THE EFFECT OF DIETARY MEDIUM CHAIN AND LONG CHAIN TRIGLYCERIDE ON ACYL GHRELIN LEVELS AND RELATIONSHIPS TO HUNGER AND SATIETY

By

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CERTIFICATE OF APPROVAL

This is to certify that the Master's thesis of

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Abstract

Background: Hunger and satiety are mechanisms that govern energy intake. Ghrelin is a gut hormone that stimulates hunger and food intake in rodents and humans. In order to become active, ghrelin is acylated by an 8-carbon fatty acid. The source of this fatty acid is unknown, but may derive from dietary fat.

Methods: Following a baseline 2-week, isocaloric low-fat diet (Lo-Fat), 21 subjects were randomized to one of two additional two-week periods in which subjects consumed isocaloric high fat diets: which were composed of equal amounts of polysaturated:saturated:monounsaturated oils (Hi-Fat) or high in medium chain triglyceride (MCT). Following completion of the first high-fat diet feeding assignment, subjects underwent a 6-week washout period and were then fed the alternative high-fat diet. At the end of each feeding period, blood was taken for measurement of ghrelin levels (acyl- and desacyl-) and subjects completed visual analog scores to measure hunger and fullness every 30 minutes over a 13.5-hour period including three standardized meals.

Results: RM-ANOVA area under the curve (AUC) for acyl ghrelin was significantly different between all three diet groups (P= 0.025). AUC on MCT was significantly greater compared to Hi-Fat (P=0.012), and the AUC on Lo-Fat was significantly higher than Hi-Fat (P=0.03). The fasting acyl ghrelin levels were also significant between groups (P=0.032). The MCT diet resulted in a significantly higher fasting acyl ghrelin than Hi-Fat

(*P*=0.009). Des acyl ghrelin levels were significantly lower on the MCT diet group than both Hi-Fat (*P*=0.016) and Lo-Fat (*P* =<0.001). The ratio of acyl ghrelin to des-acyl ghrelin was significantly higher during the MCT diet than Hi-Fat, and approached significance compared to Lo-Fat (*P*=0.05). Daily hunger scores were higher (*P*=0.02) and fullness scores were lower (*P*=0.034) during the two weeks feeding phase on MCT diet compared to Lo-Fat. There was no statistical difference between the MCT and Hi-Fat with regard to hunger or satiety during the two-week diet phases. Hunger and ghrelin levels followed a similar pattern, increasing before a meal and decreasing after the meal, in all subjects.

Conclusion: The proportion of ghrelin in its active form is higher during an MCT diet compared to a Lo-Fat diet. A diet high in MCT also increases hunger and decreases fullness. These data suggest that 1) the acyl group on ghrelin can be derived from dietary sources and 2) the increase in acyl ghrelin while on a high MCT diet may mediate the enhanced hunger experienced by participants on this diet compared to the other diets.

Introduction

The complex regulation of hunger and satiety in humans, including hormones and receptors in the brain, gut and adipose tissue, has not been fully characterized. Increased hunger, decreased satiety, or both may induce excess energy intake and lead to obesity. Thus, dysregulation of the body's systems that control appetite and energy expenditure, including gut hormones, may play a role in the development of obesity. For example, ghrelin is an endogenous gut hormone that, in its active form, stimulates appetite and food intake in rodents as well as in humans and has been proposed to act as a meal initiator. In order to be active, ghrelin requires esterification of a medium chain fatty acid, octanoate. Although the source and regulation of this acyl group is not completely understood, it has been proposed that levels of active ghrelin and the fatty acid moiety can be derived from dietary sources.

Increased consumption of specific components of food, such as fat, can lead to changes in satiety and total calories consumed in humans. Medium chain triglycerides (MCT) are a type of fat commonly found in foods such as coconut oil and palm kernel oil that are absorbed much faster and undergo different hepatic metabolism than long chain triglycerides (LCT). Fatty acids in MCT oil have chain lengths between 6 and 12 carbons, including octanoate, an eight-carbon fatty acid. If dietary fat is a source for the octanoate during generation of ghrelin to its acylated form, then it is possible to test how diets that differ in fat composition alter circulating ghrelin levels and, in turn, hunger. Therefore the goal of this project is to compare the effects of a diet high in MCT on hunger and satiety in humans to control diets either high in LCT (Hi-Fat) or low in fat

and high in carbohydrates (Lo-Fat). By measuring desacyl- and acyl (active) ghrelin, along with hunger and satiety levels before and after meals, we plan to determine if a high MCT diet will alter active ghrelin concentrations in a direction that is consistent with individual differences subjects may experience in hunger and satiety during isocaloric feedings.

This is a secondary analysis of the study "Ghrelin Regulation and Structure: Effect of Diet Composition on Ghrelin," conducted by Dr. Jonathan Q. Purnell, in which obese and lean subjects were fed three different isocaloric diets for two-weeks each: 1) a diet high in medium-chain triglycerides (MCT), 2) a diet high in total fat from long-chain triglycerides (Hi-Fat), and 3) a diet low in fat and high in carbohydrates (Lo-Fat).

We sought to complete the following aims:

Specific Aim 1: Compare the average daily hunger and satiety scores in lean and obese subjects during two-week meal cycles consisting of meals with different dietary fat and macronutrient content.

Hypothesis 1:

Isocaloric diets high in MCT will increase acyl ghrelin levels, increase hunger, and decrease satiety compared to control diets high in long-chain triglyceride and CHO (low in fat) (directional effect: MCT > Hi-Fat > Lo-Fat).

Table 1: Expected Outcomes Specific Aim One			
	Acyl ghrelin	Hunger	Satiety
Diet: Lo Fat, high carbohydrate	Neutral	Neutral	Neutral
Diet: High Fat	Neutral	↑	↓
Diet: High MCT	↑↑	↑↑	↓↓

Specific Aim 2: From measurements taken over the course of three meals in a day, correlate the changes in plasma concentrations and ratio of active (acyl) ghrelin to total immunoreactive ghrelin in response to differing fat content of meals and to the changes in hunger or satiety levels experienced by lean and obese subjects during those meals.

Hypotheses:

1) The levels of active (acyl) ghrelin will be higher following consumption of a high MCT diet compared to both the high LCT and the high carbohydrate diets.
2) The change in acyl ghrelin levels between meals will be positively associated with an increase in hunger and a decrease in satiety.

Table 2: Expected Outcomes Specific Aim Two			
	Acyl Ghrelin	Hunger	Satiety
Lean Subjects	Higher	Increased	Decreased
Obese Subjects	Lower	Decreased	Increased
High MCT Diet	Higher	Increased	Decreased
High Total Fat Diet	Lower	Decreased	Increased

Background and Significance

Obesity has become a worldwide epidemic that is a leading contributor of increased morbidity and mortality (1). Although the study of the physiology of body fat regulation has been advancing rapidly, the pathophysiological processes that lead to obesity remain incompletely understood. Body weight regulation is a complex interaction between bodily systems that control food intake and energy expenditure, such as the brain stem and hypothalamus, as well as "feedback" mechanisms from hormonal signals released from fat cells and the gut, reflecting fat mass and the timing and proportion of food intake, respectively. Ghrelin, a recently discovered ligand of the growth hormone secretagogue receptor (GHSR) secreted by the gut, has been shown to stimulate food intake in animals and humans. This project will explore the relationships of dietary macronutrients on concentrations of total and active ghrelin and the perception of hunger and satiety in human subjects.

Obesity and Diet

Environmental factors play a role in obesity. Decreased daily physical activity has been linked with increased BMI, but there is also a positive correlation found between calorie-adjusted total fat intake, especially saturated fatty acid intake, and obesity (2). The palatability of dietary fat may play a role as women have been shown to exhibit a significant correlation between greater percent body fat with an increase in the sensory preference for fat, even in normal-weight subjects (3). Dietary levels of fat have also been shown to stimulate appetite and food intake in animal models and in human studies (3).

Regulation of Hunger and Satiety

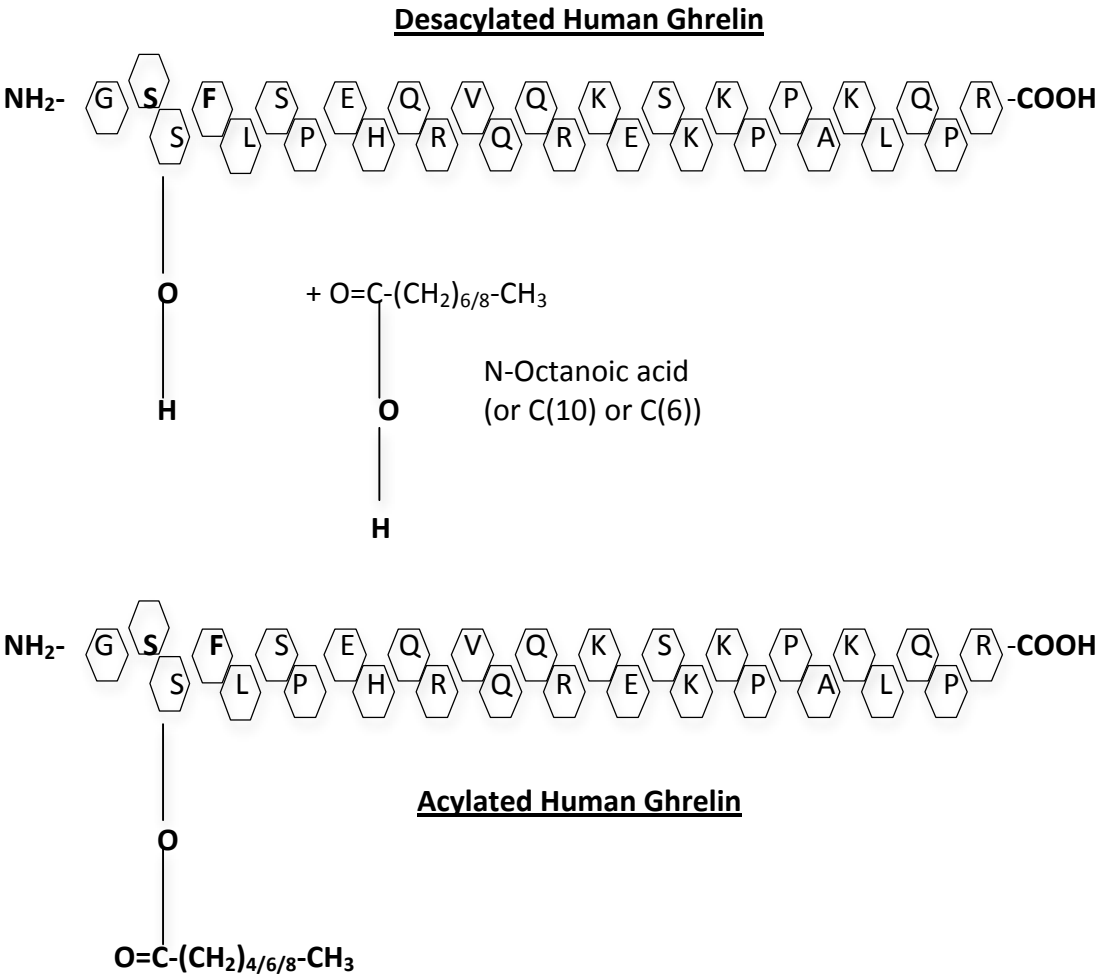
The regulation of food intake and appetite is mediated through changes in hunger and satiety. One of the major brain sites involved in food intake regulation is a region in the hypothalamus called the arcuate nucleus, which contains neurons that respond to peripheral hormonal signals through hormone receptors. In turn, these neurons alter feelings of hunger and fullness by signaling second and third-order brain centers through release of neuropeptides. Examples of such neuropeptides are the melanocyte-stimulating hormone (MSH), which suppresses hunger, neuropeptide Y (NPY), which stimulates hunger, and agouti related protein (AgRP), which inhibits the actions of MSH (4). Several peripheral hormones have been identified that regulate these neurons. Most of these are gut hormones, such as cholecystekinin (CCK), that promote satiety during meal ingestion (4).

Ghrelin Structure and Activity

Another gut hormone, ghrelin, is secreted from the stomach and duodenum and is thought to be a meal initiator through stimulation of hunger (5-8). Ghrelin is an endogenous ligand for the growth hormone secretagogue receptor (GHS-R). Ghrelin is a 28 amino acid peptide, which is modified at serine residue 3 by attachment of *n*-octanoic acid (C8:0) (5-8). This modification is essential for bioactivity (5). It was determined by Matsumoto, et al. that the highest activity for ghrelin came with the octanoyl (C8:0) modification (6). They also found that it was essential for the side chain at Ser3 to be hydrophobic in order for the GHS-R stimulating activity of ghrelin to work properly. In another example, it has been reported that acyl-ghrelin activates the GHS-R

and in primary hepatocytes it can induce an increase in glucose output (7-8). Des-acyl ghrelin, in contrast, does not bind or activate the GHS-R and inhibits glucose release in primary hepatocytes. When given together, des-acyl ghrelin completely reverses the acylated ghrelin-induced glucose output (8).

Figure 1: Two Structures of Ghrelin



Ghrelin Site of Action, Stimulated Hunger, and Meal Initiation

Ghrelin can stimulate hunger through hypothalamic brain receptors. In an animal study conducted by Banks, et al., it was found that human acyl-ghrelin is transported via saturable systems from brain-to-blood and from blood-to-brain (9) whereas des-Octanoyl ghrelin is able to enter the brain via a nonsaturable transmembrane diffusion. Once across the blood-brain barrier, acyl ghrelin can mediate a hunger response primarily through stimulation of NPY and AgRP containing arcuate neurons (4). In an animal study, ghrelin injected directly into the arcuate reduced the latency to feed and increased the amount of food consumed in the first couple of hours after the treatment was given, consistent with a meal-initiating role (10). There have been several studies, both rodent and human, that suggest the primary mode of action of ghrelin on hunger is via the vagus nerve. In one rodent study, it was found that in response to food deprivation, sham-operated rats had an increase in circulating ghrelin of approximately 60%, whereas the vagotomized rats showed no response (11). In human subjects who have undergone a vagotomy and gastrectomy, infusion of ghrelin increased growth hormone (GH) secretion but did not change appetite or energy intake (12).

Previous infusion studies in humans utilizing pharmacologic doses support a role for ghrelin as a meal initiator by increasing appetite before meals and inducing hunger (13-14). In one study, subjects who received ghrelin injections reported increased hunger scores before breakfast and lunch with an increase in food intake and no evidence of reduced hunger up to one hour after each meal (14). However, it is

important to consider effects of endogenous, physiologically relevant levels of ghrelin. In studies of changes in endogenous ghrelin levels, Cummings, et al. (13) showed that plasma ghrelin levels increased by as much as 78% one to two hours before the initiation of each meal, and fell to trough levels within an hour of meal initiation. Ghrelin levels were also found to progressively increase between meals, throughout the day. In a related study, however, it was found that with no external hunger cues, that a rise or peak pre-meal plasma ghrelin levels were not significant determinants of a meal request (15). Although there was still a rise, though not significant, in plasma ghrelin before a meal, the authors of this study suggest that ghrelin may play a role in appetite stimulation but it is not crucial for the timing of a meal request. This study also found a rapid fall in ghrelin after a meal, which led the authors to suggest that ghrelin may play a role in signaling to stop eating.

Other studies that measure ghrelin levels pre- and post-meal consistently illustrate an association between ghrelin and meal initiation. One such study compared ghrelin levels between three distinct groups: normal weight controls, diet-induced weight loss controls and gastric bypass subjects (16). In the diet-induced weight loss group, circulating ghrelin levels had a similar temporal pattern before and after weight loss. These ghrelin levels were found to rise progressively one to two hours before each meal and fell to trough levels within one to two hours post meal initiation, consistent with ghrelin's role in meal initiation. As seen in previous studies, ghrelin levels rose progressively before each meal throughout the day, with a mean of 20 percent before breakfast, 45 percent before lunch, and 51 percent before dinner. These findings were

not the case with the gastric bypass group, which demonstrated not only unusually low levels of ghrelin but also failed to show the meal-related oscillations and diurnal pattern of ghrelin that is demonstrated in the control group (16).

Ghrelin and Body Weight

Ghrelin levels have been shown to be related to weight gain and body fat. For example, there is a positive association between plasma ghrelin levels and age, which contributes to the theory that as humans age, we tend to progressively gain weight (13). Mice that have undergone a gastrectomy have been shown to have plasma ghrelin concentrations that are decreased by approximately 80%, as well as having a mean body weight that is 15% lower than in mice that had not undergone a gastrectomy (17). The gastrectomised mice that received ghrelin injections did not lose weight and had an increased body weight and fat mass. In the nongastrectomized mice, ghrelin injections increased the fat mass. These studies suggest that increases in ghrelin, especially endogenous sources, can have an impact on fat mass and weight gain, possibly due to ghrelin's capacity to increase hunger.

These conclusions about ghrelin's role in obesity, however, are tempered by a paradoxical finding that circulating ghrelin levels are lower in obese subjects than in lean subjects (18-20). This observation could indicate that during weight gain, the body "down regulates" ghrelin secretion to prevent further fat accretion, since diet-induced weight loss can increase ghrelin levels in obese persons (16). Other studies have reported that while ghrelin levels are lower in obese compared to lean subjects, obese subjects do not exhibit meal-dependent suppression of postprandial circulating ghrelin

normally seen in lean subjects (19). In a related study, however, there was significant postprandial ghrelin suppression in obese subjects, though it still remains less than in lean subjects (21).

In summary, findings from animal and human studies suggest that ghrelin is a hormone that behaves as a meal initiator through actions on the hypothalamus, the hunger/satiety center of the brain. The relationship between obesity and ghrelin is still ambiguous, yet ghrelin could play a role in weight regulation, especially in the case of caloric deprivation.

MCT, Obesity, and Weight Loss

Several studies have tested the impact of various types of dietary fat, such as medium-chain triglyceride (MCT) oil and long-chain triglyceride (LCT) oil on food intake and the accumulation of body fat (22, 23, 26, 28, 29). MCTs are considered to be saturated fatty acids with an 8 to 10 carbon chain length, where LCTs are typically fatty acids with a carbon chain length of 12 or more carbons and may be saturated or unsaturated. MCTs are absorbed in the gastrointestinal track where they are rapidly hydrolyzed into MCFAs and enter the portal circulation where they go directly to the liver (24, 25, 27). LCTs undergo more detailed processing where they have to be re-esterified into triglycerides by the enterocyte before they can be packaged into chylomicrons and enter the circulatory system, where they may be hydrolyzed back to free fatty acids and taken up into tissues other than just the liver (4), such as adipose tissue.

Most studies looking at MCT versus LCT on food intake and body weight have conflicting outcomes. In one study, conducted by Van Wymelbeke, et al (22), subjects who were served breakfast supplemented with MCT oil had greater satiation at lunch then did the subjects served breakfast supplemented with LCT oil. This study also found that the MCTs were absorbed much more quickly then was the LCTs, demonstrated by a plasma fatty acid peak of 30-60 minutes after breakfast in the MCT group versus approximately 5 hours in the LCT groups. Yet another study found that in comparing a diet high in MCT versus a diet high in LCT, the MCT diet had a smaller reduction in hunger then did the LCT diet (23). These results demonstrate the need for clarification of the effects that fatty acid chain length, especially medium chain fatty acids, has on hunger or satiety.

Also of interest, would be the role of dietary carbohydrate in comparison with dietary fatty acids, and their effect on hunger and satiety. Results of a study conducted in 2001, looking at lunches with varying carbohydrate and fat contents, showed there was no significant difference between the hunger ratings on a hedonic scale between a high carbohydrate, high LCT, or high MCT diets (24). This study looked at the effects of consuming four different lunches, which included a control, high carbohydrate, high MCT, and high LCT. The outcome of the study demonstrated that carbohydrates are more satiating and resulted in a delayed dinner request by participants. Looking at the fat content, however, the high MCT meal did not delay the dinner request but resulted in a significantly lower food intake at dinner. By looking at the time to next meal and

amount consumed at next meal, this study suggests that fat and carbohydrate play different roles in satiety and food intake.

Several studies have documented that consumption of MCT oil in the diet has been shown to be beneficial for weight loss in rodents and in humans (26-28). One study compared an intake of MCT oil with olive oil in humans for the impact on weight management and cardiovascular risk (26). The consumption of MCT oil led to a greater weight loss than that of olive oil, and there was no adverse effect on cardiovascular risk. At the beginning of this study, there were nine participants with metabolic syndrome, six in the olive oil group, and three in the MCT group. By the end of the study there were six participants with metabolic syndrome, five were in the olive oil group and one in the MCT group. This study leads to the conclusion that including MCT oil in the diet can be a part of a successful weight loss program with little if any cardiovascular side effects. There were several limitations to this study that should be taken into consideration: 1) there was no real long chain saturated fat control oil comparison, 2) the number of subjects was very small, and 3) the population was a mix of insulin sensitive and insulin resistance patients.

Seaton B, et al. reported that after consuming a meal high in MCT the resting metabolic rate in men increased significantly when compared to a meal high in LCT (27). This study concludes that this increase in metabolic rate may suggest that the liver plays a role in postprandial thermogenesis. The authors of this study suggest that by replacing LCT with MCT for extended periods of time weight loss can be produced without reduced energy intake.

In rodents, an MCT diet versus an LCT diet demonstrated a weight loss benefit for the group consuming MCT oil (28), with a weight loss of approximately 15%. Amount and size of fat cells were also measured in these rodents, showing a significantly lower amount of body fat, as well as a lower mean adipocyte size, in the MCT group when compared to the LCT group. This study also looked at the resting oxygen consumption in the two groups of rats. Rats fed MCT oil had a significantly higher resting oxygen consumption, which suggests MCT oil may alter dietary induced thermogenesis. This concept lends itself to explaining how excess calories from MCT oil can lead to smaller adipocyte size and less body fat due to increased total energy expenditure.

To solidify this concept, a study was conducted with forty-nine overweight humans in a double-blind randomized case control design to look at the effect of MCT versus LCT (olive oil) on weight loss and fat mass (29). It was concluded that when compared to LCT, the subjects in the MCT group had greater weight loss, as well as a greater loss of fat mass and trunk fat. Of note is that the subjects in the study also received counseling on topics of weight management.

