition during energy balance in previously obese individuals after a seven percent reduction in initial body weight.

Hypotheses

Energy flux following weight loss is an important physiological determinant of resting metabolic rate, the thermic effect of feeding, and perceptions of hunger and satiety.

Specific aims

1) To determine the daily effect on resting metabolic rate of five days of high energy flux versus five days of low energy flux when participants are in energy balance following a 7% loss of body weight.

2) To compare the thermic effect of feeding following five days of a high flux condition versus five days of a low flux condition

3) To examine ratings of hunger and satiety in a high flux versus low flux state.

CHAPTER II

WHAT GOES DOWN NEED NOT GO BACK UP: DECREASING THE BIOLOGICAL DRIVE TOWARD WEIGHT REGAIN BY INCREASING ENERGY FLUX

INTRODUCTION

While the problem of overweight and obesity is well recognized, a solution remains elusive [3-5,9,10,151]. Long-term weight loss results are usually modest - the more common experience among individuals who diet to lose weight is ultimately, weight regain [16]. This recidivism is often blamed on volitional behavior and pressures from an obesogenic environment [32]. However, in addition to these factors, increasing emphasis is being given to the biological/metabolic regulators of energy balance.

Two biological adjustments that appear to be driving forces in weight regain are an increased appetite and reduced energy expenditure (EE). Weight loss from caloric restriction is seen to affect appetite by up-regulating certain orexigenic peptides, and down-regulating other anorexigenic peptides, thus increasing the internal drive to eat [152,153]. Alongside this alteration in appetite, weight loss also leads to a reduction in total and daily energy expenditure (TDEE) [154,155]. Dieting lowers resting metabolic rate (RMR), which contributes to an overall reduction in caloric expenditure and, coupled with an up-regulated internal drive to eat, acts as a catalyst for regression back into positive energy balance and weight regain. Therefore, developing an approach that attenuates the biological drive to regain lost weight is central to promoting the sustainability of weight loss and its health-related benefits.

One possible method in countering the tendency towards weight regain is by influencing energy flux. The concept of energy flux pertains to the total throughput of calories. When attempting to maintain energy balance upon weight reduction, trends in dieting tend to favor a low flux (LF) state – reducing physical activity while also reducing caloric consumption. However, recent findings have made evident the potential advantage of favoring a high flux (HF) state in order to offset the biological mechanisms that drive towards weight regain. Specifically, a HF state has been associated with a greater resting metabolism when compared to LF conditions [103,144].

Given the established relationship between an elevated energy flux and attenuated reduction in RMR, describing the differences in metabolic rate (both in the rested, as well as postprandial conditions) and ratings of hunger and satiety following a post-weight loss manipulation of energy flux is pertinent when considering methods of contesting contemporary tendencies towards post-diet weight regain. In the present study, we tested the hypothesis that, following a seven percent reduction in body weight, a HF state will result in an elevated RMR, greater thermic effect of feeding (TEF), and lower perceptions of hunger as compared to a LF state. To do so, we examined the metabolic characteristics of obese adults before and after weight reduction and during alteration of energy flux.

METHODS

Study Participants

Twelve obese adults were recruited for the study. Criteria for inclusion consisted of body mass index (BMI) within the range 30-43 kg/m^2, age range within 18-55 years old, normotensive, ability to exercise, and desire to lose weight. Individuals who were currently

pregnant, breast feeding, smoking, using medications known to affect metabolism or appetite, had prior surgery for weight loss, and/or had dieted over the previous 12 months, were excluded from participation. Additionally, the presence of any contraindication at rest or during incremental exercise based on high blood pressure or an abnormal 12-lead electrocardiogram (ECG) was criteria for exclusion. Dietary counseling took place in the Colorado State University (CSU), Kendall Anderson Nutrition Center. All other procedures took place in the Human Performance Clinical Research Laboratory at Colorado State University. Experimental protocol was approved by the Institutional Review Board at Colorado State University. The nature, purpose, and risks of the study were explained to each subject before written informed consent was obtained.

Experimental Protocol

Experimental protocol involved four sequential phases: 1) baseline testing; 2) hypocaloric diet induced weight loss; 3) three weeks of weight stabilization; 4) five days of a HF or LF condition, followed by five days of the opposing HF or LF condition (order of interventions was randomly assigned). This model was based on previous designs that looked at 5-10% reductions in body weight with ~4 weeks of weight stabilization. These phases are depicted in the table below.

Table 2. Four phases (baseline, weight loss, weight stabilization, flux intervention) of experimental protocol examining the influence of energy flux on weight regain following a 7% reduction in body weight. Abbreviations: body comp - body composition (includes circumference measurements and DEXA body scan); E. balance - energy balance (based on daily measurement of body mass); PAL – physical activity level (PAL 1.3 = RMR*1.3); RMR - resting metabolic rate; TEF – thermic effect of feeding.

BASELINE TESTING 2-3 DAYS		**WEIGHT LOSS** 12-16 weeks	**WEIGHT STABILIZATION** 3 WEEKS	**HIGH FLUX** 5 DAYS		**LOW FLUX** 5 DAYS	
BODY COMP. & VO_2 peak	RMR & TEF MEASURE -MENT	HYPOCALORIC DIET. 7% LOSS OF BODY WEIGHT	INTAKE AND EXPENDITURE ADJUSTED TO MAINTAIN E. BALANCE.	E. BALANCE @ PAL 1.7 (days 1-4)	RMR & TEF MEASURE -MENT (day 5)	E. BALANCE @ PAL 1.35 (days 1-4)	RMR & TEF MEASURE -MENT (day 5)

Baseline Testing

Baseline measurements occurred on two separate days. The first visit involved completion of a health history screening form, self-reported logs of dietary intake and physical activity, and body composition analysis. Measurements of blood pressure and heart rate via 12-lead ECG at rest and during incremental stationary cycle ergometry exercise were also recorded during the initial visit. Exercise intensity continued to increase until volitional exhaustion for the calculation of peak oxygen uptake (VO_{2peak}). During the second baseline visit, participants underwent testing to determine RMR, TEF, and measures of perceived hunger and satiety. Upon completion of initial RMR measurement, participants were fed a small meal based on 20% of measured RMR. Following breakfast, ratings of hunger and satiety were collected at 30 minute intervals over the next 3 hours. During this 3-hour span, metabolic rate was also recorded for 20 continuous minutes within each 30-minute interval. Blood samples from an indwelling intravenous catheter for measurement of specific appetite hormones were taken before and at 30-min intervals after the standardized breakfast. However, these samples have not yet been

assayed, are the focus of another thesis, and therefore are not included in this write-up. A summary of the RMR and TEF testing protocol is displayed below.

