Effects of Consuming Beans Before or With a Meal on Satiety and Gastrointestinal Hormones Concentration in Obese Men and Women

Lisa Delia Griffith

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EFFECTS OF CONSUMING BEANS BEFORE OR WITH A MEAL ON SATIETY AND GASTROINTESTINAL HORMONES CONCENTRATION IN OBESE MEN AND WOMEN

By
Lisa Delia Griffith

A Dissertation in Fulfillment of the Requirements for the Degree of Doctor of Public Health in Nutrition

August 2012
Each person whose signature appears below certifies that this dissertation, in his/her opinion, is adequate in the scope and quality as a dissertation for the degree of Doctor of Public Health.

Ella Haddad, Chair
Associate Professor of Nutrition, DrPH, RD

Joan Sabaté
Professor of Nutrition, MD, DrPH

Serena Tonstad
Associate Professor of Preventive Care, MD, MPH, PhD
ABSTRACT OF THE DISSERTATION

EFFECTS OF CONSUMING BEANS BEFORE OR WITH A MEAL ON SATIETY AND GASTROINTESTINAL HORMONES CONCENTRATION IN OBESE MEN AND WOMEN

by

Lisa Delia Griffith

Doctor of Public Health Candidate in Nutrition

Loma Linda University, Loma Linda University, 2012

Ella Haddad, Chair

Background: Consuming beans and staggering meals may control energy intake. We examined the effect of consuming 0.5 cup of beans 15 minutes before a meal on gastrointestinal (GI) peptides concentrations in obese men and women.

Methods: A randomized crossover design was used to measure GI peptides response to two test meals in 28 healthy obese adults. Subjects consumed a standardized breakfast meal on each test day followed by one of two test meals: a meal incorporating 0.5 cup of beans (control bean meal), and an isocaloric meal (staggered bean meal) in which 0.5 cup of beans was consumed 15 minutes before the meal. Blood samples were obtained prior and at 30, 60, and 120 minutes following the consumption of the test meals and analyzed for unacylated ghrelin, acylated ghrelin, peptide YY, glucagon-like peptide and oxyntomodulin by ELISA. Visual analog scale for feelings of hunger and feelings of fullness were recorded at each blood draw. Dietary recalls were completed using
Nutrition Data System for Research (NDS-R). The area under the curve (AUC) was compared using t-tests.

Results: The AUC\textsubscript{30-120} for postprandial hormone GLP-1 was higher for the control bean meal compared to the control meal (P=0.03). AUC\textsubscript{30-120} for the other postprandial hormones, insulin, glucose and subjective responses showed no statistical difference between the control bean meal and staggered bean meal. Subsequent meal intake was lower after staggered bean meal, however, the difference was not statistically significant.

Conclusion: Incorporating beans and staggering meals may be useful in conjunction with behavioral modification techniques to help with weight control and reduce energy intake.
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CHAPTER 1
INTRODUCTION

A. Statement of the Problem

While obesity levels have remained steady since 2003 (Ogden, Carroll, McDowell, & Flegal, 2007), current levels are associated with substantial morbidity and mortality. According to the Centers for Disease Control, about 34% of adults over age 20 in the United States are obese (Ogden, et al., 2007).

Obesity is associated with higher morbidity and mortality worldwide (Thompson, Cook, Clark, Bardia, & Levine, 2007). Medical risk increases with the increasing degrees of obesity. Class I obesity is defined as having a Body Mass Index (BMI) 25.0 to 29.9 kg/m², Class II obesity BMI, 35.0 to 39.9 kg/m² and Class III or extreme obesity BMI 40 kg/m² (Aronne, 2002). Obesity is associated with diabetes, hypertension, coronary artery disease, certain cancers and reproductive abnormalities (Haslam & James, 2005; Venn & Mann, 2004).

Diets that promote weight loss have been studied, however, there is little scientific evidence that one is more successful than another (Dansinger, Gleason, Griffith, Selker, & Schaefer, 2005; Thompson, et al., 2007). Among diets studied, low carbohydrate and high fiber diets have been promoted in the weight loss arena (Brinkworth, Noakes, Buckley, Keogh, & Clifton, 2009).

Consuming a diet high in fiber is associated with reduced risk of cardiovascular disease, abdominal obesity, hypertension, diabetes, obesity, and certain gastrointestinal disorders. In addition, a high fiber diet promotes regularity, aids in weight loss and improves immune function (Anderson, et al., 2009; Katcher, et al., 2008; Trowell &
Dietary fiber consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants (Slavin, 2005). It has been suggested that diets rich in fiber help decrease body weight and fat, ameliorate feelings of hunger, delay gastric emptying, and decrease food intake. A diet high in natural fiber may displace unhealthy foods with healthier alternatives such as fruits and vegetables. A recent review found that increased dietary fiber can aid in the fight against the obesity epidemic (Slavin, 2005).

Typically, studies of fiber supplements ask subjects to ingest the supplements usually 10-15 minutes before a meal (Hylander & Rossner, 1983; Rigaud, Ryttig, Angel, & Apfelbaum, 1990; Salas-Salvado et al., 2008). In a well designed randomized study, subjects who consumed a high fiber cereal containing 33g of insoluble fiber 75 minutes before a meal experienced subsequent reduced appetite, food intake and glycemic response (Samra & Anderson, 2007). To our knowledge, trials have not compared the effects of fiber supplements or foods rich in fiber ingested before a meal versus fiber supplements or foods rich in fiber ingested as part of a meal. Time periods of 60-75 minutes between digestion of fiber and consumption of the usual meal may not be practicable in real life.

Very few foods contain the variety of nutrients of beans, which are a significant source of fiber, protein, and a host of vitamins and minerals (Mitchell, Lawrence, Hartman, & Curran, 2009; Wong, Mollard, Zafar, Luhovyy, & Anderson, 2009). Studies show that increased consumption of legumes results in increased fiber consumption, which in turn leads to higher satiety (Abete, Parra, & Martinez, 2009). Other studies show that consumption of beans is protective against cardiovascular disease and diabetes (Mitchell, et al., 2009). The US Department of Agriculture (USDA) recommends the
consumption of legumes of 2.5 to 3.5 cups per week. Consuming ½ cup beans may be counted as a 2 oz-equivalent from the meat and bean group on the food pyramid, providing a source of complex carbohydrate and soluble fiber (Mitchell et al., 2009).

Adipose tissue mass is controlled by a series of signals from the tissue and hypothalamic receptors. It is a change in these processes that results in an increased or decreased weight (Jequier & Tappy, 1999). In the last decade, researchers have shown interest in studying the relationship of gastrointestinal (GI) hormones on energy intake, appetite, satiety and weight loss (O. B. Chaudhri, Wynne, & Bloom, 2008; Willbond & Doucet, 2011).

Ghrelin levels are increased during fasting, before meals and at night suppressed by food intake, especially a high calorie or high carbohydrate diet. Peptide YY and oxyntomodulin are also implicated in suppressing ghrelin (Tritos & Kokkotou, 2006). It is the only known GI peptide that stimulates increased food intake (Dostalova & Haluzik, 2009). Total and active fasting ghrelin levels are decreased in obese people.

Another gastric peptide, peptide YY3-36, inhibits food intake by signaling satiety and suppressing the appetite, delaying gastric emptying and gastrointestinal transit (Essah, Levy, Sistrun, Kelly, & Nestler, 2007; le Roux & Bloom, 2005). A dose-dependent reduction in appetite and food intake response was observed in a recent study (O. B. Chaudhri, et al., 2008).

Oxyntomodulin (OXM) is co-secreted with Glucagon-like-peptide-1 amide (GLP-1) and PYY3-36 after ingesting nutrients. It signals satiety, has incretin effects and reduces food intake (O. B. Chaudhri et al., 2008; Druce, Small, & Bloom, 2004). Studies
demonstrated that OXM may be beneficial in weight loss and reduction in appetite may be maintained (Murphy, Dhillo, & Bloom, 2006).

GLP-1 is produced during the processing of the gene encoding preproglucagon in the gut and the brain (Small & Bloom, 2004), and released from the intestinal L cells in response to food (Wynne & Bloom, 2006). GLP-1 stimulates the release of insulin and decreases gastric emptying, which leads to lower food intake (Small & Bloom, 2004).

Gastrointestinal hormones may represent a novel means of fighting obesity since they regulate energy homeostasis as well serve as a signal system that could provide a pathway for anti-obesity treatments (Murphy et al., 2006).

Despite research on many popular diets, there is little known about the effects of beans on as a whole food on satiety and gastrointestinal peptides. According to Ludwig, et al. (1999), long term high fiber interventional studies are needed to determine the effects of bean consumption on obesity.

B. Purpose of the Study

Many studies have concluded with a recommendation to increase fiber in the diet in order to decrease obesity by reducing appetite and increasing satiety. However, to date we are unaware of studies that have investigated the consumption of a single food, such as beans, which has been done for many years for the treatment of diabetes and promotion of weight loss at Lifestyle Center of America versus consuming the same food within the meal. In order to understand whether a single food may influence satiety and gastrointestinal hormones, we will conduct a single meal study utilizing beans. The premise is that in the long term, society would be more aware of foods that would increase satiety and eventually lead to weight reduction.
C. Research Questions

What is the effect of a single food meal of beans on gastrointestinal peptides, ghrelin, peptide YY, oxyntomodulin and glucose-like protein-1 when the meal is consumed at once versus consuming beans 15 minutes before the rest of the meal?

D. Theoretical Justification

Satiation is the process of satisfying the appetite during the course of eating, which eventually signals the completion of the meal. Satiety is the state in which an individual feels full after eating (Isaksson, Sundberg, Aman, Fredriksson, & Olsson, 2008; Slavin, 2005). Preloading or eating a small meal before a meal is known to induce satiety and reduce the subsequent meal. Beans have the desirable quality of providing satiety by delaying gastric emptying and leading to lower food intake at the next meal. The assumption is that due to the characteristics of beans, the satiety hormones GLP-1, PYY, and oxyntomodulin will be released and their effects prolonged, leading to lesser feelings of hunger and lower caloric consumption at the following meal when beans are consumed 15 minutes prior to the lunch meal.

E. Significance to Nutrition

Obesity is an epidemic that is affecting 34% of adults in United States (Ogden, et al., 2007). Obesity is significantly linked to Type II diabetes, hypertension and CVD, the most common cause of premature mortality among the obese population (N. M. Neary, Goldstone, & Bloom, 2004). In light of this growing problem, studying and understanding the effects of a bean pre-load in increasing satiety and decreasing obesity will be beneficial for the field of nutrition and public health as a whole. Consuming a high fiber diet has been recommended for weight loss, however, the effects of eating bean
before meals on gastrointestinal hormones that delay gastric emptying, inhibit food intake and promote satiety, subsequently leading to weight loss, has not been studied. The current study will help determine whether nutrition professionals, working with weight loss patients, should promote the consumption of high fiber foods, such as beans, before a meal for reduction of obesity. Reducing obesity will in turn lead to a reduction in chronic and acute health problems associated with obesity.
CHAPTER 2
REVIEW OF THE LITERATURE

A. Overview

Obesity is the result of an imbalance between energy intake and energy out take. The energy density of food, its total energy content and the number of times an individual eats affect energy intake and thus, weight (Martins, Truby, & Morgan, 2007; Solomon, Chambers, Jeukendrup, Toogood, & Blannin, 2008). The problem of obesity is reaching epidemic proportions worldwide (Martins, et al., 2007; McCrory, Hamaker, Lovejoy, & Eichelsdoerfer, 2010).

According to data from the World Health Organization, approximately 1.6 billion adults are overweight and 400 million are obese. It is estimated that by 2015, approximately 2.3 billion adults will be overweight and 700 million will be obese if the problem continues to grow at the present rate (Kastorini, Milionis, Goudevenos, & Panagiotakos, 2010). Obesity is linked to chronic problems such as type 2 diabetes, heart disease, and some cancers (Field, et al., 2010; Kastorini, et al., 2010; McCrory, et al., 2010).

While diet and exercise remain the main areas for treatment of obesity (Abete, Goyenechea, Zulet, & Martinez, 2011), there is a growing interest in finding new strategies to combat the problem. In the early 1980s, researchers looked into the effects of fiber on body composition. It was noted that populations that consumed high amounts of fiber tended to have lower body weight (Hylander & Rossner, 1983). In recent years more research has focused on the health benefits of fiber and on its role in modulating dietary intake and satiety (Abete, et al., 2011; Olendzki, et al., 2009).
B. Dietary Fiber and Body Weight

Dietary fiber is the undigestible part of the plant which is not hydrolyzed by enzymes of the upper intestines and includes non-starch polysaccharides, lignin, resistant oligosaccharides, and resistant starches (Kaline, Bornstein, Bergmann, Hauner, & Schwarz, 2007; Venn & Mann, 2004). Non-starch polysaccharides are classified as soluble and insoluble fiber (Venn & Mann, 2004). Fiber’s qualities include its ability to reduce blood glucose, induce satiety, reduce body weight, and decrease mortality due to cardiovascular disease, (Katcher, et al., 2008; Venn & Mann, 2004), as well as lower serum cholesterol and support laxation (Kaline, et al., 2007). A reduced risk of diabetes also is seen in the consumption of fiber-rich whole grain and cereal fiber (Kaline, et al., 2007).

Early epidemiological research showed that high fiber intake, particularly insoluble fiber, is protective against cardiovascular disease (CVD) and diabetes (Gruendel, et al., 2007). More recent research has shown that high fiber intake is positively associated with a lower body mass index (BMI), not smoking, greater physical activity, lower saturated fat intake, and lower alcohol intake (Kaline et al., 2007). In the Seven Countries Study, dietary fiber was significantly correlated with subscapular skinfold thickness (Slavin, 2005). In a longitudinal study, fiber intake was inversely associated with BMI at all levels of fat intake after adjusting for lifestyle factors and other confounding factors (Slavin, 2005). Obese men and women have significantly lower fiber intakes when compared to their lean counterparts (Slavin, 2005). In the Nurses’ Health Study, weight gain was positively correlated with refined grain foods, but inversely associated with high fiber and whole grain foods (Slavin, 2005).
In a quest to determine whether consuming a high fiber diet versus a low fiber diet helped control weight, Williams, et al. (2008) completed a meta-analysis of epidemiological and interventional research studies and concluded that there is a direct link between diets high in whole grain and lower body mass index, waist circumferences and body weight, and in weight reduction and weight loss (Williams, Grafenauer, & O'Shea, 2008). Jenkins, et al. (2008) concluded from a study that looked at the consumption of plant based diet and hypertension that it was beneficial to consume a diet high in fiber due to the fact that such a diet reduces serum lipids and blood pressure (Jenkins, et al., 2008).

It is proposed that fiber acts as a physiologic obstacle to energy intake by at least three mechanisms. The first mechanism is the displacement of available calories and nutrients from the diet, by both increasing satiety and decreasing absorption efficiency. A diet high in fiber has a lower energy density than one that is high in fat, therefore increasing satiety. The bulking and viscosity properties of dietary fiber are chiefly responsible for influencing satiation and satiety (Crujeiras, Parra, Abete, & Martinez, 2007; Slavin, 2005). The second mechanism is increased chewing, which limits intake by promoting the secretion of saliva and gastric juice, resulting in an expansion of the stomach, decreased consumption, and increased satiety (Kaline et al., 2007; Slavin, 2005). The third mechanism relates to fiber’s ability to decrease the absorption efficiency of the small intestine (Slavin, 2005).

There is evidence that fiber consumption promotes satiety due to its ability to slow gastric emptying and macronutrient absorption from the gut (St-Pierre, et al., 2009).
A high fiber diet that consists of high proportion of legumes, whole grain cereals, fruits and vegetables slows digestion and nutrient absorption (Kaline, et al., 2007).

In 2000, the American Diabetes Association (ADA) recommended that diabetics consume 20-35 g of fiber daily due to the cholesterol lowering properties in soluble fiber (Chandalia, et al., 2000). Despite this, Western diets are low fiber, with the United States intake averaging approximately 17 g daily, according to the Third National Health and Nutrition Examination Survey (NHANES). Venn and Mann (2004) in a review of existing research on cereal grains and legumes, reported that a diet high in cereal and legumes produces a lower and delayed elevation of postprandial carbohydrate oxidation as well as a reduction in hunger when compared to a diet high in refined carbohydrates (Venn & Mann, 2004). Crujeiras, et al. (2007) also found that a diet high in fiber, particularly legumes, resulted in significant weight-loss compared to participants on the control diet.

