

Acute Effect of High and Low Carbohydrate Meals on  
Postprandial Concentrations of Weight Regulation Markers

By

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CERTIFICATE OF APPROVAL

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## Abstract

Persons following low carbohydrate (LC) diets often report a sense of “food disinterest”. Diets high in protein have also been shown to increase feelings of satiety after meal consumption compared to lower protein diets. Differences in the macronutrient composition of traditional low fat, low calorie and low carbohydrate diets may have different effects on the hormones that influence central regulation of appetite and energy balance. Consuming a diet that has a greater stimulatory affect upon hormones that induce short-term satiety should result in an increased feeling of fullness and a decreased feeling of hunger and lead to a decrease in energy intake.

This study used a random order, crossover design to examine how acute exposure of 10 healthy, normal weight adults to low carbohydrate (LC) and high complex-carbohydrate (HC) meals affect circulating concentrations of weight regulation markers and influence hunger and fullness. Fasting and postprandial blood samples were collected over a period of 9.5 h and analyzed for concentrations of glucose, insulin, leptin, total and active ghrelin, GLP-1 and PYY. Participant feelings of hunger and fullness were assessed by visual analog scales (VAS) before and after meal consumption. Differences in AUC after the LC and HC meals were compared using one sided paired t-tests. Pattern of postprandial change in glucose, insulin, leptin, total ghrelin, GLP-1, and PYY concentrations over time were analyzed using orthogonal polynomials (up to order 4) with components of these polynomials assessed for significance using a Wilcoxon signed-rank test. Differences between concentrations for each analyte at pre-selected time points after LC and HC meal consumption were analyzed using repeated measures analysis of variance (MANOVA) and post-hoc analysis. Differences in linear contrasts between the LC and HC meals for VAS hunger and fullness scores were analyzed using a Wilcoxon signed-rank test.

Total area under the curve for PYY ( $1107 \pm 100$  vs.  $954 \pm 72$  pg·h/ml,  $p < 0.05$ ) and GLP-1 ( $55 \pm 11$  vs.  $42 \pm 9$  pM·h/ml,  $p < 0.01$ ) was found to be higher after consumption of the LC meals than the HC meals, respectively. Total area under the curve was lower for glucose ( $793 \pm 13$  vs.  $841 \pm 20$  mg·h/dL,  $p < 0.05$ ), insulin ( $84 \pm 10$  vs.  $240 \pm 23$   $\mu$ IU·h/ml,  $p < 0.01$ ), and leptin ( $2.8 \pm 0.58$  vs.  $3.7 \pm 0.83$  ng·h/kg fat mass·ml,  $p = 0.04$ ) after LC than HC meal consumption, respectively. There was no significant difference in total area under the curve after consumption of the LC meals than the HC meals for total ( $7662 \pm 1746$  vs.  $7391 \pm 1517$  pg·h/ml,  $p > 0.05$ ) and active ghrelin ( $815 \pm 199$  vs.  $865 \pm 235$  pg·h/ml,  $p > 0.05$ ), respectively. Repeated measures ANOVA showed a significant effect of diet for at least one of the selected time points for glucose, insulin, leptin, total ghrelin, and GLP-1 ( $p < 0.05$  for all significant time points). Repeated measures ANOVA was not significant for differences between LC and HC meals for active ghrelin and PYY at any of the time points selected. Postprandial patterns of change were significant for differences after consumption of LC compared to HC meals for all analytes. There were no significant differences any of the contrasts made between participant feelings of hunger and satiety after consumption of LC and HC meals.

This study provided convincing evidence that there is a significant difference in the effect that consumption of LC meals has on the postprandial excursion of weight regulation markers compared to HC meals in healthy, normal weight individuals. The feelings of food disinterest and the resulting weight loss experienced by persons following LC diets may be related in part to changes in weight regulation hormones that affect the desire to consume food and feelings of fullness.



## Significance

Despite efforts to stop the increase in prevalence, obesity is still a growing problem in the United States and other industrialized countries. Its effects are not only felt by the adult population, but have become a problem facing today's pediatric population (1). Obesity occurs when a person consumes more energy than they expend, which leads to excess energy being stored as fat. The model that is used to explain this process and its role in the development of obesity is the Energy Balance Model. When a person is in energy balance, their energy intake is equal to their total energy expenditure, and theoretically, their fat stores remain stable. A person at risk for developing obesity would have a positive energy balance in which their energy intake exceeds their total energy expenditure, and excess energy would be stored as fat. Persons can alternately achieve negative energy balance and lose weight by increasing their energy expenditure and/or changing their diet to include less energy.

Popular diets such as the Atkins, Weight Watchers, and the Zone diets are options for people who wish to lose weight (2). Some studies have shown that low carbohydrate diets like the Atkins diet have yielded weight loss results equal to or greater than traditional low fat, low calorie diets (3, 4). Other studies have evidence that diets low in fat and high in complex carbohydrates also result in weight loss (5). Proponents of low carbohydrate diets claim that the significant decrease in carbohydrate results in an "increase in ketosis, lipid oxidation, and energy expenditure" which should lead to weight loss (6). Diets high in protein have also been shown to increase feelings of satiety after meal consumption compared to lower protein diets (7). Subjective reports from participants in ongoing studies using low and high carbohydrate diet interventions suggest that low carbohydrate diets lead to weight loss in part by causing individuals to experience a loss of interest in food (Stadler, personal communication). The sense of

food disinterest potentially leads to a reduction in food intake and a state of negative energy balance. Although the short-term weight loss effects of low carbohydrate diets are convincing, there is not a significant body of scientific evidence to support the belief that these diets offer a long-term weight loss solution (8). Low carbohydrate diets have only been shown to be more effective than traditional low fat diets up to a year; after that time there is no difference in weight loss between the two diets (9)

Differences in the macronutrient composition of traditional low fat, low calorie and low carbohydrate diets may have different effects on the hormones that influence central regulation of appetite and energy balance. Diets high in fat and protein, like popular low carbohydrate diets, have a greater stimulating effect on the release of appetite regulating hormones such as peptide YY (PYY), compared to high carbohydrate diets (10). Peripheral infusion of another appetite regulating hormone, glucagon-like peptide 1 (GLP-1), has been shown to reduce energy intake and suppress appetite in normal weight individuals (11). Consuming a diet that has a greater stimulatory effect upon hormones that induce short-term satiety should result in an increased feeling of fullness and a decreased feeling of hunger and energy intake, leading to negative energy balance and weight loss.

This study used a random order, crossover design to examine how acute exposure of individuals with a normal body mass index (BMI) ( $18-25 \text{ kg/m}^2$ ) to low carbohydrate (LC) and high complex-carbohydrate (HC) meals affect circulating concentrations of hormones known to affect central regulation of body weight (insulin, leptin, total and active ghrelin, PYY, and GLP-1), and influence hunger and satiety. Investigating the hormonal response of normal weight persons to each meal type will lead to an effective model that can be applied to understanding how these diets affect short term and long term weight regulation in overweight and obese individuals.

## Specific Aims

### Study Objectives

The purpose of this study is to investigate the acute physiological response to consuming low carbohydrate (LC) and high complex-carbohydrate (HC) meals in normal weight individuals. Circulating concentrations of hormones associated with weight regulation and hunger and satiety were measured under fasting and postprandial conditions in weight stable individuals using a random order, crossover study design.

**Primary Aim #1:** To measure circulating concentrations of glucose, insulin, leptin, ghrelin, PYY, and GLP-1 before and after consumption of low carbohydrate and high complex-carbohydrate meals.

**Hypothesis #1:** Postprandial concentrations and area under the curve of PYY and GLP-1 will be higher and postprandial concentrations and area under the curve of glucose, insulin, leptin, and ghrelin will be lower after consuming low carbohydrate meals than high carbohydrate meals of equal energy content.

**Primary Aim #2:** To measure using visual analog scales, how low carbohydrate and high complex-carbohydrate meals affect feelings of hunger and satiety.

**Hypothesis #2:** Participants will report feeling less hungry and more satiated after consuming low carbohydrate meals than high complex carbohydrate meals of equal energy content.

## Background

### Chapter 1: Obesity

The obesity epidemic continues to be a problem which threatens the health of the general public in the United States and other industrialized countries. Currently persons are categorized as normal weight, overweight, or obese depending on their body mass index: 18.5 to 24.9, 25 to 29.9, and greater than 30 kg/m<sup>2</sup>, respectively (12). An article that analyzed the most recent National Health and Nutrition Examination Survey (NHANES) data from 2004 found the prevalence of obesity in the United States had increased significantly for men from 27% in 2000 to 31.1% in 2004. The numbers stayed relatively stable for women at a prevalence of 33.4% in 2000 to 33.2% in 2004 (13). It has been projected that if the prevalence of obesity continues to increase at the current rate, 86.3% of adults will be overweight and 51.1% will be obese by the year 2030 and all adults would be considered obese by 2048 (14).

The burden that obesity related diseases place on the national healthcare system is profound. A recent study that analyzed Medicare beneficiary data from 2003 found that on average persons classified as obese spent more on prescription drugs (\$2,374 to \$2,976 per year) compared to persons considered to be normal weight (\$1,764 per year) due to increased number of chronic diseases (15). Obese workers are also 6.9% more likely to claim that physical or emotional problems cause limitations to the overall amount and type of work they are able to perform compared to only 3% of workers who are considered normal weight (16). In 2004, obesity was associated with 112,159 excess deaths due to cardiovascular disease and 13,839 excess deaths associated with obesity related cancers such as colon and breast cancer. When combined, overweight and obese status was associated with 61,248 excess deaths from diabetes and kidney disease (17).

In response to the continuing threat that obesity poses to public health the Department of Health and Human Services published the Healthy People 2010 goals of encouraging weight loss to reduce the prevalence of obesity in adults to 15% and 5% in children (18). Two commonly recommended methods to achieve weight loss include change in diet to improve quality and decrease the amount of energy consumed, and to increase energy expenditure by increasing physical activity. Analysis of NHANES 2001-2002 data by Weiss et al. found that 51% of US adults reported trying to control their weight by attempting to lose weight or not gain weight over the past 12 months. Out of that percentage, the top four practices employed were a decrease in food consumption, exercise, decreased fat consumption, and replacement of usual foods consumed with those that contained fewer calories (19). Non-prescription supplements are an unadvisable weight loss option due to concerns regarding safety and efficacy, but an estimated 15.2% of US adults surveyed in 2002 reported having used supplements at least once and 8.7% used supplements within the past year (20). A telephone survey conducted by Blanck et al. in 2002 revealed at that time 12.5% of Americans surveyed had used low carbohydrate diets for weight loss and 3.4% of those respondents were currently following a low carbohydrate diet. Use of low carbohydrate diets was found to be similar across race, education, and sex with 5.9% of those surveyed who were actively trying to lose weight currently following a low carbohydrate diet (21). The next section will focus on the use of two different diets, low and high complex carbohydrate diets, to achieve weight loss.

## **Chapter 2: High and Low Carbohydrate Diets and Weight Loss**

### **High Complex Carbohydrate Diets and Weight Loss**

Diets low in fat and high in complex carbohydrates are commonly recommended to persons trying to lose weight. The high complex carbohydrate meals that were used in this study were modeled after the standard dietary recommendations for persons following the DASH (Dietary Approaches to Stop Hypertension) diet. The DASH diet was developed by researchers and has been found in studies to lower blood pressure and prevent hypertension. The diet recommends consumption of foods high in whole grains, fruits and vegetables, low and non-fat dairy products, and lean meats and avoidance of foods high in saturated fat and sodium (22). This diet has also been evaluated for effectiveness in weight loss by various clinical studies.

One study conducted in a cohort of 658 pre-hypertensive and hypertensive adult subjects investigated the effect of a DASH dietary pattern and established guidelines to patients with hypertension on weight loss (23). Participants in this study were randomly assigned to three groups. One group received one educational session explaining how factors such as weight, physical activity, and diet affect high blood pressure; the second group received 18 intensive in person counseling sessions to lose weight, increase physical activity, and decrease alcohol consumption; the third group received the same 18 counseling sessions with the addition of recommendations to consume a DASH dietary pattern. Measurements were collected at baseline and at 6 months. After 6 months all three groups saw reductions in energy intake, energy density, and body weight with the greatest reduction in energy intake and body weight in the second and third intervention groups ( $p < 0.05$ ).

Ello-Martin et al. conducted a one year study in which they investigated the amount of weight loss experienced by 71 obese women when they were assigned to two different diets. The participants were randomly assigned to either reduce their dietary fat

intake, or to reduce their dietary fat intake and add water rich foods such as fruits and vegetables to their diet. After the one year intervention, both groups experienced significant weight loss ( $p < 0.0001$ ), but the pattern of weight loss was different between the reduced fat and the reduced fat and fruits and vegetables group ( $p = 0.021$ ). The reduced fat and fruit and vegetable group lost more weight than the reduced fat group ( $7.9 \pm 0.9$  vs.  $6.9 \pm 0.9$  kg,  $p = 0.002$ ). The reduced fat, fruit and vegetable group also reported feeling less hunger ( $p = 0.003$ ) (24).

Thomson et al. conducted a four year randomized controlled dietary intervention study in which a sub-set of breast cancer survivors were asked to consume a low-fat diet high in fruits, vegetables, and fiber. The control group was asked to follow standard dietary guidelines for cancer prevention. The results of the study showed that in the first six months, the intervention group showed a non-significant trend toward greater weight loss compared to the control group. There was no significant weight loss seen in the intervention group during the rest of the four year study (25).

### **Low Carbohydrate Diets and Weight Loss**

Although not as popular today as they were in the earlier part of the decade, the quick results achieved by persons who follow low carbohydrate diets still make them an attractive option for weight loss. The low carbohydrate meals that were served in this study were modeled after meals typically consumed during the induction phase of the Atkins diet. The induction phase restricts consumption of carbohydrate to no more than 20 g/day for the first two weeks, and gradually increases the amount allowed by 5-10 g/day of carbohydrate a week as one works towards reaching the weight maintenance phase of the diet. The most recent Atkins book states that this specific low carbohydrate weight loss diet is the best choice for a number of reasons (6). The author claims that the diet has a “metabolic advantage” over traditional low fat diets. Dr. Atkins explains

that once a person limits their carbohydrate intake, their body switches from burning glucose normally derived from carbohydrate, to burning fat instead. Since burning fat requires more energy, persons following low carbohydrate diets should be able to burn more calories without having to increase their level of physical activity. Proponents of the Atkins diet also claim that the diet is beneficial because it avoids excessive levels of insulin, which the book states are related to obesity. Persons following the low carbohydrate diet should also feel less hungry than on traditional diets because there is little calorie restriction, and will also have more energy while on the diet (6).

There are a number of studies that have investigated the effectiveness of low carbohydrate diets for weight loss. Foster et al. conducted a one-year, multicenter study involving 63 obese male and female participants. Each subject was randomized to a low-carbohydrate, high-fat and high-protein diet or a traditional low calorie, low-fat and high-carbohydrate diet. The results showed that those subjects assigned to the low carbohydrate diet lost more weight at three and six months ( $p=0.001$ ), but at 12 months the difference in weight loss between the two diet groups was not significant ( $p=0.26$ ). The researchers concluded that longer studies are necessary to determine the long-term safety and effectiveness of low carbohydrate diets (8). Brehm et al. showed similar short term weight loss results in the study they conducted in 42 healthy, obese females. Subjects in this study were randomized to either an ad libitum very low carbohydrate diet (40-60 g carbohydrate per day after the first 2 weeks) or a low fat (30% fat) energy restricted (450 kcal reduction from baseline) diet for 6 months. Measurements were taken at baseline, 3, and 6 months. By the end of the six months, participants had lost significantly more weight and body fat after following the low carbohydrate diet compared to the low fat diet ( $p<0.001$  and  $p<0.01$ , respectively) (4).

Another study involving 31 overweight and obese male and female participants investigated the weight loss differences between the low fat and low carbohydrate diets,



as well as their affect on risk factors for diabetes. The findings reported showed that after ten weeks of dietary intervention, the amount of weight loss of those on low carbohydrate vs. low fat diets was 6.8 and 7.0 kg, respectively, and was not significant ( $p>0.05$ ). The researchers also reported fasting insulin levels, and the insulin to glucose ratio to be significantly lower for the low carbohydrate group compared to the low fat diet group after the ten week intervention ( $p<0.05$ ) (26). Volek et al. recruited 28 healthy, overweight and obese men and peri-menopausal women to participate in a randomized crossover study in which they were asked to consume energy restricted low carbohydrate and traditional low fat diets. Men followed the intervention diets for a period of 50 days and women followed the intervention diets for 30 days. Their findings showed that male participants experienced more weight loss while consuming the very low carbohydrate diet than the low fat diet (8.0 vs. 5.7 kg,  $p<0.05$ ), while the results for female participants was not significant ( $p>0.05$ ) (27).

### **Chapter 3: Hypothalamic Regulation of Energy Balance**

The primary site of action for the hormones investigated in this study is the arcuate nucleus (ARC) of the ventral hypothalamus. It is located near the bottom of the third ventricle and has projections into the paraventricular nucleus as well as the lateral hypothalamic area (28). Secondary to its proximity to the median eminence, the ARC is in a unique position to receive input from peripheral signals such as hormones released into circulation from other parts of the body. Unlike the rest of the blood brain barrier, the area of the median eminence is permeable to larger molecules and allows circulating hormones access to the central nervous system.

The ARC contains two different groups of neurons that play significant but opposite roles in the regulation of feeding and energy expenditure. The first group is the neuropeptide Y (NPY) and agouti-related peptide (AGRP) releasing neurons. The

second group is the pro-opiomelanocortin (POMC) and cocaine amphetamine related transcript (CART) releasing neurons (29). The substances released by these two groups of neurons have opposite effects on appetite and metabolism. When released, NPY/AGRP bind to receptors in the paraventricular nucleus and cause an increase in hunger and a decrease in metabolic rate. In contrast the hormone alpha melanocyte stimulating hormone ( $\alpha$ -MSH) formed from the cleavage of pro-opiomelanocortin released from POMC neurons elicits the opposite effect upon binding to its receptors. The target for  $\alpha$ -MSH is the melanocortin-4 (MC4) group of receptors located in the paraventricular nucleus, lateral hypothalamic area, and other regions of the hypothalamus. The binding of  $\alpha$ -MSH to MC4 receptors results in decreased appetite and an increase metabolic rate (30).

It was postulated by Kennedy et al. in the 1950's after his study of cholecystokinin (CCK) in young rats that there may be a peripheral signal that feeds back from adipose tissue to the central nervous system to control energy intake and energy balance (31). A complementary theory was proposed by Gibbs et al. in the 1970's that there must also be signals that feedback from the digestive system to the brain that nutrients have been consumed and to stop eating (32). All of the hormones investigated in this study have receptors on one or both of the NPY/AGRP and POMC neurons and exert control over energy balance by increasing or decreasing food intake and/or metabolic rate (33-37). The actions of these hormones have since confirmed both theories and further emphasize the impact of diet on energy intake and the state of energy balance. The next chapter will focus on insulin, leptin, ghrelin, GLP-1 and PYY and the resulting effect that their binding to receptors in the ARC has on energy intake and expenditure.

## **Chapter 4: Weight Regulation Hormones and Their Effects on Appetite**

### **Insulin and Leptin**

Both insulin and leptin have played central roles in the story of weight regulation and energy balance since the 1970's and 1990's, respectively. An early experiment conducted by Woods et al. showed that when the pancreatic hormone insulin was given intercerebroventricularly (ICV) to baboons their appetite decreased and their energy expenditure increased resulting in weight loss (38). Baura et al. subsequently demonstrated that insulin actually enters the central nervous system through the blood brain barrier from the circulation via a transporter mediated mechanism in an amount that is proportional to the level circulating in plasma (39). Leptin was discovered in 1994 by Zhang et al. when they found that a gene which they designated as the ob/ob gene coded for a substance that appeared to be secreted by white adipose cells and whose deficiency resulted in obese rats (40). A study conducted by Schwartz et al. demonstrated that leptin, like insulin circulates in the blood stream in concentrations proportional to the amount of body fat and enters into the CNS from circulation (41, 42).