These studies suggest that the mode of digestion, absorption, and transport of MCTs in the body have a beneficial effect on body weight. As previously mentioned, however, ghrelin becomes active after a modification with *n*-octanoic acid, which is an eight-carbon fatty acid chain with no double bonds. These *n*-octanoic chains, or medium-chain fatty acids (MCFAs), are found in medium-chain triglycerides (MCTs) (5). It was found in rodents fed MCFAs and MCTs that stomach concentrations of acylated

ghrelin increased while total ghrelin levels remained unchanged (5). These results indicate that ingestion of MCFAs can be directly used for the acylation of ghrelin, though this has not been shown to occur in humans thus far.

Given this data, the concept of an increase in weight loss with a diet high in MCT is logically dissonant with the hypothesis that MCT in the diet will increase active ghrelin, in turn leading to an increase in hunger. This could be explained by the hypothesis by Cummings, et al. (13), that ghrelin may play a role more as a signal to not eat instead of simply being a meal initiator. However, there may be other factors to consider, especially in the case of obesity, such as roles leptin and insulin play in hunger and satiety (13).

Summary

Obesity is known to be a growing problem in our society, and is a result of many factors, many of which are still not well understood. Some of the effectors may be the components of dietary intake, such as carbohydrates or types of fat, as well as the connection between these components and hunger and satiety regulation. There have been many studies that look at correlations between hunger and satiety levels in association with dietary fat, as well as the activation of ghrelin, a hormone found to play a role as a meal initiator. There have also been studies that look at the levels of active ghrelin in lean versus obese subjects. The structure of ghrelin has also been identified as being activated with an attachment of *n*-octanoic acid, which is a C8:0 fatty acid found in medium-chain triglycerides. One source of this C8 fatty acid may include the diet.

There is a complexity that has not yet been fully studied between the connection of dietary fat, ghrelin levels and hunger and satiety levels. Therefore, the purpose of the current study is to make a study the relationship between dietary MCT, level s of endogenous active ghrelin, and expression of hunger or satiety and in humans.

Subjects and Methods

General Study Design

This is a secondary analysis of a study conducted by Dr. Jonathan Purnell entitled "Ghrelin Regulation and Structure: Effect of Diet Composition on Ghrelin." The study included a randomized crossover design assessing hunger and satiety, as well as ghrelin levels, between lean and obese subjects conducted at the OHSU Clinical and Translational Research Center (CTRC).

Once selected, study subjects began a two-week baseline feeding period with a diet consisting of 15% protein, 20% fat, and 65% carbohydrates (Lo-Fat). All meals were provided by the OHSU CTRC Bionutrition Unit. Caloric content of the diet was calculated based on the dietary history of each study participant and the Harris-Benedict formula (BEE times an activity factor) to maintain subject's weights to within 1 kg over the two-week lead in period. The subjects were instructed to not alter normal activity patterns for the duration of the study (table 4).

At the conclusion of the two-week baseline period each subject was admitted to the inpatient unit of the OHSU CTRC for a 13.5 hour period of testing. Body composition of each subject was measured by a Dual-energy X-ray absorptiometry (DXA) scan. Visual analog scales were completed by each subject to determine their level of hunger or satiety. Blood samples were taken every thirty minutes to measure plasma levels of acyl and des-acyl ghrelin. After the first CTRC visit, subjects returned to their usual diet for a period of 6 weeks before they were randomized into either the diet high in total fat (Hi-Fat) or the diet high in medium-chain triglycerides (MCT). Two weeks on the high fat

diet, LCT or MCT, was again followed by a 13.5-hour inpatient testing period. A six-week washout period of home-prepared diet was then followed by the other CTRC prepared high fat diet for two weeks (table 4).

Participants

Lean (BMI 19-24.9kg/m^2) and obese (BMI ≥ 30 kg/m^2) subjects were recruited from the Portland-Vancouver area to participate via postings on the OHSU website and advertisements in local newspapers. In equal numbers, men and women were recruited and the groups were matched for age. Of those recruited, 16 lean and 5 obese subjects completed the study. Three factors were assessed for inclusion in the study: each subject was required to be 18 years or older, had been weight stable for at least three months, and were at their lifetime maximal body weight. Subjects were excluded if they were actively losing weight, smoking, consume more than 2 alcoholic beverages per day, were on prescription medication, or had type 2 diabetes. A complete list of criteria can be seen in table 3. All eligible subjects then returned for an interview conducted by the CTRC dietitian, as well as completed food frequency and activity questionnaires. At this time the subjects tasted the foods that represent the three different diets used for the study to ensure palatability and tolerance.

Table 3: Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
• Age 18 or older • Weight stable for at least 3 months • Currently at lifetime maximal weight	• Actively losing weight by diet or exercise • Smokers • Alcohol consumption of >2 drinks per day • Exercise >30 minutes 3 times a week • Prescription drug use (except birth control, vitamins or minerals) • Food intolerance • Type 2 diabetes • Heart disease, cancer, malabsorptive states, or chronic infections that would affect body weight • Weight >300 lbs (exceeds weight limit of the DEXA machine) • Hemoglobin <12.0 g/dL for women, < 13.5 g/dL for men

Study Design

After an initial screening visit, obtained consent, and a meeting with the CTRC dietitian subjects began a baseline feeding period for two-weeks of a diet, low fat diet or control diet (Table 4). Study participants were instructed to consume all food provided, and came to the CTRC three times per week to pick-up pre-made meals and to be weighed. Participants returned their unwashed food containers, which were then weighed to quantify uneaten food portions. The calorie content of the diet was adjusted after the first week as required for the participants to meet the target for weight stability. This adjusted caloric content was used in the subsequent feeding phases and each CTRC stay. Each participant completed a brief daily diary about their intake and any symptoms they may have experienced while eating the food provided by the Bionutrition Unit.

Table 4: Study Design

Subjects	Consent and Screen	Meet with Dietitian	2 week Low Fat Diet	CTRC 1	2 Week High Fat or MCT Diet	CTRC 2	2 Week MCT or High Fat Diet	CTRC 3
Lean	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Obese	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**

Within the first week of admission, body composition of each participant was measured by a DXA scan. At the end of the initial two-week feeding period, all subjects were admitted to the inpatient unit of the CTRC overnight to ensure a fasting state and have an IV line placed. The meals provided during this inpatient stay, breakfast, lunch

and dinner, each consisted of one third of the participant's daily needs, and were eaten at the standard times of 08:00, 12:00, and 17:00. Instructions were given to the participants to eat all of their food within 30 minutes.

After completion of the CTRC 1 visit each subject returned to his or her usual diet for a 6-week washout period. The subjects were then randomized into an isocaloric diet of 15% protein, 40% fat and 45% carbohydrate that were either high in long chain saturated fat, or high in medium chain triglycerides (MCT). The participants again were provided with 3-day rotating meals prepared by the OHSU CTRC Bionutrition Unit. The high fat diet contained a ratio of polyunsaturated:saturated:monounsaturated fats matching that of the low fat diet. The MCT diet contained 30% of total calories from MCT and 10% from vegetable oil, and provided the approximate percentages of fatty acids: C:6 (0.88%), **C8:0 (63%), C10:0 (23%)**, C12:0 (0.36%), C16:0 (1.51 %), C18:0 (0.32%), C18:1 (3.2%), C18:2 (7.3%), and C18:3 (0.18%).

Study participants again followed up in the CTRC three times per week to pick up prepared meals and for body weight checks. Participants were given the same directions as before to maintain weight and consume all foods provided. At the end of this feeding phase, the participants were again admitted for the CTRC visit 2 and repeat testing with the same sample protocol and meal timing used at the CTRC visit 1. Following this visit, the participants returned to another 6-week washout period on their usual diet, and then were assigned to the alternative high-fat diet for two weeks. During this time, they remained weight stable, and then they were admitted for the final CTRC visit 3 and repeat testing.

Hunger/Satiety Scales

During the two week diet phases, each participant was required to fill out a daily visual analog scale to assess appetite (appendix 1, parts I, II) While admitted for the CTRC visits, each subject again completed visual analog scales to assess appetite (appendix 1, part III). The scales were filled out every 30 minutes beginning at 07:30 am and continue for 13 ½ hours.

Collection of Plasma Samples for Ghrelin Assay

Participants gave written informed consent during the initial screening process, and all procedures followed a protocol approved by the Institutional Review Board of the Oregon Health and Science University and the Clinical and Translational Research Center (CTRC). During the three inpatient visits, overnight fasting blood samples of 15 mL were collected starting at 07:30 am, and collected every 30 minutes continuing for 13 ½ hours. The blood samples were drawn into cold syringes and added to chilled EDTA Vacutainer tubes preloaded with 4-[2-aminoethyl benzene] sulfonyl fluoride (AEBSF; Alexis Biochemicals, San Diego, Ca) on ice. The blood was quickly centrifuged and the plasma separated and acidified with 200 µl 1N HCL. The samples were then frozen at -80° C until assayed.

Ghrelin Sandwich Assay

To measure blood levels of acyl and des acyl ghrelin we used the same methods described in Lui J, et al. (30): Plates (384-well Maxisorb; Nunc, Roskilde, Denmark) were coated with acyl-specific antiserum at 1 µg/ml overnight. The plate was then blocked, washed, and loaded with 25µl/well wetting/neutralization buffer (0.5 M phosphate

buffer with 1% BSA, pH 7.4) and 25 µl/well ghrelin standards and incubated 1 h with the biotinylated C-terminal ghrelin antiserum in blocking buffer and then for 30 minutes with streptavidin-poly-HRP80 (RDI Fitzgerald, Concord, Ma). Finally, the plate was detected with the fluorescent substrate Amplex Red (Molecular Probes, Eugene, Or). Fluorescence was read using excitation/emission wavelengths of 535/590 nm (Tecan Genios plate reader; Phenix Research, Hayward, Ca). All unknowns were run in duplicate, and all samples for each admission of each subject were run on the same plate. Standards were made up in acid/AEBSF-treated striped plasma. The protocol for the des-acyl ghrelin assay followed that used for the ghrelin sandwich assay with the substitution of affinity-purified C-terminal ghrelin antiserum for the capture step and biotinylated N-terminal des-acyl ghrelin-specific monoclonal antiserum as in the reporter. All other steps were unchanged.

My Part of the Study

The main outcomes for my analysis were the change in hunger and satiety, and levels of acyl and des-acyl ghrelin at various times of day and between the three diets tested. I organized all of the previously collected data into tables. From there I conducted all the statistical analyses and created the tables and figures that are contained within this thesis.

Statistical Analysis

Since the trends for hunger, fullness and ghrelin levels followed similar patterns between the lean and obese groups, they were combined for all statistical analyses described here.

Descriptive Statistics

The results are expressed as the mean ± SD for each variable. These variables include hourly acyl ghrelin and des-acyl ghrelin, as well as hourly and daily hunger and satiety scores. The nadir to peak was calculated and analyzed. An area under the curve (AUC) was conducted as a secondary analysis to measure the hunger and satiety in relation to ghrelin levels. The software used was SPSS for Windows (version 21; SPSS Japan Inc., Tokyo, Japan). $P < 0.05$ was considered statistically significant.

Analysis Plan

The end points that were measured were the daily hunger and satiety scores between the three diets consumed (high CHO, MCT, LCT). A repeat measure analysis of variance (RM ANOVA) test was used to compare the statistical outcomes between the three groups.

Contrast Analysis

Exploratory studies were conducted to establish a positive or negative correlation between hunger and satiety and the three diets consumed. A paired t-test was used to make comparisons between the initial diet of high CHO and each of the high fat diets, the MCT vs. LCT. Linear relationships, as well as an analysis of peak to nadir,

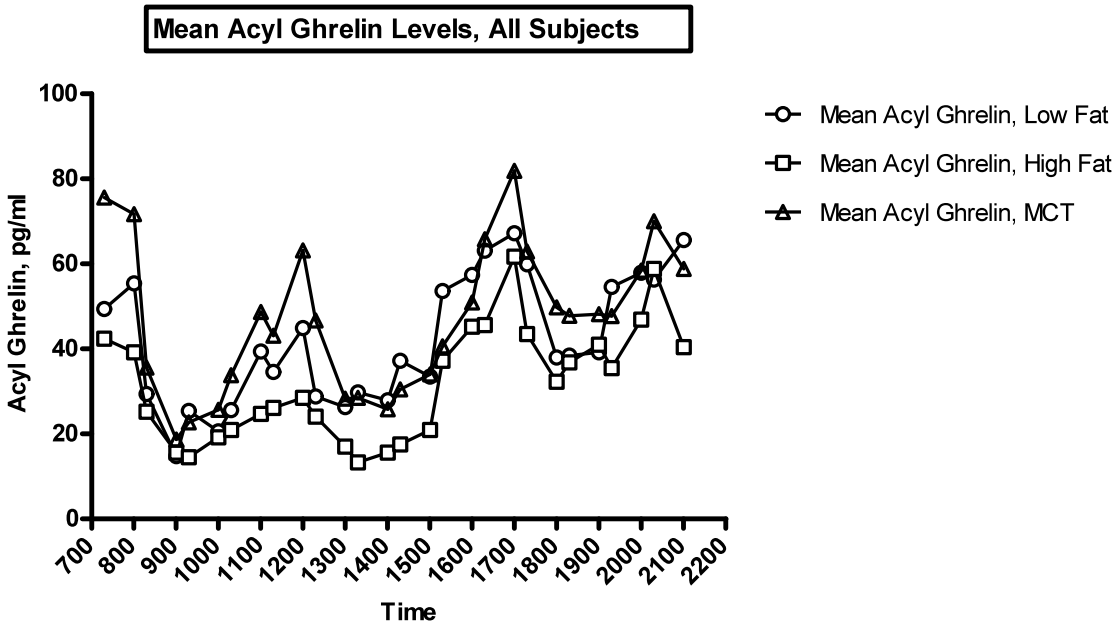
were used to determine the effect on hunger and satiety over the time specified for the study.

Results:

Effect of dietary fat and carbohydrate on ghrelin levels

Corresponding to figure 2 (below) and table 5 (appendices), the MCT diet had a significant effect on some aspects of acyl ghrelin levels.

Figure 2.



Area under the curve between groups was significantly different at *p*= 0.025 (Table 5). AUC was significantly higher on the MCT diet compared to HF diet (*P*-value =0.012), and the LF diet was significantly higher than HF diet (*P*-value =0.03).

The fasting values, measured at the beginning of the day, were significantly different between groups at *p*= 0.032. The MCT resulted in a significantly higher fasting acyl ghrelin than the HF diet (*P*-value =0.009).

At breakfast, the nadir for acyl ghrelin was significantly higher for the MCT diet than the HF diet (*P*-value =0.034) and the LF diet (*P*-value =0.042). The percent change

from the morning fasting peak to the morning nadir was in no way significant for acyl ghrelin levels by diet.

For lunch, there was more significance. The peak of acyl ghrelin before lunch was significantly different for all three diets (*P*-value =0.021). The MCT diet had significantly higher acyl ghrelin compared to the HF diet (*P*-value =0.006). The percent rise from breakfast nadir to pre-lunch peak was significantly different between groups (*P*-value =0.012). The rise in acyl ghrelin was significantly higher for the LF diet compared to the MCT diet (*P*-value =0.011) and the HF diet (*P*-value =0.009). The nadir after the lunch meal was consumed was statistically significant between groups (*P*-value =0.024). Of the three diets, only the HF diet had a statistically significant lower nadir compared to the LF diet (*P*-value =0.008). The percent from the peak at lunch to the nadir after lunch was not significant in any way.

The peak at dinner, and percent rise from nadir after lunch to peak at dinner were not statistically significant in any way. The nadir of acyl ghrelin levels after dinner were statistically different (*P*-value =0.05). The HF diet had a statistically lower acyl ghrelin nadir than the MCT diet (*P*-value =0.018). The percent change to nadir acyl ghrelin after dinner was not statistically significant in any way.

Both the acyl ghrelin peak in the evening, after dinner, and the percent rise from after dinner to evening peak were not significantly different.

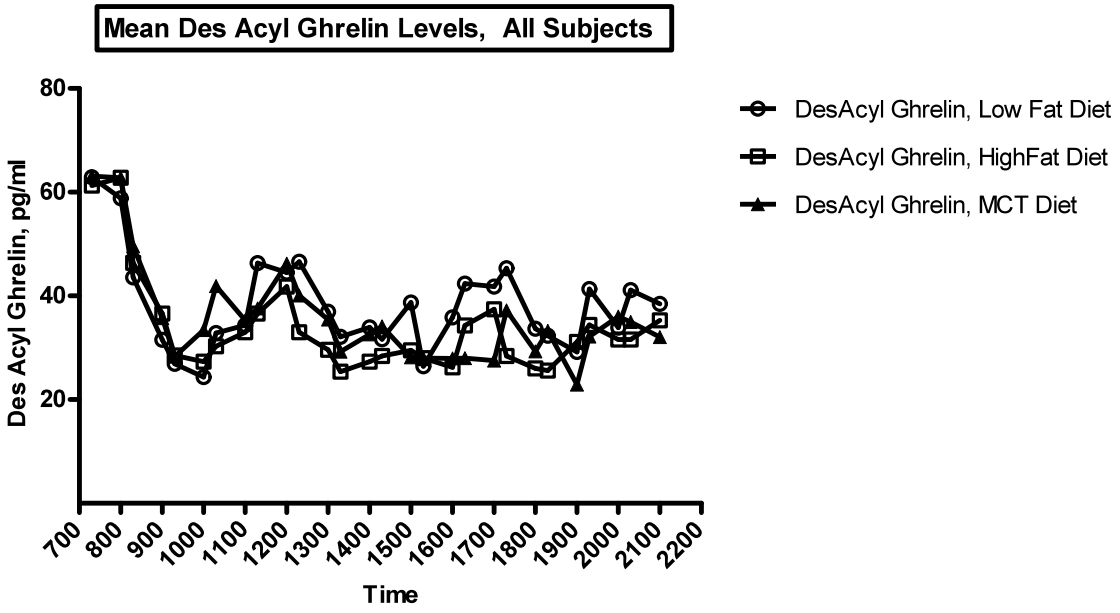
Corresponding to figure 3 (below) and table 6 (appendices), dietary fat did have a significant impact on some aspects of des acyl ghrelin levels.

There was a significant difference between diet groups and des acyl ghrelin levels (*P*-value =<0.001). The des acyl ghrelin levels were significantly lower for the MCT diet group than both the HF diet group (*P*-value =0.016) and the LF diet group (*P*-value =<0.001). The HF diet group had a lower AUC of des acyl ghrelin than the LF group that approached significance (*P*-value =0.058).

Unlike, the acyl ghrelin fasting levels, the des acyl ghrelin fasting levels were not significantly different.

The nadir of des acyl ghrelin after breakfast was consumed was significant between groups at *p*= 0.048. Of the three groups, the MCT diet group had a significantly lower des acyl ghrelin level compared to the HF diet group (*P*-value =0.018). There was no significant difference in the percent drop from breakfast peak to post-breakfast nadir.

Figure 3.



The peak in des acyl ghrelin levels at lunchtime was not significant between all three diets, but the LF diet did have a significantly higher peak than the MCT diet (*P*-value =0.033). There was no significant difference in percent rise from post-breakfast nadir to peak at lunch of des acyl ghrelin. There was also no significant difference between diets for nadir after lunch or percent drop from lunch peak to nadir.

There was a significant difference between diets on the peak of des acyl ghrelin levels at dinner (*P*-value =<0.001). The low fat diet had a significantly higher des acyl ghrelin peak at dinner than the MCT diet (*P*-value =0.008). The HF diet also had a significantly higher dinner peak than the MCT diet group (*P*-value =<0.001). Although there is no significant difference between diet groups on the percent rise at dinner, the des acyl ghrelin with the LF diet had a significantly higher percent increase than the rise on the HF diet (*P*-value =0.037).

There was not a significant difference in des acyl ghrelin levels between diet groups at the post-dinner nadir, but the MCT diet was significantly lower than the LF diet (*P*-value =0.028). There was no statistical difference between the three diet groups with respect to the percent drop from the dinner peak to post-dinner nadir.

The peak of des acyl ghrelin levels was statistically significant for the peak in the evening post-dinner (*P*-value =0.001). The two statistical differences here were that both the LF diet (*P*-value =<0.001) and the HF diet (*P*-value =0.025) were significantly higher than the MCT diet group. The percent of this rise, however, was not significant.

Corresponding to table 7 (appendices), dietary fat did have a significant impact on some aspects of the ratio of acyl to des acyl ghrelin levels. The area under the curve

for this ratio between diets was significant (*P*-value =0.02). Between groups, the MCT diet had a significantly higher ratio than the HF diet (*P*-value =0.005), but not the LF diet.

The fasting level of the acyl to des acyl ghrelin ratio was not significant, nor was the nadir after breakfast, or the percent to nadir at this time. The peak of this ratio was not significantly different, however, the peak for the MCT diet was significantly higher than the HF diet (*P*-value =0.043). The percent rise with this lunch peak was not statistically significant.

The difference between the value of the nadir after lunch for the ratio of acyl to des acyl ghrelin was significant at the *p*= <0.001 level. The nadir for this ratio was significantly lower for the HF diet compared to the MCT diet (*P*-value =<0.001). The LF diet also had a significantly lower nadir than the MCT diet (*P*-value =0.003). The percent to nadir at this time point for this ratio was not statistically significant.

The peak at the dinnertime point for this ratio was not significant between the three diets. The MCT diet did yield a significantly higher peak at dinner over the HF diet (*P*-value =0.05). There was no significance in the percent rise to the dinner peak from the lunch nadir.

The nadir after the dinner meal for this ratio was statistically significant (*P*-value =0.001). The HF diet had a significantly lower nadir than the MCT diet (*P*-value =0.001), and the LF diet also had a significantly lower nadir than the MCT diet (*P*-value = <0.001).

