Effects of macronutrient and caloric content of the diet on circulating concentrations of ghrelin and other hormones involved in energy metabolism

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ABSTRACT

Investigating the role of ghrelin and hormones involved in energy metabolism on energy balance and body composition when varying diets and caloric contents are consumed could provide insight into the etiology of obesity and related diseases. In this dissertation, I investigated the effect of Atkins and AHA diets in normal weight and overweight women and men in both the fed and fasted states on the change in circulating concentrations of ghrelin (acylated and total), adiponectin, glucagon, growth hormone, insulin, and leptin before and after consuming lunch and over waking hours (07:00 to 22:00). Eight subjects, ages 20-32, participated in each study: four normal subjects with a normal BMI and four overweight subjects with an overweight BMI. Each subject received both diets by a crossover design. Two normal and two overweight subjects were assigned to each diet. The Atkins’ diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid, and the AHA diet contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Diets were isocaloric. Each diet was fed for 14 days, and then subjects switched to the other diet. Blood was taken every hour from 7 am to 9 pm on days 13 and 27 of the study. Plasma samples were analyzed for active and total ghrelin, leptin, insulin, glucagon, growth hormone, and adiponectin. Lunch results: in women, acylated ghrelin concentrations were influenced by the interaction of diet and weight. Men consuming the AHA diet had an increase in acylated ghrelin concentrations over the lunch period, whereas, there was no change in acylated ghrelin concentrations in men consuming the Atkins diet. Women and men consuming the Atkins diet had higher glucagon concentrations before lunch. When consuming the AHA diet, overweight women had a greater increase in insulin that of normal weight women. In overweight women, the change
in insulin concentrations was greater when the AHA diet was consumed in comparison to the Atkins diet. Result for 14-hour study: acylated ghrelin did not have preprandial increases before breakfast and lunch with varied responses before dinner in overweight and normal weight women fed either the Atkins or AHA diets in both sequences. Plasma glucagon was greater after lunch through the end of the 14-hour period in women consuming the Atkins diet. Insulin concentrations were higher and had greater responses to meal ingestion in women consuming the AHA diet in comparison to the Atkins diet. Men consuming the AHA diet in period 2 had increased acylated ghrelin and total ghrelin concentrations in comparison to when the men consumed the Atkins diet. Insulin concentrations were suppressed in men consuming the Atkins diet in comparison to men consuming the AHA diet. Glucagon concentrations were higher in men consuming the Atkins diet in comparison to the AHA diet after lunch until the end of the study period (hours 7-14). Weight class affected some hormone concentrations different and thus women and men in overweight and normal weight classes were analyzed separately. Each class of macronutrients influences circulating hormone concentrations. Experimental design is critical in obtaining information on the relationship of hormones related to appetite and body composition. Our results indicate that current recommendations may not be appropriate for all individuals as men and women both normal weight and overweight have different hormone responses to the macronutrient content of the diet.
Chapter 1

General Introduction

Dissertation Organization

This dissertation is to partially fulfill the requirement of the degree of Doctor of Philosophy. This dissertation is organized into chapters. Chapter 1 consists of the dissertation organization, rationale, literature review, objectives of this research and literature cited. Chapter 2 consists of two manuscripts to be published as part I and part II in the Journal of Clinical Endocrinology and Metabolism. Chapter 3 consists of two manuscripts to be published as part I and part II in the Journal of Clinical Endocrinology and Metabolism. Chapter 4 consists of a manuscript that has been submitted to the Journal of Nutrition. The literature review introduces the background of the topics addressed in the manuscripts in the main body of the dissertation. Each manuscript is complete on its own and has an abstract, introduction, material and methods, results, discussion, literature cited, tables, and figures. Manuscripts are formatted and written according to the requirement of the journals. Following the last manuscript, the last chapter contains a general conclusion section and future research section followed by the acknowledgements.

Rationale

Obesity is a major health concern for adults and children. Obesity is associated with increased risk of many diseases such as coronary heart disease, stroke, and type II diabetes. According to the CDC, from 1999-2008, 33.8% of the total population of the United States was obese and 68 % was overweight or obese. Of total men, 32.3% were obese and 72.3%
were overweight or obese. Of total women, 35.5% were obese and 64.1% were overweight or obese. Numbers of obese and overweight individuals in the United States were stable form 1960-1980. From 1988-1994, the prevalence of obese increased by 8% and increased further in 1999-2000 for both sexes and all age groups (1). Currently, it appears that obesity rates have reached a plateau and have not changed over the past 10 years for women and the linear trend for men has no change in the last three years (2). Obesity rates vary among races, as obesity is higher in African American than in white Americans. Much of the research on ghrelin and other hormones involved in energy homeostasis has been conducted in normal weight individuals compared to obese individuals or with populations with known disease states or genetic conditions. Understanding diet and hormone interaction in the progression of obesity is crucial in finding a solution to this growing nutritional problem in America. Investigating the role of ghrelin on energy balance and body composition when varying diets are consumed could provide insight into the etiology of obesity.

**Literature Review**

While there are many hormones involved in various processes in the body, this dissertation will be focusing a small subset of hormones that are involved in energy metabolism. There are numerous hormones involved in the gastrointestinal tract and for the purpose of this literature review; we will be looking at ghrelin (total and acylated), insulin, and glucagon. Other hormones discussed in this review will be leptin and adiponectin, which are synthesized in the adipose tissue and growth hormone, which is synthesized in the anterior pituitary.
**Ghrelin**

Ghrelin is a peptide hormone that acts upon the growth hormone secretagogue receptor (GSH-R). The GSH-R was discovered in 1997. Many synthetic compounds act upon this receptor to change the animal’s body composition. Since the discovery of the GSH-R, the search for an endogenous ligand has been conducted. In 1999, ghrelin was discovered to be an endogenous ligand of this receptor (3).

Ghrelin is synthesized in the arcuate nuclei and oxyntic glands of the stomach. Stomach ghrelin is thought to be involved in physiological effects and possibly to stimulate the secretion of growth hormone. Some of the physiological effects of ghrelin are hyperglycemia in humans, adiposity in rodents (4), increased gastric acid secretion in rats (5), and increased gastric motility in rats (5).

Ghrelin can be classified into two major circulating forms, n-octanoyl ghrelin and des-acyl ghrelin. Both acylated and des-acyl ghrelin have roles in energy homeostasis (6-7). Acylation of ghrelin is necessary for the orexigenic and growth hormone stimulation activities. The majority of research published on ghrelin has been research on total ghrelin, which is all forms of ghrelin (8).

Since the discovery and isolation of ghrelin, many studies have been performed to determine the function of ghrelin in the body. Ghrelin stimulates growth hormone release independent of the growth hormone releasing hormone (3). Additionally, leptin activity is controlled by ghrelin. Ghrelin is an antagonist of leptin by acting upon the neuropeptide
Y/Y1 receptor pathway (9). Leptin causes satiety, whereas ghrelin stimulates nutrient intake (10). Leptin and ghrelin thereby regulate the action of each other in the brain.

Several studies have shown the effects of diet composition on circulating ghrelin concentration with respect to obesity. Most studies have involved ghrelin and its effects on healthy humans in comparison to obese humans or humans with specific disease states such as anorexia nervosa (AN), polycystic ovary syndrome and chronic heart failure or surgical modifications of the stomach. Patients with AN have higher plasma ghrelin concentrations than do normal humans (11). Furthermore, plasma ghrelin in AN patients was associated negatively with body mass index (BMI) values. Patients with chronic heart failure and cachexia have high plasma ghrelin concentrations (12). Ghrelin administered to patients with chronic heart failure improves hemodynamic function (13). Ghrelin could serve two roles in chronic heart failure: creating a positive energy balance and improve hemodynamic function in the patients. There have been a number of studies conducted with obese humans and the effects of gastric bypass surgery on plasma ghrelin concentrations. Patients who had gastric bypass surgery have lower plasma ghrelin concentrations (14). Lower plasma ghrelin concentrations in gastric bypass patients could be explained by the surgery. Because ghrelin is primarily produced in the stomach, gastric bypass may have an effect on the ghrelin-producing cells in the fundus of the stomach. This observation would explain the long-term weight loss with gastric bypass surgery.

Plasma ghrelin concentrations in obese humans are lower than ghrelin concentrations of normal weight individuals (15). English and colleagues demonstrated that refeeding after
fasting did not decrease the ghrelin concentrations in obese human patients (16). In normal weight humans, fasting ghrelin concentrations decreased after feeding. Salbe and colleagues showed that ghrelin has a negative association with *ad libitum* feed intake (17). Studying the effects of diet composition on ghrelin concentrations in both normal and obese patients is necessary to fully understand the mechanisms by which the body controls feed intake and body composition. Thus, understanding the regulation of ghrelin under conditions of weight gain and loss could provide insight into understanding obesity (15).

In 2002, Beck showed that rats fed a high carbohydrate diet had higher plasma ghrelin than those a low carbohydrate diet (18). This study was a long-term feeding study and demonstrated the long-term effects of diet on ghrelin concentration. In a study by Monteleone in 2003, healthy non-obese women were fed either a high fat or high carbohydrate meal (19). The high carbohydrate meal caused the greatest increase in plasma ghrelin. Also, hunger sensation of subjects fed the high carbohydrate diet was suppressed more than that of subjects fed the high fat diet. Ghrelin concentration, however, increased with weight loss of humans when eating a low fat, high carbohydrate diet (20). Diet-induced obesity, however, was not related directly to ghrelin concentration in juvenile rats prone to obesity. In the Weigle study, high fat diets decrease adiposity without increasing appetite. Most studies measure total ghrelin and not the acylated form (8). The few studies involving ghrelin and diet composition have conflicting results, leaving the relationship between ghrelin and diet composition unclear.
The research on the effects on dietary macronutrient composition on ghrelin concentrations is equivocal. The majority of research on ghrelin has been focused on total ghrelin. It is not always clear which form of ghrelin (acylated, desacylated, or total) is being reported on as the articles state ghrelin without indicating which form was measured in the methods section. Blom and colleagues showed that men consuming a high protein breakfast had decrease in total ghrelin concentrations at three and four hours post lunch than that of men consuming a high carbohydrate breakfast; however, there was no change observed in acylated ghrelin concentrations in men between the high carbohydrate and the high protein breakfast (21). Another study indicated that acylated ghrelin concentrations were lower in normal weight women consuming a high protein breakfast in comparison to a normal protein breakfast (22). However, no difference in ghrelin concentrations was observed in women consuming normal and high casein diets or in men and women consuming normal and high soy protein diets (23-24). Results vary based on what form of ghrelin is measured, composition of the dietary treatment, the form and type of the macronutrient, sex of the participants, and timing of the sample taken in the research.

Most of the high protein diets studied with respect to ghrelin concentrations contained between 20-35% of calories as protein with a range of carbohydrate of 35% to 55% of calories; (22-25). The protein source in some of the studies is in liquid form only, powders, or from specific sources such as soy; whereas, the protein in the Atkins diet in this study is from a variety of sources and forms. Most studies done on total ghrelin have measured the effect of a breakfast meal or lunch meal on total ghrelin concentrations over a 3-4 hour period (21, 25). Studies have shown the total ghrelin concentrations were lower in subjects
consuming a high protein meal 3-4 hours post meal in comparison to subjects consuming a high carbohydrate meal (26).

Caloric content of the diet has been shown to suppress postprandial acylated ghrelin concentrations proportional to the amount of calories ingested and that calories were not the only determinant of next meal initiation (27). When fasted rats were given either water or a glucose solution, ghrelin concentrations decreased after the glucose solution was administered but not when just water was administered, demonstrating that the amount ingested is not the signal for ghrelin suppression rather the composition what is ingested (28). When the pyloric sphincter was blocked, there was no difference in ghrelin concentrations between rats given water or rats given glucose solution indicating that the sensing of the caloric/nutrient content of the ingested food occurs past the stomach (28).

Despite a number of studies of plasma ghrelin and its effects on obesity, the mechanism by which ghrelin causes adiposity remains unknown. Plasma ghrelin concentrations in obese humans are lower than those of normal weight individuals (15). Cerebrospinal fluid (CSF) ghrelin is lower in obese humans than in normal weight humans (29). Obese humans have lower ghrelin concentrations both in plasma and CSF. This is contrary to the findings in rodents where ghrelin was injected and an increase in adiposity was shown (4). Studying the effects of diet composition on ghrelin concentrations in both normal and obese patients is necessary to fully understanding the mechanisms by which the body controls feed intake and body composition. Thus, understanding the regulation of ghrelin under conditions of weight gain and loss could provide insight into understanding obesity (15).
Adiponectin

Adiponectin is one of several adipokines synthesized and secreted from the adipose tissue. In addition, adiponectin is known as adipoQ, Acrp 30, apM1, GBP28. Research has shown the adiponectin increases insulin sensitivity, increases glucose uptake in muscle, decreases liver gluconeogenesis and stimulates β-oxidation of fatty acids as well as being an anti-inflammatory agent (30-32). Adiponectin increases in fatty acid oxidation through activation of AMPK in skeletal muscle, which is how adiponectin is thought to increase insulin sensitivity (33-34). Adiponectin decreases gluconeogenesis by activating AMPK in the liver, resulting in reduced rate of hepatic glucose production (33, 35).

Leptin and adiponectin regulate body weight in tandem with the effects on the brain to be additive. Concentrations of adiponectin are inversely proportional to fat mass in adults whereas leptin concentrations are elevated in obesity. In obese subjects, adiponectin concentrations are lower than in normal weight counterparts (36). Inflammation is thought to play a role in obesity. Considering the role of adiponectin as an anti-inflammatory agent, the decrease in adiponectin concentrations in obese individuals may play a role in the inflammatory aspect of obesity.

Some studies have shown that there is no effect of diet on adiponectin and that adiponectin has small changes in concentration from the fasted to postprandial states (27, 37). However, no studies have measured the effects of varying dietary compositions on the concentrations of adiponectin over the course of a day. In addition, the effects of diet on varying weight classes on adiponectin concentrations have not been well characterized.
Glucagon

Glucagon is produced by the pancreatic α-cells. Glucagon maintains acute blood glucose concentrations therefore, the lack of an increase in glucagon concentrations in the fasted state may be the result of the switch from immediate regulation of blood glucose by glucagon to short term fasting regulation of blood glucose by epinephrine and the increase in glucagon later in the day is a result of the low carbohydrate content of the diet (38-39). Glucagon is the second regulator when blood glucose concentrations are low. The first step in blood glucose regulation is the decrease in insulin concentrations then the release of glucagon from the pancreas (39-40).

Glucagon may play an important role in the maintenance of blood glucose concentrations when low carbohydrate diets are consumed. A long-term study of a low carbohydrate diet showed no differences in insulin, glucagon, or glucose at fasting (40). However, this does not take into account that epinephrine is more likely responsible for the maintenance of blood glucose after an overnight fast and thus glucagon concentrations may be different from the concentrations measured at fasting.

Insulin

Insulin has two primary roles: regulation of blood glucose concentrations and an adiposity signal. Insulin regulates plasma glucose concentrations and energy balance (41). Insulin and leptin regulate each other with respect to energy stores. Leptin inhibits insulin secretion when energy stores in the adipose tissue are sufficient whereas, insulin stimulates leptin secretion from white adipose tissue when in positive energy balance (42). However, leptin
does not regulate the short-term effects of insulin secretion, which regulates blood glucose concentrations.

Insulin concentrations have been shown to be lower when a high protein diet was consumed versus a high carbohydrate diet (43). This response would be related to the role of insulin in regulating blood glucose concentrations. Insulin sensitivity can be altered by the composition of the diet. When a low carbohydrate diet is consumed, fatty acids are released into the blood and ketone bodies are produced for use as fuel in cells that do not need glucose.

**Leptin**

Leptin is one of the two adipokines discussed in this dissertation. Adipokines or adipocytokines are synthesized and secreted by the adipose tissue. In the 1950’s, it was hypothesized that there was a factor in the blood that controlled food intake when energy store were high (44). In the late 1950s, the discovery of the ob and db loci resulted in the thought of a satiety factor associated with the ob loci (45). Leptin is a 16 kDa protein hormone secreted from the adipose tissue. Leptin is significantly higher in women than in men of the same body mass and of similar fat mass (46-48). Obese individuals have higher leptin concentrations in comparison to normal weight individuals (47). Leptin is sensitive to the fasting state and starvation than to excess energy from overfeeding (49).

Leptin concentrations peak at 02:00 and decrease over time and remains stable over waking hours when humans were fed a high fat/low carbohydrate or a high carbohydrate/low
fat diet (50). Another study demonstrated that leptin concentrations remained low from 06:00 and begin a gradual rise at 18:00 toward the 02:00 peak (51). For detection of changes of leptin in response to a diet, a 24-hour curve is the best measure of changes in leptin over time. However, measuring leptin concentrations at fasting would give an accurate picture of the leptin concentrations during waking hours (07:00-22:00) in most humans. Leptin is acutely affected by food intake (43, 52, 53). However, this effect is seen by changes in the overnight concentration, which is not correlated with fasting concentrations. Most studies measure leptin concentrations at fasting only or during the waking hours of humans. These measurements explain the lack of large changes in leptin in response to changes in macronutrient composition.

**Growth Hormone**

Growth hormone’s (GH) primary role is the promotion of growth. Growth hormone concentrations are lower in obese humans and decrease with age (54). Obese individuals have lower GH concentrations in comparison to normal weight women (54). The pulses of GH are less frequent and the half-life of GH is shorter in obese humans but can be partially recovered by weight loss (54-55).

Obesity is a risk factor for type II diabetes. Insulin resistance can be caused by long term fasting as well as obesity (56). In long-term fasting, GH concentrations are elevated as well as the concentration of free fatty acids (FFA) in the blood (57). Growth hormone promotes lipolysis, which increase the concentrations of FFA in the blood (58, 59). The products of the breakdown of FFA during fasting or a low carbohydrate diet are ketone bodies (60). Ketone bodies decrease the concentrations of specific amino acids in the blood (61, 62).
When a low carbohydrate diet is consumed, the body relies on lipids, specifically ketone bodies, for energy. Growth hormone promotes lipolysis while sparing protein thus maintaining fat free mass (63).

Growth hormone actions on lipid and glucose metabolism are opposite of the actions of insulin. Insulin inhibits lipolysis and increases glucose uptake whereas GH increases lipolysis in adipose tissue and inhibits glucose uptake in the muscle. Growth hormone impairs insulin sensitivity (38). Adiponectin increases insulin sensitivity of the muscle and increase β-oxidation of fatty acids (33, 34). The complex relationship of GH, insulin and adiponectin had yet to be clarified. Obese individuals have lower concentrations of adiponectin and GH while insulin concentrations are higher. Understanding this relationship of GH, insulin, and adiponectin may give insight into the treatment of type II diabetes.

In Chapters II and III, we investigated the effect of the American Heart Association and Atkins diet on ghrelin and other hormones involved in energy metabolism. The overall objective of these projects was to elucidate the relationship of diet composition and ghrelin concentration with respect to obesity. To accomplish the overall objective, we compared the variation of ghrelin over 15 hours in lean and overweight female and male humans. We will compare the effects of two common diets, Atkins’ and American Heart Association (AHA), on ghrelin, other hormones associated with energy metabolism, and blood metabolite concentrations in blood.
The specific aims of this project were to:

1. Analyze plasma for ghrelin (total and acylated), insulin, leptin, glucagon, adiponectin and growth hormone concentration variation during the waking hours of humans,

2. Analyze plasma for blood metabolites from normal and overweight females,

3. Compare plasma ghrelin (total and acylated), insulin, leptin, glucagon, adiponectin and growth hormone concentrations during the waking hours of humans who consume the Atkins’ and AHA diets,

4. Determine body composition of female subjects before, during, and after the diet study, and

5. Determine the effects of the consumption of breakfast (fed or fasted state) on the concentrations of ghrelin (total and acylated), insulin, leptin, glucagon, adiponectin and growth hormone before and after the noon meal.

Completion of these aims will provide necessary information on the influence of two diets with highly divergent composition on plasma ghrelin and related hormones. A long-range goal is that practical procedures may be developed to regulate obesity development through dietary manipulation of plasma ghrelin, which regulates food intake.

In Chapter IV, we investigated the effect of overnutrition and undernutrition on neonatal calves as a model for human neonates. Calves were assigned to no-, low-, and high growth groups. We measured the concentrations of ghrelin (total and acylated), insulin, and
glucagon weekly in the calves. In addition, we measured the concentrations of total and acylated ghrelin over the course of the light cycle in a subset of the calves.

**Literature Cited**


Chapter II: Part I

Effect of American Heart Association and Atkins diets on the change in circulating concentrations of ghrelin and other hormones involved in energy metabolism: comparison of the fed and fasted states over lunch period in normal and overweight women

A manuscript for submission to the Journal of Clinical Endocrinology and Metabolism

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ABSTRACT

Investigating the role of appetite-related hormones on energy balance and body composition in women when varying diets are consumed could provide insight into the etiology of obesity. In this study, we investigated the effect of Atkins and AHA diets in normal weight and overweight women in both the fed and fasted states on the change in ghrelin (acylated and total), adiponectin, glucagon, growth hormone, insulin, and leptin before and after consuming lunch. Eight female subjects, ages 20-32, participated in this study: Four normal subjects with an average BMI of 21.3 and four overweight subjects with an average BMI of 26.7. Each subject received both diets by a crossover design. In period one, two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Diets were isocaloric. Each diet was fed for 14 days, and then subjects switched to the other diet. On days 6 and 20, breakfast was consumed before 08:00 and blood was taken at 11:00 and 1 hr after the noon meal. On days 14 and 28, subjects were fasted and blood samples were taken at 11:00 and 1 hr after the noon meal. Acylated ghrelin concentrations were influenced by the interaction of diet and weight. Normal weight women consuming the AHA diet had a greater change in acylated ghrelin concentrations (P < 0.01). Women consuming the Atkins diet had higher glucagon concentrations before lunch (P < 0.0001). When consuming the AHA diet, overweight women had a greater increase in insulin than that of normal weight women (P <0.05). In overweight women, the change in insulin concentrations was greater when the AHA diet was consumed in comparison with the
Atkins diet (P < 0.05). Hormone concentrations responded differently to diet, weight class, fed or fasted state, sequence of the diets, and the interactions of these variables. These results indicate a varied relationship of appetite-related hormones with respect to diet composition.

**INTRODUCTION**

Acylated ghrelin is an orexigenic peptide hormone (1). Leptin and insulin cause satiety, whereas acylated ghrelin stimulates nutrient intake (2,3). Leptin and acylated ghrelin regulate the action of each other. Additionally, leptin can inhibit insulin secretion based on the needs of the adipose tissue; moreover, insulin signals the secretion of leptin from white adipose tissue (4).

Macronutrient composition of the diet can affect hormone concentrations in the blood. Healthy non-obese women fed a high carbohydrate meal had a greater increase in plasma ghrelin in comparison to women fed the high fat meal (5). Also, hunger sensation of subjects fed the high carbohydrate diet was suppressed more than that of subjects fed the high fat diet. However, subjects fed a high protein breakfast felt more satiated than subjects fed a high carbohydrate diet (6). Ghrelin concentration, however, increased with weight loss in humans when eating a low fat, high carbohydrate diet, whereas high fat diets decreased adiposity without increasing appetite (7).