As mentioned in the previous chapter, both insulin and leptin have receptors on the POMC/CART and NPY/AGRP neurons in the arcuate nucleus of the hypothalamus. Various studies have shown that both insulin and leptin are able to influence energy balance by increasing energy expenditure and suppressing appetite via their interaction with POMC and NPY neurons. In the ob/ob mouse model, NPY mRNA in the ARC is elevated which contributes to the characteristic obesity phenotype, but concentrations are restored to normal with leptin administration (43). In contrast, concentration of POMC mRNA is reduced in the ARC and administration of leptin was found to bring values back to normal (44). The diabetic model shows that insulin deficient diabetes is associated with an increase in NPY and a decrease in POMC synthesis, and both conditions can be corrected by insulin administration (45, 46). Due to the previously

discussed characteristics shared by both insulin and leptin, they have both been designated as “adiposity signals” as they both circulate in concentrations proportional to the amount of individual body fat and therefore provide feedback to the central nervous system that influences energy expenditure and energy intake.

The complex relationship between insulin and leptin was further investigated by an experiment conducted by Muller et al. in rat adipocytes. It was hypothesized that since insulin, glucose, and leptin concentrations are all affected by fasting energy state, there may be a mechanism by which leptin secretion is regulated by insulin and/or glucose concentrations. When treated with insulin, the cultured adipocytes increased their leptin secretion. The increase in leptin was found to be more significantly related to glucose uptake by the cells rather than the concentration of insulin they were exposed to ( $p < 0.01$ ). When the cells were treated with a glucose-uptake inhibitor it was shown that the amount of leptin inhibition was proportional to the degree of inhibition of glucose uptake by the cells. From this data the researchers concluded that the insulin mediated glucose uptake by adipose cells plays an important role in the secretion of leptin (47). The impact that insulin and leptin have on energy intake and energy balance is profound and a dietary pattern that augments the anorexogenic characteristics of both hormones would be beneficial in efforts to control the increasing obesity epidemic.

### **Ghrelin**

In 1999 Kojima et al. purified a novel protein 28 amino acid residues in length from the endocrine cells lining the stomachs of rats. That protein was found to be an endogenous ligand for the growth-hormone secretagogue receptor (GHS-R). When they administered the protein intravenously, the research team discovered that this peptide caused the release of growth hormone from the anterior pituitary comparable to response stimulated by growth hormone releasing hormone (GHRH). This peptide

hormone was subsequently named “ghrelin” after the Proto-Indo-European root word *ghre* for “grow”. Human ghrelin was found by this research group to be 82.9% homologous to rat ghrelin with a difference in only two amino acid residues (48). Ghrelin like many other hormones is cleaved from a larger protein, in this case prepro-ghrelin. Ghrelin becomes activated once it has been post-translationally modified by the addition of an n-octanoic acid group to the serine 3 residue of the N-terminal part of the protein (49). Date et al. showed that ghrelin is released from the endocrine X/A-like cells of the oxyntic glands lining the stomach into the circulation in the digestive system (50).

Subsequent studies found that ghrelin administered directly into the central nervous system and peripherally stimulates food intake, and that the response is mediated by binding of ghrelin to receptors in the ARC which leads to an increase in appetite (36, 51). In light of that information, Cummings et al. postulated that ghrelin may play a role in the initiation of meal consumption. To determine how ghrelin concentration changes in response to meal consumption, ten healthy subjects completed a 2-week controlled feeding period and were then admitted for an intervention of 24-hours. During the admission each participant was served 3 meals (35% fat, 45% carbohydrate, 20% protein) and had blood samples taken every half hour starting in the morning until night time and every hour through the night until the end of the 24-hour admission. The 38 samples collected were analyzed for concentrations of ghrelin, leptin, and insulin. Ghrelin was found to rise an average of 78% one to two hours before meals and fall to nadir within one hour of meal consumption. When the pattern of change was compared to that of insulin, it was found that they were inversely related to each other. Ghrelin decreased immediately after meal consumption while insulin exhibited an immediate increase. The relationship of ghrelin with leptin over the 24-hour period is also of note as they both exhibited a “diurnal rhythm”, both rose in concentration through 0100 and then fell until 0900 (52).

Wren et al. conducted a study to examine the effect of intravenous ghrelin vs. saline administration and its effect on appetite and amount of food consumed in a subsequent meal. Nine healthy, normal weight volunteers participated in the randomized crossover study where they were infused with ghrelin or saline at 0.2 pM/kg/min and blood samples were taken every 20 minutes starting at -20 minutes before baseline for a total of 270 minutes. The results were profound in that all participants experienced an increase in energy intake with the ghrelin infusion compared to the saline infusion ( $28 \pm 3.9\%$ ,  $p < 0.001$ ) when presented with a buffet style meal. Visual analog scales were also administered during this study and findings were significant for an increase in hunger before both breakfast and lunch during the ghrelin infusion compared to saline ( $16 \pm 10\%$  and  $46 \pm 20\%$ , respectively,  $p < 0.05$ ) (53).

Two studies were conducted by Weigle et al. to investigate the effects of consuming low and high carbohydrate meals on circulating ghrelin and leptin concentrations. The first study examined the effect of consuming a low fat, high carbohydrate diet on concentrations of leptin and ghrelin after weight loss. Eighteen subjects enrolled in the study for a total of 16 weeks. The first two weeks were a standard diet phase for weight maintenance (35% fat, 45% carbohydrate, 20% protein). During the second two weeks participants consumed a diet of a lower fat composition (15% fat, 65% carbohydrate, 20% protein) but isocaloric with the standard diet. The last 12 weeks of the study were an ad libitum phase in which the meals provided to the participants were of the same macronutrient composition as the previous two weeks. The results of the study revealed that there was no significant compensatory increase in ghrelin despite significant weight loss by the participants ( $p < 0.001$ ), regardless of macronutrient composition (54).

The second study investigated the potential effect of a high-protein diet on concentrations of leptin and ghrelin, satiety, and weight loss. Participants were first

placed on a two-week standard diet for weight maintenance containing 15% protein. The intervention diets used in this study were designed to provide the same carbohydrate composition but varied in the amount of protein provided during two separate two week long isocaloric controlled dietary periods (15% vs. 30%, respectively). The third ad libitum dietary period lasted for 12 weeks and had the same macronutrient composition of the intervention diet consumed during the second two week phase of the study.

Subjects were admitted for 24-hour inpatient visits on the last day after each of the two-week dietary intervention phases as well as at the end of the ad libitum phase. Blood samples were taken at half hourly and hourly time points to measure circulating ghrelin, leptin, and insulin concentrations throughout the 24-hour admission. The results from the study showed that participants experienced a significant increase in satiety while consuming the high protein diet validated by a decrease in energy intake during the ad libitum dietary phase ( $-441 \pm 63$  kcal/d,  $p < 0.01$ ). The decrease in energy intake was maintained throughout the end of the study despite a return to baseline of hunger and satiety scores. Body weight showed a decrease after high-protein diet consumption ( $-4.9 \pm 0.5$  kg) and area under the curve for leptin also decreased ( $402 \pm$  vs.  $259 \pm 35$  ng·24 h/ml,  $p < 0.05$ ). Area under the curve for ghrelin increased after consumption of the high-protein diet ( $13,979 \pm 1072$  vs.  $15,456 \pm 1173$  pg·24h/ml,  $p < 0.05$ ) The authors of this study concluded that the increased satiety experienced by participants on the high-protein diet was a result of increased sensitivity of the CNS to leptin which overruled the increase in ghrelin concentration which should have lead to an increase in feelings of hunger (55).

## **GLP-1 and PYY**

PYY and GLP-1 are peptides released from L cells in the small intestine, specifically the distal jejunum, ileum, and colon in response to the presence of food in the digestive tract (56, 57). GLP-1 is a 30 amino acid peptide hormone which is produced from the cleavage of proglucagon in the intestinal cells. A paper by Holst et al. states that once an increase in glucose or lipid in the intestinal lumen is sensed, GLP-1 is released from the L cells into the circulation. Once it is secreted, GLP-1 diffuses across the basal lamina into the lamina propria and capillary circulation. GLP-1 is susceptible to quick degradation by the enzyme dipeptidyl peptidase IV (DPP-IV) and is believed to act on receptors in the lamina propria, such as sensory afferent neurons originating from the nodose ganglion, before it is degraded. Although the exact mechanism is not clear, these afferent neurons are believed to have an effect on the solitary tract nucleus in the medulla oblongata, which in turn generates a response in the hypothalamus and sends impulses to the vagus nerve. There are also sensors in the liver and the hepatoportal circulatory system that have the same effect on the solitary tract nucleus. The motor neurons from the vagus nerve send inhibitory impulses to the gastrointestinal tract and excitatory signals to the pancreas (58). The result of the interaction of GLP-1 with the hypothalamus leads to an increase in insulin secretion from the pancreas, and a delay gastric emptying (57).

PYY, or PYY<sub>1-36</sub> is also a peptide hormone 36 amino acids in length and was isolated in 1980. PYY is secreted in response to the same macronutrient stimulus in the small intestine, and its release has been found to be moderated by gut peptides such as GLP-1 and gastrin. Once released from the L cells, PYY<sub>1-36</sub> travels through the blood stream to the brain and is broken down by dipeptidyl peptidase IV (DPP-IV) enzymes to produce the active form PYY<sub>3-36</sub>. PYY<sub>3-36</sub> crosses the blood brain barrier into the hypothalamus and binds to Y2 receptors on neuropeptide Y (NPY) neurons in the



arcuate nucleus of the hypothalamus. The binding of PYY to the Y2 receptor inhibits the release of NPY from the hypothalamus which leads to an increased feeling of satiety (35, 56).

Both PYY and GLP-1 are important in the feedback mechanism known as “ileal brake” (56, 58). Schirra et al. describes the mechanism of “ileal brake” as “the breaking of gastrointestinal transit and inhibition of exocrine pancreatic and gastric secretion triggered by nutrients in the distal intestine”. The macronutrient that has the greatest stimulatory effect on this mechanism is fat, but protein and carbohydrate also trigger the response. The feedback mechanism sends a signal from the distal portion of the small intestine to the proximal end and works to stop movement and secretion in the upper gastrointestinal tract. This inhibition of movement leads to a decrease in rate of absorption (59) and enhanced duration of satiety.

A recent randomized crossover study by Essah et al. investigated the response of PYY to the consumption of both low fat, high carbohydrate (25% fat, 65% carbohydrate, 10% protein) and high fat, low carbohydrate (74% fat, 6% carbohydrate, 20% protein) meals. Eighteen obese subjects were asked to consume a low fat, high carbohydrate or a high fat, low carbohydrate weight maintenance diet for one week, and one test meal of the same diet composition as their weight maintenance diet. Postprandial measurements of PYY were taken every 30 minutes for 2.5 hours. Concentrations of PYY rose within 15 minutes after consumption of the low carbohydrate, high fat meal, reached a plateau at 90 minutes, and gradually decreased through the end of the data collection period. After consumption of the high carbohydrate meal, postprandial PYY concentration rose to a peak level within 30 minutes, dropped after 60 minutes, and rose again gradually through the end of the data collection period. PYY concentration did not return to baseline after consumption of either meal type. Mean postprandial concentrations of PYY were reported to be 1.5 times higher after consumption of the low carbohydrate,

high fat test meal than after the high carbohydrate, low fat meal ( $p < 0.001$ ). Although both meals resulted in an increase in postprandial PYY concentrations from baseline, the low carbohydrate, high fat diet resulted in significantly greater PYY concentration over the time of the data collection ( $p=0.005$ ) (60). The increase in PYY concentrations should lead to a decrease in NPY secretion and a corresponding increase in satiety and decrease in hunger.

Peripheral administration of PYY and GLP-1 has been shown to reduce energy intake and suppress appetite in normal weight individuals (11, 61). In a randomized, placebo controlled, blinded, crossover study Flint et al. investigated the effect of peripheral administration of GLP-1 on 20 healthy, young male subjects. Each participant was fed an energy-fixed breakfast meal before GLP-1 administration and an ad libitum lunch meal after the infusion. They found enhanced feelings of satiety ( $p < 0.03$ ) and lower ad libitum energy intake by 12% ( $p=0.002$ ) after GLP-1 compared to saline infusion (11). Batterham et al. used a similar double blind, placebo controlled, crossover design to investigate the effect of peripheral PYY administration on appetite. Twelve obese and twelve lean subjects were given infusions of PYY and offered a buffet meal two hours after the infusion. The results showed a decrease in energy consumption by 30 and 31% in obese and lean subjects ( $p < 0.001$ ), respectively, after PYY infusion compared to placebo (61).

The additive effect of GLP-1 and PYY was also studied in both animals and humans. In the human portion of the study, ten young healthy participants were given four different infusions: saline, PYY<sub>3-36</sub>, GLP-1<sub>7-36</sub>, and an infusion of both PYY<sub>3-36</sub> and GLP-1<sub>7-36</sub>, each separated by a five day washout period. Each infusion was given for a period of 120 minutes, and each participant was served a buffet style lunch meal after 90 minutes of infusion. All participants were allowed to consume lunch for 30 minutes and then the food was taken away and the infusions were discontinued. Both pre and

postprandial blood samples were taken during the intervention. The researchers found that the combined infusion of GLP-1 and PYY resulted in a 27% reduction in buffet meal intake compared to each of the other separate infusions ( $p < 0.05$ ) (62). These results suggest that consumption of a diet that results in higher postprandial concentrations of PYY and GLP-1 should lead to a decrease in appetite, a subsequently decrease in energy consumption, and ultimately a reduction in body weight.

## Research Study Methods

### **Study Design**

The main goal of this study was to examine the effect of an acute feeding intervention of low carbohydrate (LC) and high carbohydrate (HC) meals on the circulating postprandial concentrations of insulin, leptin, ghrelin, peptide YY (PYY) and glucagon-like peptide 1 (GLP-1), and the effect on feelings of hunger and satiety. The study was conducted in a healthy, normal weight population using a randomized crossover design to measure differences in response between the two meals. Ten weight stable subjects participated in the study; 4 from a previous pilot study and 6 new recruits. Each study participant completed two 4-day controlled dietary phases separated by at least a three day wash-out phase. All study related procedures were reviewed and approved by the OHSU Institutional Review Board and performed in the Oregon Clinical & Translational Research Institute (OCTRI).

### **Subject Selection**

Study participants were healthy male and female adults aged 21-65 years, with a BMI between 19-25 kg/m<sup>2</sup>. Inclusion and exclusion criteria are detailed in Table 1. Subjects were recruited from the general population residing in the greater Portland area. All subjects remained free-living for the entirety of the study. Each subject was required to provide written informed consent before participating in any screening or intervention related activities. A copy of the consent form is provided in Appendix A.

### **Screening**

Pre-study screening visits were conducted for each potential participant to determine eligibility for the study. Each subject was provided a screening consent form, and once the potential participant fully comprehended the study requirements and gave their

written consent they continued with the established screening procedures. Participant height, weight, and blood pressure were measured at the OCTRI outpatient nursing station by trained research nursing staff, and a fasting blood sample was obtained via finger-stick for point of care hemoglobin (HemoCue  $\beta$ -hemoglobin photometer, HemoCue AB, Angelholm, Sweden) and glucose assessment (Precision Xceed Pro blood glucose monitor, Abbott Laboratories, Alameda, CA). A urine sample was obtained from female participants to rule-out pregnancy. Concentration of human chorionic gonadotropin (hCG) in the urine samples were measured using Aceava hCG Combo II test cartridges (Inverness Medical, Waltham, MA). Forms were administered at this time to assess physical activity (Baecke Activity Questionnaire (63)), medical history (Cornell Medical Index (64)), and food preferences. Subjects judged to be healthy by self-report, review of medical history and medication use, and lab screenings were considered eligible for participation. No subjects screened for this study required a second visit for a physical examination by the study physician for further determination of eligibility.

**Table 1. Inclusion and Exclusion Criteria**

<b>Inclusion</b>	<b>Exclusion</b>
<ul style="list-style-type: none"><li>• BMI 19-25 kg/m<sup>2</sup></li><li>• Age: 21-65 yrs</li><li>• Good health</li><li>• Willingness to eat both a high and low carbohydrate diet</li><li>• Willingness to stop taking multivitamins or any other dietary supplements for the duration of the study</li></ul>	<ul style="list-style-type: none"><li>• Major debilitating mental or physical illness that would interfere with participation.</li><li>• Pregnancy or lactation within the last 12 months</li><li>• Weight instability (any loss or gain of more than <math>\pm</math> 5% within last 6 months)</li><li>• Current participation in a self-directed or commercial weight loss plan</li><li>• Any self imposed food restrictions (eg: kosher, vegetarian diet) that the participant would not be willing to stop for the duration of the study.</li><li>• Any food allergies or food preferences that are not consistent with the research diets</li><li>• Prescription medication use, with the exception of birth control and intermittent over the counter analgesics.</li></ul>

### **Standardization Protocol**

On days 1-3 of the protocol each participant arrived at the OCTRI Outpatient Unit between 0700 and 1000 and had their weight measured. Each subject was fed a standard diet (50% carbohydrate, 35% fat and 15% protein) designed to meet their individual energy needs to prevent weight loss or weight gain. The purpose of the standard diet was to minimize variation related to inherent differences in dietary intake between subjects. The participants ate breakfast in the OCTRI Bionutrition Unit dining room and discussed any study related items with the study coordinator. All other meals and snacks for the remainder of the day were prepared for each participant to take home. Participants were asked to consume all the food provided to them and nothing else.

### **Intervention Protocol**

At about 0600 of the fourth study day, each subject was admitted to the inpatient unit of the OCTRI. The total length of stay for each participant was approximately 12 hours. Subjects were not allowed to eat or drink anything except for water after 2200 the night before, and were asked to refrain from significant physical activity for 24 hours prior to admission. Blood pressure and vital signs were taken, and an indwelling catheter was placed in a peripheral vein in the subject's arm. A half-normal saline gravity drip was infused to keep the system open. Fasting blood samples were taken at 0800, after which the subject consumed either a low carbohydrate or high complex-carbohydrate breakfast. A postprandial blood sample was taken at 0830 and subsequent samples were taken every hour after for nine hours and at 1300. The study subject consumed a lunch meal of the same macronutrient composition as the breakfast meal between 1300 and 1330. A detailed table of the blood sample collection schedule is shown in Appendix B. After the completion of postprandial blood sample collection, participants were

offered a dinner meal of their choice from the OHSU Food and Nutrition Services menu. Participants were discharged with orders to resume their usual diet and activity level and to return to repeat the 4 day procedure as scheduled. During their second admission, each participant completed the same measurement and blood sampling protocol, but consumed the alternative diet option of either low carbohydrate or high complex carbohydrate meals compared to their first admission.

### **Inpatient Intervention Diets**

During the inpatient admissions, participants consumed either a low carbohydrate meal, modeled after the Atkins diet induction phase, or a high complex carbohydrate meal, modeled after the Dietary Approaches to Stop Hypertension (DASH) diet. The planned macronutrient composition of the low carbohydrate meals was 66% fat, 30% protein, and 4% carbohydrate, as used in previous studies. The planned macronutrient composition of the high complex carbohydrate meals was 27% fat, 55% carbohydrate, and 18% protein, as used in previous studies. Each meal consumed during the inpatient admission provided 10 kcal/kg of body weight.



## **Measurements**

### **Body Composition:**

Body weight was measured in light clothing each morning on Days 1-4 with a digital scale (Scale-Tronix, Model 5002, Carol Stream, IL) in the Bionutrition Unit of the OCTRI. Height was measured without shoes on Day 1 by a Harpenden wall mounted stadiometer (Holtain Ltd., UK). Body composition was measured by total body dual energy X-ray absorptiometry (DEXA) scan (Discovery A Series Densitometer, Hologic Inc., Bedford, MA) before the 1<sup>st</sup> or 2<sup>nd</sup> inpatient admission.

### **Blood Pressure and Vital Signs:**

On Day 4, blood pressure and vital signs were taken with the participant in a sitting position using an automated vital signs monitor (Dinamap XL Vital Signs Monitor, Critikon Corp., Tampa, FL). Temperature was taken using an electronic thermometer (SureTemp 678, Welch Allyn, Skaneateles Falls, NY).