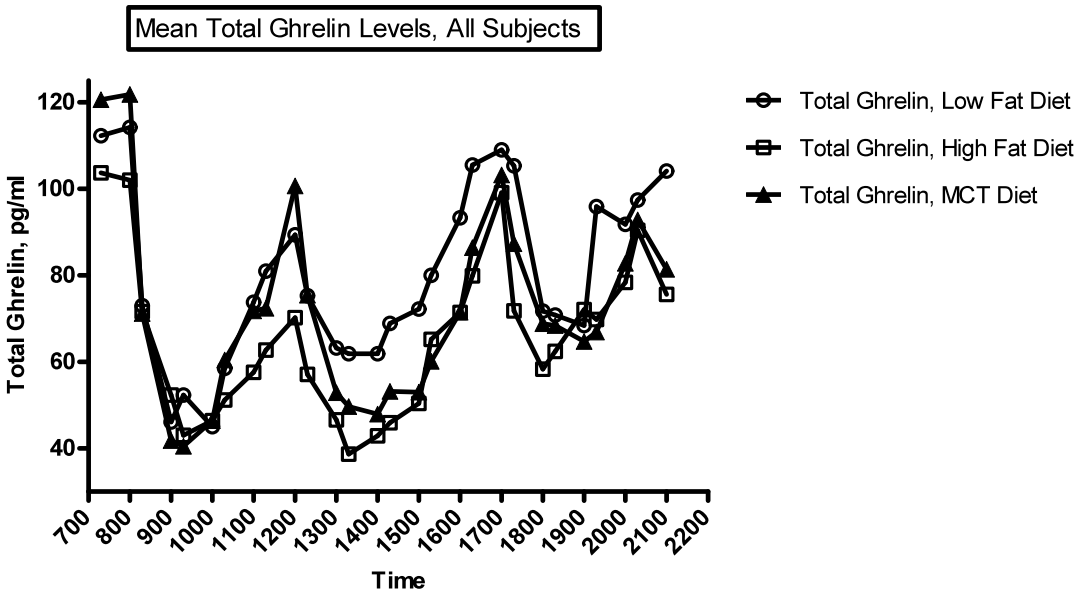
The percent change from the dinner peak to nadir was statistically significant (*P*-value =<0.001). The LF diet produced a statistically higher percent change in acyl to des

acyl ghrelin ratio from peak at dinner to nadir than the MCT diet (*P*-value =<0.001), and from the HF diet (*P*-value =<0.001).

The ratio of acyl to des acyl ghrelin peak levels in the evening, and the percent change from the dinner nadir to evening peak were not statistically significant.

Corresponding to figure 4 (below) and table 8 (appendices), dietary fat did have a significant impact on some aspects of the total ghrelin levels (combined acyl and des acyl levels).

Figure 4.



The area under the curve for total ghrelin between all three diets was found to be significantly different (*P*-value =0.011). The LF diet had a significantly higher AUC for total ghrelin level than the MCT diet group (*P*-value =0.039) and for the HF diet group (*P*-value =0.004).

The fasting values, nadir after breakfast and the percent change to nadir after breakfast were not significant.

The peak in AUC for total ghrelin at the lunchtime drawing was statistically significant (*P*-value =0.05) with the MCT diet having resulted in a significantly higher peak than the HF diet (*P*-value =0.029). The LF diet also had a significantly higher lunch peak than the HF diet (*P*-value =0.004). The percent change from nadir to lunch peak was not significant.

The nadir for total ghrelin after the lunch meal was statistically significant (*P*-value =<0.001) between groups. The MCT diet yielded a significantly lower nadir after lunch than did the LF diet (*P*-value =<0.01). The HF diet also had a significantly lower nadir after lunch than did the LF diet (*P*-value =<0.001). Correspondingly, the percent change to nadir after lunch from the breakfast peak was significant (*P*-value =0.013). From this, the only significant difference between two diets was the MCT diet produced a larger change from peak to nadir after lunch than the LF diet (*P*-value =0.003).

The peak of total ghrelin at dinner was not significant. The percent rise from post-lunch nadir to dinner peak was also not significant.

The nadir of total ghrelin after dinner was significant (*P*-value =0.016). The MCT diet yielded a significantly lower total ghrelin nadir at this time point than did the LF diet (*P*-value =0.009). The HF diet treatment also yielded a significantly lower nadir of total ghrelin than the LF diet treatment (*P*-value =0.017). The percent change from peak to nadir after dinner was not significant.

The peak of total ghrelin in the evening and the percent change to peak in the evening were both statistically insignificant.

Corresponding to table 9 (appendices), dietary fat did have a significant impact on some aspects of the ratio of acyl to total ghrelin levels. The area under the curve for the ratio of acyl to total ghrelin levels was significant (*P*-value =0.04). The MCT diet resulted in a significantly higher AUC for the ratio of acyl to total ghrelin levels than the HF diet (*P*-value =0.017). The fasting levels of this ratio were not significant.

The nadir for this ratio after breakfast was significant (*P*-value =0.036). Between diet groups, the LF diet had a significantly lower nadir than the MCT group (*P*-value =0.014). While the percent change for this nadir was not significant, the percent change to this nadir was significantly higher for the LF diet group than the MCT diet group (*P*-value =0.038).

The peak for the ratio of acyl to total ghrelin at lunch and the percent change to peak were not significant. The nadir for this ratio at lunch was significant (*P*-value =0.003). Between diet treatments, the HF diet group had a significantly lower nadir than the MCT group (*P*-value =0.001), and the LF diet treatment also had a significantly lower nadir than the MCT diet treatment (*P*-value =0.032). While the percent change to nadir was not significant, the HF diet had a significantly higher percent change to nadir than the MCT diet (*P*-value =0.034).

The peak in the ratio of acyl to total ghrelin at dinnertime was not significant. The percent rise from nadir to peak at dinner was significant (*P*-value =0.044). The HF diet treatment had the highest percent rise compared to the MCT diet (*P*-value =0.014).

Nadir after dinner for the ratio of acyl to total ghrelin was significant (*P*-value =0.006). The HF diet group had a significantly lower nadir than the MCT diet group (*P*-value =0.007). The LF diet group also had a significantly lower nadir than the MCT group (*P*-value =0.004). The percent drop to that nadir was also significant (P-value=0.005). The HF diet treatment had a significantly increased change compared to the MCT group (*P*-value =0.004). The LF diet group also had a significantly increased change compared to the MCT diet group (*P*-value =0.005).

The peak at the evening time point and the percent rise to that evening peak were not significant for the ratio of acyl to total ghrelin.

Effect of two-week diets

The results of the two-week diet treatments are expressed below in Tables 10 and 11, and Figures 5 and 6 below.

Table 10. Effect of diet fat and carbohydrate on ghrelin levels in lean and obese subjects during inpatient CTRC visit (n=21)							
	MCT	HF	LF	ANOVA *P*-value	MCT vs. HF *P*-value	MCT vs. LF *P*-value	HF vs. LF *P*-value
Acyl Ghrelin							
AUC (pg-13 hr/mL)	587 ± 371	404 ± 181	533 ± 274	**0.025**	**0.012**	0.69	**0.03**
Fasting	73.7 ± 49.4	40.8 ± 22	51.6 ± 39	**0.032**	**0.009**	0.113	0.275
Des Acyl Ghrelin							
AUC (pg-13 hr/mL)	308.4 ± 251.3	418.6 ± 266.4	480.4 ± 316.9	**<0.001**	**0.016**	**<0.001**	0.058
Fasting	47.6 ± 33	62.1 ± 46	61.1 ± 49.6	0.143	0.112	0.071	0.818
Acyl:Des Acyl Ghrelin							
AUC (pg-13 hr/mL)	47.7 ± 33.1	23.1 ± 14.6	33.2 ± 34.4	**0.02**	**0.005**	0.134	0.165
Fasting	3.1 ± 3.8	2.1 ± 3.9	1.4 ± 1.6	0.256	0.926	0.109	0.614
Total Ghrelin							
AUC (pg-13 hr/mL)	895.7 ± 544.2	822.2 ± 409.4	1008.1 ± 491	**0.011**	0.342	**0.039**	**0.004**
Fasting	121.2 ± 60.6	102.8 ± 54.3	112.6 ± 78.8	0.486	0.266	0.856	0.35
Acyl:Total Ghrelin							
AUC (pg-13 hr/mL)	8.4 ± 1.8	6.7 ± 2.1	6.6 ± 2.1	**0.04**	**0.017**	0.052	0.62
Fasting	0.6 ± 0.2	0.5 ± 0.3	0.5 ± 02	0.197	0.089	0.175	0.719
Results are mean ± SD. AUC- area under the curve. MCT-medium chain diet. HF-high fat diet. LF-low fat diet.							

There was a significant difference between groups for the acyl ghrelin morning fasting levels (*P*-value =0.032) and 13-hr AUC (*P*-value=0.0025). The MCT diet had a significantly higher AUC than the HF group (*P*-value =0.012). This is also true between the HF and LF diets (*P*-value =0.03). MCT had a significantly higher fasting acyl ghrelin level than did the HF diet (*P*-value =0.009).

For the des acyl ghrelin, the AUC ANOVA was significant at $P < 0.001$. The MCT diet group had a significantly lower AUC for des acyl ghrelin than the HF and LF diet groups (*P*-value = 0.016 and <0.001, respectively). The difference at fasting, while lowest for MCT, was not significant.

The acyl to des acyl ratio was significant for AUC (*P*-value =0.002), and is only significant between MCT and HF diets (*P*-value =0.005).

Total ghrelin AUC showed a significant difference (p=0.011), between groups AUC was significantly higher for the LF diet than the MCT diet (*P*-value=0.039). The AUC for LF diet is significantly higher than HF diet (*P*-value =0.004).

Acyl to total ghrelin ratios had a significant AUC (*P*-value = 0.004). The MCT diet had a significantly higher AUC than the HF diet (*P*-value = 0.017). The AUC for MCT was higher than the AUC for LF, approaching significance at p= 0.052.

Appetite

Table 11 Repeat Measures ANOVA on Hunger and Fullness, During Two-week home-prepared diet periods

	MCT	HF	LF	ANOVA *P*-value	MCT vs. HF *P*-value	MCT vs. LF *P*-value	HF vs. LF *P*-value
Average Hunger	44.07 ± 19.72	42.79 ± 22.86	34.69 ± 22.86	**0.047**	0.468	**0.02**	0.144
Average Fullness	48.7 ± 20.03	48.42 ± 19.54	61.29 ±16.81	**0.01**	0.568	**0.034**	**0.002**

Results are mean measure in millimeters ±SD. MCT- medium chain diet. HF- high fat diet. LF-low fat diet.

There was a significant difference between the three diets for both hunger and fullness (*P*-value = 0.047 and 0.01, respectively) for the two-week home prepared diet periods. Hunger had a significantly higher score with the MCT diet vs. the LF diet (*P*-value = 0.02). The MCT diet had a significantly lower fullness score than the LF diet (*P*-

value =0.034). The HF diet also had a significantly lower fullness score than the LF diet (*P*-value =0.002).

Figure 5. Two-week visual analog scores for hunger

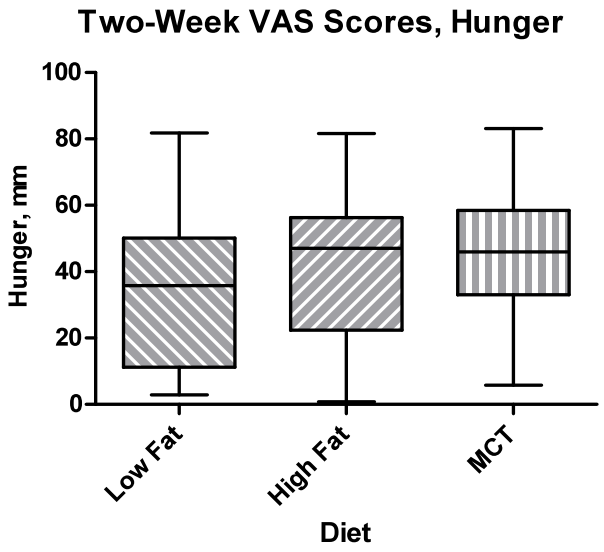
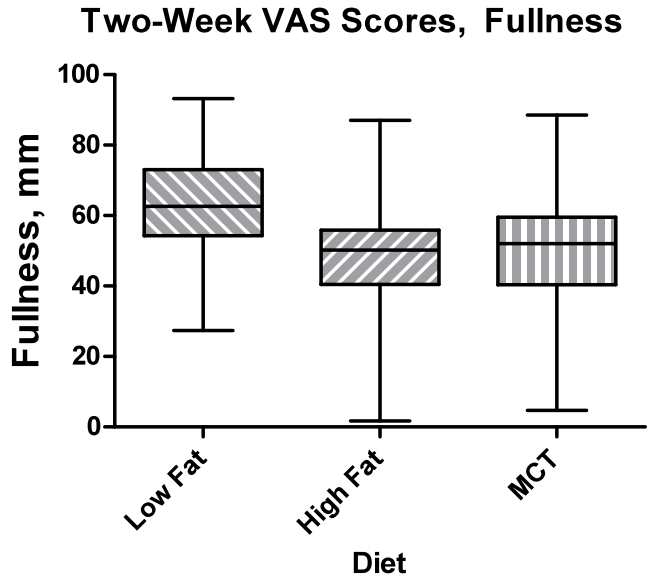


Figure 6. Two-week visual analog scores for fullness



Correlation and Regression Analysis

Hunger and Acyl Ghrelin

The results of the correlation and regression analysis between the change in ghrelin levels and change in hunger by diet and meal are expressed below in Tables 12-16 and Figures 7-11.

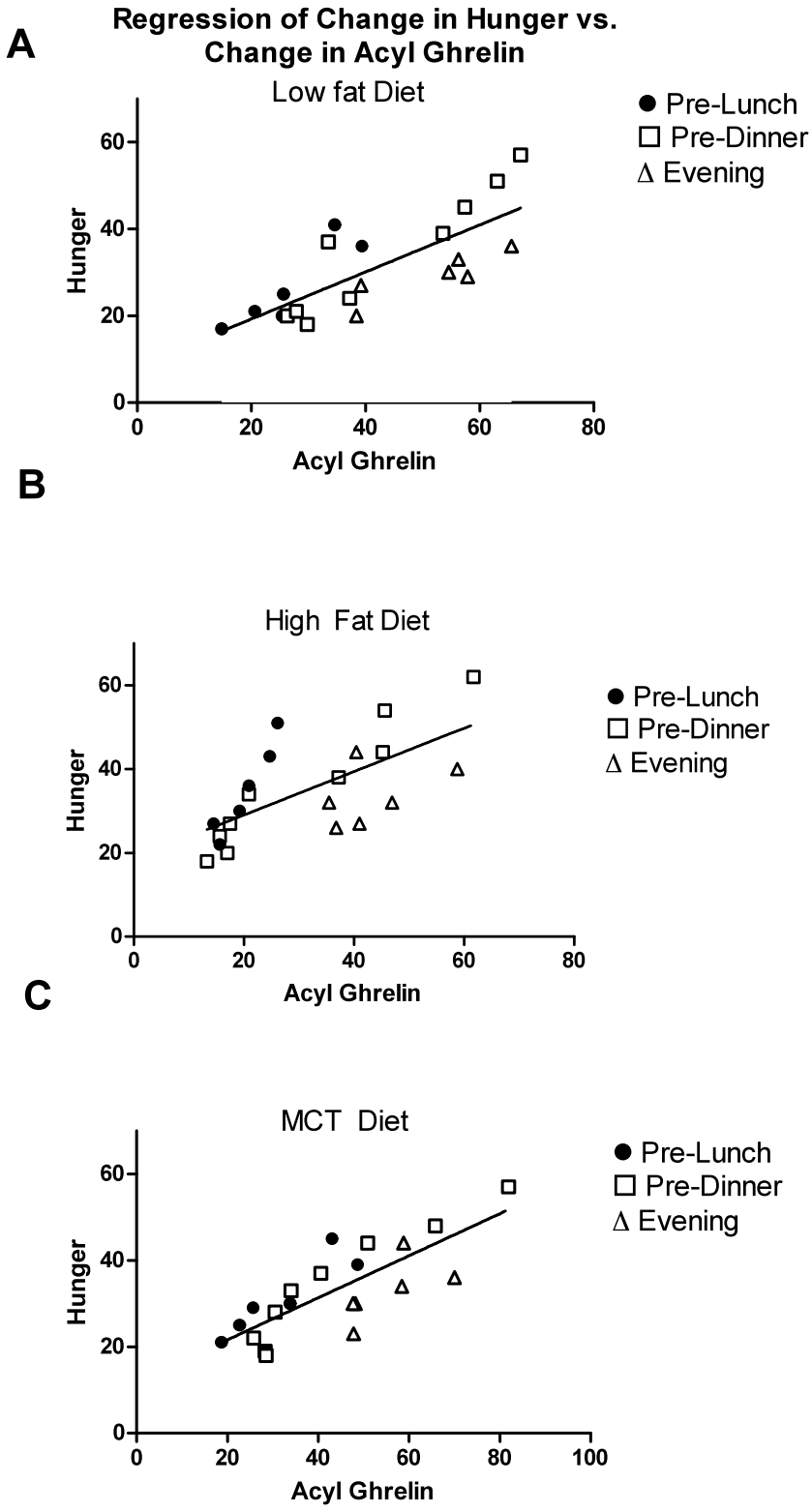
The correlation between the change in acyl ghrelin and hunger on a low fat diet is positive and significant at all meals. The correlation between change in hunger and change in acyl ghrelin on a high fat diet are positive for all meals and only significant for breakfast (*P*-value =0.003), lunch (*P*-value =<0.001), and when all meals are combined (*P*-value =0.001). The correlation for change in acyl ghrelin and the change in hunger on the MCT diet is positive, and significant at breakfast (*P*-value =0.01), lunch (*P*-value =<0.001), and when all meals are combined (*P*-value =<0.001).

Table 12. Correlation between change in hunger and change in acyl ghrelin levels, by diet and meal

	Change in AG, LF		Change in AG, HF		Change in AG, MCT	
	r	*P*-value	r	*P*-value	r	*P*-value
Change in Hunger, B	**0.902**	**0.014**	**0.956**	**0.003**	**0.918**	**0.01**
Change in Hunger, L	**0.941**	**<0.001**	**0.963**	**<0.001**	**0.948**	**<0.001**
Change in Hunger, D	**0.879**	**0.021**	0.456	0.363	0.647	0.165
Change in Hunger, all meals combined	**0.767**	**<0.001**	**0.666**	**0.001**	**0.794**	**<0.001**

Results are based on averages of all subjects at specific times: B- Breakfast (0900-1130), L-Lunch (1300-1700), D-Dinner (1830-2100). LF- low fat diet. HF-high fat diet. MCT-medium chain diet. AG-acyl ghrelin

Figure 7.


A
Regression of Change in Hunger vs. Change in Acyl Ghrelin
Low fat Diet
● Pre-Lunch
□ Pre-Dinner
Δ Evening
Hunger
Acyl Ghrelin
0
20
40
60
80
B
High Fat Diet
● Pre-Lunch
□ Pre-Dinner
Δ Evening
Hunger
Acyl Ghrelin
0
20
40
60
80
C
MCT Diet
● Pre-Lunch
□ Pre-Dinner
Δ Evening
Hunger
Acyl Ghrelin
0
20
40
60
80
100

Hunger and Des Acyl Ghrelin

The correlation between change in hunger and change in des acyl ghrelin were all positive, except the correlation between hunger and ghrelin at lunch on the MCT diet, which was not significant. The correlation between changes in des acyl ghrelin and hunger were significant at breakfast on the LF diet (*P*-value =0.038), and when the meals were combined on the LF diet (*P*-value =0.004). The correlation between changes in des acyl ghrelin and hunger were significant at lunch on the HF diet (*P*-value =0.014) and when the meals were combined (*P*-value =0.006).

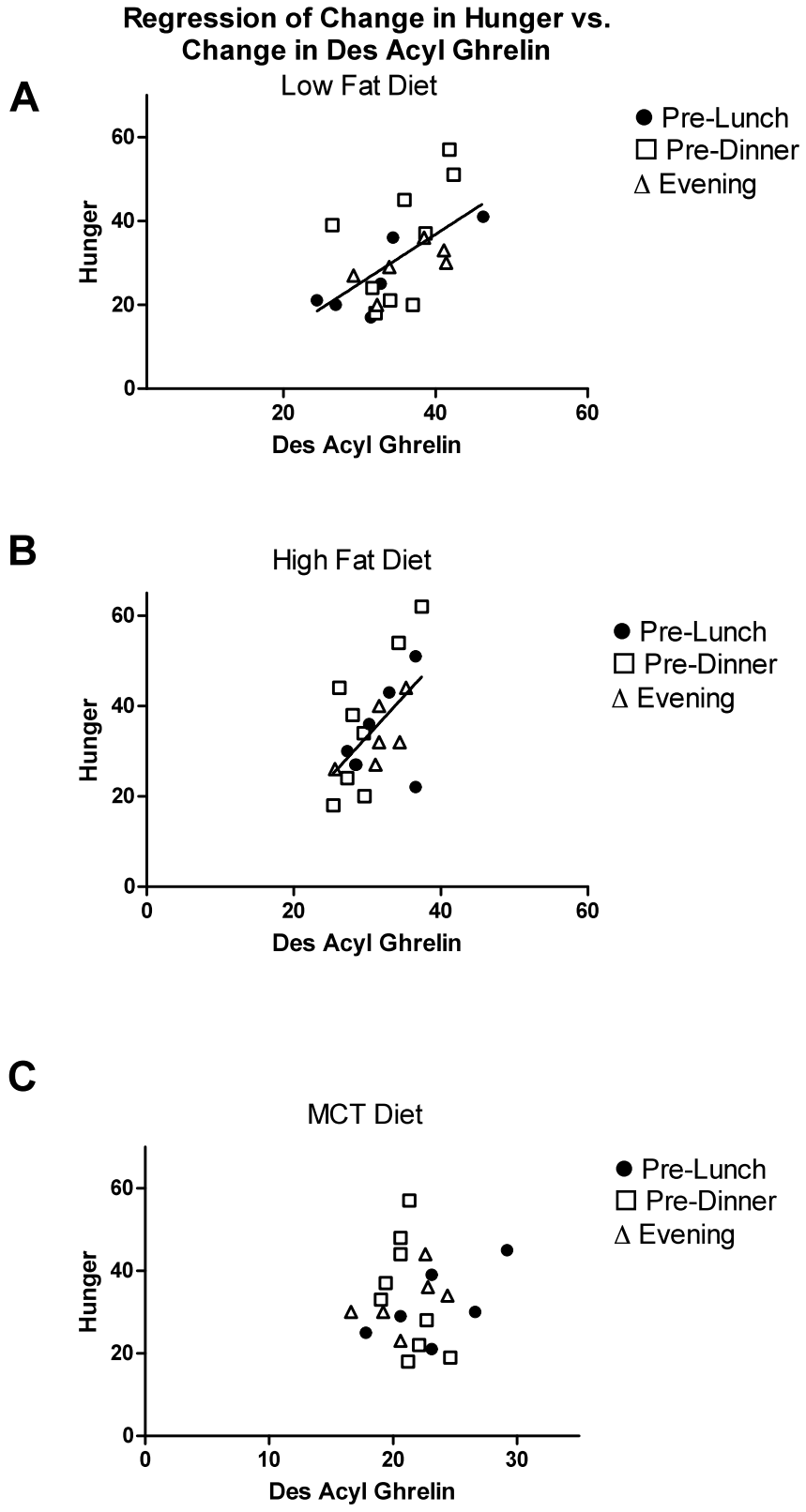
The correlation between change in des acyl ghrelin levels and change in hunger on the MCT diet was not significantly different.

