HORMONE RESPONSES TO TWO DIFFERENT ENERGY RESTRICTION PROGRAMS

BY

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LIST OF ABBREVIATIONS

Agouti-related Peptide (AgRP)

Arcuate Nucleus (ARC)

Blood Brain Barrier (BBB)

Central Nervous System (CNS)

Clinical Research Unit (CRU)

Cocaine and Amphetamine-regulated Transcript (CART)

Complete Blood Count (CBC)

Complete Metabolic Panel (CMP)

Dual X-ray Absorptiometry (DXA)

Energy Expenditure (EE)

Gastrointestinal tract (GI)

Glucagon-like Peptide 1 (GLP-1)

Low Calorie Diet (LCD)

Neuropeptide Y (NPY)

Oral Glucose Tolerance Test (OGTT)

Proopiomelanocortin (POMC)

Resting Energy Expenditure (REE)

Resting Metabolic Rate (RMR)

Tapered Reduction in Energy as a Novel approach to Dieting (TREND)

Total Energy Expenditure (TEE)

Thyroid Stimulating Hormone (TSH)

Wake Forest University Baptist Medical Center (WFUBMC)

ABSTRACT

There is a general decrease in satiety hormones (glucagon-like peptide 1 (GLP-1) and leptin) and an increase in hunger hormones (ghrelin) with weight loss, however results are conflicting. Currently, no studies have examined hormone levels comparing different approaches in calorie reductions for weight loss. PURPOSE: The primary purpose was to compare plasma levels of ghrelin, GLP-1, and leptin in obese participants undergoing a traditional low calorie diet (LCD) vs. those on a calorie tapered program (TAPER). METHODOLOGY: Eight obese participants were randomized into the LCD (immediate caloric reduction) or a taper diet (gradual caloric reduction) for 6 weeks. Fasting and postprandial hormone levels after a meal challenge were taken at week 0 and week 6. STATISTICAL ANALYSIS: T tests were performed to examine differences between groups in change of hormone levels from week 0 to week 6. Effect sizes were calculated. RESULTS: Weight loss was 6.1% in LCD and 5.3% for TAPER. The intervention had a moderate effect on fasting ghrelin, change in postprandial ghrelin and GLP-1 and a large effect on fasting GLP-1. CONCLUSION: These findings suggest that a gradual caloric reduction could lower ghrelin and increase GLP-1 after a meal more so than an immediate caloric reduction.

REVIEW OF LITERATURE

Obesity

The prevalence of adult obesity in the United States has increased dramatically from the 1970's until now (Finkelstein et al., 2012; Mokdad, Ford, Bowman, & Dietz, 2003; Ogden, Carroll, Kit, & Flegal, 2014). The most recent report on obesity, published by the Centers for Disease Control and Prevention (CDC) based on the National Health and Nutrition Examination Survey (NHANES) data, revealed that obesity was diagnosed in over one-third (36.5%) of the United States adult population from 2011-2014 (Ogden, Carroll, Fryar, & Flegal, 2015). According to the Behavior Risk Factor Surveillance System (BRFSS) survey conducted by the CDC, the prevalence of obesity in the United States adult population increased by 74% from 1990 to 2000 (Mokdad et al., 2003). Finkelstein et al. estimated adult obesity rates would rise by 51% from 2008 to 2030 (Finkelstein et al., 2012; Wang, McPherson, Marsh, Gortmaker, & Brown, 2011). Obesity prevalence remains high and has the potential to increase dramatically if no successful intervention is implemented (Finkelstein et al., 2012).

Obesity, or excess storage of lipids, is a primary concern since it puts an individual at a higher risk for cardiovascular and diabetic complications, ultimately leading to increased mortality and risk for chronic diseases (Mokdad et al., 2003). In most cases of obesity, there is a cluster of additional medical health problems such as hypertension, hyperlipidemia, dyslipidemia, atherosclerosis, asthma, arthritis, insulin resistance, diabetes, low quality of life, and general poor health (Mokdad et al., 2003; Redinger, 2007; Wang et al., 2011). The presence of central adiposity, as well as these multiple health issues, are known as the Metabolic Syndrome (Alberti, Zimmet, & Shaw,

2006). Obesity is thus a chain of adverse reactions. Due to the accumulation of multiple health disorders, obesity leads to 300,000 adult deaths every year in the United States (Mokdad et al., 2003), approximately 20% of all deaths (Anton, Rahman, Bhanushali, & Patel, 2016).

The Necessity of Weight Loss

While obesity puts the body at harm for various chronic diseases, modest weight loss can significantly lower the risk of the associated chronic diseases such as diabetes and cardiovascular complications (K. G. Murphy & Bloom, 2004; Perry & Wang, 2012a). Health practitioners who specialize in obesity treatment and have an understanding of the health benefits of weight loss have pushed prescribed weight loss programs on overweight and obese individuals. These programs are centered on behavior changes that reduce energy intake and increase energy expenditure (K. G. Murphy & Bloom, 2004). While lifestyle modifications are crucial to one's health, the problem with current weight loss programs is that only short-term weight loss is reported. Long-term weight loss maintenance is not successful in the vast majority of individuals (Greenway, 2015; Müller, Enderle, & Bosy-Westphal, 2016; K. G. Murphy & Bloom, 2004). A limitation of most behavior change centered weight loss programs is that they do not address the physiological changes that occur in the presence of weight loss. The body's system of energy balance is complex, and difficulties with weight loss and maintenance can lie with factors that influence it, e.g. hormonal and metabolic adaptations. Thus, a successful treatment will need to recognize and address the mechanisms of these elements involved (Greenway, 2015).

Energy Expenditure

Total daily energy expenditure (TEE) for adults is categorized into three components – resting, activity, and thermic effect of food, also known as dietary induced thermogenesis (R. L. Leibel, Rosenbaum, & Hirsch, 1995; Müller et al., 2016). The resting component, or resting energy expenditure (REE) comprises the highest amount of daily energy expenditure for most adults, attributing 60% of daily expenditure (R. L. Leibel et al., 1995; Müller et al., 2016). This is the amount of energy needed to maintain physiological processes during resting conditions under standard conditions, including no vigorous physical activity 24 hours prior, fasting 6 hours prior, and no stimulants taken 24 hours prior. The next largest component contributes 30% of daily expenditure and is energy needed for activity, including activities of daily living and structured exercise (R. L. Leibel et al., 1995). The smallest component is diet induced energy expenditure, which contributes to only 10% of daily expenditure (R. L. Leibel et al., 1995). This component is responsible for the energy needed for the digestion, absorption, and postprandial processing of food components (R. L. Leibel et al., 1995).

The individual's values for each of the three components of energy expenditure has been shown to predict weight gain or weight loss (Müller et al., 2016). A high TEE is associated with reduced weight gain; however, when those values are low there is an increased risk for fat storage (Müller et al., 2016). For example, weight gain was apparent in individuals with a low REE (R. L. Leibel et al., 1995; Ravussin, 1993). REE makes up such a large proportion of total daily energy expenditure, so when this resting component of energy expenditure is low, the likelihood of weight gain is increased (R. L. Leibel et al., 1995). This challenge is documented clearly in Ravussin's classic studies

on the Pima Indians (Ravussin, 1993). Pima Indians (n = 126) were divided into weight gainers and non-weight gainers; Weight gainers had a lower mean REE compared to the REE of non-weight gainers, 1694 ± 103 kcal/day and 1764 ± 109 kcal/day, respectively ($P < 0.03$) (Ravussin, 1993). After a three-year follow-up, in comparison to those with the highest REE, individuals with the lowest REE had seven times greater risk of weight gain (Ravussin, 1993). Ravussin found that a genetically determined low resting metabolic rate (RMR) or REE was a significant risk factor for weight gain; however, he also indicated that there are other factors that predict weight gain such as physical activity and food intake (Ravussin, 1993).

Regulating Body Energy Stores

It is difficult for obese individuals to maintain weight loss under the oversimplified current ideology of "Calories In vs. Calories Out" since it does not fully encompass the entire process of energy uptake and storage (Greenway, 2015). While this method of understanding incorporates both the behavioral and environmental regulation aspect, it does not consider biological regulation such as changing metabolic rate based on current energy balance (Müller, Bosy-Westphal, & Heymsfield, 2010). A more accurate assumption of body weight regulation is rooted in the understanding that the body has various biological, behavioral, and environmental regulators.

Biological regulators of energy expenditure are composed of hormones, neural stimulation, genes, and epigenetic effects (a change in how cells read genes that do not result from a change in DNA) (Hopkins & Blundell, 2016; Müller et al., 2010). These biological regulators affect behavior by initiating or inhibiting a drive to eat (R. Leibel, 2008). When the human body is subjected to states of overfeeding or starvation, the body

will respond by secreting hormones known to affect feeding responses with the goal to help regulate stored energy (Sumithran et al., 2011a). This control of body fat seems to be more effective in a state of negative energy balance than in a state of positive energy balance, therefore favoring weight gain instead of weight loss (Müller et al., 2010, 2016). This phenomenon was first seen in the famous Minnesota Starvation Study where semi-starved participants consumed 50% of their caloric intake needs and lost 66% of their body fat. The participants then underwent a refeeding period where they gained back their initial body fat plus more, adding up to 145% of their pre-starvation body fat (Müller et al., 2010). This demonstrates that during the refeeding period with positive energy balance, the body's regulatory mechanisms are not finely tuned, with follow-up body weight reaching above initial body weight. This is also observed in previously obese individuals or reduced weight individuals who have a difficult time maintaining the new body weight due to biological defenses occurring (R. Leibel, 2008). Part of this response is a 15-20% greater than predicted reduction in energy expenditure based on changes in body mass and composition as well as an increase in hunger due to the threat to survival and reproduction (R. Leibel, 2008). This is not to say that behavioral or environmental effects do not have an influence on body regulation. The Western diet, rich in calorically dense, high fat and sugar containing foods, promotes over eating and shifts the individual to a constant positive energy balanced state. This can then undermine the biological regulators and cause a gradual but constant weight gain (Müller et al., 2010). Therefore, these biological regulators work better in an individual at a healthy body weight compared to in an obese state. More research needs to be established to understand this energy regulatory process.

Adaptive Thermogenesis

Adaptive thermogenesis is the body's response to either a positive or negative energy balance (i.e. relative over- or under-feeding) (Müller et al., 2016). This concept is thought to be a significant contributor to the lack of long-term success with weight maintenance following weight loss. When undergoing a calorie restricted diet, REE decreases to offset the reduced energy intake and to prevent further weight loss and/or maintain body weight (R. L. Leibel et al., 1995). Adaptive thermogenesis can be defined as the amount of reduced energy expenditure that is different than the expected reduction in energy expenditure based on changes in body weight and/or body composition (Camps, Verhoef, & Westerterp, 2013). The greater the weight loss, the higher the metabolic adaptation the person will have to overcome (Camps et al., 2013). The biggest challenge is that this reduction in energy expenditure after weight loss is sustained throughout the weight maintenance phase as long as the weight is lower than the initial weight prior to the start of the weight loss program (Camps et al., 2013). Camps and colleagues found the reduced REE to be sustained up to 44 weeks in individuals trying to maintain weight loss. However, REE did not remain reduced in those people who were not successful in losing weight (Camps et al., 2013).

The metabolic adaptation in the body is not equal in regards to energy balance (Müller et al., 2016). Adaptive thermogenesis can also work the opposing direction, meaning there is an increase in TEE when overfeeding is involved (Müller et al., 2016). In a state of overfeeding the non-resting component of EE adapts to help energy dissipate (Müller et al., 2016). While individual variability with adaptive thermogenesis exists, obese individuals, when compared to lean individuals, are more likely to have a high

adaptive response in caloric restriction and a low adaptive response in overfeeding. This would mean a greatly reduced REE in response to negative energy balance, as well as, only a slightly increased REE in response to positive energy balance. Both these scenarios would make weight loss difficult (Müller et al., 2016). The first would make losing weight difficult, due to the continued reduction in REE, while the latter would make weight regain easier, due to a lack of substantial increase in REE when more food is welcomed back after the diet.

Hormones and Obesity

A number of hormones play a role in the pathophysiology of obesity, including interacting with adaptive thermogenesis (Sumithran et al., 2011a). These hormones are involved in feeding behaviors and energy expenditure. Secretion of selected hormones is altered with weight loss, whether the weight loss is achieved solely by lifestyle interventions or bariatric surgery procedures to create a negative energy balance (Greenway, 2015; Sumithran et al., 2011a). Hormones such as leptin, ghrelin, GLP-1, peptide YY, cholecystokinin, insulin, amylin, and pancreatic polypeptide, among others, respond to caloric restriction (Sumithran et al., 2011a). Studies support that with short-term weight loss, there is an increase in hunger hormones as well as a simultaneous decrease in satiety hormones (Adam, Jocken, & Westerterp-Plantenga, 2005; Crujeiras et al., 2010; Cummings et al., 2002; Greenway, 2015; Hopkins & Blundell, 2016; Santo et al., 2016; Sloth et al., 2008; Sumithran et al., 2011a). However, the effect of weight loss programs that vary on level of dietary intake has not been established. This thesis will address this issue by specifically examining ghrelin, GLP-1, and leptin in two weight loss programs varying in the initial degree of caloric restriction.

Brain and Periphery Communication

Regulation of body weight is a homeostatic process within the human body that requires integration of signals from peripheral components of the body to the hypothalamus of the central nervous system (CNS). These peripheral components include the gastrointestinal (GI) tract and tissues involved with energy storage, such as adipose tissue, (K. g. Murphy & Bloom, 2004). Messages originating from adipose tissue are referred to as adipostat factors. Communication between the GI tract and the hypothalamus (including GLP-1 and ghrelin) results in short-term signals to control meal initiation and termination. Communication between the adipostat factors (such as leptin) and the hypothalamus result in a slow and chronic long-term informative signals about the current fat stores within the body (K. g. Murphy & Bloom, 2004; Schwartz, Baskin, Kaiyala, & Woods, 1999). Peripheral signals affect energy balance through stimulation or inhibition of neurons that influence energy expenditure and feeding behavior (Schwartz et al., 1999). To do this, these periphery signals from the GI and adipose tissue circulate in the plasma to the arcuate nucleus (ARC), which is located at the base of the hypothalamus. The ARC contains the appropriate neurons with hormone receptors that release neuropeptides which regulate food intake (Perry & Wang, 2012a). There are two groups of neurons located in the ARC: appetite inhibiting neurons consisting of proopiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART) and appetite stimulating neurons consisting of neuropeptide Y (NPY) and agouti-related peptide (AgRP) (K. G. Murphy & Bloom, 2004). The orexigenic hormone (ghrelin) stimulates NPY and AgRP, while the anorexigenic hormones (GLP-1 and leptin) stimulate POMC and CART (K. g. Murphy & Bloom, 2004).

Hormone Response to Meal Challenge

Due to the existing relationship between the brain and periphery (GI and adipose tissue) on feeding behavior, it is important to objectively measure hormone concentrations at the pre and post time point of a meal challenge. The pre, or the fasting measurement, will give information about the body's steady-state regulation of feeding behaviors, while the postprandial measurement will provide information about how the body regulates the length of satiety, meal size, and meal termination (Graaf, Blom, Smeets, Stafleu, & Hendriks, 2004). Ingestion of a meal also induces responses from various gut hormones which have an effect on satiety; therefore a meal challenge is necessary to observe that postprandial phase (Graaf et al., 2004). Hormonal responses differ depending on the composition of macronutrient content within the meal. Therefore a variety of meal types should be used when understanding responses of hormones in the postprandial phase. For example, secretion of GLP-1 is augmented after a carbohydrate-rich meal, but GLP-1 is not altered after a meal rich in fat (De Silva & Bloom, 2012; Huda, Wilding, & Pinkney, 2006). Secretion of ghrelin is positively correlated to a low caloric load as well as meals high in protein or fat macronutrients (Carlson, Turpin, Wiebke, Hunt, & Adams, 2009; Carroll, Kaiser, Franks, Deere, & Caffrey, 2007). In contrast, ghrelin secretion is negatively correlated to high caloric loads and meals high in carbohydrates, which is likely due to a rise in glucose levels in the blood, driving insulin levels in the blood which inhibit ghrelin secretion (Carlson et al., 2009; Carroll et al., 2007).