Table 3. Timeline of events for metabolic rate and preload meal test occurring at baseline, day five of high flux, and day five of low flux conditions. Protocol consisted of initial measurement of resting metabolic rate, a standardized breakfast, and three hours of data collection.

	RMR	**BREAKFAST (LIQUID MEAL) ***	**METABOLIC RATE**	**HUNGER/SATIETY RATINGS**
TIME (min)	0-45 MINUTES	15 MINUTES TO COMPLETE	0-20 30-50 60-80 90-110 120-140 150-170	20-30 50-60 80-90 110-120 140-150 170-180

*TIME 0 BEGINS UPON COMPLETION OF BREAKFAST

Hypocaloric Diet/Weight Loss Phase

Upon completion of pre-weight loss baseline measurements, participants were counseled on how to adhere to a hypocaloric diet under free-living conditions in order to lose 7% of their baseline body mass. Nutritional content of the individual diets took participant preferences into consideration in order to achieve sustainability. Therefore, macronutrient composition was not addressed to the extent that caloric intake was. Caloric restriction was designed such that participants would lose 0.5-1.0 kg per week, based on measured RMR and reported activity levels. Participants reported weekly to the Nutrition Center to be weighed, and to receive dietary counseling from a trained graduate student in the Department of Food Science and Human Nutrition at CSU. In order to achieve a 7% reduction in body mass, the intervention focused on lowering dietary intake and participants were encouraged to maintain their typical physical activity levels, but not begin an exercise program to increase their EE.

Weight Stabilization Phase

Following a 7% reduction in body mass, participants entered a three-week period of stabilization, with caloric adjustments made to keep body mass constant. RMR and body composition were measured at the beginning and end of this three-week span, and a VO_{2peak} test was again performed to determine workload for the exercise associated with the HF condition.

High/Low Flux Conditions

Three weeks after the onset of the stabilization phase, participants began a two-week protocol: five days of a LF condition, a two day washout period, and five days of a HF condition. The order of interventions (HF *vs*. LF) was assigned randomly.

The five-day LF condition kept participants in energy balance at RMR*1.35 (Physical activity level (PAL) = 1.35). Body mass was recorded each morning in the fasted state. If body mass began to rise or fall on two consecutive days, appropriate compensations were made in the caloric content of given meals to bring mass back to starting values. Body mass and RMR were measured each morning, and daily meals were provided based on these measurements. Participants did not engage in exercise during these five days, and were instructed to maintain a low, non-exercise step count recorded by a pedometer. Day five of the LF condition included post-intervention analyses of hunger, satiety, RMR, and TEF measurements. The protocol for these analyses was the same as the RMR and standardized breakfast test described previously, with a supplementary questionnaire that examined hunger and satiety levels throughout the week.

We estimated that for participants to remain in energy balance during the HF condition they would require an EI of 1.7*RMR, i.e. PAL=1.7. In order to maintain energy balance, EI was increased to compensate for the elevated level of physical activity. Body mass and RMR

were measured every morning, and food was provided to the participant based on his or her RMR and body mass that morning in a manner similar to that described for the LF condition. Based on previously measured RMR data and oxygen uptake levels for the given population, expectations were that participants would need to expend approximately 600-750 additional kcal/day through daily exercise and elevated activities of daily living. Following morning measurements of body mass and RMR, participants used a cycle ergometer and/or treadmill to achieve a net increase in daily caloric expenditure of approximately 500 kcal based on an exercise intensity of 60% VO_{2peak}. Anticipated time for completion of each exercise session was 70-90 minutes, according to the fitness level, i.e. VO_{2peak}, of the individual. All exercise bouts took place either immediately following RMR measurements or following the work day that evening. Every exercise session was supervised by a member of the investigative team. Additionally, participants were given a pedometer and encouraged to maintain a step count of 7,500 steps per day. Day five of the HF condition began in the morning immediately following the last exercise day of the HF condition, and involved post-intervention measurements according to the same RMR and breakfast test protocol as in the LF week.

Measurements

The initial day of baseline testing included anthropometric and body composition measurements, and a test designed to elicit a VO_{2peak}. Height was recorded to the nearest millimeter and body mass to the nearest 100 grams by use of a stadiometer and beam scale (Detecto, Webb City, MO, USA). Fat mass (FM), fat free mass (FFM), and bone mineral content were measured using duel-energy X-ray absorptiometry (Hologic, Discovery W, QDR

Series, Bedford, MA, USA). Girth measurements were taken at the waist, hip, thigh, and arm according to guidelines set by the American College of Sports Medicine [156].

Peak oxygen uptake was determined through an incremental exercise test to volitional exhaustion. Heart rate was recorded using a 12-lead ECG for baseline testing, and a short-range telemetry device (FT1, Polar Electro Inc., Lake Success, NY) for testing post-stabilization. Participants were outfitted with a two-way non-rebreathing mouthpiece, valve, and headgear device (Hans-Rudolph, St. Louis, MO). Exercise was performed on a cycle ergometer (Velotron Dynafit Pro, RacerMate, Inc., Seattle, WA, USA), and consisted of a 15-30 Watt/min continuous ramp protocol from 0 Watts. The test was terminated upon volitional exhaustion or once pedal cadence fell below 40 revolutions/min. Heart rate and blood pressure were recorded at rest, in the exercise position, and every two to three minutes during the protocol. The composition of expired gases, as well as ventilation, was measured continuously with a metabolic cart (Parvo TrueOne 2400 Metabolic Measurement System, Parvo Medics, Sandy, UT). The four highest consecutive 15-second average VO_2 values were used to calculate VO_{2peak}. Peak RER was calculated by averaging the four RER values that corresponded to the VO_2 values used in calculating VO_{2peak}.