Table 13. Correlation between change in hunger and change in des acyl ghrelin levels, by diet and meal

	Change in DAG, LF		Change in DAG, HF		Change in DAG, MCT	
	r	*P*-value	r	*P*-value	r	*P*-value
Change in Hunger, B	**0.836**	**0.038**	0.332	0.52	0.646	0.166
Change in Hunger, L	0.538	0.135	**0.778**	**0.014**	-0.46	0.212
Change in Hunger, D	0.655	0.158	0.682	0.135	0.491	0.323
Change in Hunger, all meals combined	**0.600**	**0.004**	**0.577**	**0.006**	0.103	0.656

Results are based on averages of all subjects at specific times: B- Breakfast (0900-1130), L-Lunch (1300-1700), D-Dinner (1830-2100). LF- low fat diet. HF-high fat diet. MCT-medium chain diet. DAG-des acyl ghrelin

Figure 8.


Regression of Change in Hunger vs.
Change in Des Acyl Ghrelin
A
Low Fat Diet
Hunger
Des Acyl Ghrelin
Pre-Lunch
Pre-Dinner
Evening
B
High Fat Diet
Hunger
Des Acyl Ghrelin
Pre-Lunch
Pre-Dinner
Evening
C
MCT Diet
Hunger
Des Acyl Ghrelin
Pre-Lunch
Pre-Dinner
Evening

Hunger and Acyl:Des Acyl Ghrelin Ratio

The correlations between the change in hunger and the change in the ratio of acyl to des acyl ghrelin were all positively significant except for the HF dinner correlation, which was not significant (table 14).

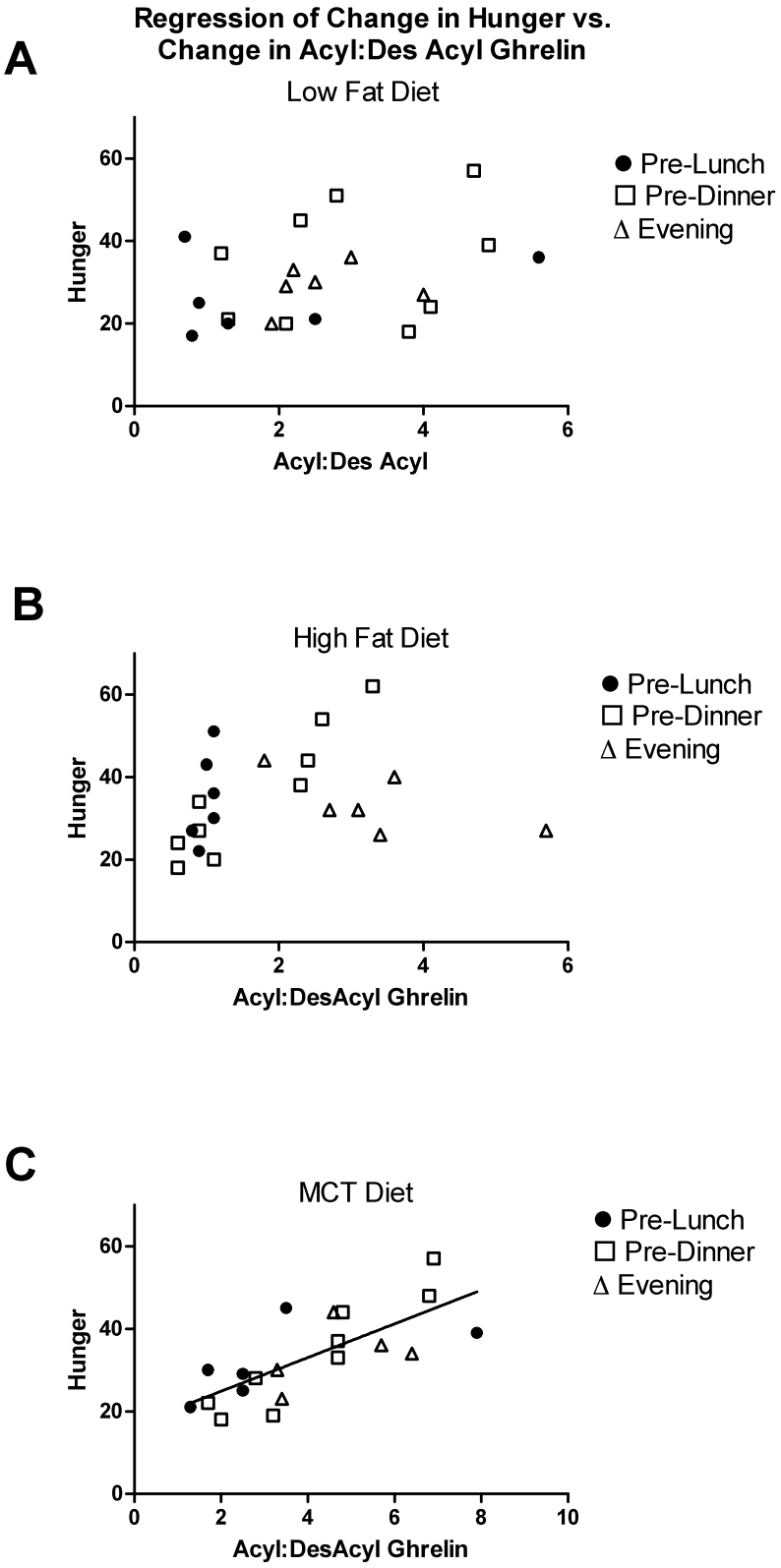
The correlation between change in AG to DAG ratio and hunger was significant at lunch on the HF diet (*P*-value <0.001). This same correlation was also significant at lunch on the MCT diet (*P*-value <0.001). For all meals combined, the only significant correlation was on the MCT diet (*P*-value <0.001).

Table 14. Correlation between change in hunger and change in acyl:des acyl ghrelin ratio levels, by diet and meal

	Change in AG:DAG, LF		Change in AG:DAG, HF		Change in AG:DAG, MCT	
	r	*P*-value	r	*P*-value	r	*P*-value
Change in Hunger, B	0.33	0.523	0.603	0.206	0.630	0.180
Change in Hunger, L	0.262	0.496	**0.931**	**<0.001**	**0.933**	**<0.001**
Change in Hunger, D	0.214	0.683	-0.622	0.187	0.519	0.291
Change in Hunger, all meals combined	0.334	0.139	0.248	0.278	**0.745**	**<0.001**

Results are based on averages of all subjects at specific times: B- Breakfast (0900-1130), L-Lunch (1300-1700), D-Dinner (1830-2100). LF- low fat diet. HF-high fat diet. MCT-medium chain diet. AG:DAG- ratio of acyl to des acyl ghrelin

Figure 9.


A
Regression of Change in Hunger vs.
Change in Acyl:Des Acyl Ghrelin
Low Fat Diet
Hunger
Acyl:Des Acyl
Pre-Lunch
Pre-Dinner
Evening
B
High Fat Diet
Hunger
Acyl:DesAcyl Ghrelin
Pre-Lunch
Pre-Dinner
Evening
C
MCT Diet
Hunger
Acyl:DesAcyl Ghrelin
Pre-Lunch
Pre-Dinner
Evening

Hunger and Total Ghrelin

Correlations between change in hunger and change in total ghrelin are all positive. The table and figures of this correlation and regression can be found in the appendices (table 15, figure 10).

The correlation between total ghrelin and hunger were significant for all meals, and the combined meals, on the LF diet (*P*-values = 0.001 for breakfast, <0.001 for lunch, 0.027 for dinner, and <0.001 for all meals)

The correlations for change in total ghrelin and hunger on the HF diet were only significant for the lunch mean (*P*-value =<0.001) and for all meals combined (*P*-value =<0.001). These correlations are also significant for the MCT diet at breakfast (*P*-value =0.008), lunch (*P*-value =<0.001), and for all meals combined (*P*-value =<0.001).

Hunger and Acyl:Total Ghrelin Ratio

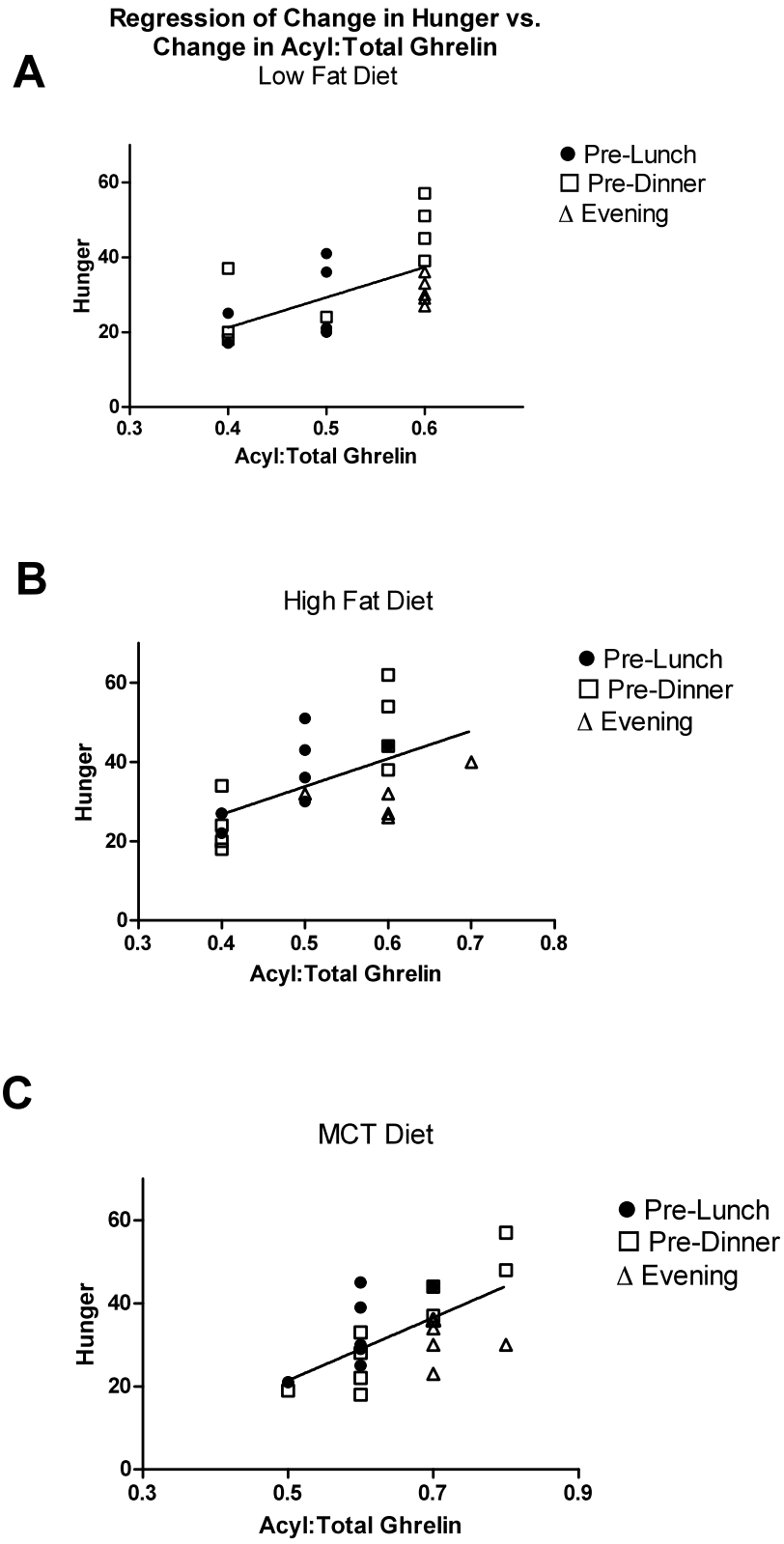
Correlations between the changes in hunger and the acyl to total ghrelin levels are all positive except for the lunch meal on the LF diet and the dinner meal on the HF diet. This can be seen below in table 16 and figure 11.

The correlation between change in hunger and change in acyl:total ghrelin ratio were significantly negative on the LF diet at the lunch meal (*P*-value =0.004), significantly positive on the dinner meal (*P*-value =0.047) and significantly positive for all meals combined (*P*-value =0.006).

There were significant correlations on the HF diet at lunch (*P*-value =0.003) and for all the meals combined (*P*-value =0.006). The significant correlations on the MCT diet were also at lunch (*P*-value =<0.001) and all meals combined (*P*-value =0.002).

Table 16. Correlation between change in hunger and change in acyl:total ghrelin ratio levels, by diet and meal						
	Change in AG:TG, LF		Change in AG:TG, HF		Change in AG:TG, MCT	
	r	*P*-value	r	*P*-value	r	*P*-value
Change in Hunger, B	0.455	0.365	0.744	0.09	0.575	0232
Change in Hunger, L	**-0.852**	**0.004**	**0.857**	**0.003**	**0.92**	**<0.001**
Change in Hunger, D	**0.818**	**0.047**	-0.039	0.941	0.171	0.746
Change in Hunger, all meals combined	**0.58**	**0.006**	**0.58**	**0.006**	**0.636**	**0.002**
Results are based on averages of all subjects at specific times: B- Breakfast (0900-1130), L-Lunch (1300-1700), D-Dinner (1830-2100). LF- low fat diet. HF-high fat diet. MCT- medium chain diet. AG:TG- ratio of acyl to total ghrelin						

Figure 11.


A
Regression of Change in Hunger vs.
Change in Acyl:Total Ghrelin
Low Fat Diet
Hunger
Acyl:Total Ghrelin
● Pre-Lunch
□ Pre-Dinner
Δ Evening
B
High Fat Diet
Hunger
Acyl:Total Ghrelin
● Pre-Lunch
□ Pre-Dinner
Δ Evening
C
MCT Diet
Hunger
Acyl:Total Ghrelin
● Pre-Lunch
□ Pre-Dinner
Δ Evening

Fullness

The results of the correlation and regression analysis between the change in ghrelin levels and change in fullness by diet and meal are expressed below in Tables 17-21 and Figures 12-16.

Fullness and Acyl Ghrelin

The correlation between the changes in fullness and acyl ghrelin are all negative, except for the breakfast on the LF diet. All of the correlations are significant on the LF diet, including breakfast (*P*-value =<0.001), lunch (*P*-value =<0.001), dinner (*P*-value =0.015) and all meals combined (*P*-value =<0.001).

The correlations between acyl ghrelin and fullness on the HF diet are significantly negative at breakfast (*P*-value =0.005), lunch (*P*-value =<0.001) and all meals combined (*P*-value =<0.001).

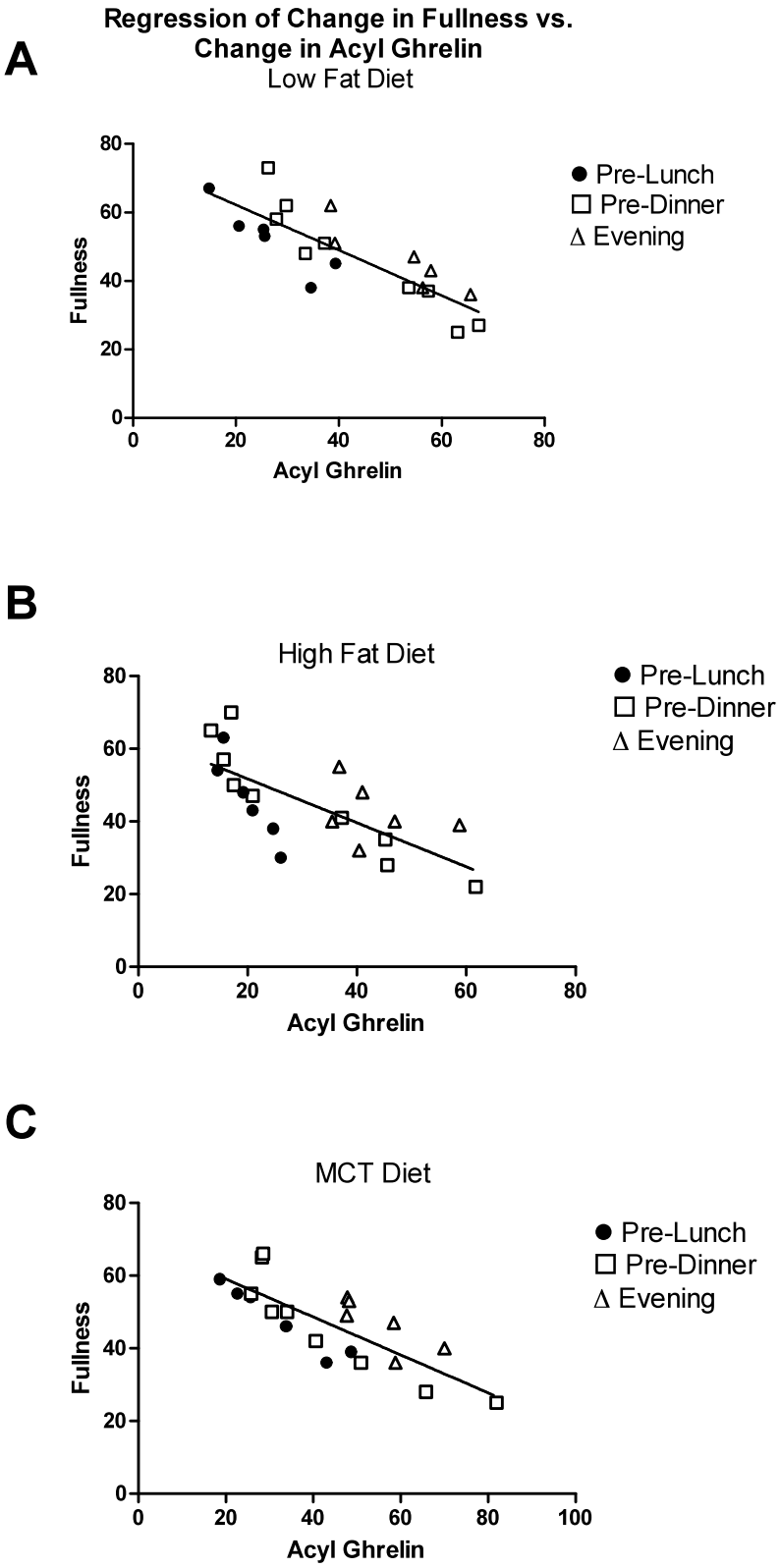
These correlations on the MCT diet are also significantly negative at breakfast (*P*-value =0.002), lunch (*P*-value =0.001) and all meals combined (*P*-value =<0.001).

Table 17. Correlation between change in fullness and change in acyl ghrelin levels, by diet and meal

	Change in AG, LF		Change in AG, HF		Change in AG, MCT	
	r	*P*-value	r	*P*-value	r	*P*-value
Change in Fullness, B	**1.000**	**<0.001**	**-0.939**	**0.005**	**-0.967**	**0.002**
Change in Fullness, L	**-0.947**	**<0.001**	**-0.923**	**<0.001**	**-0.909**	**0.001**
Change in Fullness, D	**-0.899**	**0.015**	-0.311	0.548	-0.783	0.065
Change in Hunger, all meals combined	**-0.838**	**<0.001**	**-0.719**	**<0.001**	**-0.798**	**<0.001**

Results are based on averages of all subjects at specific times: B- Breakfast (0900-1130), L-Lunch (1300-1700), D-Dinner (1830-2100). LF- low fat diet. HF-high fat diet. MCT-medium chain diet. AG- acyl ghrelin

Figure 12.


A
Regression of Change in Fullness vs.
Change in Acyl Ghrelin
Low Fat Diet
Fullness
Acyl Ghrelin
Pre-Lunch
Pre-Dinner
Evening
B
High Fat Diet
Fullness
Acyl Ghrelin
Pre-Lunch
Pre-Dinner
Evening
C
MCT Diet
Fullness
Acyl Ghrelin
Pre-Lunch
Pre-Dinner
Evening

Fullness and Des Acyl Ghrelin

The correlations between the changes in fullness and des acyl ghrelin are all negative, except for the breakfast on the LF diet and the lunch on the MCT diet. Both of which were not significant. This can be seen below in table 18 and figure 13.

The only significant correlation between des acyl ghrelin and fullness on the LF diet was when all the meals were combined (*P*-value =0.02).