Despite the documented benefits of consuming fiber rich foods, Bachman, et al. (2008) found that in the US population during the time period 2001-2002, only 10% of all grains consumed were from whole grains (Bachman, Reedy, Subar, & Krebs-Smith, 2008).

C. **Legumes in the diet**

Sources of fiber include whole grains, cereals, fruits, vegetables and legumes (Retelny, Neuendorf, & Roth, 2008). The 1999-2000 NHANES data shows that adults in the US are consuming less than a third of the recommended daily legume intake. Legumes include dry beans such as pinto beans, kidney beans, garbanzo beans, split peas, lentils, and soybeans (Duranti, 2006; Mitchell, et al., 2009). Legumes are a valuable
component of a healthy diet due to their low fat content, and high levels of vegetable protein, micronutrients and dietary fiber (Duranti, 2006; Kaline, et al., 2007). Dried beans and peas contain a variety of nutrients unlike any other foods, and are a significant source of protein, minerals, vitamins, and fiber (Flight & Clifton, 2006; Mitchell, et al., 2009). Legumes are said to contribute to a balanced diet and legume containing diets are thought to prevent diseases such as type 2 diabetes, coronary heart disease, and digestive tract diseases, as well as obesity (Duranti, 2006).

Current recommendations from the US Department of Agriculture (USDA) for fruits and vegetables doubled compared to previous serving recommendations; recommendations for specific vegetable subgroups, including legumes, target fiber and other important nutrients such as folate, iron, calcium, magnesium and potassium. The USDA recommends an intake of 2 ½ to 3 ½ cups legumes weekly (Mitchell et al., 2009).

Legumes have been studied in relation to oxidative stress (Crujeiras, et al., 2007), metabolic syndrome and proinflammatory markers (Hermsdorff, Zulet, Abete, & Martinez, 2011), total cholesterol and LDL-cholesterol (Bourdon, et al., 2001; Pittaway, Ahuja, Robertson, & Ball, 2007), diabetes and insulin resistance (Pittaway, et al., 2007; Shaheen & Fleming, 1987; Steyn, et al., 2004; Venn & Mann, 2004; Zhang, et al., 2010), cardiovascular disease (Abete, et al., 2011; Kan, et al., 2007), cancer prevention (Murthy, Mukherjee, Ray, & Ray, 2009; Schatzkin, et al., 2007), nutrient deficiency prevalence (Trinidad, Mallillin, Loyola, Sagum, & Encabo, 2010) and weight regulation (Howarth, Saltzman, & Roberts, 2001). Recently there has been increasing interest in the mechanisms underlying the relationship between legumes in the diet and weight control, specifically, do fiber-rich legumes produce similar effects on gastrointestinal hormones?
D. Role of Gastrointestinal Hormones in Satiety

There is prominent evidence to demonstrate the reaction of a gut system that recognizes the presence of food in the gastrointestinal tract and signals the brain via neural and endocrine mechanisms to regulate short-term appetite and satiety (le Roux & Bloom, 2005; Murphy, et al., 2006).

During the 1970s, more evidence clearly showed that gut hormones signaled the central nervous system in profound and at times less noticeable ways (Murphy, et al., 2006). The hypothalamus and the brain stem are thought to be the most important central nervous system target centers for these peripheral signals (le Roux & Bloom, 2005; Murphy, et al., 2006), with the hypothalamus interpreting neural and humoral inputs and connecting the information to establish homeostasis. Short-term signals that involve the gut hormones and neural signals from the higher brain centers and the gut regulate the start and termination of a meal. Long-term signals that affect the body’s energy stores, endocrine status, and general health are humoral inputs. Therefore, gut hormones play an important physiological role in postprandial satiety (Murphy, et al., 2006).

Appetite and food intake is thought to be controlled by two negative feedback mechanisms. The first mechanism is through mechanical stimuli, as gastrointestinal hormones control the size of a meal by inducing satiation. Cholecystokinin (CCK) which is found in the duodenum, and glucagon-like-peptide-1 (GLP-1) and peptide YY (PYY), hormones which are found in the lower intestine, also induce satiation. These hormones along with ghrelin communicate with the hindbrain via the afferent vagus. The second mechanism is the change in body fat through adipocyte hormone leptin and pancreatic
insulin. The absence of leptin in humans leads to significant increase in eating and morbid obesity (Borer, Wuorinen, Ku, & Burant, 2009).

1. Ghrelin

Ghrelin, also known as the “hunger hormone” or appetite-stimulating gut hormone, consists of 28 amino acids and chiefly comes from the stomach, but may be found in other peripheral cells of the gastrointestinal tract, pancreas, ovary and adrenal cortex (Barazzoni, et al., 2007; O. Chaudhri, Small, & Bloom, 2006; Klok, Jakobsdottir, & Drent, 2007; Murphy, et al., 2006). It is formed by the cleaving of pre-proghrelin, and is released from the X/A-like cells of the gastric oxyntic glands, with smaller levels expressed in the small intestines and the hypothalamus (Neary & Batterham, 2009a). Ghrelin functions include stimulation of the anterior pituitary for production of growth hormone, stimulation of the hypothalamic-pituitary-adrenal axis, increase of gastric motility, and induction of positive inotrophic effects on the heart and vasodilatation (Chaudhri, et al., 2006). Ghrelin also plays a physiological role in the regulation of food intake, with characteristically high levels of ghrelin before a meal and a decrease after the meal (Barazzoni, et al., 2007; Klok, et al., 2007; Murphy, et al., 2006; St-Pierre, et al., 2009). However, in obese humans, ghrelin levels are low and the postprandial levels are even lower or nonexistent (St-Pierre, et al., 2009). Lower ghrelin levels are found in humans with higher body weight and rise after a diet-induced weight loss. The customary drop in plasma ghrelin is absent or lessened in obese humans, indicating that ghrelin may possibly play an important role in obesity (Barazzoni, et al., 2007; Klok, et al., 2007; Murphy, et al., 2006; Neary, et al., 2004). Previous studies have shown that the increased preprandial ghrelin levels correspond with hunger scores in healthy humans initiating
meals freely in the absence of time and food related cues (Klok, et al., 2007). The level of plasma ghrelin in gastric bypass patients is said to decrease by 76% after surgical intervention compared to their healthy counterparts. These levels gradually return close to normal, supporting the case that the body utilizes ghrelin from other sources in the body (Castaneda, Tong, Datta, Culler, & Tschop).

Ghrelin exists in circulation in both acylated and un-acylated form. Acylated ghrelin is the biologically active hormone associated with orexigenic properties, energy balance and growth hormone secretion, whereas unacylated ghrelin has been linked to anti-diabetogenic functions (Goodyear, Arasaradnam, Quraishi, Mottershead, & Nwokolo; Klok, et al., 2007; Murphy, et al., 2006; St-Pierre, et al., 2009). Unacylated ghrelin, sometimes referred to as desacyl-ghrelin, is the major molecular form that is secreted into circulation, accounting for as much as 80-98% of total ghrelin in circulation (Chen, Asakawa, Fujimiya, Lee, & Inui, 2009; Inhoff, Wiedenmann, Klapp, Monnikes, & Kobelt, 2009). Unacylated ghrelin is a 27-amino acid peptide that is produced in the gastric mucosa and in plasma (Inhoff, et al., 2009) by alternatively splicing the ghrelin gene (Chen, et al., 2009). The physiological role of unacylated ghrelin was previously unclear (Perboni & Inui, 2009; Stengel, Goebel, Wang, & Tache, 2010), but it is now believed to play an active role in the intake of food, gut motility, insulin secretion and resistance, as well as body size development, adipogenesis, and cell proliferation and survival (Chen, et al., 2009). Studies have shown that unacylated ghrelin promotes a state of negative energy balance. Another study supports the idea that unacylated ghrelin stimulates feeding through a mechanism that does not use the GHS-R receptor (Perboni & Inui, 2009; Stengel, et al., 2010).
Acylated ghrelin is the endogenous ligand for the growth hormone secretagogue receptor (GHS-R) (Murphy, et al., 2006; Perboni & Inui, 2009). The acyl group increases the hydrophobicity of the peptide, increasing its ability to bind with plasma proteins (Perboni & Inui). Acylated ghrelin crosses the blood brain barrier in both directions (Perboni & Inui), however, unacylated ghrelin does not, due to the lack of the fatty acid group (Chen, et al., 2009; Inhoff et al., 2009; Stengel et al., 2010). Acylated ghrelin plays an important role in the short-term regulation of appetite, determining food intake from meal to meal as well as long-term regulation of energy balance. It also controls glucose homeostasis, gastric motility, and secretion of acid (Perboni & Inui, 2009).

According to Klok, et al. (2007), there are three proposed pathways for which ghrelin affects the intake of food. First, ghrelin may cross the blood-brain barrier and bind to the receptors in the hypothalamus after being released by the stomach into the blood stream (Klok, et al., 2007). Second, ghrelin signals may travel to the brain through the vagal nerve and nucleus tractus solitarus to mediate its orexigenic effects (Klok, et al., 2007; Murphy, et al., 2006). Third, ghrelin is produced in the hypothalamus and may directly affect the hypothalamic nuclei (Klok, et al., 2007). According to Yin, et al., the preprandial increase in ghrelin may be due to the expectation of a meal, with the central nervous system sending out a message to the efferent fiber of vagus nerve that is transmitted to the stomach (Yin, Li, Xu, An, & Zhang, 2009).

Ghrelin produces a leptin-induced reduction in food intake and body weight by regulating the expression of various hypothalamic peptides (Klok, et al., 2007; Neary, et al., 2004). Leptin activates the polypeptide pro-opiomelanocortin (POMC), which promotes the release of two anorexigenic peptides, α-MSH and CART, and inhibits
neuropeptide Y (NPY) and agouti-related protein (AgRP). This inhibition prevents the release of γ-aminobutyric, which leads to the activation of POMC (De Vriese & Delporte, 2007). It has been found that ghrelin stimulates neurons that express neuropeptide Y (NPY), agouti-related protein (AgRP) and orexin, and inhibits pro-opiomelanocortin (POMC) and corticotrophin-releasing hormone neurons, thus leading to a reduction in appetite (De Vriese & Delporte, 2007; Klok, et al., 2007).

2. Glucagon-like Peptide-1

Glucagon-like peptide-1 (GLP-1) is a gastrointestinal hormone that is released as a result of food intake from the distal small intestine (Beglinger & Degen, 2006) along with PYY and oxyntomodulin (OXM) (Jayasena & Bloom, 2008). It is 36- or 37-amino acid molecule cleaved from a 160-amino acid preproglucagon prohormone with a 20-amino acid signal sequence at the N-terminal end (Chaudhri, et al., 2006). GLP-1's primary roles are to stimulate the secretion of insulin, inhibit the production of glucagon, stimulate an increase in pancreatic β-cell mass and decrease the rate of gastric emptying (Banasch, et al., 2006; Chaudhri, et al., 2006).

GLP-1 exists in two different forms, however it is the GLP-17-36 amide form that is most important for its strong incretin effect (Chaudhri, et al., 2006; Degen, et al., 2006). GLP-1 accounts for approximately fifty percent of the incretin affect in the body (Forbes, et al., 2005). GLP-1 is also considered part of the ileal brake mechanism which inhibits the upper gastrointestinal digestive functions and affects appetite and food intake (Chaudhri, et al., 2006; Degen, et al., 2006; Jayasena & Bloom, 2008) and functions as a neurotransmitter in the central nervous system (Chaudhri, et al., 2006). Previous research has demonstrated the presence of GLP-1 receptors in parts of the brain that contribute to
the controlling of the appetite (Chaudhri, et al., 2006). Past studies have suggested that
GLP-1 is a physiological satiety factor that suppresses food intake when infused
intravenously, even in low concentrations. (Gutzwiller, Degen, Heuss, & Beglinger,
2004).

GLP-1 levels increase in two phases approximately 10 minutes after eating, with a
peak occurring at around 30 minutes. Levels will remain elevated for several hours after a
meal. Studies have shown that a slow digesting protein will elicit a slower GLP-1
response than a fast digesting protein (Neary & Batterham, 2009a); carbohydrates have a
variable effect on stimulating the release, which may be influenced by the fiber content
(Karhunen, Juvonen, Huotari, Purhonen, & Herzig, 2008).

3. Peptide Tyrosine-Tyrosine

Peptide tyrosine-tyrosine YY (PYY) is a 36-amino acid peptide that was
first isolated and characterized in 1980. PYY is found along the human small intestine, a
tissue concentration that increases distally, with the highest levels in the colon and rectum
(le Roux & Bloom, 2005; Murphy, et al., 2006; Suzuki, Simpson, Minnion, Shillito, &
Bloom, 2010). PYY is an anorexigenic hormone (Lomenick, Clasey, & Anderson, 2008;
Wynne & Bloom, 2006) and is co-secreted from the L-cells in the gastrointestinal gut
with OXM after a meal (Chaudhri, et al., 2006; Wynne & Bloom, 2006). It is released
after a meal in response to caloric intake and meal composition (Chaudhri, et al., 2006;
Gardiner, Jayasena, & Bloom, 2008; Wynne & Bloom, 2006) as well as considered part
of the ileal and colonic break mechanism since it inhibits upper gastrointestinal function
(Witte, et al., 2009; Wynne & Bloom, 2006).
PYY is found in two forms; PYY_{1-36} and PYY_{3-36}. The major form of PYY is stored in the gut and found in circulation is the N-terminally shortened PYY_{3-36}, the result of cleaving tyrosine and proline from PYY_{1-36} by dipeptidyl peptidase IV. PYY_{3-36} is said to be the biologically active satiety signal (Neary & Batterham, 2009a; Wynne & Bloom, 2006). PYY is released before nutrients interact with the L cells of the distal intestine. This could imply that the initial release of PYY may be the result of a neural reflex (Wynne & Bloom, 2006).

PYY properties include gastric emptying delay, increase satiety and reduced gastric secretion (Gardiner, et al., 2008; Murphy, et al., 2006; Ratliff, et al., 2010). PYY also inhibits gallbladder emptying (Chaudhri, et al., 2006). Although PYY may reduce postprandial intake of food, the more important effect may be the regulation of the size or timing of the following meal (Murphy et al., 2006; Ratliff et al., 2010).

There is a possible link between meal termination and PYY since while PYY levels are decreased after 2-3 days of fasting, they increase after consuming a meal and satiety is induced when PYY is injected peripherally (El Khoury, El-Rassi, Azar, & Hwalla). Plasma OXM and PYY interact with hypothalamic appetite processes and brainstem areas in order to induce satiety (Wynne & Bloom, 2006). A double-blinded crossover study showed a 30% reduction in food intake for participants in whom PYY was administered peripherally (Neary, et al., 2004).

Circulating PYY levels are lower in obese humans than lean ones; overweight human subjects have an associated deficiency of postprandial PYY with reduced satiety (Chaudhri, et al., 2006; Murphy, et al., 2006). During the fasting state, circulating PYY concentrations are low and quickly increase following a meal, peaking at 1-2 hours and
remaining elevated for several hours after a meal. Some studies have found low levels of circulating PYY in obese subjects and high levels in anorexia nervosa patients (Suzuki, et al., 2010). Presence of intraluminal bile acids, gastric acid and CCK also causes release of PYY (Chaudhri, et al., 2006). Exercise and stress are also noted to increase PYY levels (Neary & Batterham, 2009a).

PYY concentrations are noticeable approximately 15 minutes after the consumption of a meal, reaching a peak level at 1 to 2 hours. Levels remain high for several hours afterwards and are dependent on caloric intake, consistency and meal make up of the meal (Neary & Batterham, 2009a).

In a double blind randomized controlled study in which four men and six women were enrolled, it was concluded that when PYY is infused alone, it reduces meal intake by 15 percent. The reduction of energy intake increased to 27% when PYY is combined with GLP-1 before administration (N. M. Neary et al., 2005). It is for this reason that PYY administration is considered an avenue for the fight against obesity.