Ghrelin

In 1999, the hormone ghrelin was discovered in rat and human stomachs. This peptide hormone stimulates the secretion of growth hormone and is involved in energy homeostasis as it induces feeding behaviors through increasing the rate of gastric emptying. It also downregulates anorexigenic hormone receptors for the satiety hormone GLP-1 (Anton et al., 2016; Sakata & Sakai, 2010). Ghrelin is an orexigenic 28 amino acid chain peptide, with 90% of it produced and secreted from ghrelin cells that primarily reside in the stomach. However, ghrelin cells are dispersed all throughout the GI tract (Perry & Wang, 2012b; Sakata & Sakai, 2010) (Anton et al., 2016). Circulating levels of ghrelin increase during a fasted state and decrease during a postprandial state (Toshinai et al., 2001). Ghrelin levels also rise immediately prior to mealtime or in anticipation of a meal taking on a drive for meal initiation (Cummings et al., 2002). Once in circulation, ghrelin travels to the brain and binds to the growth hormone secretagogue on the orexigenic NPY and AgRP receptor in the ARC (Anton et al., 2016; Perry & Wang, 2012b; Toshinai et al., 2001). This leads to an increase in appetite as well as a decrease in energy expenditure (Anton et al., 2016; Perry & Wang, 2012b). Supporting this action is that infusions of ghrelin in rats and lean and obese humans stimulate feeding behavior (Perry & Wang, 2012b).

Ghrelin and Obesity

Obese individuals have lower fasting levels of ghrelin, possibly indicating the body is making an effort to reduce energy stores by decreasing food intake (K. G. Murphy & Bloom, 2004). In support of this, the majority of literature (Appendix I) through various cross-sectional research studies comparing obese to normal weight individuals indicate this impact of obesity. These studies report obese individuals having

lower fasted ghrelin than their normal weight counterparts (Carlson et al., 2009; Carroll et al., 2007; English, Ghatei, Malik, Bloom, & Wilding, 2002; Monti, Carlson, Hunt, & Adams, 2006; Morpurgo et al., 2003). Two studies examined the correlation of ghrelin with BMI (Carroll et al., 2007; Monti et al., 2006). Monti and colleagues (2006) found ghrelin and BMI to have a low to moderate negative correlation ($r = -.39$; $p = .001$), while Carroll and colleagues (2007) found a moderate negative correlation ($r = -.47$; $p = .005$). Carroll et al. (2007) examined fasting active ghrelin levels in obese vs. normal weight men and women. Obese men and women had lower fasting levels of active ghrelin (53.4 ± 10.9 pg/ml for men; 68 ± 14.1 pg/ml for women) compared to their normal weight counterparts (76.7 ± 17.4 pg/ml for men; 86.9 ± 10.2 pg/ml for women) (Carroll et al., 2007). Another study, Carlson et al. (2009), examined total fasting ghrelin levels in severely obese vs. normal weight women. Levels for the severely obese were significantly lower than levels in the normal weight women's, 1087 ± 187 pg/ml and 1418 ± 232 pg/ml, respectively ($p = 0.001$) (Carlson et al., 2009). Lastly, a third study comparing obese and lean individuals found obese to have fasting total ghrelin at 325 ± (204-519) pmol/l and lean at 857 ± (627-1171) pmol/l ($p = .002$) (English et al., 2002). The lower ghrelin levels seen in obese in these studies were predicted based on the individuals constantly being in a positive energy balanced state. These studies support that low ghrelin levels in obese individuals persist to bring the body back to energy homeostasis.

It is common to not only study fasting plasma ghrelin but to also examine the response of ghrelin following a meal. There is a weakened response to the fall in plasma ghrelin levels after a meal in the obese than the lean, further substantiating a role for

ghrelin in the pathophysiology of obesity (Perry & Wang, 2012b). Since ghrelin levels remain higher after a meal in the obese, this could lead to greater food intake in this cohort. Additionally, the literature shows that there is a significant difference in timing of the course of response in ghrelin. Obese have either no change, a relatively small change, or a slower change from fasting values following a meal challenge, compared to lean individuals who have a greater and quicker change from fasting values (Carlson et al., 2009; Carroll et al., 2007; English et al., 2002; Morpurgo et al., 2003). In normal weight vs. severely obese women, the postprandial ghrelin levels following a moderately high carbohydrate meal were lower when averaged over time in severely obese women compared to normal weight women ($p = .004$) (Carlson et al., 2009). Carroll et al. (2007) reported the time lapse until the nadir (lowest point) of ghrelin obtained and found that normal weight men and women dropped ghrelin relatively quicker than obese men and women (26.7 ± 3.3 minutes in normal weight women, 25.5 ± 3.7 minutes in obese women, 18.9 ± 3.1 minutes in normal weight men, and 34.4 ± 5.6 minutes in obese men). English et al. (2002) reported the obese postprandial levels showed no change from fasting, while the lean postprandial levels dropped $39.5\% \pm 10\%$ thirty minutes after ingestion of the meal. Finally, with continued support to the previous findings, Morpurgo et al. (2003) observed no change in ghrelin during the postprandial phase of a standardized meal (550 kcal) in obese subjects compared to a significant drop in ghrelin in the postprandial phase in healthy subjects (110.8 ± 69.7 pmol/l to 91.8 ± 70.2 pmol/l in obese and 352.4 ± 176.7 pmol/l to 199.0 ± 105.2 pmol/l in healthy; $p < .01$). Although ghrelin is primarily responsible for increasing feeding behaviors, the overall decreased

magnitude and slower timing of the reduction in ghrelin release in the postprandial phase in obese vs. normal weight individuals may indicate decreased satiety.

Ghrelin and Weight Loss

Appendix II presents studies that examined the response in fasting and postprandial plasma ghrelin to dietary restriction weight loss interventions. Over a sustained period of caloric restriction, fasting ghrelin levels increase (Anton et al., 2016). This is postulated to be due to the body's defense mechanism against starvation (Anton et al., 2016). In contrast, bariatric surgery and other gastrostomies decrease fasting ghrelin levels, potentially leading to the relative success in surgery-induced weight loss maintenance (Anton et al., 2016). While some studies show an increase in fasting ghrelin after weight loss, some studies do not. Studies examining short-term weight loss (3-8 weeks) (Crujeiras et al., 2010; Morpurgo et al., 2003) indicate no significant increase in fasting ghrelin, however studies examining long-term weight loss (10-62 weeks) indicate significant increases in ghrelin (Crujeiras et al., 2010; Cummings et al., 2002; Hooper et al., 2010; Sumithran et al., 2011a). It is important to examine multiple studies that look at both acute and long-term weight loss programs. For example, Morpurgo et al. (2003) indicated that a short 3 week weight loss program was not long enough to observe significant changes in fasting ghrelin. A long-term study by Cummings et al. (2002) examined a 6 month weight loss program on obese subjects with a mean weight loss of 17.4 ± 1.5 percent of the initial body weight. These obese subjects significantly increased their 24 hours ghrelin profile with weight loss (24% increase in area under the curve; $p = .006$). A cross-sectional study examining fasting ghrelin concentrations in self-reported weight loss in postmenopausal women revealed fasting ghrelin increased

with higher levels of intentional weight loss (Hooper et al., 2010). Fasting ghrelin was 11% higher in women who reported intentional weight loss > 10 lbs, 22% higher in women who reported intentional weight loss > 20 lbs, and 44% higher in women who had reported intentional weight loss > 50 lbs, compared to those who reported no intentional weight loss (Hooper et al., 2010). These last two studies support that with weight loss there is an adaption to fasting ghrelin which may predispose one to weight gain. These studies taken together indicate the necessity to examine both the acute and the long-term negative energy balance to understand ghrelin secretion.

A more clinically relevant finding of some of these studies that looked at long-term weight loss programs is that fasting ghrelin levels remained increased in the long-term (Crujeiras et al., 2010; Cummings et al., 2002; Sumithran et al., 2011a). Sumithran et al. (2011) examined fasting active ghrelin in a 10 week very low calorie (500-550 kcal/day) weight loss study in overweight or obese individuals. From baseline to after the 10 week diet, fasting active ghrelin increased significantly ($p < 0.001$) and remained elevated from baseline to 62 weeks ($p < 0.001$), indicating a hormonal adaptation to weight loss. Fasting active ghrelin at baseline was ~125 pg/mL, increased at week 10 to ~175 pg/mL, and remained higher than baseline at 62 weeks (~150 pg/mL) (Sumithran et al., 2011a). Crujeiras et al. in 2010 examined total fasting ghrelin levels in obese and overweight men and women at baseline (week 0), after an 8 week low calorie diet (week 8), and then re-examined ghrelin at follow-up (week 32). There was no significant change in fasting total ghrelin levels from week 0 to week 8 of the diet. The researchers did, however, reveal that the hormonal adaptation to weight loss was different in those who were successful (55 maintained weight loss) vs. those who regained the lost weight

(49 regained at least 10% of the lost weight). The weight regain group had significantly lower fasting total ghrelin levels compared to the weight maintenance group ($p < 0.05$). Due to these findings of increased sustained fasting ghrelin in the obese, these studies support that a disadvantageous hormonal adaptation in fasting ghrelin persists and therefore can make weight maintenance difficult. This is a clinically relevant finding, as it gives support for a physiological basis of relapse in weight regain rather than simply a resumption of previous bad habits from the individual (Crujeiras et al., 2010; Sumithran et al., 2011a).

Interestingly, the three studies that included observation of postprandial ghrelin did not show significance in two (Morpurgo et al., 2003; Sumithran et al., 2011a), with the one showing mild increased postprandial ghrelin (Cummings et al., 2002). In Sumithran et al. study the postprandial responses of ghrelin at all time points (0, 10, and 62) reported were not significantly different (Sumithran et al., 2011a). The lack of change in the postprandial responses of active ghrelin would suggest that secretion of active ghrelin after a meal was similar at weeks 0, 10, and 62. Despite a decrease in BMI, the individuals on average remained obese at the end of the 10 and 62 weeks so a similar postprandial response could be due to not enough reduction in weight (Sumithran et al., 2011a). A potential reason why the two studies showed no significance could be due to a shorter period of caloric restriction of 3-10 weeks compared to 3 months, or it could also be due to less weight loss of 5-14% body weight compared to 17% body weight lost.

Another possible mechanism underlying ghrelin adaptations with weight loss could be a resistance to ghrelin. Crujeiras at al. (2010) examined fasting ghrelin levels as

a dichotomous variable (0 = ghrelin above the median and 1 = ghrelin below the median) and found a significant risk of weight regain in those individuals who had fasting ghrelin levels below the median fasting ghrelin levels after the diet. After the 8 week diet, those who had fasting levels below the median were more than 3 times more likely to regain weight than those who's fasting ghrelin levels that were above the median fasting ghrelin levels during the diet (OR = 3.109; CI 1.346-7.181; p = 0.008) (Crujeiras et al., 2010). It would seem surprising that low ghrelin could be indicative of weight regain since ghrelin is itself a hormone inducing hunger. Here, it is postulated that there is a disruption in the sensitivity of the signals in obese and that the lower levels indicate a greater response to a given level of ghrelin. This is a novel finding since it indicates that the underlying mechanisms of ghrelin are different for successful weight maintenance vs. those who regain weight as well as a different possible mechanism of a sensitivity disruption seen in obese individuals.

Glucagon-Like Peptide 1

Glucagon-like peptide 1 (GLP-1) is a hormone synthesized by the enteroendocrine L-cells of the gut, and its release is dependent on total caloric intake, as well as ingestion of carbohydrates (Lean & Malkova, 2016; Perry & Wang, 2012b). GLP-1 is an incretin hormone, and it stimulates release of insulin from the pancreas (Huda et al., 2006). GLP-1 has anorexigenic effects such as reducing gastric emptying and delaying gastric acid secretion. Both effects are mediated by stimulating the vagus nerve (Huda et al., 2006; Lean & Malkova, 2016). Exogenous administration of GLP-1 to humans increases satiety and a reduction in caloric intake (Perry & Wang, 2012a). GLP-1 receptors are located throughout the brain, GI tract, and peripheral organs. In the

ARC of the brain, GLP-1 stimulates the anorexigenic POMC and CART neurons and inhibits the orexigenic NPY and AgRP neurons (Baggio & Drucker, 2014). The GLP-1 receptors in the brain help to control homeostasis by regulating food intake, and since GLP-1 is an incretin, stimulation of GLP-1 receptors increases insulin release and simultaneously inhibits glucagon release (Lean & Malkova, 2016). In the gut, activation of GLP-1 receptors leads to "ileal brake," which is a reduced rate of gastric emptying and absorption of nutrients (Lean & Malkova, 2016; Perry & Wang, 2012a).

GLP-1 and Obesity

In cross-sectional studies comparing obese and normal weight individuals, (Appendix III), no differences in fasting GLP-1 are seen (Adam & Westerterp-Plantenga, 2005; Carroll et al., 2007; Kim et al., 2005; Sloth et al., 2008; Verdich et al., 2001). Obesity is associated with a diminished postprandial response of GLP-1, however, results from various studies are not always consistent (Huda et al., 2006). The majority of the studies show that obese individuals have an attenuated postprandial response in GLP-1 compared to the normal weight individuals (Adam & Westerterp-Plantenga, 2005; Carroll et al., 2007; Verdich et al., 2001). In contrast, Kim et al. (2005) did not find any difference between the postprandial response of obese and normal weight individuals.

Three studies found significant findings on diminished postprandial GLP-1 response to weight loss. Carroll and colleagues examined both obese and normal weight men and women and found obese to have a decrease in GLP-1 in the beginning of the postprandial phase from 0 to 20 minutes while the normal weight had an increase in GLP-1 in the same postprandial phase ($p = 0.02$) (Carroll et al., 2007). Although Carroll and colleagues did not find a significant difference in the overall postprandial response of

GLP-1 in obese vs. normal weight adults, the lack of statistical differences between the two groups could be due to the mixed macronutrient meal challenge used (Carroll et al., 2007). GLP-1 secretion is affected by macronutrient content so the mixing of macronutrients could result in less of a GLP-1 increase. The meal challenge used was a high carbohydrate meal replacement shake at 510 kcals. However, this is not likely a strong cause since the next two following studies also used a mixed macronutrient meals and did find a significant overall postprandial response between obese and normal weight (Adam & Westerterp-Plantenga, 2005; Verdich et al., 2001). In Verdich et al., a standardized sandwich meal was used for the meal challenge consisting of approximately 597 kcal. Area under the curve was assessed after a meal challenge in obese (BMI = 34.1 ± 43.8 kg/m^2) and lean (BMI = 20.4 ± 24.7 kg/m^2) male participants. The obese had a smaller area under the curve at 2955 (2656-3255) 180min x pmol/l compared to the lean with 3678 (3309-4065) 180 min x pmol/l (Verdich et al., 2001). Verdich et al. did, however, measure only males, while Carroll et al. used a sample size of both males and females. Since there was also a slight gender effect noted in Carroll et al., where men tended to have higher fasting GLP-1 concentrations than women ($p = .06$), the use of only male samples in Verdich et al. study could have given support in finding a statistical difference between groups (Carroll et al., 2007; Verdich et al., 2001). Adam and colleagues also investigated GLP-1 response after a standard breakfast in overweight and obese compared to normal weight. Overweight and obese subjects had a lower GLP-1 response than normal weight subjects at 30 minutes (4.2 pmol/l x h in overweight and obese and 6.42 pmol/l x h in normal weight; $p = .02$) (Adam & Westerterp-Plantenga, 2005). The area under the curve for the postprandial response of GLP-1 was significantly

lower in the obese and overweight than the normal weight ($p = .03$) (Adam & Westerterp-Plantenga, 2005). While Adam et al. measured both men and women similar to Carroll et al., their sample size was larger (58 vs. 39) which could have helped gain significance.