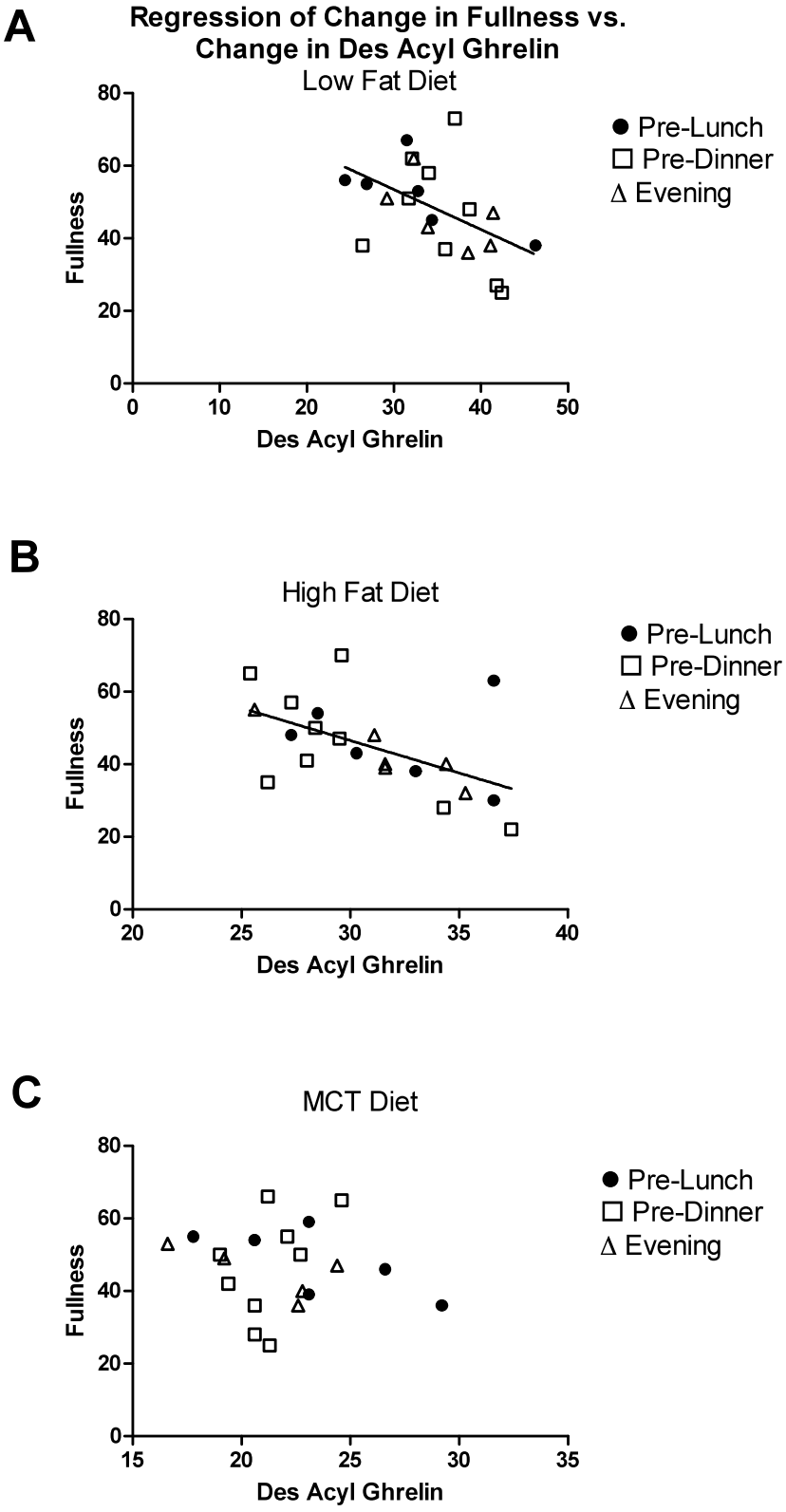
There were two significant correlations on the HF diet, the dinner meal (*P*-value =0.013) and when the meals were combined (*P*-value =0.012).

Table 18. Correlation between change in fullness and change in des acyl ghrelin levels, by diet and meal

	Change in DAG, LF		Change in DAG, HF		Change in DAG, MCT	
	r	*P*-value	r	*P*-value	r	*P*-value
Change in Fullness, B	0.585	0.222	-0.152	0.774	-0.697	0.124
Change in Fullness, L	-0.361	0.34	-0.663	0.052	0.452	0.222
Change in Fullness, D	-0.616	0.193	**-0.907**	**0.013**	-0.619	0.19
Change in Hunger, all meals combined	**-0.503**	**0.02**	**-0.537**	**0.012**	-0.136	0.557

Results are based on averages of all subjects at specific times: B- Breakfast (0900-1130), L-Lunch (1300-1700), D-Dinner (1830-2100). LF- low fat diet. HF-high fat diet. MCT-medium chain diet. AG- acyl ghrelin. DAG- des acyl ghrelin

Figure 13.


A
Regression of Change in Fullness vs.
Change in Des Acyl Ghrelin
Low Fat Diet
Fullness
Des Acyl Ghrelin
Pre-Lunch
Pre-Dinner
Evening
B
High Fat Diet
Fullness
Des Acyl Ghrelin
Pre-Lunch
Pre-Dinner
Evening
C
MCT Diet
Fullness
Des Acyl Ghrelin
Pre-Lunch
Pre-Dinner
Evening

Fullness and Acyl:Des Acyl Ghrelin Ratio

All of the correlations between the changes in fullness and the ratio of acyl to des acyl ghrelin were negative, except the breakfast meal on the LF diet, and the dinner meals on the HF diet. Neither of these were significant. This can be seen below in table 19 and figure 14.

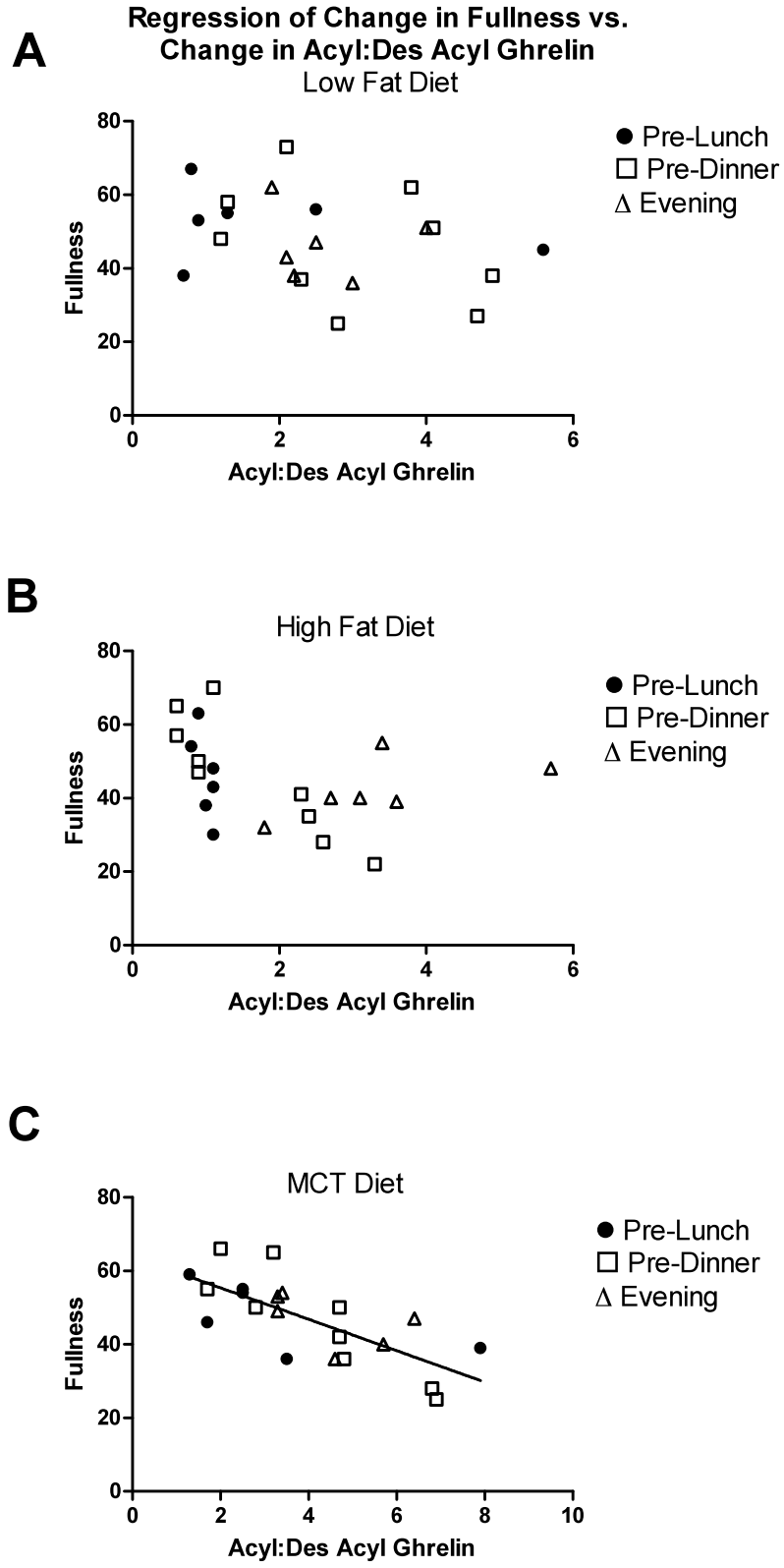
There were three significant correlations between change in fullness and change in acyl to des acyl ghrelin ratio. These were at lunch on the HF diet (*P*-value =0.002), lunch on the MCT diet (*P*-value =0.001) and with the meals combined on the MCT diet (*P*-value =<0.001).

Table 19. Correlation between change in fullness and change in acyl:des acyl ghrelin ratio levels, by diet and meal

	Change in AG:DAG, LF		Change in AG:DAG HF		Change in AG:DAG , MCT	
	r	*P*-value	r	*P*-value	r	*P*-value
Change in Fullness, B	0.571	0.237	-0.675	0.141	-0.649	0.163
Change in Fullness, L	-0.368	0.33	**-0.882**	**0.002**	**-0.89**	**0.001**
Change in Fullness, D	-0.097	0.854	0.599	0.209	-0.556	0.252
Change in Hunger, all meals combined	-0.329	0.146	-0.364	0.105	**-0.732**	**<0.001**

Results are based on averages of all subjects at specific times: B- Breakfast (0900-1130), L-Lunch (1300-1700), D-Dinner (1830-2100). LF- low fat diet. HF-high fat diet. MCT-medium chain diet. AG:DAG- ratio of acyl to des acyl ghrelin

Figure 14.


A
Regression of Change in Fullness vs.
Change in Acyl:Des Acyl Ghrelin
Low Fat Diet
Fullness
Acyl:Des Acyl Ghrelin
Pre-Lunch
Pre-Dinner
Evening
B
High Fat Diet
Fullness
Acyl:Des Acyl Ghrelin
Pre-Lunch
Pre-Dinner
Evening
C
MCT Diet
Fullness
Acyl:Des Acyl Ghrelin
Pre-Lunch
Pre-Dinner
Evening

Fullness and Total Ghrelin

Correlations between change in fullness and change in total ghrelin are all negative, except the breakfast meal on the LF diet. The table and figures of this correlation and regression can be found in the appendices (table 20, figure 15).

All of the correlations for the LF diet were significant. The breakfast meal, the only positive correlation in this cohort, was significant at p= 0.012. The correlations for the LF diet at lunch (*P*-value =<0.001), dinner (*P*-value =0.026) and for all the meals combined (*P*-value =<0.001) were all negatively correlated.

On the HF diet, lunch was significantly negative (*P*-value =<0.001) as well as all meals combined (*P*-value =<0.001).

On the MCT diet, breakfast (*P*-value =-.001), lunch (*P*-value =0.001), and all meals combined had a significantly negative correlation.

Fullness and Acyl:Total Ghrelin Ratio

The correlations between the change in fullness and the change in ratio of acyl to total ghrelin are positive for the breakfast on LF and MCT diets, and dinner on the MCT diet. All other correlations in this cohort are negative (table 21).

The correlation between the acyl to total ghrelin and fullness on the LF diet are significant at lunch (*P*-value =0.004), dinner (0.049), and for all meals combined (*P*-value =<0.001).

The correlation for this cohort on the HF diet is significant at breakfast (*P*-value =0.042), lunch (0.004), and for all meals combined (*P*-value =0.001).

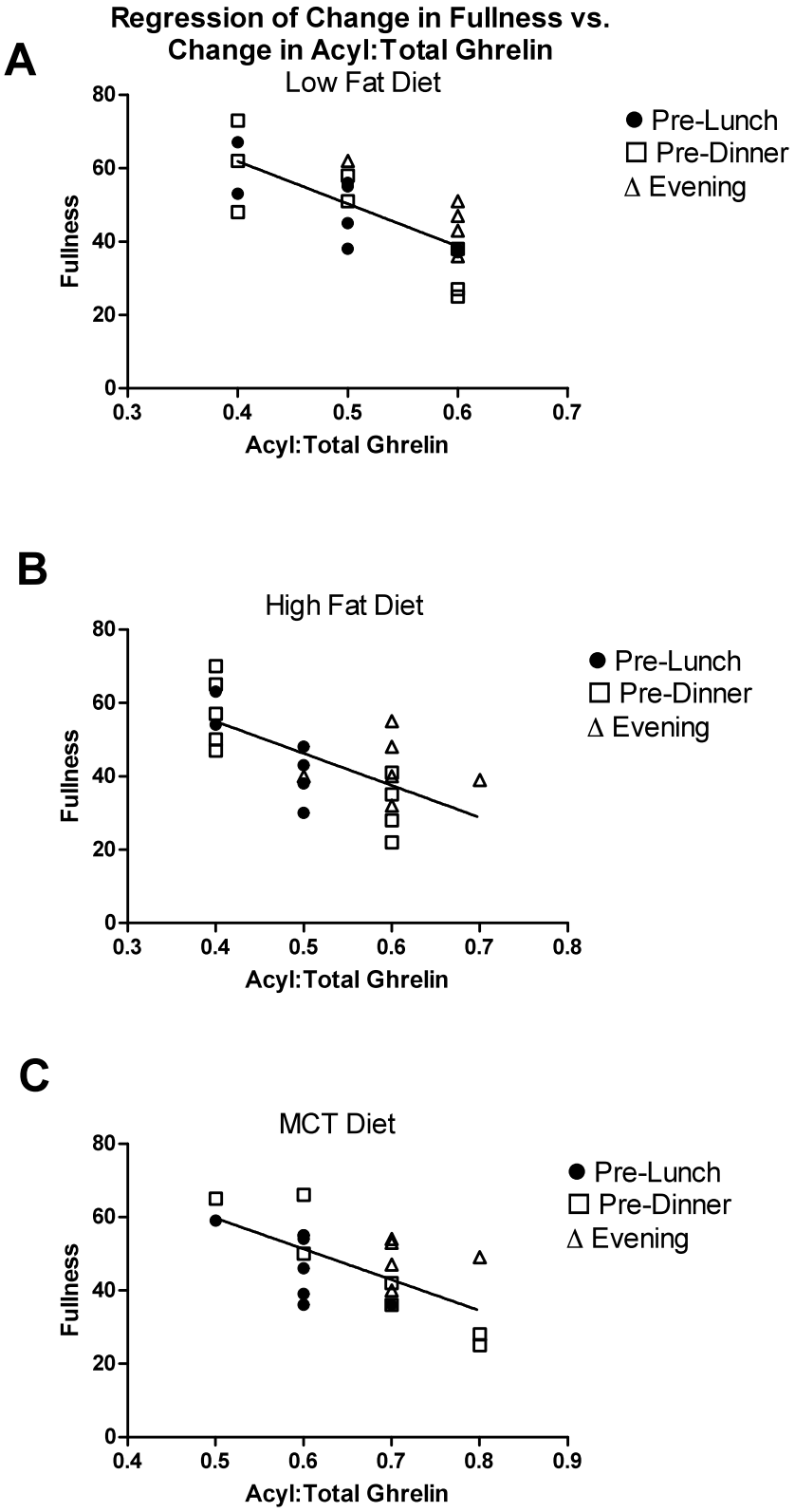
The correlation for this cohort on the MCT diet was significant for the lunch meal (*P*-value =<0.001) and when all meals were combined (*P*-value =0.001).

Table 21. Correlation between change in fullness and change in acyl:total ghrelin ratio levels, by diet and meal

	Change in AG:TG, LF		Change in AG:TG, HF		Change in AG:TG, MCT	
	r	*P*-value	r	*P*-value	r	*P*-value
Change in Fullness, B	0.562	0.246	**-0.827**	**0.042**	0.171	0.746
Change in Fullness, L	**-0.852**	**0.004**	**-0.851**	**0.004**	**-0.938**	**<0.001**
Change in Fullness, D	**-0.813**	**0.049**	-0.039	0.941	0.171	0.746
Change in Hunger, all meals combined	**-0.745**	**<0.001**	**-0.666**	**0.001**	**-0.661**	**0.001**

Results are based on averages of all subjects at specific times: B- Breakfast (0900-1130), L-Lunch (1300-1700), D-Dinner (1830-2100). LF- low fat diet. HF-high fat diet. MCT-medium chain diet. AG:TG- ratio of acyl to total ghrelin

Figure 16.


A
Regression of Change in Fullness vs.
Change in Acyl:Total Ghrelin
Low Fat Diet
Fullness
Acyl:Total Ghrelin
● Pre-Lunch
□ Pre-Dinner
Δ Evening
B
High Fat Diet
Fullness
Acyl:Total Ghrelin
● Pre-Lunch
□ Pre-Dinner
Δ Evening
C
MCT Diet
Fullness
Acyl:Total Ghrelin
● Pre-Lunch
□ Pre-Dinner
Δ Evening

Discussion

Ghrelin becomes active in its ability to stimulate hunger when a fatty acyl group (most commonly C8) is attached to the serine 3 by ghrelin O-acyltransferase (GOAT) (5-8). The source of the acyl group is thought to be from either endogenous synthesis, or incorporation from circulating fatty acids or dietary sources from the lumen of the gastrointestinal tract. Incorporation of dietary sources of fatty acids had previously been demonstrated in a rodent model (5), but to our knowledge this has not been studied in humans. We therefore hypothesized that consumption of a high MCT diet would lead to increased availability of C8, C10, and C12 fatty acids for attachment to ghrelin and would lead to increased acylated ghrelin compared to the LF and HF diets. Because acyl ghrelin stimulates hunger and food intake in animal models and in humans, we also predicted that the higher levels of acyl ghrelin with the MCT diet would be associated with greater hunger levels. Due to the low number of participants, we were not able to assess the differences of ghrelin levels or hunger and satiety between lean and obese subjects. However, the effects of each diet were consistent in both the lean and obese groups, so all subjects were combined for our analyses.

<u>Two-week isocaloric feeding:</u>

In fact, we did find a significant effect of the MCT diet on both acyl ghrelin levels and hunger during two weeks of isocaloric feeding on each diet (table 11). The directionality of the effect was such that subjects consuming the high MCT diet had the highest average daily levels of acyl ghrelin and reported greater hunger compared to the control diets. In the case of acyl ghrelin levels, significance was achieved for MCT > HF,

and while the average levels were higher on MCT than LF, this difference did not reach statistical significance. Interestingly, we found a stronger directionality for des-acyl ghrelin levels such that des-acyl ghrelin levels were significantly lower during the MCT diet than both the HF and LF diets (with HF non-significantly greater than LF). Des-acyl ghrelin is inactive with regard to hunger and its true biological activity is unknown. However, it does appear to exert opposite action to acyl ghrelin in some cases (31). If we looked at the ratio of acyl to total ghrelin (acyl + desacyl), we found that the proportion of acyl ghrelin was greatest on the MCT diet (P=0.017 vs. HF, P=0.054 vs. LF) and lowest on the LF diet.

The average daily hunger scores were greatest during the MCT diet (MCT>HF>LF; P=0.02 compared to LF, P=0.468 vs. HF diet). Our findings of increased hunger on the MCT diet compared to the HF and LF diets differs from what has been reported in the literature. Van Wymelbeke, et al (22) demonstrated that hunger scores and energy intake were lower on a high MCT diet. The authors also concluded that the MCT diet enhanced satiety, where in our study the LF diet had a significantly higher fullness score compared to the MCT diet. Although both our study and this contrasting study are randomized cross-over designs, our study differs in several ways. First, our participants were aware of the time of day, there was no absence of time cues, as in this other study. Second, our participants consumed the specific diet for two weeks prior to admission. Third, hunger in this study was measured by time to next meal and amount of food consumed, in an ad libitum fashion.

In our study, the average daily fullness scores were also significantly higher during the low fat diet compared to both the high fat and MCT diets during the two-week diet periods (table 11). The fullness scores for the MCT diet and high fat diet were not different. This result supports several studies which have found that a high carbohydrate diet is more satiating, or cause a decrease in hunger, than high fat diets (24, 32, 33).

In summary, during two weeks of isocaloric feeding, acyl ghrelin levels and average daily hunger scores were highest while average daily fullness scores were lowest during the high MCT diet compared to the HF and LF diets. The strongest directionality relationship between diet, ghrelin levels, and hunger and fullness, however, was with des-acyl ghrelin levels, which were lowest on the high MCT diet, intermediate on the HF diet, and highest on the LF diet. Given that des-acyl ghrelin is inactive and may be antagonistic to acyl-ghrelin, it may be that the ratio of acyl to total ghrelin is a better biologic predictor of ghrelin effects on appetite.

Acyl Ghrelin, Hunger and Fullness

Our findings that hunger levels and acyl ghrelin rise before meals and fall to trough levels within two hours after the meal is consistent with previous studies that measured total ghrelin (13, 14, 16) and consistent with a role for this form of ghrelin in mediating between meal hunger. When we looked at each meal separately by diet (Figure 17-19), there are significant correlations between change in acyl ghrelin and change in hunger both before lunch and before dinner for all three diet treatments. After dinner, however, the only significance between hunger and acyl ghrelin is on the

LF treatment. The directionality of the strengths and significance for these correlations are MCT>LF>HF. A previous study also found that there was a significant correlation between change in hunger and the change in ghrelin levels (33). The correlations from this study looked at both HF and LF diets, and the authors do not elaborate on the difference between the two diets, only stating that the correlation was significant. The consistency of these relationships suggests that rises in acyl ghrelin levels within physiological ranges do play a role in hunger, which supports the findings of our study.