The breakfast protocol required participants to consume RMR*0.2 kcal of a commercially available, liquid-mixed meal (Ensure, Ross Laboratories, Abbott Park, IL; 64% CHO, 22% fat, 14% protein). Prior to breakfast, RMR was determined in the morning, after a 12-hour fast and 24-hour abstention from exercise (except during the HF condition when RMR was determined 14-22 hours after the last exercise bout). Participants were studied under quiet, resting conditions in a dimly lit room. Metabolic rate was measured before and after the standardized breakfast. Initial measurement of metabolic rate, prior to the liquid meal, was

recorded for 45 minutes, with the first 15 minutes being considered a habituation period. For the remaining 30 minutes, oxygen consumption and carbon dioxide production were averaged each minute using either a ventilated canopy, indirect calorimetry system (Perkin Elmer MGA 1100, MA Tech Services, Inc., St. Louis, MO) or a metabolic cart (Parvo). Respective metabolic measurement systems were kept consistent within subjects, and energy expenditure was calculated using the Weir formula [157]. The systems were calibrated each morning, prior to data collection, using precision-mixed gases. The RMR measures for the indirect calorimetry system have been previously shown to be reproducible with a typical margin of error of 2.5%. [158]. During the 30 minutes of actual RMR measurement, respiratory gas exchange outliers were identified and excluded from final analyses if greater than two standard deviations from the mean, and/or if RER values fell outside of physiological values. Exclusions were made in real time, and were minimal – approximately 1-2 minutes of data excluded for every 135 minutes of data collected.

The caloric load of breakfast for each participant was standardized at a value of 20% of RMR. The breakfast was consumed within a 15 minute time interval. TEF represents the postprandial rise in EE above fasting values, and was measured for three hours. Indirect calorimetry measurements were performed for 20 min every 30-min period, providing a break from the ventilated canopy for ten minutes out of each half hour. TEF was calculated as the increase in EE above RMR over each time point for the 180 minutes. TEF data are presented both as adjusted for initial metabolic rate, as well as adjusted for caloric content of the standardized meal.

Daily meals during HF and LF weeks were provided based on RMR*1.7 and RMR*1.3 respectively. Each morning, food was divided into three meals per day, with an additional snack

portion and a food module option if hunger persisted. Meals were based on participant food requirements and preferences, and maintained a macronutrient composition of 50/35/15 CHO/fat/protein. Following each five-day HF and LF intervention periods, subjects completed questionnaires based on hunger and satiety levels for that particular day (the standardized breakfast day), as well as hunger and satiety levels over the entire week.

Exercise was performed on either a cycle ergometer (Ergomedic 874E, Monark Exercise, Sweden) or motorized treadmill (MedTrack ST65, Quinton, Bothell, WA) based on participant preference. While continuous exercise was encouraged, participants were allowed a short break upon request, or if changing the exercise modality prior to exercise completion. Intensity was kept at approximately 60% VO_{2peak} and was monitored by measurements of heart rate (Polar), VO_2 and ratings of perceived exertion (Borg) every 10-15 minutes. Non-exercise activity was measured using a pedometer (NL-1000 pedometer, NEW-LIFESTYLES, Inc., Lees Summit, MO) that was continuously attached to participants when not engaging in the mandatory supervised exercise bout during the HF condition.

Calculations & Statistical Analysis

Rates of fat oxidation (g/min) were calculated from VO_2 and VCO_2 values collected during minutes 15-45 of morning RMR measurements in the HF and LF conditions. The calculations were made according to a previously published equation [159]:

$$Fat\ oxidation\ (g/min) = 1.695\ VO_2 - 1.701\ VCO_2$$

Data were analyzed with SPSS software version 22. Student's *t*-tests were used to examine variables between the two flux conditions including RMR, TEF, and RER. A repeated measures analysis of variance was used to examine specific variables across baseline, HF, and

LF conditions. In accounting for a covariate with paired observations, a mixed models approach was performed for the adjustment of RMR to FFM. Because the covariate was not significant, a paired *t*-test was also used to compare RMR values. Statistical significance was set at $P < 0.05$. Data are presented as mean ± SE, unless otherwise stated.

RESULTS

Subject characteristics

A total of 12 subjects were initially enrolled, but 6 were unable to complete the protocol because of failure to lose 7% of initial body weight (n=3); work conflicts (n=2), and medication use (n=1). The baseline physical characteristics of the study participants are shown in **Table 4**. All individuals (4 males, 2 females) were obese as reflected by BMI greater than 30 kg/m^2, and in accord with their largely sedentary lifestyles, exhibited low levels of cardiorespiratory fitness. The order of interventions (HF first vs. LF first) was assigned randomly, except for one participant who, due to a conflict with a work schedule, requested the HF intervention be performed during the first week.

Energy intake and body composition

As per the experimental protocol, average four-day energy intake for the HF condition (3191 ± 239 kcal/day) was significantly greater than for the LF condition (2449 ± 166 kcal/day; P=0.001). Also consistent with the study protocol, the higher energy intake for HF was coupled with greater energy expenditure owing to daily monitored exercise, which resulted in no significant changes in body weight over the course of the HF and LF conditions. (**Figure 2**).

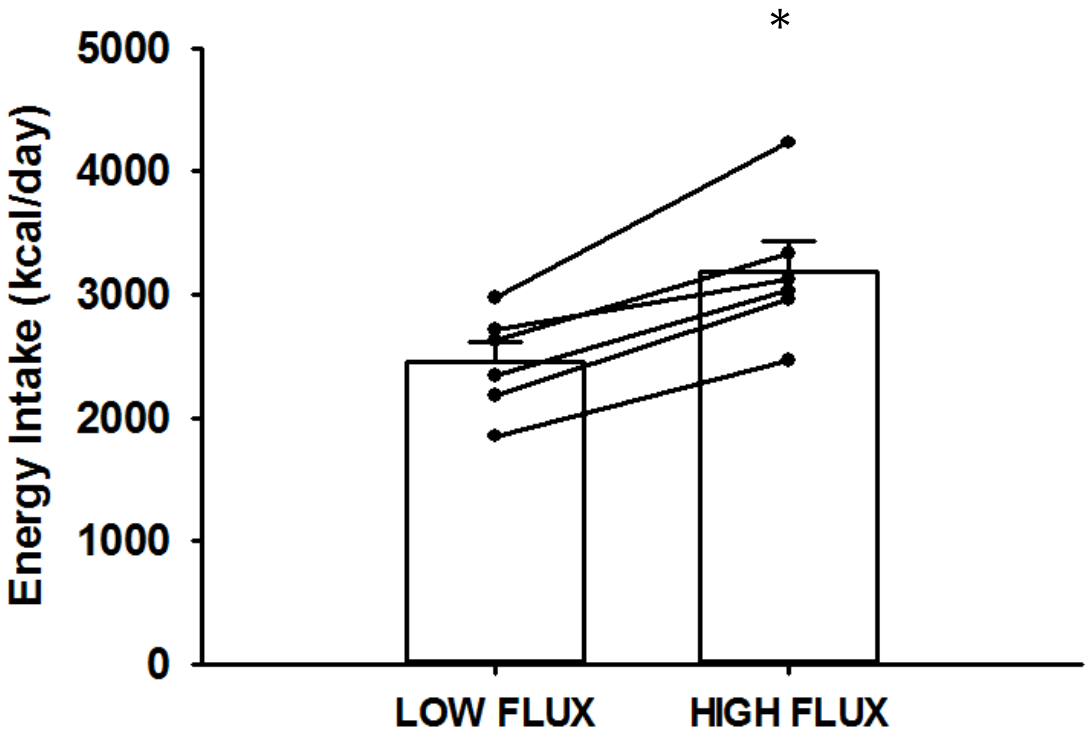
The magnitude of difference in dietary intake represents the additional energy expended in the HF condition resulting from exercise and an increased daily step count (data not shown).