Results from studies with high protein diets are mixed. Increasing specific protein sources (soy and casein) in the diet did not affect ghrelin concentrations (8,9). Al Awar and colleagues showed that a high protein diet decreased acylated ghrelin concentrations,
whereas Blom and colleagues showed that a high protein diet resulted in no change in acylated ghrelin concentrations \((10,11)\).

In addition, weight class can influence the concentrations of hormones in plasma. Plasma ghrelin concentrations in obese humans are lower than those of normal weight individuals \((12)\). One study demonstrated that refeeding after fasting did not decrease the ghrelin concentrations in obese human patients \((13)\). In normal weight humans, fasting ghrelin concentrations decreased after feeding. In 2004, Salbe and colleagues showed that ghrelin has a negative association with \textit{ad libitum} feed intake \((14)\). Studying the effects of diet composition on ghrelin concentrations in both normal weight, overweight, and obese patients is necessary to understand the mechanisms by which the body controls food intake and body composition. Thus, understanding the regulation of ghrelin under conditions of weight gain, maintenance, and loss could provide insight into understanding obesity \((12)\).

The overall objective of this research was to elucidate the relationship of diet composition and ghrelin concentration with respect to obesity. We compared the effects of macronutrient composition of two common diets, Atkins New Diet Revolution (Atkins) and American Heart Association (AHA), on ghrelin and selected hormones associated with energy metabolism \((15,16)\). We hypothesized that overweight females will have greater ghrelin concentration in plasma. Furthermore, we hypothesized that subjects consuming the Atkins diet will have lower plasma ghrelin and lower insulin concentrations than subjects consuming the AHA diet.
MATERIALS AND METHODS

Subjects

Approval for all screening and study procedures was obtained from the Iowa State University Institutional Review Board. Each subject read and signed an informed consent document. Subjects were screened for BMI, eating disorders, and major health problems through an interview and physical examination. Volunteers were excluded if they have or had an eating disorder or have a major health problem such as diabetes, polycystic ovary syndrome, heart conditions, and hypoglycemia. Eight women were chosen for the study; four with normal weight BMIs and four with overweight BMIs (17, Table 1). Female subjects in each weight class were assigned randomly to treatment diets. One participant discontinued participation the 2nd day of the study for personal reasons and an alternate participant continued on the study.

Each subject received both treatment diets by a crossover design. Subjects were given 2000 kcal/day based on average energy intake of females of that age group (18). For the first period, two normal and two overweight subjects were assigned to the Atkins diet and the others to the AHA diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The AHA diet contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Diets were formulated using Nutritionist Pro (version 2.3; First DataBank, Inc.; San Bruno, CA) by a registered dietitian. All diets were prepared and served by personnel at the Human Metabolic Unit at Iowa State University. Participants consumed each diet for 14 days with a repeating 7-day menu for each diet. Meals were served between 08:00-09:00, 12:00-13:00, and 17:00-18:00 in the dining area of Human Metabolic Unit.
On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and 1 hr after completion of the noon meal (Fed state). On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00 (Fasted state). Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the completion of the noon meal. All blood was collected in EDTA-containing vacutainer tubes (BD Scientific, Franklin Lakes, NJ) and kept at 4 °C during processing. Samples were centrifuged at 3000 × g for 20 min. Plasma was collected and analyzed for adiponectin, ghrelin (acylated and total), glucagon, growth hormone, insulin, and leptin.

**Hormone Analysis**

Adiponectin concentrations were measured by using a commercially available radioimmunoassay (Linco Research, St. Charles, MO). The lower limit of detection was 1 ng/mL, and the intra-assay coefficient of variation was < 10%.

Total ghrelin concentrations were measured by using a commercially available radioimmunoassay kit (Linco Research, St. Charles, MO). The lower limit of detection was 93 pg/mL, and the intra-assay coefficient of variation was < 10%.

For acylated ghrelin samples, 50 µL of 1 N hydrochloric acid and 10 µL of 10 mg/mL phenylmethylsulfonyl fluoride (PMSF, Sigma, St. Louis, MO) in methanol were added for every 1 mL of plasma immediately after centrifugation. Acylated ghrelin concentrations were measured using a commercially available radioimmunoassay kit (Linco Research, St. Charles, MO). This assay has been found to be highly specific for acylated ghrelin with less than 0.1 % cross-reactivity for desoctanoyl ghrelin and no cross reactivity with ghrelin 14-28,
motilin-related peptide, leptin, insulin, glucagon, or GLP-1 (7-36). The lower detection limit was 7.8 pg/mL. The intra-assay coefficient of variation was < 10%.

For glucagon samples, 50 µL of aprotinin (5500 KIU/mL, Sigma, St. Louis, MO) were added to 1 mL of plasma with a final concentration of 550 KIU aprotinin per mL of plasma. All plasma samples were stored at -20 °C until assayed. Glucagon concentrations were measured by using a commercially available radioimmunoassay (Linco Research, St. Charles, MO). The lower limit of detection was 20 pg/mL, and the intra-assay coefficient of variation was < 10%.

Growth hormone concentrations were measured by using a commercially available radioimmunoassay (Diagnostic Products Corporation (DPC), Los Angeles, CA). The lower limit of detection was 0.9 ng/mL, and the intra-assay coefficient of variation was < 10%.

Insulin concentrations were measured by using a commercially available radioimmunoassay (Linco Research, St. Charles, MO). The lower limit of detection was 0.2 µU/mL (1.389 pmol/L), and the intra-assay coefficient of variation was < 10%.

Leptin concentrations were measured by using a commercially available radioimmunoassay (Linco Research, St. Charles, MO). The lower limit of detection was 0.05 ng/mL, and the intra-assay coefficient of variation was < 10%.

Statistics

Hormone data were analyzed by using the MIXED procedure in SAS (Version 9.1, SAS Institute, Cary, NC). All hormone data were analyzed as a split-plot with repeated measures ANOVA. The hormone concentrations for before lunch were analyzed. In addition, the change score of before lunch concentrations subtracted from 1 hr after lunch concentrations
were analyzed. The model included fixed effects of diet, weight (weight class), state (fed or fasted), sequence, diet*weight, weight*sequence, sequence*state, diet*weight*state, and diet*weight*state*sequence, while person(weight*sequence) was included in the model as the random effect. Significance of the interaction terms is listed in Table 2. No multiple comparison adjustments were made because this is an exploratory study. For all tests, P < 0.05 was considered significant and P < 0.1 was considered a trend.

RESULTS

Comparison of Fed and Fasted States Before Lunch

Acylated Ghrelin

The two-way interaction term of weight*diet in the model was significant (P = 0.016, Fig. 1). Normal weight women consuming the Atkins diet had lower ghrelin in comparison to normal weight women consuming the AHA diet (85.3 ng/L vs. 205.5 ng/L, P = 0.0029). Overweight women tended to have lower active ghrelin concentrations than normal weight when consuming the AHA diet (130.6 ng/L vs. 205.5 ng/L, P = 0.0818).

Total Ghrelin

The four-way interaction (diet*weight*timepoint*sequence) term in the model was significant (P = 0.016, Fig. 2). Normal weight women consuming the Atkins diet who ate breakfast had higher total ghrelin concentrations than did normal weight women consuming the AHA diet who ate breakfast when the Atkins diet was given before the AHA diet (1,492 ng/L vs 1,084 ng/L, P = 0.0293). The order in which the participants consumed the diets altered responses as well. Fasted normal weight women consuming the Atkins diet had
lower total ghrelin concentrations than did fasted normal weight women consuming the AHA diet when the AHA diet was administered before the Atkins diet (1,076 ng/L vs. 1,522 ng/L, P = 0.0192).

**Insulin**

The three-way interaction (diet*weight*state) term in the model was significant (P = 0.002, Fig. 3). In the fed state, overweight women consuming the Atkins diet had lower insulin concentrations that did overweight women consuming the AHA diet (58.6 pmol/L vs. 153.2 pmol/L, P = 0.0001). Overweight women consuming the AHA had higher insulin concentrations in the fed state in comparison to the fasted state (153.2 pmol/L vs. 42.9 pmol/L, P < 0.0001). In the fed state, overweight women consuming the AHA diet had higher insulin concentrations than did normal weight women consuming the AHA diet (153.2 pmol/L vs. 77.4 pmol/L, P = 0.0029).

**Glucagon**

Glucagon concentrations differed between the AHA and Atkins diets (P < 0.0001, Fig. 4). Women consuming the Atkins diet had higher glucagon concentrations than those consuming the AHA diet (122.5 ng/L vs. 89.1 ng/L). There were no significant effects of weight class, fed/fasted state, sequence or interactions on glucagon concentrations 1 hr before lunch in women.
Leptin

The three-way interaction (diet*weight*state) term in the model was significant (P = 0.004, Fig. 5). When consuming the Atkins diet after eating breakfast, overweight women had higher leptin concentrations than normal weight women (12.2 µg/L vs. 4.9 µg/L, P = 0.009). Normal weight women consuming the Atkins diet had lower leptin concentrations than did overweight women consuming the Atkins diet in the fasted state (4.4 µg/L vs. 11.2 µg/L P = 0.0139). In the fasted state, overweight women fed the AHA diet had higher leptin concentrations than did normal weight women fed the AHA diet (15.0 µg/L vs. 3.9 µg/L P = 0.0005).

In the fed state, overweight women consuming the Atkins diet had higher leptin concentrations than did overweight women fed the AHA diet (12.2 µg/L vs. 8.2 µg/L, P = 0.0224). In the fasted state, overweight women had lower leptin concentrations when consuming the Atkins diet in comparison to the AHA diet (11.2 µg/L vs. 15.0 µg/L P = 0.0281). Overweight women consuming the AHA diet had lower leptin concentrations in the fed state in comparison to the fasted state (8.2 µg/L vs. 15.0 µg/L P = 0.0008).

Adiponectin

There was a significant difference in adiponectin concentrations between the fed and fasted states (P = 0.0037, Fig. 6). Adiponectin concentrations were greater in the fed state (14,350 µg/L) than in the fasted state (10,347 µg/L). There were no significant effects of weight class, diet, sequence, or interactions on adiponectin concentrations 1 hr before lunch in women.
**Growth hormone**

Growth hormone concentrations only differed between the fed and fasted states and were not affected by weight class, diet, sequence, or interactions 1 hr before lunch in women (Fig. 7). Growth hormone concentrations were higher in the fasted state than in the fed state in women (6.6 µg/L vs. 3.5 µg/L, P = 0.0340).

**Before and After Lunch Change in Hormone Concentrations**

Results reported in this section are the change scores of before lunch concentrations subtracted from after lunch concentrations of individual hormones. The changes were measured to test the effects of consuming breakfast or skipping breakfast on hormone concentrations. In addition, the changes were measured to test the effect of macronutrient composition of the diet and weight class on hormone concentrations.

**Active Ghrelin**

The two-way interaction term of weight*diet in the model was significant (P = 0.020, Fig. 8). Normal weight women consuming the AHA diet had a postprandial decrease in ghrelin concentrations in comparison to normal weight women consuming the Atkins diet (-147.4 ng/L vs. 19.7 ng/L, P = 0.0005). Normal weight women had a greater decrease in active ghrelin concentrations than overweight women when consuming the AHA diet (-147.4 ng/L vs. -34.4 ng/L, P = 0.0099).
**Total Ghrelin**

There were no differences in the change before and after lunch in total ghrelin concentrations with respect to weight, diet, fed/fasted state, sequence, or interactions (Fig. 9).

**Insulin**

The four-way interaction (diet*weight*timepoint*sequence) term in the model was significant (P = 0.04, Fig. 10). Overweight women consuming the AHA diet had a greater increase in insulin concentrations in comparison to overweight women consuming the Atkins diet when the AHA diet was given before the Atkins diet when breakfast was not consumed (244.5 pmol/L vs. 1.9 pmol/L, P<0.0001). Overweight women who did not consume breakfast fed the AHA diet had greater insulin concentrations than did overweight women who did not consume breakfast fed the Atkins diet when the Atkins diet was given before the AHA diet (166.0 pmol/L vs. 81.9 pmol/L, P=0.0346). Normal weight women who did not consume breakfast fed the AHA diet had a greater increase in insulin concentrations than did normal weight women who did not consume breakfast fed the Atkins diet when the AHA diet was administered before the Atkins diet (222.2 pmol/L vs. 15.3 pmol/L, P<0.0001). From the fasted state, normal weight women fed the AHA diet had a greater increase in insulin concentrations in comparison to normal weight women fed the Atkins diet when the Atkins diet was given before the AHA diet (176.4 pmol/L vs. 41.7 pmol/L, P=0.0025). In the fed state, the change in insulin concentrations was different between the AHA and Atkins diets in overweight women when the Atkins was given before the AHA diet (94.5 pmol/L vs. -53.5 pmol/L, P = 0.0013).
Overweight women fed the AHA diet had a significant difference in the change in insulin concentrations between the fed and the fasted states when the Atkins diet was administered before the AHA (-53.5 pmol/L vs. 166.0 pmol/L, P < 0.0001). Normal weight women fed the AHA diet had a significant difference in the change in insulin concentrations between the fed (36.1 pmol/L) and fasted (176.4 pmol/L) states when the Atkins diet was given before the AHA (P=0.0019). Overweight women consuming the AHA diet had a significant difference in the change in insulin concentrations between the fed (-26.4 pmol/L) and fasted (244.5 pmol/L) states when the AHA diet was given before the Atkins diet (P < 0.0001). Moreover, normal weight women fed the AHA diet had a significant difference in the change in insulin concentrations between the fed (117.4 pmol/L) and fasted (222.2 pmol/L) states when the AHA diet was given before the Atkins diet (P=0.0119).

The change in insulin concentrations was significantly different between normal weight and overweight women consuming the AHA diet in the fed state when the AHA diet was given before the Atkins diet (117.4 pmol/L vs. -26.4 pmol/L, P=0.0023). The change in insulin concentrations was significantly different between normal weight and overweight women in the fed state consuming the AHA diet when the Atkins diet was given before the AHA diet (36.1 pmol/L vs. -53.5 pmol/L, P=0.0325).

In the fed state, normal weight women consuming the AHA diet had a greater increase in insulin concentrations when the AHA diet was given before the Atkins diet (117.4 pmol/L) in comparison to when the Atkins was given before the AHA (36.1 pmol/L, P=0.0498).
Glucagon

The four-way interaction (diet*weight*state*sequence) term in the model was significant (P = 0.01, Fig. 11). Overall, the change in glucagon concentrations is affected by the Atkins diet in the fed state in both overweight and normal weight women when the Atkins diet was given before the AHA diet (sequence effect). Overweight women fed the Atkins diet when the Atkins diet was given before the AHA diet had significant difference in the change in glucagon concentrations between the fed and fasted states (93.6 ng/L vs. -34.0 ng/L, P=0.0002). However, there was a significant difference in the change in glucagon concentrations between the fasted and fed states in normal weight women fed the Atkins diet when the Atkins diet was administered before the AHA diet (-46.6 ng/L vs. 45.0 ng/L, P = 0.0030).

There was a significant difference in the change in glucagon concentrations between when the Atkins diet was given before the AHA diet (93.6 ng/L) and when the AHA diet was given before the Atkins diet (-30.3 ng/L) in overweight women fed the Atkins diet in the fed state (P=0.0002). There was a significant difference in the change in glucagon concentrations between when the Atkins diet was given before the AHA diet (45.0 ng/L) and when the AHA diet was given before the Atkins diet (-34.5 ng/L) in normal weight women fed the Atkins diet in the fed state (P=0.0061).

Overweight women in the fed state had significant differences in the changes in glucagon concentrations between the Atkins (93.6 ng/L) and AHA (-24.0 ng/L) diets when the Atkins diet was given before the AHA diet (P=0.0005) and between the Atkins (-30.3 ng/L) and AHA (23.48 ng/L) diets when the AHA diet was given before the Atkins diet (P=0.0497). There was a tendency for there to be a difference in the change in glucagon in concentrations
in normal weight women in the fed state between the Atkins (45.0 ng/L) and AHA (-6.55 ng/L) diets when the Atkins diet was administered before the AHA diet (P=0.0585). The change in glucagon was significantly different in normal weight women in the fasted state between the Atkins (-46.6 ng/L) and AHA (8.1 ng/L) diets when the Atkins diet was administered before the AHA diet (P=0.0465).

**Leptin**

There were no differences in the change before and after lunch in leptin concentrations (Fig. 12). There was no effect of weight, diet, physiological state or sequence on the change in leptin concentrations in women.

**Adiponectin**

There were no differences in the change before and after lunch in adiponectin concentrations with respect to weight, diet, fed/fasted state, sequence, or interactions (Fig. 13).

**Growth hormone**

There was a significant difference in the change in GH concentrations between the fed and fasted states (Fig. 14). The change in GH concentrations was greater in the fasted state (-4.92 µg/L) than in the fed state (-1.58 µg/L, P = 0.0197). However, there was no effect of weight, diet, or sequence on the change in GH concentrations in women.
DISCUSSION

In this study, we investigated the effects of consuming breakfast on hormone concentration changes before and after lunch. Consumption of breakfast in obese subjects has been shown to be a key element to successful weight loss (19). The nutrient composition of breakfast has been shown to influence feelings of satiety (6). In this study, visual analog scale of hunger and fullness were not taken but informally reported feeling less hunger and increased fullness when consuming the Atkins diet versus the AHA diet.

Acylated ghrelin concentrations were higher before lunch in normal weight women consuming the AHA diet and decreased after lunch where as normal weight women consuming the Atkins diet saw little change in acylated ghrelin concentrations over the lunch period. Blom et al. showed that men consuming a high protein breakfast had decreased total ghrelin concentrations at 3 and 4 hr post lunch than that of men consuming a high carbohydrate breakfast; however, there was no change observed in acylated ghrelin concentrations in men between the high carbohydrate and the high protein breakfast (11). Another study indicated that acylated ghrelin concentrations were lower in normal weight women consuming a high protein breakfast in comparison to a normal protein breakfast (10). In normal weight women, acylated ghrelin concentrations may be influenced by the high protein content of the diet. However, no difference in ghrelin concentrations was observed in women consuming normal and high casein diets or in men and women consuming normal and high soy protein diets (8, 9). It is important to note that the diets used in this study were more extreme in the differences in macronutrient composition than diets of other studies. Most of the high protein diets studied contained between 20-35% of calories as protein with a range of carbohydrate of 35% to 55% of calories, whereas our Atkins diet had 45% of
calories from protein and 10% of calories from carbohydrate (8-10, 27) and our AHA had 12% of calories from protein and 63% of calories from carbohydrate. The protein source in some of the studies is in liquid form only, or powders, or from specific sources such as soy. The protein in the diets in this study is from a variety of sources and textures, which may explain the differences among the studies.

In the fed state, normal weight women consuming the Atkins diet had higher total ghrelin concentrations than did normal weight women fed the AHA diet. In normal weight women given a high carbohydrate meal, total ghrelin concentrations were lowest between 90 to 120 min. after a meal (28). In our study, the before blood sample in the women was at least 3 hr after consuming breakfast as participants were to have consumed the breakfast meal prior to 08:00. Total ghrelin concentrations increased more after consuming breakfast in the women consuming the Atkins diet in comparison to women fed the AHA diet in the fed state. In the fasted state, normal weight women consuming the Atkins diet had lower total ghrelin than did women consuming the AHA diet. Men consuming high protein breakfast had lower total ghrelin concentrations in comparison to men consuming a normal protein breakfast (11). There were no changes in total ghrelin from 1 hr before lunch to 1 hr after consuming lunch in our study. The period of time was between 2.5 to 3 hr for the lunch period depending on the rate of consumption of the lunch meal. The period between before and after lunch may have not been long enough to detect differences in total ghrelin concentrations seen in other studies as changes in total ghrelin have been observed in men and women three to four hr after consuming a meal (11, 27). The observed decrease in total ghrelin concentrations at 90 to 120 min. after consuming a meal may not have been observed in this study as the sampling times were chosen to investigate acute effects on hormone concentrations (28).
Obese humans have lower acylated ghrelin concentrations than those of normal weight humans (12, 13, 29). In our study, overweight women tended to have lower ghrelin than did the normal weight women when consuming a high carbohydrate diet. There was no difference in ghrelin concentrations between normal and overweight women when a low carbohydrate, high protein diet was consumed. The macronutrient content of the diet influenced the acylated ghrelin concentrations in normal weight women consuming the AHA but not normal weight women consuming the Atkins diet, nor overweight women consuming the AHA or Atkins diets. Obese women have a smaller change in acylated ghrelin from the preprandial to the postprandial state (13). In our study, normal weight and overweight women consuming the Atkins diet had no change in acylated ghrelin from the preprandial to postprandial state. Weight class may be less important than diet in determining the response of acylated ghrelin in women.

In short fasting times, ghrelin is suppressed, whereas, in long-term undernutrition, ghrelin concentrations increase. The lack of differences between the fed and fasted states in ghrelin concentrations may be influenced by the GH/IGF-1 axis (30). Growth hormone concentrations were higher in the fasted state in women in comparison to the fed state. In addition, the change in GH concentrations was greater in the fasted state in comparison to the fed state. Growth hormone can be a negative feedback on total ghrelin (30). During short term fasting, GH can serve as a feedback to ghrelin thus decreasing the concentration of ghrelin the blood (31). Physiological state before lunch affected plasma adiponectin concentrations in women independent of diet or weight class. Women in the fed state had higher adiponectin than those in the fasted state. With the increase in blood glucose from the
breakfast meal, adiponectin concentrations may be higher to increase glucose uptake in muscle and inhibit endogenous glucose production in the liver (20, 21).

Glucagon concentrations were influenced by dietary composition. Women consuming the Atkins diet had higher glucagon in comparison to women consuming the AHA diet. The low carbohydrate content of the Atkins diet may have caused the increased glucagon concentrations as a result of low blood glucose concentrations. Glucagon is responsible for the maintenance of acute blood glucose concentrations (32). We hypothesize that the lack of an increase in glucagon concentrations in the fasted state may be the result of the switch from immediate regulation of blood glucose by glucagon to short-term fasting regulation of blood glucose by epinephrine (32-34). In men, a high protein breakfast resulted in higher glucagon concentrations at 30 min. after consumption with no change in glucagon concentrations in men consuming a high carbohydrate breakfast (11).

Insulin concentrations were influenced by weight, diet, and physiological state. Insulin concentrations were lower in overweight women consuming the Atkins diet in comparison to the AHA diet. In another study, insulin concentrations increased at 30 min. postprandial in men consuming a high carbohydrate diet whereas men consuming a high protein diet did not have a change in insulin concentrations (11). Insulin concentrations were higher in the fed state versus the fasted state in overweight women consuming the AHA diet. When food is digested and absorbed, blood glucose concentrations increase and in response to increased blood glucose concentrations, insulin concentrations increase as well. Insulin concentrations were higher in overweight women, which is similar to the findings in obese women (35, 36).