There are two possible pathways by which PYY may exhibit its anorectic effects. It may directly act through an incomplete blood brain barrier in the median eminence of the hypothalamus, or via vagal-brainstem-hypothalamic stimulation or both (Suzuki, et al., 2010). Other hormones such as gastrin and CCK are thought to be mediators in the release of PYY prior to the passage of the food to the distal gut (Gardiner, et al., 2008).

4. **Oxyntomodulin**

Oxyntomodulin (OXM) is a by-product of the preproglucagon gene that is released from the L-cells in the intestines into circulation after a meal. OXM reduces food intake, inhibits gastric acid secretions, and may also increase energy expenditure.
OXM is made up of 37 amino acids and has the 29 amino acid structure of glucagon with an octapeptide C-terminal extension (Neary & Batterham, 2009a; Wynne & Bloom, 2006). OXM is secreted into blood circulation with GLP-1 and PYY (Chaudhri, et al., 2008). Five to ten minutes after a meal, OXM is released, with levels peaking at 30 minutes in response to caloric intake levels remain elevated for several hours (Neary & Batterham, 2009a; Wynne & Bloom, 2006). The hormone also has a daily change with levels highest at night and lowest in the early mornings (Neary & Batterham, 2009a; Wynne & Bloom, 2006).

OXM and GLP-1 utilize the same receptors, despite the biological difference between the two hormones. Studies have shown that OXM has a less potent incretin effect than GLP-1 and works through different CNS pathways. However, it has a stronger effect on food intake than GLP-1 (Chaudhri et al., 2008; Neary & Batterham, 2009a).

Reduced food intake has been noted in studies done by Cohen et al., (2003) and Wynne et al., (2006), when OXM was administered subcutaneously (Neary & Batterham, 2009a). Elevated plasma levels in weight loss illness provides a possible connection to OXM and appetite control (Chaudhri, et al., 2006). There is a possible link between weight loss secondary to gastric bypass and elevated levels of OXM (Chaudhri, et al., 2006; Gardiner, et al., 2008).

Lean volunteers reduced their intake by 19.3% at a buffet after the infusion of OXM. It is speculated that the mechanism by which OXM reduces appetite may be through the suppression of ghrelin, the orexigenic hormones (Chaudhri, et al., 2006).
E. Dietary Factors and Satiety

Satiety refers to the factors that control eating during the postprandial period, in contrast to satiation, which involves factors leading to meal termination (Rolls, 2009). Satiety is influenced by the macronutrients in food, which stimulate the release of gastrointestinal hormones leading to satiation and by the size of the meal (Almiron-Roig, Grathwohl, Green, & Erkner, 2009; Borer, et al., 2009). It is also influenced by the sensory and physical attributes of food and previous experience with the food (Rolls, 2009). Dietary fat has been associated with the increase release of cholecystokinin, a satiety hormone. The viscosity properties of fiber are positively related to its effect on satiety (Almiron-Roig, et al., 2009; Karhunen, et al., 2010). Some studies have concluded that protein has the highest satiating effect due to the altered production of ghrelin, GLP-1 and PYY (Karhunen, et al., 2010). The satiation power of carbohydrate foods is linked to the glycemic index of the food; thus low glycemic food leads to lower food intake (Johnson, Thomas, & Hall, 2005).

Satiety has been studied using visual analog scales to rate preload and post-preload feelings of fullness (VAS) (Rolls, 2009). VAS scales have been used by numerous researchers to point to the sensory and perceptual dimensions of satiety, hunger, fullness and desire to eat (Cardello, Schutz, Lesher, & Merrill, 2005; Stubbs, et al., 2001). VAS scores have been shown to predict the intake of the subsequent meal (Cardello, et al., 2005; Parker, et al., 2004). They avoid the measurement problems of unequal intervals and the central tendency or regression effect that comes from the under use of the end categories, normally associated with simple category scales of satiety (Cardello, et al., 2005). A VAS consists of a question followed by a horizontal line with
extreme descriptions at each end to anchor the line (Flint, Raben, Blundell, & Astrup, 2000; Zabel, Ash, King, & Bauer, 2009). The lines are designed to capture the state of the participant at the time and the questions are repeated over a time period (Zabel, et al., 2009). Participants are asked to make a mark along the line to answer the questions (Flint, et al., 2000; Stubbs, et al., 2000).

1. **Studies on the Effect of Fiber in the Diet on Satiety and Weight**

There has been interest in the role of fiber in promoting satiety based on its contribution to food volume and ability to slow absorption. The aim of Delargy, et al. (1997) was to compare the effects of a light, a low fiber, a high insoluble fiber and a high soluble fiber breakfast on energy intake, hunger motivation, and gastrointestinal sensations over the course of the day, in a randomized cross over design study. The low fiber cereal contained 3 grams of fiber, the high insoluble fiber contained 22 g of fiber with wheat bran as the insoluble fiber and the high soluble fiber cereal contained 22 g of fiber with psyllium as the soluble fiber (Delargy, O'Sullivan, Fletcher, & Blundell, 1997).

An *ad libitum* test snack was provided one and half hours following breakfast. Participants were given a fixed energy meal for lunch. No other foods were consumed between meals. Food boxes that contained a variety of foods were provided for the remaining meals. These items were to be consumed *ad libitum* as wanted by the participants. Food items were recorded and visual analog scales used to assess hunger, desire to eat, fullness and prospective consumption were completed prior to breakfast, after breakfast, an hour after breakfast, before and after lunch and every two hours for the remainder of the day (Delargy, et al., 1997).
There were no significant differences in hunger ratings after the high insoluble and high soluble fiber breakfast, but there was a greater hunger score after the high soluble fiber compared to the high insoluble fiber breakfast. Participants rated themselves significantly less hungry after the low fiber breakfast than after the high insoluble fiber one. The hunger rating remained lower at the pre-snacking rating. Compared to the low, high soluble and high insoluble cereals, participants rated themselves significantly hungrier after the light breakfast (p<0.01). Participants ate significantly less of the test snack after the high insoluble fiber breakfast than after the high soluble fiber breakfast (p<0.001). Participants ate more of the test snack after the light breakfast than after the low fiber (p<0.01) and high insoluble fiber breakfast (p<0.001). The study found evidence that eating a breakfast high in soluble fiber did not reduce the motivation to eat again in 1 ½ to 2 hours as effectively as a high insoluble fiber breakfast (Delargy, et al., 1997).

In a study conducted at Hospital Bichat in France, 52 overweight patients were randomly assigned either a fiber supplement or placebo along with a hypocaloric diet for the 6-month study period. Participants were given routine medical examination which included anthropometrics and blood pressure (BP) measurements. During a 2-week run-in period, daily habitual energy intake was calculated using 7-day recall method. The dietary fiber supplements or placebos were taken 3 times daily with 120 ml of water 20-30 minutes before meals. The fiber supplements provided an additional seven grams of fiber, while the placebo provided one gram of fiber. The participants were given the visual analogue scale (VAS) for evaluating hunger feelings immediately before the three main meals during the last week of the run-in period, and each month during the
treatment period. Adherence to the treatment was verified each month by counting the remaining tablets. Weight, BP and treatment effects were recorded at the visits (Rigaud, et al., 1990).

Although both groups lost weight, the weight reduction in the fiber treated group was 5.5±0.7 kg, significantly higher than that of the placebo group: 3.0±0.5 kg (P=.005). The greater weight reduction was evident throughout the entire trial period. Feelings of hunger were initially lower in both groups, however 2 months into the study, the placebo group’s hunger feelings were significantly higher (p-value <0.02) than the fiber group. Even though the feelings of hunger among the fiber group increased, they remained significantly lower (p-value < 0.0008) compared to the initial value. Mean energy intake at the end of the trial was 8354± 462 kJ in the fiber group, compared to 9169 ± 9169 ± 647 kJ in the placebo group (p-value > 0.1) so the difference between two groups was not significant (Rigaud, et al., 1990).

Conclusions drawn from the study include the fact that despite the lack of significance in energy intake between the 2 groups, reductions in weight and hunger feelings were higher in the fiber treated group. The participants in the fiber group also adhered better to the diet than those in the placebo group (Rigaud, et al., 1990).

The above studies suggest that eating a breakfast rich in insoluble fiber or taking fiber supplements given as part of a hypocaloric diet may inhibit food intake and contribute to weight reduction.

2. **Studies on the Effect of Preloads on Hunger and Satiety**

Studies have also examined the effect of various preloads on hunger and satiety. Hylander and Rössner reported in 1983 on a study that examined the effects of
dietary fiber intake before meals on weight loss and hunger in a weight reducing club. The study’s participants were members of a commercial weight reducing group called “The Slim Club” in Stockholm, Sweden. Slim Club members attended weekly group sessions for weighing, food preparation instructions, nutrition education, and support for 8 weeks. Members were given booklets with recipes and food items and amounts permitted while taking part in the study. Participants were instructed to consume three meals daily (Hylander & Rossner, 1983).

One hundred and thirty-five members were instructed to record their hunger ratings during the first week of study at breakfast, lunch, dinner, and 3 pm. They also recorded number and type of bowel movements and any gastrointestinal discomfort during the week (Hylander & Rossner, 1983). At the end of the first run-in week, participants were divided blindly into groups. They were given either a sachet of bran or ispaghula to be mixed with ½ glass of water and consumed 15 minutes before meals. Thirty-five participants initially were placed in the control group, receiving no additional dietary fiber and also recording bowel movements and hunger ratings. The study was for 3 weeks (Hylander & Rossner, 1983).

Of the original 135 members, a total of 110 participants completed the study: 43 in the ispaghula (soluble fiber) group, 44 in the bran (non-soluble fiber) group, and 23 in the control group. All groups had similar weight loss with no significant differences. All groups showed a low hunger rating in the morning at breakfast and reported that their hunger rating increased during the day, peaking just before dinner. Adding dietary fiber to their intake showed a significant reduction in hunger ratings. Hunger ratings in both fiber groups were also lower before meals and at the 3 pm point. The bran preload
showed significantly lower hunger ratings than the control at lunch (p <0.01) (Hylander & Rossner, 1983).

In a study that looked at the effects of a high fiber breakfast on lunch given ad libitum 3½ hours later, researchers found a decrease in energy intake during the lunch meal after the high fiber breakfast (Levine, et al., 1989).

Berti, et al. (2005) investigated the effect of alternative crop formulations containing various types of carbohydrates and fiber on specific and general satiety. All test foods were given as preloads. Fifteen healthy male subjects were enrolled in Experiment 1, 14 subjects were enrolled in Experiment II and 12 subjects were enrolled in Experiment III (Berti, Riso, Brusamolino, & Porrini, 2005).

Test foods for the study were white bread, oat bread, spaghetti, oat spaghetti, lasagna, carboxymethyl cellulose buckwheat lasagna, buckwheat lasagna, quinoa and rice. Participants were asked to fast for 22 hours prior to arriving at the test site. Each participant were given 500 ml of water asked to evaluate the amount of food needed to reach satiety prior to eating in order to apply the preloading paradigm and were given 500 ml water. A satiety rating questionnaire was given (Berti et al., 2005).

No significant difference was seen between white bread or consuming a preload of oat bread on subsequent energy intake or weight of food eaten. Both breads were rated similarly for pleasantness. Weight and energy intake were unaffected by the time of preload consumption. The oat bread, however, did bring out a different test meal energy intake depending on the preload energy level. The higher the preload, the lower the energy intake (p=0.022). The study concluded that even though total energy intake for
white bread and oat was comparable, the higher satiating efficiency of oat makes it a better food option (Berti, et al., 2005).

Moorhead, et al. (2006) conducted a study whose aim was to determine if the structure and fiber content of carrots would alter the postprandial satiety and subsequent food intake when consumed as part of a mixed meal (Moorhead, et al., 2006).

The study was a randomized repeated measure, cross-over design with 34 non-obese women from the University of Ulster. All participants were given meals with carrots in three different forms. A standardized breakfast and a carrot lunch were given. Lunch was served at 12:30 pm, which included boiled rice with sweet and sour sauce chicken and the carrots in various forms (whole carrots, blended carrots and carrot nutrients). An ad libitum buffet style meal was served at 4 pm, with a choice of two meals (Moorhead, et al., 2006).

Food intakes for the afternoon meal were calculated by the difference between the leftovers and what was served at the meal. The food diaries were converted to weights using manufacturers’ data or standard portion sizes. A dietary analysis program was used to calculate the energy, macronutrients, and fiber intake (Moorhead, et al., 2006).

There was a significant difference in the amount of time it took to consume the whole carrots compared to the blended carrots, compared to the carrot nutrients. Participants were significantly less hungry, fuller, with little desire to eat and prospective consumption after the whole and blended carrot meals compared to the carrot nutrient meal. However, there was no statistical difference between the whole carrots and the blended carrot meals for satiety (Moorhead, et al., 2006).
Total energy intakes for the day after the whole carrot meals was less than the blended after the blended carrot and carrot nutrient meals. Significant differences were reported between the three conditions for total energy intake, food energy, and carbohydrate and protein intakes. Whole carrots had a 1212 kJ lower energy intake compared to carrot nutrients, and blended carrots had a 634 kJ lower energy intake compared to carrot nutrients. The energy intake for the remainder of the day followed the same pattern as after lunch: energy intake after the whole carrot meal was less than after the blended carrot, meal, which in turn was less than the meal made up of carrot nutrients. These intakes were significantly lower for weight of food, total energy, food energy, carbohydrates, and fat (Moorhead, et al., 2006).

Whole carrots and blended carrots contain the same amount of fiber, therefore, the authors concluded that it is the fiber, not the structure of the food, that affects satiety. On the other hand, the fiber content and the structure were influential in decreasing intakes after eating the carrots in the mixed meals. Consuming whole or blended carrots may be beneficial in increasing satiety and decreasing subsequent food intake (Moorhead, et al., 2006).

To determine whether or not a low energy density preload affects postprandial satiety, Flood and Rolls (2007) examined the effects of consuming different forms of a low energy dense soup as a preload on the subsequent test meal and total energy intake at meal. All the soups were made from the same ingredients (Flood & Rolls, 2007).

Participants were residents of the community near Pennsylvania State University between the ages 18-45 years and had a BMI of 18-40 kg/m². A crossover design with repeated measures was used. The study’s 60 participants attended five test sessions for
breakfast and lunch at the university’s laboratory. A standardized breakfast of bagels and yogurt was given *ad libitum* on each test day, with lunch served 3 hours after breakfast. One of the four preloads or no preloads was given before lunch. Preloads were randomized for participants. The preload was to be consumed within 12 minutes and when no preload was given, participants were asked to sit quietly for 12 minutes and then rate the hunger and satiety feeling within the next 3 minutes. The same test meal, cheese tortellini with parmesan cheese and one liter of water, was then served 15 minutes after the preload each test day. Participants were given the option to eat as little or as much as they wanted of the test meal (Flood & Rolls, 2007).

The preloads were broth with a plate of vegetables in butter; chunky soup made from broth; vegetables and butter combined in a chunky soup; and chunky pureed soup make up of broth, butter and half of the vegetables blended for 10 seconds and remainder of vegetables added after blending, and pureed soup made from broth, vegetables and butter blended together for 15 seconds. Women were given 1 ½ cups of soup and men were given 2 cups (Flood & Rolls, 2007).

Visual analog scales were given at each test session to assess hunger, thirst, fullness, prospective consumption, and nausea. The test meal and preloads were rated on appearance and pleasantness of taste. Food was also rated on perceived caloric content, how filling they thought the preload would be, pleasantness of the texture, thickness of the preloads, perceived consumption, and portion size of the test meal (Flood & Rolls, 2007).

Results from the study showed that participants ate significantly less of the test meal when they were given a preload compared to no preload (*p*<0.0001). Men had a
significantly higher energy intake from the test meal than women (p<0.001). Lunch energy intake did not differ among the different types of soup, but the total energy intake at lunch—soup and test meal—was significantly less when the preload soup (p<0.0001) was consumed compared to no preload. Energy intake was decreased by 20% (134 ± 25kcal) during lunch when the soup preload was consumed. Participants ate a significantly greater total weight of food at the meal when preload (p<0.0001) was consumed compared to no preload (Flood & Rolls, 2007).