These three studies support the hypothesis that the postprandial GLP-1 response is attenuated in overweight and obese compared to normal weight (whether it be in the immediate rise or the overall secretion) (Adam & Westerterp-Plantenga, 2005; Carroll et al., 2007; Verdich et al., 2001). The results of both of these studies are however in contrast to Kim and colleagues where no significant difference was found in the response of GLP-1 after administration of an oral glucose tolerance test (OGTT) (Kim et al., 2005). This discrepancy may be due to a much lower caloric value of the OGTT challenge (300 kcals) compared to the meal challenge used by others (500-650 kcals).

GLP-1 and Weight Loss

Appendix IV represents studies that examine the response in fasting and postprandial GLP-1 to dietary restriction weight loss interventions. When undergoing a calorie restricting diet, fasting and postprandial GLP-1 levels decrease in the body making weight loss and weight maintenance even more challenging (Huda et al., 2006; Lean & Malkova, 2016). Two weight loss studies in particular show this reduction in GLP-1 with weight loss (Adam et al., 2005; Sumithran et al., 2011a), while a third shows the opposite, specifically with an increase in GLP-1 postprandial response (Verdich et al., 2001).

Two studies supported a general decrease in GLP-1 with accompanied weight loss. For fasting GLP-1 reductions with weight loss, both studies supported this overall reduction in fasting GLP-1. Sumithran et al. examined fasting levels of active GLP-1

during a very low-calorie diet of 500 kcal/day for 10 weeks, followed by a 52 week weight maintenance phase. Fasting active mean GLP-1 levels did not significantly change at week 10 from week 0, however, at week 62 fasting mean GLP-1 levels were significantly reduced compared to week 0 ($p = 0.005$) (Sumithran et al., 2011a). While surprising that the fasting GLP-1 levels were not significantly different at week 10 from week 0, the decrease in fasting GLP-1 levels in the body at week 62 could represent a significant roadblock in the desired goal of weight loss. This study supports the notion that after caloric restriction, fasted active GLP-1 secretion is attenuated up to 12 months, which can have the potential to influence weight maintenance negatively. The second supporting study by Adam and colleagues (2005) reported similar findings for GLP-1 responses after weight loss in a reduction in fasting GLP-1. This study examined GLP-1 response after 6 weeks of a very low-calorie meal replacement diet. In support of the previous study, the fasting GLP-1 levels reduced at week 6 compared to week 0; however, these results only approached significance ($P = 0.07$). If the intervention were longer like Sumithran et al. study, perhaps more significance could be found (Adam et al., 2005).

For postprandial changes accompanied with weight loss, the studies are conflicting. Sumithran et al.'s study did not find any differences in postprandial responses of active GLP-1 at either time point (0, 10, 62). As stated earlier when examining ghrelin, the lack of significant weight loss could indicate as to why all postprandial responses were similar at each time point. In contrast to Sumithran et al., but in support of the overall literature, the postprandial GLP-1 levels observed in Adam et al. significantly dropped below week 0 at week 6 by showing a reduced area under the

curve of GLP-1 secretion at every time point ($P < .05$) (Adam et al., 2005). This study supports the overall hypothesis that after a short-term weight loss period and when the body is in negative energy balance, there is an attenuated GLP-1 postprandial response. A reason as to why Adam et al. found stronger results at the postprandial responses could be due to the timespan the second time point was measured, since 6 weeks is a general time frame for the completion of the first phase of weight loss and the body is in a greater change in body composition and energy expenditure (Heymsfield et al., 2011). Finally, Verdich et al. (2001) contradicted the previous studies indicating the GLP-1 secretion is increased with weight loss. The researchers examined obese (BMI = 34.1 ± 43.8 kg/m^2) individuals on a 6 month weight reduction program and found the GLP-1 postprandial response significantly increased from an area under the curve of 2955 ± (2656-3255) pmol/l to an area under the curve of 3228 ± (2934-3521) pmol/l ($p<.05$) (Verdich et al., 2001). This study is unique from the other two studies, however, due to the long-term intervention (6 months vs. 6-10 weeks), an included weight maintenance phase, as well as only a moderately restricted diet (1000 kcal/day vs. 500-607 kcal/day) these studies are not easily comparable. More studies should examine weight loss and GLP-1 secretion that can be comparable, therefore measurements should be taken both immediately after energy restriction as well as after a weight maintenance period to better understand GLP-1 secretion with weight loss.

Leptin

In 1994 a circulating hormone called leptin was discovered which was found critical in maintaining energy homeostasis in the body (Lean & Malkova, 2016). Leptin is a long-term signaling hormone in the body that is released from the white adipose

tissue adipocytes and circulates to the ARC of the hypothalamus. Leptin activates leptin receptors found in anorexigenic hypothalamic POMC neurons and also inhibits orexigenic NPY/AgRP neurons to induce satiety (Perry & Wang, 2012b). Ultimately, leptin communicates the fat storage levels in the body to the hypothalamus; therefore serum concentrations of leptin are directly related to fat stores in the body. Leptin is not altered acutely after meal consumption like that of GLP-1 and ghrelin, due to leptin being a more long-term signal (Schwartz et al., 1999). The primary functions of leptin are reduced food intake and increased energy expenditure (Lean & Malkova, 2016). In the absence of leptin, the body has few fat stores, and this absence of leptin will induce hunger for the body to increase its fat stores (Lean & Malkova, 2016).

Leptin and Obesity

After the discovery of the satiety effects of leptin, exogenous administration of leptin was initiated in the obese as a hopeful method of treatment (K. G. Murphy & Bloom, 2004; Perry & Wang, 2012a). Exogenous leptin given both peripherally and centrally have been seen to decrease appetite and increase energy expenditure in rodents (Perry & Wang, 2012a). Leptin administration has also been seen to suppress appetite in the ob/ob mice, which are deficient in the hormone, and in children who have a genetic leptin deficiency (K. G. Murphy & Bloom, 2004). In rare instances, some obese adults have low levels of leptin despite excessive body fat; these adults benefited from long-term leptin replacement therapy by significantly reducing their body weight (Lean & Malkova, 2016). Leptin deficiency is associated with rapid early onset obesity. However, most obese adults do not have leptin deficiency, but instead leptin resistance (Lean & Malkova, 2016). One important finding was that exogenous administration of

leptin to leptin resistant obese adults did not suppress appetite (Hopkins & Blundell, 2016). This resistance is thought to occur due to constant overstimulation of leptin receptors which activated a negative feedback loop ultimately blocking the desired anorexigenic signals from leptin (Perry & Wang, 2012b). Another proposed mechanism of leptin deficiency is the lack of transport of leptin across the blood brain barrier (BBB) seen in studies with obese rodents (Perry & Wang, 2012b). Leptin has a strong effect on inhibiting appetite when there are lower amounts of adiposity in the body, but leptin is quite inefficient with a vast amount of adiposity (Hopkins & Blundell, 2016). Leptin resistance is then directly proportional to the amount of excess body fat (Hopkins & Blundell, 2016).

Appendix V includes studies comparing obese and non-obese leptin concentrations. The following studies support obese individuals having higher leptin concentrations than their leaner counterparts (Carlson et al., 2009; Carroll et al., 2007; English et al., 2002; Monti et al., 2006; Morpurgo et al., 2003). Two studies examined the relationship between leptin levels and BMI and found there to be a strong correlation (English et al., 2002; Monti et al., 2006). Both Monti and colleagues and English and colleagues found leptin to be correlated with BMI in men and women ($r = .72$; $p = .001$; $r = .79$; $p < .0001$). Carlson et al. (2009) examined the baseline values of leptin as well as the postprandial response of leptin in severely obese adults (BMI = 40–49 kg/m^2) and compared it to normal weight adults (BMI = 21–24 kg/m^2). The baseline leptin values were significantly higher in the severely obese group (48.1 ± 16.2 ng/ml) as compared to the normal weight group (10.1 ± 4.5 ng/ml). Although most studies do not see postprandial responses with leptin, this study reported that the postprandial response of

leptin in the severely obese contrasted with the postprandial response of the normal weight. In over a two hour period, leptin seemed to decrease gradually in the severely obese while leptin levels in the normal weight group remained relatively constant (Carlson et al., 2009).

Another study by Carroll et al. examined the same baseline and postprandial leptin levels in obese (BMI = > 30 kg/m^2) and normal weight adults (BMI < 25 kg/m^2) (Carroll et al., 2007). Their results in fasting baseline leptin levels were similar to Carlson et al. in that the obese group had significantly higher fasting leptin as compared to the normal weight group. They found the obese women to have a fasting leptin level of 26.1 ± 2.5 ng/ml and the obese men to have a fasting leptin level of 18.1 ± 1.4 ng/ml compared to the normal weight women with 10.8 ± 1.2 ng/ml and the normal weight men with 4.4 ± 1.0 ng/ml. One unique finding of this study was that there was a gender and BMI group interaction on fasting leptin concentrations. Obese women had raw data 142% higher than the normal weight women, while obese men had raw data 311% higher than the normal weight men ($p = .01$) (Carroll et al., 2007).

While both studies support that there is significantly higher fasting leptin concentrations in obese compared to normal weight individuals, the results in the postprandial response were conflicting. Carroll et al. (2007) supported the lack of acute change in leptin after a meal challenge for either group. However, Carlson et al. (2009) did find a significant postprandial change in leptin in the SO group. One hypothesis as to why this occurred could be that Carlson et al. (2009) included a much higher BMI range and therefore could see that with more fat stores there is more leptin deficiency; therefore the difference in leptin patterns would be increasingly different.

Leptin and Weight Loss

As stated earlier, there is a direct relationship between BMI and leptin; therefore when an individual undergoes either a dietary-induced or exercise-induced weight loss, there is an associated decrease in fasting leptin concentrations (Hopkins & Blundell, 2016). The literature on leptin in weight loss (Appendix VI) are in agreement due to this strong association (Crujeiras et al., 2010; Morpurgo et al., 2003; Sumithran et al., 2011a).

The following two studies report decreased fasting leptin with concurrent weight loss. Sumithran et al. (2011) conducted a study where overweight or obese men and women underwent a 10 week very low-calorie diet with the goal of losing 10% of their bodyweight at baseline while periodically getting their hormonal adaptations to weight loss measured. During the weight loss period, leptin levels dropped below baseline by 64.5 ± 3.4%. Leptin levels rose at the completion of the study at week 62 but were still 35.5 ± 4.7% below baseline levels. The researchers concluded that the leptin adaptations to weight loss remain below baseline up to 12 months which may make weight maintenance increasingly difficult (Sumithran et al., 2011b). Crujeiras et al. also examined leptin levels after the 8 week hypocaloric diet and found plasma leptin levels to significantly decrease: levels prior to the diet were at 22.5 ± 14.7 ng/ml and after the diet were 14.9 ± 14.5 ng/ml ($p < 0.001$) (Crujeiras et al., 2010). The researchers also reported that there were greater reductions of leptin levels in those who lost at least 5% of their body weight (-51 ± 17 ng/ml) than those who lost < 5% body weight (-28 ± 26 ng/ml; $p < 0.001$) (Crujeiras et al., 2010). The researchers included a follow-up period (32 weeks) and reported the change in leptin levels during the 8 week weight loss intervention to determine if change in leptin would predict weight regain later on. They found that those

who's drop in leptin after the 8 week diet was below the median had an 86% lower risk of weight regain and were more successful in weight loss maintenance (OR = 0.141; CI 0.44-0.454; p = 0.001) (Crujeiras et al., 2010).

Both of these studies support the notion that there is a significant decrease in leptin concentrations in overweight an obese individuals when undergoing diet-induced weight loss. Crujeiras et al. (2010) did also support the Carroll et al. (2007) study in that there is a gender associated response with leptin, finding women leptin levels were higher in the weight regain than in the weight maintenance group in all time periods, while men showed no differences. Despite these agreements, the crucial findings of each study were seemingly contradictory. The crucial finding in Sumithran et al. (2011) was that leptin decreases remain decreased for up to one year, which then indicated that this decrease could predispose the individual to future weight regain. On the other side, the crucial finding of Crujeiras reported that when there is low leptin after weight loss, there is less of a risk of weight regain. Their seemingly contradictory findings proposed that there is some sensitivity to the leptin signal either in centrally or peripherally which means that the leptin concentrations will not have to reach such high levels. It is postulated from Crujeiras study that those who had high levels of leptin were predisposed to weight regain due to resistance to of the hormonal signal, while those with low leptin levels were able to maintain low levels since there was no hormonal resistance. Either way, both studies indicate that there is a physiological alteration in leptin which can lead to difficult weight maintenance.

In summary, ghrelin, GLP-1, and leptin have physiological differences in obese vs. normal weight individuals through the absolute levels as well as the responses after a

meal (Table I). Weight loss also induces changes in these hormones that have the potential to make weight maintenance increasingly difficult. Ghrelin levels are lower in the obese than normal weight individuals but will increase with weight loss, making hunger more prominent. In response to a meal, obese individuals have less reduction in ghrelin than normal weight individuals. Fasting GLP-1 levels are similar in obese and normal weight individuals, however, with weight loss, these levels will decrease, making an environment of less satiety. The literature is conflicting in GLP-1 response to a meal; however, there is a clear different time course in GLP-1 response in obese vs. normal weight individuals, with the majority of literature reporting a diminished response in GLP-1 after weight loss. Leptin levels are higher in obese vs. normal weight individuals and will decrease with weight loss, possible reducing satiety. Leptin generally does not respond to a meal.

Table I: SUMMARY OF HORMONE LEVELS IN OBESITY AND HORMONE RESPONSES TO WEIGHT LOSS

Hormone	Origin and Target	Physiological Effect	Hormone Levels in Obese vs. Normal Weight (NW)	Obese Levels after Weight Loss/during Caloric Restriction
Leptin	Produced from white adipocytes; circulates to ARC to regulate appetite/energy expenditure	↑ satiety ↑ EE	Obese: Fast: High PP: No change NW: Fast: Low PP: No Change	Obese: Fast: ↓ PP: No change
Glucagon-Like Peptide 1	Produced in L cells of GI Tract; circulates to ARC to regulate appetite	↑ satiety	Obese: Fast: Low/Same PP: Weakened ↑ NW: Fast: High PP: ↑	Obese: Fast: ↓ PP: More of a Weakened ↑
Ghrelin	Produced in ghrelin cells in stomach; circulates to ARC to regulate appetite/energy expenditure	↑ hunger ↓ EE	Obese: Fast: Low PP: Weakened ↓ NW: Fast: High PP: ↓	Obese: Fast: ↑ PP: More of a Weakened ↓

EE = Energy Expenditure; Fast = Fasting state; PP = Postprandial state; NW = Normal Weight; ↑ = Increase; ↓= Decrease; Weakened ↑ = Weakened Increase; Weakened ↓ = Weakened Decrease

Tapering Caloric Restriction

It is not a new theory that the human body has a tendency to remain in a homeostatic state. This is apparent with energy metabolism and is seen clearly in the body's response to overfeeding and underfeeding (Müller et al., 2010). Konarzewski and Diamond (1994) found that in mice when the environmental temperature is reduced, mice reduce their body mass to a critical point. The mice subsequently increased their caloric intake, which eventually helped to maintain their weight (Konarzewski & Diamond, 1994). The idea of gradually reducing caloric intake is based on the idea that the human body works better in a homeostatic environment. Even though the body can adapt to extreme conditions, one could hypothesize that abrupt adaptations would do more damage to the body than putting the body through gradual changes where any adaptation the body must make would be gradual and much smaller. This concept of gradually increasing a stimulus is seen in many forms of treatments. For example, in the treatment of hypertension, blood pressure medications are given at a low dose and slowly increased until a desired blood pressure is achieved (Kessler & Joudeh, 2010). If severe hypertension is dropped abruptly, hypotension can occur and the brain would suffer from lack of adequate perfusion resulting in ischemia and infarction (Kessler & Joudeh, 2010). The reverse is seen in treatments for addictions to nicotine and alcohol, where there is a gradual decrease of the stimulus so as to counter dangerous side effects when taking away the stimulus abruptly (Stead et al., 2012; Stehman & Mycyk, 2013). Gradual

increases in physical activity are even seen as a treatment to sedentary lifestyles as well as for training purposes. One would not expect an unfit individual to be able to perform in a high endurance race the very next day, however this has never been the case with dieting. Traditional dieting includes abrupt drops in caloric intake that can result in very low calorie diets of 500 kcal/day to low calorie diets of 1300 kcal/day (Crujeiras et al., 2010; Sumithran et al., 2011b). The aim of this paper is to look at the pattern of hormonal response in hunger hormone ghrelin and satiety hormones leptin and GLP-1 to a traditional drop in calories vs. a gradual taper in reduction of calories.