Insulin concentrations are influenced by physiological state and composition of the diet. Insulin concentrations should be lower in the fasted state than insulin concentrations in the
fed state. Insulin concentrations are lower when dietary carbohydrates are decreased and replaced by dietary lipid (37). In our study, we also saw a and lipid increased as decrease in insulin concentrations when dietary carbohydrate was decreased and protein evidenced by women consuming the AHA diet having greater increases in insulin concentrations in comparison to the Atkins diet. The increase in insulin concentrations was higher in normal weight women in comparison to overweight women consuming the AHA diet in the fed state. Some research shows that insulin resistance increases with increasing BMI (38). The increase in insulin concentrations was seen in overweight women but only when consuming the AHA diet, which indicates that consuming a higher protein/lower carbohydrate diet such as the Atkins diet results in lower insulin concentrations.

Overweight women consuming the AHA diet had a decrease in insulin concentrations over lunch in the fed state in comparison to an increase in insulin concentrations in the fasted state over lunch. Consuming breakfast may improve the insulin response to the lunch meal and decrease insulin resistance, whereas skipping breakfast increases insulin concentrations over the lunch period when consuming a high carbohydrate diet such as the AHA diet. However, in normal weight women fed the AHA diet, insulin concentrations increased in the women who were fasted or who had consumed breakfast. Breakfast did not have the same effect in normal weight women as consuming breakfast did in overweight women.

Overweight women had higher leptin concentrations in comparison to normal weight women in both physiological states when fed the Atkins diet and in the fasted state when fed the AHA diet. Leptin concentrations are higher in obese individuals in comparison to normal weight individuals. Overweight women had higher leptin when consuming the Atkins diet versus the AHA diet in the fed state, whereas the opposite effect was observed in the fasted state.
state. Some studies have indicated that subjects fed a high protein diet felt more satiated than subjects fed a high carbohydrate diet, which agrees with the hormone results of our study (6).

Adiponectin has been shown to have small changes in concentration from the fasted to postprandial states (22, 23). In this study, there was no significant change in adiponectin concentrations from the fasted or fed pre-prandial state to the post-prandial state. In addition, some research shows that adiponectin concentrations are lower in obese individuals in comparison to normal weight individuals (24). However, we did not observe a significant difference in adiponectin concentrations between normal weight and overweight women, which may indicate that overweight women do not respond similarly to obese women. Other research shows that adiponectin was lower in obese men but not in obese women in comparison to their normal weight counterparts (25). The difference in BMIs between the overweight and normal weight women may not be enough to influence adiponectin concentrations. Our research indicates that grouping overweight and normal weight women may not be appropriate when measuring adiponectin responses between physiological states at one time point. Adiponectin has not been shown to be influenced by dietary composition; rather, adiponectin concentrations are inversely proportional to the amount of calories in the diet, which agrees with our data (22, 23, 26).

Universal dietary guidelines may not be appropriate for all humans to achieve optimal health. Grouping normal weight, overweight, and obese women would not be appropriate based on results in this study and previous research. Weight class affected some hormone concentrations differently, and thus women in overweight and normal weight classes should be analyzed separately. Also, dietary composition influences hormone concentrations. High protein diets affected hormone concentrations differently. The differences in findings
between studies lie with the total dietary composition. Several high protein diets increased the percent of calories from protein by lowering the percent calories from lipid or by lowering calories from both carbohydrate and lipid. Each class of macronutrients influences hormone concentrations, and care should be taken when drawing conclusions about the general effects of high protein diets and more generally any diet based on changing a single macronutrient. Experimental design is critical in obtaining information on the relationship of hormones related to appetite and body composition.

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Table 1: Characteristics of Female Participants

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<th>Weight Class</th>
<th>Age (yr)</th>
<th>BMI</th>
<th>Total Mass (kg)</th>
<th>Body Fat (%)</th>
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<td>24.25 ± 5.06</td>
<td>21.3 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.2 ± 4.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.9 ± 3.3&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>a,b</sup> Values are means ± std. dev.  
<sup>a,b</sup> Means with different superscripts are different.
Table 2: Significance of Terms in Model

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<tr>
<th>Before Lunch Only</th>
<th>Adiponectin</th>
<th>AG</th>
<th>TG</th>
<th>GH</th>
<th>Glucagon</th>
<th>Insulin</th>
<th>Leptin</th>
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<td>Diet</td>
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<td>0.0019</td>
<td>0.54</td>
<td>0.43</td>
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<td>0.020</td>
<td>0.35</td>
<td>0.81</td>
<td>0.27</td>
<td>0.047</td>
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<td>0.17</td>
<td>0.97</td>
<td>0.24</td>
<td>0.22</td>
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<td>0.36</td>
<td>0.60</td>
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<td>0.02</td>
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<td>Seq*state</td>
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<tr>
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<td>0.21</td>
<td>0.39</td>
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<td>0.57</td>
<td>0.22</td>
<td>0.0069</td>
<td>0.036</td>
<td>0.2003</td>
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</table>

1 P < 0.05 was considered significant and 0.05 > P < 0.1 was considered a trend. AG = Acylated ghrelin, TG = Total ghrelin, GH = growth hormone.
Figure 1. Acylated ghrelin concentrations (mean ± SEM) in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets (upper panel). Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 2000 kcal/d. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. The interaction of diet*weight was significant (P = 0.0084). The acylated ghrelin concentrations in normal weight and overweight women fed the AHA and Atkins diets are shown in the lower panel. a–b Treatment means with different superscripts differ, P < 0.05 with δ referring to the difference is between diets and ω referring to the difference is between weights.
Figure 1
Figure 2. Total ghrelin concentrations (mean ± SEM) in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets (upper panel). Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 2000 kcal/d. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. The interaction of diet*weight*state*sequence was significant (P = 0.0159). The total ghrelin concentrations in normal weight and overweight women fed the AHA and Atkins diets between sequence 1 and sequence 2. a–b Treatment means with different superscripts differ, P < 0.05 with δ referring to the difference is between diets and the number 1 or 2 indicating the sequence.
Figure 2

The figure shows the total ghrelin concentration (ng/L) for two periods: Fed and Fasted. The bars represent different groups: Overweight Atkins, Normal weight Atkins, Overweight AHA, and Normal weight AHA. The figure includes error bars indicating the standard deviation.
Figure 3. Insulin concentrations (mean ± SEM) in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets (upper panel). Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 2000 kcal/d. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. The interaction of diet*weight was significant (P = 0.0024). The insulin concentrations in normal weight and overweight women fed the AHA and Atkins diets in the fed and fasted states are shown in the lower panel. a–b Treatment means with different superscripts differ, \( P < 0.05 \) with \( \delta \) referring to the difference is between diets, \( \omega \) referring to the difference is between weights and \( \zeta \) referring to the difference between states.
Figure 3
Figure 4. Glucagon concentrations (mean ± SEM) in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets (upper panel). Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 2000 kcal/d. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. Only the main effect of diet was significant ($P < 0.0001$). The difference in glucagon concentrations between the Atkins and AHA diet in women are shown in the lower panel. $a$–$b$ Treatment means with different superscripts at specific times differ, $P < 0.05$. 
Figure 4
Figure 5. Leptin concentrations (mean ± SEM) in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets (upper panel). Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 2000 kcal/d. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. The interaction of diet*weight was significant (P = 0.0043). The leptin concentrations in normal weight and overweight women fed the AHA and Atkins diets in the fed and fasted states are shown in the lower panel. a–b Treatment means with different superscripts differ, $P < 0.05$ with $\delta$ referring to the difference is between diets, $\omega$ referring to the difference is between weights and $\xi$ referring to the difference between states.
Figure 5

![Graph showing leptin concentration (μg/L) for different periods and conditions.](image)
Figure 6. Adiponectin concentrations (mean ± SEM) in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets (upper panel). Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 2000 kcal/d. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. Only the main effect of physiological state was significant (P = 0.0037). The adiponectin concentrations in the fed and fasted state in women are shown in the lower panel. a–b Treatment means with different superscripts at specific times differ, P < 0.05.
Figure 7. Growth hormone concentrations (mean ± SEM) in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets (upper panel). Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 2000 kcal/d. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. Only the main effect of physiological state was significant (P = 0.0340). The GH concentrations in the fed and fasted state in women are shown in the lower panel. a–b Treatment means with different superscripts at specific times differ, P < 0.05.
Figure 7
**Figure 8.** Changes in acylated concentrations (mean ± SEM) in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets (upper panel). Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 2000 kcal/d. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. The diet*weight interaction was significant (p = 0.020). The changes in acylated ghrelin concentrations in normal weight and overweight women fed the AHA and Atkins diets are shown in the lower panel. a–b Treatment means with different superscripts differ, *P* < 0.05 with δ referring to the difference is between diets and ω referring to the difference is between weights.
Figure 8
Figure 9. Changes in total ghrelin concentrations (mean ± SEM) in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 2000 kcal/d. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. There were no differences in the changes in total ghrelin concentrations. a–b Treatment means with different superscripts at specific times differ, $P < 0.05$. 
Figure 9

Change in Total Ghrelin Concentrations (ng/L)

Fed  Fasted  Fed  Fasted
Period 1  Period 2

-400  0  200  400  600  800

Overweight Atkins
Normal weight Atkins
Overweight AHA
Normal weight AHA
**Figure 10.** Changes in insulin concentrations (mean ± SEM) in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets in the fed and fasted states. Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 2000 kcal/d. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. The interaction of diet*weight*state*sequence was significant (P = 0.0356). a–b Treatment means with different superscripts differ, $P < 0.05$ with $\delta$ referring to the difference is between diets, $\omega$ referring to the difference is between weights, $\theta$ referring to the difference between sequences, and $\zeta$ referring to the difference between states.
**Figure 11.** Changes in glucagon concentrations (mean ± SEM) in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets in the fed and fasted states. Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 2000 kcal/d. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. The interaction of diet*weight*state*sequence was significant (P = 0.0069). $a$–$b$ Treatment means with different superscripts differ, $P < 0.05$ with $\delta$ referring to the difference is between diets, $\omega$ referring to the difference is between weights, $\theta$ referring to the difference between sequences, and $\xi$ referring to the difference between states.
Figure 11
Figure 12. Changes in leptin concentrations (mean ± SEM) in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 2000 kcal/d. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. There were no differences in the changes in leptin concentrations. a–b Treatment means with different superscripts at specific times differ, $P < 0.05$. 
Figure 12

Change in Leptin Concentrations (µg/L)

-6 -5 -4 -3 -2 -1 0 1 2 3 4

Fed  Fasted  Fed  Fasted
Period 1  Period 2

- Overweight Atkins
- Normal weight Atkins
- Overweight AHA
- Normal weight AHA
Figure 13. Changes in adiponectin concentrations (mean ± SEM) in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 2000 kcal/d. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. There were no differences in the changes in adiponectin concentrations. a–b Treatment means with different superscripts at specific times differ, *P* < 0.05.
Figure 13
Figure 14. Changes in growth hormone concentrations (mean ± SEM) in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets (upper panel). Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 2000 kcal/d. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. Only the main effect of physiological state was significant (P = 0.0197). The changes in GH concentrations in the fed and fasted state in women are shown in the lower panel. a–b Treatment means with different superscripts at specific times differ, $P < 0.05$. 
Figure 14

[Graph showing change in growth hormone concentrations between Fed and Fasted states for different groups: Overweight Atkins, Normal weight Atkins, Overweight AHA, Normal weight AHA.]

[Graph showing a comparison between Fed State and Fasted State, with annotations a and b.]
Chapter II: Part II

Effect of American Heart Association and Atkins diets on the circulating concentration profiles of ghrelin and other hormones involved in energy metabolism in normal and overweight women

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ABSTRACT

Investigating the role of hormones on energy balance and body composition when varying diets are consumed could provide insight into the development of obesity and other metabolic disorders. Eight female subjects, ages 20-32, participated in this study: four normal subjects with an average BMI of 21.3 and four overweight subjects with an average BMI of 26.7. Each subject received both diets by a crossover design. Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Women were fed 2,000 kcal/day. Each diet was fed for 14 days, and then subjects switched to the other diet. Blood was taken every hour from 07:00 to 09:00 on days 13 and 27 of the study. Plasma samples were analyzed for active and total ghrelin, leptin, insulin, glucagon, growth hormone, and adiponectin. Acylated ghrelin did not have preprandial increases before breakfast and lunch with varied responses before dinner in overweight and normal weight women fed either the Atkins or AHA diets in both sequences. Plasma glucagon was higher after lunch through the end of the 14-hr period in women consuming the Atkins diet. Insulin concentrations were higher and had greater responses to meal ingestion in women consuming the AHA diet in comparison to the Atkins diet. All hormones were affected by diet *period (sequence), weight class, and diet. Our results indicated that overweight and normal weight women have different hormone responses to diet and should be studied separately. In addition, sequence effect was significant in this study, indicating that the dietary habits of subjects prior to the study may influence the outcomes of controlled
experiments and that study design is critical in understanding the effect of diet on hormone concentrations in women.

INTRODUCTION

Understanding diet and hormone interaction in the progression of obesity is crucial in finding a solution to this growing nutritional problem in United States. Investigating the role of ghrelin on energy balance and body composition when varying diets are consumed could provide insight into the etiology of obesity.

Plasma ghrelin concentrations in obese humans are lower than those of normal weight individuals (1). English and colleagues demonstrated that refeeding after fasting did not decrease the ghrelin concentrations in obese human patients (2). In normal weight humans, fasting ghrelin concentrations decreased after feeding. Salbe et al showed that ghrelin has a negative association with *ad libitum* feed intake (3). Studying the effects of diet composition on ghrelin concentrations in both normal and obese patients is necessary to fully understand the mechanisms by which the body controls feed intake and body composition. Thus, understanding the regulation of ghrelin under conditions of weight gain and loss could provide insight into understanding obesity (1). Rats fed a high carbohydrate diet had higher plasma ghrelin than those fed a low carbohydrate diet (4). Healthy non-obese women were fed a high fat or high carbohydrate meal and the greatest increase in plasma ghrelin were seen in subjects consuming the high carbohydrate meal (5). In addition, hunger sensation of subjects fed the high carbohydrate diet was suppressed more than that of subjects fed the high fat diet. Ghrelin concentration, however, increased with weight loss of humans when eating a low fat, high carbohydrate diet (6). In the Weigle and colleagues study, high fat
diets decrease adiposity without increasing appetite. Most studies measure total ghrelin and not the acylated form (7). The few studies involving ghrelin and diet composition have conflicting results, leaving the relationship between ghrelin and diet composition unclear.

The overall objective of this research was to elucidate the relationship of diet composition and ghrelin concentration with respect to obesity. To accomplish the overall objective, we compared the variation of ghrelin over 15 hrs in lean and overweight women. We compared the effects of two common diets, Atkins and American Heart Association (AHA), on ghrelin, other hormones associated with ghrelin, and blood metabolite concentrations. We hypothesized that overweight females have greater ghrelin concentration in plasma over 15-hour period, which stimulates greater food intake to cause obesity. Furthermore, we hypothesized that subjects consuming the Atkins diet will have lower plasma ghrelin concentrations than subjects consuming the AHA diet.

**MATERIAL AND METHODS**

**Subjects**

Approval for all screening and study procedures was obtained from the Iowa State University Institutional Review Board. Approval for the use of dual-energy x-ray absorptiometry (DXA) was obtained from the State of Iowa Department of Public Health. Each subject was screened for BMI, eating disorders, and major health problems through an interview and physical examination. Volunteers were excluded if they have or had an eating disorder, or have a major health problem such as diabetes, polycystic ovary syndrome, heart conditions, and hypoglycemia. After successful completion of the screening, eight female subjects were assigned randomly to treatment diets (Table 1). One participant discontinued
participation the second day of the study for personal reasons and an alternate participant continued on the study. One participant was unable to be successfully catheterized and thus seven venapuncture samples were taken in period 1 and eight venapuncture samples were taken in period 2.

Each subject received both treatment diets by a crossover design. Subjects were given 2,000 kcal/day based on average energy intake of females of that age group (8). Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the American Heart Association (AHA) diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Diets were formulated using Nutritionist Pro (version 2.3; First DataBank, Inc.; San Bruno, CA) by a registered dietitian. All diets were prepared and served by personnel at the Human Metabolic Unit at Iowa State University. Each diet was fed for 14 days with a repeating 7-day menu for each diet. Meals were served between 08:00-0900, 12:00-13:00, and 17:00-18:00 in the dining area of Human Metabolic Unit.

Three DXA scans were performed during the study per subject to monitor body composition. The scans were on the Friday before the study began, day 14, and day 28 of the study. Scans were performed at the ISU metabolic unit by trained personnel using a Hologic Delphi W dual-energy X-ray absorptometer (DXA) (Hologic Inc.; Bedford, MA).

A fasted blood serum sample was taken and a basic metabolic panel analysis was performed by Laboratory Corporation of America (LabCorp, Omaha, NE).

From day 12 at 22:00, subjects fasted until 8:00 on day 13 of each diet. Subjects arrived at the ISU Human Metabolic unit at 06:30. A 23 gauge peripheral intravenous catheter was
placed in each subject. At each blood timepoint, 1 ml of blood was taken from the catheter and then the blood sample for analysis was taken. After the blood sample was taken, 3 mL of saline was administered to flush the catheter. Blood samples were taken every hour for 14 hr. All blood was collected in 3 mL tuberccline syringes and placed in EDTA-containing vacutainer tubes (BD Scientific, Franklin Lakes, NJ) and kept at 4 °C during processing. Samples were centrifuged at 3000 × g for 20 min. Plasma was collected and analyzed for ghrelin (acylated and total), adiponectin, glucagon, growth hormone, insulin, and leptin.

**Hormone Analysis**

Total ghrelin concentrations were measured by using a commercially available radioimmunoassay kit (Linco Research, St. Charles, MO). The lower limit of detection was 93 pg/mL, and the intra-assay coefficient of variation was < 10%.

For acylated ghrelin samples, 50 µL of 1 N hydrochloric acid and 10 µL of 10 mg/mL phenylmethylsulfonyl fluoride (PMSF, Sigma, St. Louis, MO) in methanol were added for every 1 mL of plasma immediately after centrifugation. Acylated ghrelin concentrations were measured using a commercially available radioimmunoassay kit (Linco Research). This assay has been found to be highly specific for acylated ghrelin with less than 0.1 % cross-reactivity for desoctanoyl ghrelin and no cross reactivity with ghrelin 14-28, motilin-related peptide, leptin, insulin, glucagon, or GLP-1 (7-36). The lower detection limit was 7.8 pg/mL. The intra-assay coefficient of variation was < 10%.

Adiponectin concentrations were measured by using a commercially available radioimmunoassay (Linco Research). The lower limit of detection was 1 ng/mL, and the intra-assay coefficient of variation was < 10%.
For glucagon samples, 50 µL of aprotinin (5,500 KIU/mL, Sigma) were added to 1 mL of plasma with a final concentration of 550 KIU aprotinin per mL of plasma. All plasma samples were stored at -20 °C until assayed. Glucagon concentrations were measured by using a commercially available radioimmunoassay (Linco Research). The lower limit of detection was 20 pg/mL, and the intra-assay coefficient of variation was < 10%.

Growth Hormone concentrations were measured by using a commercially available radioimmunoassay (Diagnostic Products Corporation (DPC), Los Angeles, CA). The lower limit of detection was 0.9 ng/mL, and the intra-assay coefficient of variation was < 10%.

Insulin concentrations were measured by using a commercially available radioimmunoassay (Linco Research). The lower limit of detection was 0.2 µU/mL (1.389 pmol/L), and the intra-assay coefficient of variation was < 10%.

Leptin concentrations were measured by using a commercially available radioimmunoassay (Linco Research). The lower limit of detection was 0.05 ng/mL, and the intra-assay coefficient of variation was < 10%.

Statistics

Hormone data were analyzed by using the MIXED procedure in SAS (Version 9.1, SAS Institute, Cary, NC). All hormone data were analyzed as a split-plot with repeated measures ANOVA. The model included fixed effects of diet, weight (weight class), timepoint, sequence, diet*weight, weight*sequence, sequence*timepoint, diet*weight*timepoint, and diet*weight*timepoint*sequence, while person(weight*sequence*period) was included in the model as the random effect. Significance of the interaction terms is listed in Table 2. No
multiple comparison adjustments were made because this is an exploratory study. For all tests, $P < 0.05$ was considered significant and $P < 0.1$ was considered a trend.

RESULTS

Body Composition and Blood Metabolites

There were no significant changes in total body mass, total body fat percentage, trunk mass, and trunk fat percentage between the Atkins diet and AHA diet within a weight class (Table 4). Blood metabolite data is summarized in Table 3. Blood urea nitrogen was higher in women consuming the Atkins diet in comparison to the AHA diet ($P<0.0001$). Total, HDL and LDL cholesterol was higher in women consuming the Atkins diet in comparison to the AHA diet; however, there was no significant difference in the ratio of LDL/HDL. Blood glucose tended to be higher in overweight women consuming the Atkins diet in comparison to normal weight women consuming the Atkins diet ($P = 0.0669$) and overweight women consuming the AHA diet ($P = 0.0656$).

Hormone Concentrations

Acylated Ghrelin

Acylated ghrelin concentrations over waking hours in overweight and normal weight women (individual data presented) are shown in Figure 1.

Timepoint Comparisons

Overweight women consuming the Atkins diet had higher acylated ghrelin concentrations before and after dinner (hours 9, 11-14, $P \leq 0.0490$) in comparison to normal weight women consuming the Atkins diet when the AHA diet was administered before the Atkins diet.
When the Atkins diet was administered before the AHA diet, overweight women tended to have higher acylated ghrelin concentrations before 1 hr before dinner (hour 8, $P = 0.0576$) in comparison to normal weight women consuming the Atkins diet. Overweight women consuming the Atkins diet had higher acylated ghrelin concentrations after lunch (hour 6, $P = 0.0143$) and after dinner (hours 11-14, $P \leq 0.0022$) when the AHA diet was fed before that Atkins diet in comparison to when that Atkins diet was fed before the AHA diet. Acylated ghrelin concentrations tended to be higher at hour 7 ($P = 0.0747$) when the AHA diet was fed before the Atkins diet in comparison to when the Atkins diet was fed before the AHA diet. There were no differences in acylated ghrelin concentrations between sequences in normal weight women fed the Atkins diet.

There were no differences in acylated ghrelin concentrations between normal weight and overweight women fed the AHA diet when the AHA diet was given before the Atkins diet. However, when the Atkins diet was given before the AHA diet, normal weight women fed the AHA diet had lower acylated ghrelin concentrations in comparison to overweight women fed the AHA diet before dinner (hour 9, $P = 0.0109$). Acylated ghrelin concentrations tended to be higher in overweight women consuming the AHA diet after breakfast (hour 2, $P = 0.0563$) in comparison to normal weight women consuming the AHA diet when the Atkins diet was given before the AHA diet. There were no differences in acylated ghrelin concentrations between the sequences in overweight women fed the AHA diet. Normal weight women consuming the AHA diet when the AHA diet was administered before the Atkins diet had lower acylated ghrelin concentrations after breakfast (hour 2, $P = 0.0348$) and before dinner (hour 9, $P = 0.0183$) when compared to normal weight women consuming the AHA diet when the Atkins diet was administered before the AHA diet.
Atkins diet to AHA Comparisons

There were no differences in acylated ghrelin concentrations between the AHA and Atkins diets in normal weight women when the AHA diet was given before the Atkins diet. Normal weight women consuming the Atkins diet had lower acylated ghrelin concentrations before dinner (hour 9, \( P = 0.0006 \)) that did normal weight women consuming the AHA diet when the Atkins diet was given before the AHA diet. When the AHA diet was given before the Atkins diet, overweight women consuming the Atkins diet had higher acylated ghrelin concentrations after breakfast (hour 2-3), after lunch (hours 6-7, \( P \leq 0.0435 \)), and after dinner (hours 11-14, \( P \leq 0.0034 \)) and tended to have higher acylated ghrelin concentrations before breakfast (hour 0, \( P = 0.0898 \)) and dinner (hours 9-10, \( P \leq 0.0721 \)) in comparison to overweight women consuming the AHA diet. There were no differences between the Atkins diet and AHA diets in overweight women when the Atkins diet was given before the AHA diet.