Body composition differed only slightly on day five between the two conditions, with HF resulting in higher FFM (63.4 ± 4.5 kg vs. 62.1 ± 4.5 kg; $P<0.05$), and less FM (39.4 ± 2.5 kg vs. 39.5 ± 2.2 kg; $P<0.05$) compared to LF. Bone mineral content was unaffected by energy flux ($P>0.05$; **Figure 3**).

Table 4. Baseline subject characteristics (n=4 males, 2 females)

Variable	Mean ± SE
Age	42 ± 5
Height (cm)	176 ± 4
Body mass (kg)	110.7 ± 4.7
Body mass index (kg/m^2)	35.7 ± 1.5
Body fat (%)	42 ± 2
Resting metabolic rate (kcal/day)	1933 ± 118
Respiratory exchange ratio (VCO_2/VO_2)	0.86 ± .04

A


*
5000
4000
3000
2000
1000
0
Energy Intake (kcal/day)
LOW FLUX
HIGH FLUX

B

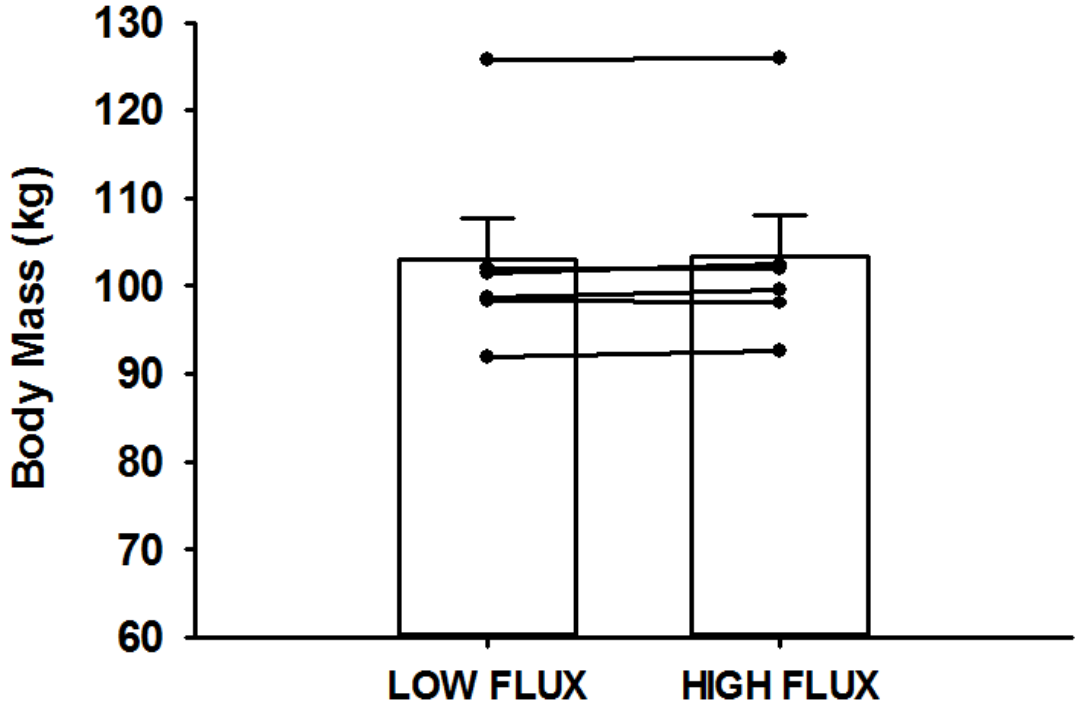


Figure 2. Energy (dietary) intake and body mass during the HF and LF conditions. (A) Mean kilocalories per day consumed in each of the flux states. (B) Mean group body mass over the course of the four-day LF condition and four-day HF condition. *, $P < 0.05$ compared to the low flux state.

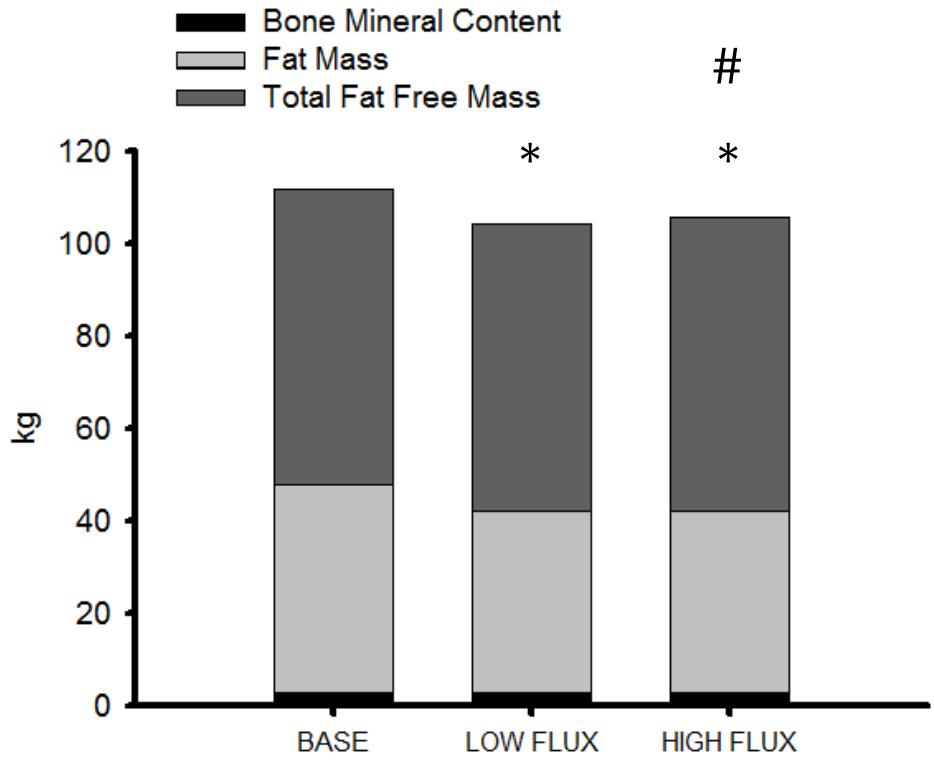


Figure 3. Body composition at baseline and during the 5th day of low and high energy flux. *, $P < 0.05$ for body mass compared with baseline values. #, $P < 0.05$ for FFM and FM in the HF condition compared with FFM and FM in the LF condition.