Summary

In response to a negative energy balance, the body uses biological defenses such as increasing oregixenic hormones like ghrelin and suppressing anorexigenic hormones like GLP-1 and leptin to promote an energy storage environment. These biological defenses make weight maintenance increasingly difficult when attempting to remain at a reduced weight. Understanding the underlying mechanisms of hormonal and metabolic adaptation involved with energy regulation could hopefully lead to a safe and practical anti-obesity treatment. If a treatment is found, it would need to be a versatile method of inhibiting the orexigenic response of ghrelin while also enhancing the anorexigenic response of leptin, GLP-1 and many more anorexigenic hormones, and thus promoting a state of energy dissipation rather than energy storage (Perry & Wang, 2012b). Since reduced weight individuals experience an increased biological defense of the body to regain body weight, a treatment that promotes energy dissipation could be especially useful after or during short-term weight loss. Clinically, many health professionals still refer to obesity as a simple consequence of too much energy intake with concurrent

reduced energy expenditure, putting the body in a positive energy state (Hopkins & Blundell, 2016).

Specific Aims

Therefore, the specific aims of this study were to examine the levels of GLP-1, leptin and ghrelin both in a fasted state and a postprandial state in response to a gradually decreased caloric restricted diet compared to the traditional abrupt drop in caloric energy intake. This included obtaining effect size for the response of these hormones to the interventions. Secondary aims were to examine the relationships between hormones and measures of body fatness.

Hypotheses

Based on the more gradual approach to calorie restriction, it is hypothesized that after 6 weeks of intervention, the tapered caloric intake group as compared to the traditional abrupt drop in caloric intake group, will have a dampened response in ghrelin (both fasting and in response to a meal), an increase in GLP-1 (both fasting and in response to a meal), and dampened lower leptin (fasting only). It was also hypothesized that leptin would be positively correlated, and ghrelin would be negatively correlated to body fatness.

METHODOLOGY

The dataset for this project was obtained from, the TREND Study: Tapered Reduction in Energy as a Novel approach to Dieting (IRB Number: IRB00037795). TREND was a two arm, prospective randomized trial with aims of comparing the impact of a dietary caloric taper versus a traditional abrupt dietary caloric decrease on ghrelin, leptin and GLP-1, body composition, weight loss, and resting energy expenditure at 6, 24, and 52 weeks after diet initiation. The current thesis project analyzed select data obtained at baseline and during the initial 6 weeks of the TREND study. TREND, including data for the current analysis was conducted in the Clinical Research Unit (CRU) of the Wake Forest University Baptist Medical Center (WFUBMC) located in Winston-Salem, NC.

Recruitment

Participants were recruited through flyers, internet, and Wake Forest University Baptist Medical Center (WFUBMC) intranet. Flyers were hung up around the WFUBMC hospital with a brief description of the study as well as the study email account (trendstudy@wakehealth.edu). The research study was listed on a clinical research website "Be Involved" under WFUBMC where interested applicants could request to participate. This website was also accessed through the employee-facing intranet under the "Research and Education Tab." The online request to participate was sent to the study email account and the participants were contacted by a study staff member to complete a 20-30 minute phone screening process to ensure eligibility. See Table II for the inclusion and exclusion criteria.

Table II: INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria	Exclusion Criteria
Age 18-45 years	Already participating in another weight loss program or research study
BMI 30-40 kg/m^2	History of bariatric surgery (or currently planning)
Currently engaging in ≤ 60 minutes of exercise per week	History of eating disorders
Interested in weight loss	History of cancer (other than non-melanoma skin CA)
	History of thyroid disease
	History of severe mental illness
	Diabetes
	Pregnant, breastfeeding, planning pregnancy
	Peri- or post-menopausal women
	Any medical contraindication to exercise
	Food allergies
	Night shift worker (> 2 nights per week)
	Planning prolonged travel in next 6-8 months
	Resting metabolic rate < 1000 kcal/day for women and < 1200 kcal/day for men

Informed Consent

The informed consent for TREND was a two part process. Participants were first screened over the telephone and asked for self-reported data as well as verbally consenting for their medical chart to be reviewed by the medical director. If the participants had a Wake One record with WFUBMC the record was reviewed by the principal investigator to verify inclusion/exclusion criteria. If the participant did not have a Wake One record they were asked to bring in pertinent medical information to the baseline visit. The second part of the informed consent was in written form at the baseline visit. The participants were walked through the informed consent document face-to-face in detail by a study staff member and asked to sign indicating their understanding of the study. This study was approved by the Wake Forest University School of Medicine Institutional Review Board.

Study Design

The current project was a randomized controlled trial with participants randomized to one of two dietary programs.

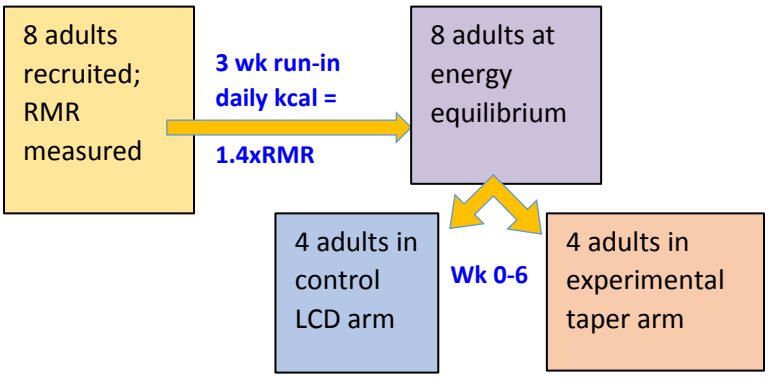
Baseline Visit

After consent was established at this baseline visit and participants met all inclusion criteria, the participants completed multiple tests including height, weight, resting metabolic rate, vitals (blood pressure and heart rate), fasted safety bloodwork, surveys, as well as placing a food order. An explanation of each measure is listed in a section below.

Run-In Period

Following the baseline visit, participants returned to the CRU one week later to start a three week run in period. During this three-week period, participants were given all their meals and snacks at a calorie level specific to their body's needs based off the measured RMR value x 1.4 activity factor for light physical activity. The purpose of the run-in period was to achieve weight stability / energy-balanced state prior to the initiation of the study and to provide a basis for determining adherence to the dietary procedure of picking-up and consuming prepared meals that would occur once the study began. Participants had to maintain their body weight during the run-in period within 2% above or below their initial weight prior to run-in in order to be eligible to move on to the main study. See Figure 1 for the schematic of the study design.

Fig 1: SCHEMATIC OF STUDY DESIGN – INITIAL 6 WEEKS OF FEEDING STUDY OVERVIEW



Randomization

At the beginning of the third week of the run in period the participants were randomized into either the low calorie diet arm (LCD) or the taper arm upon confirmation that the body weight did not drop or increase more that 2% from their initial body weight

prior to run-in. The randomization was stratified by gender using an online randomization tool in RedCap®.

Interventions

The 6 week intervention period started immediately after the three week run-in. Once participants were randomized into either of the two arms they were instructed to follow the specific guidelines of the appropriate study arm. The two diet arms only differed in the pattern of calorie intake during the first 6 weeks of the weight loss period of the study. Although not part of the current analysis, in the overall TREND study, both interventions were identical in their dietary intake patterns starting at week 6. They each maintained the low calorie limit of 1200 kcal for men and 1000 kcal for women from weeks 6-24. A detailed description of each arm is listed below.

Both groups were given a binder with education material provided by OPTIFAST on physical activity recommendations. The recommendations for a reduction in chronic diseases were at least 30 minutes of physical activity on most days of the week. An additional 30-60 minutes of physical activity was recommended for prevention of excess weight regain and maintenance of weight loss. This accumulation was recommended to be accrued in at least 10 minute increments throughout the day. Both weight loss diets were based off traditional clinic diets using OPTIFAST products.

Low Calorie Diet (LCD) Arm

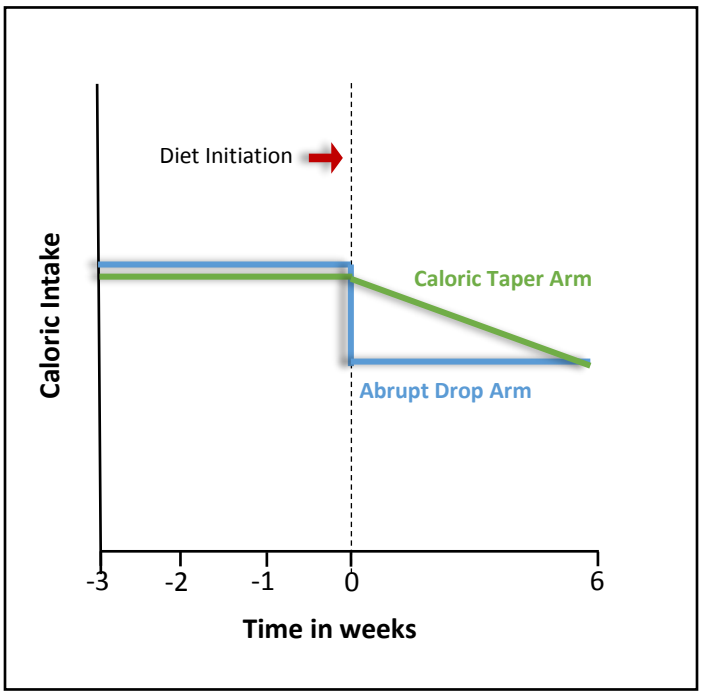
The LCD group reduced caloric intake abruptly from the RMR based caloric daily value used in run-in to 1200 kcal/day for men or 1000 kcal/day for women, i.e. from the last day of the run-in to the first day of the energy restricted diet. The LCD group

continued at this low caloric limit for the full 6 week period. All the food and meal replacements, which the participants picked up twice a week, were provided by the CRU. The daily diet for this arm of the study consisted of 5 OPTIFAST meal replacement shakes and 1 Lean Cuisine dinner. Women had a 200 kcal Lean Cuisine dinner while men had a 400 kcal Lean Cuisine dinner to make up for the extra 200 kcals the men needed. See Figure 2 for a visual representation of the two study arms.

Taper Arm

The taper group differed in the time it took to achieve the final calorie level goal (1200 calories for men and 1000 calories for women). The rate of caloric reduction was calculated by finding the difference between the measured RMR based caloric daily level and the low calorie level of 1000 kcal/day for women or 1200 kcal/day for men. This difference was then divided by 6 and distributed evenly across the 6 weeks to gradually decrease the caloric level to 1000 kcal/day for women or 1200 kcal/day for men at the end of week 6. For example, if a male participant was prescribed 2000 kcal per day during the run-in period for weight maintenance, and his goal daily energy intake for weight loss is 1200 kcal, the difference would be 800 kcal. This man would gradually decrease his daily caloric intake by ~130 kcal (=800/6) per week, over 6 weeks, in order to reach that 1200 kcal/day goal. The food content of this diet each day consisted of a variety of OPTIFAST meal replacement shakes and Lean Cuisine dinners, as well as CRU prepared snacks and lunches to make up for the additional calories granted during the taper period. See Figure 2 for a visual representation of the two study arms.

Fig 2: TWO STUDY ARMS



Meal Challenge

At the week 0 and week 6 visits, participants were instructed to arrive at the CRU in the morning between 7:00AM and10:00AM fasted, i.e. having no food or drink except water for at least 6 hours prior to the visit. Participants were asked to indicate how hungry they were on a visual analog scale from extremely hungry/starving to somewhat hungry to not at all hungry (corresponding to 0-100 score) prior to the administration of the gut hormone shake. The CRU nursing staff obtained heart rate and blood pressure followed by a blood draw. Immediately after the blood draw the participant had a meal challenge and was given a shake made by the metabolic kitchen in the CRU. They were instructed to finish the shake within 15 minutes. This shake consisted of 41 g of evaporated milk, 9 g of canola oil, 25 g of SolCarb, 130 g of bottled water, and 44 g of EAS 100% vanilla whey protein. The caloric content of the shake was 405 kcals. The

time was noted once the participant finished the shake and exactly one hour after the shake was consumed the nursing staff took another blood draw.

Measures

Height

Height was measured at the baseline visit only, using the 235A model of the Heightronic digital stadiometer by QuickMedical. This stadiometer was calibrated and documented daily by the CRU staff using a meter stick. The participant was measured wearing light socks or in bare feet with his/her back to the stadiometer. Their hands hung freely with palms facing the thighs. Weight was placed evenly on both feet and the head was positioned in the Frankfort Horizontal Plane, thus the participant was centered with the heels, buttocks, scapulae, and posterior aspect of the cranium placed in one vertical plane and at the same level of the left tragion (the deepest point in the notch superior to the tragus of the auricle) and parallel to the floor. The participant was asked to inhale and hold their breath while maintaining a fully erect positon without altering the load on the heels while the measurement was made by moving the headpiece until it rested on the top of the head.

Weight

Weight was recorded at baseline, two times per week during the run in period, and once per week during the main study. Weight was obtained by the CRU nursing staff using a Cardinal Detecto 758C digital scale. This scale was calibrated daily by the CRU staff with the use of 25 pound weights, as well as yearly by hospital clinical engineering.

Participants had their weight measured wearing only light clothing and no heavy items in their pockets. The scale was first zeroed out (tared) before the participant stepped on it.

Body Mass Index (BMI)

BMI was determined by using the equation of weight in kg divided by height in meters squared. The BMI had to be within 30-39.9 kg/m^2 in order to be eligible for the study.

Body Composition

Total body fat (kg and percent total body mass) was measured at week 0 using a GE enCORE-based X-ray Bone Densitometer Lunar iDXA machine (Madison, WI) at Wake Forest University. Participants were instructed not to wear metal and be still while lying down for the full body scan.

Resting Metabolic Rate (RMR)

Once BMI was confirmed to be in the appropriate range, participants had their resting metabolic rate (RMR) measured using indirect calorimetry. This test was conducted in the CRU in the WFUBMC by the nutrition staff. The metabolic cart used was a MedGraphics ULTIMA series by MGC Diagnostics. This cart was calibrated before each test through gas and flow calibration. Prior to the test the CRU nursing staff confirmed that the participant had fasted for at least 6 hours, had taken no stimulants for the past 12 hours, and had done no vigorous physical activity 24 hours before the test. The participant was instructed to lie down in a dark room under a heated blanket and rest with soothing music for 30 minutes with clear guidance on not to fall asleep. Once the participant was in a rested state, the nutrition staff started the RMR test by placing the

tube in a tight seal against the participant's mouth and plugging their nose. The participant was instructed to relax while breathing in and out of the tube and not to remove the tube or nosepiece. The test took around 30 minutes to complete per participant. The study staff then confirmed that the RMR for women was > 1000 kcal or the RMR for men was > 1200 kcal in order for the participant to be eligible to continue.