In a separate study looking at dietary influence on ghrelin levels, Tentolouris et al. compared lean and obese subjects on a carbohydrate rich (CHO) meal versus a fat rich meal (35). Their outcomes included a decreased suppression of acyl ghrelin on the fat rich meal compared to the CHO rich meal. After the CHO meal it was found that plasma ghrelin was significantly lowered in the lean but not in the obese subjects. Although this study did not differentiate types of fat, such as in our study, this does support our findings that the CHO diet had a lower acyl ghrelin level compared to the high fat meals. Due to the low number of participants in our study, we could not compare the lean to the obese as has been done in previous studies.

A more recent study looking at the effect of dietary components on ghrelin levels in relation to food intake found that high fat and high protein diet stimulated increased acyl ghrelin levels compared to a high CHO diet. (34). Similar to our study, this study also used visual analog scores to measure the hunger levels of the participants, however, the difference from our study was the scores were measured every fifteen minutes, and for only four hours. The results of that study found a significant change in hunger and

satiety ratings from baseline to three hours after ingestion of the meal, however, their results differed from our study in that there was no significant difference in magnitude of hunger and satiety between the fat and protein rich diets compared to the CHO rich diet. Although there were similarities between this study and our study, such as weight-maintenance diets throughout the experiment and similar visual analog scales, there were also many differences. Again, the subjects were only on specific diets for two days at a time, it was an ad libitum food study, and other dietary components were considered, such as protein, fruits/vegetables, as well as measurement of insulin and glucose. The different dietary components and hormones, which we did not measure or consider, do play a role in hunger, fullness and digestion. For example, protein and fiber may affect fullness. Other studies have also considered glucose, leptin and insulin interaction with dietary components such as dietary fat (35).

While some of these other factors, such as fiber, protein, leptin and insulin, were not measured for our study, it is important to recognize that other factors may play a role in meal initiation, such as other hunger or satiety hormones in the body. For example, in the study conducted by Callahan, et al., a rise in ghrelin before a meal did not specifically relate to the timing of meal initiation or request for meal (15). The authors of this study suggest that ghrelin is involved in meal initiation, supporting our study, but that meal initiation and hunger are complex and could be affected by other factors, some listed above.

Another potentially important observation is that fasting acyl ghrelin level is significantly higher on the MCT diet (73.7 pg/mL) compared to the high fat diet (which

had 40.8 pg/mL, p= 0.009). This fasting level is even higher than the general upward trend we see during the day with peak levels continuing to increase from breakfast to lunch to dinner, as has been reported in previous studies (16). This suggests that ghrelin synthesis continues to increase overnight when no exposure to dietary sources of fatty acids is occurring; in other words, the acyl portion of active ghrelin is likely coming from endogenous synthesis. While it was not within the scope of this study to address this question, our data is consistent with a dual role of absorption of dietary MCT and endogenous synthesis contributing to acylation of ghrelin during the day, but primarily endogenous synthesis contributing during the prolonged overnight postprandial state. It would be interesting to know the proportion of C8 vs. C10 and C12 acyl groups making up the active ghrelin levels after the MCT vs. high fat or low-fat meals, as well as their relative potency to bind and activate the ghrelin receptor. Likewise, it is known that high carbohydrate diets increase endogenous lipogenesis, which may explain why acyl ghrelin levels were higher than expected on the LF diet and not statistically different from the MCT diet.

Assessing the correlations between change in acyl ghrelin levels and change in fullness, there are similarities to the hunger correlations. All but one correlation between fullness and acyl ghrelin levels is negative. The negative correlation between change in fullness and change in acyl ghrelin follows our hypothesis, although we are still unsure of why the correlation was positive for the CHO diet in the morning. Overall, however, when using the combined meals data for this correlation analysis there was a statistically significant negative correlation. Overall, by combining the meals and looking

at the regression throughout the day, there was a stronger correlation. These data are consistent with a separate effect of ghrelin to inhibit fullness, though mechanisms mediating this relationship are less well studied and likely include changing postprandial levels of other GI hormone levels, including PYY and GLP-1.

The strengths and significance for the correlations between change in fullness and change in acyl ghrelin are LF>MCT>HF. When compared to the hunger versus acyl ghrelin correlations, in which the strength and significance was higher for the MCT diet compared to the LF diet, it follows the outcomes we found that fullness is significantly higher for LF diet and hunger is significantly higher for the MCT diet. This result may be explained by the effect that the MCT diet, compared to the LF diet, has on total ghrelin levels, suggesting that acyl ghrelin could possibly play a greater role in hunger, and less of a role in fullness.

Desacyl Ghrelin, Hunger and Fullness

Desacyl ghrelin is the inactive form of the hormone as it has been shown that this form of the hormone does not bind to the active receptor sites (7, 31). In a recent study looking at the effects of an infusion of acyl and des acyl ghrelin in humans, it was found that the acyl ghrelin infusion raised both forms of ghrelin in circulation, however, the des acyl ghrelin infusion only caused des acyl ghrelin in circulation to rise (31). The authors suggest that this may be due to the process of acylation, metabolism and endogenous synthesis of plasma ghrelin levels. Although this study looked at exogenous ghrelin, there can be connections made to the outcomes of our study. Interestingly, our results show that des acyl ghrelin levels were significantly lower (AUC, table 6) when the

participants were on the MCT diet compared to both the LF (*P*=<0.001) and the HF diet (*P*=0.016). Because our study focused more on the dietary impact of endogenous sources of ghrelin, there was a change in both acyl and des acyl ghrelin with the different diets. A difference that can be noted between our study and this recent exogenous ghrelin study is that with the MCT diet, there was a decrease, compared to the other diets, in des acyl ghrelin levels. This may suggest that dietary components, such as fat, may play a role in the acylation process and metabolism of endogenous ghrelin.

When the correlations between des acyl ghrelin and hunger or fullness are evaluated, the consistency of the associations with individual meals as well as when data from all the meals were combined, were weaker than those with acyl ghrelin (tables 13 and 18). The only significant correlations between hunger and des acyl ghrelin are at breakfast on the low fat diet and lunch on the high fat diet. This might be explained by the relative consistency of des acyl ghrelin levels throughout the day. Our results show that the fasting levels of des acyl ghrelin in the morning were the highest for the day, and remained within approximates a 20 pg/ml range throughout the day. The fasting peak is consistent with the literature (30). It is important to note that our study looked at three different diets and the study conducted by Lui, et al (30) simply looked at one diet, with caloric content based on the Harris-Benedict equation, similar to ours. These outcomes and correlations suggest that acyl and des acyl ghrelin levels are not affected the same by either diet or exogenous sources of ghrelin.

Ratio of Acyl to Desacyl Ghrelin, Hunger, and Fullness

When comparing acyl and des acyl ghrelin levels before individual meals, the correlations are strong for acyl ghrelin and weak for des acyl ghrelin for both hunger and fullness. Similarly, when we compared the ratio of acyl to des acyl ghrelin levels to appetite throughout the day, the results indicate that change in the ratio of acyl to desacyl ghrelin levels correlated with hunger and fullness only weakly. Our study does reveal, however, that when on a higher MCT diet, there is a stronger correlation between the ratio of acyl to des acyl ghrelin and hunger and fullness (tables 14 and 19). These results do not support use of this ratio as a superior indicator of ghrelin action compared to the levels of each hormone separately. Unfortunately few studies have included both measures of acyl and desacyl ghrelin, and of these, most are in patients that underwent acyl ghrelin infusions, which may create artificial ratios of the two ghrelin forms (31).

Total Ghrelin, Hunger and Fullness

Total ghrelin is a combination of acyl ghrelin and desacyl ghrelin and therefore may simply reflect the contributions of one or the other hormone form that has a dominant biologic activity. So, it is not surprising that evaluating the correlations between total ghrelin and hunger or fullness, they have similar significance levels as the acyl ghrelin levels. All of the correlations between total ghrelin and hunger are positive, while all but one of the correlations with fullness, are negative.

It is of interest that the biggest change in acyl ghrelin levels occurs in the afternoon and evening, while the biggest changes in des acyl ghrelin levels occur in the

morning. Another area to look into would be the breakdown of both acyl and des acyl ghrelin and their clearance from the body. From the data collected here, it indicates that des acyl ghrelin might have a higher clearance rate. The fact that acyl ghrelin continues to increase throughout the day, despite the desacylation process that occurs supports this supposition (31).

Ratio of Acyl to Total Ghrelin, Hunger and Fullness

The relationships between the ratio of acyl/total ghrelin levels, hunger, and fullness are similar to those seen for acyl ghrelin alone and, like the ratio of acyl/des acyl ghrelin, does not appear to be a better indicator of ghrelin action than either hormone alone.

Limitations

One of the major limitations to this study is the small sample size, especially with regard to examining whether differences by sex or obesity status are present. Another limitation was not being able to examine food intake results in order to compare results to other studies. Many studies analyzed hunger based on food intake at the next meal, when food was provided ad libitum. Our study provided set meals with set calorie counts for each participant and measured hunger by visual analog scales. This provided a challeng in comparing the outcomes with the ad libitum studies. Another limitation was having the participants eat at specific meal times during their 24 hour admission to the CTRC and not while they were completing the two week feeding trials at home. This may or may not have changed the increase or decrease of hunger, satiety, or ghrelin levels at meal times. For example, if a person normally ate their meals at 9 am, 2 pm and 8pm rather than the 8 am, noon, and 5 pm, their normal hunger patterns may deviate from what was measured during the inpatient visit to the CTRC. A final limitation is that because we did not use tracers, we still do not know if the rise in ghrelin was due to incorporation of the dietary FA's or endogenous synthesis. Also, without mass spectroscopy measures, even if we know that the acyl groups on the higher active ghrelin molecules did come from the diet, we don't know if C10 and C12 fatty acids were included in addition to C8's.

Conclusion:

In summary, during and after two weeks of isocaloric feeding, acyl ghrelin levels and subjective hunger were highest, and subjective fullness was lowest during the high MCT diet compared to the HF and LF diets. The strongest directionality relationship between diet, ghrelin levels, and hunger and fullness over the two weeks, however, was with the fasting des-acyl ghrelin levels, which were lowest on the high MCT diet, intermediate on the HF diet, and highest on the LF diet. When individual meals were examined, changes in acyl ghrelin levels were mostly strongly correlated with rise in hunger and reduction in fullness prior to consuming a meal (before lunch, before dinner, and the rise after dinner), regardless of diet treatment assignment. Our data support a role for incorporation of dietary fatty acids directly onto endogenous ghrelin and furthermore, the rise in acyl ghrelin levels that occurs during a high MCT diet may mediate the increased hunger and suppressed fullness compared to control diets.

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Appendix 1: Participant Visual Analog Scores

I. Participant VAS and Daily Diary

ID # _____

Filled out for Study Date__ __ / __ __ / __ __ __ __

Reviewed by (staff member)_____

Effect of Diet Composition on Ghrelin– eIRB Protocol #3224
Participant VAS and Daily Diary

FOODS			**PLEASE ANSWER THE FOLLOWING QUESTIONS CONSIDERING THE FOODS AND BEVERAGES THAT YOU CONSUMED TODAY.**
1.	Circle your answer:		Was the amount of food provided to you overall: way too much 1 a little too much 2 just right 3 not quite enough 4 not enough 5
	Yes	**No**	
2.			Is there anything you would like us to know regarding your participation in this study? _____ _____
3.			Did you have any coffee, tea, or soda? If yes, what kind and how much you consumed: What Kind Amount _____ _____ _____ _____
4.			Did you drink any alcoholic beverages? If yes, what kind and how much you consumed: What Kind Amount _____ _____ _____ _____
5.			Did you eat one meal in the CRC dining room?

6.			Did you eat or drink any foods that are not part of the study diet? If yes, please list the kind and amount, and the reason for eating them. What kind Amount Reason (please explain) _____ _____ _____ _____ _____ _____
7.			Did you take any over-the-counter medicines such as Tylenol, cold medications, allergy medications, or any vitamins or other dietary supplements? What kind Amount Reason (please explain) _____ _____ _____ _____ _____ _____

	Yes	**No**	**THINK ABOUT HOW YOU HAVE FELT OVER THE LAST 24-HOURS. DURING THAT TIME DID YOU EXPERIENCE ANY OF THE FOLLOWING?**
20.			Lost the desire to eat (Loss of appetite)
22.			Feeling of more energy
24.			Feeling more bloated than usual
25.			Abdominal pain
27.			Vomiting
29.			Diarrhea (number of episodes:)
30.			Constipation
31.			Number of bowel movements different from normal If yes, please circle correct description Decreased 1 Increased 2
32.			Volume of bowel movements different from normal If yes, please circle correct description Decreased 1 Increased 2

33.			Dry skin (overall)
34.			Headache
35.			Drowsiness
37.			Decreased energy level (lethargy)
39.			Light headedness
40.			Difficulty sleeping
42.			For Women: Were you menstruating?

How hungry did you feel today?

Not at all hungry _____ Extremely hungry

How thirsty did you feel today?

Not at all thirsty _____ Extremely thirsty

How nauseated did you feel today?

Not at all nauseated _____ Extremely nauseated

How full did you feel today?

Not at all full _____ Extremely full

II. Participant Daily VAS

Subject ID#:_____
Date: _____
Day of Week:_____

APPROVED: Jul. 25, 2006

Hunger and Satiety Scale

The following questions ask you to rate on a line scale how strongly you feel specific sensations. A vertical mark on the far left side of the line indicates that you do not feel that sensation at all. A mark on the far right side of the line indicates that you feel that sensation very, very strongly. Please do the ratings carefully, giving each question some thought.

Place a **single vertical mark** on the line that indicates how you are feeling. Please **do not** mark the lines with an X.

I. How hungry have you felt between meals today?

Not at all _____Extremely
hungry hungry

II. How full have you felt after eating meals today?

Not at _____ Extremely
all full full

3. How easy or hard was it for you to eat your diet today?

Very _____ Very
easy hard

III. Participant VAS for CTRC Visit

PI: Purnell
eIRB# 3224
Subject ID: _____
Date: _____

INSTRUCTIONS ON HOW TO DO RATINGS

Below is an example of the type of question that you will be asked during your meals in the lab:

How happy are you right now?

Not at all happy _____ Extremely happy

Place a **single vertical mark** on the line that indicates how you are feeling. Please **do not** mark the lines with an X.

Each question asks you to rate on a scale, such as the examples above, how strongly you feel specific sensations. A vertical mark on the far left side of the scale indicates that you do not feel that sensation at all. A mark on the far right side of the scale indicates that you feel that sensation very, very strongly. Please do the ratings carefully, giving each question some thought. Also, make sure that you are only turning one page in the booklet at a time so that you do not miss any questions.

Please note:
The above instructions are given to the subjects prior to completing any VAS questions.

The 5 VAS questions are given in booklet form. They are scored by measuring the distance to the vertical mark in centimeters. These values are then analyzed for differences between conditions.

Appetite Scale 7:30 AM*

How hungry do you feel right now?

Not at all hungry _____ Extremely hungry

How thirsty do you feel right now?

Not at all thirsty _____ Extremely thirsty

How much food do you think you could eat right now?

Nothing at all _____ A large amount

How nauseated do you feel right now?

Not at all nauseated _____ Extremely nauseated

How full do you feel right now?

Not at all full _____ Extremely full

***** This scale was given in a booklet that included time points corresponding to all thirty minute increments between 7:30 am and 9:00 pm.

Appendix 2: Tables

Table 15: Correlation between change in hunger and change in total ghrelin levels, by diet and meal

	Change in TG, LF		Change in TG, HF		Change in TG, MCT	
	r	*P*-value	r	*P*-value	r	*P*-value
Change in Hunger, B	**0.978**	**0.001**	0.805	0.053	**0.928**	**0.008**
Change in Hunger, L	**0.966**	**<0.001**	**0.975**	**<0.001**	**0.927**	**<0.001**
Change in Hunger, D	**0.863**	**0.027**	0.661	0.153	0.644	0.168
Change in Hunger, all meals combined	**0.807**	**<0.001**	**0.728**	**<0.001**	**0.802**	**<0.001**

Results are based on averages of all subjects at specific times: B- Breakfast (0900-1130), L-Lunch (1300-1700), D-Dinner (1830-2100). LF- low fat diet. HF-high fat diet. MCT- medium chain diet. TG- total ghrelin

Table 20: Correlation between change in fullness and change in total ghrelin levels, by diet and meal

	Change in TG, LF		Change in TG, HF		Change in TG, MCT	
	r	*P*-value	r	*P*-value	r	*P*-value
Change in Fullness, B	**0.909**	**0.012**	-0.692	0.127	**-0.981**	**0.001**
Change in Fullness, L	**-0.923**	**<0.001**	**-0.918**	**<0.001**	**-0.888**	**0.001**
Change in Fullness, D	**-0.865**	**0.026**	-0.608	0.20	-0.786	0.064
Change in Hunger, all meals combined	**-0.837**	**<0.001**	**-0.766**	**<0.001**	**-0.811**	**<0.001**

Results are based on averages of all subjects at specific times: B- Breakfast (0900-1130), L-Lunch (1300-1700), D-Dinner (1830-2100). LF- low fat diet. HF-high fat diet. MCT- medium chain diet. TG- total ghrelin

Table 5: Effect of diet fat and carbohydrate on **acyl** ghrelin levels in lean and obese subjects (n=21).

	MCT	HF	LF	ANOVA *P*-value	MCT vs. HF *P*-value	MCT vs. LF *P*-value	HF vs. LF *P*-value
AUC (pg-13 hr/mL)	587 ± 371	404 ± 181	533 ± 274	**0.025**	**0.012**	0.69	**0.03**
Fasting (pg/mL)	73.7 ± 49.4	40.8 ± 22	51.6 ± 39	**0.032**	**0.009**	0.113	0.275
Nadir B (pg/mL)	15.8 ± 11.6	10.5 ± 7.6	10.2 ± 9.3	0.057	**0.034**	**0.042**	0.925
% Nadir B	-75.1 ± 17.1	-73.5 ± 11.7	-76.7 ± 17	0.465	0.78	0.367	0.239
Peak L (pg/mL)	72.5 ± 59.8	35.1 ± 18.7	54.8 ± 40.6	**0.021**	**0.006**	0.25	0.089
% Rise L	356.3 ± 283.9	312.7 ± 202.3	989.3 ± 1420.1	**0.012**	0.923	**0.011**	**0.009**
Nadir L (pg/mL)	15.4 ± 11.1	9.9 ± 8.3	16.8 ± 14.4	**0.024**	0.053	0.433	**0.008**
% Nadir L	-72.6 ± 19.1	-72.4 ± 17.5	-65.6 ± 18.3	0.744	0.993	0.505	0.0511
Peak D (pg/mL)	94.8 ± 68.2	76.3 ± 43.8	85.2 ± 42.7	0.389	0.184	0.70	0.342
% Rise D	624.2 ± 459	1075.7 ± 1230.4	750.9 ± 897	0.284	0.115	0.467	0.386
Nadir D (pg/mL)	26.2 ± 18.5	17.1 ± 9.1	22.5 ± 14.2	**0.05**	**0.018**	0.495	0.083
% Nadir D	-68.5 ± 16.3	-73.9 ± 16.4	-70.7 ± 14.8	0.65	0.434	0.413	.0971
Peak Eve (pg/mL)	100.6 ± 80.3	78.6 ± 50.2	93.8 ± 68.8	0.384	0.211	0.903	0.258
% Rise Eve	338.8 ± 351.6	366.8 ± 235.8	555.3 ± 1131.4	0.488	0.967	0.293	0.312

Results are mean ± SD. MCT-medium chain diet. HF-high fat diet. LF-low fat diet. B-breakfast. L-lunch. D-dinner. Eve-evening.

Table 6: Effect of diet fat and carbohydrate on **desacyl** ghrelin levels in lean and obese subjects (n=21).

	MCT	HF	LF	ANOVA *P*-value	MCT vs. HF *P*-value	MCT vs. LF *P*-value	HF vs. LF *P*-value
AUC (pg-13 hr/mL)	308.4 ± 251.3	418.6 ± 266.4	480.4 ± 316.9	**<0.001**	**0.016**	**<0.001**	0.058
Fasting (pg/mL)	47.6 ± 33	62.1 ± 46	61.1 ± 49.6	0.143	0.112	0.071	0.818
Nadir B (pg/mL)	13.1 ± 15.7	21.9 ± 19.7	18.6 ± 18.1	**0.048**	**0.018**	0.08	0.501
% Nadir B	-78.6 ± 20.6	-69.9 ± 16	-73.8 ± 19.1	0.204	0.109	0.893	0.141
Peak L (pg/mL)	42.3 ± 29.1	50.7 ± 31.5	54.8 ± 35.3	0.10	0.233	**0.033**	0.326
% Rise L	339.3 ± 439.9	198.7 ± 160.9	356.4 ± 499.7	0.515	0.773	0.412	0.27
Nadir L (pg/mL)	8.1 ± 12.8	11.2 ± 12.5	13.3 ± 11.3	0.161	0.312	0.057	0.356
% Nadir L	-86.8 ± 15.8	-81.5 ± 21	-74.5 ± 19.9	0.484	0.452	.0236	0.66
Peak D (pg/mL)	37.2 ± 29.4	53 ± 25.9	58.3 ± 31	**<0.001**	**0.008**	**<0.001**	0.154
% Rise D	491.8 ± 453.3	320.6 ± 239.3	555.3 ± 591.6	0.103	0.484	0.156	**0.037**
Nadir D (pg/mL)	10.4 ± 15.6	14 ± 17.1	18.8 ± 18.7	0.083	0.381	**0.028**	0.17
% Nadir D	-77.5 ± 24.4	-78.8 ± 22	-71 ± 21	0.85	0.863	0.703	0.58
Peak Eve (pg/mL)	37.2 ± 34.2	53.2 ± 33	62.8 ± 45.7	**0.001**	**0.025**	**<0.001**	0.07
% Rise Eve	332.1 ± 184.8	350.2 ± 351.8	401.4 ± 548.4	0.422	0.892	0.233	0.288

Results are mean ± SD. MCT-medium chain diet. HF-high fat diet. LF-low fat diet. B-breakfast. L-lunch. D-dinner. Eve-evening.