Total Ghrelin

Total ghrelin concentrations over waking hours in overweight and normal weight women (individual data presented) are shown in Figure 2.

Timepoint Comparisons

When the AHA diet was administered before the Atkins diet, normal weight women consuming the Atkins diet had lower total ghrelin concentrations before and after breakfast (hours 0-1, \( P \leq 0.0694 \)) and tended to have lower total ghrelin concentrations after lunch (hour 6, \( P = 0.0092 \)) in comparison to overweight women consuming the Atkins diet. When the Atkins diet was administered before the AHA diet, normal weight women had higher
total ghrelin concentrations at hour 14 (P = 0.0427) in comparison to overweight women consuming the Atkins diet. Overweight women consuming the Atkins diet when the Atkins diet was given before the AHA diet had lower total ghrelin concentrations at before breakfast (hour 0, P = 0.0049), before lunch (hours 3-4, P ≤0.0289) and after lunch (hour 6, P <0.0001) and tended to have lower total ghrelin concentrations after breakfast (hours 1-2, P ≤ 0.0626) in comparison to overweight women consuming the Atkins diet when the AHA diet was given before the Atkins diet. Normal weight women consuming the Atkins diet when the AHA diet was given before the Atkins diet had higher total ghrelin concentrations before lunch (hour 4, P = 0.0138) and after dinner (hour 10, P = 0.0453) in comparison to normal weight women consuming the Atkins diet when the Atkins diet was given before the AHA diet.

Overweight women consuming the AHA diet tended to have lower total ghrelin concentrations after lunch (hour 5, P = 0.0703) than did normal weight women consuming the AHA diet when the AHA diet was given before the Atkins diet. When the Atkins diet was administered before the AHA diet, normal weight women consuming the AHA diet tended to have lower total ghrelin concentrations before lunch (hour 4, P = 0.0495) and higher total ghrelin concentrations before dinner (hour 8, P = 0.0495) in comparison to overweight women consuming the AHA diet. Overweight women consuming the AHA diet had higher total ghrelin concentrations before lunch (hour 4, P = 0.0223) when the Atkins diet was fed before the AHA diet in comparison to when the AHA diet was fed before the Atkins diet. Normal weight women consuming the AHA diet higher total ghrelin concentrations after lunch (hour 6, P = 0.0139) when the Atkins diet was administered before the AHA diet in comparison to when the AHA diet was administered before the Atkins diet.
but had lower total ghrelin concentrations after dinner (hour 14, \( P = 0.0101 \)) and tended to have lower total ghrelin concentrations after dinner (hour 13, \( P = 0.0905 \)) when the Atkins diet was administered before the AHA diet in comparison to when the AHA diet was administered before the Atkins diet.

**Atkins diet to AHA Comparisons**

Normal weight women consuming the Atkins diet had higher total ghrelin concentrations before lunch (hours 3-4, \( P \leq 0.0183 \)) in comparison to normal weight women consuming the AHA diet when the AHA diet was administered before the Atkins diet. However, normal weight women consuming the Atkins diet had lower total ghrelin concentrations after dinner (hour 14, \( P = 0.0074 \)) in comparison to normal weight women consuming the AHA diet when the AHA diet was administered before the Atkins diet. Normal weight women consuming the Atkins diet had higher total ghrelin concentrations after dinner (hour 13, \( P = 0.0352 \)) in comparison to normal weight women consuming the AHA diet when the Atkins diet was administered before the AHA. However, normal weight women consuming the Atkins diet had lower total ghrelin concentrations immediately after dinner (hour 10, \( P = 0.0286 \)) and tended to have lower total ghrelin concentrations after lunch (hour 6, \( P = 0.0765 \)) and before dinner (hour 8, \( P = 0.0818 \)) in comparison to normal weight women consuming the AHA diet when the AHA diet was administered before the Atkins diet.

Overweight women consuming the Atkins diet had higher total ghrelin concentrations before, after breakfast (hour 0-1, \( P \leq 0.0128 \)), before, and after lunch (hours 3, 4, 6, \( P \leq 0.0281 \)) than did overweight women consuming the AHA diet when the AHA diet was given before the Atkins diet. Conversely, when the Atkins diet was administered before the AHA diet, overweight women fed the Atkins diet had lower total ghrelin concentrations before and
after lunch (hour 4 and 6, $P \leq 0.0102$) in comparison to overweight women consuming the AHA diet.

**Insulin**

Insulin concentrations over waking hours in overweight and normal weight women (individual data presented) are shown in Figure 3.

**Timepoint Comparisons**

Overweight women consuming the Atkins diet had lower insulin concentrations after breakfast (hour 1, $P = 0.0364$) and lunch (hour 5-6, $P \leq 0.0166$) in comparison to normal weight women consuming the Atkins diet when the AHA diet was given before the Atkins diet. Overweight women tended to have higher insulin concentrations after dinner (hour 10, $P = 0.0495$) in comparison to normal women consuming the Atkins diet when the Atkins diet was given before the AHA diet. Overweight women consuming the Atkins diet tended to have higher insulin concentrations before dinner (hour 7, $P = 0.0652$) when the AHA diet was given before Atkins diet in comparison to when the Atkins diet was given before the AHA diet. Normal weight women consuming the Atkins diet had higher insulin concentrations after lunch (hour 6, $P = 0.0114$) and after dinner (hour 11, $P = 0.0371$) and tended to have higher insulin concentrations after lunch (hour 5, $P = 0.0623$) and after dinner (hour 13, $P = 0.0614$) when the AHA diet was given before the Atkins diet in comparison to when the Atkins diet was given before the AHA diet.

Overweight women consuming the AHA diet had lower insulin concentrations at hour 2 ($P = 0.0459$) in comparison to normal weight women consuming the AHA diet when the AHA diet was administered before the Atkins diet. When the Atkins diet was administered
before the AHA diet, overweight women had lower insulin concentrations after breakfast (hour 2, \( P = 0.0058 \)) and after lunch (hours 5-6, \( P \leq 0.0317 \)) in comparison to normal weight women consuming the AHA diet. Overweight women consuming the AHA diet had lower insulin concentrations when the AHA diet was given before the Atkins diet after breakfast (hour 2, \( P = 0.0033 \)), after lunch (hours 5-6, \( P \leq 0.0425 \)), before dinner (hour 8, \( P = 0.0386 \)) and after dinner (hours 12-14, \( P \leq 0.0425 \)) and a tendency to be higher before dinner at hour 7 (\( P = 0.0617 \)) in comparison the when the Atkins diet was given before the AHA diet. However, insulin concentrations were higher when the AHA diet was given before the Atkins diet immediately after dinner (hour 10) in comparison to when the Atkins diet was given before the AHA diet in overweight women consuming the AHA diet. Normal weight women consuming the AHA diet had higher insulin concentrations after breakfast (hour 2, \( P = 0.0493 \)) and after dinner (hours 10 and 13, \( P \leq 0.0258 \)) when the AHA diet was administered before the Atkins diet in comparison the when the Atkins diet was administered before the AHA diet.

*Atkins diet to AHA Comparisons*

When the AHA diet was administered before the Atkins diet, normal weight women consuming the Atkins diet had lower insulin concentrations after breakfast (hour 2, \( P = 0.0452 \)) and after dinner (hours 10-12, \( P \leq 0.0492 \)) and higher insulin concentrations at hour 1 (\( P = 0.0482 \)) and after lunch (hours 6 and 8, \( P \leq 0.0163 \)) in comparison to normal weight women consuming the AHA diet. Insulin concentrations tended to be higher at hour 9 (\( P = 0.0944 \)) in normal weight women consuming the Atkins diet in comparison to the AHA diet when the AHA diet was administered before the Atkins diet. Normal weight women consuming the Atkins diet had lower insulin concentrations in comparison to normal weight
women consuming the AHA diet after dinner (hours 11-13, \( P \leq 0.0002 \)) and tended to have lower insulin concentrations after breakfast (hour 1, \( P = 0.0768 \)) when the Atkins diet was given before the AHA diet. When the AHA diet was administered before the Atkins diet, overweight women consuming the Atkins diet had lower insulin concentrations after breakfast (hours 1 and 3, \( P \leq 0.0405 \)) and after dinner (hours 10-12, \( P \leq 0.0093 \)) and higher insulin concentrations before and after dinner (hours 7-9, \( P \leq 0.0472 \)) in comparison to overweight women consuming the AHA diet. Overweight women consuming the Atkins diet had lower insulin concentrations after breakfast (hours 1-3, \( P \leq 0.0056 \)), after lunch (hour 5, \( P = 0.0002 \)), and after dinner (hours 11-13, \( P = 0.0011 \)) in comparison to overweight women consuming the AHA diet when the Atkins diet was administered before the AHA diet.

**Glucagon**

Glucagon concentrations over waking hours in overweight and normal weight women (individual data presented) are shown in Figure 4.

**Timepoint Comparisons**

There were no differences between normal weight and overweight women consuming the Atkins diet when the AHA diet was given before the Atkins diet. Overweight women consuming the Atkins diet had higher glucagon concentrations at baseline (\( P = 0.0375 \)) and before lunch (hour 3, \( P < 0.0001 \)) than did normal weight women consuming the Atkins diet when the Atkins diet was given before the AHA diet. Overweight women consuming the Atkins diet when the AHA diet was administered before the Atkins diet had lower glucagon concentrations at baseline (\( P = 0.0292 \)) and after dinner (hour 10, \( P = 0.0046 \)) in comparison to overweight women consuming the Atkins diet when the Atkins diet was given before the
AHA diet. Normal weight women consuming the Atkins diet when the AHA diet was given before the Atkins diet had lower glucagon concentrations after dinner (hour 10, \( P = 0.0044 \)) and tended to have lower concentrations before dinner (hour 8, \( P = 0.0771 \)) than did women who consumed the Atkins diet when the Atkins diet was given before the AHA diet.

There was no difference in glucagon concentrations between normal weight and overweight women consuming the AHA diet when the AHA diet was given before the Atkins diet. Overweight women consuming the AHA diet tended to have higher glucagon concentrations after dinner (hour 10, \( P = 0.0834 \)) than did normal weight women consuming the AHA diet when the Atkins diet was given before the AHA diet. There were no differences between sequences in glucagon concentrations in normal weight or overweight women consuming the AHA diet.

*Atkins diet to AHA Comparisons*

Normal weight women consuming the Atkins diet had higher glucagon concentrations after lunch through the end of the study period (hours 5-14, \( P \leq 0.0336 \)) in comparison to normal weight women consuming the AHA diet when the AHA diet was administered before the Atkins diet. When the Atkins diet was administered before the AHA diet, normal weight women consuming the Atkins diet had higher glucagon concentrations after lunch to after dinner (hours 7-11, 13, \( P \leq 0.0074 \)) and a tendency for glucagon concentrations to be higher at hour 12 (\( P = 0.0615 \)) in comparison to normal weight women consuming the AHA diet.

Overweight women consuming the Atkins diet had higher glucagon concentrations from hours 6-13 (\( P = 0.0045 \)) with a tendency for glucagon concentrations to be higher at hour 5 (\( P = 0.961 \)) in comparison to overweight women consuming the AHA diet when the AHA diet was administered before the Atkins diet. Glucagon concentrations were higher at
baseline, before dinner (hours 7-8, $P \leq 0.0343$) and after dinner (hours 10-13, $P \leq 0.0167$) with a tendency for higher concentrations at hours 6 ($P = 0.0634$) and 14 ($P = 0.0576$) in overweight women consuming the Atkins diet in comparison to the AHA diet when the Atkins diet was administered before the AHA diet.

**Leptin**

Leptin concentrations over waking hours in overweight and normal weight women (individual data presented) are shown in Figure 5.

**Timepoint Comparisons**

There were no differences in leptin concentrations between normal weight and overweight women consuming the Atkins diet when the AHA diet was administered before the Atkins diet. Overweight women consuming the Atkins diet had higher leptin concentrations than did normal weight women consuming the Atkins diet at all timepoints in the 14-hour study when the Atkins diet was given before the AHA diet ($P = 0.0200$). Overweight women consuming the Atkins diet had lower leptin concentrations after breakfast (hours 1 and 2, $P \leq 0.0002$), before lunch (hour 4, $P = 0.0001$), after lunch (hour 5, $P = 0.0469$), and after dinner (hours 10-14, $P = 0.0303$) when the AHA diet was given before the Atkins diet in comparison to when the Atkins diet was given before the AHA diet. Overweight women consuming the Atkins diet tended to have lower leptin concentrations before and after dinner (hours 7-9, $P \leq 0.0915$) when the AHA diet was given before the Atkins diet in comparison to when the Atkins diet was given before the AHA diet. Normal weight women consuming the Atkins diet had higher leptin concentrations at hour 13 ($P = 0.0368$) and tended to have lower leptin concentrations at hour 12 ($P = 0.0949$) and 14 ($P = 0.0618$) when the AHA diet
was given before the Atkins diet in comparison to when the Atkins diet was given before the AHA diet.

Overweight women consuming the AHA diet had lower leptin concentrations hour 11 (P = 0.0274) and tended to have lower leptin concentrations after dinner (hour 9, P = 0.0539) than did normal weight women consuming the AHA diet when the AHA diet was given before the Atkins diet. Overweight women consuming the AHA diet had higher leptin concentrations over the 14-hour study than did normal weight women consuming the AHA diet when the Atkins diet was given before the AHA diet (P ≤ 0.0001). Overweight women consuming the AHA diet had higher leptin concentrations over the 14-hr study when the Atkins diet was given before the AHA diet in comparison to when the AHA diet was given before the AHA diet (P ≤ 0.0034). Normal weight women consuming the AHA diet had higher leptin concentrations at baseline and after dinner (hours 11 and 13, P ≤ 0.0312) and tended to have higher leptin concentrations after dinner (hour 9 and 12, P ≤ 0.0985) when the AHA diet was given before the Atkins diet in comparison to when the Atkins diet was given before the AHA diet.

**Atkins diet to AHA Comparisons**

Normal weight women consuming the Atkins diet had lower leptin concentrations at baseline and after dinner (hours 9 and 11, P ≤ 0.0031) in comparison to normal weight women consuming the AHA diet when the AHA diet was administered before the Atkins diet. When the Atkins diet was administered before the AHA diet, normal weight women consuming the Atkins diet had lower leptin concentrations before lunch (hour 4, P = 0.0149) and tended to have lower leptin concentrations before and after dinner (hours 7 and 9, P ≤ 0.0719) in comparison to normal weight women consuming the AHA diet.
When the AHA diet was administered before the Aktins diet, overweight women consuming the Atkins diet had lower leptin concentrations after dinner (hours 12-14, $P \leq 0.0352$) in comparison to overweight women consuming the AHA diet. Overweight women consuming the Atkins diet had lower leptin concentrations at baseline ($P = 0.0062$), before lunch (hour 4, $P < 0.0001$), after lunch (hours 5-7, $P < 0.0001$), before dinner (hours 8-9, $P < 0.0001$), and after dinner (hours 10-14, $P \leq 0.0006$) in comparison to overweight women consuming the AHA diet when the Atkins diet was given before the AHA diet.

**Adiponectin**

Adiponectin concentrations over waking hours in overweight and normal weight women (individual data presented) are shown in Figure 6.

**Timepoint comparisons**

When the AHA diet was administered before the Atkins diet, overweight women consuming the Atkins diet had lower adiponectin concentrations before lunch (hour 4, $P = 0.0197$) and 3 hr after dinner (hour 13, $P = 0.0158$) and a tendency for adiponectin concentrations to be higher after lunch (hour 5, $P = 0.0607$) in comparison to normal weight women. When the Atkins diet was given before the AHA diet, there were no differences in adiponectin concentrations between timepoint in overweight and normal weight women consuming the Atkins diet. Fasting adiponectin concentrations were lower in overweight women fed the Atkins diet when the AHA diet was given before the Atkins diet in comparison to overweight women consuming the Atkins diet when the Atkins diet was given before the AHA diet ($P = 0.0443$). In normal weight women fed the Atkins diet, adiponectin concentrations were higher an hour before dinner (hour 8, $P = 0.0377$) and tended to be
higher 3 hr after dinner (hour 13, \( P = 0.0623 \)) when the AHA diet was given before the Atkins diet in comparison to when the Atkins diet was given before the AHA.

When the AHA diet was administered before the Atkins diet, overweight women consuming the AHA diet had lower adiponectin concentrations after breakfast (hour 3, \( P = 0.0428 \)) and a tendency for adiponectin concentrations to be lower at baseline (\( P = 0.0727 \)) and 2 hr after dinner (hour 12, \( P = 0.0897 \)) in comparison to normal weight women. When the Atkins diet was given before the AHA diet, adiponectin concentrations tended to be lower 1 hr before dinner (hour 8, \( P = 0.0645 \)) in overweight women consuming the AHA diet in comparison to normal weight women. There were no differences between timepoints in overweight women fed the AHA diet when the AHA diet was given before the Atkins diet in comparison to when the Atkins diet was given before the AHA diet. Adiponectin concentrations tended to be higher after breakfast (hour 1, \( P = 0.0571 \)) in normal weight women when the AHA diet was given before the Atkins diet. In addition, adiponectin concentrations tended to be lower before dinner (hour 8, \( P = 0.0942 \)) in normal weight women when the AHA diet was given before the Atkins diet.

_Atkins diet to AHA diet comparisons_

When the AHA diet was administered before the Atkins diet, normal weight women fed the AHA diet had higher adiponectin concentrations at fasting (\( P = 0.0004 \)) and after breakfast (hour 1, \( P = 0.0255 \)) in comparison to normal weight women fed the Atkins diet, whereas, normal weight women fed the Atkins diet had higher adiponectin concentrations over lunch (hours 4-6, \( P \leq 0.0211 \)) and after dinner (hours 13-14, \( P \leq 0.0328 \)). When the Atkins diet was given before the AHA diet, normal weight women consuming the Atkins diet had lower adiponectin concentrations than did normal weight women consuming the AHA.
diet before (hour 8, P < 0.0001) and tended to have lower concentrations after dinner (hours 10 and 13, P ≤ 0.0831).

There was no difference in timepoints between the AHA and Atkins diets in overweight women when the AHA diet was given before the Atkins diet. However, when the Atkins diet was given before the AHA diet, overweight women consuming the Atkins diet had higher adiponectin concentrations in comparison to overweight women consuming the AHA diet at fasting and after breakfast (hours 0-1, P < 0.0326). When the Atkins diet was administered before the AHA diet, overweight women had lower adiponectin concentrations when consuming the Atkins diet in comparison to the AHA diet before dinner (hours 7 and 9, P ≤ 0.0380). Adiponectin concentrations tended to be higher in overweight women consuming the AHA diet in comparison to the Atkins diet over lunch (hours 4 and 6, P ≤ 0.0671).

Growth Hormone

Growth hormone concentrations over waking hours in overweight and normal weight women (individual data presented) are shown in Figure 7.

Timepoint Comparisons

Overweight women consuming the Atkins diet had lower GH concentrations than that of normal weight women consuming the Atkins diet when the AHA diet was given before the Atkins diet before and after dinner (hours 8, 10, P ≤ 0.0337). When the Atkins diet was given before the AHA diet, overweight women consuming the Atkins diet had lower GH concentrations in comparison to normal weight women after dinner (hour 11, P = 0.036). Overweight women consuming the Atkins diet had higher GH concentrations after dinner (hours 10-11, P ≤ 0.036) when the AHA diet was administered before the Atkins diet in
comparison to when the Atkins diet was administered before the AHA diet. Normal weight women consuming the Atkins diet had higher GH concentrations after dinner (hours 8, 10, 11, \( P \leq 0.0325 \)) and tended to be higher at hour 9 (\( P = 0.0502 \)) when the AHA diet was administered before the Atkins diet in comparison to when the Atkins diet was administered before the AHA diet.

Overweight women consuming the AHA diet had lower GH concentrations at baseline (\( P = 0.0137 \)) and at hour 14 (\( P = 0.0148 \)) in comparison to normal weight women consuming the AHA diet when the AHA diet was given before the Atkins diet. When the Atkins diet was given before the AHA diet, overweight women had lower GH concentrations before lunch (hour 3, \( P = 0.0373 \)) in comparison to normal weight women consuming the AHA diet. Normal weight women consuming the AHA diet tended to have lower GH concentrations after dinner (hour 11, \( P = 0.055 \)) in comparison to overweight women consuming the AHA diet when the Atkins diet was given before the AHA diet. Overweight women consuming the AHA diet had lower GH concentrations at baseline (\( P = 0.0246 \)) and before and after dinner (hours 8, 10, 11, \( P \leq 0.0293 \)) and higher concentrations at hour 14 (\( P = 0.0122 \)) when the AHA diet was given before the Atkins diet in comparison to when the Atkins diet was given before the AHA diet. Normal weight women consuming the AHA diet had lower GH concentrations before lunch (hour 3, \( P = 0.0405 \)) and before dinner (hour 8, \( P = 0.0246 \)) when the AHA diet was given before the Atkins diet in comparison to when the Atkins diet was given before the AHA diet. GH concentrations tended to be lower at hour 9 (\( P = 0.0824 \)) in normal weight women fed the AHA diet when the AHA diet was administered before the Atkins diet in comparison to when the Atkins diet was administered before the AHA diet.
Atkins diet to AHA Comparisons

When the AHA diet was fed before the Atkins diet, normal weight women consuming the Atkins diet had higher GH concentrations before and after dinner (hours 8, 9, 10, 11, \(P \leq 0.0386\)) in comparison to normal weight women consuming the AHA diet. When the Atkins diet was given before the AHA diet, normal weight women consuming the Atkins diet tended to have lower GH concentrations before and after dinner (hours 7, 8, 9, \(P \leq 0.0975\)) and higher GH concentrations at hour 11 (\(P = 0.0629\)) than did normal weight women consuming the AHA diet. Overweight women consuming the Atkins diet had higher GH concentrations at hours 10 and 11 (\(P \leq 0.0323\)) and lower GH concentrations at hour 14 (\(P = 0.0191\)) in comparison to overweight women consuming the AHA diet when the AHA diet was given before the Atkins diet. When the Atkins diet was given before the AHA diet, overweight women consuming the Atkins diet had lower GH concentrations before and after dinner (hours 8, 10, 11, \(P \leq 0.0177\)) than did overweight women consuming the AHA diet.