Safety blood draws

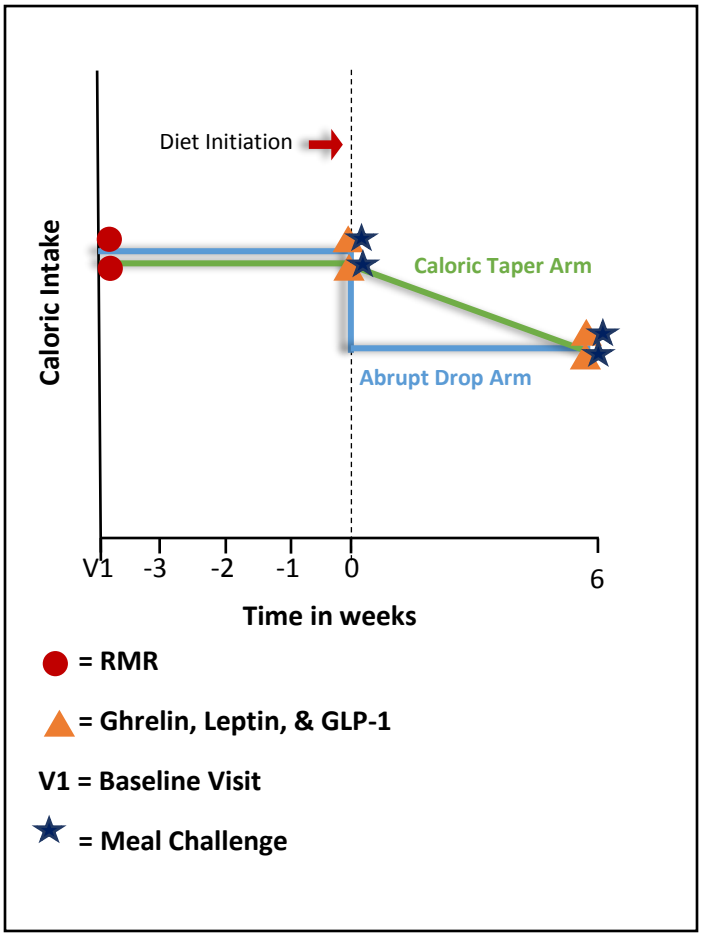
All fasted blood draw visits were performed at the CRU in the mornings between 7:00AM-10:00AM. Vital signs (heart rate, blood pressure) were taken prior to the any fasting blood draws. If the heart rate was > 110 or < 55 or the blood pressure was > 150/90 or < 110/70 the medical director was notified before continuing with the blood draw. At the baseline visit, a complete metabolic panel (CMP), complete blood count (CBC), and a Hemoglobin A1c test was performed for each participant to confirm individuals were in reasonably good health and did not have metabolic conditions targeted in the exclusion criteria. If participants did not have any thyroid stimulating hormone (TSH) bloodwork performed in their medical history, a TSH test was also added at the baseline visit. The blood work was reviewed by the medical director and discussed with the participant if any abnormally high or low levels were found. Additional CMP & CBC tests could be performed at any time during the study at the medical director's discretion.

Blood draws for hormone analysis

Vitals and fasted blood draws were taken at baseline, week 0, week 1, and week 6 of the larger 1-year study. During weeks 0, 1, and 6 ghrelin, leptin, and GLP-1 tests were

assessed from the blood samples. At week 0 and week 6 a meal challenge was initiated and hormones ghrelin and GLP-1 were analyzed one hour after the meal was consumed. A detailed description about the meal challenge procedure is listed above. See Figure 3 for a visual representation of the collected measures taken during the study.

Fig 3: TIMELINE OF COLLECTED MEASURES



Collection, storage, and analysis of ghrelin, leptin, and GLP-1 samples

All blood draws were obtained by a trained and certified phlebotomist in the CRU in the mornings between 7:00 and 10:00AM. Whole blood samples were collected in 1 x 10 mL K3 EDTA treated tubes for plasma and Vacutainer® serum tubes or non-specific binding (NSB) tubes for serum. After collection, the tubes were gently inverted 8-10

times and immediately placed on ice. One mL of whole blood was removed and transferred to 2 mL Sarstedt cryovial. 10uL of AEBSF was added to the 1 mL of whole blood and mixed by gentle inversion 8-10 times. Centrifugation was immediately carried out at 12,500 rpm x 60 seconds. 100uL K3 EDTA plasma was transferred to 2 x .5 mL Sarstedt vials. To each of these two vials, 5 uL of 1N HCL was added. Plasma was stored at – 70 ˚C. The remaining whole blood stored on ice in the 10 mL K3 EDTA tube was centrifuged at 3000 rpm x 15 minutes at 4 ˚C. Plasma leptin aliquots were stored in 2 x 500 uL EDTA tubes. Plasma GLP-1 aliquots were stored in 2 x 250 uL EDTA tubes. All blood samples were analyzed by the Biomarker Analytical Core at WFUBMC. A description of the analysis of each hormone is listed below.

Ghrelin

Fasting and postprandial concentrations of total ghrelin were determined with an ELSA 96-Well Plate EZGRT-89K, EZGRT-90BK radioimmunoassay kit (St. Charles, MO).

Leptin

Fasting concentrations of total leptin were determined by EMD Millipore human leptin radioimmunoassay HL-81HK kit (St. Charles, MO).

GLP-1

Fasting and postprandial concentrations of total GLP-1 were determined using an enzyme-linked immunosorbent assay, Thermo Scientific™ Pierce™ Human GLP 7-36a ELISA Kit (Frederick, MD).

Monitoring adherence

Participants responded to a 16 question weekly questionnaire to assess adherence to the interventions, and evaluate for possible adverse events. For the adherence of physical activity: this questionnaire asked 4 questions about the number of hours of moderate and vigorous physical activity performed each day, 2 questions on how many days/week and minutes/day of walking the participant did, and 1 question about the number of sedentary hours per week. For monitoring adherence to the diet: 2 questions were asked how often the participant ate/drank something off plan or skipped a meal. Question 1 asked: "During the past 7 days, how often did you eat or drink something that was not provided to you by the TREND study?" and Question 2 asked, "During the past 7 days, how many times did you skip a meal or snack that was provided to you by the TREND study?" The participants could respond with the following selections: "never, only once or twice, several times in the week, but not every day, once a day, and more than once a day."

STATISTICAL ANALYSIS

All data were checked for normality using Shapiro-Wilk. Concentrations of ghrelin, GLP-1, and leptin were not normally distributed and therefore were transformed into logarithm values. For ease of interpretation, the non-transformed data are given in the tables and figures, but the log transformed data were used in the analyses. Descriptive statistics (including means, standard deviations, and minimum and maximum values) were calculated for age, gender, race, weight, BMI, resting metabolic rate, and total body fat percentage. Descriptive statistics were completed for all participants in the study (n = 8) as well as for each arm of the study: taper arm (n = 4) and LCD arm (n = 4). Comparisons between groups for these variables at baseline were performed using independent T tests. Mean values (SD) were calculated at week 0 and week 6 for body weight, BMI, and fasting concentrations of ghrelin, GLP-1, and leptin. Mean values (SD) were calculated at week 0 and week 6 for both pre and postprandial (0 min to 60 minutes after a meal challenge) concentrations of ghrelin and GLP-1. The change and percent change from week 0 to week 6 were calculated and listed as mean values (SD) for the following variables: fasting ghrelin, fasting GLP-1, fasting leptin, postprandial ghrelin, postprandial GLP-1, body weight, and BMI. Group differences for these variables were determined by independent T tests. With the purpose to utilize these results for designing future, larger research trials, effect sizes were calculated and presented for the response of the intervention for these hormones. Cohen's d was calculated to indicate the effect size. This provides a standardized difference between two intervention means. The formula used for this calculation was the difference between groups for the change value from week 0 to week 6 divided by the standard deviation of the LCD group. In this

design, the LCD group corresponds to the control group. Additionally, Spearman correlations examined the relationships of the following at week 0 prior to the initiation of the intervention: fasting leptin and total body fat percentage and fasting ghrelin and total body fat percentage. Lastly, participant diet adherence was examined using Chi Square analysis from the responses to the weekly questionnaire on food eaten off plan and skipped meals. Statistical significance was set at $p < 0.05$ for all statistical tests. All analyses were performed using SPSS 24.0 (IBM Corp).

RESULTS

A total of 8 participants were consented and had completed the initial six weeks of the study by the time of this manuscript. Out of the 8 participants, 4 were randomized into the LCD study arm and 4 were in the taper arm. Baseline characteristics of all 8 participants as well as each individual study arm are shown in Table III. Body weight, BMI, and total body fat percentage at week 0 for all participants as well as individual study arms are shown in Table IV. Participants were predominantly Caucasian (87.5%), with one Hispanic individual. The mean age of the participants was 38.3 (5.1) years with a range from 30 to 40 years, body weight was 94.0 (4.3) kg (range: 85.0-98.8 kg), and the mean BMI was 33.7 (2.9) kg/m^2 (range: 30.2-37.6 kg/m^2). All participants completed every testing visit over the 6 week analyses. BMI at the start of the study was significantly higher in the LCD study arm 36.0 (1.6) kg/m^2 (range: 34.7 - 38.1 kg/m^2), compared to the taper arm 30.9 (1.7) (range: 29.4 - 33.2 kg/m^2) ($p = .004$). There were no other differences between groups in these variables.

Change and Percent Change in Body Weight

Change and percent change in body weight for the taper group compared to the LCD group from week 0 to week 6 are shown in Table IV. Taper group lost an average of 5.3 (2.2) percent body weight and LCD group lost an average of 6.1 (1.9) percent body weight. No significant differences in change and percent change in body weight between the groups were observed.

Hormone Concentrations

Fasting (ghrelin, GLP-1, and leptin) and postprandial (ghrelin and GLP-1) hormone concentrations obtained at week 0 and week 6, as well as change and percent change (week 0 – week 6) are displayed as means (SD) by group in Table V (for ghrelin), Table VI (for GLP-1), and Table VII (for leptin). No significant differences in the change or percent change from week 0 to week 6 were observed in fasting levels of ghrelin, GLP-1, and leptin between the two study arms. Effect size for the interventions was also calculated from the change between week 0 and week 6 values for each of the fasting hormone levels. The Cohen's d effect sizes for the interventions were 0.5 for ghrelin, 1.6 for GLP-1, and 0.1 for leptin.

Pre (0 minute) and postprandial (60 minute) concentrations of ghrelin (Table V) and GLP-1 (Table VI) in response to a meal challenge at week 0 and a meal challenge at week 6 are shown as means (SD). The change between week 0 and week 6 for the difference in pre and postprandial measures were calculated and presented in Figure 4 for ghrelin and Figure 5 for GLP-1. No significant differences were observed in the change between week 0 and week 6 between groups in pre-post meal challenge ghrelin or GLP-1. The Cohen's d effect sizes for the interventions on the change from week 0 to week 6 on the effect of the meal challenge was 0.7 for ghrelin and 0.7 for GLP-1.

Correlations between Leptin and Ghrelin with Total Body Fat Percentage at Week 0

The correlation between leptin and total body fat percentage achieved statistical significance ($r = 0.881$; $p = 0.004$) (Figure 6). Individuals with higher body fat had higher levels of leptin. The correlation between ghrelin and total body fat percentage did not achieve statistical significance ($r = -.262$; $p = 0.53$) (Figure 7).

Participant Diet Adherence

No statistical significance was observed between groups in diet adherence. On a weekly average 37.5% of all participants indicated that they never ate nor drank additional calories (45.8% of taper group and 29.2% of LCD group). The mean weekly average of 45.8% of all participants indicated that they never skipped a meal or snack that was provided (50.0% of taper group and 41.7% of LCD group) (Table VIII).

Tables and Figures

Table III: BASELINE PARTICIPANT CHARACTERISTICS

Characteristics	Total (n = 8)	Taper Arm (n = 4)	LCD (n = 4)
Gender:			
Female	6	3	3
Male	2	1	1
Race:			
Non-Hispanic White	7	3	4
Hispanic	1	1	0
Age (Years)	38.3 (5.1)	35.8 (5.1)	40.8 (4.3)
	30-45	30-42	35-45
Baseline Resting	1525 (190)	1602 (142)	1448 (220)
Metabolic Rate	1278-1797	1457-1797	1278-1768
(kcals)			
Total Body Fat	43.0 (5.5)	40.1 (4.0)	45.3 (5.9)
Percentage	36-53	36-44	40-53

Values for race and gender are frequency. Age, baseline resting metabolism rate, and total body fat percentage are presented as means (S.D.) and minimum and maximum.

Table IV: BODY WEIGHT AND BMI AT WEEK 0 AND WEEK 6

Variable	Taper Arm (n = 4)	LCD (n = 4)
Weight (kg) – Week 0	90.1 (4.7) 84.8-94.9	96.4 (2.2) 94.3-99.5
Weight (kg) – Week 6	85.4 (4.1) 81.1-91.0	90.5 (3.5) 87.5-95.2
Change in Body Weight (kg)	-4.8 (2.2) -8.0- -3.4	-5.8 (1.8) -8.3- -4.4
% Change in Body Weight	-5.3 (2.2) -8.6- -3.9	-6.1 (1.9) -8.6- -4.4
Body Mass Index (kg/m^2) – Week 0	30.9 (1.7)* 29.4-33.2	36.0 (1.6) 34.7-38.1
Body Mass Index (kg/m^2) – Week 6	29.3 (2.0)* 26.9-31.8	33.8 (0.8) 33.0-34.8
Change in Body Mass Index (kg/m^2)	-1.6 (0.6) -2.5- -1.2	-2.2 (0.8) -3.3- -1.6
Body Fat (%) – Week 0	40.1 (4.0) 36-44	45.3 (5.9) 40-53

Change in body weight and body mass index calculated as: (week 6 – week 0)

% change in body weight calculated as: (week 6 – week 0)/(week 0) x 100. Statistical comparisons are between groups at the corresponding variable. Values are presented as means (S.D.) and minimum and maximum. *statistically different between groups at $p < .05$

Table V: GHRELIN CONCENTRATIONS

Variable	Taper Arm (n = 4)	LCD (n = 4)
Fasting Ghrelin (pg/ml) – Week 0	856.72 (290.52)* 563.24-1227.94	472.83 (54.86) 426.47-550.86
Fasting Ghrelin (pg/ml) – Week 6	1002.28 (321.90)* 622.60-1401.43	552.86 (138.65) 366.92-698.40
Change in Fasting Ghrelin (pg/ml)	145.56 (72.74) 59.36-229.31	80.03 (143.79) -78.06- 229.40
% Change in Fasting Ghrelin	17.54 (10.16) 10.54-32.61	17.76 (31.78) -17.54- 48.91
Postprandial Ghrelin (pg/ml) – Week 0	653.23 (172.55) 407.60-805.29	398.35 (116.88) 297.14-562.22
Postprandial Ghrelin (pg/ml) – Week 6	723.90 (182.63) 459.71-878.63	489.85 (110.94) 362.12-622.11

Statistical comparisons are between groups for the corresponding variable. Change in fasting ghrelin calculated as: (week 6 – week 0). % change in fasting ghrelin calculated as: (week 6 – week 0)/(week 0) x 100. Values are presented as means (S.D.) and minimum and maximum. *statistically different between groups at $p < .05$

Table VI: GLP-1 CONCENTRATIONS

Variable	Taper Arm (n = 4)	LCD (n = 4)
Fasting GLP-1 (pg/ml) – Week 0	396.53 (117.88) 239.66-525.30	275.54 (32.63) 242.40-317.12
Fasting GLP-1 (pg/ml) – Week 6	395.69 (138.90) 273.36-578.30	333.95 (50.56) 292.07-407.43
Change in Fasting GLP-1 (pg/ml)	-.84 (164.40) -222.02- 174.79	58.42 (36.42) 7.90-90.31
% Change in Fasting GLP-1	4.39 (35.54) -42.27- 43.32	21.42 (13.15) 2.78-32.43
Postprandial GLP-1 (pg/ml) – Week 0	358.07 (106.17) 270.51-485.45	289.39 (54.04) 226.16-356.56
Postprandial GLP-1 (pg/ml) – Week 6	406.08 (89.90) 303.88-514.78	352.59 (63.11) 292.14-408.76

Statistical comparisons are between groups for the corresponding variable. No statistical significance was obtained for any of the comparisons. Change in fasting GLP-1 calculated as: (week 6 – week 0). % change in fasting GLP-1 calculated as: (week 6 – week 0)/(week 0) x 100. Values are presented as means (S.D.) and minimum and maximum.