Table 7: Effect of diet fat and carbohydrate on **acyl/desacyl** ghrelin levels in lean and obese subjects (n=21).

	MCT	HF	LF	ANOVA *P*-value	MCT vs. HF *P*-value	MCT vs. LF *P*-value	HF vs. LF *P*-value
AUC (pg-13 hr/mL)	47.7 ± 33.1	23.1 ± 14.6	33.2 ± 34.4	**0.02**	**0.005**	0.134	0.165
Fasting (pg/mL)	3.1 ± 3.8	2.1 ± 3.9	1.4 ± 1.6	0.256	0.926	0.109	0.614
Nadir B (pg/mL)	0.8 ± 0.8	0.6 ± 0.7	0.4 ± 0.4	0.188	0.265	0.071	0.474
% Nadir B	-53.3 ± 34.6	-51.6 ± 28.2	-64.8 ± 23.8	0.157	0.675	0.153	0.068
Peak L (pg/mL)	11.6 ± 20.9	1.8 ± 2.9	5.9 ± 13.5	0.124	**0.043**	0.256	0.354
% Rise L	1104.7 ± 1775.6	285.8 ± 312.8	1976.3 ± 4265	0.166	0.387	0.295	0.06
Nadir L (pg/mL)	0.8 ± 0.6	0.3 ± 0.2	0.4 ± 0.4	**<0.001**	**<0.001**	**0.003**	0.247
% Nadir L	-69.3 ± 22.6	-69.8 ± 20.3	-64.5 ± 25.3	0.966	0.938	0.858	0.798
Peak D (pg/mL)	12.7 ± 11.9	6 ± 5.7	10.2 ± 13.2	0.13	**0.05**	0.547	0.166
% Rise D	1678.3 ± 2086	3669.6 ± 6048.7	2888.4 ± 4184.5	0.358	0.172	0.297	0.741
Nadir D (pg/mL)	1.5 ± 0.9	0.7 ± 0.6	0.6 ± 0.3	**0.001**	**0.001**	**<0.001**	0.712
% Nadir D	-76.6 ± 20.6	-79.3 ± 25.7	-82.6 ± 16.4	**<0.001**	0.699	**<0.001**	**<0.001**
Peak Eve (pg/mL)	12.4 ± 13	10.1 ± 9	8.6 ± 12.8	0.655	0.545	0.37	0.769
% Rise Eve	1003.6 ± 1682.9	1395.7 ± 1648.4	1845.8 ± 3591.9	0.477	0.679	0.234	0.433

Results are mean ± SD. MCT-medium chain diet. HF-high fat diet. LF-low fat diet. B-breakfast. L-lunch. D-dinner. Eve-evening.

Table 8: Effect of diet fat and carbohydrate on **total** ghrelin levels in lean and obese subjects (n=21).

	MCT	HF	LF	ANOVA *P*-value	MCT vs. HF *P*-value	MCT vs. LF *P*-value	HF vs. LF *P*-value
AUC (pg-13 hr/mL)	895.7 ± 544.2	822.2 ± 409.4	1008.1 ± 491	**0.011**	0.342	**0.039**	**0.004**
Fasting (pg/mL)	121.2 ± 60.6	102.8 ± 54.3	112.6 ± 78.8	0.486	0.266	0.856	0.35
Nadir B (pg/mL)	32.6 ± 23	36.1 ± 24.4	34.1 ± 23.7	0.504	0.295	0.333	0.936
% Nadir B	-73.4 ± 13.3	-66.2 ± 9.8	-68.4 ± 14.6	0.271	0.126	0.734	0.23
Peak L (pg/mL)	107.9 ± 62.2	79.4 ± 39.6	101.1 ± 53	**0.05**	**0.029**	0.892	**0.04**
% Rise L	298 ± 241.1	170.2 ± 129.2	453.8 ± 1019.7	0.253	0.542	0.299	0.103
Nadir L (pg/mL)	32.3 ± 25.9	32 ± 23.6	47.8 ± 31	**<0.001**	0.95	**<0.001**	**<0.001**
% Nadir L	-70 ± 16.5	-61 ± 19.8	-51.5 ± 20.1	**0.013**	0.088	**0.003**	0.181
Peak D (pg/mL)	117.9 ± 76.2	113.8 ± 52.6	126.6 ± 58.3	0.381	0.774	0.301	0.189
% Rise D	326.3 ± 200.2	417.9 ± 499.5	209.6 ± 149	0.175	0.306	0.39	0.064
Nadir D (pg/mL)	42.5 ± 28	43.7 ± 30.1	53.5 ± 32.8	**0.016**	0.808	**0.009**	**0.017**
% Nadir D	-61.7 ± 16.8	-62.1 ± 16.9	-57.8 ± 15.3	0.969	0.96	0.853	0.814
Peak Eve (pg/mL)	125 ± 103.8	119.9 ± 75.3	139.3 ± 101.6	0.427	0.811	0.322	0.221
% Rise Eve	200.2 ± 119	190.3 ± 116.1	178.7 ± 105.4	0.871	0.606	0.738	0.856

Results are mean ± SD. MCT-medium chain diet. HF-high fat diet. LF-low fat diet. B-breakfast. L-lunch. D-dinner. Eve-evening.

Table 9: Effect of diet fat and carbohydrate on **acyl/total** ghrelin levels in lean and obese subjects (n=21).

	MCT	HF	LF	ANOVA *P*-value	MCT vs. HF *P*-value	MCT vs. LF *P*-value	HF vs. LF *P*-value
AUC (pg-13 hr/mL)	8.4 ± 1.8	6.7 ± 2.1	6.6 ± 2.1	**0.04**	**0.017**	0.052	0.62
Fasting (pg/mL)	0.6 ± 0.2	0.5 ± 0.3	0.5 ± 02	0.197	0.089	0.175	0.719
Nadir B (pg/mL)	0.4 ± 0.2	0.3 ± 0.2	0.2 ± 0.2	**0.036**	0.057	**0.014**	0.537
% Nadir B	-33.2 ± 27.9	-37.8 ± 23.6	-49.8 ± 26.7	0.094	0.596	**0.038**	0.114
Peak L (pg/mL)	0.7 ± 0.2	0.5 ± 0.3	0.6 ± 0.3	0.163	0.058	0.35	0.321
% Rise L	93.6 ± 77.4	136.2 ± 150.7	296 ± 637	0.227	0.704	0.102	0.204
Nadir L (pg/mL)	0.4 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	**0.003**	**0.001**	**0.032**	0.177
% Nadir L	-42.1± 20.5	-57.4 ± 20.5	-48 ± 21	0.103	**0.034**	0.25	0.312
Peak D (pg/mL)	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.2	0.462	0.621	0.22	0.459
% Rise D	159.9 ± 158.6	415.2 ± 416	280.9 ± 289.7	**0.044**	**0.014**	0.135	0.296
Nadir D (pg/mL)	0.5 ± 0.2	0.4 ± 0.2	0.3 ± 0.1	**0.006**	**0.007**	**0.004**	0.798
% Nadir D	-39.5 ± 18.9	-58.1 ± 15.3	-54.8 ± 15.5	**0.005**	**0.004**	**0.005**	0.921
Peak Eve (pg/mL)	0.9 ± 0.1	0.8 ± 0.2	0.8 ± 0.2	0.645	0.493	0.374	0.837
% Rise Eve	89 ± 119.7	139.9 ± 104.8	262.5 ± 666.4	0.328	0.734	0.156	0.275

Results are mean ± SD. MCT-medium chain diet. HF-high fat diet. LF-low fat diet. B-breakfast. L-lunch. D-dinner. Eve-evening.

Appendix 3: Figures

Figure 10.

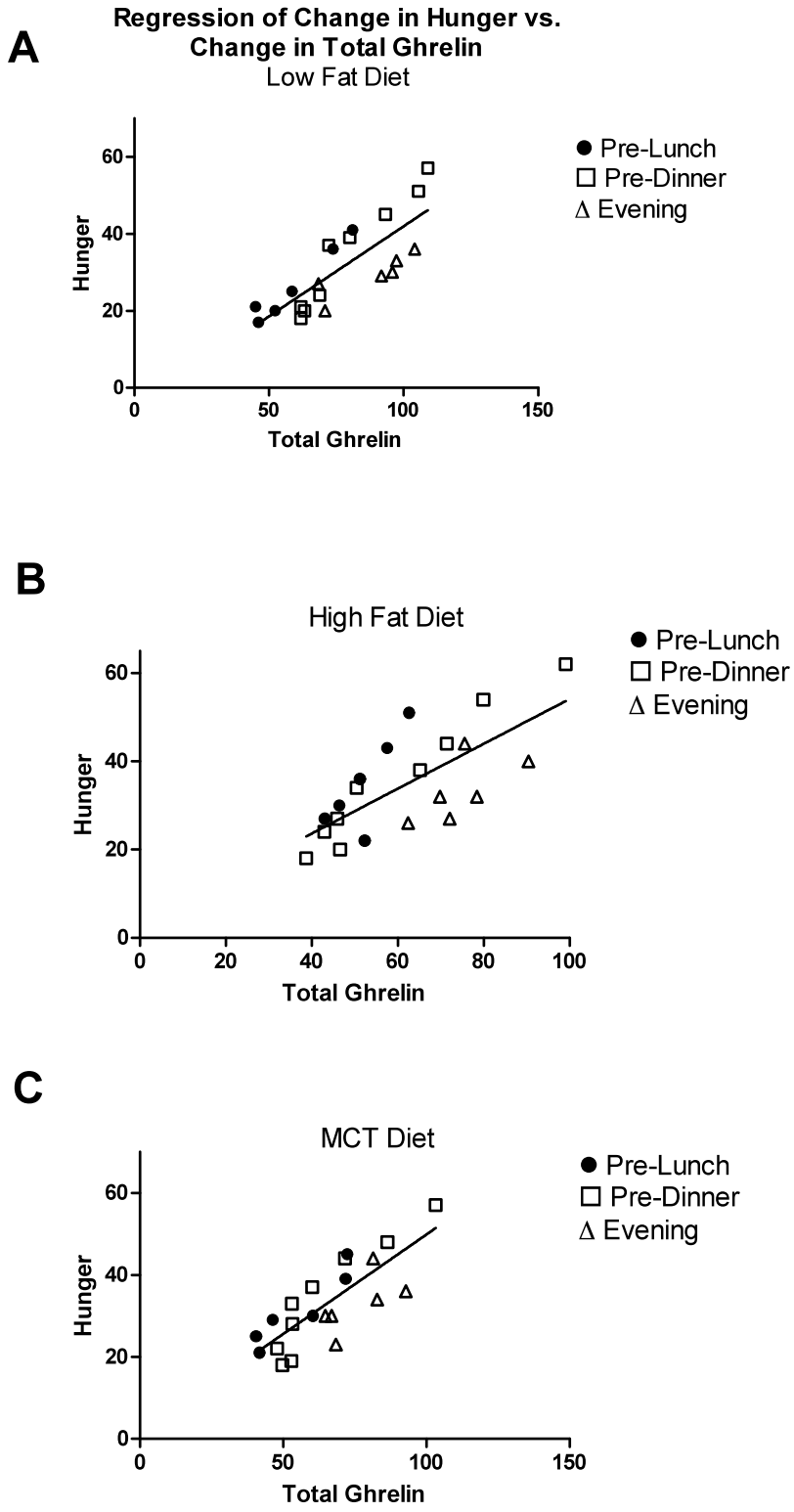


Figure 15.

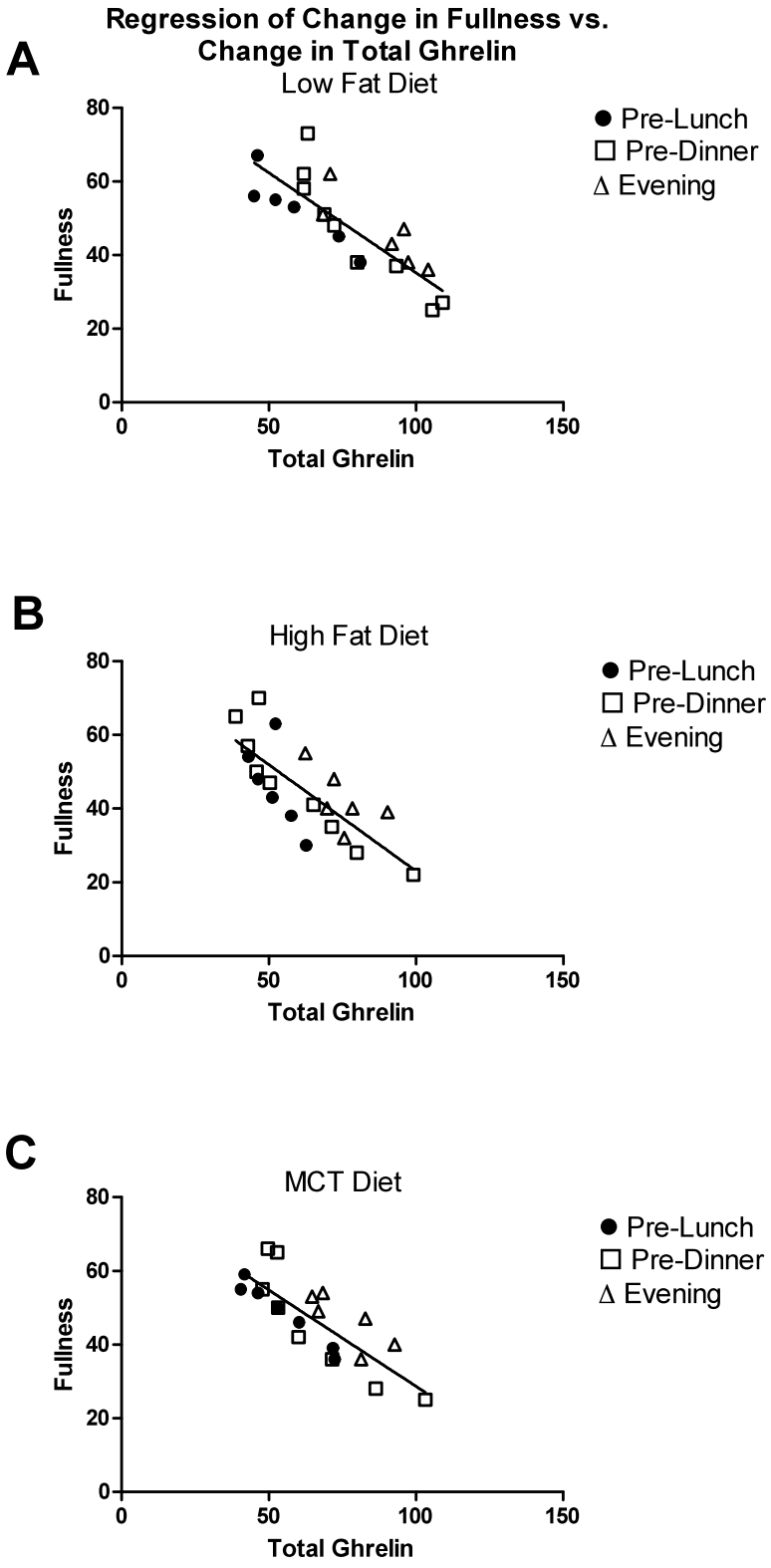

A
Regression of Change in Fullness vs.
Change in Total Ghrelin
Low Fat Diet
Fullness
Total Ghrelin
Pre-Lunch
Pre-Dinner
Evening
B
High Fat Diet
Fullness
Total Ghrelin
Pre-Lunch
Pre-Dinner
Evening
C
MCT Diet
Fullness
Total Ghrelin
Pre-Lunch
Pre-Dinner
Evening

Figure 17.

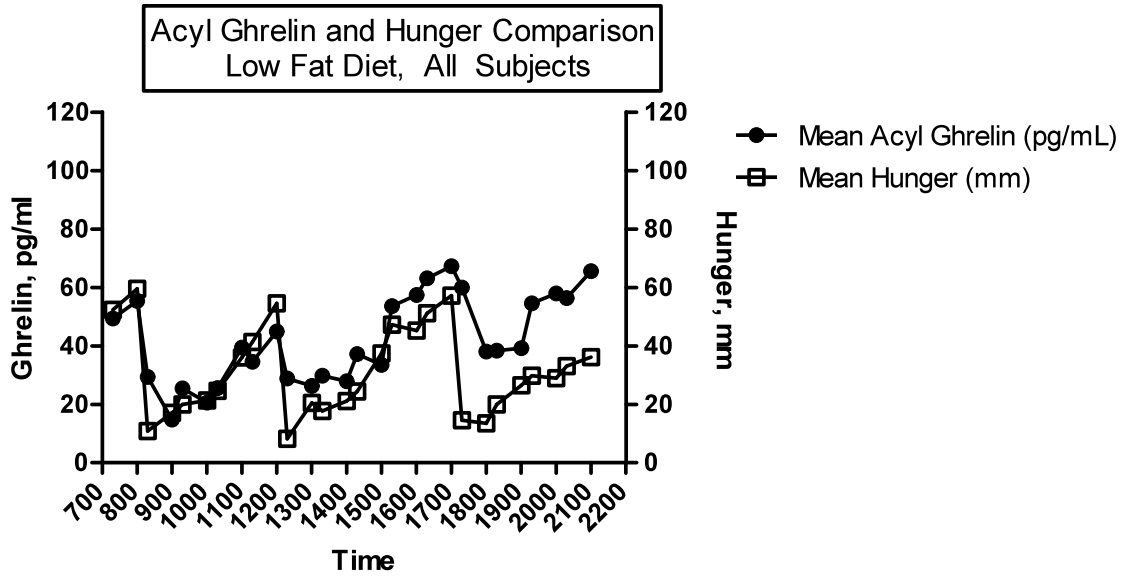


Figure 18.

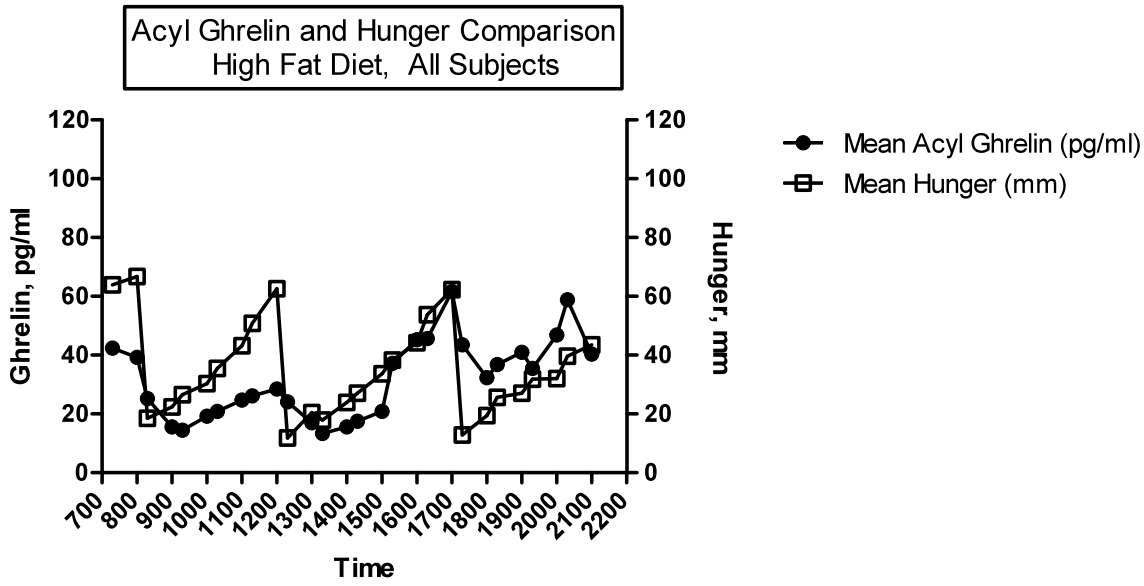
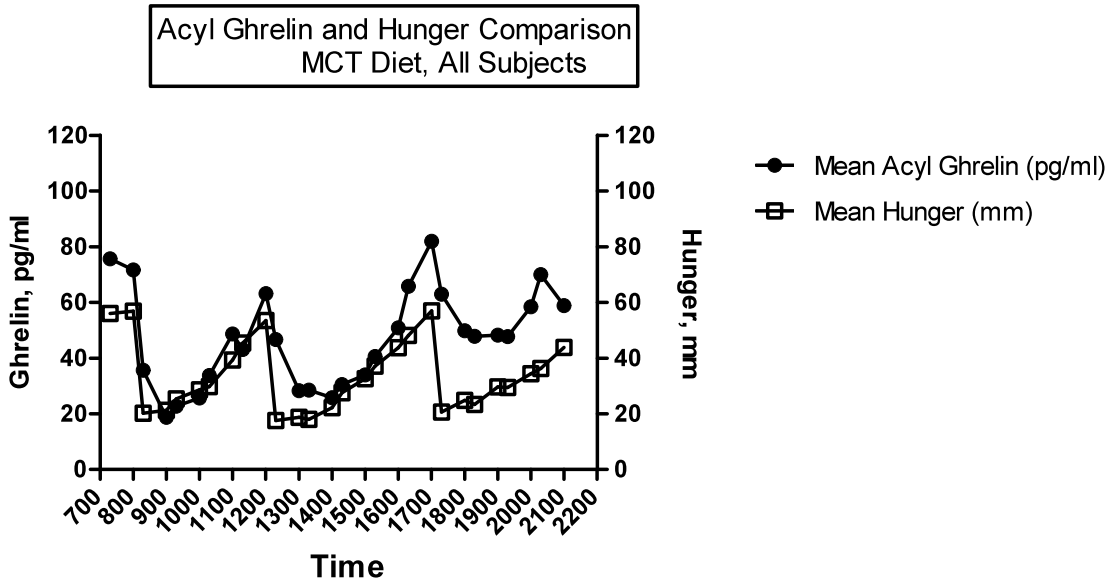


Figure 19.