Hunger ratings right after lunch was served were significantly lower with the preload (p<0.001) compared to no preload. Satiety was significantly higher immediately before the test meal was served with preload (p=0.04) compared to when there were no preload. Prospective consumption was significantly lower before the test meal was served when there was a preload (p<0.04) compared to no preload meal. At no time point was there significant difference in hunger, thirst, fullness, or prospective consumption between the preload vs no preload (Flood & Rolls, 2007).

Researchers concluded that consuming a low energy dense soup as a preload resulted in a reduction in intake at test meal compared to no preload. They suggested that adding such a soup would be beneficial in the weight loss and management epidemic (Flood & Rolls, 2007).

Also tested was whether the form of a food given as a preload affected outcomes. J. Flood-Obbagy and B. Rolls (2009) conducted a study whose aims were to determine whether food served in different forms – in this case, apples -- affects satiety and energy intake independent of variations of energy or fiber content and whether fruit consumption as a preload affects satiety and meal energy intake (Flood-Obbagy & Rolls, 2009).
On each test day, participants were given a standardized breakfast of bagels and yogurt *ad libitum*. Lunch was served 3 hours after breakfast. Prior to eating lunch, participants were given one of the four forms of apples as a preload or no preload. The test meal was served 15 minutes after the preload was served. The same test meal was served at each session. The preload foods were apples, applesauce, apple juice with fiber, and apple juice without fiber. The test meal was cheese tortellini and tomato sauce with 1 liter of water. Participants were given the choice to eat and drink as much or little as they wanted (Flood-Obbagy & Rolls, 2009).

Participants were asked to rate their hunger, satiety, thirst, and characteristics of the preload using a 100-mm visual analog scale before and after breakfast, preload time, and lunch (Flood-Obbagy & Rolls, 2009).

Participants consumed significantly less at the test meal after consuming an apple compared to applesauce and the two apple juices. Hunger ratings were significantly lower after preloads than after the control (*p* < 0.001). Hunger after the apple preload was significantly lower than after applesauce, apple juice with fiber, and apple juice without fiber (*p* <0.0001). After lunch, hunger feelings were similar to those after consumption of preloads (Flood-Obbagy & Rolls, 2009).

Almiron-Roig hypothesized that a fiber enriched yogurt would be more satiating than a regular drinking yogurt, due to the fact that the enriched yogurt contained fruit pieces and a more viscous texture to the regular yogurt (Almiron-Roig et al., 2009).

Results showed that the fiber yogurt, regular yogurt, and banana were more satiating than the crackers and water during the first 45 minutes. The fiber yogurt remained satiating at 60 minutes compared to the crackers. The fiber yogurt and regular
yogurt energy intake on the subsequent meal was significantly lower (p<0.001) compared to water, and consumption after the banana was significantly lower (p<0.05) compared to water (Almiron-Roig, et al., 2009).

From this the researchers concluded that foods with low energy density are often more satiating in the short-term. The study also showed that low energy, low volume, fiber enriched yogurt is linked to low hunger, higher satiety, and more accurate energy compensation for up to 60 minutes compared to isoenergenic water and crackers. These findings may be helpful in enhancing satiating properties in everyday food items to aid in the short term management of appetite and food intake (Almiron-Roig, et al., 2009).

Freeland, et al., (2009) examined the effects of wheat bran fiber on appetite and short-term energy intake. They enrolled nine participants for two repeated measure crossover design experiments. The four preloads were a high fiber cereal containing 41 grams of insoluble fiber, a high fiber plus glucose cereal, a low fiber cereal and a low fiber plus glucose cereal. Preloads were consumed within 15 minutes. The preload was followed by an ad libitum meal consisting of pizza squares (Freeland, Anderson, & Wolever, 2009).

Total energy intake of the preload plus meal was lower after the high fiber cereal compared to the low fiber and the low fiber plus glucose preload (Freeland, et al., 2009).

It may therefore be concluded that consuming a preload inhibits subsequent food intake especially over a relatively short period. Preloads that are most effective in lowering overall caloric intake are those characterized by low energy density either because of their high water content (soups) or because of their high fiber content.
3. **Studies on the Effect of Meals on Postprandial GI Hormones**

A number of studies have examined the impact of meals on fluctuations in the release of GI hormones. Meals studied have differed in their macronutrient composition and fiber content. In a study looking at the effect of macronutrients on postprandial PYY responses in obese hyperinsulinemic women, it was found that high protein and fat in a meal results in an immediate increase and longer PYY response, thereby increasing satiety and prolonging hunger (Helou, Obeid, Azar, & Hwalla, 2008).

The study enrolled eight obese women and provided them with one of three meals: a high carbohydrate meal, a high fat meal, or a high protein meal on test-day morning. Participants were restricted from eating additional food for three hours after eating the test meal. Blood samples were drawn from intravenous catheter at intervals up to 180 minutes after the meal (Helou, et al., 2008).

There was a significant increase in PYY concentration following all three meals; however, the high carbohydrate meal sustained an elevated postprandial PYY concentration throughout the experiment. Comparison of the three diets showed that the high fat meal elicited a significantly higher increase in postprandial PYY concentrations at 15 and 30 minutes compared to the high protein meal (p<0.05). Postprandial PYY levels for the high protein meal were significantly higher than the postprandial PYY level following the high fat meal at 120 minutes. Study researchers concluded that increasing protein and fat content in meals may result in increased satiety and its maintenance for a longer period of time because of the immediate and prolonged response of PYY to these nutrients (Helou. et al., 2008).
Weickert, et al. (2006) investigated the effects of fiber on postprandial PYY and ghrelin and their relation to satiety ratings. The study was a randomized, controlled, single blind crossover design that enrolled 14 healthy women. The participants consumed one of three test meals at three different times throughout the day. A 24-hour diet recall was recorded. The test meals were low fiber white bread, bread enriched with wheat fiber, or oat fiber bread (Weickert et al., 2006).

The results showed no statistical difference between baseline PYY and ghrelin postprandial concentrations. However, PYY and ghrelin levels were attenuated after ingesting the wheat fiber meal. The area under the curve (AUC) for PYY after consumption of wheat fiber at the 180 minute time point was reduced significantly compared to the control (P=0.016), but this was not the case for the oat fiber (P=0.597). Ghrelin at 180 minutes AUC was increased after wheat fiber (P=0.003) but not oat fiber (P=0.127) compared to the control. After all meals, hunger was significantly decreased (p < 0.001) with no difference between the meals. The author concluded that insoluble fiber significantly affects postprandial PYY and ghrelin compared to insoluble oat fiber and low fiber wheat bread (Weickert, et al., 2006).

Gruendel, et al. (2007) conducted a postprandial study to determine the delayed effects of carob fiber consumption on fasting levels, postprandial response of glucose, insulin, non-esterified fatty acid, triglycerides, leptin, and total and acylated ghrelin concentration. Using a randomized crossover design, on Day 1 of the study healthy young people were randomly assigned a diet consisting of 52 g of fiber (high fiber diet) versus 14 g of fiber (control diet). The fiber came from the carob food item in pulp form.
On Day 2 of the study, all participants were given 103 g of standardized white bread after a 10 h overnight fast (Gruendel, et al., 2007).

Results from the study indicated that carob fiber consumed on the day prior did not significantly affect fasting blood glucose, triglycerides, or serum non esterified fatty acid. However, the standardized bread meal did increase plasma glucose (P=0.020), and lower triglycerides (P=0.033) and serum non esterified fatty acid (P=0.001). Fasting serum insulin and postprandial response after ingestion of bread was similar to responses after ingestion of foods with or without having consumed carob fiber (Gruendel et al., 2007).

Fasting acylated plasma ghrelin concentrations were higher on Day 2 following the consumption of carob fiber, compared to when the control diet (P=0.046) was consumed. The fasting total plasma ghrelin was unchanged. However, absolute total and acylated plasma ghrelin responses to bread consumption were higher on the day following the carob fiber in comparison to the control. When plasma ghrelin concentrations were normalized to baseline, no changes were observed with the total and acylated plasma ghrelin concentrations after the carob fiber intake (Gruendel, et al., 2007).

In summary, findings include an elevated total and acylated plasma ghrelin and higher postprandial plasma glucose response following a meal after consuming carob fiber on the previous day (Gruendel, et al., 2007).

Vitaglione, et al. (2004) looked at the effects of β-glucan enriched bread on plasma ghrelin and PYY concentrations. The study enrolled 14 participants in a randomized crossover design study. The study’s foods were bread with no glucan, β-
glucan bread, pasta with sauce, cold rice salad, meat, fish, green salad, chips, bread, and fruits. There were two treatments with a 1-week washout period. Participants were given breakfast and an *ad libitum* lunch. Blood samples and VAS were taken at baseline and continued until 180 minutes after consuming breakfast. Desire to eat, fullness, satiety, glucose, insulin, ghrelin, and PYY blood concentrations were calculated (Vitaglione, Lumaga, Stanzione, Scalfi, & Fogliano, 2009).

There was a significant reduction in hunger and increase in fullness and satiety with the β-glucan bread compared to the no glucan bread at 120 minutes to 180 minutes after consuming breakfast. The AUC at 60 – 180 minutes showed a significant reduction in hunger from −3863± 2312 with control bread to −5761± 2944 with β glucan bread, p < 0.05; increase in fullness from 3285± 101 with control bread to 4105± 1898 with β-glucan bread, p < 0.05); and increase of satiety from 2221± 1375 with control bread to 3444± 1980 with β-glucan bread, p < 0.05. A significant reduction in energy intake (-172 ± 8.5 kcal) was seen in subsequent lunches after participants ate the β-glucan breakfast compared to when the control bread was consumed. Over the post breakfast period, a significantly lower blood glucose concentration was seen with the β-glucan bread breakfast: p<0.05. A reduction in plasma ghrelin concentrations was seen in both breads; the β-glucan bread saw a reduction from 90±12 to 33± 10 pg/mL, compared to control bread from 89±5 to 37±7 pg/mL. PYY concentrations were higher after the β-glucan bread with significant effect at all points except at 120 minutes. The study concluded that barley β-glucans added to bread is able to decrease hunger and increase fullness and satiety, while at the same time reducing energy intake of the subsequent meal (Vitaglione, et al., 2009).
In a study reported by Beck, et al., whose objective was to test the effect of increasing doses of β-glucan in extruded cereals on PYY levels in overweight adults, it was concluded that PYY levels increase in proportion to the amount of fiber in the form of β-glucan consumed (Beck, Tapsell, Batterham, Tosh, & Huang, 2009).

A randomized crossover study was conducted on nine healthy participants (six healthy men and three women) to determine the effects of barley compared to white rice mix on postprandial plasma glucose, insulin and unacylated ghrelin. The participants were given five different test meals each separated by a 7-day washout period. They were instructed to eat and drink the same prescribed foods the day before test day. Blood samples and appetite visual analog scale (VAS) were collected 20 minutes before test meals were given and at intervals up to 240 minutes after the meals (Sakuma, et al., 2009).

The five test meals were glucose, white rice, white rice with 30% rolled barley, 50% rolled barley, and 100% rolled barley. The VAS were used to measure subjective feelings of hunger and fullness (Sakuma, et al., 2009).

The results showed that there was a marked suppression of postprandial unacylated ghrelin after glucose and white rice intake. However, plasma unacylated ghrelin tended to be lower at different time points following the barley containing meals compared to glucose and white rice (Sakuma, et al., 2009).

Fullness scores at 180 and 240 min after ingestion of 100% barley meal were significantly higher than after intake of glucose and white rice at the same points (P<0.01). After 240 min fullness scores for glucose and white rice returned to fasting level values, but remained above fasting level for 100% barley meal. Fullness scores at
45 min for 50% barley meal were significantly higher than white rice and 30% barley meal (P<0.05). Thirty percent and 50% barley meal fullness scores at 180 and 240 min were significantly higher than white rice (P<0.05; 180 min, P<0.01; 240 min) respectively. The pattern of the hunger scores was the reverse of the fullness scores (Sakuma, et al., 2009).

The study concluded that postprandial satiety is maintained longer after eating a high fiber meal compared to a low fiber intake. Barley intake at different levels showed prolonged postprandial fullness as well as an attenuated reduction of unacylated ghrelin level compared to white rice (Sakuma, et al., 2009).

Willis, et al. (2010) argued that while gut hormone changes after consumption of carbohydrate, protein, and fat have been evaluated, there were very few studies that looked at the effect of fiber on GI hormones such as ghrelin, GLP-1, and PYY.

Twenty participants were enrolled and completed the study. They were asked to consume a low fiber diet prior to test days. Participants were given VAS for appetite assessment prior to eating one of the four test muffins containing 0, 4, 8 or 12 g of mixed fiber. Ghrelin samples were collected at baseline and at intervals up to 90 minutes after baseline. GLP-1 and PYY were drawn at 30 and 60 minutes after baseline. Statistical analysis were calculated on changes from baseline, area under the curve for gut hormones, and appetite questions (Willis, et al., 2010).

The results showed that there was no statistical difference between hunger and prospective food intake, and satisfaction and fullness showed slight differences with the test meal. The area under the curve for hunger showed that the test meal with 4 g of fiber was more filling than the test meal with 0 grams. Test meals did not affect food intake at
the lunch buffet or the post intervention period. Ghrelin is known as the hunger hormone, however, the ghrelin area under the curve was higher after the test meal with 12 g of fiber compared to the lower grams test meals, and the VAS scores showed no difference in hunger amongst the four treatments. The author attributed this unexpected finding to the viscosity of the fiber used in the research and suggested further research be conducted to better understand this effect. Another unexpected finding was the GLP-1 area under the curve was higher after the test meal with 0 grams than after the other treatments, and the test meal with 8 g was higher than test meal with 12 g. The researchers hypothesized that the fiber may have slowed down the gastric emptying and nutrient absorption, causing fewer nutrients in the gut to promote the release of GLP-1. PYY area under the curve showed no difference between any of the treatments (Willis, et al., 2010).

Karhunen, et al., (2010) carried out a research project that examined the relationship of food enriched with soluble fiber in the form of psyllium and vegetable protein on post-prandial satiety related peptides and satiety in subjects 26 ± 1 years of age. The study enrolled 16 healthy participants who were given one of five test meals. The test meals were white wheat bread, a low fiber low protein meal, a high fiber low protein meal, a meal low in fiber and high in protein, and a high fiber and high protein meal. Each participant was instructed to keep a detailed 24-hour food diary the day before the test day as well as the test day. An ad libitum meal was provided 2 hours after the test meal. Test meals were consumed within 20 minutes. Blood samples and VAS scores for appetite and pleasantness were taken (Karhunen, et al., 2010).

The study showed that glucose and insulin responses were blunted after both high fiber meals compared to the low fiber and white wheat bread meals (p<0.001). The low
fiber meals resulted in lower glucose and insulin than the white wheat bread meals (p<0.05). Glucose response was lower after the high fiber-high protein meal than after the high fiber-low protein meal (p<0.05). Area under the curve for glucose for the white wheat bread was larger than the AUC for the low fiber and high fiber-high protein meal (p<0.01). The high protein meals had a smaller AUC compared to the high fiber-low protein (p<0.05). Insulin AUC was significantly larger for white bread compared to the other meals (p<0.01). Insulin AUC after the low fiber meals was larger than the high fiber meals (p<0.001) (Karhunen, et al., 2010).

Ghrelin levels decreased for 40 minutes after the low fiber meals and for 60 minutes after white wheat bread meals, then increased toward baseline. The decrease in ghrelin after the high fiber meals was significantly different from white wheat bread and low fiber low protein (p<0.05). There was a greater decrease in ghrelin after the low fiber high protein meal compared to the high fiber, high protein meal (p = 0.05). GLP-1 concentrations increased for 20 minutes after the consumption of white wheat bread, and low fiber and high fiber low protein meals, then returned gradually to baseline. After the high fiber high protein meal, GLP-1 decreased below baseline at time 40 minutes (p<0.05). White wheat bread and low fiber meals had a PYY concentration increase for 40 minutes after consumption and then gradually decreased towards baseline. After the high fiber meals, PYY concentrations increased and remained high throughout the study period (Karhunen, et al., 2010).