Resting metabolic rate and resting substrate oxidation

Daily RMR during flux conditions is presented in **Figure 4**. Average RMR during the four days of HF (1926±138 kcal/day) was significantly greater (P=0.046) than average RMR recorded over the four days of LF (1847±126 kcal/day) (**Figure 5**). Individual data showed five of six participants with a greater average RMR during the four days of HF compared to the four days of LF (**Figure 5**).

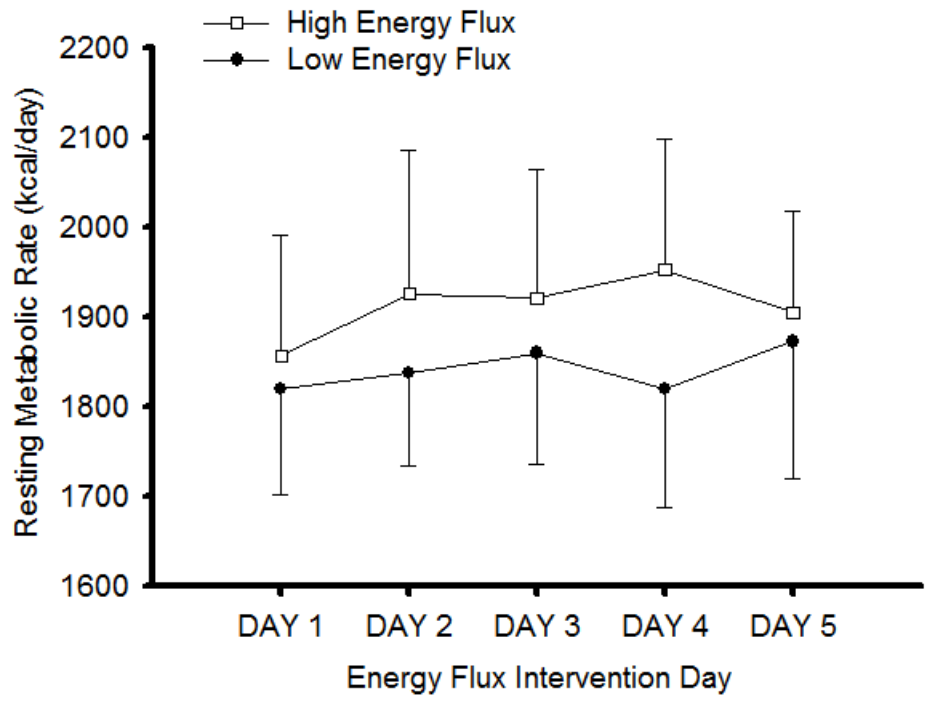


Figure 4. Influence of energy flux on resting metabolic rate. *P*>0.10 for comparison between corresponding days of different conditions (i.e. LF1 compared with HF1).

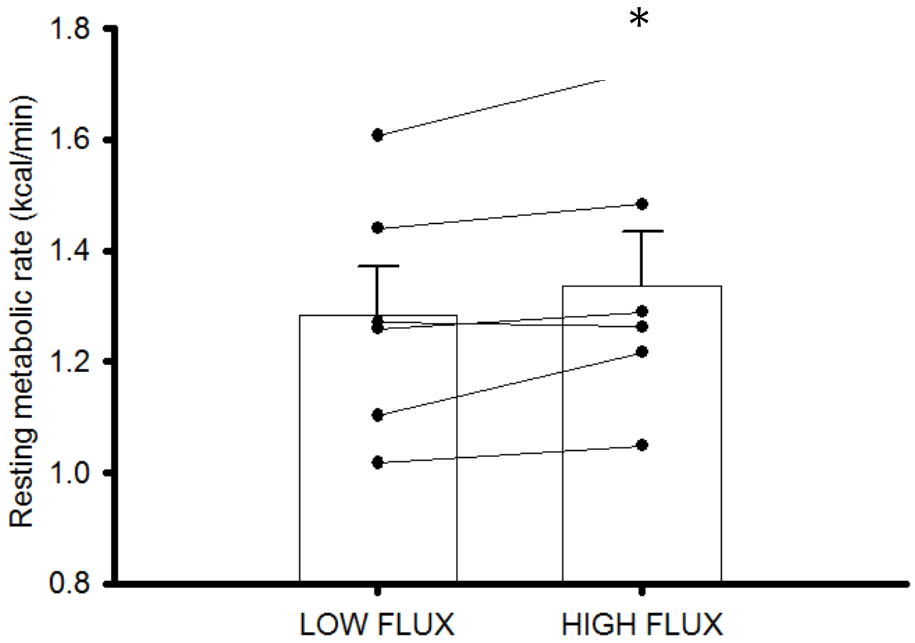


Figure 5. Individual data and group differences in resting metabolic rate for the four LF days compared with the four HF days. *, $P < 0.05$ compared to LF. Data are expressed as mean ± SE.

The four-day average resting RER was lower in HF compared to the four days of LF, but the magnitude of difference did not attain statistical significance (P=0.086) (**Figure 6**). However, owing to the lower RER value and the higher VO_2 measured at rest, fat oxidation (g/min) during HF (0.07g/min) was significantly greater (P=0.015) than that of the LF condition (0.06 g/min) (**Figure 7**).