### **Hunger/Satiety Level:**

Participants reported levels of hunger and fullness using 100-mm visual analog scales (VAS) 15 minutes before and after each meal was consumed during each inpatient admission. A copy of the visual analog scale questionnaire is provided in Appendix C. The visual analog scales consisted of two questions: one asking how hungry and one asking how full the participant felt when the form was administered. The participant was asked to make a mark on the 100 mm line that corresponded with how hungry or full they felt at that time. Each 100 mm line was anchored by the phrases “Not at all hungry” or “Not at all full” on the left side of the line and “Extremely hungry” or “Extremely full” on the right side of the line. The magnitude of the participant responses was measured from

the left anchor of the line to the left side of the mark made using a ruler. Visual analog scales were administered by either a designated nurse or the study coordinator.

### **Blood sample analysis**

Blood samples were collected in pre-chilled phlebotomy tubes, except for serum samples which were collected in tubes maintained at room temperature. All plasma tubes were centrifuged under 4°C refrigerated conditions immediately after each collection for ten minutes at a speed of 1500 rcf. The plasma was harvested and divided into polypropylene aliquot tubes to be stored for batched analysis at the end of the study. Serum samples were centrifuged and processed for storage using the same process as plasma samples after 20-30 minutes of clotting time. All storage tubes were frozen at -20°C and then transferred within 24 hours to -80°C freezers until the time of analysis. Insulin, glucose, leptin, total and active ghrelin, PYY, and GLP-1 concentrations were measured in fasting and post-prandial blood samples obtained during each inpatient admission.

Glucose concentrations were measured in duplicate in sodium-fluoridated plasma using a colorimetric assay (Stanbio Laboratory, Boerne, TX). The lowest concentration able to be detected by the glucose assay is 70 mg/dl. The average intra-assay % coefficient of variation (CV) was 1.6, 1.9, and 2.4% for three controls after six assays. If the percent difference for a sample was >10% between duplicates, it was re-analyzed. Insulin concentrations were measured in singlet in serum using a chemiluminescent immunoassay on the automated immulite system (Siemens Medical Solutions Diagnostics, Los Angeles, CA). The lowest concentration of insulin able to be detected by the assay is 2.0  $\mu$ IU/ml. The average intra-assay %CV for insulin was 2.6, 4.8, 0.4, and 3.5% for four controls after four assays. Leptin concentrations were measured in duplicate in serum using an immunoradiometric assay (IRMA) (Diagnostic

Systems Laboratories Inc., Webster, TX). The lowest concentration of leptin able to be detected by the assay is 0.5 ng/ml. The average intra-assay %CV for leptin was 2.5 and 4.2% for two sets of controls after four assays. If the percent difference for a sample was >10% between duplicates, it was re-analyzed.

Total ghrelin and active ghrelin concentrations were measured in duplicate in ethylenediaminetetraacetic acid (EDTA) plasma using a radioimmunoassay (RIA) (LINCO Research, St. Charles, MO). A volume of 25  $\mu$ l of hydrochloric acid (HCl) and 5  $\mu$ l of phenylmethanesulphonyl fluoride (PMSF) was added to 500  $\mu$ l plasma for active ghrelin analysis to inhibit the action of serine proteases. The lowest concentrations of total and active ghrelin able to be detected by the assays are 93 pg/ml and 7.8 pg/ml, respectively. The average intra-assay %CV was 9.0 and 3.5% for two sets of controls after six total ghrelin assays. If the percent difference for a sample was >10% between duplicates, it was re-analyzed. The average intra-assay %CV was 7.2 and 9.3% for two sets of controls after five assays for active ghrelin. For active ghrelin, if the percent difference between duplicates for a sample was greater than 15%, it was re-analyzed.

GLP-1 concentrations were measured in duplicate in EDTA and dipeptidyl peptidase-IV (DPP-IV) inhibitor (15  $\mu$ l) treated plasma using enzyme-linked immunosorbent assay (ELISA) (LINCO Research, St. Charles, MO). The lowest concentration of GLP-1 able to be detected by the assay is 2 pM/ml. The average intra-assay %CV was 4.1 and 7.6% for two sets of controls after seven assays for GLP-1. If the percent difference for a sample was >10% between duplicates, it was re-analyzed. PYY concentrations were measured in duplicate in EDTA, DPP-IV inhibitor (15  $\mu$ l), and aprotinin (90  $\mu$ l) treated plasma using a RIA (LINCO Research, St. Charles, MO). The lowest concentration of PYY able to be detected by the assay is 20 pg/ml. The average intra-assay CV was 12.1 and 5.7% for two sets of controls after six assays for PYY. For PYY, if the percent difference between duplicates for a sample was greater than 15%, it

was re-analyzed. A constant internal control sample was not run with each assay; therefore inter-assay %CV was not able to be calculated for any of the analytes.

## **Calculations**

### **Estimated Energy Requirements:**

The energy intake necessary to maintain the body weight of participants during the run-in phase of the study was calculated using the Harris-Benedict energy prediction equation and multiplied by an activity factor (65). The activity factor was determined by the average amount of physical activity (1.3 for very inactive to 1.6 for very active) for each participant as estimated by the Baecke activity questionnaire and participant interview. The Harris-Benedict equation listed below takes into consideration a person's sex, age, height and weight.

### **Harris-Benedict equation:**

Basal Energy Requirements (male) =  $66 + 13.7 (\text{weight in kg}) + 5 (\text{height in cm}) - 6.8$   
(age in years)

Basal Energy Requirements (female) =  $665 + 9.6 (\text{weight in kg}) + 1.8 (\text{height in cm}) -$   
4.7 (age in years)

### **Leptin Normalization:**

Leptin concentration for each participant was normalized to his/her respective fat mass by dividing leptin concentration (ng/ml) by fat mass in kilograms. Fat mass for each participant was obtained from the results of their DEXA scan.

### **Percent Suppression and Time to Nadir of Total and Active Ghrelin:**

The nadir for total and active ghrelin was defined as the minimum concentration of ghrelin between the first postprandial blood sample after breakfast at 0830 and the 1300 blood sample before lunch was consumed. Percent suppression of total and active ghrelin was calculated by subtracting the nadir concentration from baseline (fasting) concentration, then dividing the result by the nadir concentration. Time to nadir for both total and active ghrelin was calculated as the time in hours from the first postprandial time point after breakfast at 0830 to the nadir value for each participant before the lunch meal was consumed at 1300.

### **Contrasts between Meals for Visual Analog Scales:**

Linear contrasts were created to test for differences in feelings of hunger and fullness after LC and HC meals were consumed. Contrasts were created by subtracting the post-meal hunger and fullness scores from the pre-meal hunger and fullness scores. The mean of the difference obtained for each participant for each contrast was tested using Wilcoxon signed-rank analysis to determine if that change was different from zero. The four linear contrasts that were created are shown in Table 2. The abbreviations  $A_1$  and  $B_1$  correspond to pre- and post-meal hunger or satiety scores for breakfast. The abbreviations  $A_2$  and  $B_2$  correspond to the pre- and post-meal hunger or satiety scores for lunch. Contrasts LC1 and HC1 were calculated to be the difference between pre- and post-breakfast hunger and fullness scores for the LC and the HC meals, respectively. Contrasts LC2 and HC2 were calculated to be the difference between pre- and post-lunch hunger and fullness scores for the LC and the HC meals, respectively. Contrasts LC3 and HC3 were calculated to be the difference in the change in the breakfast and lunch scores. This third contrast was calculated separately for both the LC and HC meals for both hunger and fullness. The linear contrasts LC4 and HC4 were

calculated to be the difference in the average change between the breakfast and lunch scores. The fourth contrast was calculated separately for both the LC and HC meals for both hunger and fullness. Contrast L4 was only analyzed for significance if the change for L3 was not significantly different from zero. Contrasts 1-3, and contrast 4 if necessary, for the LC and HC meals were compared to each other for both hunger and fullness scores using Wilcoxon signed-rank tests to determine if there was a difference in the responses between the two diets.

**Table 2. Linear Contrasts for Visual Analog Scales**

Contrasts	Meals	
	Breakfast (1)	Lunch (2)
LC1/HC1 and LC2/HC2	$A_1 - B_1$	$A_2 - B_2$
LC3/HC3	$(A_2 - B_2) - (A_1 - B_1)$	
LC4/HC4	$(A_2 + A_1)/2 - (B_2 + B_1)/2$	
A = post-meal hunger/fullness score, B = pre-meal hunger/fullness score LC = contrast for low carbohydrate meals HC = contrast for high carbohydrate meals		

### **Data Management**

All subject data collected as a result of participation in this study was kept completely confidential. Forms, except a master copy, identify patients only by their specific study ID and were kept in a locked office in the OHSU Hatfield Research Building. Specific forms were developed for each data set including patient demographics and history, and each discrete outcome variable. Computer databases were developed to store information and were password protected; only those study staff with assigned passwords were allowed to access participant data.

### **Missing Data**

At the 1430 time point during one admission, blood samples were not drawn for one participant due to loss of IV access. One sample at one time point (1430) for insulin for one participant was also unavailable for analysis. Missing data for blood samples were interpolated by taking the average of the values at the time points before and after the missing data point. There was one missing value for baseline hunger response for one participant. The missing data point was imputed as the mean before breakfast hunger score for the rest of the participants on the same LC diet day.

### **Statistical Analysis**

The change in the concentrations of weight regulation markers from baseline were analyzed using area under the curve (AUC) from breakfast to lunch, lunch to discharge and breakfast to discharge (total) calculated by the trapezoidal method (66). Differences in AUC after the LC and HC meals were compared using one sided paired t-tests. Patterns of postprandial changes in glucose, insulin, leptin, total ghrelin, GLP-1, and PYY concentrations over time were modeled by orthogonal linear, quadratic, cubic and quartic polynomial analyses. Components of the polynomial equations were assessed for significance using a Wilcoxon signed-rank test. Differences between concentrations for each analyte at pre-selected time points after LC and HC meal consumption were analyzed using repeated measures analysis of variance (MANOVA). Fixed effects included type of diet (LC or HC), order of diet consumption, and time. If the MANOVA revealed a significant effect, follow-up comparisons of differences at the individual time points were assessed using a t-critical value corrected for multiple comparisons by the Bonferroni procedure (67). Contrasts between pre-selected time points were also analyzed for differences between LC and HC meals, with differences assessed using two sided, paired t-tests.

The change in hunger and fullness after consumption of LC and HC meals were analyzed using four linear contrasts. Contrasts were created between the hunger and fullness scores obtained before and after the breakfast and lunch meals. The calculations section explained the details of how each contrast was constructed and the model for each individual contrast is shown in Table 2. Differences in the linear contrasts between the LC and HC meals for VAS hunger and fullness scores were analyzed using a Wilcoxon signed-rank test. In all instances, a p-value of  $< 0.05$  was considered statistically significant and data analyses were performed using STATA (version 10.0; StataCorp LP, College Station, Texas).



## Results

### **Sample Characteristics**

The acute response of weight regulation markers to the consumption of low carbohydrate and high carbohydrate meals was studied in healthy, weight stable male and female subjects. A total of 11 participants were enrolled in the study and all were Caucasian. Four male and six female participants completed the study (n=10). One participant dropped out due to time constraints. All participants met the inclusion criteria described in Table 1. The average age was  $24.5 \pm 2.7$  yr and the average BMI was  $22.8 \pm 2.1$  kg/m<sup>2</sup>. The average fat mass and percent body fat of participants were  $16.1 \pm 3$  kg and  $23.7 \pm 4.5\%$ , respectively. Body weight for all subjects remained stable throughout the three day standard diet phase and during the washout period between dietary phases (data not shown).

The 3-day standard diet provided  $2775 \pm 43$  kcal/d and was comprised of 51% carbohydrate, 14% protein, and 35% fat. All subjects tolerated meals served and compliance was high as assessed by visual inspection of food containers upon return to the OCTRI kitchen. The macronutrient composition of the LC test meals was 4% carbohydrate, 30% protein, and 66% fat. The macronutrient composition of the HC test meals was 55% carbohydrate, 18% protein, and 27% fat. The average amount of energy consumed by participants during the inpatient admissions was  $1366 \pm 241$  kcal (10 kcal/kg) for both meals and was the same for both the LC and HC test meals. Complete consumption of test meals was verified by visual inspection of participant trays by study coordinators during each inpatient admission. Further description of the macro and micronutrients consumed by participants during the standardization diet and test meals is presented in Tables 3 and 4.

**Table 3: Average Energy and Nutrient Consumption during the Three Day Standardization Phase**

<b>Component</b>	<b>Mean ± SD</b>	<b>mg or g/1000 kcal</b>
<b>Energy (kcal/d)</b>	2775 ± 43	
<b>Carbohydrate (g)</b>	360 ± 57	130
<b>Protein (g)</b>	101 ± 16	36
<b>Fat (g)</b>	111 ± 18	40
<b>Cholesterol (mg)</b>	228 ± 51	104
<b>Total Dietary Fiber (g)</b>	32 ± 0.43	9
<b>Sodium (mg)</b>	4035 ± 709	1454
<b>Potassium (mg)</b>	3022 ± 646	1089
<b>Calcium (mg)</b>	1876 ± 86	557
<b>Phosphorous (mg)</b>	1601 ± 399	557

**Table 4. Average Energy and Nutrient Consumption during the Low and High Carbohydrate Test Meals**

<b>Dietary Component</b>	<b>HC Meals</b>	<b>LC Meals</b>
<b>Energy (kcal/d)</b>	1366 ± 241	
<b>Carbohydrate</b>		
mean ± SD (g)	194 ± 34	12 ± 2
g/1000 kcal	142	9
<b>Protein</b>		
mean ± SD (g)	63 ± 11	103 ± 18
g/1000 kcal	46	75
<b>Fat</b>		
mean ± SD (g)	41 ± 7	99 ± 17
g/1000 kcal	30	73
<b>Cholesterol</b>		
mean ± SD (mg)	181 ± 32	895 ± 157
mg/1000 kcal	133	655
<b>Total Dietary Fiber kcal)</b>		
mean ± SD (g)	16 ± 3	2 ± 0.35
g/1000 kcal	12	1.5
<b>Sodium</b>		
mean ± SD (mg)	1716 ± 304	2793 ± 494
mg/1000 kcal	1256	2044
<b>Potassium</b>		
mean ± SD (mg)	1835 ± 327	1459 ± 254
mg/1000 kcal	1343	1068
<b>Calcium</b>		
mean ± SD (mg)	660 ± 120	721 ± 129
mg/1000 kcal	483	528
<b>Phosphorus</b>		
mean ± SD (mg)	1090 ± 194	1298 ± 229
mg/1000 kcal	798	950

## Postprandial Analyte Concentrations and Areas Under the Curve

Mean concentration of each analyte at each time point and AUC analysis for each analyte are presented in Tables 5 and 6, respectively.

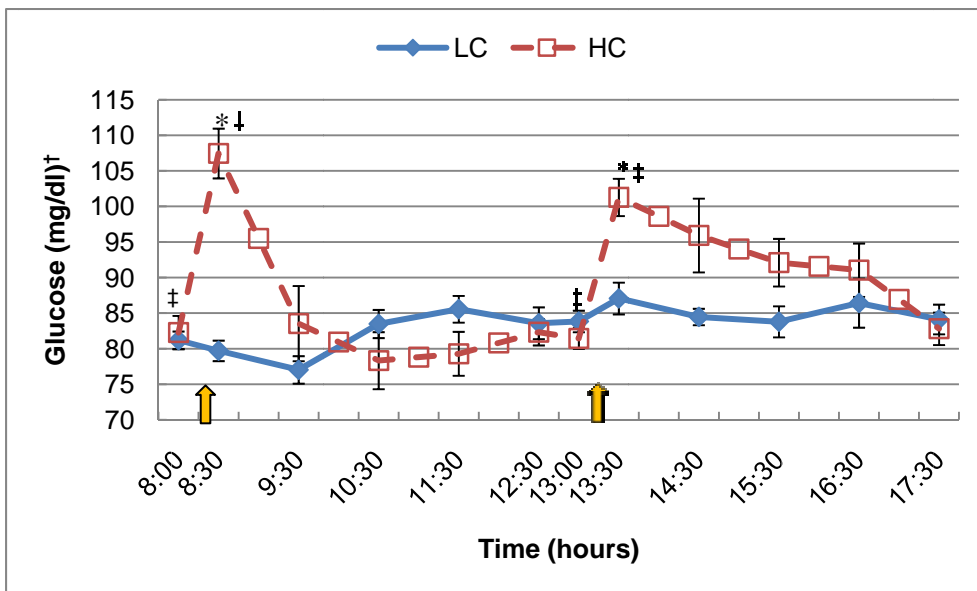
### **Postprandial Glucose Concentration and Area Under the Curve**

A graph illustrating differences in the patterns of change over time for glucose after the LC and the HC meals is depicted in Figure 1. Mean fasting glucose concentrations were similar at  $82.3 \pm 2.3$  and  $81.2 \pm 1.2$  mg/dl before the HC and LC meals, respectively ( $p=0.6$ ). Mean glucose concentration increased by 31% from baseline 30 minutes after the HC breakfast meal to  $107.5 \pm 3.5$  mg/dl, then returned to baseline within 90 minutes. Mean glucose concentration after the LC meal decreased by 6% from baseline to  $77.0 \pm 1.9$  mg/dl within 90 minutes then rose to just above baseline for the remainder of the observation period. There was no significant difference in AUC of glucose for the postprandial period after the LC or HC breakfast meals ( $410 \pm 7$  vs.  $425 \pm 13$  mg·h/dL, respectively,  $p=0.33$ ). The AUC for the postprandial period after lunch was significantly lower after the LC compared to the HC meal ( $425 \pm 8$  vs.  $457 \pm 12$  mg·h/dL, respectively,  $p<0.05$ ). The total AUC for glucose was also significantly lower after the LC compared to the HC meals ( $793 \pm 13$  vs.  $841 \pm 20$  mg·h/dL, respectively,  $p<0.05$ ).

Repeated measures ANOVA for time points 0800, 0830, 1300, 1330 and 1730 revealed a significant effect of diet on glucose concentration for at least one of the time points selected ( $p<0.01$ ). Post-hoc analysis at 0830 and 1330 showed that glucose concentration was significantly higher immediately after the HC breakfast and lunch meals than the LC meals ( $p<0.05$ , adjusted for 4 comparisons). There was no difference in glucose concentration between HC and LC meals at the 1300 or 1730 time points

( $p > 0.05$ ). Contrast analysis between the before and after meal time points of 0800 and 0830, and 1300 and 1330 showed a significantly greater change in glucose concentration after HC than LC meal consumption ( $p < 0.05$ ). Consumption of HC meals resulted in significantly greater postprandial increases in glucose concentration and AUC compared to LC meals.