Feelings of hunger and desire to eat initially decreased and fullness and satiety increased after all meals (p<0.001). Desire to eat decreased significantly after the high fiber meals compared to the white bread and low fiber-low protein meals (p<0.05). High
fiber meals were rated less pleasant than the low fiber meals (p<0.05). There was no significant difference between total energy intake among test meals (Karhunen, et al., 2010).

Willbound and Doucet (2011) conducted a study that examined whether eating high protein preloads at different times before a meal would maximize premeal fullness, leading to a lower total energy intake. They also examined whether individually timed preloads would influence energy intake, appetite, and gut peptides by testing the participants under different conditions (Willbond & Doucet, 2011).

Participants arrived at the test site on three different occasions, where they were given a standardized breakfast. In Condition One, or the control, participants were given a list of snacks that were allowed between breakfast and lunch and told that they may have as many as they wanted. In Condition Two, a high protein preload was given 15 minutes before lunch and dinner, which participants ate within 5 minutes. In Condition Three, the preload was given to participants 20-80 minutes before lunch and dinner. The time that they received the preload was based on the time that they needed to reach fullness peak. The preloads were made up of 40% carbohydrates, 40% protein, and 20% fat. Lunch and dinner were self-selected by participants from a menu of five meal items and six drinks. Participants were asked to eat the food items until they felt full. Participants were given the VAS to rate their appetites, and blood samples were collected at various times throughout the test day (Willbond & Doucet, 2011).

Results show that there was no significant difference between daily energy intake for the three different conditions. A significantly greater protein intake was seen in conditions 2 and 3 (p <0.01) in absolute and relative values (Willbond & Doucet, 2011).
The daily profiles and premeal levels for PYY and GLP-1 were not different between the three conditions. However, Condition One, in which participants selected the timing and choice of snacks, resulted in the lowest scores for desire to eat, hunger, and prospective food consumption (Willbond & Doucet, 2011).

Researchers concluded that the timing of the high protein preload had no effect on energy intake and that during the control condition, the desire to eat, hunger and perceived food consumption was at its lowest (Willbond & Doucet, 2011).

Zwirska-Korczala, et al. (2007) conducted a study whose aims were to analyze the plasma levels of certain gastric hormones under basal conditions and in response to a standard mixed meal, and to provide clarification of the relationship between the gastric hormones and metabolic syndrome (Zwirska-Korczala, et al., 2007).

Control group study participants were eight lean, healthy women; 12 moderately obese women with metabolic syndrome, and 17 morbidly obese women with metabolic syndrome (Zwirska-Korczala, et al., 2007).

After a 12-hour fast, participants reported to the test site where anthropometrics were taken, baseline blood samples were drawn and participants were given a standardized mixed breakfast. Additional blood samples were taken at 30, 60 and 120 minutes after consuming a meal (Zwirska-Korczala, et al., 2007).

Fasting total ghrelin levels were significantly lower in the morbidly obese with metabolic syndrome and the moderately obese with metabolic syndrome groups compared to the control group (p<0.05). The total plasma ghrelin significantly fell in the control group at 30 minutes after the meal and remained low, but this drop was not seen in either of the obese groups. The moderately obese with metabolic syndrome group had
a small non-significant drop in total ghrelin levels after the meal, with the concentration significantly lower than the control and morbidly obese with metabolic syndrome groups. Fasting acylated-ghrelin levels were significantly decreased in the moderately obese with metabolic syndrome group, however, no differences were seen between the control and morbidly obese with metabolic syndrome groups. Levels dropped in the control after the meal and remained lower until end of the study. No significant changes were seen in the morbidly obese with metabolic syndrome or moderately obese with metabolic syndrome groups (Zwirska-Korczala, et al., 2007).

Results showed no difference between the control group and moderately obese with metabolic syndrome for fasting PYY plasma level, however the morbidly obese with metabolic syndrome women had a significantly lower fasting PYY than the control group and the moderately obese with metabolic syndrome group. After the test meal, the mean plasma PYY level increased in the control group by approximately 40 percent at 60 minutes and remained elevated to the end of study. The morbidly obese with metabolic syndrome group showed small but significantly increased concentrations of PYY from 30 to 60 minutes postprandial; however, the increase was significantly lower than the increase in the control group. The highest level was at 120 minutes. When compared to the control group, the increase in the PYY level for the moderately obese with metabolic syndrome was not significant. Both obese groups showed a significantly lower postprandial PYY compared to the control group (Zwirska-Korczala, et al., 2007).

Fasting cholecystokinin (CCK) concentrations were lower in the morbid obese group compared to control and moderately obese with metabolic syndrome group (p<0.05). In the control group, CCK concentrations after the meal increased at 30
minutes, decreased slightly at 60 minutes, then and increased at 120 minutes. On the other hand, CCK showed a steady increase in the moderately obese with metabolic syndrome and morbidly obese with metabolic syndrome groups, peaking at 60 minutes after the meal. The increase in the morbidly obese with metabolic syndrome group was significantly lower than that found in the control and moderately obese with metabolic syndrome groups (Zwirska-Korczala, et al., 2007).

The hormonal response to meals may be influenced by factors other than simply the composition of the meal itself. Factors such as age, gender, and body weight may modulate the effect.

4. Studies Using Legumes

The fiber- and protein-rich make up of legumes have spurred researchers to take a closer look at their role in the diet and their relationship to health. A study examined the benefits of consuming legumes by comparing two high fiber diets and a low fiber diet. The study enrolled six normal weight participants who reported to the testing lab, where they ate the required meals and had blood samples drawn after breakfast and lunch. The participants were given three different breakfasts that contained equal amounts of carbohydrate, protein, and fat. The two high fiber breakfasts contained the same amount of fiber but different fiber-rich foods. The first test meal was cooked beans with tomato and banana. The second test meal was bran cereal with skim milk, dry cottage cheese, tomato and a banana. The low fiber breakfast was white bread with butter, dry cottage cheese, skim milk, raw tomato, and a banana. This was followed by a standard lunch composed of whole-wheat bread, dry cottage cheese, tomato and a banana (Shaheen & Fleming, 1987).
Participants were given a test meal and blood samples for insulin and glucose measurements were drawn at intervals up to 240 minutes following breakfast and 120 minutes following lunch (Shaheen & Fleming, 1987).

Postprandial glucose was reduced in all breakfasts; there were no significant differences between the three meals. The authors alluded to the fact that a larger dose of beans may have elicited a lower glucose compared to the other meals. While glucose response at lunch was lower following the bean meal, it was not significantly different from the other test meals. Insulin postprandial responses were not significantly different. Lunch was not significantly affected by the different breakfasts but the maximum insulin concentration was higher after the bean meal than the other meals (Shaheen & Fleming, 1987).

In 2000, researchers Kirkmeyer and Mattes enrolled 24 healthy adults in a study that looked at the relationship between hunger and peanuts, hypothesizing that the high fiber, protein content, energy density, and solid nature of peanuts would suppress the appetite. The study had eight preloads: unsalted cocktail peanuts, low sodium peanut butter, almonds, chestnuts, milk chocolate, dill pickles, salt free and fat free rice cakes, and a no load condition. Desire to eat and hunger ratings were recorded, and a 24-hr diet record was kept for the study day (Kirkmeyer & Mattes, 2000).

Results showed no statistical differences for hunger ratings at baseline. All preloads except pickles and rice cakes lead to a suppression of hunger. The difference from baseline to the 180 minute point was significantly lower for hunger ratings for the peanut, peanut butter, almonds, chestnuts, and chocolate preloads. The area under the curve was significantly lower for these items compared to the control (p <0.001). The
energy intake from fat on days peanuts, peanut butter, and almonds were eaten were significantly higher than each of the control preloads (p < 0.001). The study concluded that it is the energy of the preload that affects the hunger more than the macronutrients, energy density, fiber content, weight, volume, sensory properties or rheology (Kirkmeyer & Mattes, 2000).

Schäfer, et al. (2003) examined the glycemic and insulinenic responses of type II diabetic patients in treatment to three different mixed meals that contained dried peas, potatoes, or both as carbohydrate sources. Nine participants completed the study, and had a mean age of 61±3 years with a mean body mass index of 29.9±1.7. Mean hemoglobin A1C was 6.3% on the first study visit (Schäfer, Schenk, Ritzel, Ramadori, & Leonhardt, 2003).

Study participants attended the testing facility on three different days with a one week washout period for the randomized crossover study. They were given a standardized breakfast at 8:00 am and a snack at 10:00 am. At 12:30 PM, participants were given one of three of the test meals in random order. All meals were similar in carbohydrate, protein, fat and water content, but the carbohydrate source for each meal was different. The carbohydrate for Meal 1 was dried peas, the carbohydrate for Meal 3 was potatoes and Meal 2 included a combination of dried peas and potatoes. Blood samples were collected at intervals up to 180 minutes (Schäfer, et al., 2003).

Study results showed that plasma glucose and serum insulin did not differ significantly before the test meals. After all test meals a significant increase in glucose was observed at 30 and 60 minutes respectively (p=0.0002; p<0.0001). The initial increase in insulin at 30 minutes was not significant (p=0.0826) for any of the test meals.
Mean insulin increase was significant (p=0.0037) at 60 minutes for all meals; insulin peak remained at the same concentration up to 120 minutes in test meal 3. From the AUC, data showed that the potatoes-alone meal produced a significantly higher (p<0.05) glucose and insulin response than the dried peas at 120 and 180 minutes.

The investigators concluded that this study reinforced the recommendations for type II diabetics, particularly obese persons, to increase legumes in their diet. It also suggests that when counting carbohydrates, that only ½ of the beans consumed should be counted as a carbohydrate (Schafer, et al., 2003).

Pittaway and colleagues (2007) launched a study that examined the effect of substituting wheat-based foods with chickpeas on serum lipids, long term glucose tolerance, bowel function, and satiety. Using a randomized crossover design, the study consisted of a 10 week intervention period with a washout period of six to eight weeks between the two dietary periods. The chickpea diet consisted of 140 grams of chickpeas with bread and shortbread biscuits made with chickpeas. The control diet was made up of whole wheat bread and higher fiber wheat breakfast cereals. The low fiber diet used white bread and low wheat fiber breakfast cereals (Pittaway, et al., 2007).

Participants were given a VAS for satiety and the consistency of stools and bowel function at the first and last week of the dietary intervention period. Blood samples were collected after overnight fasting. Serum was collected for total cholesterol, triacylglycerols, HDL-cholesterol, insulin, and plasma for glucose. Stata software was used for statistical analysis. Repeated measure ANOVA was used to compare diet results and to examine the effect of diet on lipid profile and glucose tolerance (Pittaway, et al., 2007).
The study results show that there was a significant reduction in total cholesterol (p<0.01) and LDL-cholesterol (p=0.02) with the chickpea diet compared to the wheat diet. Glucose, insulin, and insulin resistance were not significant between the diets but were reduced in the chickpea diet. Bowel function was not significantly different from the wheat fiber diet. Participants experienced satiety on both the wheat and chickpea diets; however, there was a significant reduction in satiety with the lower wheat fiber meals (15.2±1.6grams/day) compared to the other diets (27.9±7.1grams/day), which the authors attributed to the unplanned reduction in energy intake. They concluded that chickpeas may be added to an individual’s current diet because there was a reduction in the sweet and fatty cravings that are common in the traditional American eating pattern (Pittaway, et al., 2007).

Zhang, et al.’s (2010) study, the Legume Inflammation Feeding Experiment, that examined the effects of a legume-rich, high soluble fiber, low glycemic index diet on inflammation biomarkers and insulin resistance in men at risk for colorectal cancer. The study was an 8 week randomized controlled crossover design in which participants were assigned a healthy American diet or an isocaloric legume diet. The legume diet required participants to eat 250 g of cooked pinto beans, navy beans, kidney beans, lima beans or black beans. All meals were prepared and given to participants. Each Friday, participants were given packed meals for the weekend (Zhang, et al., 2010).

Results showed a significant reduction in total cholesterol and LDL cholesterol (p<0.001) and triglycerides (p<0.01) from the legume meal over the course of the time period. The healthy American diet also showed a reduction in total cholesterol and
triglycerides but had less effect on LDL cholesterol. Researchers concluded that incorporating legumes in the diet may help reduce blood lipids (Zhang, et al., 2010).

Mollard, et al. (2011) hypothesized that pulses would reduce food intake, appetite and blood glucose at the present meal as well as the subsequent meal. The study’s aim was to determine the effect of ad libitum pulse meals compared to a non pulse meal on satiation with a current meal and a second ad libitum meal, as well as appetite and blood glucose after both meals.

Twenty-four male participants from the University of Toronto completed the within-subject, balanced, repeated-measure design study. The participants received one of five treatments with a 1 week interval between each treatment. The treatments consisted of isoenergetic meals containing chickpeas, lentils, navy beans/haricot beans, yellow peas, or a control meal. All meals contained macaroni pasta and homemade tomato sauce. The control contained more pasta to accommodate for the pulse energy (Mollard, et al., 2011).

Participants were asked to eat a standardized breakfast before coming to the testing center. A questionnaire to assess sleep, stress, and compliance with fasting and activity patterns were given and answered by each participant. Subjects were given one of the study’s treatment meals in random order. They ate alone and were given 18 minutes to finish the meal. Meals were served in excess to allow participants to reach satiation. VAS was given for appetite, pleasantness, taste, and texture of the meals. Blood glucose and appetite VAS were completed at intervals up to 260 minutes. At 260 minutes, an ad libitum pizza test meal was given to participants and told to eat until they were full, which was followed by blood glucose and VAS measurements up to 340 minutes after the beginning of the test day (Mollard, et al., 2011).
Food intake was affected by the treatment, with lentils having the strongest satiating effect compared to chickpeas and navy beans, which had a lower food intake compared to chickpeas. There was no significant difference for food intake (p=0.13) at the pizza meal, however, lentils significantly reduced cumulative food intake (p<0.02) compared to the control sauce and pasta meal. There was no difference in palatability ratings for pizza. Pre- and post- pizza meal appetite were affected by time (p<0.001), but not by treatment (p=0.23). Participants were hungry when they arrived but that hunger decreased when they finished eating and returned to baseline values by 260 minutes.

Blood glucose was affected by time both pre- and post- pizza (p<0.0001). Pre- and post-pizza blood glucose were affected significantly by treatment (p<0.0001, p=0.03 respectively). At time point 260 minutes, there was a significant effect of treatment on pre-pizza meal blood glucose. Blood glucose was lower for all bean treatments than for pasta and sauce at time 20 and 40 minutes. At time 60 minutes, lentils and navy beans had a lowering affect on blood glucose compared to the yellow peas. Lower blood glucose was seen with navy beans at 110 minutes. At 200 minutes, lentils and navy beans led to lower blood glucose compared to pasta and sauce. At 260 minutes only navy beans produced a lower blood glucose concentration compared to chickpeas and pasta and sauce (Mollard, et al., 2011).

The study concluded that with the reduction on food intake and blood glucose, as well as increase in satiety after consuming beans, it may be beneficial to incorporate a variety of pulses into the diet because they have varied effects on appetite and satiety (Mollard, et al., 2011).
F. Conclusions

Consuming legumes on a regular basis is recommended by health professionals to assist in the reduction of obesity, heart disease, diabetes, and other diet related diseases (Johnson, et al., 2005).

Ghrelin, PYY, and GLP-1 affect the hypothalamus and play an important role in the regulation of hunger, satiety and energy intake (Kokkinos, et al., 2010). The responses of the hormones are dependent on the calories and macronutrients of a meal (Essah, et al., 2007; Willbond & Doucet, 2011).

Studies have demonstrated that preloads, beans, and fiber all play a role in gastric emptying, reducing appetite, and lower energy intake at the subsequent meal. Many researchers have studied beans in relation to satiety and chronic diseases. No study has yet examined the effects of a bean control meal versus staggered bean meal on gastrointestinal hormone concentrations, glucose and insulin levels, feelings of hunger and fullness, and subsequent meal intake.
CHAPTER 3

METHODS

A. Overview/Design

This substudy was conducted in a randomized, crossover design fashion and involved 28 non-diabetic subjects drawn from the pool of subjects who took part in a High Fiber vs. Low Carbohydrate Study for weight reduction. The subjects participated in the current study prior to randomization into the main study’s dietary group. The subjects who volunteered for this study were randomized to consume the same standardized high fiber meal twice (once on Tuesday and again on Thursday), except that a ½ cup of beans was consumed 15 minutes prior to the rest of the meal on one day, while on the other day the ½ cup of beans was consumed with the rest of the meal. Participants served as their own controls. Both meals contained the same macronutrient composition and fiber content (see Table 4.1).