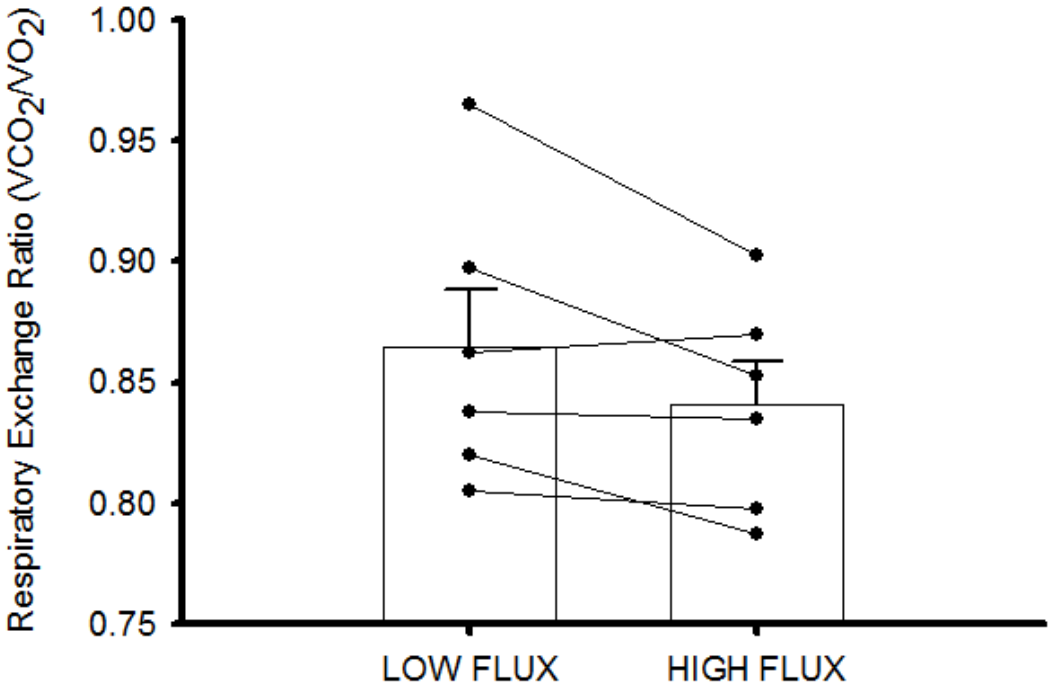


Figure 6. Individual and group means for RER during four days of LF and HF. Values were recorded simultaneously with RMR (P=0.086).

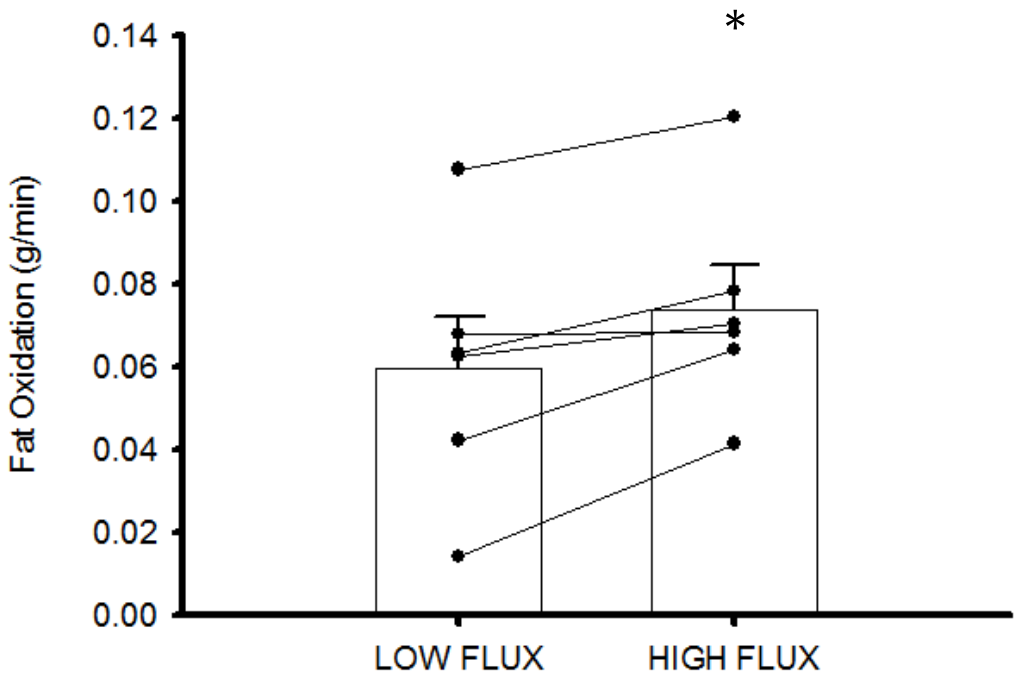
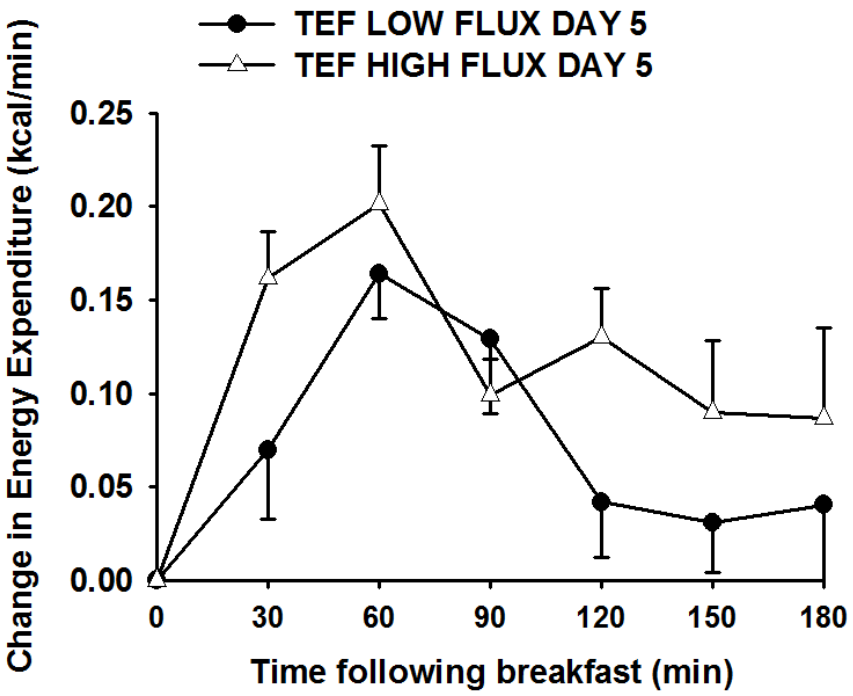


Figure 7. Individual data and group means for the rate of fat oxidation (g/min) during four days of low and high flux. Values based on VO_2 and VCO_2 recorded during RMR measurement. *, $P < 0.05$ between HF and LF conditions.

Thermic effect of feeding and postprandial substrate oxidation

The TEF of breakfast (liquid meal, RMR*0.2) at the end of both LF and HF conditions is presented in **Figure 8A**. Data represent the caloric expenditure above resting metabolism for the three hours following the standardized breakfast. Group averages were not statistically significant between HF and LF, but demonstrated a trend for TEF to be greater in the HF condition (P=0.069; **Figure 8B**).