Acyl Ghrelin and Hunger Comparison
MCT Diet, All Subjects
Ghrelin, pg/ml
Hunger, mm
Time
Mean Acyl Ghrelin (pg/ml)
Mean Hunger (mm)

Appendix 4: Evidence Table

Study Identification	Participants	Duration	Design	Outcomes
Mokdad A, et al. The spread of the obesity epidemic in the United States, 1991-1998. JAMA 1999;282: 1519	>100,000 per year	7 years	Cross-sectional random-digit telephone survey	The prevalence of obesity increased from 12% in 1991 to 17.9% in 1998. A steady increase in all states, w/ both sexes, across all age groups, races and education levels, regardless of smoking status. Greatest magnitude of increase: 18-29y/o (7.1% to 12.1%), those w/ some college education (10.6 to 17.8%) and Hispanics (11.6 to 20.8%). the amount of leisure time physical activity has remained relatively the same. Obesity is spreading at a rate similar to that of a communicable disease epidemic. May be more prevalent d/t self-reporting (people increase ht, and decrease wt when asked)
Romieu I, et al. Energy intake and other determinants of relative weight. Am J Clin Nutr 1988; 47:406	141 females, age 34-59	4 weeks over one year period	Original research article (cross-sectional)	Age was positively correlated w/ obesity and negatively correlated with vigorous activity. A weak inverse correlation between energy intake and BMI. A strong inverse correlation between daily activity (stairs, walking, sports) and BMI. Alcohol intake inversely related to obesity. Fatty acid intake correlated to total energy intake. Calorie-adjusted total fat intake was positively correlated to obesity, mostly d/t sat fatty acids.*suggest that increased intake of fat may be associated w/ obesity independent of total energy intake.

Study Identification	Participants	Duration	Design	Outcomes
Mela DJ, Sacchetti DA. Sensory preferences for fats: relationships with diet and body composition. Am J Clin Nutr 1991;53:908	30 healthy adults (21 F, 9M)	2 weeks	Original research article	There were no consistent correlations in preferred fat level among the various stimuli. There was no correlation btwn %fat in the diet and the overall most preferred level of fat. Measures of relative adiposity were all positively correlated w/ the most preferred level of fat across all stimuli, sig in women, not men (in women, increase in % body fat ->increase in sensory preference for fat). There was no sig relationship between dietary fat intake and measures of adiposity.* There is a consistent positive correlation between overall fat preference and measures of adiposity in normal-wt subjects.
Nishi Y, et al. Ingested medium-chain fatty acids are directly utilized for the acyl modification of ghrelin. Endocrinology 2005; 146:2255	male C57BL/6J mice	14 day	animal study	Gastric concentrations of n-octanoyl ghrelin increased significantly in mice fed n-octanoic acid. Ingestion of either MCFAs or MCTs increased the stomach concentrations of acylated ghrelin w/o changing the total ghrelin amounts. After the ingestion of either MCT or MCFA, the carbon chain lengths of the acyl groups attached to nascent ghrelin molecules correspond to that of the ingestion of MCT or MCFAs. This indicates that ingestion of MCFAs are directly used for the acylation of ghrelin

Study Identification	Participants	Duration	Design	Outcomes
Matsumoto M, et al. Structure-activity relationship of ghrelin: pharmacological study of ghrelin peptides. Biochemical and Biophysical Research Comm 2001; 287:142	Sprague-Dawley male rats	unspecified	in vitro	They identified the minimum active core of ghrelin as the N-terminal tetrapeptide possessing the octanoyl modification at the third serine. The octanoyl ester bond can be effectively replaced by a more stable ether or thioether bond, an advantageous substitution creating long-term stability in pharmaceuticals. Hydrophobicity surrounding the third amino acid residue, regardless of the aromaticity or aliphaticity, is essential in GHS activity (growth hormone secretagogue)
Hosoda H, et al. Ghrelin and des-acyl ghrelin:two major forms of rat ghrelin peptide in gastrointestinal tissues. Biochemical and Biophysical Research Communications 2000;279:909	Tissue samples from 5 6-week old male rats	unspecified	Animal study	Two major molecular forms exist: ghrelin and des-n-octanoyl ghrelin. Ghrelin activates the GHS receptor, des-acyl ghrelin does not. High concentrations of ghrelin in the stomach and small intestines
Guana C, et al. Ghrelin stimulates, whereas des-octanoyl ghrelin inhibits, glucose output by primary hepatocytes. J Clin Endocrinol Metab 2005; 90:1055	6-month old female pigs	unspecified	in vitro study	Acylated ghrelin (AG) induced glucose output dose dependently after 20 min of incubation, unacylated ghrelin (UAG) inhibited glucose release also dose dependently after 20 min. UAG completely reversed AG-induced glucose output.

Study Identification	Participants	Duration	Design	Outcomes
Banks WA, et al. Extent and direction of ghrelin transport across the blood-brain barrier is determined by its unique primary structure. JPET 2002; 302:822	mice		animal	Human ghrelin was readily transported by a saturable system across the blood brain barrier in both directions, whereas the mouse ghrelin was only saturable in the brain-to-blood direction. (mouse ghrelin differs from human in two of the 28 residues.) Des-octanoyl ghrelin entered the brain by nonsaturable transmembrane diffusion, and was sequestered once in the CNS.
Faulconbridge LF, et al. Hyperphagic effects of brainstem ghrelin administration. Diabetes 2003;52:2260	rats	days	animal study	Delivery of ghrelin to the brainstem induces a hyperphagic response similar to that obtained with the same dose of ghrelin delivered to the forebrain. There were similar response profiles from third intracerebroventricular placements, providing optimal stimulation of hypothalamic structures, including ARC, bearing more directly on feeding relevant structures in the caudal brainstem. Ingestive responses to ghrelin can be elicited from more than one location; including the ARC and at least one site within the caudal brainstem.

Study Identification	Participants	Duration	Design	Outcomes
Williams DL, et al. Vagotomy dissociates short- and long-term controls of circulating ghrelin. Endocrinology 2003; 144:5184	Naïve sprague dawley rats	unspecified	animal study	Baseline ghrelin levels were not altered by subdiaphramatic vagotomy; sham-operated rats increased circulating ghrelin levels in response to food deprivation, vagotomized rats failed to show this response. The results show a role for the vagus nerve in the plasma ghrelin response to long term changes in energy stores, but not in the response to short-term fluctuations in nutritive status. Vagal mediation is indicated for the food deprivation-induced elevation of plasma ghrelin. Subdiaphramatic vagotomy completely prevented the otherwise robust deprivation-related increase in circulating ghrelin.
le Roux CW, et al. Ghrelin does not stimulate food intake in patients with surgical procedures involving vagotomy. J Clin Endocrinol Metab 2005; 90:4521	6 men and 1 woman	9 + days	double-blind, randomized, cross-over design	A significant and dose dependent rise in GH was assoc with ghrelin infusion, confirming that the ghrelin was metabolically active Results: IV ghrelin did not alter appetite or energy intake in pts with vagotomy and partial or total gastrectomy. There was a significant reduction in postprandial fullness score after the ghrelin infusion. Ghrelin stimulated Gh release and may have stimulated gastric motility but did not alter appetite or energy intake. In humans, an intact vagus nerve may be required for exogenous ghrelin to increase appetite and food intake.

Study Identification	Participants	Duration	Design	Outcomes
Cummings D, et al. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 2001;50:1714	10 healthy subjects (9 women, 1 man)	2 weeks prior (prep/diet period) and 1-24 hr collection period	Cohort study	Plasma ghrelin levels rose by average of 78% 1-2 hr before the onset of each meal, and fall to trough levels w/in 60 min after first consumption of food. Ghrelin levels between meals increased progressively throughout the day. There was a significant positive correlation between levels of plasma ghrelin and age
Wren AM, et al. Ghrelin enhances appetite and increases food intake in humans. J. Clin. Endocrinol. Metab. 2001;86:5992.	9 non-obese Caucasian volunteers, age 21-32, BMI 19.8-26.8	Two days, with one week in between	Randomized double-blind cross-over study	Subjects with ghrelin infusion consumed more energy (kcals), increased hunger scores before breakfast/lunch, no evidence of reduced hunger 1 hr after meal, no change in gastric emptying
Callahan HS, et al. Postprandial suppression of plasma ghrelin level is proportional to ingested caloric load but does not predict intermeal interval in humans. J Clin Endocrinol Metab 2004;89:1319	10 healthy subjects	3 weeks	randomized blinded study	The depth and duration of the ghrelin suppression after preload ingestion were directly proportional to the caloric content of the preload. Plasma ghrelin profile was not a critical determinant of the meal request. The relative risk of requesting a meal actually decreased with increasing ghrelin level. absolute ghrelin level is not primarily responsible for meal size. The stimulatory effect of the rise in ghrelin before a meal, may not be critical for the timing of the meal request.

Study Identification	Participants	Duration	Design	Outcomes
Cummings DE, et al. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. N Engl J Med 2002;346:1623	28 humans: 13 obese on wt loss program, 5 after gastric bypass, 10 normal wt controls	6 months	Case control	Plasma ghrelin rose before and fell after meals. A diet-induced wt loss of 17% of initial body wt was assoc w/ 24% increase in area under the curve for 24 hr ghrelin profile. However, w/ gastric bypass, the area under the curve was 77% lower then the normal wt controls, and 72% lower then the obese. The meal related fluctuations and diurnal pattern of ghrelin was absent in the bypass group.
Dornonville de la Cour C, et al. Ghrelin treatment reverses the reduction in weight gain and body fat in gastrectomised mice. Gut 2005; 54:907	young female mice	eight weeks	Animal study	Gastrectomy reduced plasma concentrations of total ghrelin by ~80%. At endpoint, the mean body wt was 15% lower in gastrectomised mice than in sham operated mice; daily ghrelin injections for 8 weeks partially prevented this wt loss. The wt of fat was reduced in gastro. mice; this effect was reversed by ghrelin, enhancing the wt of fat in sham mice. Ghrelin replacement prevented the gastro. induced decrease in lean body mass. In sham mice, ghrelin only increased fat mass.
Tschöp M, et al. Circulating ghrelin levels are decreased in human obesity. Diabetes 2001;50:707	15 Caucasians and 15 Pima Indians	One measurement	Cohort Study	Fasting plasma ghrelin was found to be negatively associated with percent body fat. Plasma ghrelin was decreased in obese whites in comparison with lean whites. Plasma ghrelin was lower in Pima Indians when compared to whites

Study Identification	Participants	Duration	Design	Outcomes
le Roux CW, et al. Postprandial plasma ghrelin is suppressed proportional to meal calorie content in normal-weight but not obese subjects. J Clin Endocrinol Metab 2005; 90: 1068	40 subjects: 20 lean, 20 obese	1 week	Randomized, blinded study	Fasting plasma ghrelin levels were significantly lower in the obese group compared to normal wt group. Reduction in the ghrelin levels of obese after consumption of meals was less than the normal wt group. Obese individuals do not show a calorie-dependant suppression in postprandial circulating ghrelin, but there was a calorie-dependent. suppression in normal-wt people.
English PJ, et al. Food fails to suppress ghrelin levels in obese humans. J Clin Endocrinol Metab 2002; 86:2984	13 lean and 10 obese subjects	1 day	Original research article	Fasting ghrelin was significantly higher in lean than obese subjects, and fell by 39.5% thirty minutes after eating in the lean group before returning rapidly toward baseline. There was no change in circulating ghrelin in the obese group. Obese subjects do not exhibit the decline in plasma ghrelin and leptin seen after a meal in the lean.
Haqq AM, et al. Circulating ghrelin levels are suppressed by meals and octreotide therapy in children with Prader-Willi Syndrome. J Clin Endocrinol Metab 2003;88:3573	15 human subject total; 4 treated with octreotide	1 week	human	Elevated ghrelin levels in children with PWS can be lowered to a normal range using a modest dose of octreotide. Subjects with PWS demonstrate meal-related ghrelin suppression and this suppression is still present, but blunted, after octreotide therapy. In short term, the octreotide did to adversely impact levels of insulin, leptin or parameters of Gh secretion.

Study Identification	Participants	Duration	Design	Outcomes
Van Wymelbeke V, et al. Influence of medium-chain and long-chain triacylglycerols on the control of food intake in men. Am J Clin Nutr 1998; 68: 226	12 healthy adult males	A week	Randomized near cross-over design	Energy intake at the free-choice lunch in session 1 was significantly lower after the MCT breakfast than after the other types of breakfasts. B-hydroxybuterate concentrations were 6x higher after the MCT breakfast until lunch. Neither the MCT nor the LCT-U conditions postponed the first meal after their ingestion, although MCTs enhanced satiation at the next meal.
Feinle C, et al. Fat digestion modulates gastrointestinal sensations induced by gastric distention and duodenal lipid in humans. Gastroenterology 2001;120:1100	15 healthy humans	5 weeks	double-blind, placebo-controlled, crossover randomized study	Increase in gastric volume was higher in LCT emulsion than in the MCT emulsion. Effect of the LCT emulsion was greater than the MCT emulsion. Symptom scores changed most for LCT (fullness, bloating, nausea, pressure; decrease in hunger) The effect of MCT emulsion on sensations was smaller than that of the LCT, reaching stat sig for hunger and nausea. LCT caused an increase in CCK. * The effect of MCT on the reduction of hunger was smaller than the effect of LCT.

Study Identification	Participants	Duration	Design	Outcomes
Van Wymelbeke V, Louis-Sylvestre J, and Fantino M. Substrate oxidation and control of food intake in men after a fat-substitute meal compared with meals supplemented with an isoenergetic load of carbohydrate, long-chain triacylglycerols, or medium-chain triacylglycerols. Am J Clin Nutr 2001; 74:620	10 men	4 sessions	randomized control study	When fed lunches with high CHO, MCT or LCT, the CHO group had increased satiety and delayed time of dinner request; however, food intake of the MCT group was significantly lower then the control or CHO group.
Odle J. New insight into the utilization of medium-chain triglycerides by the neonate: observations from a piglet model. J Nutr 1997; 127:1061	various aged piglets	multiple studies	review	Differences in digestion, absorption and metabolism of MCT and LCT (and their fatty acids MCFA and LCFA) stem mostly from diff in their physical chemistry. MCT are more rapidly digested, and resulting MCFA are more rapidly absorbed via portal circulation, and are predominantly oxidized. LCFAs are re-esterified and packaged into chylomicrons for lymphatic absorption, may be directly deposited into adipose triglyceride stores. * MCT are better utilized then LCT. The first organ profused by MCFA is the liver, whereas LCFA enter systemic circulation via chylomicrons in the thoracic duct and may profuse other tissue prior to the liver.

Study Identification	Participants	Duration	Design	Outcomes
St-Onge MP, et al. Medium chain triglyceride oil consumption as part of a weight loss diet does not lead to an adverse metabolic profile when compared to olive oil. J Am Col Nutr 2008;27:547	31 men and women age 19-50, BMI 27-33	16 weeks	Randomized controlled weight loss program	Weight loss was greater in the MCT consuming group compared to the olive oil group. Participants fulfilling criteria for metabolic syndrome: o.o 6 at start and 5 at end; MCT group 3 at start and 1 at end. This study shows that consuming moderate amounts of MCT does not have adverse effects on CVD risk.
Seaton T, et al. Thermic effect of medium-chain and long-chain triglycerides in man. Am J Clin Nutr 1986; 44:630	7 male humans	several days	randomized control trial	Average oxygen consumption increased more after the MCT meal than after LCT meal. Respiratory quotient gradually decreased after both MCT and LCT consumption. 3-hydroxybuterate conc. were higher after MCT then LCT ingestion. Small increase in insulin conc. after the MCT meal. Average glycerol conc. decreased after MCT but not LCT ingestion. Triglyceride conc. increased after LCT meal but no change after MCT meal. Overall: increase in resting metabolic rate after MCT ingestion, suggest that liver plays role in postprandial thermogenesis. Suggests that replacing LCT with MCT over long run can produce wt loss in absence of reduced energy intake.

Study Identification	Participants	Duration	Design	Outcomes
Baba N, et al. Enhanced thermogenesis and diminished deposition of fat in response to overfeeding with diet containing medium chain triglyceride. Am J Clin Nutr 1982;35:678	15 male Sprague - Dawley rats	6 weeks	Animal study	MCT rats gained 15% less weight then the LCT controls. Total dissectible fat was significantly lower in MCT group, as was mean adipocyte size. Resting oxygen consumption in the MCT fed rats was sig. higher than that of the LCT rats. Excess kcals from MCT led to smaller adipocyte size and to less body fat, then excess kcals from LCT.
St-Onge MP, Bosarge A. Weight-loss diet that includes consumption of medium-chain triacylglycerol oil leads to a greater rate of weight and fat mass loss than does olive oil. Am J Clin Nutr 2008;87:621	49 overweight men and women, 31 subjects completed	16 weeks	Randomized double-blind case control study	Consumption of MCT oil as part of a weight-loss plan improves weight loss compared to olive oil. MCT oil consumption resulted in lower endpoint body wt, greater loss of fat mass and trunk fat mass then consumption of olive oil.
Liu J, et al. Novel ghrelin assays provide evidence for independent regulation of ghrelin acylation and secretion in healthy young men. J Clin Endocrinol Metab 2008; 93: 1980	8 male volunteers	27 hours plus fasting days	Case control	In the fed state, ghrelin and des-acyl ghrelin showed similar dynamics: both sharply inhibited by meals and increased at night. During fasting, ghrelin decreased to nadir levels seen postprandially, and des-acyl ghrelin remained near peak levels seen preprandially. Meals inhibited secretion of both, yet long-term fasting inhibited acylation but not total secretion. Acylation may be regulated independently of secretion by nutrient availability in the gut or by esterases that cleave the acyl group. ** It is important to measure both forms simultaneously.

Study Identification	Participants	Duration	Design	Outcomes
Tong J, et al. The pharmacokinetics of acyl, desacyl and total ghrelin in healthy human subjects. Eur J Endocrinol 2013;13-0072v1(168/6/821-828)	29 healthy humans	4 different days	Randomized, single-blinded study design	Study 1: Infusions of AG at 1, 3, and 5 mcg/kg/h raised plasma conc. of AG to peak concentrations corresponding to 118-, 355- and 594- fold increases from baseline. Study 2: AG infusion increased both plasma AG and DAG concentrations significantly. The infusion of DAG exclusively increased the plasma DAG. Infusion of AG/DAG combined raised both AG and DAG. The AG infusion reversed the DAG:AG ratio. BuChE is the enzyme that is responsible for deacylation in humans, which in this study was not effected by either infusion time or treatment.** Indicates that Ag is deacylated in the plasma. Also suggests that AG is metabolized to DAG in peripheral circulation while there is little acylation of exogenous DAG. Another suggestion is that there is a production of new DAG from Ag breakdown, and DAG elimination was also increased in proportion to load. Ag seems to be extracted from the liver, and DAG appears to go through significant renal clearance. Also Suggested: half-life of DAG is 3x longer than AG.** implication for biological effects: ghrelin isoforms are relatively long-lived in circulation.

Study Identification	Participants	Duration	Design	Outcomes
Cotton JR, et al. Dietary fat and appetite: similarities and differences in th e satiating effect of meals supplemented with either fat or carbohydrate. J Hum Nutr Dietet 2007; 20:186	16 lean healthy male volunteers	5 weeks (5 test days)	Within subject design	Experiment 1: Each meal brought a sharp suppression of hunger which gradually recovered during intermeal intervals. Clear sig difference between CHO and fat supplements, no difference detected between normal and fat conditions. Experiment 2: Subjects were less hungry and had less desire to eat following the high CHO breakfast when compared to the high fat and normal breakfasts. There was a sig diff in intake following the three types of breakfast. Intake following the high CHO breakfast was sig lower than after the high fat or normal breakfast. Intake on high CHO or normal breakfast days were similar, but intake on high fat breakfast days was elevated by about 800 kcal. Conclusions: the weaker action of fat on satiety then CHO or protein appear to be consistent with the measured metabolic response to nutrients and w/ the concept of food balance. Weak effect of fat on satiety indicate how easily a positive fat balance could lead to fat storage. Individualized results, behavioral action

Study Identification	Participants	Duration	Design	Outcomes
Monteleone P, et al. Differential responses of circulating ghrelin to high fat or high-carbohydrate meal in healthy women.J Clin Endocrinol Metab 2003;88:5510	14 healthy women	2 months	within-subject repeated measure design	Mean plasma ghrelin levels significantly dropped after both meals, but the drop after the CHO meal was greater than that observed after the fat meal. Hunger change after the two meals: 120 min after fat meal the hunger ratings had already returned to baseline values, whereas after the CHO meal, they were significantly decreased at the end of the observation period. Plasma ghrelin changes were sig associated with hunger changes (increased hunger correlated with increased ghrelin).
Erdmann J, et al. Postprandial response of ghrelin levels to various test meals in relation to food intake, plasma insulin, and glucose. J Clin Endocrinol Metab 2004;89:3048	14 healthy volunteers	2 weeks	randomized cross-over study	Considering all five test meals, there was no strong relationship between the changes of early postprandial plasma ghrelin levels and hunger and satiety. Increase in ghrelin levels is associated with only a small or moderate increase of plasma insulin, during fat/pro high meals. Greater rise of insulin and glucose after bread ingestion is associated with an inhibition of ghrelin. Inverse relationship between ghrelin and insulin. Fat and protein had a stimulatory and not an inhibitory effect on plasma ghrelin levels.

Study Identification	Participants	Duration	Design	Outcomes
Tentolouris N, et al. Differential effects of high-fat and high-carbohydrate content isoenergetic meals on plasma active ghrelin concentrations in lean and obese women. Horm metab Res 2004;36:559	16 humans: 8 lean, 8 obese	2 days	randomized cross-over study	Lean women had sig higher fasting plasma ghrelin concentrations then their obese counterparts. Plasma active ghrelin conc were negatively and sig associated with BMI, % of body fat,, plasma glucose, plasma insulin, plasma leptin levels, and RMR. After CHO-rich meal, plasma ghrelin declines sig in lean but not obese individuals, while a fat rich meal fails to suppress plasma active ghrelin conc in either lean or obese subjects.