In order to control for differences in eating habits, participants were asked to eat standardized meals on Monday, Wednesday, Tuesday morning, and Thursday morning of the week of the single meal study. Details of the standardized meals for men and women may be found in the Appendix. In addition, a Visual Analog Scale (VAS) for Feelings of Fullness and Feelings of Hunger were given at each blood draw to look at differences in hunger and satiety between the two meals. A copy of the appetite VAS is located in the Appendix.

Participants met at the clinic of the Diabetes Treatment Center (Loma Linda, CA) at 11:30 AM on the first test day, where their blood was drawn and they received a VAS form. Participants were instructed to complete the VAS form each time their blood
samples were collected. Participants were also instructed not to consume any food following the standardized breakfast at home. Following the test meals, blood samples were drawn by venipuncture at 30, 60, 120 and 180 minutes (only for 14 participants) after consuming the meal. Samples were centrifuged, aliquoted and stored at -80°C until study completion, when all samples were analyzed.

Participants were called on the morning following each visit to the test site and a dietary recall to record all foods and beverages consumed after leaving the test site and until they retired for the night. This was performed using Nutrition Data System for Research (NDS-R), software version 8 developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN.

Participants were provided with movies or were able to work on their personal laptops at the test site for approximately 3 hours while the substudy was being conducted on each of the two days. A compensation of $50 was paid to each participant for their time upon completion of the two clinic visits.

Participants for the high-fiber versus low carbohydrate study were recruited from the San Bernardino area in southern California. Flyers were placed at local businesses such as Starbucks and the Loma Linda University campus, as well as at local physicians’ offices, churches, and trade schools. The flyers included a telephone number and website that potential subjects may call if they were interested. In addition, patients attending the Diabetes Treatment Center were informed about the study by the center personnel and patients who were interested were given an appointment for further information. The study’s protocol, flyers and recruitment materials were approved by the Institutional Review Board of Loma Linda University (Loma Linda, CA).
B. Blood Collection

1. Acylated Ghrelin

Ghrelin circulates in two different forms throughout the blood system. Acylated ghrelin is found bound to larger molecules while unacylated ghrelin circulates as free peptides (Murphy, et al., 2006). For this reason specimens were drawn to ensure that both forms may be measured. The half life of total ghrelin is between 10 and 31 minutes in the bloodstream (Castaneda, et al., 2010). Because the half life of acylated ghrelin is 9-13 min (Inhoff, et al., 2009), blood samples were drawn and an additive was used to prevent degradation. One mL of blood was drawn in an EDTA tube (BD Vaccutainers) and 10 μL of 100 mM p-hydroxymercuribenzoic (PHMB containing 0.50% of sodium hydroxide 10N (v/v)) acid was added immediately following the blood draw to prevent the degradation of the specimen during storage. Samples were centrifuged at 3,500 rpm for 10 minutes at 4°C and then supernatants were pulled off and transferred into separate tubes. Immediately after centrifuging, 100 μL of 1N HCL per mL was added to the plasma and centrifuged for an additional 5 minutes at 3,500 rpm at 4°C.

2. Unacylated Ghrelin

The half life of unacylated ghrelin is 27-31 min (Inhoff, et al., 2009), allowing time to capture the peptides after a blood draw. Blood samples were drawn in EDTA tubes with no additive and centrifuged at 3,500 rpm for 10 minutes at 4°C. Supernatants were then removed and transferred to separate tubes and stored.
3. PYY

Plasma levels of endogenous PYY rise within 15 minutes of a meal, plateau at approximately 90 minutes and peak within the second hour after a meal depending on the caloric intake (Essah, et al., 2007; Murphy, et al., 2006). One mL of blood was collected in EDTA tubes and 20 μL of 100 mM dipeptidyl peptidase IV (DPP-IV) inhibitor was added immediately after blood draw to prevent the degradation of the hormone. Samples were centrifuged at 3,500 rpm for 10 minutes at 4°C. Supernatants were pipette off and transferred to separate tubes for storage.

4. GLP-1

GLP-1 blood specimens were collected in EDTA tubes and 20 μL of dipeptidyl peptidase IV (DPP-IV) inhibitor was added to each tube immediately after the blood draw to prevent degradation. Samples were centrifuged at 3,500 rpm for 10 minutes at 4°C. Supernatants were aliquoted and stored at -20°C until end of the study.

5. OXM

OXM samples were collected in EDTA tubes with no additive. Tubes were centrifuged for 15 minutes at 3,500 rpm. Plasma was aliquoted and stored at -20°C until end of study.

All blood specimens were drawn at the clinic and stored in the refrigerator until transported to the Department of Nutrition laboratory for further aliquoting. All tubes with additives were inverted approximately ten times to ensure proper mixing. All assays were completed at the Department of Nutrition, Loma Linda University laboratory.
C. Blood Assays

Blood specimens collected for acylated ghrelin were drawn in EDTA tubes with p-hydroxymercuribenzoic acid added to prevent the degradation of acylated ghrelin by protease. Assaying of acylated ghrelin was completed using human acylated ghrelin EIA Kit #A05106 (ALPCO, USA). Unacylated ghrelin, OXM specimens were drawn in EDTA tubes with no additive. Assays for unacylated ghrelin were completed using human unacylated ghrelin EIA Kit # A05119 (ALPCO, USA). OXM was assayed using an ELISA kit (CUSABIO, USA). GLP-1 and PYY$_{3-36}$ blood specimens were collected in EDTA tubes with dipeptidyl peptidase IV (DPP-4) protease inhibitor added to tubes. Samples were assayed for GLP-1 using an ELISA kit (ALPCO, USA). PYY$_{3-36}$ samples were assayed using YK080 Human PYY EIA kit (ALPCO, USA).

D. Data Analysis

All analyses were performed using SAS (Version 9.3: SAS Institute Inc.). Descriptive statistics are given as mean, standard error, and standard deviation for each variable. The mixed model procedure was used in the analysis to test the effect of diet type, time point, and diet x time interaction on each outcome variable. Post hoc tests were done on least-squares means using Tukey adjustment for multiple comparisons.

Area under the curve (AUC) was calculated for each subject using three time points (30 min, 60 min, and 120 min). The paired sample T tests were used to compare the mean scores of AUC of the two diets for each outcome variable. For measurements at time 5 (180 min), we used paired sample T tests to compare the mean scores of the two diets for each outcome variable. Log transformation was used when the assumption of normality was not met. Alpha was set at 0.05 significance level.
E. Power Analysis

For the single meal feeding sub-study, sample size was determined on the basis of previous literature (Esposito, 2003). A sample size of 24 subjects is satisfactory to achieve a type 1 error rate of 5% and 80% power in order to detect a difference of 10% in gastrointestinal peptide levels after consumption of the high fiber meal with a single pre-meal item such as beans versus the high fiber meal with no pre-meal beans. A sample size of 28 allows for attrition or technical problems.

F. Limitations

The sub-study took place one week before the randomization of the weight loss study, limiting the time frame between the administration of the two diets. Due to limited personnel and liability, central intraluminal catheters could not be used and as a result participants had to be stuck each time blood was drawn.

G. Research Ethics

Informed written consent (see Appendix) was obtained from all participants and the study protocol was approved by the Loma Linda University Institutional Review Board.
CHAPTER 4
PUBLISHABLE PAPER

Effect of consuming a serving of beans 15 minutes before or with a meal on gastrointestinal peptide concentrations in obese women and men

Authors:
Lisa Griffith¹, Ella Haddad¹, Neal Malik², Joan Sabaté¹, and Serena Tonstad²

Loma Linda University, School of Public Health, Loma Linda CA, 92354
¹Department of Nutrition, School of Public Health, Loma Linda University, Loma Linda, CA 92354
²Department of Health Promotion and Education, School of Public Health, Loma Linda University, Loma Linda, CA 92354

Corresponding author: Ella Haddad, R.D., DrPH. Department of Nutrition, School of Public Health, Loma Linda University, Loma Linda, CA 92354. Telephone: 909-558-4598. Fax: 909-558-4095. email: ehaddad@llu.edu

Running Header: Effect of beans on gastrointestinal peptides

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KEYWORDS: beans, food intake, GI hormones, obesity

The formatting and reference style is not in accordance with dissertation guidelines and is according to journal specifications
Abstract

Staggering meals with a short pause may affect control of energy intake. The aim of the study was to examine the effect of eating a serving of beans before or with a meal on gastrointestinal (GI) peptide, glucose, and insulin concentrations and subsequent energy intake in obese women and men. A randomized within-subjects crossover design was used to measure hormonal responses to 2 test meals in 28 healthy obese adults. Subjects consumed a standardized breakfast meal on each test day followed by 1 of 2 test meals: 1) a control meal incorporating 86 g (0.5 cup) of beans with lunch, and 2) a staggered meal incorporating 86 g (0.5 cup) of beans 15 minutes before the rest of the meal. Blood samples were obtained prior and at 30, 60, and 120 minutes following the consumption of the test meals and analyzed for acylated ghrelin, unacylated ghrelin, glucagon-like peptide -1 (GLP-1), peptide YY (PYY), and oxyntomodulin (OXM) using enzyme-linked immunosorbent assay kits. Energy intake following the test meal was obtained by computer assisted dietary recalls from participants on the day following testing. Repeated measures analysis of variance showed no differences due to meal staggering on any of the outcome parameters. The area under the curve from 30-120 minutes (AUC30-120) following the end of the meal for acylated and unacylated ghrelin, PYY, OXM, insulin, glucose, and subjective ratings of hunger and fullness showed no differences due to meal condition. However, GLP-1 AUC30-120 was attenuated following the staggered meal (P=0.03).

Subsequent energy intake did not differ between the two test meals (P=0.25) except for a meal x time interaction in those who ate a staggered meal on the second test day (P=0.04). In conclusion, consuming beans before meal may moderate GLP-1 responses. Whether a staggered meal may reduce subsequent food intake cannot be ascertain from this study.
Further research is needed that clearly defines whether consuming a serving of beans before a meal affects appetite peptides, subjective responses and subsequent energy intake.
Obesity is one of the greatest public health challenges with serious implications both on the healthcare system and the U.S. economy\(^1\)\(^-\)\(^3\). Obesity has been linked to cardiovascular disease, type II diabetes, sleep apnea, musculoskeletal disorders, some cancers\(^1\)\(^,\)\(^4\) hypertension, and reduction in life expectancy\(^5\)\(^-\)\(^7\). Obesity may be the result of an increase in food intake paired with a decrease in physical activity\(^1\)\(^,\)\(^8\).

Diet and exercise have been recommended to treat obesity\(^9\), however, despite the initial weight loss attained by different diets, continual and sustainable weight management have proven to be less effective as evidenced by the growth of obesity in adults and children\(^5\)\(^,\)\(^10\)\(^,\)\(^11\). A key reason for the yo-yo effect of body weight after weight loss is the homeostatic system which stimulates the changes in appetite and energy expenditure to boost weight regain\(^12\).

Identifying strategies and methods to aid in the reduction of energy intake, increase in satiety and maintenance of weight loss are important in weight management\(^13\). Some researchers have shown that the rate of eating, staggering meals, and meal frequency\(^14\)\(^-\)\(^18\) results in lower energy intake by having an impact on gut intestinal hormones.

Gastrointestinal peptides are important in the regulation of food intake\(^19\)\(^,\)\(^20\). Previous research has shown that gut peptides such as PYY, glucagon-like peptide 1 and oxyntomodulin inhibit food intake, and ghrelin stimulates the appetite\(^21\). Previous studies have shown that food influences the release of postprandial hormones\(^22\)\(^,\)\(^23\).

Bean consumption has been associated with reduced risk of obesity due to their satiating effect and lowering of food intake\(^24\). The satiation effect (the termination of a meal due to fullness) and satiety (postprandial feeling of fullness that delays the start or reduces the
intake of the next meal) attributes of beans increase their benefits in the prevention and treatment of obesity\(^{(25-27)}\).

The aim of the present study was to determine the effect of beans consumed 15 minutes before a meal compared to within the meal on postprandial circulating concentrations of gastrointestinal peptides ghrelin, PYY, glucagon-like peptide 1, oxyntomodulin, glucose and insulin, and on feelings of hunger and fullness.
Methods

Participants

Twenty-eight non-diabetic obese participants (7 men and 21 women) age 48±13 y (mean ± SD) with a BMI of 35 ± 3.85 kg/m² participated in this study. They were recruited for this study from a group of individuals who had volunteered to participate in a low-carbohydrate versus high fiber diet weight loss study. Participants were recruited by advertisements in local newspaper and on notice boards at the university’s diabetes treatment center and the surrounding community. Exclusion criteria included pregnancy, lactation, active cancer, participation in another study/trial, dieting within the last 3 months with weight loss exceeding four pounds, following a vegan diet, type 1 diabetes, type 2 diabetes, having an active eating disorder, currently suffering from an acute or chronic infection, using glucagon-like-peptide-1 analogue or weight loss medication, or gastrointestinal problems that may be worsened by a high fiber diet. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Institutional Review Board of Loma Linda University. Written informed consent was obtained from all participants.

Study design

The study was conducted in a randomized crossover design. Participants came to the university’s diabetes treatment center clinic twice on 2 nonconsecutive days within the same week, usually Tuesdays and Thursdays one week prior to randomization into the low-carbohydrate versus high fiber diet weight loss study. They consumed the same standardized lunch meal twice except that ½ cup of refried beans was consumed 15
minutes prior to the rest of the food at one test meal (staggered bean meal), while at the other meal the beans were consumed with the rest of the food (control bean meal). The order of the two test meals was randomized across the participants to avoid any order effects. The food and nutritional composition of the test meals is shown in Table 1.

On the morning of the study, participants consumed a standardized breakfast (2.17 MJ for women and 2.67 MJ for men) at home and were instructed not to consume any other food or beverages prior to the test meal. Participants arrived at the clinic venue at 11:45 h, at which time they completed an initial VAS form followed by baseline blood sampling for measurement of biochemical parameters. This was followed by consumption of either the ½ cup of beans (for the staggered bean meal) or the complete lunch meal (for the control bean meal) according to the order protocol. Those with the staggered bean meal were given their lunch meal 15 minutes after they began eating the beans.

Additional VAS forms were completed and blood drawn at 30, 60, 120 minutes following the completion of the test meals. In addition, VAS forms and blood draws were collected at 180 minutes following the completion of the test meals on 14 of the 28 participants. Participants remained at the clinic venue until all blood draws and VAS forms were completed and were allowed to read, work on their computers or watch movies. After leaving the test clinic, participants were free to eat or drink as desired.

On the morning following each test day, participants were telephoned and dietary recalls were collected and entered into using Nutritional Data System Research software (University of Minnesota, 2007) for all foods and beverages consumed after leaving the clinic venue and until they retired for the night.
Appetite profile

A 100-mm VAS was used to assess the appetite profile components of hunger and fullness. The hunger question was anchored with “I don’t want anything to eat” at one end and “I am starving, I want to eat right now” at the other end. The fullness question was anchored with “I am not full at all. I can eat a lot more” at one end and, “I am stuffed! I can’t eat anything else” at the other end. The VAS lines were completed before lunch and at 30, 60 and 120 minutes following lunch completion for all participants and at 180 minutes for 14 of the 28 participants.

Blood sample collections and analysis

Venous blood samples were collected by venipuncture during the test day before lunch and at 30, 60 and 120 minutes following lunch completion for all participants and at 180 minutes for 14 of the 28 participants.