A



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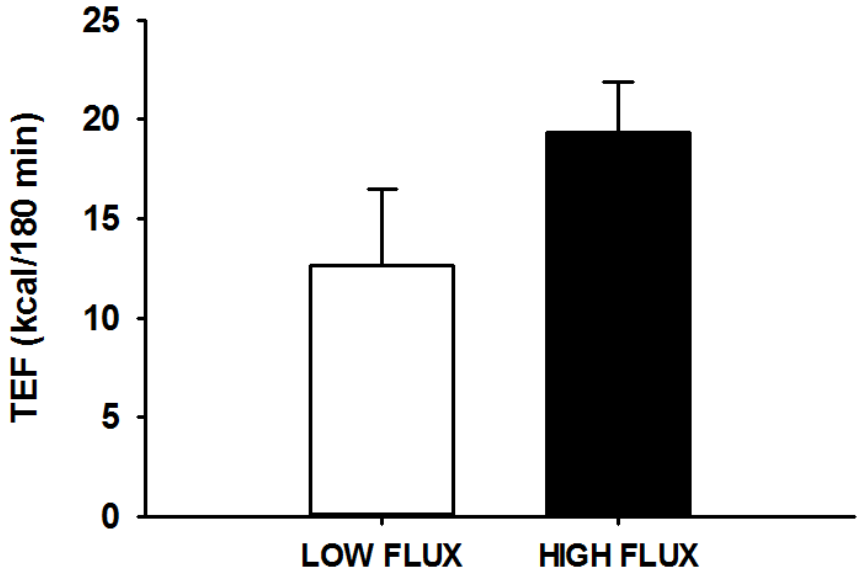

Figure 8. Thermic effect of feeding following breakfast (RMR*0.2). (A) TEF for LF and HF conditions averaged over 30 minute intervals during the 180 minute time span following

consumption of the standardized breakfast. (B) Area under the curve for TEF as a percent of caloric value of the breakfast. Percent was calcualted by dividing caloric expenditure above RMR for the 180 minutes following the meal, by the calories ingesting in the meal [(EE/EI)*100]. All values are for postprandial thermogenesis *above* RMR. Data are expressed as mean ± SE ($P > 0.05$).

Perceived hunger

End of day hunger was reduced (P=0.02) by ~ 81% in the HF state (**Figure 9**).

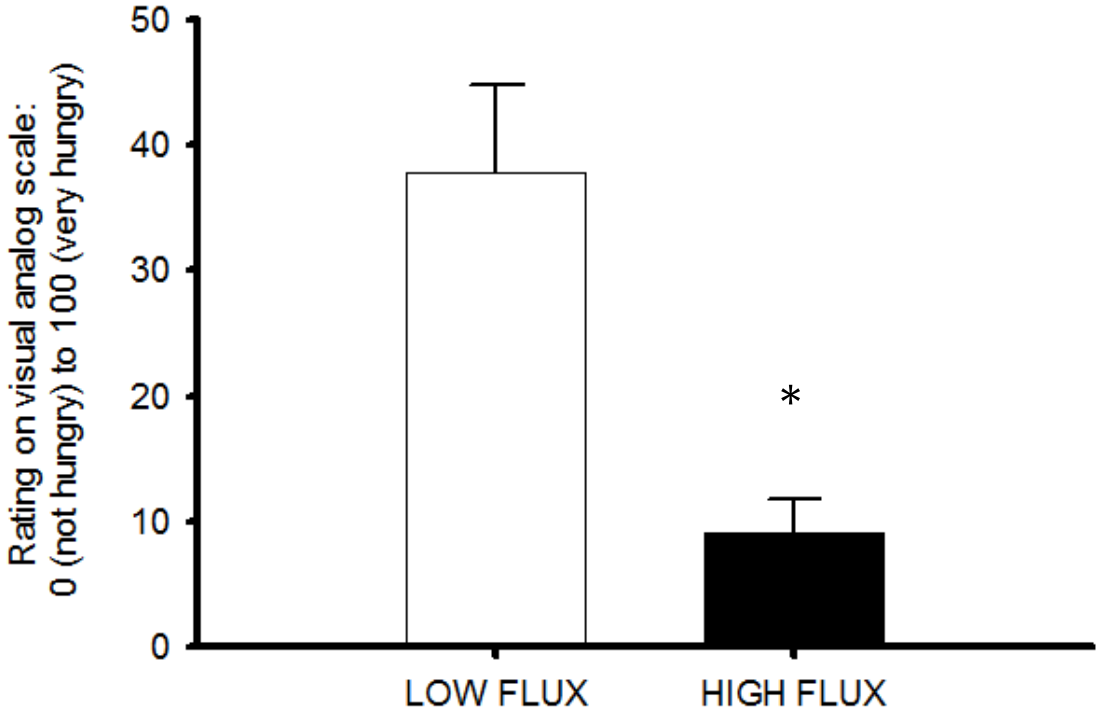

Figure 9. Response to question, "On average, how hungry did you feel at the end of these 4 days" for each of the HF and LF conditions? A value of 0 corresponded with, I was "not hungry, plenty of food" at the end of the four days. A value of 100 corresponded with, I was "very hungry, not enough food" at the end of the four days. *, $P < 0.05$ between HF and LF.

DISCUSSION

This study was undertaken to determine if a HF state following weight loss could narrow the energy gap by attenuating the decline in RMR and reducing the subjective feelings of hunger that accompany weight loss. These pilot data suggest that, consistent with our previous studies [103,144], RMR is higher under HF compared to LF. Additionally, when in the HF state, individuals experience less hunger compared with the LF state. To our knowledge, these data are the first to identify the influence of HF on RMR in obese, weight-reduced individuals.

Resting Energy Expenditure

We made no attempt in this pilot study to identify mechanisms for the elevation of RMR during the four days of HF compared to the four days of LF. However, our previous work supports the idea that an elevated sympathetic drive contributes to this increase in RMR. Previous literature has established the relationship between habitual exercise and β-adrenergic support of resting metabolism [160], as well as the role of HF in mediating this relationship and therefore elevating RMR [103,144]. β-Adrenergic responsiveness has also been examined, and while past studies have centered around endurance-trained, habitually exercising individuals, some research suggests that previously sedentary obese adults may have an elevated β-adrenergic response to aerobic exercise training [161] -

In addition to sympathetic drive, the key determinant of resting metabolic rate is FFM. Exercise in a HF state may conserve FFM to a greater degree than a LF state, such that reductions in RMR would be slightly offset [145]. In the present study, FFM was measured on the last day of each condition and was two percent greater in the HF state. Though our data showed no relationship between the change in FFM and the change in RMR (data not shown),

the possibility exists that small increases in FFM associated with HF could beneficially affect RMR over a long-term period and help attenuate the weight loss associated energy gap. As the duration of HF was only four days, a more plausible explanation as to the change in FFM is had in examining the components other than skeletal muscle that contribute to this measurement. Specially, the component of altered water acts as a likely candidate for the explanation as to why FFM was greater in the HF vs. LF state.