**Figure 1.** Postprandial changes in glucose concentrations after low and high carbohydrate meal consumption<sup>†</sup>



<sup>†</sup>Mean  $\pm$  SEM, meal times indicated by arrows

LC = low carbohydrate

HC = high carbohydrate

Repeated Measures ANOVA @ 0800, 0830, 1300, 1330, 1730: \*significantly different between meals ( $p < 0.05$ )

Contrast analysis between 0800 and 0830, 1300 and 1330: <sup>‡</sup>significantly different between meals ( $p < 0.05$ )

**Table 5. Fasting and Postprandial Concentrations of Weight Regulation Markers before and after Low and High Carbohydrate Meals\***

Analyte	Meal	0800	0830	0930	1030	1130	1230	1300	1330	1430	1530	1630	1730
Glucose (mg/dl)	LC	81±1	80±1	77±2	84±2	86±2	84±2	84±2	87±2	85±1	84±2	86±4	84±2
	HC	82±2	106±4	84±5	78±4	79±3	82±2	81±2	101±3	96±5	92±3	91±4	83±2
Insulin (µU/ml)	LC	4±0.6	9±1	8±1	8±0.7	9±0.9	8±1	8±1	11±1	10±2	9.1±2	10±1	8±2
	HC	4±0.6	51±11	39±6	22±3	12±2	7±2	6±1	24±3	35±5	33±3	26±4	16±3
Leptin (ng/kg fat mass·ml)	LC	0.36±0.1	0.34±0.1	0.33±0.1	0.30±0.1	0.29±0.1	0.28±0.1	0.28±0.1	0.28±0.1	0.29±0.1	0.29±0.1	0.28±0.1	0.29±0.1
	HC	0.36±0.1	0.34±0.1	0.36±0.1	0.37±0.1	0.40±0.1	0.38±0.1	0.37±0.1	0.37±0.1	0.39±0.1	0.42±0.1	0.44±0.1	0.46±0.1
Total Ghrelin (pg/ml)	LC	971±232	953±213	912±211	887±226	795±180	807±188	767±169	793±180	717±147	732±171	728±167	699±152
	HC	923±192	843±152	650±126	680±140	836±203	974±229	923±189	842±133	709±135	686±147	746±160	836±179
Active Ghrelin (pg/ml)	LC	128±52	88±18	69±11	84±22	103±40	81±14	93±26	78±12	73±18	76±18	90±22	111±35
	HC	115±47	124±35	86±34	88±23	83±12	97±21	100±22	115±40	83±22	81±26	75±21	81±19
GLP-1 (pM/ml)	LC	2.5±0.5	4.1±0.8	5.9±1.2	5.9±1.1	5.7±1.3	5.9±1.4	5.1±1.1	8.5±1.3	7.2±1.5	5.7±1.2	4.9±1.1	4.3±0.8
	HC	2.5±0.7	7.7±1.9	4.2±0.8	4.1±1.0	3.7±0.9	3.9±0.9	3.2±0.7	6.5±1.5	5±1	3.0±0.8	4±0.9	3.5±0.8
PYY (pg/ml)	LC	83±9	92±9	108±11	110±11	116±12	108±9	109±8	136±17	134±12	126±12	130±12	116±10
	HC	85±10	96±9	98±7	103±7	100±8	101±7	99±9	109±10	106±9	101 ± 8	96±8	101±9

\*Mean ± SEM  
LC = low carbohydrate  
HC = high carbohydrate

**Table 6. Area Under the Curve for Glucose, Insulin, Leptin, Total and Active Ghrelin, GLP-1, and PYY\***

Analyte	AUC 1 <sub>(0800-1300)</sub>			AUC 2 <sub>(1300-1730)</sub>			Total AUC <sub>(0800-1730)</sub>		
	LC	HC	Δ	LC	HC	Δ	LC	HC	Δ
<b>Glucose (mg-h/dl)</b>	410 ± 7	425 ± 13	-15 ± 14	425 ± 8	457 ± 12	-32 ± 16 <sup>a</sup>	793 ± 13	841 ± 20	-48 ± 26 <sup>a</sup>
<b>Insulin (μU-h/ml)</b>	41 ± 4	118 ± 12	-77 ± 11 <sup>b</sup>	47 ± 7	125 ± 13	-78 ± 8 <sup>b</sup>	84 ± 10	240 ± 23	-156 ± 16 <sup>b</sup>
<b>Leptin (ng-h/ml·kg fat mass)</b>	1.6 ± 0.33	1.9 ± 0.41	-0.30 ± 0.16 <sup>a</sup>	1.4 ± 0.28	2.0 ± 0.46	-0.62 ± 0.23 <sup>a</sup>	2.8 ± 0.58	3.7 ± 0.83	-0.87 ± 0.36 <sup>a</sup>
<b>Total Ghrelin (pg-h/ml)</b>	4739 ± 1101	4422 ± 921	317 ± 228	3313 ± 734	3409 ± 677	-96 ± 124	7662 ± 1746	7391 ± 1517	270 ± 333
<b>Active Ghrelin (pg-h/ml)</b>	482 ± 120	530 ± 137	-47 ± 26	376 ± 88	390 ± 113	-14 ± 37	815 ± 199	865 ± 235	-51 ± 41
<b>GLP-1 (pM-h/ml)</b>	31 ± 6	25 ± 5	7 ± 2 <sup>b</sup>	27 ± 5	20 ± 4	7 ± 2 <sup>b</sup>	55 ± 11	42 ± 9	13 ± 3 <sup>b</sup>
<b>PYY (pg-h/ml)</b>	594 ± 54	547 ± 42	47 ± 33	574 ± 52	459 ± 37	115 ± 39 <sup>b</sup>	1107 ± 100	954 ± 72	153 ± 64 <sup>a</sup>

\*Mean ± SEM; LC = low carbohydrate; HC = high carbohydrate; Δ = AUC<sub>LC</sub> – AUC<sub>HC</sub>  
<sup>a</sup>Significantly different between meals (p<0.05)  
<sup>b</sup>Significantly different between meals (p<0.01)

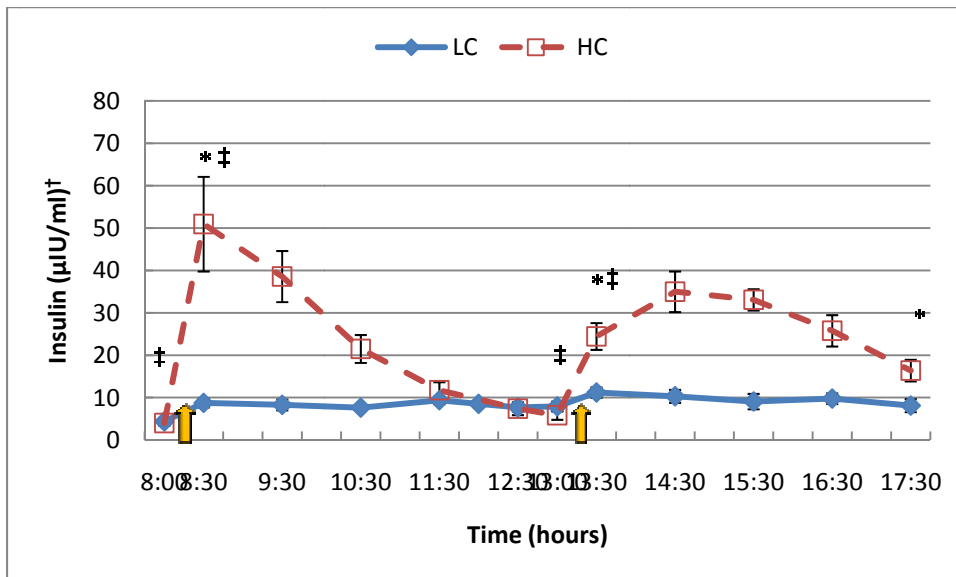
## Postprandial Insulin Concentration and Area Under the Curve

The postprandial patterns of change in insulin concentration were also different after consumption of the LC and HC meals (Figure 2). Mean fasting insulin concentration was  $4.4 \pm 0.6$  and  $4.0 \pm 0.6$   $\mu\text{IU/ml}$  before the LC and HC meals, respectively, and were similar ( $p=0.4$ ). Mean insulin concentration rose to  $51.0 \pm 11.2$   $\mu\text{IU/ml}$ , an 1175% increase, 30 minutes after the HC breakfast was consumed and gradually returned to baseline 3 hours after meal consumption. After eating the HC lunch, mean insulin concentration rose significantly and fell more gradually but did not return to baseline by the end of the sampling period. In contrast, after eating LC meals insulin concentrations rose by 98% from baseline to  $8.7 \pm 1.0$   $\mu\text{IU/ml}$  and remained stable throughout the sampling period. The post breakfast and post lunch AUC as well as total AUC for insulin were significantly higher after the HC than the LC meals ( $p<0.01$ ) (Table 6).

Repeated measures ANOVA for time points 0800, 0830, 1300, 1330 and 1730 revealed a significant effect of diet on at least one of the time points selected ( $p<0.01$ ). Post-hoc analysis showed a significantly higher insulin concentrations after the HC meals than the LC meals at 0830, 1330, and 1730 ( $p<0.05$ , adjusted for 4 comparisons) which corresponded to samples taken immediately after each meal and at the end of the sampling period. The significant difference in insulin concentration at 1730 shows that insulin concentrations remained higher at the end of the sampling period after HC compared to LC meal consumption ( $p<0.05$ ). There was no difference in insulin concentration at 1300 between LC and HC meals ( $p>0.05$ ). Contrast analysis between the before and after meal time points of 0800 and 0830, and 1300 and 1330 confirmed a significantly greater increase in insulin concentration after HC meal consumption than LC meal consumption ( $p<0.05$ ). Consumption of HC meals resulted in greater postprandial increases in insulin concentration and AUC compared to LC meals.



**Figure 2.** Postprandial changes in insulin concentrations after low and high carbohydrate meal consumption<sup>†</sup>



<sup>†</sup>Mean ± SEM, meal times indicated by arrows

LC = low carbohydrate

HC = high carbohydrate

Repeated Measures ANOVA @ 0800, 0830,1300,1330,1730:\* significantly different between meals (p<0.05)

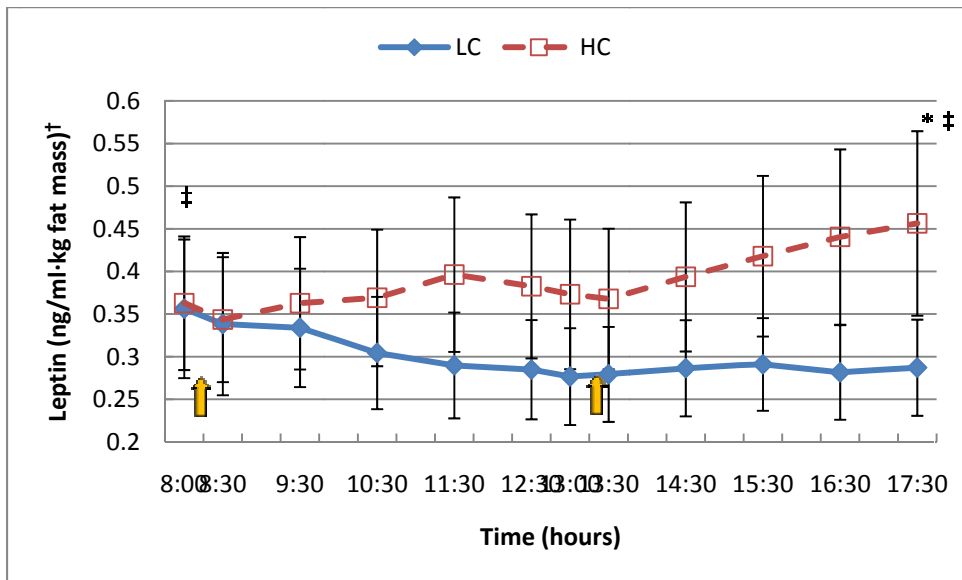
Contrast analysis between 0800 and 0830, 1300 and 1330:<sup>†</sup> significantly different between meals (p<0.05)

### **Postprandial Leptin Concentration and Area Under the Curve**

Differences in the pattern of change over time for leptin concentration indexed to body fat mass are depicted in Figure 3. Mean fasting leptin concentration was 0.36 ng/ml·kg fat mass before both the LC and HC meals. After consumption of the LC and HC meals, leptin levels decreased by 9.0% from baseline within the first half hour. Postprandial leptin concentration after the LC meals continued to decrease through the rest of the sampling period. In contrast, leptin concentration increased after the HC meals for the duration of the sampling period. Area under the curve was significantly lower after the LC breakfast ( $1.6 \pm 0.33$  vs.  $1.9 \pm 0.41$  ng·h/kg fat mass·ml,  $p < 0.05$ , respectively) and lunch meals ( $1.4 \pm 0.28$  vs.  $2.0 \pm 0.46$  ng·h/kg fat mass·ml,  $p < 0.05$ , respectively). The total AUC was also lower after the LC than the HC meals ( $2.8 \pm 0.58$  vs.  $3.7 \pm 0.83$  ng·h/kg fat mass·ml,  $p = 0.04$ , respectively) (Table 6).

Repeated measures ANOVA for time points 0800 and 1730 revealed a significant effect of diet on at least one of the time points selected ( $p < 0.01$ ). Post-hoc analysis showed a significantly higher leptin concentration after the HC than the LC meals at 1730 ( $p < 0.01$ , adjusted for two comparisons) corresponding to the sample taken at the end of the sampling period. There was no significant difference found between meals at the baseline time point of 0800. Contrast analysis between the baseline sample at 0800 and the ending time point of 1730 confirmed that the change in leptin concentration was significantly different ( $p < 0.01$ ) after the HC and LC meals. Consumption of LC meals resulted in a decrease in leptin concentration from baseline and lower AUC compared to HC meals.

**Figure 3.** Postprandial changes in leptin concentrations after low and high carbohydrate meal consumption<sup>†</sup>



<sup>†</sup>Mean  $\pm$  SEM, meal times indicated by arrows

LC = low carbohydrate

HC = high carbohydrate

Repeated Measures ANOVA @ 0800 and 1730: \*significantly different between meals ( $p < 0.05$ )

Contrast analysis between 0800 and 1730: <sup>†</sup>significantly different between meals ( $p < 0.05$ )

## Postprandial Total Ghrelin Concentration and Area Under the Curve

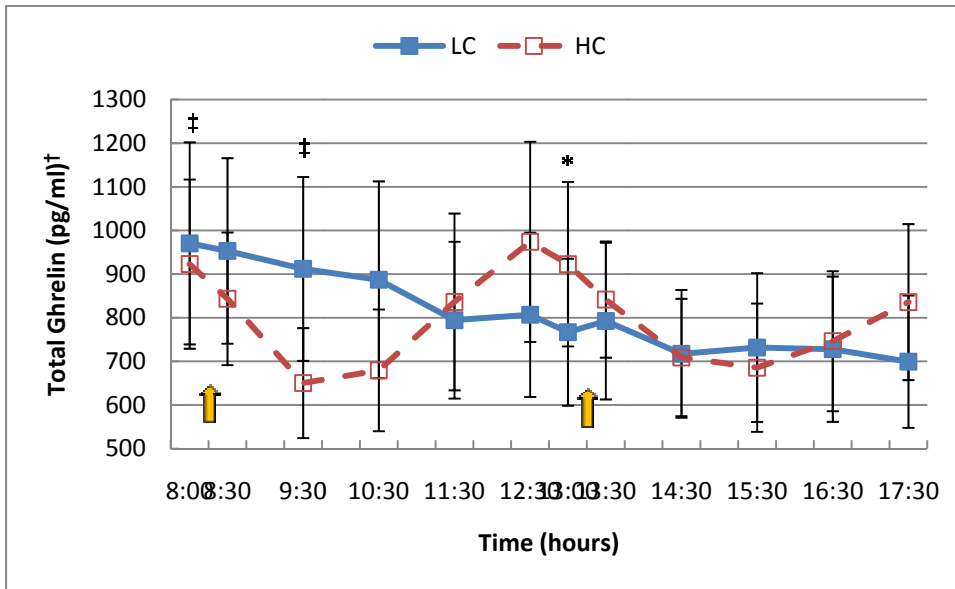
Differences in postprandial changes in concentration of total ghrelin over time after LC and HC meals are shown in Figure 4. Mean fasting total ghrelin concentrations were similar ( $p=0.3$ ) at  $970.6 \pm 231.6$  and  $923 \pm 123.9$  pg/ml before the LC and HC meals, respectively. After eating the HC breakfast, mean total ghrelin concentration decreased 30% from baseline within 60 minutes, and then rose to slightly above baseline before lunch. This pattern was repeated after the HC lunch meal was consumed. In contrast, after eating the LC meals, total ghrelin concentration decreased gradually from baseline until the end of the observation period by 301 pg/ml, or 31%.

Maximal percent suppression and time to nadir of total ghrelin concentration were also analyzed in the postprandial period after breakfast. After the LC meal and the HC meal, the mean maximal percent suppression of total ghrelin from baseline was 22% and 24%, respectively, which was not different ( $p=0.5$ ). The mean time to nadir after the LC vs. the HC breakfast meal was  $4.6 \pm 0.2$  and  $1.8 \pm 0.2$  hours ( $p<0.01$ ), respectively. There were no significant differences in AUC of total ghrelin after the LC and the HC meals for the post-breakfast ( $p=0.9$ ), post-lunch ( $p=0.2$ ), or total time periods ( $p=0.8$ ) (Table 6).

Repeated measures ANOVA for time points 0800, 0930, 1230, 1300, and 1430 revealed a significant effect of diet for at least one of the time points selected ( $p<0.01$ ). Post-hoc analysis showed a significant difference in total ghrelin concentration just before the LC and HC lunch meals at 1300, only ( $p<0.05$ , adjusted for 5 comparisons). Contrast analysis between the baseline sample at 0800 and the time point at 0930 confirmed a significantly greater decrease in total ghrelin concentration after the HC meal compared to the LC meal ( $p<0.05$ , adjusted for two comparisons). The second contrast comparing the 1230 and 1330 time points was not significant for differences in the changes in total ghrelin concentration between the LC and HC meals ( $p>0.05$ ).

There was no significant difference in AUC for total ghrelin between LC and HC meals. Total ghrelin concentrations decreased more rapidly and to a greater extent in the first postprandial period after the HC breakfast, but were not suppressed to any greater extent compared to the LC meal.

**Figure 4.** Postprandial changes in total ghrelin concentration after low and high carbohydrate meal consumption<sup>†</sup>



<sup>†</sup>Mean ± SEM, meal times indicated by arrows

LC = low carbohydrate

HC = high carbohydrate

Repeated Measures ANOVA @ 0800, 0930, 1230, 1300, 1430: \*significantly different between meals (p<0.05)

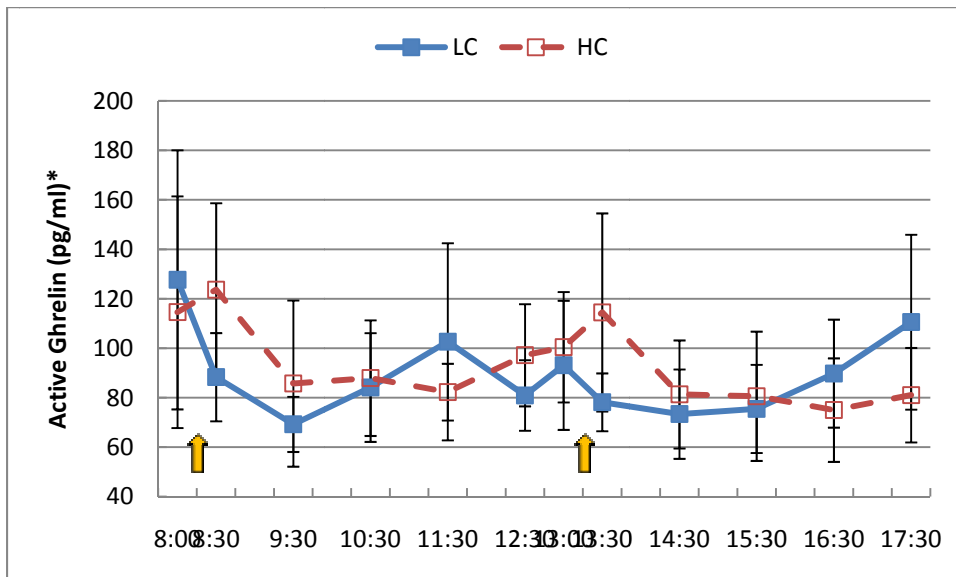
Contrast analysis between 0800 and 0930, 1230 and 1300: <sup>‡</sup>significantly different between meals (p<0.05)

## **Postprandial Active Ghrelin Concentration and Area Under the Curve**

Differences in postprandial changes in concentration of active ghrelin over time after LC and HC meals are shown in Figure 5. Mean fasting active ghrelin concentrations were similar at  $127.7 \pm 52.4$  and  $114.6 \pm 46.9$  pg/ml before the LC and HC meals, respectively ( $p=0.1$ ). After consuming the LC breakfast meal the mean active ghrelin concentrations fell immediately and to a greater extent (31%) than after the HC breakfast meal. Active ghrelin concentration increased slightly after the HC meal before decreasing by 31% by 0930 (Figure 8). A similar but less pronounced change in mean concentration was repeated after the LC and HC lunch meals were consumed. Maximal percent suppression of active ghrelin concentration and time to nadir for active ghrelin were also analyzed for the postprandial period after the breakfast meal was consumed. After the LC and HC meals, the mean maximal percent suppression of active ghrelin from baseline was similar at 31% and 27%, respectively ( $p=0.5$ ). The mean time to nadir for active ghrelin after the LC vs. the HC breakfast meals was also similar at  $3.0 \pm 0.5$  and  $2.6 \pm 0.5$  hours ( $p=0.6$ ), respectively. There was no significant difference in AUC between meal type after the breakfast, lunch, or total time periods ( $p>0.05$ ) (Table 6).