Blood for acylated ghrelin was drawn in EDTA tubes with p-hydroxymercuribenzoic acid (1 mM in final volume) added to prevent the degradation of acylated ghrelin by protease enzymes whereas no additive was used for unacylated ghrelin and oxyntomodulin. For GLP-1 and PYY3-36 analysis, blood was collected in EDTA tubes to which dipeptidyl peptidase IV inhibitor (20 µl/ml of blood) was added. After collection, blood samples were centrifuged for 10 min at 4°C at 1800 x g. Serum samples for insulin and glucose were allowed to clot and then centrifuged at 2400 x g for 10 min. All samples were stored at -80°C until assayed. ELISA methods were employed for the analysis of acylated ghrelin, unacylated ghrelin, total PYY3-36, GLP-1, insulin and oxyntomodulin with kits from Alpco Diagnostics (Salem, NH) except for oxyntomodulin which was from Cosmo.
Bio USA (Carlsbad, CA). Serum glucose was measured using reagents from Cayman Chemical Co (Ann Arbor, MI).

Statistical Analysis

All analyses were performed using SAS version 9.3 (Statistical Analysis Systems, SAS Institute, Cary, NC). Descriptive statistics are given as mean, standard error, and standard deviation for each variable. The mixed models procedure was used in the analysis to test the effect of the test meal, time, and meal x time interaction on each outcome variable, adjusting for baseline measurements as covariate. Post hoc tests were done on least-squares means using Tukey adjustment for multiple comparisons.

Area under the curve (AUC) was calculated for each subject using 3 time points (30 min, 60 min, and 120 min). The paired sample t-tests were used to compare the mean scores of AUC of the two diets for each outcome variable. For measurements at time 5 (180 min), paired sample t-tests were used to compare the mean scores of the two diets for each outcome variable. Log transformation was used when the assumption of normality was not met. Alpha was set at 0.05 significance level.

Results

Energy intake on testing days

The rate of consumption for the staggered bean meal and control bean meal averaged 40 ± 2 and minutes 20 ± 2 minutes respectively. There was no effect of the staggered bean meal compared to the control bean meal at lunch on the ad libitum energy intake (3.99 MJ; 95 % CI 3.12, 4.85; 4.66 MJ; 95 % CI 3.52, 5.81; P=0.25) of participants at supper. Supper is defined as all food consumed following lunch and before retiring. Although not
significant, the energy intake was approximately 14% lower for supper on the staggered bean meal test days. Analysis of variance showed that supper intake was not affected by meal condition (P=0.35) or time (P=0.40), but there was a meal x time interaction (P=0.04).

*Appetite profile*

VAS scores for hunger and fullness were lowest and highest respectively at 30 min following the both test meal. Scores for fullness declined and those of hunger increased thereafter at similar rates independent of diet condition. Hunger was affected by time (P=0.002), but not by meal (P=0.61) and there was no meal x time interaction (P=0.75; Fig 3). No significant difference in AUC$_{30-120}$ (P=0.99; Table 3).

Fullness was affected by time (P<0.0001), but not affected by the meal (P=0.63) and there was no meal x time interaction (P=0.94; Fig. 3). No difference was observed in AUC$_{30-120}$ (P=0.53; Table 3).

*Ghrelin*

Baseline values of plasma acylated and unacylated ghrelin immediately before start of the test meals did not differ between the two meals. Both acylated and unacylated ghrelin fell following lunch and tended to rise after 60 min with peak concentrations occurring at 120 min. Although not statistically significant, plasma concentration of acylated ghrelin tended to increase sooner following the staggered bean meal lunch and remain higher throughout the testing period. Plasma ghrelin was not affected by meal (P=0.78), time (P=0.15) and there was no meal x time interaction (P=0.49; Fig. 1). No statistical difference in AUC$_{30-120}$ (P=0.85)
Plasma unacylated ghrelin was affected by time (P<0.0001), but not by meal (P=0.91) and there was no meal x time interaction (0.52; Fig. 1). No differences in AUC\textsubscript{30-120} (P=0.75; Table 3) observed between the two meal conditions.

GLP-1

Plasma GLP-1 concentrations increased following lunch for both test meals but remained elevated following the control bean meal. The control bean meal had a higher GLP-1 AUC\textsubscript{30-120} compared to the staggered bean meal (P=0.026; Table 3). GLP-1 response was affected by time (P=0.003), however, not by meal (P=0.89), and there was no meal x time interaction (P=0.10; Fig. 1).

PYY and oxyntomodulin

PYY concentrations increased after both meal conditions reaching a peak at 120 min with no significant difference affect due to meal (P=0.86), time (P=0.75) or, meal x time interaction (P=0.61; Fig. 1). There was no differences between the test meal AUC\textsubscript{30-120} (P=0.88).

The postprandial oxyntomodulin response was not affected by meal (P=0.86), time (P=0.58) and there was no meal x time interaction (P=0.65; Fig.1). There was no difference in AUC\textsubscript{30-120} (P=0.29; Table 3) for the two meal conditions.

Glucose and insulin

Postprandial glucose concentration rose following both test meals and reached their peak at 30 min. A significance time effect was noted (P<0.0001), but there was no meal affect (0.31) and no meal x time interaction (P=0.08; Fig. 2). AUC\textsubscript{30-120} was higher following the
staggered bean meal compared to the control bean meal but there was no difference (P=0.07).

Postprandial insulin concentrations rose following both test meals showing a peak at 30 minutes. Insulin was affected by time (P<0.0001), but not by meal (P=0.59) and there was no meal x time interaction (P=0.86; Fig 2). AUC_{30-120} was not different (P=0.63) between the two meal conditions.

**Discussion**

The current study was designed to determine whether consuming beans 15 minutes before a meal versus with a meal would influence postprandial peptides and subsequent meal energy intake in obese non-diabetic adults. Previous studies have indicated that the length of time to consume a meal might influence physiological feedback signals produced in the hypothalamus and peripheral organs and thus affect subsequent energy intake^{16,17}. We hypothesized that the release of postprandial GI hormones would be moderated following a longer meal duration when part of the meal was consumed 15 minutes before the rest of the meal (staggered) compared to the same meal consumed at once (control). This would sustain satiety over a longer time period and decrease ensuing energy intake. A small portion of beans was selected as the food item to be consumed because beans have been associated with increased satiety and reduced food intake and therefore consuming them before a meal might enhance this effect^{24,28,29}.

In the current study, no differences in postprandial ghrelin fluctuations (either acylated or unacylated) were noted between the two meal conditions That there were no differences in ghrelin responses between the two identical meals suggests that factors other than the rate of eating, such as the energy content of a meal, its macronutrient composition, or its
viscosity, play a more prominent role in post meal ghrelin responses as other studies have reported\(^{16, 18, 30}\). Postprandial ghrelin levels are partially or totally impaired in the obese population which may also have influenced the results in this study in obese participants\(^{31}\).

GLP-1 is characteristically low in the fasting state, rising quickly after a meal\(^{32}\). GLP-1 is known to induce feelings of fullness by inhibiting gastric emptying, reducing gastric motility, and decreasing intestinal transit time\(^{33}\). In the current study, GLP-1 AUC\(_{30-120}\) was attenuated after the staggered bean meal compared to the control bean meal—a response similar to the one observed by Lemmens, et al.\(^{16}\) following their staggered meal condition. This is in contrast to Kokkinos, et al.\(^{15}\) or Karl, et al.\(^{13}\) who found that eating slowly causes a higher postprandial GLP-1 response or no difference in response, respectively, compared to eating the same meal rapidly. A slower rate of eating is expected to slow the process of gastric emptying, leading to lower albeit more sustained nutrient absorption, thereby inducing decreased GLP-1 release in the short term.

Observing GLP-1 responses over a longer postprandial time period than the one followed in this study might be helpful in understanding the role of GLP-1 in appetite regulation following a slow meal.

Similar to GLP-1, PYY\(_{3-36}\) concentrations rise with the intake of food. It is affected by the caloric content and the macronutrient composition of the meal\(^{32}\). Since the test meals were isocaloric, the current study did not show differences in post meal concentrations of PYY\(_{3-36}\) between meal conditions. Our findings are in contract to Lemmens, et al.,\(^{16}\) who found a reduced response in postprandial PYY\(_{3-36}\) following a staggered meal versus a non-staggered meal. Post meal PYY\(_{3-36}\) levels tend to be inhibited in obese
individuals, and the fact that participants in the current study were obese compared to those in the Lemmens study, whose participants had BMI of $25 \pm 3.1 \text{ kg/m}^2$, may explain some of the discrepancies.

Our OXM findings are novel because to our knowledge no study has looked at OXM with the present study’s protocol. In previous studies, OXM was administered intravenously and subcutaneously, where a reduction in energy intake was observed, as well as increases in circulating concentrations of OXM. More research is needed to understand the OXM responses following food intake and factors influencing those responses.

As in similar studies, both diet patterns showed an increase in subjective ratings of fullness and a decrease in feelings of hunger following the test meal, but no difference related to meal conditions. This is in contrast to Lemmens, et al. who showed a sharp decrease in hunger and increase in fullness immediately following the non-staggered meal compared to the staggered meal, which was still in progress. Whether or not sensations of fullness or satiety are influenced by eating rate may also be impacted by non-physiological factors related to the meal condition, such as expectations about foods that may still be provided in the meal.

In the current study, although the staggered eating condition at lunch did not influence subsequent energy intake at supper, there was a meal x time interaction. The group that consumed the control meal on the first experimental day showed reduced ad libitum energy intake following the staggered intervention on the second experimental day. Why the testing order may have influenced postmeal energy intake is not clear. Regulation of
energy balance in humans is complex and in addition to neuronal and hormonal influences, external factors may play a role. One such factor may be habituation with the test meal or the testing conditions.

Epidemiological studies both support and oppose the premise that frequent small meal intake reduces energy intake compared to fewer larger meals\(^ {35}\). As in previous reports\(^ {14, 32} \), in this study, subsequent meal energy intake was not affected by the inserting a short 15 minute waiting period in the previous meal thus lengthening its eating time.

The strength of the current study is in utilizing the same meal equivalent in energy intake and composition for both diet conditions. It reduces the effect of confounding factors that may have influenced the GI peptides and subjective responses due to variable macronutrient intake.

Limitations to the current study include the lack of control over what participants ate for breakfast, the short wash-out period between testing days, and the and non-isolation of subjects. The subjects were given a standardized menu to be consumed at breakfast however, all food items were not consumed by all of the participants. The study took place one week prior to randomization into the weight loss study, providing a narrow margin between testing days, whereas other studies have had at least 2 days between the various test conditions. Participants were allowed to sit together while they ate and this may have affected responses and eating behavior at subsequent meals.

To our knowledge, there has been no research that has looked at the relationship between gut hormones and a food consumed before the meal compared to within the meal.
In conclusion, beans are important in weight management, the control of chronic diseases, and the reduction of mineral deficiencies\(^{(36)}\). There may be some clinical and practical implications in the fight against obesity for the consumption of beans prior to eating, however, those benefits were not clearly detected in this study. Further research is needed to examine the effect of beans on appetite peptides.

**Acknowledgements**

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Thank you to McLean Endowment Fund for financial assistance. The authors declare no conflict of interest.
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### Table 4.1 Composition of test meal

<table>
<thead>
<tr>
<th>Amount</th>
<th>Energy (MJ)</th>
<th>Carbohydrate (g)</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
<th>Fiber (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Refried beans ½ cup</td>
<td>0.51</td>
<td>0.51</td>
<td>22</td>
<td>22</td>
<td>0.6</td>
</tr>
<tr>
<td>Enchilada ¾/1 ea</td>
<td>0.83</td>
<td>1.11</td>
<td>26</td>
<td>35</td>
<td>12</td>
</tr>
<tr>
<td>Brown rice 1/3 cup</td>
<td>0.30</td>
<td>0.30</td>
<td>15</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Corn ½ cup</td>
<td>0.28</td>
<td>0.28</td>
<td>15</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>Rice pudding ½ cup</td>
<td>0.48</td>
<td>0.48</td>
<td>21</td>
<td>21</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2.40</strong></td>
<td><strong>2.68</strong></td>
<td><strong>99</strong></td>
<td><strong>108</strong></td>
<td><strong>25</strong></td>
</tr>
</tbody>
</table>

### Table 4.2 Baseline concentrations of GI hormones, glucose, and insulin (Mean values with standard deviations)

<table>
<thead>
<tr>
<th></th>
<th>Staggered bean meal</th>
<th>Control bean meal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Acylated ghrelin</td>
<td>50.26</td>
<td>57.04</td>
</tr>
<tr>
<td>(ng/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unacylated ghrelin</td>
<td>179.30</td>
<td>69.79</td>
</tr>
<tr>
<td>(ng/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLP-1 (pmol/mL)</td>
<td>1.51</td>
<td>2.91</td>
</tr>
<tr>
<td>PYY (ng/mL)</td>
<td>521.16</td>
<td>642.06</td>
</tr>
<tr>
<td>OXM (ng/mL)</td>
<td>3.03</td>
<td>5.66</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>82.15</td>
<td>28.17</td>
</tr>
<tr>
<td>Insulin (uIU/mL)</td>
<td>26.06</td>
<td>28.28</td>
</tr>
</tbody>
</table>

Premeal baseline values did not differ between treatments.
Table 4.3 Postmeal AUC (30-120 min) for plasma GI peptides, insulin, glucose, and subjective responses in obese women and men after consuming staggered and control meals (Mean values with standard deviations)

<table>
<thead>
<tr>
<th></th>
<th>Staggered bean meal</th>
<th>Control bean meal</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>GI Peptides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acylated ghrelin (pg/mL x min)</td>
<td>4190</td>
<td>3990</td>
<td>4500</td>
</tr>
<tr>
<td>Unacylated ghrelin (pg/mL x min)</td>
<td>13400</td>
<td>5580</td>
<td>13100</td>
</tr>
<tr>
<td>GLP-1 (pmol/mL x min)</td>
<td>116</td>
<td>139</td>
<td>151</td>
</tr>
<tr>
<td>PYY (ng/mL x min)</td>
<td>52400</td>
<td>55700</td>
<td>51200</td>
</tr>
<tr>
<td>OXM (ng/mL x min)</td>
<td>188</td>
<td>365</td>
<td>245</td>
</tr>
<tr>
<td>Glycemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dL x min)</td>
<td>9840</td>
<td>2520</td>
<td>9030</td>
</tr>
<tr>
<td>Insulin (µIU/mL x min)</td>
<td>5250</td>
<td>2750</td>
<td>5710</td>
</tr>
<tr>
<td>Subjective response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feelings of hunger (mm x min)</td>
<td>1230</td>
<td>1090</td>
<td>1180</td>
</tr>
<tr>
<td>Feelings of fullness (mm x min)</td>
<td>6860</td>
<td>1470</td>
<td>6670</td>
</tr>
</tbody>
</table>

Acylated ghrelin, PYY, GLP-1, OXM and Insulin p-value calculated based on log transformed variables. Subjective response recorded on 100 mm line VAS.
Figure 4.1 GI peptide concentrations following meal consumption of test meals. Time course of (A) acylated ghrelin, (B) unacylated ghrelin, (C) glucagon-like peptide-1 (GLP-1), (D) protein tyrosine tyrosine and (E) oxyntomodulin (OXM) responses following consumption of staggered bean and control bean meals. Data point are means ± SE (n=28 except at 180 min when n=14). a,b,c Values with unlike letters are significantly different at each time point P < 0.05.
Figure 4.2 Glucose and insulin concentrations following consumption of test meals. Time course of (A) glucose and (B) insulin responses following consumption of staggered bean and control bean meals. Data point are means ± SE (n=28). a,b,c Values with unlike letters are significantly different at each time point P < 0.05.

Figure 4.3 Feelings of hunger and fullness following meal consumption of test meals. Time course of subjective responses (A) fullness (B) hunger responses following consumption of staggered bean and control bean meals. Data point are means ± SE (n=28 except at 180 min when n=14). a,b,c Values with unlike letters are significantly different at each time point P < 0.05.
Figure 4.4 Energy consumed at supper. Mean ± SEM energy intake at supper following the consumption of a staggered bean meal or a control bean meal on Tuesday or Thursday of the week. Supper kcals are defined as that of all food consumed following the test meal and before retiring. Participants in the control first followed by the staggered meal sequence consumed less energy following the staggered meal p 0.04 (at each meal, n=14).
CHAPTER 5
GENERAL SUMMARY

A. Summary

The current study was the first of its kind to examine a food consumed before a meal versus within the meal. GLP-1 AUC$_{30-120}$ showed that the control bean meal was statistically different from the staggered bean meal. No significant statistical differences were observed between the two meal conditions on acylated and unacylated ghrelin, PYY, OXM, insulin, glucose, and subjective responses. However, the study did find that when the staggered bean meal was consumed, the participants’ energy intake was reduced by 14 percent compared to the control bean meal. There was also a significant time x meal interaction, which suggests that the sequence in how the meals were consumed made a difference in energy intake at the next meal.