Table VII: LEPTIN CONCENTRATIONS

Variable	Taper Arm (n = 4)	LCD (n = 4)
Fasting Leptin (ng/ml) – Week 0	41.97 (27.95) 6.06-72.35	50.78 (15.99) 33.71-69.60
Fasting Leptin (ng/ml) – Week 6	24.03 (23.11) 3.27-57.10	34.62 (6.66) 25.97-40.22
Change in Fasting Leptin (ng/ml)	-17.93 (12.66) -33.39- -2.79	-16.16 (15.06) -36.79- -2.66
% Change in Fasting Leptin	-46.40 (18.37) -63.79- -21.08	-28.10 (19.31) -52.86- -6.31

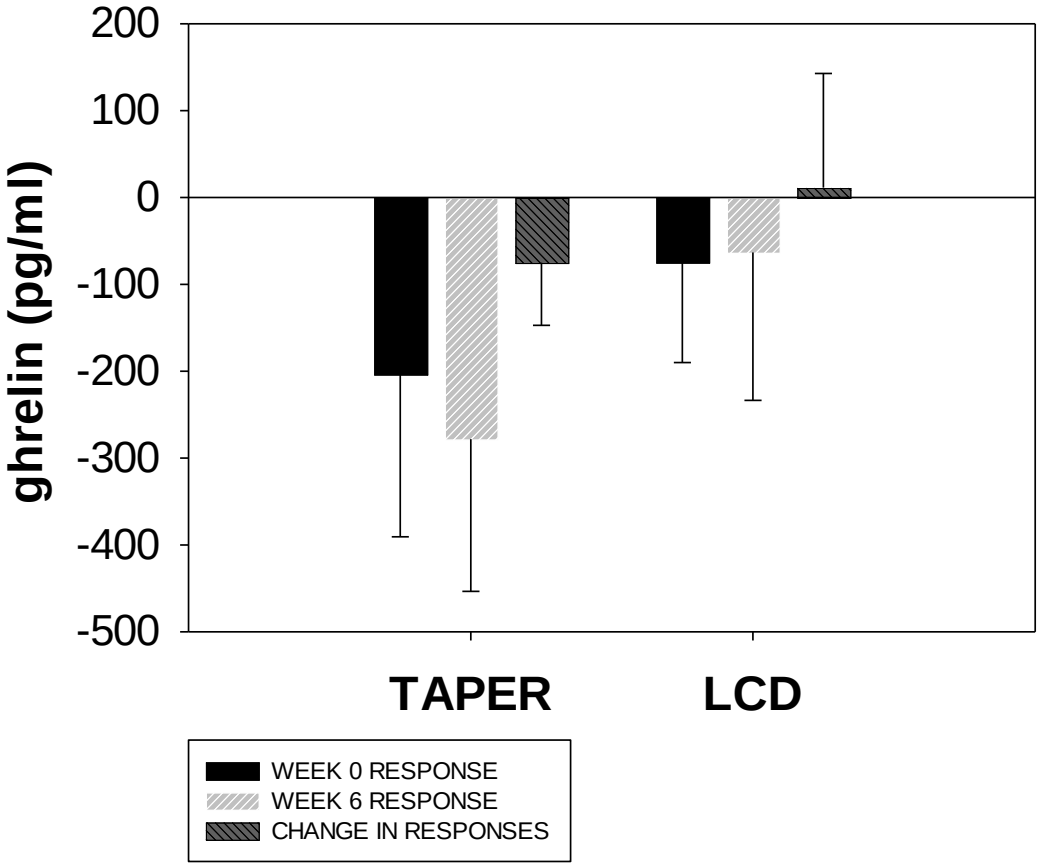
Statistical comparisons are between groups for the corresponding variable. No statistical significance was obtained for any of the comparisons. Change in fasting leptin calculated as: (week 6 – week 0). % change in fasting leptin calculated as: (week 6 – week 0)/(week 0) x 100. Values are presented as means (S.D.) and minimum and maximum.

Table VIII: PARTICIPANT DIET ADHERENCE

Question from weekly questionnaire	Study Arm	"Never"	"Only once or Twice"	"Several Times in a week"	"Once a day"	"More than once a day"
Q1	TAPER	11	10	1	2	0
	LCD	7	15	1	1	0
Q2	TAPER	12	11	1	0	0
	LCD	10	10	4	0	0

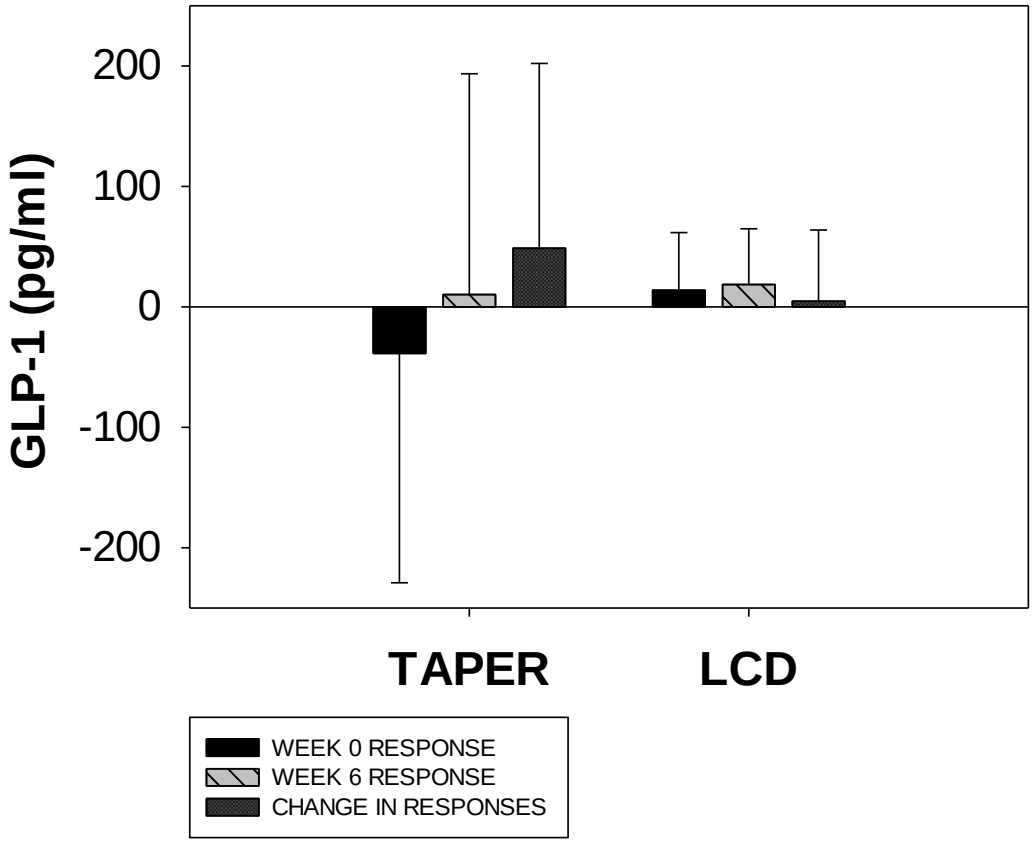
Q1: "During the past 7 days, how often did you eat or drink something that was not provided to you by the TREND study?" Q2: "During the past 7 days, how many times did you skip a meal or snack that was provided to you by the TREND study?" Values are presented as frequencies.

FIG 4: THE CHANGE IN GHRELIN RESPONSES TO A MEAL CHALLENGE FROM WEEK 0 TO WEEK 6



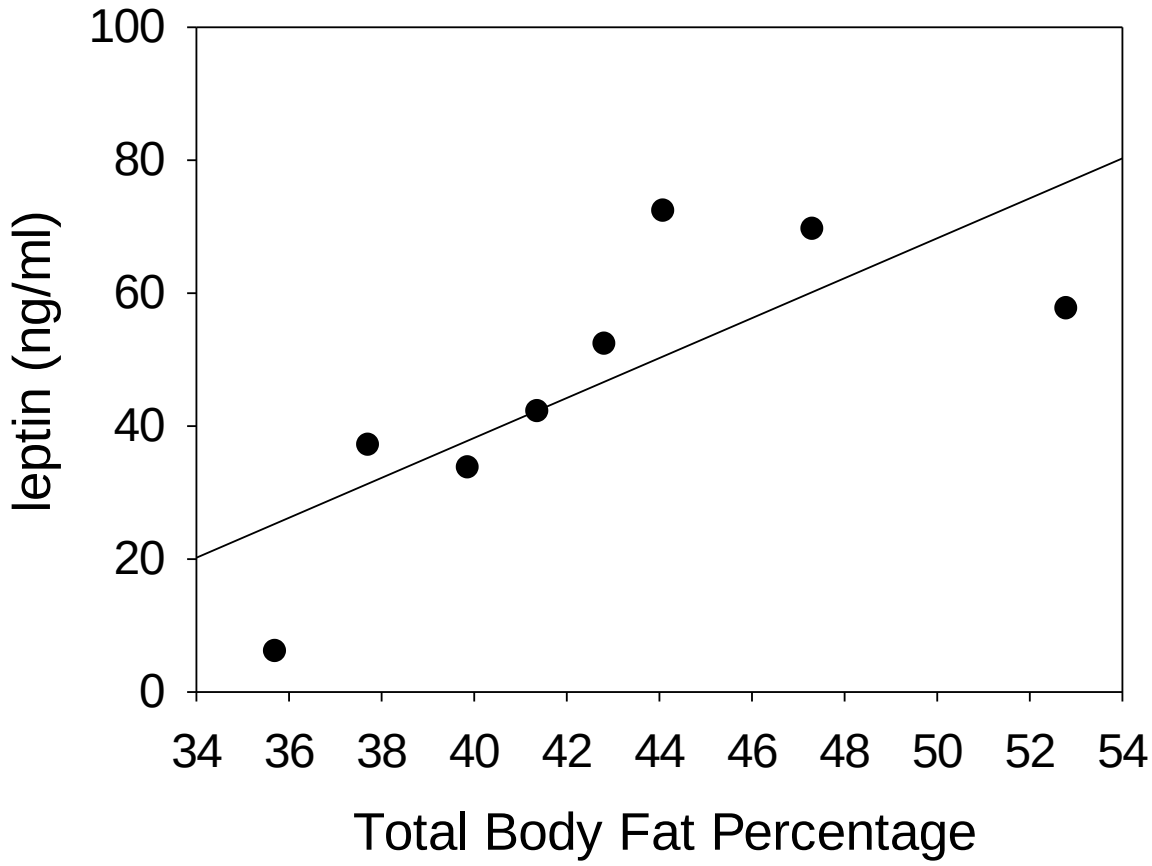
Statistical comparisons are between groups for the corresponding variable. No statistical significance was obtained for any of the comparisons. Week 0 and week 6 responses calculated as: (60 minute ghrelin levels – 0 minute ghrelin levels). Change in responses calculated as: (week 6 response – week 0 response). Values are presented as means (S.D.).

FIG 5: CHANGE IN GLP-1 RESPONSES TO A MEAL CHALLENGE FROM WEEK 0 TO WEEK 6



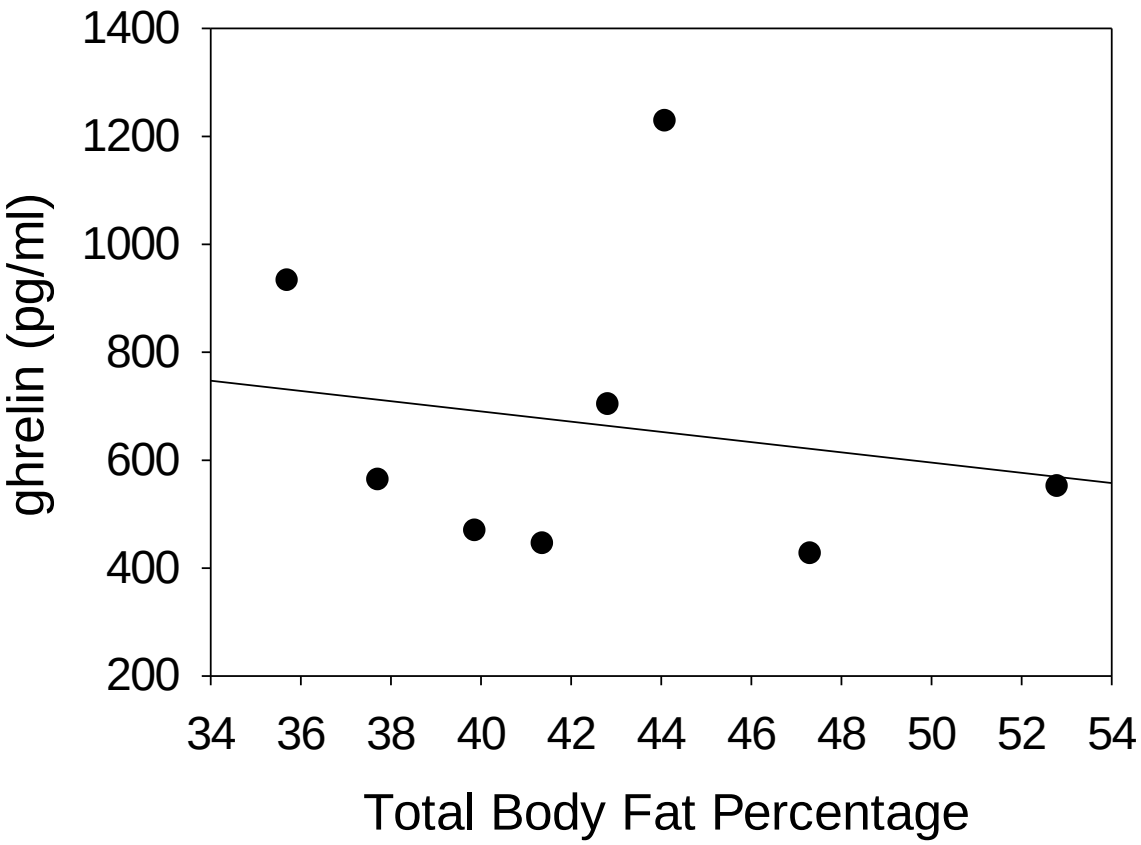
Statistical comparisons are between groups for the corresponding variable. No statistical significance was obtained for any of the comparisons. Week 0 and week 6 responses calculated as: (60 minute ghrelin levels – 0 minute ghrelin levels). Change in responses calculated as: (week 6 response – week 0 response). Values are presented as means (S.D.).

FIG 6: CORRELATION OF FASTING LEPTIN AND TOTAL BODY FAT PERCENTAGE AT WEEK 0



For the Correlation: r = 0.88; p = .028

FIG 7: CORRELATION OF FASTING GHRELIN AND TOTAL BODY FAT PERCENTAGE AT WEEK 0



For the Correlation: r = -0.26; No statistical significance was found.

DISCUSSION

The purpose of the present study was to examine the difference in plasma levels of hormones that regulate feeding behaviors in response to either a traditional abrupt decrease in calories or a gradual tapered reduction of calories. The proposed rationale was that a gradual reduction in calories will keep the body close to homeostasis by being in less of a negative energy balance than the traditional weight loss diet. It was hypothesized that the two different diets, gradual tapered caloric deficit vs. abrupt decrease in caloric intake, will have differential effects on circulating levels of hormones linked with feeding behaviors. Specifically, in taper, less of an increase in fasting ghrelin, less of a decrease in fasting GLP-1 and fasting leptin, more of a reduction in ghrelin response to a mean, and more of an increase in GLP-1 in response to a meal was proposed for the group compared to the LCD group. Ultimately, understanding and optimizing these responses could have clinical ramifications on the success for weight loss and weight loss maintenance. Although these analyses only examined the short-term (6 weeks) effect of this dietary approach, continued observations over the long-term could lead to better understanding of these effects. Tailoring the diet to provide a more gradual reduction in calorie intake could potentially benefit long-term feeding behaviors and weight loss success. This preliminary study was unique in that it was a rigorously controlled feeding study that was the first to examine plasma levels of these hormones while in a fasted state and following a meal challenge in response to different ways of initiating a calorie restricted weight loss program.