Repeated measures ANOVA for time points 0800, 930, 1230, 1300, and 1430 revealed no significant effect of diet on active ghrelin for any the time points selected. Contrast analysis showed no significant differences in change in active ghrelin concentration after LC and HC meal consumption between 0800 and 0930 or 1300 and 1430 time points. There was no significant difference in AUC for active ghrelin between LC and HC meals. There was not a significant difference in the rate of decrease in concentration for the first postprandial period after the HC and LC breakfast meals. Active ghrelin concentrations were not suppressed to any greater extent after consumption of either breakfast meal.

**Figure 5.** Postprandial changes in active ghrelin concentrations after low and high carbohydrate meal consumption<sup>†</sup>



<sup>†</sup>Mean  $\pm$  SEM, meal times indicated by arrows

LC = low carbohydrate

HC = high carbohydrate

Repeated Measures ANOVA @ 0800, 0930, 1230, 1300, 1430: \*significantly different between meals ( $p < 0.05$ )

Contrast analysis between 0800 and 0930, 1300 and 1430: <sup>‡</sup>significantly different between meals ( $p < 0.05$ )

## Postprandial GLP-1 Concentration and Area Under the Curve

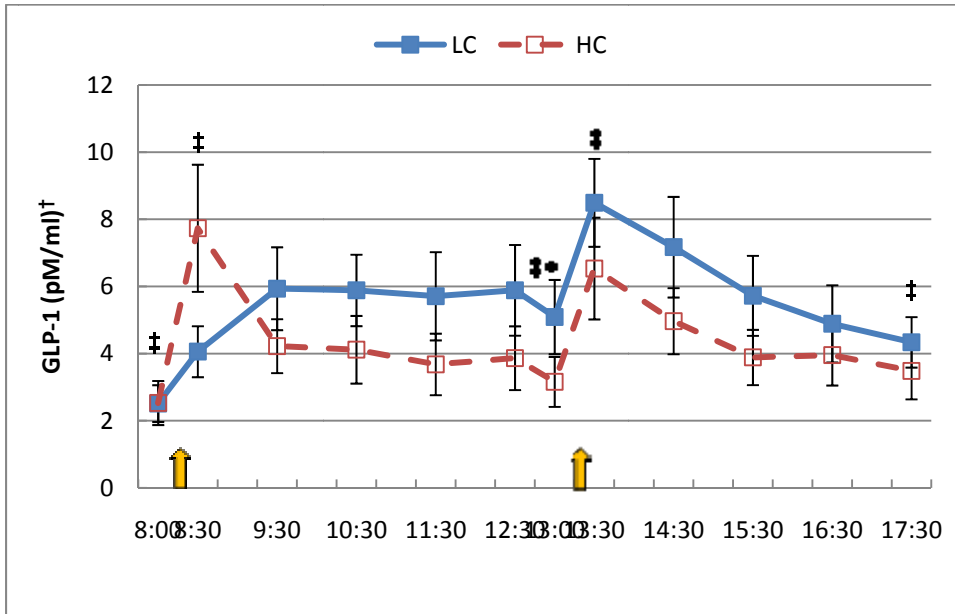
Differences in postprandial change in concentration over time for GLP-1 after LC and HC meals are shown in Figure 6. Mean fasting GLP-1 concentration was the same at 2.5 pM/ml before the LC and HC meals ( $p=0.9$ ). After the HC breakfast was consumed, GLP-1 concentration rose sharply by 208% within 30 minutes, and then decreased sharply by 45% within 60 minutes, and then remained stable until 1300. In contrast, GLP-1 concentrations rose gradually after the LC breakfast meal and remained elevated through 1300. The sharp rise in GLP-1 concentration after HC breakfast consumption was likely due to a larger increase in concentration demonstrated by two subjects at the 0830 time point. The range for the concentration for eight of the 10 participants at the 0830 time point was 2.27-10.46 pM/ml compared to 14.0 and 20.47 pM/ml for the two other participants. The LC and HC curves demonstrated a similar pattern of change after the lunch meal with an immediate increase in GLP-1 followed by a gradual return to baseline by the end of the sampling period. Area under the curve for GLP-1 after breakfast, lunch, and for the total duration of the sampling period was significantly higher after LC than HC meals ( $p<0.01$ ) (Table 6).

Repeated measures ANOVA for time points 0800, 0830, 1300, 1330, and 1730 revealed a significant effect of diet for at least one of the time points selected ( $p<0.05$ ). Post-hoc analysis showed a significantly higher GLP-1 concentration after the LC than the HC meals at 1300 only ( $p<0.05$ , adjusted for 5 comparisons). Further, contrast analysis was performed on the 0830, 1300, 1330, and 1730 time points to determine if the change over time in GLP-1 concentration after the 0830 time point was consistently different between LC and HC meals. The GLP-1 concentrations at the specified time points were found to be significantly different between the LC and HC meals ( $p<0.05$ , adjusted for 4 comparisons). These results confirm that although there was a significant



difference in GLP-1 concentration at each time point between meals, the difference was not consistent throughout the end of the sampling period.

**Figure 6.** Postprandial changes in GLP-1 concentration after low and high carbohydrate meal consumption<sup>†</sup>



<sup>†</sup>Mean ± SEM, meal times indicated by arrows

LC = low carbohydrate

HC = high carbohydrate

Repeated Measures ANOVA @ 0800, 0830, 1300, 1330, 1730: \*significantly different between meals (p<0.05)

Contrast analysis between 0830, 1300, 1330 and 1730: † significantly different between meals (p<0.05)

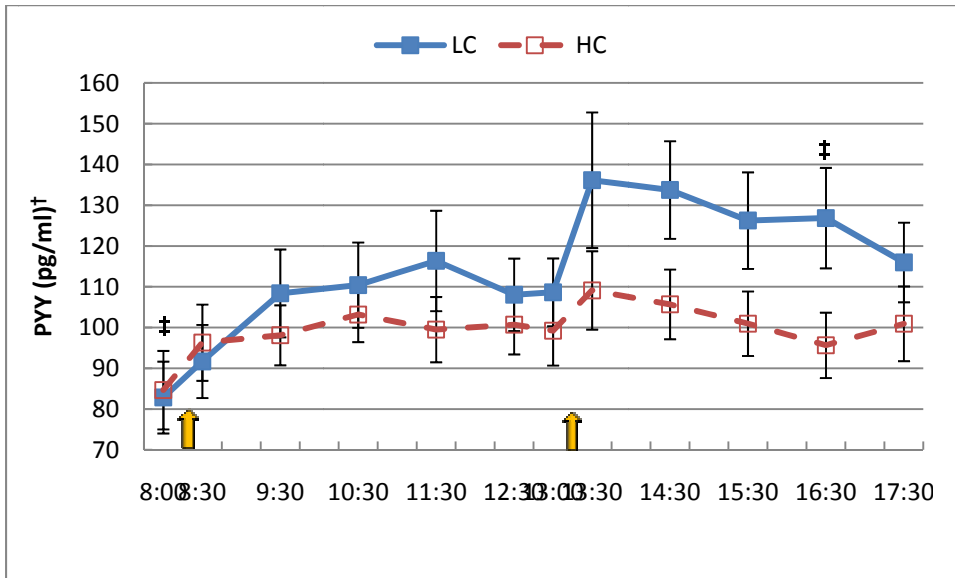
### **Postprandial PYY Concentration and Area Under the Curve**

As illustrated in Figure 7, PYY concentrations rose after consumption of the LC and HC meals and remained above baseline throughout the sampling period.

Consumption of the LC meals resulted in higher PYY concentrations especially after the lunch meal, but concentrations were not significantly different at any of the selected time points. Area under the curve was not significantly different after the LC and HC breakfast meals, but was higher after the LC lunch meal ( $574 \pm 52$  vs.  $459 \pm 37$  pg/ml,  $p=0.02$ ), and overall ( $1107 \pm 100$  vs.  $954 \pm 72$  pg/ml,  $p=0.04$ ) compared to the HC meals (Table 6). Contrast analysis between the time points of 0800 and 1630 showed a significantly greater increase in PYY concentration after the LC than the HC meals ( $p<0.05$ ).

Consumption of LC meals resulted in a greater AUC and higher PYY concentration at the end of the sampling period compared to HC meals.

**Figure 7.** Postprandial changes in PYY concentration after low and high carbohydrate meal consumption<sup>†</sup>



<sup>†</sup>Mean ± SEM, meal times indicated by arrows

LC = low carbohydrate

HC = high carbohydrate

Repeated Measures ANOVA @ 0800, 0830, 1330, 1430, 1630: \*significantly different between meals (p<0.05)

Contrast analysis between 0800 and 1630:† significantly different between meals (p<0.05)

## Orthogonal Polynomial Analysis

To determine whether there was a significant difference in the pattern of change over time after consumption of the LC and HC meals, the polynomial characteristics of each participant's curve for glucose, insulin, leptin, total ghrelin, active ghrelin, GLP-1, and PYY were analyzed. A polynomial analysis of a line defines the number of stationary points the line has before it changes direction. For example, a pure linear ( $x^1$ ) contribution indicates that there are no points in which the line changes direction, while a significant quartic ( $x^4$ ) contribution indicates that there are three points where the line changes direction. In comparison to the previous graphs that illustrate the change in the mean concentration at each time point, the graphs representing the dominant polynomial terms take into account all the individual changes in concentration throughout the day and therefore better represent the overall pattern of change. The mean contribution of each of the polynomial terms to the curves generated for the change over time for glucose, insulin, leptin, total ghrelin, active ghrelin, GLP-1 and PYY after the LC and HC meals is presented in Table 7.

Graphs representing the fitted polynomial curves for the change over time of each analyte are shown in Figures 8-14. To construct the graphs each participant's data for all 12 time points was standardized by subtracting the individual's mean concentration and dividing by the individual's standard deviation. A polynomial (up to order 4) was then separately fitted to each person's standardized concentrations. This analysis generates four coefficients per person, with each coefficient showing the contribution of the linear, quadratic, cubic, and quartic terms of the polynomial fit. These components were averaged across the 10 subjects to yield the "typical" effect for each component. Graphs were drawn based on the four average components from each of the two diets and represent the dominant polynomial characteristics found to be significant for the change over time for each analyte after consumption of the LC and HC

meals. The solid line represents the LC meals and the dashed line represents the HC meals. The Y-axis of each graph was changed to reflect the standardized analyte concentration; the X-axis of each graph represents time from the start of the sampling period, 0800, which was set at 0.

**Table 7. Orthogonal Polynomial Analysis Results**

Terms	Glucose		Insulin		Leptin		Total Ghrelin		Active Ghrelin		GLP-1		PYY	
	LC	HC	LC	HC	LC	HC	HC	HC	LC	HC	LC	HC	LC	HC
<b>Linear (x)</b>	6±7*	1 ± 11	3 ± 4	-3 ± 16	-0.7 ± 0.1*	0.1 ± 0.1*	-300±73*	-48±82	-8.4 ± 40	-34±58	2 ± 2 <sup>†</sup>	-0.81 ± 2	40 ± 22 <sup>†</sup>	9 ± 22
<b>Quadratic (x<sup>2</sup>)</b>	-3 ± 7	3 ± 8	-2 ± 2 <sup>†</sup>	8 ± 11	0.05±0.05 <sup>†</sup>	0.03±0.04 <sup>†</sup>	68±99	-18 ± 52	32 ± 64*	4±37	-4 ± 3 <sup>†</sup>	-0.37 ± 2	-23±19 <sup>†</sup>	-12 ± 8 <sup>†</sup>
<b>Cubic (x<sup>3</sup>)</b>	-1 ± 4	-16±14 <sup>†</sup>	-0.14±2	-5 ± 8	-0.01±0.03	0.02±0.03	5±83	-35 ± 96	1 ± 11	-17±54	0.1 ± 1	-0.58±0.93	-4 ± 6	4 ± 12
<b>Quartic (x<sup>4</sup>)</b>	3 ± 6	-7±8*	-2 ± 2*	-35 ± 13 <sup>†</sup>	-0.01±0.03	-0.004±0.03	-16±51	297±208*	35 ± 68	22±20*	-0.84±2	-1 ± 1 <sup>†</sup>	-13±16*	-2 ± 9

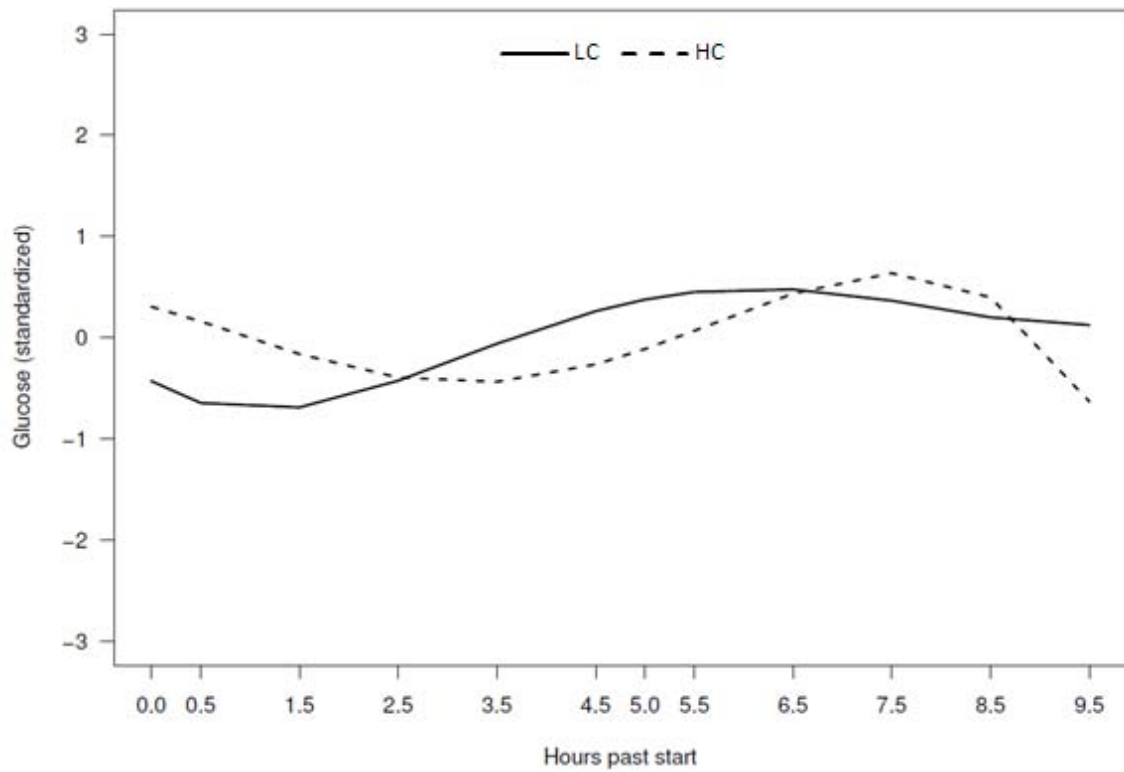
Mean ± SD; LC = low carbohydrate; HC = high carbohydrate  
 \*Significant contribution to the curve (p<0.05)  
<sup>†</sup>Significant contribution to the curve (p<0.01)

## Orthogonal Polynomial Analysis of Glucose Curves

The only component found to contribute significantly to the shape of the LC glucose curves was the linear term ( $p < 0.05$ ) (Table 7). The significance of the linear term indicates that the rate of change in mean glucose concentrations remained relatively stable throughout the sampling period. The two components that were found to be significant for the HC glucose curve were the cubic and the quartic terms ( $p < 0.01$  and  $p < 0.05$ , respectively). The significance of the cubic term indicates that curve for mean glucose concentration changed direction twice throughout the sampling period. As seen in Figure 8, on average concentration after HC meal consumption decreased gradually to 3.5 hours after baseline, increased until 6.5 hours after the start of sampling, and decreased from 7.5 hours until the end of the day.

The significance of the quartic term for the HC meals indicates that although overall the HC curve increased and decreased in a manner characteristic of a third degree polynomial, the length of time over which the change in concentration occurred was extended compared to what would be typical of a cubic term. Figure 8 shows the decrease of the HC curve from baseline during the first postprandial period occurred over approximately 4 hours compared to the decrease in concentration after the lunch meal which occurred over approximately 3 hours. To represent a typical cubic polynomial, the curve should be much more symmetric around the points where the line changes direction. The difference in the polynomial terms that were significant for the LC and HC curves indicate that there was a distinct difference in the way each type of meal affected postprandial glucose concentrations.

**Figure 8.** Fitted polynomial curves for glucose concentrations after low and high carbohydrate meal consumption



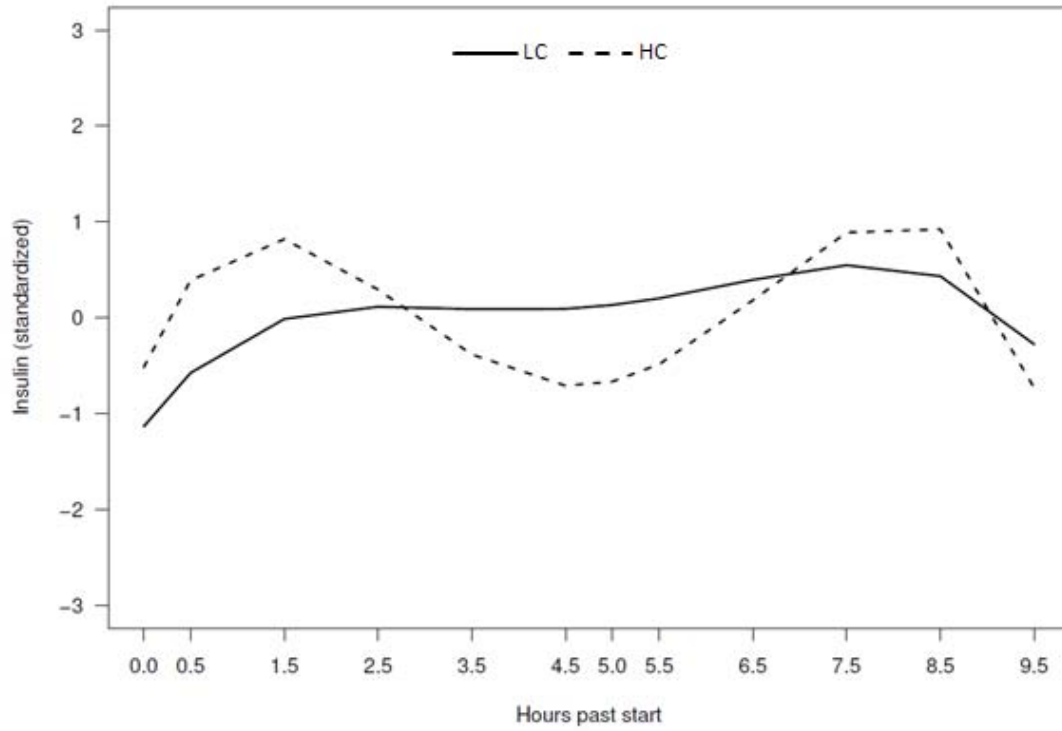


## **Orthogonal Polynomial Analysis of Insulin Curves**

The components found to contribute significantly to the LC curve for insulin were the quadratic and the quartic terms ( $p < 0.01$  and  $p < 0.05$ , respectively) (Table 7).

Although it is not easily seen in Figure 9, the significance of the quartic term indicates that on average the postprandial curve changed direction three times after consumption of LC meal at approximately 1.5, 4.5 and 7.5 hours from the beginning of the sampling period. The significance of the quadratic term indicates that insulin curve makes at least one other change in direction. As seen in Figure 9, the mean insulin concentration increased from baseline and ended at a concentration above baseline which indicates one overall turn or increase in the curve from baseline. The quartic term was also significant for the insulin curve generated by the HC meals ( $p < 0.01$ ) and was the only contributor to the postprandial HC curves. Figure 12 shows that after increasing from baseline to 1.5 h mean concentrations of insulin decreased to 4.5 h, then increased from 7.5 h, and then decreased to the end of the sampling period. The terms that were significant for the LC and HC curves indicate that there was a distinct difference in the way each type of meal affected postprandial insulin concentrations.