Other findings were the lower GLP-1 response and higher fullness ratings when the staggered meal was consumed, which suggested a possible delay in the next meal. The lower response in the staggered meal may be due to the beans consumed 15 minutes before the meal, slowing gastric absorption and inhibiting gastric emptying by the time the second phase of meal was given.

B. Application of Results

The fact that participants had lower energy intake following the staggered meal is important to the struggle against obesity because it could possibly prove to be a way to reduce energy intake using an inexpensive commodity such as beans. In this study it was applied to a single meal, however, it may be beneficial for obese adults and children to consume a filling, high fiber food such as beans prior to their meals to stimulate appetite
peptides and induce feelings of fullness by inhibiting gastric emptying, reducing gastric motility, and decreasing intestinal transit time (Carroll, et al., 2007).

C. Conclusion

Obesity is a problem that continues to escalate in developed and developing countries. It is unlikely to retreat without input from multidisciplinary groups that include physicians, psychologists, physical therapists, and dietitians. The problem is now evident in children and adults alike, prompting America’s First Lady to establish the “Let’s Move” program, which is geared towards helping children deal with obesity.

Beans are inexpensive, nutrient-rich commodities widely available in a variety of types. Vegetarians and vegans worldwide incorporate beans in many dishes as part of their daily eating pattern. Beans have the potential to be a food that aids in displacing fatty and unhealthy foods that promote obesity. Previous research showed that, among other qualities, beans are filling and lower cholesterol.

Daniel 1 tells the story of the four Hebrew teenagers who refused to eat and drink from the king’s table. After much protest, Daniel and his friends offered to conduct the first case-control nutrition study in history. They hypothesized that after 10 days of eating pulses and vegetables they would be healthier than those who were eating the king’s rich food and drinking wine. At the end of the study, Daniel, Hananiah, Mishael and Azariah not only looked healthier than their counterparts who ate from the king’s table; they were also more knowledgeable and skillful than the other boys. As a result, all the young men in the king’s courts were fed as the four young Hebrew boys were. The value of legumes/pulses has not changed from those times. The current study suggests that there may be some benefit to consuming beans before a meal.
D. Future Application

The fight against obesity can no longer be taken lightly. It has been linked to many health issues and reduced lifespan. Further research is needed which incorporates beans to examine the effects of beans on long term weight control, appetite hormones, and satiety. Future research should utilize a third meal condition that does not include beans as a control.

The study was limited by the fact it took place one week prior the subjects beginning a high fiber, low carbohydrate diet. In the future, the diets should be repeated with a washout period of at least one week between treatments instead of one day, as in this study. Other adjustments that may be useful in future studies are providing the participants with standardized meals instead of giving them a choice of foods to consume, excluding participants who already include large amounts of beans in their daily eating pattern, those who need their food prepared in a particular method, and those who eat at a very slow rate. Due to the time sensitive blood draws, future studies should seek to have the necessary personnel to ensure that the blood is drawn at the specified time through an indwelling cannula.
REFERENCES


APPENDIX A

IRB APPROVAL

INSTITUTIONAL REVIEW BOARD
Initial Approval Notice - Expedited Review

TO: Tonstad, Serena
Department: Health Promotion & Education
Protocol: A very high fiber diet versus a low carbohydrate diet for weight loss in obese men and women with and without type 2 diabetes

This study was review and approved administratively on behalf of the IRB. This decision includes the following determinations:

Risk to research subjects: Minimal
Situations of approval: Exclude individuals weighing

Consent Form
Unless IRB has given a specific waiver of informed consent (as documented in the approval stipulations above) the IRB approved and stamped consent form accompanies this letter. This now becomes the official master consent form for making copies to provide to study participants.

Adverse Events / Protocol Changes
The IRB should be notified in writing of any modifications to the approved research protocol. Adverse effects must be reported to the IRB in accordance with institutional policy. If sponsor or contractual adverse event reporting requirements differ from requirements for reporting to IRB, all reporting requirements must still be met.

Protocol Review
Your protocol is tentatively scheduled for review and renewal at least two weeks prior to the approval expiration date indicated above. To assure uninterrupted approval of this project, you will be sent a report form to request renewal by completing and timely returning to Office of Sponsored Research. Anticipate the approval expiration so your study does not lapse, contact IRB for assistance if necessary. In addition to reporting the requested renewal status information, you may also use the form to close the study at that time, if applicable.

Records
All records relating to this project, including signed consent forms, must be kept on file for three years following completion of the study. Please note the PI’s name and the IRB number assigned to this IRB protocol (as indicated above) on any future communications with the IRB. Direct all communications to the IRB at the Office of Sponsored Research. Thank you for your cooperation in LLU’s shared responsibility for the ethical use of human subjects in research.

Signature of IRB Chair/Designee

Loma Linda University Advanced Health Science Center holds Federalwide Assurance (FWA) No. 000147 with the U.S. Office for Human Research Protections, and the IRB registration no. 4, IRB00298. This Assurance applies to the following institutions: Loma Linda University, Loma Linda University Medical Center (including Loma Linda University Children’s Hospital, LLU Community Medical Center), Loma Linda University Behavioral Medicine, and affiliated medical practice groups.

IRB Chair: Rhoder L. Rigby, M.D.
Department of Medicine
(909) 558-2941, rigbyrl@lhu.edu

IRB Administrator: Linda G. Halverson, M.A., Director
Office of Sponsored Research
Ext 430570, Fax 80131, halverson@lhu.edu

IRB Specialist: Mark Tendlerman
Office of Sponsored Research
Ext 430401, Fax 80131, mrtendlerman@lhu.edu

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APPENDIX B

INFORMED CONSENT

LOMA LINDA UNIVERSITY
School of Public Health

Effect of pre-meal fiber ingestion versus no pre-meal fiber ingestion on gastrointestinal hormones in obese men and women substudy

Informed Consent Document

This is a substudy of the long-term diet research project, which is also by Dr. Serena Tonstad, Medical Doctor of Preventive Medicine in the School of Public Health at Loma Linda University. The information below will inform you about a substudy in which you may be interested in participating. This substudy is optional, if you take part in the long-term diet study, you are not obligated to partake in this substudy. Please take your time and read the information carefully before deciding whether or not you wish to participate in this study.

Why is this study being done?
You are invited to participate in this substudy because we are drawing participants from the long-term study and because your body mass index falls within the desired range for this study (BMI 30-42 kg/m²). The aim of this study is to evaluate the effects of pre-meal fiber ingestion versus no pre-meal fiber ingestion on gastrointestinal hormones in obese individuals. The study will require 2 sessions of 5 hours each and will include 26 participants.

What is involved in this study?
This is a trial to study the effects of pre-meal fiber on gastrointestinal hormones. Participants in this substudy will be required to eat two standardized meals that contain 18 grams of fiber for men and 14 grams of fiber for women with and without a pre-meal dose of fiber. This will involve eating the lunch provided on two different days, a Tuesday and the following Thursday. Whether you get the pre-meal fiber on the first or second day will be decided at random. There will be 5 hours of monitoring blood hormone levels before and after the meal. Blood samples will be taken at pre-set intervals from an indwelling catheter.

For 24 hours prior to participating in the substudy, you will be required to prepare and eat all meals from breakfast the day before through breakfast the day of the substudy. The menu will be as follows:

<table>
<thead>
<tr>
<th>Initials</th>
<th>Date</th>
<th>Page 1 of 3</th>
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Loma Linda University
Adventist Health Sciences Center
Institutional Review Board
Approved 10/23/09, Valid after 10/31/2010

A Seventh-day Adventist Institution
SCHOOL OF PUBLIC HEALTH | Loma Linda, California 92350
(909) 558-4546 · fax (909) 558-4087 · www.llu.edu
Breakfast – Blueberry bagel, cream cheese, strawberry yogurt, banana, orange juice
Lunch – Ham or turkey & Swiss sandwich, pretzels, grapes, chocolate chip cookie
Dinner – Spaghetti with meat sauce, tossed salad with Italian dressing, breadstick
Breakfast - Rice Krispies with milk, blueberry muffin, strawberry yogurt, orange juice

Lunches in substudy will be provided for you.

A needle for drawing blood will be inserted into a vein in your forearm prior to the start of the meal. The needle will stay there for the entire session and will be used to draw blood samples throughout the session. A blood sample will be taken prior to the meal. Then blood samples will be taken at 30, 60, 120, and 240 minutes after the meal. Each time a sample is taken, 4 tubes of about 1 teaspoon each will be drawn. This will be performed each of the two days of the study. This will add up to be about 11 tablespoons in all. The results of the blood tests will not be provided to participants until after the end of this study.

Who can take part?
Obese men and women (BMI 30-42 kg/m²) over the age of 18 who are not pregnant or lactating, do not have active cancer, are not participating in other research, have not dieted within the past 3 months with weight loss of more than 4 lbs, and are not vegans can take part in this study. If you have been placed on a special treatment by your doctor or you are being treated for other medical or surgical problems, you may need the consent of your doctor to certify that you are fit to participate in this study.

What are the risks of the study?
Pain and minor bleeding or bruising at the site of needle placement are possible risks of participating.

Are there benefits to participating in the study?
Although you are unlikely to benefit personally, the information obtained from this study may help us understand how the body reacts to different types of meals and develop better alternatives for weight management.

What are my rights as a participant?
Participation in this study is voluntary. Your decision whether or not to participate or terminate at any time will not affect your present or future medical care/relationship to the school, medical center, or Diabetes Treatment Center. If you decide to stop, please inform the study investigator.

How will my privacy be protected?

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</table>
Effect of pre-meal fiber ingestion versus no pre-meal fiber ingestion on appetitive hormones in obese men and women.

Informed Consent Document

All of the information collected during this study will be kept strictly confidential. Your personal information will be kept in a secured place including password protection in a computer database. Additionally, after all information is collected, your name will be deleted from the information and it will be replaced with a serial number called an identifier. Any publication resulting from this study will refer to the participants as a group.

Are there any costs or other payments?
There are no costs to you for participating in this study. The blood test will be performed at no cost to you. You will be paid the sum of $50 for completing the substudy.

Whom do I call if I have questions about my rights as a research participant?
If you wish to contact an impartial third party not associated with this study regarding any question or complaint you may have about this study, you may contact the Office of Patient Relations, Loma Linda University Medical Center, Loma Linda, CA 92354, call the Office of Patient Relations at (909) 559-4647, or e-mail patientrelations@llu.edu for information and assistance.

Informed consent statement
I have read the contents of this consent form, and have listened to the verbal explanation given by the investigator. My questions concerning this study have been answered to my satisfaction. I hereby give voluntary consent to participate in this study. This consent does not waive my rights or the does it release the investigator, institution or sponsors from their responsibilities. I may call the faculty advisor, Serena Tonstad, MD, PhD, at Loma Linda University, Department of Health Education and Promotion during routine office hours at (909) 558-4741 or Debbie Clausen, RN, MSN, nurse manager for the Diabetes Center at (909) 558-3022 if I have additional questions or concerns. I have been given a copy of this form for future reference.

I hereby give voluntary consent to participate in this study.

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<th>Subject name (print)</th>
<th>Signature</th>
<th>Date</th>
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**Investigator’s Statement**
I have reviewed the contents of the consent form with the person signing above. I have explained potential study risks and benefits.

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<tr>
<th>Investigator name (print)</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
</table>

Initials __________
Date __________

Page 3 of 3
APPENDIX C

STANDARDIZED MEALS FOR MEN

Standardized Meals

Thank you for participating in the pre-meal fiber study. Below are menus for Monday, Wednesday meals and for breakfast on Tuesday and Thursday. What you eat for Dinner on Tuesday and Thursday is up to you.

Thank you.
Lisa

Monday and Wednesday Meals for Men

Breakfast
1 medium size bagel or 2 slices of toast
2 tablespoons cream cheese
6 oz of yogurt
1 medium size banana or other fruit
1 cup orange juice or other fruit juice

Lunch
1 turkey (or ham) sandwich: 2 slices bread, 2 slices turkey, 2 slices cheese
1 bag (single servings) Pretzels
1 cup grapes or other fruit
2 small cookies

Dinner
1 1/2 cup spaghetti with meat sauce
1 cup lettuce salad with Italian dressing
1 medium breadstick or bread roll

Tuesday and Thursday Breakfast for Men

3/4 cup dry cereal (corn flakes, rice krispies, cheerios, etc.) with 1/2 cup 2% milk
1 medium size muffin or a slice toast with margarine and jelly
6 oz yogurt
1 cup orange juice or other fruit juice
APPENDIX D

STANDARDIZED MEALS FOR WOMEN

Monday and Wednesday Meals for Women

**Breakfast**
½ medium size bagel or 1 slice of toast
2 tablespoons cream cheese
6 oz (3/4th cup) yogurt
1 small size banana or other fruit
1 cup orange juice or other juice

**Lunch**
1 turkey (or ham) sandwich: 2 slices bread, 2 slices turkey, 1 slice cheese
1 small bag Pretzels (1 serving)
1 cup grapes or other fruit
1 small cookie

**Dinner**
1 cup spaghetti with meat sauce
1 cup lettuce salad with Italian dressing
1 medium breadstick or bread roll

**Tuesday and Thursday Breakfast for Women**
½ cup dry cereal (corn flakes, rice krispies, cheerios, etc.) with ½ cup 2% milk
1 small muffin or 1 slice toast with margarine and jelly
6 oz (3/4th cup) yogurt
1 cup orange juice or other fruit juice
APPENDIX E

TEST LUNCH MENU

Test Day Lunch

½ cup Refried Beans
1 High Fiber Enchilada (3/4 for women)
1/3 cup Brown Rice
½ cup Corn
½ cup Rice Pudding
APPENDIX F

HIGH FIBER ENCHILADA

High Fiber Enchilada

8 oz lean beef
1 onion chopped
1 tomato chopped
½ cup frozen corn kernels
2 cloves garlic minced
2 tsp chili powder
1 tsp dried oregano
1 tbsp whole wheat flour
6 multigrain tortilla
10 oz mild red enchilada sauce
1/3 cup shredded cheese, Mexican blend, reduce fat

Preheat oven to 350°F. Coat a 13” x 9” baking pan with cooking spray. Heat a large nonstick skillet over medium high heat and add the beef; cook, stirring with wooden spoon to break up chunks, until no longer pink, 3 to 4 minutes. Stir in onion, tomato, corn and garlic; cook until the onion starts to soften, 1 to 2 minutes. Stir in chili powder and oregano and cook, stirring for 30 seconds. Sprinkle with flour and cook, stirring occasionally until thickened, about 1 to 2 minutes. Remove from heat. Wrap the tortilla in between damp paper towels and microwave until warm, 20 to 40 seconds.

Place ½ cup of beef mixture in a line across the center, parallel to the edge of counter closest to you. Roll up jelly roll style and place seam side down in the prepared baking dish. Repeat with remaining tortillas and filling. Pour the sauce over the enchilada and sprinkle with cheese. Bake until hot and cheese has melted, 18 to 20 minutes.
Name: ___________________

Visual Analogue Scale 1

Please indicate your answer to the questions below by making an “X” along the line (eg. X-)

I don’t want anything to eat!

How hungry do you feel? I am starving. I want to eat right now!

I am not full at all. I can eat a lot more!

How full do you feel? I am stuffed! I can’t eat anything else!

Time: ___________________

Visual Analogue Scale 2

Please indicate your answer to the questions below by making an “X” along the line (eg. X-)

I don’t want anything to eat!

How hungry do you feel? I am starving. I want to eat right now!

I am not full at all. I can eat a lot more!

How full do you feel? I am stuffed! I can’t eat anything else!

Time: ___________________
Table A-1. Postmeal AUC (30-180 min) for plasma GI peptides, insulin, glucose, and for subjective responses in obese women and men after consuming staggered and control meals for 14 participants

(Mean values with standard deviations)

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<th>Control bean meal</th>
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