Body Weight and BMI

As expected with calorie restriction, there was a general reduction in body weight of about 1 kg per week (~5.5 kg over the 6 weeks) for both groups. This level of weight loss is consistent with supported literature based on the intervention time, caloric restriction, and baseline BMI of participants (Adam et al., 2005; Crujeiras et al., 2010; Cummings et al., 2002; Morpurgo et al., 2003; Sumithran et al., 2011a; Verdich et al., 2001). Surprisingly, there were no significant differences in body weight or BMI changes (week 0 - week 6) between the two groups. It was hypothesized that the LCD group would have a larger reduction in body weight compared to the taper group, due to the LCD group having a larger energy deficit. While no previous studies have examined differences in initiating a calorie restricted weight loss program, this hypothesis is partly based on examining studies with severely constricted diets vs mildly restricted diets. The studies examining short-term weight loss (3-10 weeks) following a highly restricting caloric diet of 500-1000 kcals/day had greater weight loss (Adam et al., 2005; Morpurgo et al., 2003; Sloth et al., 2008; Sumithran et al., 2011a) compared to those studies with a moderate caloric restricting diet with over 1000 kcals/day (Crujeiras et al., 2010; Howard et al., 2006). Based on the differences in caloric deficits between the two groups the LCD group was expected to lose 2.7 kg more than the taper group, however the LCD group lost only 1.0 kg more than the taper group. Possible explanations for the lack of difference in weight loss between groups include: 1) differential adherence to the dietary prescription; 2) variance in exercise participation; 3) disparity in the conservation of metabolic rate (i.e. metabolic adaptation); and 4) miscalculation of relative energy deficit for taper diet prescription. This study was unable to investigate each of these possible explanations, however the feeding study design employed is considered the gold standard

for adherence in these types of studies. Individuals were also surveyed at each visit about eating off-plan. Data from self-reported compliance to the diet showed nothing statistically significant between groups, however, there was a pattern for more compliance in the taper group compared to the LCD group. Interestingly, the starting weights and BMI were different between groups. Since the LCD diet was an absolute amount (1000 and 1200 kcals per day for women and men, respectively), the relative energy imbalance would be different between groups since metabolism is largely dependent on body composition and size. With a larger sample size and complete data, should this imbalance in baseline BMI remain, it would be important to adjust for baseline BMI in analyses comparing outcomes between groups.

Earlier work has demonstrated that obesity and/or dietary induced weight loss programs affect ghrelin, GLP-1, and leptin (Crujeiras et al., 2010; Sumithran et al., 2011a; Verdich et al., 2001). Although, this was not the primary aim of the study and hypotheses were not developed for this, it was expected that both weight loss programs would: 1) increase ghrelin (both fasting and postprandial); 2) decrease GLP-1 (both fasting and postprandial); and 3) decrease leptin. This analysis specifically addressed the impact of the different dietary approaches for weight loss. Each hypothesis for the three hormones are addressed in detail below regarding the impact of the two intervention arms. The analysis for each of these is restrained by the small sample size, which limits the use of covariates to adjust the analytical model. Thus, as the purpose of this preliminary study is to power a larger clinical trial, effect sizes were calculated and discussed.

Ghrelin

It was hypothesized that the LCD group would have a greater increase in the change of fasting ghrelin levels (from week 0 – week 6) due to a greater negative energy balance created by the LCD vs. the taper intervention. Individuals in negative energy balance have increased ghrelin levels (Crujeiras et al., 2010; Cummings et al., 2002; Hooper et al., 2010; Morpurgo et al., 2003; Sumithran et al., 2011a). The studies with the most caloric restriction and the highest weight loss showed an increase in ghrelin (Cummings et al., 2002; Hooper et al., 2010; Sumithran et al., 2011a), while the mildly restricted diets show no significant increase in ghrelin (Crujeiras et al., 2010; Morpurgo et al., 2003). Thus, it was reasoned that the taper diet group would have a dampened response in ghrelin. The findings do not support this hypothesis. However since the weight loss in the two study arms were not significantly different, this could have been a factor into why the change in fasting ghrelin levels between groups was not significant. Interestingly, the two study arms also had different fasting ghrelin levels at week 0, with the taper arm being higher than the LCD arm. This may be related to the higher BMI for LCD at week 0 as the literature suggests that lower fasting ghrelin is seen in those with higher BMI (Carlson et al., 2009; Carroll et al., 2007; English et al., 2002; Monti et al., 2006; Morpurgo et al., 2003).

The meal challenge was used to examine the response of the hormones to the consumption of a set number of calories and nutrient content. It was hypothesized that the LCD group would have less of a decrease in the postprandial ghrelin response than the taper group. This proposed different response from the interventions is supported by studies examining ghrelin responses in obese individuals in a chronic positive energy balance vs. normal weight individuals in a somewhat stable energy balance (Carlson et

al., 2009; Carroll et al., 2007; English et al., 2002; Morpurgo et al., 2003). This hypothesis was not supported as statistical analysis did not show an intervention effect on the change from week 0 to week 6 for the ghrelin response to a meal.

To provide a more detailed look at the magnitude of the effect of the interventions on ghrelin responses, Cohen's d was calculated to estimate effect size. A value of 0.5 was observed for fasting ghrelin, which is considered an effect of moderate magnitude (Cohen, 1992). Similarly, the effect size for ghrelin's response to the meal was of moderate magnitude, with a value of 0.7.

GLP-1

It was hypothesized that the LCD group would have a greater reduction in fasting GLP-1 levels than the taper group. Furthermore, it was hypothesized that the LCD arm would have a less of an increase in GLP-1 levels from fasting to postprandial over the 6 week intervention period compared to the taper arm. These hypotheses were based on previous work that reported reductions in fasting GLP-1 with a higher degree of negative energy balance (500-600 kcals/day diets) (Adam et al., 2005; Sumithran et al., 2011a). compared to the study with moderate restriction (1000-1500 kcal/day) which did not show significant reductions in GLP-1 (Verdich et al., 2001). The different response among groups to the meal challenge was postulated based on the different level of negative energy balance. It was shown earlier that obese individuals in a chronic positive energy balance, as compared to normal weight individuals in a stable energy balance, have a diminished postprandial response (Adam & Westerterp-Plantenga, 2005; Carroll et al., 2007; Verdich et al., 2001). Although there was not a statistically significant group difference for GLP-1 (both fasting and meal response), calculation of Cohen's d for these

variables showed that the effect size of the interventions was moderate for the meal response and large for fasting GLP-1.

Leptin

There was no significant difference between the two groups in the change in leptin from week 0 to week 6. It was hypothesized that the LCD arm would show a greater decrease in leptin due to the expectation of greater weight loss than the taper arm. This is also based on the well-established correlation between leptin and measures of body fat (Carlson et al., 2009; Carroll et al., 2007; Monti et al., 2006). However, since both interventions lost a similar amount of weight it is not surprising there was no statistical group difference for leptin. Finally, and in contrast to the effect size for ghrelin and GLP-1, the effect size for the intervention on fasting leptin was low at 0.1.

Summary

Although no statistically significant group effects were observed for the 3 hormones, the effect size of the interventions for the hormones is noteworthy. Overall, the present study found a moderate-to-large effect size in the response to both interventions on ghrelin and GLP-1. The lack of statistical significance between groups could be due to the small sample size and to the significant differences in the groups at baseline. Importantly, this pilot study was meant to assess feasibility of these interventions, and was not powered to find significance. The calculation of effect size was employed so that a larger, appropriately powered study could be designed. Additionally, the small sample size limits the use of covariates to adjust the statistical model. Other potential limitations that confound the interpretation of the findings include

the short duration of follow-up and the difference in initial BMI between groups. Some strengths of the present study include the following: the inclusion of a run-in period so that all participants were in a priori defined energy balance before randomization and the start of the interventions; the measurement of both fasting and postprandial responses in multiple appetite hormones; well-controlled feeding study that provided 100% of the food required by the intervention; and the randomization allocation by gender.

Clinical Importance and Future Directions

While diet induced weight loss works well in the short-term, focus must not be shifted away from the most clinically relevant issue to reducing obesity, which is weight regain after weight loss (Crujeiras et al., 2010). Solving obesity has seemed to be impossible due to the various mechanisms in the body that drive energy intake as well as energy expenditure. The literature has indicated that with obesity there is a disruption or altered response of appetite hormones. Research also demonstrates a change in hormone responses when undergoing weight loss, which remain altered over the long-term. As discussed in the literature review, these hormonal alterations are directed towards fighting against weight loss and to promote weight regain by restoring homeostasis to the weight prior to the initiation of weight loss. The relatively strong effect sizes observed in the study for postprandial ghrelin and GLP-1 are consistent with the premise that a progressive tapering in the calorie content of a weight loss diet may provide a more favorable metabolic environment to promote and sustain weight loss. In addition, the excellent weight loss of the taper group despite less caloric restriction than the LCD group could have clinical ramifications for a more successful approach to initiating a diet. These findings need to be substantiated with a larger sample size and for a longer period

of time. Larger randomized controlled studies are also necessary to help clarify these physiological processes and gain an understanding of how energy intake is driven, as well as the mechanisms that affect energy expenditure (Hopkins & Blundell, 2016). Due to the somewhat recent discovery of these various appetite hormones, further research is needed in evaluating hormonal responses under different conditions with many different samples. This thesis adds to the literature by suggesting that a gradual reduction of caloric intake as opposed to an immediate reduction in caloric intake could keep the body closer to homeostasis and therefore help prevent weight regain.

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APPENDIX

Appendix I: Studies Comparing Fasting and Postprandial Levels of Ghrelin in Normal Weight and Obese

Authors & Study Design	Population	Intervention	Results	Time points measured	Conclusion
English et al. 2002; experimental	13 non-obese (19.2-26.6 kg/m^2) and 10 obese (31.9-57.5 kg/m^2)	Mixed meal (714 kcal for men and 551 kcal for women; 56.8% carbohydrate, 12.2% protein, 31% fat)	Obese fasting ghrelin at 325±(204-519) pmol/l and lean at 857±(627-1171) pmol/l (p = .002); Obese postprandial levels no change, lean postprandial levels dropped 39.5%± 10% 30 min after meal.	0, 15, 30, 60, 120, 180 minutes after meal	Fasting ghrelin lower in obese, and postprandial response of ghrelin is less reduced in obese than in lean, possibly indicating a lack of satiety in obese after feeding
Carroll et al. 2007; experimental	19 NW (BMI < 25kg/m^2) and 20 obese subjects (BMI ≥ 30kg/m^2) male and female	Standard liquid diet (mixed macronutrients; 510 kcal, chocolate soy meal replacement drink, high carbohydrate around 58%)	Fasted obese female ghrelin: 68.0±14.1pg/ml, Fasted NW female ghrelin: 86.9±10.2 pg/ml; Fasted obese male ghrelin: 53.4±10.9 pg/ml, Fasted NW male ghrelin: 76.7±17.4 pg/ml, Ghrelin trended to being lower in obese (p=.07); ghrelin negatively correlated w/ BMI (r=-.47, p=.005) and body fat (r=-.32, p=.05); Obese men had a delayed nadir ghrelin response (p = .0004); the percent change in ghrelin after meal tended to be reduced in obese (p=.07)	0, 10, 20, 30, 40, 50, 60 minutes after meal	There was a trend for lower ghrelin in Obese. Ghrelin was negatively correlated to BMI and body fat. The ghrelin time course of response in obese is different from NW (more reduction seen in NW)
Carlson et al. 2009; experimental	10 NW (BMI = 21-24kg/m^2) and 13 SO (BMI = 40-49kg/m^2) women	Moderately High Carb Meal (60% Carbohydrate, 20% protein, 20 % fat, ¼ of the participant's kcal needs; 567-804 kcals for NW and 101-706 kcals for SO) kcal less in SO (p=.038)	Fasted ghrelin SO women: 1087±187 pg/ml, Fasted ghrelin NW women: 1418±232 pg/ml (p<.001); trend for different ghrelin response patterns btwn groups (p=.062); averaged over time SO had lower ghrelin response than NW (p=.004)	0, 15, 30, 60, 90, 120	Fasted ghrelin in SO lower than NW; postprandial ghrelin response is less pronounced in SO; less of a drop seen in SO; may indicate less satiety
Morpurgo et al. 2003; experimental	10 obese subjects (3 male, 7 female; mean age: 35±9.3 yr; BMI: 45.2±10.6 kg/m2); 5 healthy subjects (2 male, 3 female; mean age: 45±15; BMI: 21.6±2 kg/m2)	Meal challenge: Standard solid-liquid meal test (550 kcal, 48% carbohydrates, 33% fat, 19% protein)	Wk0 Fasted ghrelin in healthy: 352.4+176.7 pmol/l, Obese subjects 110.8±69.7 pmol/l (p<.05) Healthy AUC at wk0 meal: 18790±10790, ghrelin nadir at 120 time of 199.0+105.2 pmol/l (p<.01); Obese AUC at week 0 meal: 3949±3385, week 0 meal did not affect postprandial in obese, nadir at 120 min of 91.8+70.2 pmol/l (p=ns); Healthy AUC higher after meal at wk0 vs. Obese AUC (p<.05)	0, 30, 60, 90, 120 minutes after meal.	Obese have lower levels of fasting ghrelin than their healthy counterparts; postprandial ghrelin significantly reduced in healthy but did not reduce in obese

Appendix II: Studies Examining Weight Loss and Ghrelin Levels in Obese

Authors & Study Design	Population	Intervention	Results	Time points measured	Conclusion
Sumithran et al. 2011; experimental	50 overweight/obese men and women (BMI = 27-40kg/m^2)	10 week VLC Diet (500-550 kcal/day) and a standardized breakfast (550kcal)	From wk0-wk10: ghrelin increased from ≈ 125pg/ml to 175pg/ml ($p<.001$), From wk10-wk62 ghrelin decreased ($p<.001$), At week 62: ghrelin still above wk0 at ≈ 150pg/ml ($p<.001$); No differences in postprandial response at week 0, 10, or 62. Weight loss after 10 weeks: 13.5±0.5 kg (14%) Weight loss after 62 weeks: 7.9±1.1 kg	Week 0, 10, 62 and time points 30, 60, 120, 180, 240 minutes after meal	Ghrelin significantly increasing after weight loss and remaining high for up to 12 months can negatively impact continued weight loss or maintenance
Hooper et al., 2010; cross-sectional	173 postmenopausal women age 50-75 (BMI = 25–45 kg/m^2)	Questionnaires about history of weight loss in an exercise intervention study	Ghrelin was 11% higher in participants who had intentionally lost ≥10 lb ($p=0.37$), 22% higher in participants who had intentionally lost ≥20 pounds ($p=0.05$), and 44% higher for those who had lost ≥50 pounds ($p=0.03$)	N/A	The more the self-reported weight loss, the higher ghrelin levels are.
Morpurgo et al. 2003; experimental	10 obese subjects (3 male, 7 female; mean age: 35±9.3 yr; BMI: 45.2±10.6 kg/m2);	3 wk weight reduction program: (1200-1800 kcal/day) + exercise training 5 days/wk Meal challenge: Standard solid-liquid meal test (550 kcal, 48% carbohydrates, 33% fat, 19% protein)	Fasting ghrelin at wk0: 110.8±69.7 pmol/l Fasting ghrelin at wk3: 126.4±108.6 pmol/l (p=ns) Obese AUC at week 0 meal: 3949±3385 pmol/l, week 0 meal did not affect postprandial in obese, nadir at 120 min of 91.8+70.2 pmol/l (p=ns) Obese AUC at week 3 meal: 3242±2012 pmol/l (p=ns) No correlation btwn ghrelin and BMI or percent body fat Total Weight loss after 3 weeks: 5% (~5 kg)	Week 0, Week 3 and time points 30, 60, 90, 120 minutes after meal.	The postprandial response in obese does not change significantly from 3 weeks of weight loss with modest weight loss; ghrelin did not decrease
Crujeriras et al. 2010; experimental	104 overweight/obese men and women volunteers (mean BMI = 31.1±2.9 kg/m^2)	8 wk hypocaloric diet (70% of daily EE; 55% carbohydrate, 15% protein, 30% fat)	From wk0-wk8: No difference in ghrelin levels; ghrelin levels higher in females ($p\leq.001$); ghrelin higher in wk0 and wk8 in WR males compared to WM males, but no diff in women; A decrease in ghrelin from the median from wk0-wk8 was related to an increased risk of weight regain (OR=3.109; $p=.008$) Total weight loss after 8 weeks: 5.0±2.2% (~ 4.8 kg) Total weight loss after 32 weeks: (~ 5.1 kg)	Wk 0, 8, 32	Those not successful and regained weight had lower fasted ghrelin levels, revealing a possible disruption in ghrelin receptor sensitivity