DISCUSSION

There was an effect of sequence (period*diet) in all hormones. In this exploratory study, we did not have an adjustment period before period 1 and food logs were not taken. Hormones react relatively quickly to changes in diet, however, it is not know how long humans take to adjust to a meal schedule and set amount of calories per day when coming from varied diet and prior habits. We demonstrated that there are confounding factors that play into differences in hormone concentrations. These confounding factors may explain the discrepancy in results related to studies of single macronutrient effects on hormone concentrations.
Blom and colleagues showed that men consuming a high protein breakfast had decrease in total ghrelin concentrations at three and four hours post lunch than that of men consuming a high carbohydrate breakfast; however, there was no change observed in acylated ghrelin concentrations in men between the high carbohydrate and the high protein breakfast (16). There were no differences in acylated ghrelin concentrations between the Atkins and AHA diet in normal weight women when the AHA diet was given before the Atkins diet, which agree with the findings of Blom et al (16). We did see higher acylated ghrelin concentrations in normal weight women fed the AHA diet in comparison the Atkins diet when the Atkins diet was given before the AHA diet. In this study, there were no clear increases in acylated ghrelin concentrations before meals. There was an increase before dinner in acylated ghrelin at hour 8 or 9 in overweight women consuming the Atkins diet in both sequences and in normal weight women consuming the AHA diet when the Atkins diet was given before the AHA diet. Caloric content of the diet has been shown to suppress postprandial acylated ghrelin concentrations proportional to the amount of calories ingested and that calories were not the only determinant of next meal initiation (17). Women on this study consumed 2,000 kcal/day and maintained body weight, which may be the reason we did not see strong preprandial ghrelin responses. Another study indicated that acylated ghrelin concentrations were lower in normal weight women consuming a high protein breakfast in comparison to a normal protein breakfast (18). However, no difference in ghrelin concentrations was observed in women consuming normal and high casein diets or in men and women consuming normal and high soy protein diets (19, 20). Most of the high protein diets studied contained between 20-35% of calories as protein with a range of carbohydrate of 35% to 55% of calories; whereas, our Atkins diet had 45% of calories from protein and 10% of
calories from carbohydrate, which would be considered low carbohydrate/high protein (18-21). The protein source in some of the studies is in liquid form only, powders, or from specific sources such as soy; whereas, the protein in the Atkins diet in this study is from a variety of sources and forms.

Total ghrelin concentrations differed after breakfast to 1 hr after lunch in normal weight women starting on the AHA diet and in all overweight women. Most studies done on total ghrelin have measured the effect of a breakfast meal or lunch meal on total ghrelin concentrations over a 3-4 hr period (16, 21). When the Atkins diet was given before the AHA diet, total ghrelin concentrations were lower in overweight women consuming the Atkins diet in comparison to overweight women consuming the AHA diet, however, when the AHA diet was given before the Atkins diet, total ghrelin concentrations were lower in overweight women consuming the AHA diet in comparison to overweight women consuming the Atkins diet. Studies have shown the total ghrelin concentrations were lower in subjects consuming a high protein meal 3-4 hours post meal in comparison to subjects consuming a high carbohydrate meal (22). Total ghrelin finding of this study may differ from previous studies for several reasons. First, the sequence in which the diets were given may be affecting total ghrelin concentrations. Second, the high protein diet in our study is also low carbohydrate whereas in other studies, the carbohydrate content is double or triple the percent calories from carbohydrate in our study (16, 21). Lastly, we studied specific weight classes in this study with each group having a tight BMI range in comparison to the broad range of BMIs used in previous studies (16, 19, 20, 21).

Insulin concentrations in response the macronutrient content of diet reacted similarly to previous studies (26). Overall, overweight and normal weight women consuming the Atkins
diet had lower insulin concentrations in comparison to overweight and normal weight women consuming the AHA diet after breakfast and after dinner. There were differences in the response of insulin to the diet in the different sequences around lunchtime and before and right after dinner. When the AHA diet was consumed, insulin concentrations had more profound spikes after meals in comparison to when the Atkins diet was consumed. Insulin concentrations have been shown to be lower when a high protein diet was consumed versus a high carbohydrate diet (27). Normal weight and overweight women responded differently to the AHA or Atkins diet when the sequence in which the diets were administered was different. Our data suggest that overweight women and normal weight women should be studied separately when looking at the response of insulin to dietary manipulations.

Increased glucagon concentrations in women consuming the Atkins diet may be a result of lower blood glucose concentrations caused by the low carbohydrate content of the Atkins diet. Glucagon maintains acute blood glucose concentrations therefore, we hypothesize that the lack of an increase in glucagon concentrations in the fasted state may be the result of the switch from immediate regulation of blood glucose by glucagon to short term fasting regulation of blood glucose by epinephrine and the increase in glucagon later in the day is a result of the low carbohydrate content of the diet (23-25). The increased glucagon concentrations were for a longer duration in women consuming the Atkins diet when the AHA diet was given before the Atkins diet in comparison to when the Atkins diet was given before the AHA diet. The order in which these diets are administered on the impact on glucagon concentrations needs further study.

In addition, leptin concentrations in response to the macronutrient content of the diet were similar to previous research (26). Leptin concentrations remained relatively stable over
the 14-hr period, which agrees with the previous findings of the 24-hr pattern of leptin concentrations. One of these studies showed that leptin concentrations peak at 02:00 and remains stable over waking hours when humans were fed a high fat/low carbohydrate or a high carbohydrate/low fat diet (28). Another study had leptin patterns in agreement with those of this study as leptin concentrations begin a gradual rise at 18:00 (26). For detection of changes of leptin in response to a diet, a 24-hr curve is the best measure of changes in leptin over time. However, measuring leptin concentrations at fasting would give an accurate picture of the leptin concentrations during waking hours (07:00-22:00) in most humans. In our study, overweight women consuming the Atkins and the AHA diets when the Atkins diet was given before the AHA had higher leptin concentrations in comparison to normal weight women and overweight women in the opposite sequence.

Adiponectin concentrations were not necessarily lower in overweight women in comparison to normal weight women. In obese subjects, adiponectin concentrations are lower than in normal weight counterparts (9). Adiponectin concentrations responded differently in overweight and normal weight women consuming the AHA or Atkins diets in each sequence. Some studies have shown that there is no effect of diet on adiponectin and that adiponectin has small changes in concentration from the fasted to postprandial states, however, in our study, adiponectin concentrations are influenced by diet, weight, timepoint, and sequence (10-12).

Growth hormone concentrations increased in normal weight women at the same time or after adiponectin concentrations appeared to be higher. Research has shown the adiponectin increases insulin sensitivity, increases glucose uptake in muscle, decreases liver gluconeogenesis and stimulates β-oxidation of fatty acids (13, 14). The increase in GH
concentrations near increases in adiponectin concentrations may be a result of GH stimulating FFA release from adipose tissue and adiponectin stimulating the use of the free fatty acids by muscle for β-oxidation (15). Further study into the possible relationship of GH and adiponectin is needed to understand fully the role of GH on glucose and fatty acid metabolism.

Universal dietary guidelines may not be appropriate for all humans to achieve optimal health. Consumption of enough calories to maintain weight resulted in no clear preprandial increase in ghrelin concentrations in women regardless of macronutrient composition of the diet thus indicating that energy balance influences the preprandial response of ghrelin concentrations. Both macronutrient and caloric content of the diet are important in modulating acylated ghrelin concentrations in women. Prediabetic and individuals with Type II diabetes may benefit from a high protein diet to control insulin profiles over the day. In addition, this study indicates that the order in which the diets are administered affects hormone concentrations in the blood. Experimental design is critical in obtaining information on the relationship of hormones related to appetite and body composition.

ACKNOWLEDGEMENTS

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thank K. Hanson for her support, advice, and assistance throughout all aspects of the study. MBB, LLA, AHT and DCB designed the research; MBB conducted the research; MBB analyzed the data; MBB wrote the paper; LLA, AHT, and DCB had responsibility for final content.

REFERENCES


Table 1: Characteristics of Female Participants

<table>
<thead>
<tr>
<th></th>
<th>Age (yr)</th>
<th>BMI (± std. dev.)</th>
<th>Total Mass (kg)</th>
<th>Body Fat (%)  (± std. dev.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Weight</td>
<td>24.25</td>
<td>21.3 ± 0.6</td>
<td>58.2 ± 4.9</td>
<td>22.9 ± 3.3</td>
</tr>
<tr>
<td>Overweight</td>
<td>25.75</td>
<td>26.7 ± 2.5</td>
<td>74.5 ± 3.0</td>
<td>32.7 ± 6.1</td>
</tr>
</tbody>
</table>

*Values are means ± std. dev.*
*a,b Means with different superscripts are different.*
Table 2: Significance of the Terms in the Model

<table>
<thead>
<tr>
<th>Term</th>
<th>Adiponectin</th>
<th>AG</th>
<th>TG</th>
<th>GH</th>
<th>Glucagon</th>
<th>Insulin</th>
<th>Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>0.29</td>
<td>0.0003</td>
<td>0.41</td>
<td>0.46</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Weight (wt)</td>
<td>0.31</td>
<td>0.40</td>
<td>0.90</td>
<td>0.66</td>
<td>0.95</td>
<td>0.95</td>
<td>0.008</td>
</tr>
<tr>
<td>Timepoint (tp)</td>
<td>0.77</td>
<td>0.007</td>
<td>0.031</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sequence (seq)</td>
<td>0.84</td>
<td>0.18</td>
<td>0.26</td>
<td>0.58</td>
<td>0.34</td>
<td>0.67</td>
<td>0.04</td>
</tr>
<tr>
<td>Diet*wt</td>
<td>0.67</td>
<td>&lt;0.0001</td>
<td>0.08</td>
<td>0.08</td>
<td>0.003</td>
<td>0.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Wt*seq</td>
<td>0.47</td>
<td>0.19</td>
<td>0.51</td>
<td>0.81</td>
<td>0.46</td>
<td>0.105</td>
<td>0.005</td>
</tr>
<tr>
<td>Tp*seq</td>
<td>0.72</td>
<td>0.73</td>
<td>0.98</td>
<td>0.83</td>
<td>0.0006</td>
<td>0.0023</td>
<td>0.39</td>
</tr>
<tr>
<td>Diet<em>wt</em>tp</td>
<td>0.025</td>
<td>0.802</td>
<td>0.25</td>
<td>0.33</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.32</td>
</tr>
<tr>
<td>Diet<em>wt</em>seq*tp</td>
<td>&lt;0.0001</td>
<td>0.038</td>
<td>0.0006</td>
<td>0.0002</td>
<td>0.0091</td>
<td>0.0016</td>
<td>&lt;0.0001</td>
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</table>

\(^1\) P < 0.05 was considered significant and 0.05 > P < 0.1 was considered a trend. AG = Acylated ghrelin, TG = Total ghrelin, GH = growth hormone.
<table>
<thead>
<tr>
<th>Measurement</th>
<th>Period 1</th>
<th></th>
<th></th>
<th>Period 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>99.0 ± 22.6</td>
<td>77.0 ± 9.9</td>
<td>81.5 ± 9.2</td>
<td>79.0 ± 9.9</td>
<td>71.0 ± 1.4</td>
<td>77.0 ± 1.4</td>
</tr>
<tr>
<td>Blood Urea Nitrogen (mg/dL)</td>
<td>23.5 ± 2.1</td>
<td>11.0 ± 1.4</td>
<td>11.0 ± 0.0</td>
<td>22.5 ± 0.7</td>
<td>22.0 ± 1.4</td>
<td>11.0 ± 0.0</td>
</tr>
<tr>
<td>AST (IU/mL)</td>
<td>27.0 ± 2.8</td>
<td>25.5 ± 2.1</td>
<td>31.5 ± 10.6</td>
<td>23.0 ± 2.8</td>
<td>24.0 ± 0.0</td>
<td>26.0 ± 2.8</td>
</tr>
<tr>
<td>ALT (IU/mL)</td>
<td>23.5 ± 0.7</td>
<td>19.0 ± 0.0</td>
<td>30.5 ± 0.7</td>
<td>17.5 ± 0.7</td>
<td>25.0 ± 2.8</td>
<td>21.5 ± 0.7</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>178.0 ± 17</td>
<td>127.0 ± 1.4</td>
<td>165.5 ± 26.2</td>
<td>148.5 ± 30.4</td>
<td>185.0 ± 2.8</td>
<td>156.5 ± 26.1</td>
</tr>
<tr>
<td>Triacylglycerols (mg/dL)</td>
<td>61.0 ± 22.6</td>
<td>55.0 ± 9.9</td>
<td>80.0 ± 9.2</td>
<td>52.5 ± 9.9</td>
<td>67.5 ± 1.4</td>
<td>94.5 ± 2.1</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dL)</td>
<td>59.5 ± 26.2</td>
<td>47.5 ± 4.9</td>
<td>60.0 ± 11.3</td>
<td>51.5 ± 2.1</td>
<td>77.5 ± 29.0</td>
<td>55.0 ± 24.0</td>
</tr>
<tr>
<td>VLDL Cholesterol (mg/dL)</td>
<td>12.5 ± 2.1</td>
<td>11.0 ± 0.0</td>
<td>16.0 ± 0.0</td>
<td>10.5 ± 2.1</td>
<td>13.5 ± 10.6</td>
<td>19.0 ± 4.2</td>
</tr>
<tr>
<td>LDL Cholesterol (mg/dL)</td>
<td>106.0 ± 11.3</td>
<td>68.5 ± 6.4</td>
<td>89.5 ± 37.5</td>
<td>86.5 ± 30.4</td>
<td>94.0 ± 21.2</td>
<td>82.5 ± 2.1</td>
</tr>
<tr>
<td>LDL/HDL ratio</td>
<td>2.05 ± 1.06</td>
<td>1.5 ± 0.28</td>
<td>1.55 ± 0.92</td>
<td>1.70 ± 0.71</td>
<td>1.35 ± 0.78</td>
<td>1.65 ± 0.78</td>
</tr>
</tbody>
</table>

*Values are means ± std. dev. ^Missing values for both participants for period 1.

Δ indicates difference between diets only with no effect of period or weight.

Δ* indicates the difference in weight*diet with no effect of period.
Table 4: Body Composition

<table>
<thead>
<tr>
<th></th>
<th>Trunk Mass (kg)</th>
<th>Trunk Fat (%)</th>
<th>Total Mass (kg)</th>
<th>Total Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Period 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight Atkins(^a)</td>
<td>34.7 ± 3.3</td>
<td>37.3 ± 4.3</td>
<td>73.4 ± 3.6</td>
<td>37.4 ± 1.7</td>
</tr>
<tr>
<td>Normal Weight Atkins(^b)</td>
<td>25.9 ± 3.5</td>
<td>13.9 ± 1.8</td>
<td>56.8 ± 7.4</td>
<td>18.6 ± 2.0</td>
</tr>
<tr>
<td>Overweight AHA(^a)</td>
<td>31.6 ± 1.2</td>
<td>22.5 ± 3.0</td>
<td>70.1 ± 0.7</td>
<td>26.2 ± 0.0</td>
</tr>
<tr>
<td>Normal Weight AHA(^b)</td>
<td>24.7 ± 1.4</td>
<td>19.9 ± 3.3</td>
<td>56.0 ± 3.1</td>
<td>25.1 ± 0.5</td>
</tr>
<tr>
<td><strong>Period 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight Atkins(^a)</td>
<td>30.7 ± 1.2</td>
<td>21.1 ± 2.2</td>
<td>69.3 ± 0.2</td>
<td>25.1 ± 0.2</td>
</tr>
<tr>
<td>Normal Weight Atkins(^b)</td>
<td>24.1 ± 2.1</td>
<td>19.1 ± 2.6</td>
<td>55.4 ± 3.8</td>
<td>24.8 ± 0.1</td>
</tr>
<tr>
<td>Overweight AHA(^a)</td>
<td>35.0 ± 2.3</td>
<td>36.6 ± 5.4</td>
<td>73.2 ± 3.0</td>
<td>36.9 ± 2.5</td>
</tr>
<tr>
<td>Normal Weight AHA(^b)</td>
<td>25.3 ± 3.0</td>
<td>13.8 ± 1.3</td>
<td>56.0 ± 7.1</td>
<td>19.2 ± 2.3</td>
</tr>
</tbody>
</table>

\(^1\)Values are means ± std. dev.
\(^a,b\) Means with different superscripts are different. Significant difference re between overweight and normal weight in trunk mass, trunk fat, total mass and total fat. There was no difference between diets or sequence.
Figure 1. Acylated ghrelin concentrations in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet and two normal and two overweight subjects were assigned to the AHA diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid and the AHA diet contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid of a 2,000 kcal/day diet. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. Breakfast was served after hour 1, lunch after hour 4, and dinner after hour 9. Significance is $P < 0.05$ with $\delta$ referring to the difference is between diets and $\omega$ referring to the difference is between weights with 1 or 2 referring to the sequence when the difference is between the diets or weights, and $\zeta$ referring to the difference between sequences.
Figure 2. Total ghrelin concentrations in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet and two normal and two overweight subjects were assigned to the AHA diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid and the AHA diet contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid of a 2,000 kcal/day diet. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. Breakfast was served after hour 1, lunch after hour 4, and dinner after hour 9. Significance is $P < 0.05$ with $\delta$ referring to the difference is between diets and $\omega$ referring to the difference is between weights with 1 or 2 referring to the sequence when the difference is between the diets or weights, and $\zeta$ referring to the difference between sequences.
Figure 3. Insulin concentrations in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet and two normal and two overweight subjects were assigned to the AHA diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid and the AHA diet contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid of a 2,000 kcal/day diet. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. Breakfast was served after hour 1, lunch after hour 4, and dinner after hour 9. Significance is $P < 0.05$ with $\delta$ referring to the difference is between diets and $\omega$ referring to the difference is between weights with 1 or 2 referring to the sequence when the difference is between the diets or weights, and $\varsigma$ referring to the difference between sequences.
Figure 4. Glucagon concentrations in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet and two normal and two overweight subjects were assigned to the AHA diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid and the AHA diet contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid of a 2,000 kcal/day diet. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. Breakfast was served after hour 1, lunch after hour 4, and dinner after hour 9. Significance is $P < 0.05$ with $\delta$ referring to the difference is between diets and $\omega$ referring to the difference is between weights with 1 or 2 referring to the sequence when the difference is between the diets or weights, and $\zeta$ referring to the difference between sequences.
Figure 5. Leptin concentrations in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet and two normal and two overweight subjects were assigned to the AHA diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid and the AHA diet contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid of a 2,000 kcal/day diet. Each diet was fed for 14 days, and then subjects Switched to the other diet, which is indicated by period 1 and period 2. Breakfast was served after hour 1, lunch after hour 4, and dinner after hour 9. Significance is $P < 0.05$ with $\delta$ referring to the difference is between diets and $\sigma$ referring to the difference is between weights with 1 or 2 referring to the sequence when the difference is between the diets or weights, and $\zeta$ referring to the difference between sequences.
Figure 6. Adiponectin concentrations in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet and two normal and two overweight subjects were assigned to the AHA diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid and the AHA diet contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid of a 2,000 kcal/day diet. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. Breakfast was served after hour 1, lunch after hour 4, and dinner after hour 9. Significance is \( P < 0.05 \) with \( \delta \) referring to the difference between diets and \( \omega \) referring to the difference between weights with 1 or 2 referring to the sequence when the difference is between the diets or weights, and \( \zeta \) referring to the difference between sequences.
Figure 7. Growth Hormone concentrations in the peripheral blood of normal weight women and overweight women fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet and two normal and two overweight subjects were assigned to the AHA diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid and the AHA diet contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid of a 2,000 kcal/day diet. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. Breakfast was served after hour 1, lunch after hour 4, and dinner after hour 9. Significance is $P < 0.05$ with $\delta$ referring to the difference is between diets and $\omega$ referring to the difference is between weights with 1 or 2 referring to the sequence when the difference is between the diets or weights, and $\zeta$ referring to the difference between sequences.
Chapter III: Part I

Effect of American Heart Association and Atkins diets on the change in circulating concentrations of ghrelin and other hormones involved in energy metabolism: comparison of the fed and fasted states over lunch period in normal and overweight men

A manuscript for submission to the Journal of Clinical Endocrinology and Metabolism

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Author disclosures: M. Bohan Brown, A. Trenkle, L. Anderson, D. Beitz, no conflicts of interest.
ABSTRACT

Investigating the role of hormones that regulate energy metabolism on energy balance and body composition in men when varying diets are consumed could provide insight into the etiology of obesity. In this study, we investigated the effect of Atkins and AHA diets in normal weight and overweight men when breakfast was consumed or skipped on the change in ghrelin and other energy metabolism hormones before and after consuming lunch. Eight male subjects, ages 19-24, were used in this study: four normal subjects with an average BMI of 21.3 and four overweight subjects with an average BMI of 27.9. Each subject received both treatments by a crossover design. Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Men on both diets were fed 3,000 kcal/day. Each diet was fed for 14 days, and then subjects switched diets. On days 6 and 20, breakfast was consumed before 08:00 and blood was taken at 11:00 and 1 hr after finishing the noon meal. On days 14 and 28, subjects were fasted and blood samples were taken at 11:00 and 1 hr after finishing the noon meal. Plasma samples were analyzed for acylated and total ghrelin, leptin, insulin, glucagon, growth hormone, and adiponectin. When consuming the Atkins diet, both normal weight and overweight men had higher adiponectin concentrations in the fed state in comparison to the fasted state. Men consuming the AHA diet had an increase in acylated ghrelin concentrations over the lunch period, whereas there was no change in acylated ghrelin concentrations in men consuming the Atkins diet. Both
normal weight and overweight men had increased glucagon concentrations in the fed state when consuming the Atkins diet when the AHA diet was administered before the Atkins diet. When consuming the Atkins diet, overweight men in the fed and fasted states had lower leptin when consuming the Atkins diet in comparison to the AHA diet when the Atkins was administered before the AHA diet. Grouping overweight and normal weight men would not be appropriate based on results in this study. Consumption of breakfast may be beneficial in overweight men in the decreasing the development of insulin resistance in conjunction with consumption of a lower carbohydrate diet.

INTRODUCTION

Ghrelin (acylated) is a 28 amino acid peptide with an octanoyl group on Ser3 (1). Acylated ghrelin stimulates nutrient intake, whereas leptin and insulin cause satiety (2, 3). Leptin and acylated ghrelin regulate the action of each other. Additionally, leptin can inhibit insulin secretion based on the needs of the adipose tissue; moreover, insulin signals the secretion of leptin from white adipose tissue (4).

Dietary macronutrient composition can affect hormone concentrations in the blood. Healthy non-obese men fed a high carbohydrate meal had a greater increase in plasma ghrelin in comparison to men fed the high fat meal (5). In addition, hunger sensation of subjects fed the high carbohydrate diet was suppressed more than that of subjects fed the high fat diet. However, subjects fed a high protein breakfast felt more satiated that subjects fed a high carbohydrate diet (6). Ghrelin concentration, however, increased with weight loss in humans when eating a low fat, high carbohydrate diet, whereas high fat diets decrease adiposity without increasing appetite (7).
Results from studies on high protein diets are mixed. Increasing specific protein sources (soy and casein) in the diet did not affect ghrelin concentrations (8, 9). Al Awar and colleagues showed that a high protein diet decreased acylated ghrelin concentrations, whereas Blom and colleagues showed that a high protein diet resulted in no change in acylated ghrelin concentrations (10, 11). However, men fed a high protein breakfast had no change in acylated ghrelin in comparison to an increase in acylated ghrelin concentrations in men fed a high carbohydrate breakfast (12).