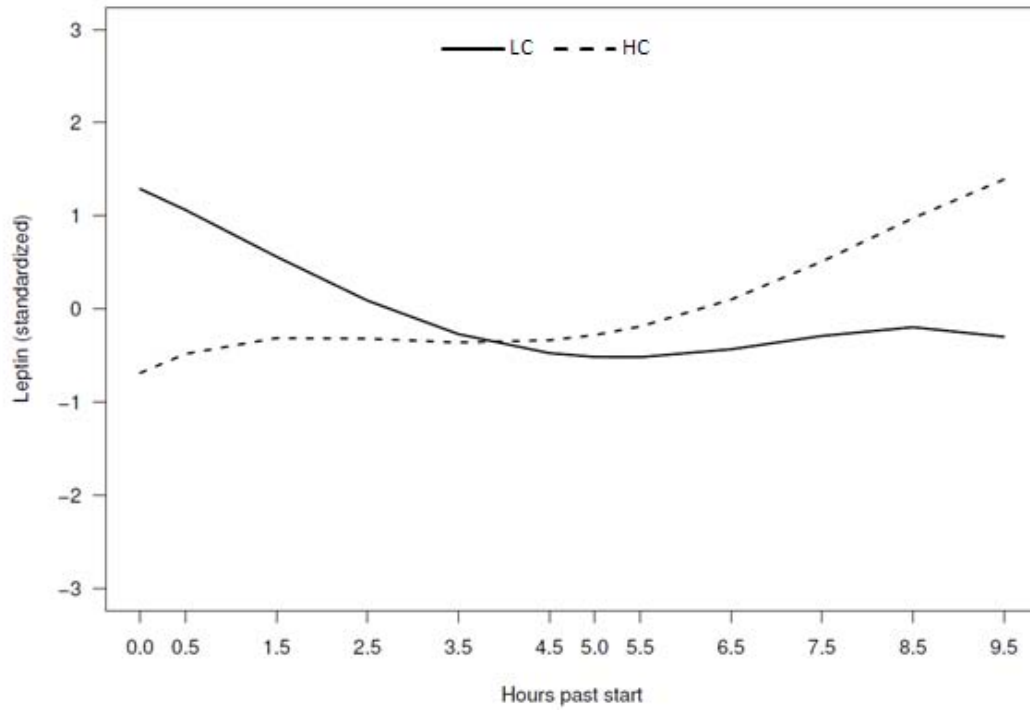
**Figure 9.** Fitted polynomial curves for insulin concentrations after low and high carbohydrate meal consumption



### **Orthogonal Polynomial Analysis of Leptin Curves**

The linear and quadratic components were significant contributors to LC and HC curves for leptin, respectively, at the  $p < 0.05$  and  $p < 0.01$  levels (Table 7). The sign of the linear component for the LC curve was negative indicating that concentrations generally decreased from baseline, while the sign for the HC curve was positive indicating that concentrations generally increased from baseline. The significance of the quadratic term for change in leptin concentration after both diets indicates that at one point in time each curve changed direction. As illustrated in Figure 10, starting from baseline the LC curve decreases until 5.0 h and then remains stable until the end of the sampling period. The HC curve increases gradually 3.5 h, then begins to increase more rapidly until the end of the sampling period. The significance of the linear and the quadratic terms for the different leptin curves support the visual appearance that consumption of LC and HC meals caused postprandial leptin concentrations to decrease and increase, respectively.

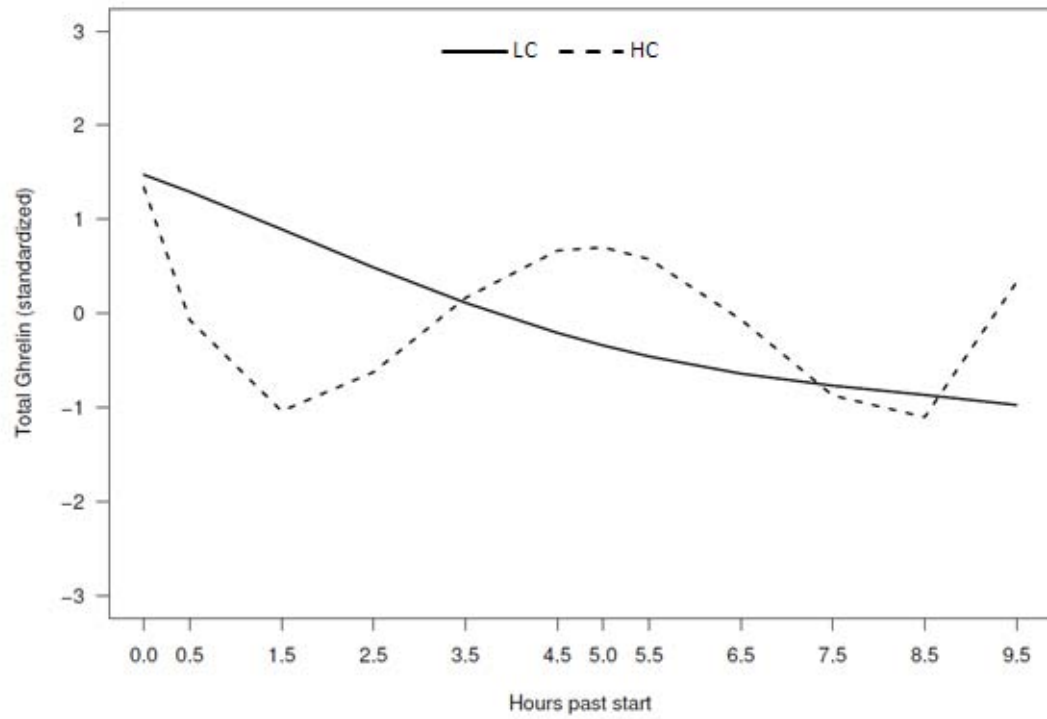
**Figure 10.** Fitted polynomial curves for leptin concentrations after low and high carbohydrate meal consumption



### **Orthogonal Polynomial Analysis for Total Ghrelin Curves**

For total ghrelin, the only term that contributed significantly to the nature of the postprandial LC curve was the linear term ( $p < 0.05$ ) (Table 7). As illustrated in Figure 4 and confirmed in Figure 11, the total ghrelin concentration started out high at baseline and decreased steadily throughout the day with no change in direction. The only term found to contribute significantly to the nature of the postprandial HC curve was the quartic term ( $p < 0.01$ ), suggesting that there were three points at which the total ghrelin curve changed direction. After decreasing from baseline to 1.5 h, mean concentrations increased to 5 h, then decreased to 7.5, and then increased through the end of the sampling period. The dominance of the quartic term for HC meals shows that over time total ghrelin concentration generally decreases after meal consumption, followed by an increase back to baseline concentration before the next meal. The terms that were significant for the LC and HC curves indicate that there was a distinct difference in the way each type of meal affected postprandial total ghrelin concentrations.

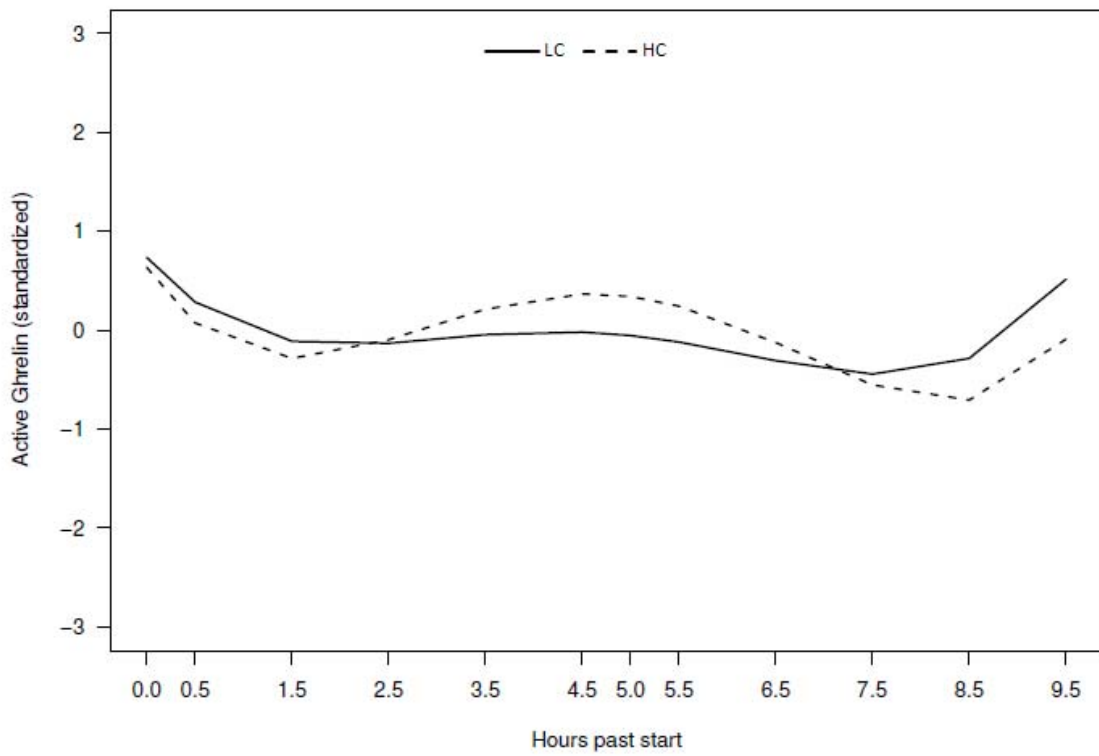
**Figure 11.** Fitted polynomial curves for total ghrelin concentrations after low and high carbohydrate meal consumption



### **Orthogonal Polynomial Analysis for Active Ghrelin Curves**

For active ghrelin, the only term that contributed significantly to the nature of the postprandial LC curve was the quadratic term ( $p < 0.05$ ) (Table 7). The active ghrelin concentration started out high at baseline then decreased and remained stable throughout the day until concentrations started to increase again at 7.5 h, making one overall change in direction (Fig. 12). The only term found to contribute significantly to the nature of the postprandial HC curve was the quartic term ( $p < 0.01$ ), suggesting that there were three points at which the active ghrelin curve changed direction. After decreasing from baseline to 1.5 h, mean concentrations increased to 4.5 h, then decreased to 8.5, and then increased through the end of the sampling period. The significance of the quadratic term for the LC curve shows that overall active ghrelin concentration decreased after breakfast meal consumption and remained stable until it increased towards the end of the sampling period. The dominance of the quartic term for the HC meal curve was the same as for total ghrelin, and showed that over time total ghrelin concentration generally decreases after meal consumption, followed by an increase in concentration before the next meal. The terms that were significant for the LC and HC curves indicate that there was a distinct difference in the way each type of meal affected postprandial active ghrelin concentrations (Table 7).

**Figure 12.** Fitted polynomial curves for active ghrelin concentrations after low and high carbohydrate meal consumption

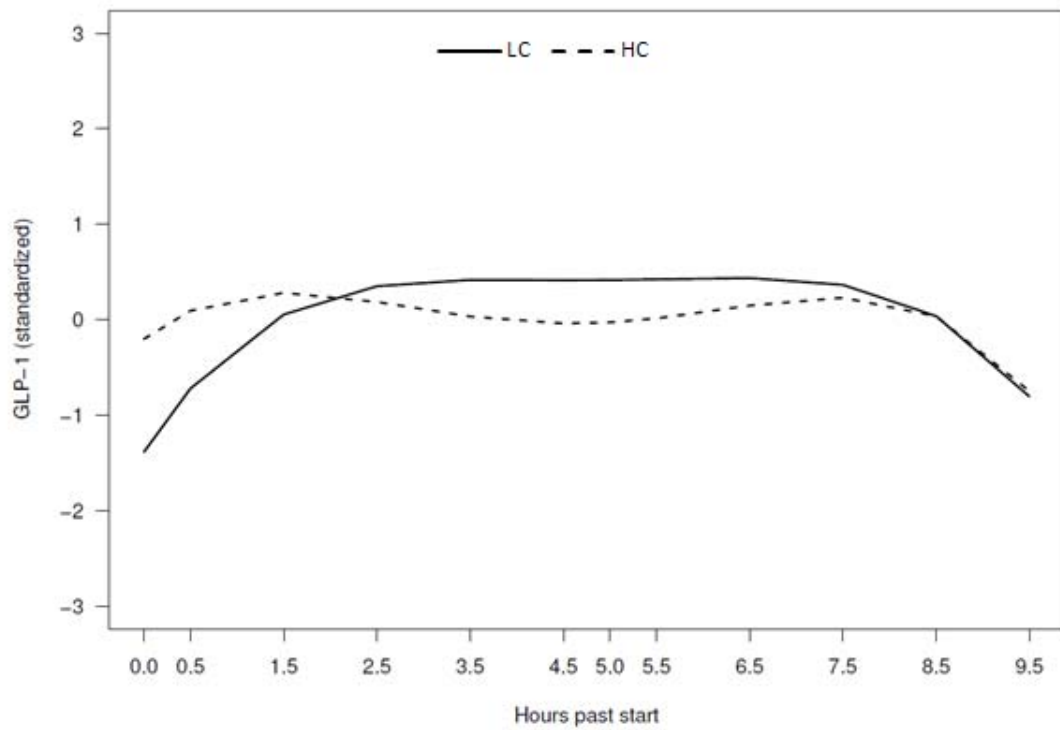




### **Orthogonal Polynomial Analysis for GLP-1 Curves**

The terms found to be significant for participant GLP-1 LC curve were the linear and quadratic terms ( $p < 0.01$ ) (Table 7). The terms that were significant imply that on average participant GLP-1 concentration rose from baseline until 1.5 h, remained flat until 7.5 h, and decreased gradually until the end of the sampling period (Fig. 13). The only term found to contribute significantly to the HC GLP-1 curve was the quartic term ( $p < 0.01$ ). The significance of the quartic term implies that on average GLP-1 concentrations increased from baseline until 1.5 h, decreased until 4.5 h, and rose until 7.5 h when concentration fell until the end of the sampling period. The significance of the linear and quadratic terms for the LC meal curve indicate that over time GLP-1 concentrations increased after the breakfast meal and remained higher compared to the HC curve until the decrease in concentration at the end of the sampling period. The dominance of the quartic term for HC meals shows that over time GLP-1 concentration generally increases after meal consumption, followed by a decrease in concentration before the next meal. The different terms that were significant for the LC and HC curves indicate that there was a distinct difference in the way each meal type affected postprandial concentrations of GLP-1.

**Figure 13.** Fitted polynomial curves for GLP-1 concentrations after low and high carbohydrate meal consumption

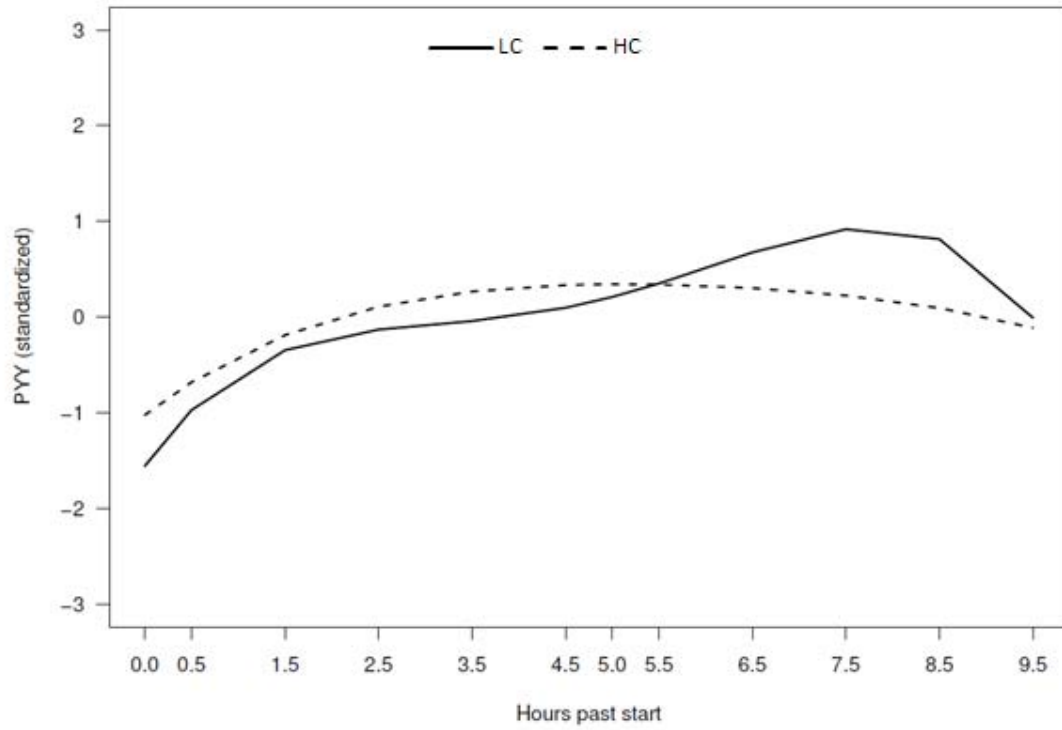


### **Orthogonal Polynomial Analysis for PYY Curves**

Polynomial analysis of the LC PYY curves showed a significant contribution was made by the linear ( $p < 0.01$ ), quadratic ( $p < 0.01$ ), and quartic terms ( $p < 0.05$ ). As shown in Figure 14, the LC curve increases from baseline and changes direction at 1.5, 4.5 and 7.5 h, showing dominance of the quartic term. The linear nature of the increase from 4.5 to 7.5 hours is represented by the significance of the linear term. The significance of the quadratic term indicates that the curve makes one other overall change in direction from the start of the sampling period. As seen in Figure 14, the mean PYY concentration increased from baseline and ended at a concentration above baseline which indicates one overall change in the curve from baseline.

The only term found to contribute significantly to the HC PYY curve was the quadratic term. PYY concentration gradually increased from baseline until 4 h and remained stable until concentrations started to decrease slightly from 6.5 h until the end of the sampling period. The significance of the linear, quadratic, and quartic terms for the LC PYY curve show that over time PYY concentrations increased after each meal was consumed and increased overall from baseline. The dominance of the quadratic term for the HC PYY curve indicates that PYY concentrations generally increased after the breakfast meal was consumed, but remained stable until the slight decrease at the end of the sampling period. The different terms that were significant for the LC and HC curves indicate that there was a distinct difference in the way each meal type affected postprandial concentrations of PYY.

**Figure 14.** Fitted polynomial curves for PYY concentrations after low and high carbohydrate meal consumption



## Visual Analog Scales

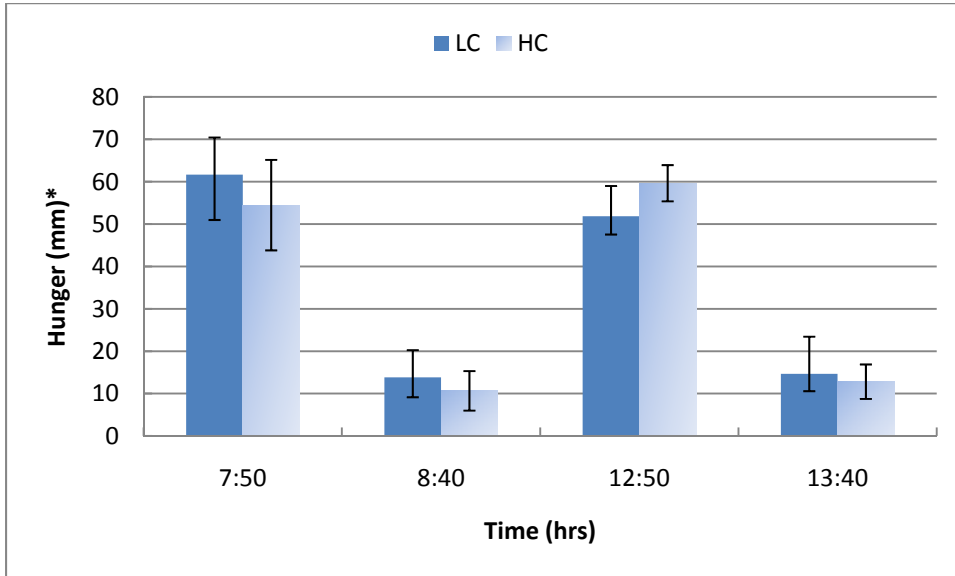
Visual analog scale data were available for six out of the ten participants and were analyzed for differences in participant feelings of hunger and fullness before and after LC and HC meal consumption. Data were not available from the four initial participants because they did not complete visual analog scales. Figure 16 illustrates the change over time for participant feelings of hunger and Figure 17 shows the change over time in participant feelings of fullness. Linear contrasts were generated between after and before meal hunger and fullness scores as described in the methods section (Table 2). The contrasts created were used to analyze changes in participant feelings of hunger and fullness after eating the LC and HC meals.