Appendix III: Studies Comparing Fasting and Postprandial Levels of GLP-1 in Normal Weight and Obese

Authors & Study Design	Population	Intervention	Results	Time points measured	Conclusion
Carroll et al. 2007; experimental	19 NW (BMI < 25kg/m^2) and 20 obese subjects (BMI $\geq$ 30kg/m^2) male and female	Standard liquid diet (mixed macronutrients; 510 kcal, chocolate soy meal replacement drink, high carbohydrate around 58%)	Fasted obese women: 2.8+1.0pM; Fasted NW women: 2.8+0.9pM; Fasted obese men: 4.2+0.8pM; Fasted NW men: 4.5+1.0pM; men tended to have higher GLP-1 than women (p=.06); obese had a decrease in GLP-1 from 0-20min while NW had increase in GLP-1 (p=0.02)	10, 20, 30, 40, 50, 60 minutes after meal	No difference in fasted GLP-1 in NW and Obese; There was a slight gender effect on fasting GLP-1 (men had tended to have higher GLP-1); No overall difference in postprandial GLP-1, but time course of GLP-1 was different from 0-20 minutes between obese and normal weight (obese lowered GLP-1 while NW increased GLP-1)
Adam & Westerterp-Plantenga 2005; experimental	30 normal weight and 28 overweight/obese subjects (BMI = 30.3$\pm$2.8 kg/m^2)	2 different meals (654kcals) (galactose/guar gum-GG+ standard breakfast, OR water + standard breakfast-W; 48% carbohydrate, 29% protein, 23% fat)	Baseline fasting GP-1: No difference between groups; Obese had lower postprandial GLP-1 at 30 minutes compared to NW after W meal (p=.02); AUC was sig lower in obese than NW after W meal only (p=.03)	0, 30, 60, 90, 120 minutes	No difference in fasting GLP-1 in NW and obese; There was an attenuated postprandial GLP-1 response in overweight/obese compared to NW; guar gum helps to increase GLP-1 sensitivity.
Verdich et al. 2001; experimental	19 obese (BMI = 34.1 ± 43.8 kg/m2) and 12 lean (BMI = 20.4 ± 24.7 kg/m2) males	A standardized meal (597 kcal; 20% protein, 50% carbohydrate, 20% fat)	Lean fasting GLP-1: 11.9 (8.8-15.0) pmol/l; AUC: 3687 (3309-4065) pmol/l Obese fasting GLP-1: 9.5 (7.6-11.5) pmol/l; AUC 2955 (2656-3255)pmol/l AUC: p <.05	0, 15, 30, 45, 60, 90, 120, 150 and 180 min after meal	No difference in fasting GLP-1 in Obese and lean. There was an attenuated GLP-1 postprandial response in the obese compared to the lean.
Kim et al. 2005; experimental	19 obese (BMI $\geq$ 30kg/m^2) and 19 lean (BMI < 25kg/m^2) age matched men and women	OGTT (75g carbohydrate) (300kcal)	No difference in the postprandial response of GLP-1 in obese and NW. No significant difference in fasted GLP-1 between the groups.	0, 30, 60, 90, 120 minutes	No difference in fasted GLP-1 having no difference based on BMI. No differemce in postprandial response of GLP-1

Appendix IV: Studies Examining Weight Loss and GLP-1 Levels in Obese

Authors & Study Design	Population	Intervention	Results	Time points measured	Conclusion
Sumithran et al. 2011; experimental	50 overweight/obese men and women (BMI = 27-40kg/m^2)	10 week VLC Diet (500-550 kcal/day) and a standardized breakfast (550kcal)	From wk0-wk10 = no change in postprandial secretion of GLP-1. In wk0-wk62 = postprandial GLP-1 was lower in wk62 (p=.005) Weight loss after 10 weeks: 13.5±0.5 kg (14%) Weight loss after 62 weeks: 7.9±1.1 kg	Week 0, 10, 62 and time points 30, 60, 120, 180, 240 minutes after meal	Reduced GLP-1 secretion after long term (62week) weight loss could be a significant roadblock to weight maintenance
Adam et al. 2005; experimental	32 overweight/obese men and women	6 week VLED Diet (607 kcal); a standardized breakfast (454kcal)	From wk0 to wk6: fasted GLP-1 was lower but not significant (p = .07); the AUC for GLP-1 at wk 0 was sig higher than wk 6 (p<.05) Total weight loss after 10weeks: (~ 6.1 kg)	6 weeks; 0, 30, 60, 90, 120 minutes	After weight loss there is a reduced fasting GLP-1 as well as an attenuated postprandial GLP-1 response
Verdich et al. 2001; experimental	19 obese (BMI = 34.1 ± 43.8 kg/m2)	6 month weight reduction (8 weeks 1000 kcal/day and 8 weeks 1500 kcal/day and 8 weeks weight maintenance)	Obese fasting GLP-1 pre: 9.5 (7.6-11.5) pmol/l; AUC 2955 (2656-3255) Obese fasting GLP-1 post: 12.4 (9.2-15.7) pmol/l; AUC: 3228 (2934-3521); AUC increased in stepwise manner from obese to reduced obese to lean (p=.003) Reduced Obese had 36% lower GLP-1 response to meal than lean Total weight loss after 6 months: 18.8 kg 14.8%	0, 15, 30, 45, 60, 90, 120, 150 and 180 min after meal	After weight loss GLP-1 response was increased

Appendix V: Studies Comparing Fasting and Postprandial Levels of Leptin in Normal Weight and Obese

Authors & Study Design	Population	Intervention	Results	Time points measured; type of blood	Conclusion
Carroll et al. 2007; experimental	19 NW (BMI < 25kg/m^2) and 20 obese subjects (BMI $\geq$ 30kg/m^2) male and female	Standard liquid diet (mixed macronutrients; 510 kcal, chocolate soy meal replacement drink, high carbohydrate around 58%)	Fasted obese female leptin = 26.1 ± 2.5 ng/ml, Fasted NW female leptin = 10.8 ± 1.2 ng/ml; fasted obese male leptin = 18.1 ± 1.4 ng/ml, fasted NW male leptin = 4.4 ± 1.0 ng/ml. Obese women had raw data 142% higher than NW women; obese men had raw data 311% higher than NW males (p=.01)	10, 20, 30, 40, 50, 60 minutes after meal	Fasted leptin in obese is higher than fasting leptin in NW. There is a gender x BMI group interaction on fasting leptin (obese women had slightly higher fasting leptin than NW women and obese men had significantly higher fasting leptin than NW men). No significant differences in postprandial response of leptin between groups.
Carlson et al. 2009; experimental	10 NW (BMI = 21-24kg/m^2) and 13 SO (BMI = 40-49kg/m^2) women	Moderately High Carb Meal (60% Carbohydrate, 20% protein, 20 % fat, ¼ of the participant's kcal needs; 567-804 kcals for NW and 101-706 kcals for SO) kcal less in SO (p=.038)	SO fasted leptin = 48.1$\pm$16.2 ng/ml, NW fasted leptin = 10.1$\pm$4.5 ng/ml, SO leptin sig higher than NW leptin (p<.001); Postprandial SO leptin dropped more than NW leptin at 15, 60, 90, 120; leptin decreased more over time in SO group than in NW group (p<.001).	0, 15, 30, 60, 90, 120	Fasted leptin higher in SO than NW. Postprandial response in NW was unchanged and similar to the normal leptin response, postprandial in SO decreased over time. The hormonal differences between BMI groups may impact weight regain or resistance to weight loss
English et al. 2002; experimental	13 non-obese (19.2-26.6 kg/m^2) and 10 obese (31.9-57.5 kg/m^2)	Mixed meal (714 kcal for men and 551 kcal for women; 56.8% carbohydrate, 12.2% protein, 31% fat)	Obese fasting leptin at 30.5$\pm$(17.7-52.5) ng/ml and lean at 1.9$\pm$(1.0-3.4) ng/ml (p < .0001); postprandial leptin fell by 37.4+6.7% 15 min after meal then returned to baseline in lean group; postprandial levels were unchanged from baseline in obese; Leptin correlated with BMI (r=0.79, p<.0001)	0, 15, 30, 60, 120, 180 minutes after meal	Fasting leptin is lower in lean than obese; postprandial leptin does not change much but did drop more so in the lean group
Morpurgo et al. 2003; experimental	10 obese subjects (3 male, 7 female; mean age: 35±9.3 yr; BMI: 45.2±10.6 kg/m2); 5 healthy subjects (2 male, 3 female; mean age: 45$\pm$15; BMI: 21.6$\pm$2 kg/m2)	Meal challenge: Standard solid-liquid meal test (550 kcal, 48% carbohydrates, 33% fat, 19% protein)	Wk0 Fasted leptin in healthy: 5.6$\pm$5.3 ng/ml Wk0 Fasted leptin in obese: 33.4$\pm$17.8 ng/ml (p<.0005)	0, 30, 60, 90, 120 minutes after meal.	Fasting leptin is lower in healthy individuals than obese individuals.

Appendix VI: Studies Examining Weight Loss and Leptin Levels in Obese

Authors & Study Design	Population	Intervention	Results	Time points measured; type of blood	Conclusion
Sumithran et al. 2011; experimental	50 overweight/obese men and women (BMI = 27-40kg/m^2)	10 week VLC Diet (500-550 kcal/day) and a standardized breakfast (550kcal)	From wk0-wk10: leptin decreased by 64.5±3.4% (P<.001). From wk10-wk62: leptin increased. At wk62 leptin was 35.5±4.7% below wk0 (P<.001). Weight loss after 10 weeks: 13.5±0.5 kg (14%) Weight loss after 62 weeks: 7.9±1.1 kg	Week 0, 10, 62 and time points 30, 60, 120, 180, 240 minutes after meal	Compensatory mechanisms such as a decrease in leptin could predispose one to weight regain, this adaptation lasts over 1 year
Crujeiras et al. 2010; experimental	104 overweight/obese men and women volunteers (mean BMI = 31.1±2.9 kg/m^2)	8 wk hypocaloric diet (70% of daily EE; 55% carbohydrate, 15% protein, 30% fat)	Leptin at wk0 = 22.5±14.7, leptin at wk8 = 14.9±14.5 (p<.001); Those who increased leptin from the median fromut wk0-wk8 had a 86% higher risk of weight regain than those who did not decrease leptin (OR=0.141, p=.001); In women leptin levels were higher in WR than in WM group at all 3 time points Total weight loss after 8 weeks: 5.0±2.2% (~ 4.8 kg) Total weight loss after 32 weeks: (~ 5.1 kg)	Wk 0, 8, 32	Fasting leptin decreases with weight loss. Subjects who regain more weight after diet induced weight loss compared to those who maintain their weight present w/ different central response to leptin signaling
Morpurgo et al. 2003; experimental	10 obese subjects (3 male, 7 female; mean age: 35±9.3 yr; BMI: 45.2±10.6 kg/m2);	3 wk weight reduction program: (1200-1800 kcal/day) + exercise training 5 days/wk Meal challenge: Standard solid-liquid meal test (550 kcal, 48% carbohydrates, 33% fat, 19% protein)	Wk0 Fasted leptin in obese: 33.4±17.8 ng/ml Wk6 Fasted leptin in obese: 21.5±13.4 (p<.0005) Circulating leptin decreased by 38% Total Weight loss after 3 weeks: 5% (~5 kg)	Week 0, Week 3 and time points 0, 30, 60, 90, 120 minutes after meal.	Fasting leptin decreases with a 3 wk calorie restricted weight loss program

CURRICULUM VITAE

EDUCATION

Wake Forest University, Winston-Salem, NC

- Masters in Health and Exercise Science, May 2017

Towson University, Towson, MD

- Bachelor of Science in Exercise Science, May 2015

RESEARCH EXPERIENCE

- ***Research Study Coordinator,*** Wake Forest University Baptist Medical Center; Winston-Salem, NC (June 2016 – May 2017)
    - Prepared material prior to the start of the TREND Study
    - Recruited participants over telephone
    - Met with and conducted consults and assessments with participants
- ***Research Assistant***, Johns Hopkins Bayview Medical Center, Baltimore, MD (January 2014 – January 2015)
    - Assisted in explaining informed consents, conducting 6 minute walk testing, obtaining heart rate and blood pressure vitals, and organizing data for the Move It Study with the Cardiology department

WORK EXPERIENCE

- ***Lab Coordinator***, The Healthy Exercise and Lifestyle ProgramS, Wake Forest University; Winston-Salem, NC (March 2016 - Current)
    - Conducted lab assessments (skinfolds, circumferences, 6 minute walk test, blood lipid testing, etc...)
    - Prepped and placed 12 lead EKG on participants
    - Directed exercise stress tests using a Medgraphics metabolic cart
    - Developed individualized exercise prescriptions based on the results of the test
    - Managed and instructed the lab staff on proper technique of assessments and conducting stress tests

- ***Exercise Physiologist,*** St. Agnes Hospital Center; Baltimore, MD (June 2015 - August 2015)
    - Conducted assessments with patients in Cardiac Rehab
    - Took multiple blood pressures and evaluated patients during exercise

    - Prepped EKG placement on participants and monitored the EKG rhythms while participants exercised
    - Conducted education sessions for participants on nutrition, exercise, CVD
    - Met with participants individually to focus on goals and write an exercise prescription
- ***Intern,*** St. Agnes Hospital Center; Baltimore, MD (January 2015 - May 2015)
    - Took blood pressures and instructed certain exercises for patients
- ***Physical Therapy Technician***, Life Fitness Physical Therapy; Towson, MD (June 2014-April 2015)
    - Worked closely with the PT in assisting patients in proper form and assessing the progress of each client

TEACHING EXPERIENCE

- ***Graduate Teaching Assistant,*** Wake Forest University; Winston-Salem, NC (August 2015 – May 2017)
    - Prepared and taught lectures and labs for HES101 to undergraduates
- ***Guest Lecturer***, Wake Forest University; Winston-Salem, NC (Fall 2016 – Spring 2017)
    - Created two lesson plans for the undergraduate Nutrition course. Lead discussion based lectures on the RDA guidelines and protein consumption
- ***Teaching Assistant,*** Towson University; Towson, MD (Spring 2015)
    - Assisted instructor with course design and course supplemental activities for the undergraduate course Functional Anatomy
    - Tutored study sessions and prepared a lecture on the use of proper footwear and foot kinematics

VOLUNTEER

- WFEMS, Volunteer EMT (2017)

CERTIFICATIONS

- BLS Provider (CPR and AED), American Heart Association
- ACLS Provider, American Heart Association
- National EMT Certification, NREMT
- ACSM Certified Clinical Exercise Physiologist, ACSM
- Collaborative Institutional Training Initiative (CITI) Training in Human Subjects Research