Plasma concentrations of hormones can be influenced by weight class. Plasma ghrelin concentrations in obese humans are lower than ghrelin concentrations of normal weight individuals (13). One study demonstrated that refeeding after fasting did not decrease the ghrelin concentrations in obese human patients (14). In normal weight humans, fasting ghrelin concentrations decreased after feeding. Another study showed that ghrelin has a negative association with *ad libitum* feed intake (15). Studying the effects of diet composition on ghrelin concentrations in both normal weight, overweight, and obese patients is necessary to understand the mechanisms by which the body controls energy homeostasis. Thus, understanding the regulation of ghrelin under conditions of weight gain, maintenance, and loss could provide insight into understanding obesity (13).

The overall objective of this research was to elucidate the relationship of diet composition and ghrelin concentration with respect to obesity. We compared the effects of the macronutrient composition of two common diets, Atkins and American Heart Association (AHA), on ghrelin, other hormones associated with ghrelin, and blood metabolite concentrations (16, 17). We hypothesized that males with a greater propensity to obesity have greater ghrelin concentration in plasma, which stimulates greater food intake to cause
obesity. Furthermore, we hypothesized that subjects consuming the Atkins diet will have lower plasma ghrelin concentrations than subjects consuming the AHA diet.

MATERIAL AND METHODS

Subjects

Approval for all screening and study procedures was obtained from the Iowa State University Institutional Review Board. Each subject was screened for BMI, eating disorders, and major health problems through an interview and physical examination. Volunteers were excluded if they have or had an eating disorder, or have a major health problem such as diabetes, heart conditions, and hypoglycemia. After successful completion of the screening, eight male subjects, four with normal weight BMIs and four with overweight BMIs (18, Table 1) were assigned randomly to treatment diets.

Each subject received both treatment diets by a crossover design. Subjects were given 3,000 kcal/day based on average energy intake of males of that age group (19). For the first period, two normal and two overweight subjects were assigned to the Atkins diet and the others to the AHA diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The AHA diet contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Diets were formulated using Nutritionist Pro (version 2.3; First DataBank, Inc.; San Bruno, CA) by a registered dietitian. All diets were prepared and served by personnel at the Human Metabolic Unit at Iowa State University. Participants consumed each diet for 14 days with a repeating 7-day menu for each diet. Meals were served between 08:00-09:00, 12:00-13:00, and 17:00-18:00 in the dining area of Human Metabolic Unit at Iowa State University.
On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and 1 hr after completion of the noon meal (Fed state). On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00 (Fasted state). Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the completion of the noon meal. All blood was collected in EDTA-containing vacutainer tubes (BD Scientific, Franklin Lakes, NJ) and kept at 4 °C during processing. Samples were centrifuged at 3000 × g for 20 min. Plasma was collected and analyzed for adiponectin, ghrelin (acylated and total), glucagon, growth hormone, insulin, and leptin.

**Hormone Analysis**

Adiponectin concentrations were measured by using a commercially available radioimmunoassay (Linco Research, St. Charles, MO). The lower limit of detection was 1 ng/mL, and the intra-assay coefficient of variation was < 10%.

Total ghrelin concentrations were measured by using a commercially available radioimmunoassay kit (Linco Research). The lower limit of detection was 93 pg/mL, and the intra-assay coefficient of variation was < 10%.

For acylated ghrelin samples, 50 µL of 1 N hydrochloric acid and 10 µL of 10 mg/mL phenylmethylsulfonyl fluoride (PMSF, Sigma, St. Louis, MO) in methanol were added for every 1 mL of plasma immediately after centrifugation. Acylated ghrelin concentrations were measured using a commercially available radioimmunoassay kit (Linco Research). This assay has been found to be highly specific for acylated ghrelin with less than 0.1 % cross-reactivity for desoctanoyl ghrelin and no cross reactivity with ghrelin 14-28, motilin-related peptide, leptin, insulin, glucagon, or GLP-1 (7-36). The lower detection limit was 7.8
pg/mL. The intra-assay coefficient of variation was < 10%.

For glucagon samples, 50 µL of aprotinin (5500 KIU/mL, Sigma) were added to 1 mL of plasma with a final concentration of 550 KIU aprotinin per mL of plasma. All plasma samples were stored at -20 °C until assayed. Glucagon concentrations were measured by using a commercially available radioimmunoassay (Linco Research). The lower limit of detection was 20 pg/mL, and the intra-assay coefficient of variation was < 10%.

Growth Hormone concentrations were measured by using a commercially available radioimmunoassay (Diagnostic Products Corporation (DPC), Los Angeles, CA). The lower limit of detection was 0.9 ng/mL, and the intra-assay coefficient of variation was < 10%.

Insulin concentrations were measured by using a commercially available radioimmunoassay (Linco Research). The lower limit of detection was 0.2 µU/mL (1.389 pmol/L), and the intra-assay coefficient of variation was < 10%.

Leptin concentrations were measured by using a commercially available radioimmunoassay (Linco Research). The lower limit of detection was 0.05 ng/mL, and the intra-assay coefficient of variation was < 10%.

Statistics

Hormone data were analyzed by using the MIXED procedure in SAS (Version 9.1, SAS Institute, Cary, NC). All hormone data were analyzed as a split-plot with repeated measures ANOVA. The hormone concentrations for before lunch were analyzed. In addition, the change score of before lunch concentrations subtracted from 1 hr after lunch concentrations was analyzed. The model included fixed effects of diet, weight (weight class), state (fed or fasted), sequence, diet*weight, weight*sequence, sequence*state, diet*weight*state, and
diet*weight*state*sequence, while person(weight*sequence) was included in the model as the random effect. Significance of the interaction terms is listed in Table 2. No multiple comparison adjustments were made because this is an exploratory study. For all tests, \( P < 0.05 \) was considered significant and \( P < 0.1 \) was considered a trend.

**RESULTS**

**Comparison of the Fed and Fasted States before Lunch**

*Acylated ghrelin*

Acylated ghrelin concentrations in men were influenced by the diet, physiological state, and sequence (Fig. 1). Men consuming the Atkins diet had higher acylated ghrelin concentrations 1 hr before lunch than did men consuming the AHA diet (135.5 ng/L vs. 75.2 ng/L, \( P = 0.0025 \)). Acylated ghrelin concentrations were higher 1 hr before lunch in men that were fasted in comparison to men that were in the fed state (122.6 ng/L vs. 88.0 ng/L, \( P = 0.0492 \)). Acylated ghrelin concentrations were higher in the men who went from the AHA diet (145.7 ng/L) to the Atkins diet in comparison to men who went from the Atkins diet to the AHA diet (64.89 ng/L, \( P = 0.0499 \)).

*Total Ghrelin*

Insulin concentrations in men were influenced by the diet (Fig. 2). Men consuming the Atkins diet had higher total ghrelin concentrations in comparison to men who consumed the AHA diet (958 ng/L vs. 761 ng/L, \( P = 0.0034 \)). There were no significant differences in total ghrelin concentrations before lunch in the fasted or fed states. There was no effect of weight, physiological state, or sequence on total ghrelin concentrations 1 hr before lunch in men.
**Insulin**

Insulin concentrations in men were influenced by the diet and physiological state (Fig. 3). Men consuming the Atkins diet had lower insulin concentrations than did men consuming the AHA diet (55.6 pmol/L vs. 178.1 pmol/L, P = 0.0150). Insulin concentrations were higher in men in the fed state in comparison to the fasted state (183.6 pmol/L vs. 50.0 pmol/L, P = 0.0094). There was no effect of weight or sequence on insulin concentrations 1 hr before lunch in men.

**Glucagon**

The four-way interaction (diet*weight*state*sequence) term in the model was significant (P = 0.0051, Fig. 4). Overweight men consuming the Atkins diet had significantly higher glucagon concentrations in the fed state (321 ng/L) in comparison to the fasted state (97.9 ng/L) when the Atkins diet was given before the AHA diet (P < 0.0001). In the fed state, overweight men fed the Atkins diet had higher glucagon when the Atkins diet was given before the AHA diet (321.9 ng/L) in comparison to when the AHA diet was given before the Atkins diet (130.6 ng/L, P = 0.0002). Overweight men in the fed state had higher glucagon when consuming the Atkins diet in comparison to the AHA diet when the Atkins diet was administered before the AHA diet (321.9 ng/L vs. 106.4 ng/L, P < 0.0001).

Normal weight men responded similarly to overweight men with respect to the effects of diet, state, and sequence on glucagon concentrations. Normal weight men consuming the Atkins diet had higher glucagon concentrations in the fed state (276.6 ng/L) in comparison to the fasted state (65.4 ng/L) when the Atkins diet was given before the AHA diet (P = 0.0001). In the fed state, normal weight men consuming the Atkins diet had higher glucagon
concentrations when the Atkins diet was given before the AHA diet (276.6 ng/L) in comparison to when the AHA diet was given before the Atkins diet (135.6 ng/L, P = 0.0020). Normal weight men in the fed state had higher glucagon concentrations when consuming the Atkins diet (276.6 ng/L) in comparison with consuming the AHA diet (73.7 ng/L) when the Atkins diet was administered before the AHA diet (P = 0.0002).

**Leptin**

The four-way interaction (diet*weight*state*sequence) term in the model was significant (P = 0.0291, Fig. 5). In the fasted state, overweight men had lower leptin concentrations when consuming the Atkins diet (5.39 µg/L) in comparison to the AHA diet (8.52 µg/L) when the Atkins diet was given before the AHA diet (P = 0.0026). In the fasted state, overweight men had lower leptin concentrations when consuming the Atkins diet (5.00 µg/L) in comparison to the AHA diet (8.45 µg/L) when the AHA diet was given before the Atkins diet (P = 0.0013). In the fed state, overweight men had lower leptin concentrations when consuming the Atkins diet (4.91 µg/L) in comparison to the AHA diet (7.98 µg/L) when the AHA diet was given before the Atkins diet (P = 0.0029).

Overweight men consuming the AHA diet had lower leptin concentrations in the fed state (4.62 µg/L) in comparison to the fasted state (8.52 µg/L) when the Atkins diet was given before the AHA diet (P = 0.0005). In the fasted state, overweight men fed the AHA diet had higher leptin concentrations than that of normal weight men fed the AHA diet when the Atkins diet was given before the AHA diet (8.52 µg/L vs. 1.06 µg/L, P = 0.0362).
**Adiponectin**

The four-way interaction (diet*weight*state*sequence) term in the model was significant (P = 0.0007, Fig. 6). Overweight men consuming the Atkins diet had higher adiponectin concentrations in the fed state when compared to the fasted state when the Atkins diet was given before the AHA diet (8,825 µg/L vs. 4,580 µg/L, P = 0.0068). Normal weight men fed the Atkins diet had higher adiponectin concentrations in the fed state (11,500 µg/L) in comparison to the fasted state (6,606 µg/L) when the Atkins diet was given before the AHA diet (P = 0.0027). Overweight men in the fasted state consuming the AHA diet had lower adiponectin concentrations in the fed state in comparison to the fasted state when the Atkins diet was administered before the AHA diet (2,548 µg/L vs. 5,737 µg/L, P = 0.0305) and when the AHA diet was administered before the Atkins diet (7,336 µg/L vs. 3,928 µg/L, P = 0.0244).

When breakfast was consumed, overweight men fed the Atkins diet had higher adiponectin concentrations in comparison to overweight men fed the AHA diet when the Atkins diet was given before the AHA diet (8,825 µg/L vs. 2,548 µg/L, P = 0.0004). Normal weight men who ate breakfast had lower adiponectin concentrations when consuming the Atkins diet (5,005 µg/L) that normal weight men who ate breakfast consuming the AHA diet (7,133 µg/L) when the Atkins diet was given before the AHA diet (P = 0.0057) and when the AHA diet was given before the Atkins diet (5,005 µg/L vs. 8,193 µg/L, P = 0.0305). In the fasted state, normal weight men fed the Atkins had lower adiponectin concentrations in comparison to normal weight men fed the AHA diet when the Atkins diet was given before the AHA diet (6,606 µg/L vs. 9,555 µg/L, P = 0.0426).
**Growth Hormone**

Men tended to have higher GH concentrations in the fasted state in comparison to the fed state (1.48 µg/L vs. 1.28 µg/L, \( P = 0.0566 \), Fig. 7). There was no effect of weight, diet, or sequence on growth hormone concentrations 1 hr before lunch in men.

**Before and After Lunch Change in Hormone Concentrations**

**Acylated ghrelin**

There was a significant three-way interaction of diet*weight*state \( (P = 0.0465 , \text{Fig. 8}) \).

Overweight men in the fed state consuming the Atkins diet had a decrease in acylated ghrelin concentrations from 1 hr before lunch to 1 hr after lunch \((-27.4 \text{ ng/L})\), whereas overweight men in the fed state had an increase in acylated ghrelin concentrations from 1 hr before lunch to 1 hr after lunch when consuming the AHA diet \((44.9 \text{ ng/L}, P = 0.0246)\). Overweight men consuming the AHA had an increase in acylated ghrelin concentrations in the fed state \((44.9 \text{ ng/L})\) in contrast to a decrease in acylated ghrelin concentrations in the fasted state \((-47.2 \text{ ng/L})\) from 1 hr before lunch to 1 hr after lunch \((P = 0.0067)\). Acylated ghrelin increased in the fed state \((22.1 \text{ ng/L})\) and decreased in the fasted state \((-80.6 \text{ ng/L})\) from 1 hr before lunch to 1 hr after lunch in normal weight men consuming the AHA diet \((P = 0.0033)\).

**Total Ghrelin**

There were no significant differences in the change in total ghrelin concentrations before lunch in the fasted or fed states (Fig. 9). There was no effect of weight, diet, physiological state, or sequence on the change before and after lunch in total ghrelin concentrations.
**Insulin**

There was a significant three-way interaction of diet*weight*state (P = 0.004, Fig. 10). In the fasted state, the change in insulin concentrations was greater in overweight men consuming the AHA diet in comparison to the Atkins diet (536.8 pmol/L vs. 32.6 pmol/L, P = 0.0005). Overweight men consuming the AHA diet had a significant difference in the change in insulin concentrations between the fed and fasted states (-163.9 pmol/L vs. 536.8 pmol/L, P < 0.0001). Overweight men consuming the AHA diet had a greater increase in insulin concentrations from 1 hr before lunch to 1 hr after lunch than did normal weight men consuming the AHA diet (536.8 pmol/L vs. 223.7 pmol/L, P = 0.0241).

**Glucagon**

The change in glucagon concentrations was higher in men in the fasted state (48.6 ng/L) in comparison to men in the fed state (-0.3916 ng/L, P = 0.0410, Fig. 11). There was no effect of weight, diet, or sequence on the change before and after lunch in glucagon concentrations.

**Leptin**

The four-way interaction (diet*weight*state*sequence) term in the model tended to be significant (P = 0.0510, Fig. 12). Men in the fed state had a decrease in leptin concentrations (-0.39 µg/L) 1 hr before lunch to 1 hr after lunch; in contrast, men in the fasted state had a increase in leptin concentrations (48.6 µg/L) 1 hr before lunch to 1 hr after lunch (P = 0.0222). There was a tendency for the 3 way interaction of diet*weight*state (P = 0.0510). There was a significant difference in the change in leptin concentration between the AHA
(1.25 µg/L) and Atkins (-0.70 µg/L) diets in overweight men in the fed state (P = 0.0168). The change in leptin concentrations was significantly different between the fed (1.25 µg/L) and fasted (-1.62 µg/L) states in overweight men consuming the AHA diet (P = 0.0015).

**Adiponectin**

There was a significant two-way interaction of diet*weight (P = 0.0362, Fig. 13). Overweight men fed the Atkins diet had a decrease in adiponectin concentrations from 1 hr before lunch to 1 hr after lunch (-896 µg/L), however, overweight men fed the AHA diet had an increase in adiponectin concentrations from 1 hr before lunch to 1 hr after lunch (1869 µg/L, P = 0.0103). When fed the AHA diet, overweight men had an increase in adiponectin concentrations over the lunch period (1869 µg/L), whereas normal weight men had a decrease in adiponectin concentrations over the lunch period (-630 µg/L, P = 0.0132)

**Growth Hormone**

There were no significant differences in the change in growth hormone concentrations before lunch in the fasted or fed states (Fig. 14). There was no effect of weight, diet, physiological state, or sequence on the change before and after lunch in growth hormone concentrations.

**DISCUSSION**

Breakfast consumption in obese humans is a key element to successful weight loss (20). The nutrient composition of breakfast has been shown to increase feelings of satiety (6). In our study, visual analog scale of satiety was not taken but informally male subjects reported
feeling increased hunger when consuming the AHA diet versus the Atkins diet.

Plasma adiponectin concentrations before lunch in men were influenced by diet, weight class, sequence and the consumption of breakfast. When consuming the Atkins diet, both normal weight and overweight men had higher adiponectin concentrations in the fed state in comparison to the fasted state. Adiponectin has not been shown to be influenced by dietary composition but by the amount of calories in the diet, which is not in agreement with our data (21-23). In our study, dietary composition affected adiponectin concentrations before lunch and the change in adiponectin concentrations. The influence of the dietary composition was different between overweight men and normal weight men. Normal weight men had lower adiponectin when consuming the Atkins diet in comparison to the AHA diet after consuming breakfast. The carbohydrate content of the diet may have influenced the adiponectin concentrations. With the increase in blood glucose from the high carbohydrate breakfast meal, adiponectin concentrations may be higher to increase glucose uptake in muscle and inhibit endogenous glucose production in the liver (24, 25), whereas the lower adiponectin concentration observed with the Atkins diet may be a result of the low carbohydrate content of the diet. In addition, the change in adiponectin concentrations in overweight men was influenced by dietary composition. Adiponectin concentrations decreased when the Atkins diet was consumed and increased when the AHA diet was consumed. There was no effect of fed or fasted states on the change adiponectin concentrations from pre-prandial to postprandial state in men in our study. Overweight men consuming the AHA diet had a significant change in adiponectin from the pre-prandial to the postprandial state. Some research has shown that adiponectin has small changes in concentration from the fasted to postprandial states (21, 22).
In addition, some research shows that adiponectin concentrations are lower in obese individuals in comparison to normal weight individuals (26). Some research shows that adiponectin was lower in obese men in comparison to their normal weight counterparts (27). However, in our research, overweight men had high adiponectin concentrations than did normal weight men when consuming the AHA diet. Our research indicates that grouping overweight and normal weight men may not be appropriate when measuring adiponectin concentrations.

Men had higher acylated ghrelin in the fasted state in comparison to the fed state. Many studies have studied the effects of a high protein breakfast on ghrelin concentrations (10, 11). Another study measured the effects of a high protein in comparison to normal protein diet on the changes in acylated ghrelin over lunch but only in the postprandial state after breakfast (10). In our study, we measured the response on acylated and total ghrelin response over the lunch period both in a fasted and fed state. There was no change in total ghrelin concentrations from pre-prandial to postprandial state. One study showed that men consuming a high protein breakfast had decrease in total ghrelin concentrations at three and four hours post lunch than that of men consuming a high carbohydrate breakfast; however, there was no change observed in acylated ghrelin concentrations in men between the high carbohydrate and the high protein breakfast (11). Another study indicated that acylated ghrelin concentrations were lower in normal weight women consuming a high protein breakfast in comparison to a normal protein breakfast (10). However, no significant difference in ghrelin concentrations was observed in women consuming normal and high casein diets or in men and women consuming normal and high soy protein diets (8, 9). We observed a difference in acylated ghrelin concentrations between the Atkins and AHA diets.
before lunch. In a recent study, men fed a low carbohydrate diet for weight loss did not see
the increase in acylated ghrelin that is associated with weight loss (12). However, men eating
the Atkins diet had higher acylated ghrelin than did men consuming the AHA diet.

Most of the high protein diets compared to our Atkins diet contained between 20-35 % of
calories as protein with a range of carbohydrate of 35% to 55% of calories and various food
matrices and protein sources (8-10, 28). Men consuming high protein breakfast had lower
total ghrelin concentrations in comparison to men consuming a normal protein breakfast (11).
The period between before and after lunch may have not been long enough to detect
differences in total ghrelin concentrations seen in other studies as changes in total ghrelin
have been observed in men and women three to four hours after consuming a meal, however,
the time interval of this study was chosen to investigate acute effects on hormone
concentrations (11, 28).

Men consuming the AHA diet had an increase in acylated ghrelin concentrations over the
lunch period, whereas there was no change in acylated ghrelin concentrations in men
consuming the Atkins diet. In a recent study, men who consumed a high carbohydrate
breakfast had an increase in ghrelin concentrations over 3 hours and had increased caloric
intake over 24 hours; whereas men who consumed a high protein breakfast did not have an
increase in acylated ghrelin concentrations and consumed less calories over 24 hours (12). A
high carbohydrate breakfast led to a greater increase in acylated ghrelin over lunch in both
overweight and normal weight men.

In women consuming the AHA and Atkins diets, GH concentrations were significantly
different in the fed and fasted states (Chapter 2 Part I). However, men consuming the Aktins
and AHA diets tended to have higher GH concentrations in the fasted state in comparison to
In short fasting times, ghrelin is suppressed, whereas in long-term undernutrition, ghrelin concentrations increase. The lack of differences between the fed and fasted states in ghrelin concentrations may be influenced by the GH/IGF-1 axis (29). Growth hormone concentrations were higher in the fasted state in women in comparison to the fed state (Chapter 2 Part I). However, there was no significant difference in acylated ghrelin concentrations between the fed and fasted states in men. In addition, the change in GH concentrations was greater in the fasted state in comparison to the fed state in women and only tended to be higher in men (Chapter 2 Part I). Growth hormone can be a negative feedback on total ghrelin (29). During short term fasting, GH can serve as a feedback to ghrelin thus decreasing the concentration of ghrelin the blood (30). In men, GH concentrations may not have been high enough to regulate ghrelin concentrations as seen in women under the same conditions.

In men, glucagon concentrations were influenced by dietary composition, weight class, sequence and physiological state. Both normal weight and overweight men had increased glucagon concentrations in the fed state when consuming the Atkins diet when the AHA diet was administered before the Atkins diet. Glucagon concentrations were lower in men fed the Atkins diet when the Atkins diet was given before the AHA diet. The low carbohydrate content of the Atkins diet may have caused the increased glucagon concentrations because of low blood glucose concentrations. Glucagon maintains acute blood glucose concentrations (31). The lack of an increase in glucagon concentrations in the fasted state may be the result of the switch from immediate regulation of blood glucose by glucagon to short term fasting regulation of blood glucose by epinephrine (31-33). In addition, switching from a high carbohydrate diet to a low carbohydrate diet may affect glucose metabolism. A high protein
breakfast resulted in higher glucagon concentrations in men at 30 min. after consumption with no change in glucagon concentrations in men consuming a high carbohydrate breakfast (11). Glucagon concentrations increased in men in the fasted state in comparison with the fed state from the pre-prandial to the postprandial states. The increase in glucagon in men when lunch was consumed after fasting could be the result of glucagon regulation of glucose homeostasis.