To determine if the change in hunger and fullness was significantly different from zero, sign-rank analysis was performed for contrasts L1-L4 for both the LC and HC meals separately. The contrasts of L1 and L2 created for both the LC and HC meals showed that there was a significant change in participant feelings of hunger and fullness after both breakfast and lunch meals were consumed ( $p=0.03$ ), meaning that participants felt less hungry and more full after both meal types were consumed. The linear contrast L3 was constructed to answer the question of whether the change in hunger and fullness after consumption of LC and HC meals was different after breakfast compared to after lunch. The results of the LC and HC L3 contrasts were not significant for changes in hunger ( $p=0.3$  and  $p=0.6$ , respectively), meaning that there was no difference in participant feelings of hunger after lunch compared to after breakfast consumption for either meal type. The results of the LC and HC L3 contrasts for fullness were not significant ( $p=0.1$  and  $p=0.2$ , respectively), meaning that there was no difference in participant feelings of fullness after lunch compared to after breakfast consumption for either meal type. Since the linear contrasts for L3 were not significant for change from zero, the contrast L4 was used to answer the question of whether the average change in

hunger and fullness over the meal periods was significantly different from zero after consumption of both the LC and HC meals. The average change in hunger for both the LC and HC meals for L4 was significant ( $p=0.03$ ) meaning that although the change in hunger was not different from zero after lunch and after breakfast separately, the average change was significant after consumption of both test meals. The average change in fullness after both the LC and HC meals for L4 was significant ( $p=0.03$ ) meaning that although the change in fullness was not different from zero after lunch and after breakfast separately, the average change in fullness was significant after consumption of both test meals.

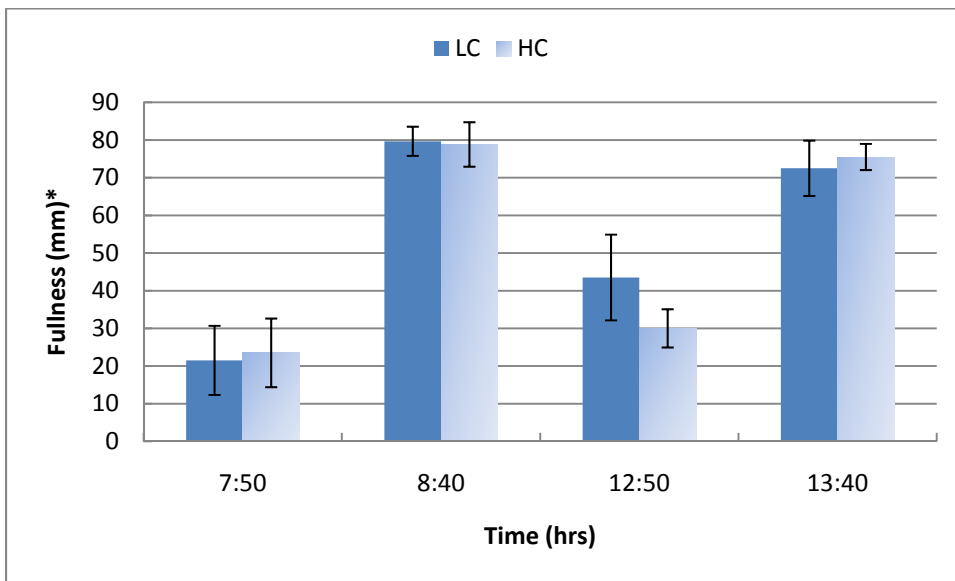
To determine if there was a significant difference between LC and HC meals for any of the linear contrasts constructed, comparisons between meals were made for each contrast. There was not a significant difference in participant feelings of hunger or satiety after breakfast or lunch consumption between the LC or HC meals when contrasts L1 and L2 were tested ( $p>0.05$ ). For hunger and fullness, the L3 contrast was also found to be not significant for a difference for either after lunch compared to breakfast between LC and HC meals ( $p>0.05$ ). Since the L3 contrast for hunger and fullness was not significant, the L4 contrast for the average change over time for both was investigated for a difference between the two meal types. The L4 contrast was also not significant for differences between the LC and HC meals ( $p>0.05$ ), meaning that although there was an significant change in the average hunger and fullness scores after consumption of both the LC and HC meals the change was similar for both meal types.

**Figure 15.** Participant hunger scores before and after low and high carbohydrate meal consumption\*



\*Mean  $\pm$  SEM, n = 6  
LC = low carbohydrate  
HC = high carbohydrate

**Figure 16.** Participant fullness scores before and after low and high carbohydrate meal consumption\*



\*Mean  $\pm$  SEM, n = 6  
LC = low carbohydrate  
HC = high carbohydrate

## Discussion

### **Summary**

This randomized, crossover, controlled feeding study was conducted in a healthy, normal-weight sample to measure the difference in concentrations of weight regulation hormones and participant feelings of hunger and fullness after consumption of low and high complex carbohydrate meals. Ten participants completed the study- six new recruits and four from a previous study. Fasting and postprandial blood samples were collected over a period of 9.5 h and analyzed for concentrations of glucose, insulin, leptin, total and active ghrelin, GLP-1 and PYY. Participant feelings of hunger and fullness were assessed by visual analog scales before and after meal consumption.

The primary hypothesis that postprandial concentrations and area under the curve would be higher for GLP-1 and PYY, and lower for glucose, insulin, leptin and total and active ghrelin was accepted for five out of the seven weight regulation hormones analyzed. A summary of the overall effect of LC and HC meal consumption on each analyte and participant feelings of hunger and fullness is presented in Table 8. Total area under the curve for PYY and GLP-1 was higher after consumption of the LC meals than after the HC meals and was lower for glucose, insulin, and leptin after LC meal consumption than HC meals. In this study, total area under the curve was calculated and compared between dietary interventions. Total area under the curve was calculated because in most cases the fasting concentration of the analyte under consideration was the same before each research meal. Other studies have calculated integrated area under the curve, which gives a summary measure of the absolute change in concentration over time after taking into account differences in baseline values between meal types. Repeated measures ANOVA also showed a significant effect of diet for at least one of the selected time points for glucose, insulin, leptin, total ghrelin, and GLP-1.



The time points that were significant for differences between the two meals usually occurred immediately after meal consumption and were consistent with our expectations of the response of the analytes to LC and HC meal consumption.

The results for PYY and GLP-1 are consistent with previously reported results from studies conducted by Essah (60) and Lejeune et al. (68). One notable difference in the results for PYY from our study compared to those from Essah et al. was the more gradual increase in mean PYY concentration in the first postprandial period after the LC breakfast meal. This difference may be due at least in part to the difference in the time of sampling as our samples were taken hourly after 0830 and versus half hourly for the duration of their study. It is possible that hourly sampling resulted in missing the peak in PYY concentration during the first hour after the breakfast meal was consumed. The studies also differed in the amount of fat provided in the breakfast meals. The LC test meals in the study presented here were comprised of 66% fat whereas the meal provided in the Essah study was comprised of 74% fat. Since fat is most potent stimulator of PYY release this 8% difference in fat content could contribute to the more gradual rise in PYY concentration exhibited by our participants.

**Table 8. Effect of Low and High Carbohydrate Meal Consumption on Analytes and Visual Analog Scale Parameters\***

Analyte/Visual Analog Parameter	Low Carbohydrate	High Carbohydrate
Glucose	↓	↑
Insulin	↓	↑
Leptin	↓	↑
Total Ghrelin	-	-
Active Ghrelin	-	-
GLP-1	↑	↓
PYY	↑	↓
Hunger	-	-
Fullness	-	-
*overall effect of meals determined by results from primary outcome analysis of AUC or contrasts ↓ = suppression or decrease in concentration after meals ↑ = increase in concentration after meals (-) = no effect		

Total area under the curve was lower for glucose, insulin and leptin after consumption of the LC meals than the HC meals. The lower glucose and insulin response to LC meals compared to HC meals is consistent with the small amount of carbohydrate contained in the LC meals. This pattern is also consistent with results from studies by Havel (69) and Essah et al. (60). Both studies reported lower postprandial glucose and insulin concentrations, and the Havel study also showed a decrease in leptin concentration after consumption of high fat, low carbohydrate meals compared to low fat, high carbohydrate meals, respectively. The reduction in leptin concentration after LC meal consumption contradicts to our hypothesis and presents a paradox in the conventional understanding of the role that leptin plays in weight regulation, e.g. that lower leptin concentrations stimulate increased eating behavior to replace depleted fat stores resulting from weight loss. Our finding can be explained at least in part by the previously mentioned relationship between glucose, insulin, and leptin.. Studies conducted by Mueller (47) and Wellhorne et al. (70) provided evidence that leptin

release by adipocytes is dependent upon the insulin-mediated uptake of glucose into the adipocyte. The limited amount of carbohydrate in the LC meals resulted in a negligible rise in circulating glucose and insulin concentrations for adipocyte metabolism and as a result may have contributed to the reduction in circulating leptin concentration. Our results suggest that leptin may play a role in the short-term regulation of energy balance, although one that is different from the conventional model.

In contrast, the increase in circulating leptin concentration, and greater glucose and insulin concentrations associated with HC meal consumption was also seen in study reported by Weigle et al. (54). During the two week period in which participants consumed a low fat, high carbohydrate diet, both glucose and insulin concentrations increased after meal consumption and decreased before the next meal. Leptin concentration decreased within the first hour after breakfast was consumed, then increased until the time when the dinner meal was served. The patterns of change for glucose, insulin, and leptin were similar to those seen in the study reported here.

There was no significant difference in total area under the curve after consumption of the LC meals or HC meals for total and active ghrelin concentration. Repeated measures ANOVA was also not significant for differences between LC and HC meals for total and active ghrelin at any of the time points selected. There was no significant difference in the maximal percent suppression of total and active ghrelin after LC and HC breakfast meals. Consumption of the HC breakfast meal resulted in a more rapid decrease to nadir concentration in the first postprandial period for total ghrelin compared to the LC meal, but the same difference was not seen for active ghrelin concentration. . There was also a high degree of inter-individual variability in the response of total and active ghrelin to both the LC and the HC meals. The higher degree of variability could have masked any significant differences in the AUC between the two meals and most likely masked significant differences in the post-hoc analysis for total

and active ghrelin due to the large standard error compared to the effect size of diet at each time point.

The effect that consumption of LC meals had on the change in total ghrelin concentrations over time was also different from what was expected. We hypothesized that the LC meals would contribute to a sense of food disinterest in part by decreasing ghrelin concentrations faster and to a greater extent than HC meals. Ghrelin concentrations were expected to remain suppressed after the breakfast meal until the lunch meal, to rise to a lesser extent before the lunch meal and remain lower until the end of the sampling period due to the subsequent consumption of a LC lunch meal. In reality LC meal consumption did result in a reduction in total ghrelin concentration, but the decrease was a consistent downward trend from baseline to the end of the day. This response may blunt the stimulus to consume the next meal. In contrast, the decrease in total ghrelin concentration after HC breakfast consumption was more rapid compared to the LC meal. However, the concentration of total ghrelin increased back to baseline before the lunch meal was consumed and at the end of the sampling period. Consumption of a low fat, high carbohydrate diet in the previously mentioned Weigle study resulted in a pattern of change in concentration for total ghrelin that was similar to the results presented here (54). This difference in the pattern of change between the LC and HC meals for total ghrelin was confirmed in subsequent polynomial analysis of each curve.

Other studies have also shown that dietary fat is a much less potent suppressor of postprandial ghrelin release compared to carbohydrate and protein. Monteleone et al. conducted a randomized crossover study in healthy, normal weight women that investigated the response of ghrelin to consumption of high fat (75%) compared to isocaloric HC (77%) meals. Fasting and postprandial blood samples were taken at six time points throughout the day and analyzed for ghrelin, leptin, insulin and glucose

concentrations. Percent suppression of ghrelin was found to be significantly greater after consumption of the HC meals compared to the high fat meals ( $63.0 \pm 9.3$  vs.  $49.7 \pm 18.4\%$ ,  $p=0.02$ ). The percent suppression for total and active ghrelin after LC and HC meal consumption in the results presented here was 22 vs. 24% and 31 vs. 27%, respectively. The HC meals provided in the Monteleone et al. study were also found to result in a greater decrease in feelings of hunger compared to the high fat meals as measured by visual analog scales ( $p<0.02$ ) (71). These findings were not confirmed in our study as percent suppression of total and active ghrelin was not greater after HC meals, and our participants did not report any difference in feelings of hunger after consumption of HC meals compared to LC meals.

A more recent study investigated the response of total and active ghrelin to consumption of liquid meals composed of fat, carbohydrate, and protein in different proportions. Sixteen healthy, normal weight participants completed the randomized crossover study and the results showed that overall protein was the most potent suppressor of total and active ghrelin in the postprandial period, followed by carbohydrate then fat. Within the first three hours after meal consumption however, the most potent suppressor of total and active ghrelin was carbohydrate, and after 6 hours concentrations had returned to baseline (72). Those results are consistent with the change in total and active ghrelin in the study reported here. As our LC meals were much higher in both fat and protein than the HC meals, this could explain the lower suppression of total and active ghrelin after LC meals compared to HC meals.

The polynomial analysis of the curves for each analyte was conducted to determine which terms characterized the typical change in concentration over the course of the sampling period. The results of the analysis showed that the postprandial patterns of change for all analytes were significantly different after consumption of the LC and HC meals. The terms that were significant for the glucose, insulin, and leptin curves showed

that consumption of LC meals suppressed or decreased the postprandial concentrations of these markers compared to HC meals. The significant terms for the PYY and GLP-1 curves illustrated that consumption of LC meals resulted in higher concentrations following meals that remained elevated compared to HC meals.

The terms that were significant for total and active ghrelin showed LC meal consumption resulted in a lack of dramatic change in ghrelin concentration throughout the sampling period compared to HC meals. It is possible that although consumption of LC meals did not suppress ghrelin concentrations to a greater extent than HC meals as we hypothesized, the lower amount of change in total and active ghrelin concentration may lead to a reduction in the stimulus to consume food. The lack of increase in pre-meal ghrelin concentration before the lunch meal, typically associated with meal initiation, could contribute to the loss of interest in consuming food and the reduced food intake experienced by persons following LC diets. Taken together, these findings support our hypothesis that there is a significant difference in the effect that LC and HC meals have on postprandial concentrations of each of the weight regulation markers studied despite the lack of significance in AUC for total and active ghrelin.

The secondary hypothesis of this study that participants would report feeling less hungry and more satiated after consuming LC meals than HC meals was not accepted. There were no significant differences in any of the contrasts evaluated between participant feelings of hunger and fullness after consumption of LC and HC meals. Likely explanations for the lack of significance in the visual analog scale data could be the time and frequency at which questions were administered, as well as the subjective nature of the questions that were asked. It is possible that administering the questionnaire 15 minutes before and after meals was not appropriate to detect differences in feelings of hunger and fullness. At these time points all participants, regardless of meal type, reported a significant reduction in sense of hunger and a significant increase in sense of

fullness after breakfast; as well as a significant increase in sense of hunger and decrease in sense of fullness before lunch meals. If the questionnaire had been administered at an intermediate time point, for instance two to three hours after meal consumption, there may have been more of an opportunity to discern differences in hunger and fullness depending on the previous meal consumed.

It is also possible that the visual analog scale was not the appropriate tool to adequately assess differences in the feelings of hunger and fullness. Each participant interpreted the meaning of hunger and fullness individually, and it is possible that the terms "hunger" and "fullness" were not sensitive enough to accurately quantify the feelings they experienced after consuming either the LC or HC meals. For example, the term "fullness" can be interpreted as a pleasant or comfortable feeling after eating a meal. Some participants reported feeling significantly full to the point of being uncomfortable after meal consumption. The visual analog scale was not able to distinguish between the two very different feelings.

### **Strengths of the Study**

The study design and subject demographics allowed for great control over many factors that could confound the relationship between the response of the selected weight regulation markers to consumption of the LC and HC intervention meals. The crossover design decreased if not omitted the effect of confounding variables such as age, BMI, and race since each subject served as their own control. All participants enrolled in the study completed the entire protocol; therefore eliminating any potential bias associated with subject attrition. The randomized order of the meals also minimized any bias that could have been introduced by consuming one type of meal before the other. This was a controlled feeding study so the exact macro and micronutrient intake of participants was known and accounted for in both the standard and intervention dietary phases. The

three day standard diet minimized the effect that self-chosen diets may have had on baseline concentrations of each analyte; by standardizing nutrient intake prior to consumption of both the LC and HC meals baseline concentrations for each analyte were similar for each participant. The macronutrient composition of the LC and HC test meals also added strength to the study. Rather than examine the response of weight regulation hormones to increases in single macronutrients like fat or protein, this study used whole meals which allowed the effects to reflect what free living subjects would experience. Due to the study design characteristics, the results presented are thought to indicate the response that would be seen in others in the general healthy, normal weight population.

The demographics of our subject population added to the strength of the study in two ways. The majority of studies conducted to investigate the effects of meal composition on markers of weight regulation have been performed in overweight and obese subjects. The investigation of the responses of healthy normal weight, weight stable subjects to the selected test meals adds to the knowledge of what the normal response of weight regulation hormones, insulin and leptin for example, would be without the confounding variables of increased weight or other disease states associated with obesity such as diabetes. Our subjects remained weight stable throughout the study which eliminated the confounding effects of weight loss or gain. An additional strength of the study design was the addition of visual analog scales to assess participant feelings of hunger and fullness. This addition of visual analog scores confirm that the increases or decreases in short-term fullness and hunger signals like ghrelin, PYY, and GLP-1 were associated with increased feelings of fullness and decreased feelings of hunger experienced by the participant after meal consumption, despite the fact that there were no differences in the response between meal types.



## **Limitations of the Study**

Due to the recruitment techniques used in this study, there was a potential ascertainment bias associated with the subjects enrolled in the study. Although our subject recruitment pool was open to the greater Portland area, all of our subjects except one were either medical professionals or students from OHSU. The visual analog scales were a limitation as well as strength of our study. The terms "hunger" and "fullness" used in the questionnaire and the times the questionnaires were administered may not have been appropriate to quantify differences in participant feelings of hunger and fullness between the two diets.

Due to the nature of conducting controlled feeding studies, there was also a lack of blinding of research staff and subjects to aspects of the intervention phase such as the composition of study meals consumed during the inpatient admissions. Although participants were not told which diet they were consuming during each inpatient admission, blinding to the type of meal was not achieved due to the stark contrast in food items served for the LC and HC meals. One source of bias associated with the data analysis portion of our study included secondary analysis of data collected in the previous pilot study. Biochemical analysis of glucose, insulin, and leptin samples of four out of the ten participants was performed by the OCTRI core lab personnel prior to the start of this sub-study. Differences in technique between lab technicians could have contributed to some of the variability in the data. Since the laboratory technicians were blinded to the intervention, the difference in personnel did not impact the interpretation of the assay results.

## **Future Directions**

The next and most important step for further investigation of the relationship between dietary micronutrient composition and weight regulation markers would be to extend the time frame of the dietary intervention. Since significance was achieved in this crossover study with a sample size of ten and acute consumption of LC and HC meals, long term consumption of diets of the same macronutrient composition could have an even more profound effect on circulating concentrations of weight regulation markers. Future studies should investigate the use of alternative tools or modified administration schedule to assess differences in participant feelings of hunger and fullness. The population studied could also be expanded to include groups that would benefit more than a healthy, normal weight population from increases and decreases in hormones associated with hunger and satiety. Conducting this study in a population with obesity, diabetes, or metabolic syndrome could reveal the effects consumption of LC and HC meals has on circulating concentrations of weight regulation markers for those individuals, and could help direct the development of better dietary interventions to treat these conditions.