Fat Oxidation

In addition to the increase in RMR in the HF condition, resting fat oxidation was also higher in HF compared to LF. Several studies have observed a reduction in RER up to one day following exercise, indicating elevated fat oxidation [162-164]. Our findings coincide with this past research. Following vigorous exercise, elevated catecholamine concentrations may result in the increased release of fatty acids for up to 24 hours post exercise [146], which could extend fat oxidation in the face of glucose being shuttled towards repletion of muscle glycogen [165]. A notable aspect of the energy gap is that following weight loss, the improvement in metabolic flexibility and heightened insulin sensitivity brought on by weight loss results in the preferential oxidation of carbohydrates, and the shuttling of dietary fats to adipose and hepatic stores [122]. Directing dietary fat to triglyceride stores is energetically more efficient than converting carbohydrates and proteins into fats and storing them accordingly [166]. Therefore, less energy is expended, adipose deposition is resumed, and the drive towards weight regain is increased. Thus, the higher fat oxidation under resting HF conditions may be of clinical importance in attenuating the biologic drive to regain lost weight and body fat by helping to minimize positive

fat balance and subsequent storage of lipids while still maintaining the metabolic health benefits associated with weight reduction.

Hunger Ratings

In addition to its effect on RMR we found that a HF state also lowered end of day hunger. A reduction in hunger could play a major role in countering recidivism into positive energy balance as higher hunger ratings are associated with predicted weight regain [167]. Weight reduction, while beneficial to metabolic health, may actually contribute to the energy gap by increasing the rate of nutrient clearance, making transient the markers of satiety, and moving individuals more rapidly into a post absorptive state [51,168,169]. The role of exercise in regulating intake has been studied extensively, but many questions still remain. In response to initiating exercise, individuals often experience compensatory responses resulting in increased EI [131]. However, research supports a loose coupling between exercise and EI, such that, on a short-term scale, an increase in EI is only a fraction of the energy expended in physical activity. On a long-term scale, EI compensation is likely elevated [132]. Exercise has also been observed to result in an acute loss of appetite in the initial hours post exercise, and an increased onset of satiety in the postprandial condition – both of which would lessen the possibility of positive energy balance [134,135]. In the present design, the final exercise bout of the HF state was performed 12-21 hours prior to measurement of hunger and satiety, and so this acute anorexia may not have been captured. Because of these alterations in hunger and satiety, a HF state may better regulate EI in the weight-reduced condition compared with dietary regulation alone, as observed in the end of day hunger ratings. The idea of intake regulation via physical activity is far from novel, and supports Mayer's work from the 1950's which showed that, compared with

the sedentary state, physical activity actually resulted in decreased energy intake, with caloric consumption elevating to coincide with intensity of activity [130].

Thermic Effect of Feeding

Weight maintenance via HF resulted in a TEF more closely resembling basal conditions than the lowered TEF observed in the LF condition. Because TEF contributes to approximately 10% of TDEE, elevating TEF exists as an additional avenue for caloric expenditure and weight maintenance. While research regarding post exercise TEF is largely variable, some evidence shows that exercise may raise TEF through elevated glucose uptake, thermogenic hormone levels, and sympathetic activity [170]. A power analysis based on the magnitude and direction of our TEF data with six subjects suggests that an additional eight participants would be necessary to achieve statistically significant differences in TEF between HF and LF states.

Experimental Strengths and Limitations

In the current study we used a within-subjects design to measure the role of energy flux in mitigating the energy gap. The design included a period of weight stabilization to ensure measurements were not taken in the dynamic phase of weight loss. The provision of all daily meals and snacks while in the respective flux conditions removed the possibility of food intake errors based on subjects preparing their own food. Additionally, while participants were in the HF state, every exercise bout was monitored such that appropriate time and intensity were achieved. Regardless, experimental limitations did exist, and need to be addressed.

Past research examining weight regain in rodent models used a rapid, energy-restriction approach to weight loss [128]. In our study, while weight loss was primarily achieved via caloric

restriction, the rate of weight loss tended to be more gradual and thus may not have reflected rapid weight loss due to severe caloric restriction. The slower velocity of weight loss may not have elicited the magnitude of discrepancy between EI and caloric requirements as described by the energy gap seen in rodent models. Additionally, past studies have been criticized for their inclusion of only those subjects that were successful at weight loss – excluding those who were not, and potentially missing data from those with the strongest metabolic drive to regain [128]. While this is a valid concern, weight loss was a necessary component of the study design, and therefore we could only include individuals able to lose 7% of initial body weight. Regardless, this is a potential limitation of the current study.

Once the target weight loss was achieved and maintained for three weeks, energy balance was assessed based on daily, morning body weight measurements following a 12 hour fast. Because acute energy balance can influence subjective hunger and satiety measures, confirming energy balance with circulating triiodothyronine concentrations would have strengthened the design of the study. However, if participants were overfed in the HF state, we would expect carbohydrate balance to be maintained but with fat deposition – thereby raising RER values. As RER was actually lower in the HF state, further confidence can be had in the maintenance of energy balance, or at least that the higher RMR occurring under HF conditions was not the result of the subjects being overfed. Following flux conditions, hunger and satiety were determined using a VAS based on previously validated scales, although the particular scale used here has not yet been validated.

Because of the small sample size, this study is yet underpowered. Remarkably though, even with only six study participants, there were statistically significant differences in both RMR

and hunger ratings between the HF and LF states. Nevertheless, we acknowledge that greater confidence could be place in these key findings if the sample size were larger.

The two different flux states used in the present study were of short duration—only four days each. Thus, it is unclear as to the long-term influence of energy flux and the sustainability of a HF state for the given population. Previous research has shown the role of chronic HF in energy metabolism [103, 144] as well as the role of exercise in maintaining lost weight [167], which would support the continual impact of HF as well as its feasibility of adoption on a long-term scale.

Conclusions

These preliminary data suggest that, following weight loss, elevated energy flux is associated with a greater RMR and less hunger. HF may also promote fat oxidation in the rested state, and elevate TEF in the postprandial condition. Collectively, these data support the role of HF in attenuating the energy gap brought on by weight loss and in lessening the biological drive towards weight regain.

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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