Insulin concentrations were influenced by the main effects of diet and physiological state. As expected, insulin concentrations were higher in men who had consumed breakfast in comparison to men who were fasted. When food is digested and absorbed, blood glucose concentrations increase and in response to increased blood glucose concentrations, insulin concentrations increase as well. In addition, men consuming the Atkins diet had lower insulin concentrations in comparison with the men consuming the AHA diet. Insulin concentrations are lower when dietary carbohydrates are decreased and replaced by dietary lipid (34). Insulin concentrations were higher in overweight women, which is similar to the findings in obese women (35, 36), whereas in men, there was no significant difference in insulin concentrations between overweight and normal weight men.

The change in insulin concentrations over lunch were influenced by the interaction weight, diet, and physiological state. Fasted overweight men had an increase in insulin concentration over lunch when consuming the AHA diet in comparison to fasted overweight men consuming the Atkins diet. Our findings agree with another study where insulin concentrations increased at 30 min. postprandial in men consuming a high carbohydrate diet, whereas men consuming a high protein diet did not have a change in insulin concentrations (11).
In our study, we saw little change in insulin concentrations when dietary carbohydrate was decreased and protein and lipid increased as evidenced by overweight men consuming the AHA diet having greater increases in insulin concentrations in comparison to the Atkins diet. The increase in insulin concentrations was greater in overweight men in comparison to normal weight men consuming the AHA diet in the fasted state. Some research shows that insulin resistance increases with increasing BMI (37). The increase in insulin concentrations when lunch was consumed after fasting was seen in both normal weight and overweight men only when consuming the AHA diet, which indicates that consuming a higher protein/lower carbohydrate diet such as the Atkins diet results in lower insulin concentrations.

Overweight men consuming the AHA diet had a decrease in insulin concentrations over lunch in the fed state in comparison to an increase in insulin concentrations in the fasted state over lunch. Consuming breakfast may improve the insulin response to the lunch meal and decrease insulin resistance, whereas skipping breakfast increases insulin concentrations over the lunch period when consuming a high carbohydrate diet such as the AHA diet. There was no significant difference in the change in insulin concentrations in normal weight men between the fed and fasted states when consuming the AHA diet. Normal weight and overweight men had different insulin responses, and thus should be studied separately to better understand the changes in insulin concentrations in men of different BMI classes.

Leptin concentrations are higher in obese individuals in comparison to normal weight individuals. In our study, leptin concentrations were higher in overweight men in comparison to normal weight men when consuming the AHA diet when the AHA diet was given before the Atkins diet. Consuming the Atkins diet before the AHA diet may have influenced the response of leptin to the AHA diet. Overweight men had higher leptin
concentrations in the fasted state in comparison to the fed state. One study has shown participants felt hungrier at lunch when breakfast had been consumed (38). When consuming the Atkins diet, overweight men in the fed and fasted states had lower leptin when consuming the Atkins diet in comparison to the AHA diet when the Atkins was administered before the AHA diet. Subjects fed a high protein diet felt more satiated than subjects fed a high carbohydrate diet (6). However, the concentrations of leptin in overweight men in our study were lower, indicating a possible decrease in satiety.

Leptin concentrations increased over lunch when overweight men had consumed breakfast, whereas leptin concentrations decreased in overweight men over lunch when breakfast was not eaten. Another study has shown that consuming breakfast increases food intake at the lunch meal (38). Leptin concentrations increase in overweight men consuming the AHA diet in comparison to a decrease in leptin concentrations on the Atkins diet. The low carbohydrate in the diet may have suppressed the insulin-mediated stimulation of leptin secretion (4).

Universal dietary guidelines may not be appropriate for all humans to achieve optimal health. Grouping overweight and normal weight men would not be appropriate based on results in this study. Weight class affected some hormone concentrations differently and should be studied and analyzed separately. In addition, dietary composition influences hormone concentrations. High protein diets among multiple studies affected hormone concentrations differently. The differences in findings between studies lie with the total dietary composition. Several high protein diets increased the percent of calories from protein by lowering the percent calories from lipid or by lowering calories from both carbohydrate and lipid. Each class of macronutrients influences hormone concentrations and care should
be taken when drawing conclusions about the general effects of any diet based on changing a single macronutrient. Consumption of breakfast may be beneficial in overweight men by decreasing the development of insulin resistance in conjunction with consumption of a lower carbohydrate diet. Experimental design is critical in obtaining information on the relationship of hormones related to appetite and body composition.

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REFERENCES


Table 1: Characteristics of Male Participants

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<sup>a,b</sup> Values are means ± std. dev.
<sup>a,b</sup> Means with different superscripts are different.
Table 2: Significance of the Terms in the Model

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<td>0.41</td>
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*P < 0.05 was considered significant and 0.05 > P < 0.1 was considered a trend. AG = Acylated ghrelin, TG = Total ghrelin, GH = growth hormone.*
**Figure 1.** Acylated ghrelin concentrations (mean ± SEM) in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets (upper panel). Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 3,000 kcal/day. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. The main effects significant of diet (P = 0.0025), state (P = 0.0492), and sequence (P = 0.0499) were significant. The acylated ghrelin concentrations in normal weight and overweight men fed the AHA and Atkins diets are shown in the lower panel. a–b Treatment means with different superscripts differ, P < 0.05.
Figure 1
Figure 2. Total ghrelin concentrations (mean ± SEM) in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets (upper panel). Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 3,000 kcal/day. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. The main effect of diet was significant (P = 0.0034). The total ghrelin concentrations in normal weight and overweight men fed the AHA and Atkins diets between sequence 1 and sequence 2. a–b Treatment means with different superscripts differ, P < 0.05.
Figure 3. Insulin concentrations (mean ± SEM) in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets (upper panel). Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 3,000 kcal/day. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. The main effects of diet and state were significant (P = 0.0150, P = 0.0094). The insulin concentrations in normal weight and overweight men fed the AHA and Atkins diets in the fed and fasted states are shown in the lower panel. 

Treatment means with different superscripts differ, \( P < 0.05 \).
Figure 3
Figure 4. Glucagon concentrations (mean ± SEM) in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 3,000 kcal/day. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. The interaction of diet*weight*state*sequence was significant (P = 0.0051). a–b Treatment means with different superscripts at specific times differ, \( P < 0.05 \) with \( \delta \) referring to the difference is between diets, \( \theta \) referring to the difference between sequences, and \( \xi \) referring to the difference between states.
Figure 4

The graph shows the glucagon concentration (ng/L) in different conditions and groups. The x-axis represents the periods (Fed and Fasted) and the y-axis represents the glucagon concentration. The graph distinguishes between different groups: Overweight Atkins, Normal weight Atkins, Overweight AHA, and Normal weight AHA. The bars indicate the mean values with error bars representing the standard deviation.
Figure 5. Leptin concentrations (mean ± SEM) in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets (upper panel). Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 3,000 kcal/day. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. The interaction of diet*weight*state*sequence was significant (P = 0.0291). a–b Treatment means with different superscripts differ, P < 0.05 with δ referring to the difference is between diets, ω referring to the difference is between weights and ξ referring to the difference between states.
Figure 5

Leptin Concentration (µg/L)

Overweight Atkins
Normal weight Atkins
Overweight AHA
Normal weight AHA

δ1a, δ1b, δ2a, δ2b, ε2a, ε2b

Fed Fasted Fed Fasted

Period 1 Period 2
Figure 6. Adiponectin concentrations (mean ± SEM) in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 3,000 kcal/day. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. The interaction of diet*weight*state*sequence was significant (P = 0.0007). a–b Treatment means with different superscripts at specific times differ, P < 0.05 with δ referring to the difference is between diets and ζ referring to the difference between states.
Figure 6

[Graph showing adiponectin concentration (μg/L) across different conditions and periods.]
Figure 7. Growth hormone concentrations (mean ± SEM) in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets (upper panel). Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 3,000 kcal/day. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. The main effect of physiological state tended to be significant (P = 0.0566). The GH concentrations in the fed and fasted state in men are shown in the lower panel. a–b Treatment means with different superscripts at specific times differ, $P < 0.05$. 


Figure 7

The upper graph shows the growth hormone concentration (μg/L) for different periods and dietary conditions. The lower graph illustrates the concentration differences between fed and fasted states.
Figure 8. Changes in acylated concentrations (mean ± SEM) in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets (upper panel). Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 3,000 kcal/day. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. The diet*weight interaction was significant (p = 0.0202). The changes in acylated ghrelin concentrations in normal weight and overweight men fed the AHA and Atkins diets are shown in the lower panel. a–b Treatment means with different superscripts differ, P < 0.05 with δ referring to the difference is between diets and ω referring to the difference is between weights.
Figure 8

[Bar chart showing changes in acylated ghrelin concentrations (ng/L) for different weight groups across fed and fasted periods.]
**Figure 9.** Changes in total ghrelin concentrations (mean ± SEM) in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 3,000 kcal/day. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. There were no significant differences in the changes in total ghrelin concentrations. a–b Treatment means with different superscripts at specific times differ, $P < 0.05$. 
Figure 9

[Bar chart showing changes in total ghrelin concentrations (ng/L) for different conditions and periods.]

Legend:
- Overweight Atkins
- Normal weight Atkins
- Overweight AHA
- Normal weight AHA

Y-axis: Change in Total Ghrelin Concentrations (ng/L)
X-axis: Fed, Fasted (Period 1, Period 2)
**Figure 10.** Changes in insulin concentrations (mean ± SEM) in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets in the fed and fasted states. Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 3,000 kcal/day. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. The interaction of diet*weight*state was significant (P = 0.0040). a–b Treatment means with different superscripts differ, P < 0.05 with δ referring to the difference is between diets, ω referring to the difference is between weights, θ referring to the difference between sequences, and ξ referring to the difference between states.
Figure 11. Changes in glucagon concentrations (mean ± SEM) in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets in the fed and fasted states. Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 3,000 kcal/day. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. The main effect of state was significant (P = 0.0410). The glucagon concentrations in the fed and fasted state in men are shown in the lower panel. a–b Treatment means with different superscripts differ, P < 0.05.
Figure 11

![Bar chart showing changes in glucagon concentrations between fed and fasted states for different groups.](image)

- Fed vs. Fasted in Period 1:
  - Overweight Atkins
  - Normal weight Atkins
  - Overweight AHA
  - Normal weight AHA

- Fed vs. Fasted in Period 2:
  - Overweight Atkins
  - Normal weight Atkins
  - Overweight AHA
  - Normal weight AHA

Legend:
- Black: Fed
- Grey: Fasted

Notations:
- a
- b
**Figure 12.** Changes in leptin concentrations (mean ± SEM) in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 3,000 kcal/day. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. The interaction of diet*weight*state tended to be significant (P = 0.0510). a–b Treatment means with different superscripts at specific times differ, $P < 0.05$ with $\delta$ referring to the difference is between diets and $\zeta$ referring to the difference between states.
Figure 12
Figure 13. Changes in adiponectin concentrations (mean ± SEM) in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 3,000 kcal/day. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. The interaction of diet*weight was significant (P = 0.0362). The adiponectin concentrations in normal weight and overweight women fed the AHA and Atkins diets are shown in the lower panel. a–b Treatment means with different superscripts at specific times differ, $P < 0.05$ with $\delta$ referring to the difference is between diets and $\omega$ referring to the difference is between weights.
Figure 13
Figure 14. Changes in growth hormone concentrations (mean ± SEM) in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was given at 3,000 kcal/day. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. On days 6 and 20, breakfast was consumed between 07:00-08:00 and blood was taken at 1 hr before and after the noon meal. On days 14 and 28, subjects were fasted from 22:00 on day 13 to 12:00 on day 14 and then fed their meal at 12:00. Two blood samples were taken on days 14 and 28 at 11:00 and 1 hr after the meal. There were no significant differences in the changes in total ghrelin concentrations. a–b Treatment means with different superscripts at specific times differ, $P < 0.05$. 


Chapter III: Part II

Effect of American Heart Association and Atkins diets on the circulating concentration profiles of ghrelin and other hormones involved in energy metabolism in normal and overweight men

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Author disclosures: M. Bohan Brown, P. Dixon, A. Trenkle, L. Anderson, D. Beitz, no conflicts of interest.
ABSTRACT

Investigating the role of appetite-related hormones on energy balance and body composition when varying diets are consumed could provide insight into the etiology of obesity. Eight male subjects, ages 20-30, were used in this study: Four normal weight subjects with BMI of 19-24 and four overweight subjects with of BMI of 27-30. Each subject received both treatments by a crossover design. Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the AHA diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Each diet was fed for 14 days, and then subjects switched to the other diet. Blood was taken every hour from 07:00 to 09:00 on days 13 and 27 of the study. Plasma samples were analyzed for active and total ghrelin, leptin, insulin, glucagon, growth hormone, and adiponectin. We hypothesized that subjects consuming the Atkins diet will have different plasma hormone concentrations over a 14-hr period than subjects consuming the AHA diet. Men consuming the AHA diet in period 2 had increased acylated ghrelin and total ghrelin concentrations in comparison to when the men consumed the Atkins diet. Insulin concentrations were suppressed in men consuming the Atkins diet in comparison to men consuming the AHA diet. Glucagon concentrations were higher in men consuming the Atkins diet in comparison to the AHA diet after lunch until the end of the study period (hours 7-14). Universal dietary guidelines for both men and women together along with weight classes may not achieve optimal health. This study indicates that the order in which the diets are administered affects hormone concentrations in
the blood. Overweight men do not respond the same as normal weight men. Experimental design is critical in obtaining information on the relationship of hormones related to energy metabolism and body composition.

INTRODUCTION

Understanding diet and hormone interaction in the progression of obesity is crucial in finding a solution to this growing nutritional problem in America. Investigating the role of ghrelin on energy balance and body composition when varying diets are consumed could provide a better understanding of the control of energy metabolism. Understanding the regulation of ghrelin under conditions of weight gain, weight maintenance, and weight loss could provide insight into understanding obesity (1).

Plasma ghrelin concentrations in obese humans are lower than those of normal weight individuals (1). English and colleagues demonstrated that refeeding after fasting did not decrease the ghrelin concentrations in obese human patients (2). In normal weight humans, fasting ghrelin concentrations decreased after feeding. Salbe et al showed that ghrelin has a negative association with ad libitum feed intake (3). Rats fed a high carbohydrate diet had higher plasma ghrelin than did rats fed a low carbohydrate diet (4). Healthy non-obese women were fed a high fat or high carbohydrate meal and the greatest increase in plasma ghrelin were seen in subjects consuming the high carbohydrate meal (5). In addition, hunger sensation of subjects fed the high carbohydrate diet was suppressed more than that of subjects fed the high fat diet. Ghrelin concentration, however, increased with weight loss of humans when eating a low fat, high carbohydrate diet (6). In the Weigle and colleagues study, high fat diets decrease adiposity without increasing appetite. Most studies measure
total ghrelin and not the acylated form (7). The few studies involving ghrelin and diet composition have conflicting results, leaving the relationship between ghrelin and diet composition unclear.

The overall objective of this research was to elucidate the relationship of diet composition and ghrelin concentration with respect to obesity. To accomplish the overall objective, we compared the variation of ghrelin over 15 hr in lean and overweight men. We compared the effects of two common diets, Atkins and American Heart Association (AHA), on ghrelin, other hormones associated with ghrelin, and blood metabolite concentrations. We hypothesized that males with a greater propensity to obesity have greater ghrelin concentration in plasma over 15-hr period, which stimulates greater food intake to cause obesity. Furthermore, we hypothesized that subjects consuming the Atkins diet will have lower plasma ghrelin concentrations than subjects consuming the AHA diet.

MATERIAL AND METHODS

Subjects

Approval for all screening and study procedures was obtained from the Iowa State University Institutional Review Board. Approval for the use of dual-energy x-ray absorptiometry (DXA) was obtained from the State of Iowa Department of Public Health. Each subject was screened for BMI, eating disorders, and major health problems through an interview and physical examination. Volunteers were excluded if they have or had an eating disorder or have a major health problem such as diabetes, heart conditions, and hypoglycemia. After successful completion of the screening, eight male subjects were assigned randomly to treatment diets.
Each subject received both treatment diets by a crossover design. Subjects were given 3,000 kcal/day based on average energy intake of males of that age group (8). Two normal and two overweight subjects were assigned to the Atkins diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid. The remaining two normal and two overweight subjects were assigned to the American Heart Association (AHA) diet that contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid. Diets were formulated using Nutritionist Pro (version 2.3; First DataBank, Inc.; San Bruno, CA) by a registered dietitian. All diets were prepared and served by personnel at the Human Metabolic Unit at Iowa State University. Each diet was fed for 14 days with a repeating 7-day menu for each diet. Meals were served between 08:00-09:00, 12:00-13:00, and 17:00-18:00 in the dining area of Human Metabolic Unit.

Three DXA scans were performed during the study per subject to monitor body composition. The scans were on the Friday before the study began, day 14, and day 28 of the study. Scans were performed at the ISU metabolic unit by trained personnel using a Hologic Delphi W dual-energy X-ray absorptometer (DXA) (Hologic Inc.; Bedford, MA).

A fasted blood serum sample was taken and a basic metabolic panel analysis was performed by Laboratory Corporation of America (LabCorp, Omaha, NE).

From day 12 at 22:00, subjects fasted until 8:00 on day 13 of each diet. Subjects arrived at the ISU Human Metabolic unit at 06:30. A 20 gauge peripheral intravenous catheter was placed in each subject. At each blood timepoint, 1 ml of blood was taken from the catheter and then the blood sample for analysis was taken. After the blood sample was taken, 3 mL of saline was administered to flush the catheter. Blood samples were taken every hour for 14 hours. All blood was collected in 3 mL tubercine syringes and placed in EDTA-containing
vacutainer tubes (BD Scientific, Franklin Lakes, NJ) and kept at 4 °C during processing. Samples were centrifuged at 3000 x g for 20 min. Plasma was collected and analyzed for adiponectin, ghrelin (acylated and total), glucagon, growth hormone, insulin, and leptin.

**Hormone Analysis**

Adiponectin concentrations were measured by using a commercially available radioimmunoassay (Linco Research, St. Charles, MO). The lower limit of detection was 1 ng/mL, and the intra-assay coefficient of variation was < 10%.

Total ghrelin concentrations were measured by using a commercially available radioimmunoassay kit (Linco Research, St. Charles, MO). The lower limit of detection was 93 pg/mL, and the intra-assay coefficient of variation was < 10%.

For acylated ghrelin samples, 50 µL of 1 N hydrochloric acid and 10 µL of 10 mg/mL phenylmethylsulfonyl fluoride (PMSF, Sigma, St. Louis, MO) in methanol were added for every 1 mL of plasma immediately after centrifugation. Acylated ghrelin concentrations were measured using a commercially available radioimmunoassay kit (Linco Research, St. Charles, MO). This assay has been found to be highly specific for acylated ghrelin with less than 0.1 % cross-reactivity for desoctanoyl ghrelin and no cross reactivity with ghrelin 14-28, motilin-related peptide, leptin, insulin, glucagon, or GLP-1 (7-36). The lower detection limit was 7.8 pg/mL. The intra-assay coefficient of variation was < 10%.

For glucagon samples, 50 µL of aprotinin (5500 KIU/mL, Sigma, St. Louis, MO) were added to 1 mL of plasma with a final concentration of 550 KIU aprotinin per mL of plasma. All plasma samples were stored at -20 °C until assayed. Glucagon concentrations were measured by using a commercially available radioimmunoassay (Linco Research, St.
Charles, MO). The lower limit of detection was 20 pg/mL, and the intra-assay coefficient of variation was < 10%.

Growth Hormone concentrations were measured by using a commercially available radioimmunoassay (Diagnostic Products Corporation (DPC), Los Angeles, CA). The lower limit of detection was 0.9 ng/mL, and the intra-assay coefficient of variation was < 10%.

Insulin concentrations were measured by using a commercially available radioimmunoassay (Linco Research, St. Charles, MO). The lower limit of detection was 0.2 µU/mL (1.389 pmol/L), and the intra-assay coefficient of variation was < 10%.

Leptin concentrations were measured by using a commercially available radioimmunoassay (Linco Research, St. Charles, MO). The lower limit of detection was 0.05 ng/mL, and the intra-assay coefficient of variation was < 10%.

Statistics

Hormone data were analyzed by using the MIXED procedure in SAS (Version 9.1, SAS Institute, Cary, NC). All hormone data were analyzed as a split-plot with repeated measures ANOVA. The hormone concentrations for before lunch were analyzed as well as the change score of before lunch concentrations subtracted from one hour after lunch concentrations. The model included fixed effects of diet, weight (weight class), timepoint, sequence, diet*weight, weight*sequence, sequence*timepoint, diet*weight*timepoint, and diet*weight*timepoint*sequence, while person(weight*sequence*period) was included in the model as the random effect. Significance of the interaction terms is listed in Table 1. No multiple comparison adjustments were made because this is an exploratory study. For all tests, P < 0.05 was considered significant and P < 0.1 was considered a trend.
RESULTS

**Body Composition and Blood Metabolites**

There were no significant changes in total body mass, total body fat percentage, trunk mass, and trunk fat percentage between the Atkins diet and AHA diet within a weight class. Blood urea nitrogen was higher in men consuming the Atkins diet in comparison to the AHA diet (P<0.0001). Serum ALT concentrations were higher in men consuming the AHA diet in comparison to men consuming the Atkins diet (P = 0.0188). Serum TAGs were higher in overweight men in comparison to normal weight men (P = 0.0493). HDL cholesterol was higher in men consuming the Atkins diet in comparison to the AHA diet (P = 0.0214) and was higher in men consuming the AHA diet in comparison to the Atkins (P = 0.0169); however, there was only significant difference in the ratio of LDL/HDL by weight class (overweight < normal weight, P = 0.0045).

**Hormone Concentrations**

**Ghrelin Active**

Acylated ghrelin concentrations over waking hours in overweight and normal weight men (individual data presented) are shown in Figure 1.

**Timepoint Comparisons**

Normal weight men consuming the Atkins diet had higher acylated ghrelin concentrations at after breakfast (hour 1, P = 0.0371) and tended to be higher fasting (P = 0.0538), after lunch (hour 5, P = 0.0583), and after dinner (hour 12, P = 0.0736) in comparison with acylated ghrelin concentrations in overweight men consuming the Atkins diet when the Atkins diet was given before the AHA diet. There were no significant differences in acylated
ghrelin concentrations between overweight and normal weight men consuming the Atkins
diet when the AHA diet was administered Atkins diet. Normal weight men had higher
acylated ghrelin concentrations at fasting (P = 0.0205), after breakfast (hour 1, P = 0.019),
before lunch (hour 4, P = 0.0308), after lunch (hour 5, P = 0.0067) and before dinner (hour
10, P = 0.0443) when the AHA diet was given before the Atkins diet in comparison to when
the Atkins diet was administered before the AHA diet.