Additional research in this area could also include more investigation into different macronutrient compositions of LC and HC diets and their effect on circulating concentrations of weight regulation markers. The survey conducted by Blanck et al. (21) found that out of those persons following the LC diet, 30% of females and 40% of males were using the diet long term to maintain weight loss. However like any other diet regimen, compliance with a LC diet is very hard for the majority of persons to maintain. The LC meals used in our study were modeled after the induction phase of the Atkins diet which allows a maximum of 20 grams of carbohydrate per day. To achieve the same results seen in our study a person would need to consume the minimum amount of carbohydrate which could prove to be an extremely difficult diet to follow long term. The

Atkins diet recommends restriction of carbohydrate to about 20 grams for two weeks before increasing to the amount allowed during the ongoing weight loss phase of the diet. Future study design should be longer, and could include multiple phases of the study in which participants would consume meals of increasing carbohydrate content. This could help to determine a more realistic macronutrient composition for a LC diet that may achieve the same response in short-term hunger and satiety hormones without the severe carbohydrate restriction.

## **Conclusions**

This study provides convincing evidence that there is a significant difference in the effect that consumption of LC meals has on the postprandial excursion of weight regulation markers compared to HC meals in healthy, normal weight individuals. Although a difference in the change in participant feelings of hunger and fullness between the LC and HC meals was not confirmed by our use of visual analog scale scores, the significant differences in AUC and postprandial patterns of change suggest that consumption of LC compared to HC meals should result in differences in participant feelings of hunger and fullness and eating behavior. The more stable reduction in total and active ghrelin concentrations in combination with the increase in postprandial PYY and GLP-1 concentrations is consistent with decreased NPY release from the hypothalamus, decreased stimulus to consume food, and delayed gastric emptying. These findings provide evidence that the feelings of food disinterest and the resulting weight loss experienced by persons following LC diets may be attributable in part to changes in weight regulation hormones known to effect central regulation of appetite and weight regulation. Larger and longer-term studies are necessary to confirm these novel findings.

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## Appendix A

IRB# e1867  
Protocol Approval Date: 8/29/2008

OREGON HEALTH & SCIENCE UNIVERSITY  
Consent Form and HIPPA Authorization- Student Research Project-2006

**Study Title: Energy and Appetite Regulation by High and Low CHO Diets (a.k.a., The Energy Balance Study) Graduate Student Research Project sub-study**

**Principal Investigator:** Diane Stadler, PhD, RD (503) 494-0168  
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**Sponsor:** National Institutes of Health, National Center for Complimentary and Alternative Medicine

**PURPOSE:**

Very low carbohydrate diets and high-complex carbohydrate, low-fat diets are popular weight-loss methods in the United States. The purpose of this study is to compare the impact of these diets on factors that influence energy balance. You are invited to participate in this research because you are healthy and between 21 and 65 years of age and have met the screening criteria established for this study. We plan to enroll up to 10 subjects into this study over the next six months. All study related procedures will take place at the Oregon Clinical and Translational Research Institute (OCTRI) at OHSU.

**PROCEDURES:**

**Summary of Procedures:**

One or two pre-study screening visits are required to determine eligibility for this research project.

The first screening visit will take up to 60 minutes to complete and will involve:

- Having your weight, height, and blood pressure measured.
- Completing a medical history questionnaire and other study related forms.
- Providing a fasting blood sample of less than 2 tsp. [Note: You will need to stop eating or drinking any food or beverages (except for water) after 7 p.m. the night before the blood sample is drawn.]
- Reviewing your medical history and having a brief physical examination with the study physician.
- Meeting with the study dietitian to review food preference and activity patterns. This part of the screening may be performed at a separate visit if you prefer.

You will be offered a complimentary breakfast in the OCTRI after all the procedures for the screening visit are complete.

The main study will involve two 4-day controlled dietary phases separated by at least three days.

During days 1-3 of the first controlled dietary phase you will be asked to:

- Have your weight measured, complete study related forms, and eat breakfast in the OCTRI outpatient unit between 7 and 10 a.m.
- Take prepackaged meals and snack foods prepared for you by the OCTRI kitchen staff to eat during the rest of the day (arrangements can be made to eat other meals at the OCTRI if preferred).
- Eat all of the food provided and nothing else so that you do not gain or lose weight.
- Return all of your food containers (and any uneaten food) to the OCTRI the day after the food was to be eaten.
- Wear an activity monitor for up to 7 days.

On Day 3 of either the first or the second controlled dietary phase you will also be asked to:

- Have your body composition (the amount of fat and muscle tissue you have) measured by:
  - Bioelectrical Impedance Analysis (BIA): The BIA procedure passes a very small, unnoticeable electrical current between electrode pads attached to your hands and feet.
  - DXA scan: A DXA scan passes a very small amount of X-rays through your body while you are lying on your back on a scanning bed. You will be asked to take off any jewelry or metal items that are part of your clothing during this measurement; hospital gowns will be available for you to use. Because of the exposure to X-rays all women will be asked to provide a urine sample for a pregnancy test on the day of the measurement.

A subgroup of participants will have internal body temperature measured:

On day 3 of both controlled dietary phases you will swallow a single-use, disposable, sensor capsule (about the size of a vitamin tablet). The capsule moves through your stomach and intestines in about 1 to 3 days. Every 15 seconds, the capsule sends information to a pager-sized monitor that you wear at your waist. You will need to wear the monitor for about 3 days until the capsule passes through your intestinal tract. The monitor can be placed within 3 feet of your body while you sleep. You will need to wear a wrist-band that states "MRI Risk: DO NOT Perform MRI; a metal-containing thermometer was swallowed on Day-MO-YR for research purposes. Contact Dr. Diane Stadler at 503-706-2074 in an emergency" until the capsule has passed from your intestinal tract.)

Early in the morning of Day 4 (by 6 am) you will be admitted to the OCTRI Inpatient Unit. When you arrive in the morning it should be before eating or drinking foods (except water) or performing any significant exercise.

During your visit to the Inpatient Unit you will:

- Be asked to start a 24-hour urine collection.
- Have your blood pressure, pulse, heart rate, and temperature measured.
- Have a blood sampling tube placed in your arm vein so that blood samples can be drawn 12 times (each sample will contain about 1 1/3 TB of blood for a total of about 1 cup of blood)

over a period of about 10 hours.

- Have your resting energy (calorie) use measured. This process involves placing a lightweight, clear, Plexiglas canopy, with an adjustable air flow rate, over your head and chest to collect samples of the air that you breathe out while you rest on a hospital bed for 45 minutes.
- Eat very-low carbohydrate breakfast and lunch meals or high complex carbohydrate breakfast and lunch meals. The very low carbohydrate meals will include foods like meat, poultry, fish, eggs, cheese, small amounts of vegetables but no fruits, cereals or bread products. The high carbohydrate/low fat meals will include foods like fruits, vegetables, cereal, bread, and low-fat meat and dairy products.
- Have your calorie use associated with meals measured for 45 minutes each hour for 10 hours. This process involves placing the same breath-collection canopy over your head and chest after you eat the research meals and while you rest on a hospital bed. You will need to remain awake during this process but you will not be allowed to engage in any activities other than quiet pursuits such as listening to music or watching TV. You will only be allowed to get up for very light activity (stretch, walk to the bathroom, etc) for 15 minutes each hour.
- Select a dinner meal from the OHSU hospital menu to eat in the OCTRI or to take with you.

You will then be discharged from the OCTRI Inpatient Unit to follow your typical diet and activities.

After the activity monitoring period is finished, you will return the physical activity monitor and core body temperature monitor.

At least three days later you will repeat the study procedures described for days 1-3 except that you will not have your body composition measured by DXA, if you did so during the first controlled dietary phase.

The following day you will be readmitted to the OCTRI Inpatient Unit and you will repeat the procedures described for day 4 except that you will eat the other combination of breakfast and lunch meals (very low carbohydrate or high complex carbohydrate meals).

Sample Storage:

- Blood and urine will be stored for other analyses if additional funds become available.
- Potential measurements will include heart, kidney, bone, and indicators of weight regulation.
- No samples will be used for genetic testing.

Study Visits and Procedures														
Screening Visit	1	2 optional												
Weight	X													
Height	X													
Blood Pressure	X													
Medical history form	X													
Other study forms	X													
Fasting blood sample	X													
Discussion with physician	X													
Discussion with dietitian	X													
Controlled Dietary Phase														
	Dietary Phase 1							Dietary Phase 2						
Day	D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 1	D 2	D 3	D 4	D 5	D 6	D 7
Weight	X	X	X	X				X	X	X	X			
Self-reports	X	X	X	X				X	X	X	X			
Visual Analog Scales				X							X			
Standard Diet	X	X	X					X	X	X				
Accelerometer	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Sample (24-hour collection)				X-----X							X-----X			
Bioelectrical Impedance (BIA)			X							X				
DXA Body Scan			X							X (if not done before)				
Core Body Temperature Measurement (subgroup)			X-----X-----X							X-----X-----X				
Repeated Blood Sampling				X							X			
High or Low Carbohydrate Research Meals				X							X			
Vital signs (BP, pulse, HR, temperature)				X							X			
Resting Energy Expenditure (calorie use)				X							X			
Meal Related Energy Expenditure (calorie use)				X							X			

If you have any questions about this study, now or in the future, please contact Dr. Stadler at (503) 494-0168.

**RISKS AND DISCOMFORTS:**

**Very low carbohydrate meals:** Risks associated with consuming a very low carbohydrate diet for a day are very low. Because the very low carbohydrate diet is low in fiber, you may experience transient changes in your bowel movement frequency and/or consistency that may result in constipation or diarrhea. Because this diet has a very low water content you will be provided with non-carbohydrate containing beverages and reminded to consume adequate fluid during the inpatient admissions.

**High complex carbohydrate/low fat diet:** Risks associated with consuming a high carbohydrate/low fat diet for a day are very low. This diet may have a higher fiber content than your typical diet and you may notice changes in your bowel movement pattern and/or consistency. You will be encouraged to consume water with and between meals throughout each inpatient admission.

**Whole Body DXA Measurement:** The procedure takes about 5 minutes to complete. You will be exposed to a small amount of radiation (x-rays) from the whole body DXA scans. While no amount of radiation has been proven safe, there is no direct evidence that small doses of radiation, similar to those used in the body scan, cause harmful effects in the persons who are exposed. Before each whole body DXA scan, every female subject must have a urine pregnancy test because of the exposure to x-rays. The reason we do this is to be as careful as possible to not scan a woman who is pregnant. The results of the urine pregnancy test will remain private. We will inform you of the results and, if positive, refer you to your regular doctor or health care provider for ongoing care.

**Internal Body Temperature Measurement:** There are minimal risks associated with measuring internal body temperature. Internal temperature information will be transferred by radio frequency transmission from the capsule to the external monitor. The capsule will be administered by the CTRC nursing staff. The capsule may be swallowed with water or other beverages. The capsule must be swallowed without chewing. There is a small chance that choking may occur when the capsule is swallowed. Ingestion of the capsule may result in gastrointestinal discomfort including nausea, vomiting, or pain. To minimize these risks, you will be screened for abnormalities in swallowing, esophageal or bowel strictures, fistulas, or gastrointestinal obstructions. If you have any one or combinations of these conditions, you will not be allowed to participate in this procedure. If medically necessary for non-study related purposes, an MRI should not be conducted until the capsule has passed from the digestive system. Study participants will be asked to wear a "MRI Warning" wristband until the capsule has passed through the digestive system. The study physician will provide on-going oversight and follow-up throughout this procedure.

**Repeated Blood Samples:** You will have 13 blood samples of about 1 tablespoon each drawn from a catheter (tube) placed in an arm vein two times during the study. Approximately 7/8<sup>th</sup> cup of blood will be collected during each inpatient admission. If the catheter stops working at any time during the inpatient admission, you may need to have a new catheter placed in your other arm. You may get an infection where the tube is placed. This would cause swelling, redness, and pain. You may bleed or get a bruise. There is a small chance your blood stream or heart valves might get a serious infection. You may get a blood clot that could go to your lungs. These problems are very rare. If you have these problems, you will need hospital care. Your blood-drawing catheter will be in place in your arm for about 11 hours.

**Single Fasting Blood Samples:** You will have a single fasting blood sample of about 2 teaspoons drawn from an arm vein or a few drops drawn by fingerstick once during the screening phase of the study. You may feel some pain when your blood is drawn. There is a small chance the needle will cause bleeding, a bruise, or an infection. This process will take about 5 minutes to complete.

**Estimation of Resting and Meal-Related Energy (Calorie) Use:** There are no risks associated with having resting or meal-related calorie use measured by the proposed methods. Some people may feel "closed-in" while lying under the plastic canopy or the air may feel "stuffy". This procedure takes about an hour to complete. These measurements will be performed over about 11 hours during each inpatient admission.

**Bioelectrical Impedance Measurement:** The electrical conductivity tests are painless to the extent that you will not feel any procedure taking place other than having the electrode pads placed on and removed from your ankles and wrists. The electrical conductivity test takes less than 1 minute to complete.

**BENEFITS:**

You may or may not notice any health or personal benefits from your participation in this study. However, by serving as a subject in this study, you may contribute new information that may benefit other patients in the future. You will be informed of any clinically significant abnormalities and the safety monitoring blood test results will be provided to your physician upon request and discussed with you at the conclusion of the study.

**ALTERNATIVES:**

You may choose not to be in this study.

**CONFIDENTIALITY:**

We will not use your name or your identity for publication or publicity purposes. Research records may be reviewed and/or copied by all investigators listed on page one of this consent form, others at OHSU who are participating in the conduct of this research protocol, the OHSU Institutional Review Board, and the Oregon Clinical and Translational Research Institute.

**COSTS:**

There will be no cost to you for participating in this study. The study will pay for all study-related examinations and laboratory procedures. In addition, the study will pay for the costs of your food and its preparation.

**LIABILITY:**

It is not the policy of the U.S. Department of Health and Human Services, or any federal agency funding the research project in which you are participating to compensate or provide medical treatment for human subjects in the event the research results in physical injury.

You have not waived your legal rights by signing this form. If you are harmed by the study procedures, you will be treated. Oregon Health & Science University does not offer to pay for the cost of the treatment. Any claim you make against Oregon Health & Science University may be limited by the Oregon Tort Claims Act (ORS 30.260 through 30.300). If you have questions on this subject, please call the OHSU Research Integrity Office at (503) 494-7887.

**PARTICIPATION:**

Dr. Diane Stadler (503) 494-0168 has offered to answer any questions you may have about this study. If you have any questions regarding your rights as a research subject, you may contact the OHSU Research Integrity Office at (503) 494-7887. You do not have to join this or any research study. If you do join, and later change your mind, you may quit at any time. If you refuse to join or withdraw early from the study, there will be no penalty or loss of any benefits to which you are otherwise entitled.

The investigators may withdraw you from this research study at any time if they believe it is in your best interest. You may be asked to withdraw from the study at the investigator's discretion, sponsor's discontinuation, or because of pregnancy or serious side effects, or because of your failure to comply

with instructions or unwillingness to participate in study procedures. If you decide to withdraw from this study, we will ask you to complete one final follow-up and discharge visit. We will inform you of any new findings that may affect your willingness to continue or to withdraw from this research study. We will give you a copy of this consent form.

The participation of OHSU students or employees in OHSU research is completely voluntary and you are free to choose not to serve as a research subject in this protocol for any reason. If you do elect to participate in this study, you may withdraw from the study at any time without affecting your relationship with OHSU, the investigator, the investigator's department, or your grade in any course.

**SIGNATURES:** Your signature below indicates that you have read this entire form and that you agree to be in this study.



\_\_\_\_\_  
Signature of Subject Date

\_\_\_\_\_  
Signature of Person Obtaining Consent Date

\_\_\_\_\_  
Signature of Investigator Date



## Appendix B

Collection Tube	Analyte	Aliquot Vol (μl)	Time Points (hr)											
			0800	0830	0930	1030	1130	1230	1300	1330	1430	1530	1630	1730
In order of priority	In order of priority	2 sets/analyte if possible												
<i>10 ml red top, deliver 6.0 ml whole blood</i>	TSH*	500	X											
	hsCRP	100	X			X		X			X		X	
	Osteocalcin	500	X	X	X	X	X	X	X	X	X	X	X	X
	Carboxylated osteocalcin	500	X	X	X	X	X	X	X	X	X	X	X	X
	C-peptide	500	X	X	X	X	X	X	X	X	X	X	X	X
	Insulin	500	X	X	X	X	X	X	X	X	X	X	X	X
	Leptin	500	X	X	X	X	X	X	X	X	X	X	X	X
2-ml grey top (NaFI/K-oxalate)	Glucose	500	X	X	X	X	X	X	X	X	X	X	X	X
<i>6-ml purple top (K3-EDTA)</i>	Total Triglyceride	500	X	X	X	X	X	X	X	X	X	X	X	X
	Total ghrelin	500	X	X	X	X	X	X	X	X	X	X	X	X

	Active ghrelin	500	X	X	X	X	X	X	X	X	X	X	X	X
	TNF- $\alpha$	500	X			X		X			X		X	
	IL-6	500	X			X		X			X		X	
	Fatty acid profile	100	X			X		X			X		X	
<i>3-ml purple top (K3-EDTA) no vacuum, pretreated with 15 <math>\mu</math>l DPP-IV and 90 <math>\mu</math>l aprotinin deliver 1.5 ml whole blood</i>	Active PYY (3-36)	300	X	X	X	X	X	X	X	X	X	X	X	X
<i>3-ml purple top (K3-EDTA) no vacuum, pretreated with 15 <math>\mu</math>l DPP-IV, deliver 1.5 ml whole blood</i>	Active GLP-1	300	X	X	X	X	X	X	X	X	X	X	X	X
<i>3-ml purple top (K3-EDTA) no vacuum, pretreated with 20 <math>\mu</math>l THL, deliver 2.0 ml whole blood</i>	Non-esterified Free Fatty Acid (NEFA)	400	X	X	X	X	X	X	X	X	X	X	X	X
<i>Total volume drawn per time point (ml)</i>			19	19	19	19	19	19	19	19	19	19	19	19

NOTE: All vacutainers, except for red top, should be pre-chilled on ice before collecting blood samples. Once blood is collected, all tubes (except red top) should be returned to ice and spun in a refrigerated centrifuge within 15 minutes. Red top tube should be allowed to sit at RT for 15-20 minutes before spinning. Make second set of aliquots if additional serum/plasma is available. Aliquot tubes for total and active ghrelin should be treated with HCL and PMSF. Aliquots should be frozen immediately at -20 C for up to 72 hours and then transferred to a -80 C freezer. \*TSH is drawn with fasting sample during first inpatient admission, only. All samples will be stored at -80 C at the GCRC Core Lab for EOS analysis. Total blood Volume = 228 ml (~1 cup total).

## Appendix C

### Appetite with Meals

ID:	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
Date:	<input type="text"/>	/	<input type="text"/>	/	<input type="text"/>
Study Week:	<input type="text"/>	<input type="text"/>			

For each item, place a single vertical mark on the line that indicates how you are feeling. Mark anyplace on the line. Please do not mark the lines with an X.

FOR EXAMPLE: How happy did you feel today? Not at all _____   _____ Extremely happy happy
--

You should answer the questions immediately before you eat each meal that is indicated above the questions (breakfast, lunch, and dinner) and immediately after you have finished eating each meal that is indicated above the questions (breakfast, lunch, and dinner).

**Before Breakfast:** \_\_\_\_\_ Time of rating

How hungry do you feel right now?

Not at all \_\_\_\_\_ Extremely  
hungry hungry

How full do you feel right now?

Not at all full \_\_\_\_\_ Extremely full

**After Breakfast:** \_\_\_\_\_ Time of rating

How hungry do you feel right now?

Not at all \_\_\_\_\_ Extremely  
hungry hungry

How full do you feel right now?

Not at all full \_\_\_\_\_ Extremely full

**Before Lunch:** \_\_\_\_\_ Time of rating

How hungry do you feel right now?

Not at all \_\_\_\_\_ Extremely  
hungry hungry

How full do you feel right now?

Not at all full \_\_\_\_\_ Extremely full

**After Lunch:** \_\_\_\_\_ Time of rating

How hungry do you feel right now?

Not at all \_\_\_\_\_ Extremely  
hungry hungry

How full do you feel right now?

Not at all full \_\_\_\_\_ Extremely full