There was no difference in acylated ghrelin concentrations between overweight and
normal weight men fed the AHA diet when the AHA diet was given before the Atkins diet.
Overweight men had lower acylated ghrelin concentrations after lunch (hour 7, P = 0.0176)
and higher acylated ghrelin concentrations before dinner (hour 9-10, P ≤ 0.0006) in
comparison to normal weight men consuming the AHA diet when the Atkins diet was given
before the AHA diet. Overweight men consuming the AHA diet had lower acylated ghrelin
concentrations before dinner (hours 8-10, P ≤ 0.0397) when the AHA diet was given before
the Atkins diet in comparison to when the Atkins diet was given before the AHA diet.
Normal weight men consuming the AHA diet had lower acylated ghrelin concentrations
between lunch and dinner (hours 7-8, P ≤ 0.026) when the AHA diet was administered before
the Atkins diet in comparison to when the Atkins diet was administered before the AHA diet.

Atkins to AHA Comparisons

Normal weight men consuming the Atkins diet tended to have higher acylated ghrelin
concentrations before dinner (hour 10, P = 0.0676) and after dinner (hour 13-14, P ≤ 0.0682)
in comparison to normal weight men consuming the AHA diet when the AHA diet was given
before the Atkins diet. When the Atkins diet was administered before the AHA diet, normal
weight men consuming the Atkins diet had higher acylated ghrelin concentrations at fasting,
after breakfast (hours 1-2, \( P \leq 0.0152 \)), before and after lunch (hours 4-5, \( P \leq 0.0247 \)) and after dinner (hour 12, \( P = 0.014 \)) but had lower acylated ghrelin concentrations before dinner (hours 7-8, \( P \leq 0.0105 \)) in comparison to normal weight men consuming the AHA diet when the Atkins diet was given before the AHA diet. When the AHA diet was given before the Atkins diet, overweight men consuming the Atkins diet had higher acylated ghrelin concentrations after dinner (hour 12, \( P = 0.0243 \)) in comparison to overweight men consuming the AHA diet. When the Atkins diet was administered before the AHA diet, overweight men consuming the Atkins diet had lower acylated ghrelin concentrations before dinner (hours 8-10, \( P \leq 0.0039 \)) than did overweight men consuming the AHA diet.

**Total Ghrelin**

Total ghrelin concentrations over waking hours in overweight and normal weight men (individual data presented) are shown in Figure 2.

**Timepoint Comparisons**

There were no significant differences in total ghrelin concentrations between overweight and normal weight men consuming the Atkins diet in either sequence. Overweight men consuming the Atkins diet had higher total ghrelin concentrations at fasting (\( P = 0.0081 \)) when the Atkins diet was given before the AHA diet in comparison to when the AHA diet was given before the Atkins diet. Normal weight men consuming the Atkins diet had higher total ghrelin concentrations at fasting (\( P = 0.0019 \)) and after breakfast (hour 1, \( P = 0.0186 \)) when the Atkins diet was given before the AHA diet in comparison to when the AHA diet was given before the Atkins diet.

Overweight men consuming the AHA diet tended to have higher total
ghrelin concentrations before dinner (hour 8, \( P = 0.0528 \)) than did normal weight men consuming the AHA diet when the AHA diet was fed before the Atkins diet. When the Atkins diet was fed before the AHA diet, overweight men consuming the AHA diet tended to have higher total ghrelin after breakfast (hour 1, \( P = 0.0531 \)) in comparison to normal weight men consuming the AHA diet. There were no significant differences in total ghrelin concentrations when the Atkins diet was fed before the AHA diet and when the AHA diet was fed before the Atkins diet in overweight men consuming the AHA diet. Normal weight men consuming the AHA diet had higher total ghrelin concentrations before dinner (hour 9, \( P = 0.0733 \)) when the Atkins diet was administered before the AHA diet in comparison to when the AHA diet was administered before the Atkins diet.

**Atkins to AHA Comparisons**

Normal weight men consuming the Atkins diet had higher total ghrelin concentrations before and after lunch (hour 4-5, \( P \leq 0.0083 \)) and tended to have lower total ghrelin concentrations before dinner (hour 10, \( P = 0.0577 \)) in comparison to normal weight men consuming the AHA diet when the AHA diet was given before the Atkins diet. When the Atkins diet was administered before the AHA diet, normal weight men consuming the Atkins diet had higher total ghrelin concentrations at fasting, after breakfast (hour 1), and before lunch (hours 3, 4) and lower total ghrelin before dinner (hours 7-9) in comparison to normal weight men consuming the AHA diet (\( P \leq 0.0167 \)).

Overweight men consuming the Atkins diet had lower total ghrelin concentrations at fasting (\( P = 0.0442 \)) and tended to have lower total ghrelin concentrations at hour 8 (\( P = 0.0802 \)) in comparison to overweight men consuming the AHA diet when the AHA diet was administered before the Atkins diet. When the Atkins diet was administered before the AHA
diet, overweight men consuming the Atkins diet had higher total ghrelin at fasting (P = 0.0002) and hour 3 (P = 0.0355) and had lower total ghrelin concentrations before dinner (hour 9, P = 0.0067) in comparison to overweight men consuming the AHA diet.

**Insulin**

Insulin concentrations over waking hours in overweight and normal weight men (individual data presented) are shown in Figure 3.

*Timepoint Comparisons*

Overweight men consuming the Atkins diet had lower insulin after breakfast (hour 1, P = 0.0279) in comparison to normal weight men consuming the Atkins diet when the AHA diet was given before the Atkins diet. When the Atkins diet was administered before the AHA diet, overweight men consuming the Atkins diet had higher insulin concentrations before dinner (hour 10, P = 0.0401) in comparison to normal weight men consuming the Atkins diet. Overweight men consuming the Atkins diet had higher insulin concentrations before and after dinner (hours 10-14, P ≤ 0.0014) when the Atkins diet was administered before the AHA diet in comparison to when the AHA diet was administered before the Atkins diet. Normal weight men consuming the Atkins diet had lower insulin concentrations after breakfast (hour 1, P = 0.0317) and higher insulin concentrations before and after dinner (hours 10-14, P ≤ 0.0209) when the Atkins diet was given before the AHA diet in comparison to when the Atkins diet was given before the AHA diet.

Overweight men consuming the AHA diet had lower insulin concentrations at hour 13 (P = 0.0106) in comparison to normal weight men consuming the AHA diet when the AHA diet was given before the Atkins diet. When the Atkins diet was given before the AHA diet,
overweight men had higher insulin concentrations at fasting (P = 0.0148) and tended to have
higher insulin concentrations after breakfast (hour 2, P = 0.0528) in comparison to normal
weight men consuming the AHA diet.

Overweight men consuming the AHA diet had higher insulin concentrations at fasting (P = 0.0158), after lunch (hour 5, P = 0.0428) and tended to have higher insulin concentrations after breakfast (hour 1 and 4, P ≤ 0.078) when the Atkins diet was administered before the AHA diet in comparison to when the AHA diet was administered before the Atkins diet, however, insulin concentrations were lower after dinner (hours 11-14, P ≤ 0.0095) when the Atkins diet was given before the AHA diet in comparison to when the AHA diet was given before the Atkins diet in overweight men consuming the AHA diet. Normal weight men consuming the AHA diet had higher insulin concentrations after breakfast (hour 1, P = 0.0096) when the Atkins diet was administered before the AHA diet in comparison to when the AHA diet was administered before the Atkins diet, however, insulin concentrations were lower after dinner (hours 10-14, P ≤ 0.0004) when the Atkins diet was given before the AHA diet in comparison to when the AHA diet was given before the Atkins diet in overweight men consuming the AHA diet.

Atkins to AHA Comparisons

When the AHA diet was administered before the Atkins diet, normal weight men consuming the Atkins diet had lower insulin concentrations in comparison to normal weight men at hour 3 (P = 0.0134) and after dinner (hours 11-14, P < 0.0001). Normal weight men consuming the Atkins diet had lower insulin concentrations after breakfast to after lunch (hours 1-5, P ≤ 0.0441), before dinner (hour 10, P = 0.002), and tended to be lower after dinner (hour 12, P = 0.0641) in comparison to normal weight men consuming the AHA diet.
when the Atkins diet was given before the AHA diet. Overweight men consuming the Aktins diet had lower insulin concentrations after breakfast (hours 2-3, $P \leq 0.0316$) and after dinner (hours 11-14, $P < 0.0001$) in comparison to overweight men consuming the AHA diet when the AHA diet was given before the Atkins diet. When the Atkins diet was given before the AHA diet, overweight men consuming the Atkins diet had lower insulin concentrations at fasting ($P = 0.017$), after breakfast (hours 1-3, $P \leq 0.0193$), before lunch (hour 4, $P = 0.0347$), after lunch (hour 5, $P = 0.0033$), and before dinner (hour 10, $P < 0.0001$) in comparison to overweight men consuming the AHA diet.

**Glucagon**

Glucagon concentrations over waking hours in overweight and normal weight men (individual data presented) are shown in Figure 4. There were no sequences effects in overweight and normal weight men consuming either the Atkins or AHA diets in glucagon concentrations. Glucagon concentrations were higher hours 7-14 ($P \leq 0.0473$) in overweight men and hours 6-12 ($P \leq 0.0003$) and hour 14 ($P < 0.0001$) in normal weight men consuming the Atkins diet in comparison to fasting concentrations. Overweight men consuming the Atkins diet had higher glucagon concentrations at hours 7 ($P = 0.0003$) and 13 ($P = 0.0124$) in comparison to normal weight men consuming the Atkins diet.

Normal weight men consuming the Atkins diet had higher glucagon concentrations after breakfast (hour 2, $P = 0.0031$) and after lunch through after dinner (hours 6-14, $P \leq 0.0019$) in comparison to normal weight men consuming the AHA diet. Overweight men consuming the Atkins diet had higher glucagon concentrations at all timepoints in comparison to overweight men consuming the AHA diet.
Leptin

Leptin concentrations over waking hours in overweight and normal weight men (individual data presented) are shown in Figure 5.

Timepoint Comparisons

There were no significant differences in leptin concentrations between overweight and normal weight men consuming the Atkins diet when the AHA diet was given before the Atkins diet. When the Atkins diet was given before the AHA diet, overweight men consuming the Atkins diet had higher leptin at fasting (P = 0.0191), after breakfast (hour 1, P = 0.036) and tended to have higher leptin concentration before lunch (hours 2-4, P ≤ 0.0967) and at hour 8 (P= 0.0987) in comparison to normal weight men consuming the Atkins diet. Overweight men consuming the Atkins diet had higher leptin at fasting (P = 0.0152) when the Atkins diet was given before the AHA diet in comparison to when the AHA diet was given before the Atkins. Overweight men consuming the AHA diet had higher leptin concentrations before dinner (hours 7-9, P ≤ 0.0640) and lower leptin concentrations at hour 13 (P = 0.696) in comparison to normal weight women consuming the AHA diet when the AHA diet was given before the Atkins diet. Leptin concentrations tended to be higher in overweight men consuming the AHA diet when the Atkins diet was given before the AHA diet before lunch (hour 3, P = 0.0962), after lunch (hour 6, P = 0.0965), before dinner (hour 8 and 10, P ≤ 0.0888) and after dinner (hours 13-14, P ≤ 0.0806). Overweight men consuming the AHA diet had higher leptin concentrations after dinner (hours 11, 13-14, P ≤ 0.0669) when the Atkins diet was given before the AHA diet in comparison to when the AHA diet was given before the Atkins diet. Normal weight men tended to have lower leptin concentrations at hour 13 (P = 0.0806) when the AHA diet was given before the Atkins diet.
in comparison to when the Atkins diet was given before the AHA diet.

*Atkins to AHA Comparisons*

Normal weight men consuming the AHA diet had higher leptin concentrations at hour 13 (P = 0.0001) in comparison to normal weight men consuming the Atkins diet when the AHA diet was administered before the Atkins diet. Normal weight men consuming the Atkins diet had lower leptin before dinner (hour 9, P = 0.0894) in comparison to normal weight men consuming the AHA diet when the Atkins diet was given before the Atkins diet. Overweight men consuming the Atkins diet had lower leptin at fasting (P = 0.0004), before lunch (hour 4, P = 0.0264), after lunch (hour 5, P = 0.0083), and before dinner (hours 7-9, P ≤ 0.0008) in comparison to overweight men consuming the AHA diet when the AHA diet was given before the Atkins, however, leptin concentrations were lower at hour 14 (P = 0.0481) in overweight men consuming the Atkins diet in comparison to the AHA diet when the AHA diet was given before the Atkins diet. When the Atkins diet was given before the AHA diet, fasting leptin concentrations were higher in overweight men consuming the Atkins diet in comparison to the AHA diet, whereas leptin concentrations were lower in overweight men consuming the Atkins diet in comparison to the AHA diet before and after dinner (hours 10-13, P ≤ 0.0009) and tended to be lower at hour 14 (P = 0.0598).

*Adiponectin*

Adiponectin concentrations over waking hours in overweight and normal weight men (individual data presented) are shown in Figure 6.

*Timepoint Comparisons*

There were no significant differences between overweight and normal weight men
consuming the Atkins diet when the AHA diet was administered before the Atkins diet. When the Atkins diet was administered before the AHA diet, overweight men consuming the Atkins diet had lower adiponectin concentrations after breakfast (hour 2, $P = 0.0005$), before lunch (hour 3, $P = 0.0003$) and after lunch (hour 5, $P = 0.0135$) in comparison to normal weight men consuming the Atkins diet. There were no significant differences in timepoints between the sequences in overweight men consuming the Atkins diet. Normal weight men consuming the Atkins diet had lower adiponectin concentrations after breakfast (hours 1-2, $P \leq 0.0463$), before lunch (hour 3, $P < 0.0001$), and after lunch (hour 5, $P = 0.0268$) when the AHA diet was given before the Atkins diet in comparison to when the Atkins diet was given before the AHA diet.

Overweight men consuming the AHA diet had lower adiponectin concentrations at hour 12 ($P = 0.0152$) in comparison to normal weight men consuming the AHA diet when the AHA diet was given before the Atkins diet. There were no significant differences in timepoints between normal weight and overweight men consuming the AHA diet when the Atkins diet was given before the AHA diet. Overweight men consuming the AHA diet had higher adiponectin concentrations after breakfast (hours 2-3, $P \leq 0.0323$) and tended to have higher adiponectin concentrations before and after breakfast (hours 0-1, $P \leq 0.0797$) when the AHA diet was administered before the Atkins diet and when the AHA diet was administered before the Atkins diet. Normal weight men consuming the AHA diet had higher adiponectin concentrations after dinner (hour 12, $P = 0.0023$) when the AHA diet was administered before the Atkins diet in comparison to when the Atkins diet was administered before the AHA diet.
**Atkins to AHA Comparisons**

When the AHA diet was administered before the Atkins diet, normal weight men consuming the Atkins diet had lower adiponectin concentrations after breakfast (hours 1-2, $P \leq 0.0228$), after dinner (hours 10, 12, and 14, $P \leq 0.0052$), and tended to have lower adiponectin concentrations after dinner (hours 11, $P = 0.0588$) in comparison to men consuming the AHA diet. Normal weight men consuming the Atkins diet had higher adiponectin concentrations after breakfast (hours 2-3, $P < 0.0001$) and after lunch (hour 5, $P = 0.0036$) in comparison to normal weight men consuming the AHA diet when the Atkins diet was administered before the AHA diet. Overweight men consuming the Atkins diet had lower adiponectin concentrations at fasting ($P = 0.0062$), after breakfast (hours 1-3, $P \leq 0.0032$), before dinner (hours 7-8, $P \leq 0.0867$) and after dinner (hour 13, $P = 0.0371$) in comparison to overweight men consuming the AHA diet when the AHA diet was administered before the Atkins diet. There was no difference between diets in overweight men when the Atkins diet was administered before the AHA diet.

**Growth Hormone**

Growth Hormone concentrations over waking hours in overweight and normal weight men (individual data presented) are shown in Figure 7.

*Timepoint Comparisons*

Overweight men consuming the Atkins diet had higher GH concentrations at hour 3 ($P = 0.003$) and before dinner (hour 9, $P = 0.0092$) in comparison to normal weight men consuming the Atkins diet when the AHA diet was administered before the Atkins diet. When the Atkins diet was given before the AHA diet, overweight men had lower GH
concentrations after lunch (hour 6, \( P = 0.0477 \)) in comparison to normal weight men consuming the Atkins diet. Overweight men consuming the Atkins diet had higher GH concentrations before and after dinner (hours 7-12, \( P < 0.0001 \)) when the AHA diet was given before the Atkins diet in comparison to when the Atkins diet was given before the AHA diet. Normal weight men consuming the Atkins diet had higher GH concentrations at hour 3 (\( P = 0.009 \)) and before and after dinner (hours 7-12, \( P \leq 0.0114 \)) and lower GH concentrations after lunch (hour 6, \( P = 0.0360 \)) when the AHA diet was given before the Atkins diet in comparison to when the Atkins diet was given before the AHA diet.

When the AHA diet was given before the Atkins diet, there was no difference in GH concentrations between normal weight and overweight men consuming the AHA diet. When the Atkins diet was given before the AHA diet, normal weight men consuming the AHA diet had higher GH concentrations before dinner (hours 8-9, \( P \leq 0.0004 \)) in comparison to overweight men consuming the AHA diet. Overweight men consuming the AHA diet had higher GH concentrations before and after dinner (hours 7-12, \( P \leq 0.0049 \)) when the Atkins diet was given before the AHA diet in comparison to when the AHA diet was given before the Atkins diet. Normal weight men consuming the AHA diet had higher GH concentrations before and after dinner (hours 8-12, \( P < 0.0001 \)) when the Atkins diet was given before the AHA diet in comparison to when the AHA diet was given before the Atkins diet.

*Atkins to AHA Comparisons*

Normal weight men consuming the Atkins diet had higher GH concentrations at hour 3 (\( P = 0.0054 \)), before, and after dinner (hours 7-12, \( P \leq 0.0447 \)) in comparison to normal weight men consuming the AHA diet when the AHA diet was given before the Atkins diet. Normal weight men consuming the Atkins diet had higher GH concentrations after lunch (hour 6, \( P = \)
0.0317) and lower GH concentrations before and after dinner (hours 7-12, $P < 0.0001$) in comparison to normal weight men consuming the AHA diet when the Atkins diet was administered before the AHA diet. Overweight men consuming the Atkins diet had higher GH concentrations before and after dinner (hours 7-12, $P \leq 0.0198$) in comparison to overweight men consuming the AHA diet when the AHA diet was administered before the Atkins diet. Overweight men consuming the AHA diet had higher GH concentrations before and after dinner (hours 7-12, $P < 0.0001$) in comparison to overweight men consuming the Atkins diet when the Atkins diet was administered before the AHA diet.

**DISCUSSION**

There was an effect of sequence (period*diet) in all hormones with the exception of glucagon. In this exploratory study, we did not have an adjustment period before period 1 and food logs were not taken before the start of the study. Concentrations of circulating hormones adjust quickly to changes in diet, however, it is not known how long humans take to adjust to a rigid meal schedule and set amount of calories per day when coming from varied diet and prior habits. We demonstrated that there are confounding factors that play into differences in hormone concentrations. These confounding factors may explain the discrepancy in results related to studies of single macronutrient effects on hormone concentrations.

Men consuming a high protein breakfast had greater decrease in total ghrelin concentrations at three and four hours post lunch in comparison to men consuming a high carbohydrate breakfast; however, there was no change observed in acylated ghrelin concentrations in men between the high carbohydrate and the high protein breakfast (14).
our study with women, there were no significant increases in acylated ghrelin concentrations before meals and a however, in our study with men, there is a dramatic increase in acylated ghrelin concentrations before dinner in normal weight and overweight men consuming the AHA diet when the Atkins diet was given before the AHA diet (Chapter II Part II). The consumption of the Atkins diet before the AHA diet may have altered the response of the body to the AHA diet in period 2. Another study indicated that acylated ghrelin concentrations were lower in normal weight women consuming a high protein breakfast in comparison to a normal protein breakfast (15). However, no difference in ghrelin concentrations was observed in women consuming normal and high casein diets or in men and women consuming normal and high soy protein diets (16, 17). Most of the high protein diets studied contained moderate protein increases with a moderate decrease in carbohydrate (15-18). Our Atkins diet had 45% of calories from protein and 10 % of calories from carbohydrate, which would be considered low carbohydrate/high protein. The protein source and food matrix may be influencing the differences in studies.

Total ghrelin concentrations increased in men consuming the AHA diet in period 2 and the peak is at the same time as the increase in acylated ghrelin concentrations. Overweight men consuming the Atkins diet when the AHA diet was given before the Atkins diet had an increase in total ghrelin concentrations after breakfast in comparison to when the AHA diet was consumed. Most studies done on total ghrelin have measured the effect of a breakfast meal or lunch meal on total ghrelin concentrations over a 3-4 hour period (14, 18). Studies have shown the total ghrelin concentrations were lower in subjects consuming a high protein meal 3-4 hours post meal in comparison to subjects consuming a high carbohydrate meal (19). Total ghrelin finding of this study may differ from previous studies for several reasons.
First, the sequence in which the diets were given may be affecting total ghrelin concentrations. Second, the high protein diet in our study is also low carbohydrate whereas in other studies, the carbohydrate content is a more moderate percent calories from carbohydrate than in our study (14, 18). Lastly, we studied specific weight classes in this study with each group having a tight BMI range in comparison to the broad range of BMIs used in previous studies (14, 16-18).

Increased glucagon concentrations in men consuming the Atkins diet may be a result of lower blood glucose concentrations caused by the low carbohydrate content of the Atkins diet. Glucagon maintains acute blood glucose concentrations therefore, we hypothesize that the lack of an increase in glucagon concentrations in the fasted state may be the result of the switch from immediate regulation of blood glucose by glucagon to short term fasting regulation of blood glucose by epinephrine and the increase in glucagon later in the day is a result of the low carbohydrate content of the diet (20-22). The increased glucagon concentrations were higher in overweight men at hours 8 and 14 in comparison to normal weight men consuming the Atkins diet. Unlike our previous study with women, sequence did not affect the response of glucagon concentrations to diet and weight.

Insulin concentrations in response the macronutrient content of diet reacted similarly to previous studies (23). Overall, overweight and normal weight men consuming the Atkins diet had lower insulin concentrations in comparison to overweight and normal weight men consuming the AHA diet. There were differences in the response of insulin to the diet in the different sequences and weight classes around meals. Insulin concentrations have been shown to be lower when a high protein diet was consumed versus a high carbohydrate diet (24). Normal weight and overweight men responded differently to the AHA or Atkins diet
when the sequence in which the diets were administered was different. Our data suggests that overweight men and normal weight men should be studied separately when looking at the response of insulin to dietary manipulations and prior dietary habits may influence the response of insulin to diet treatments.

Leptin concentrations in response to the macronutrient content of the diet were similar to previous research (23). However, overweight men in period 1 had a decrease in leptin concentrations from hours 10-14 which is not what has been previously reported (23, 25). Overall, leptin concentrations remained relatively stable over the 14-hour period, which agrees with the previous findings of the 24-hour pattern of leptin concentrations. One of these studies showed that leptin concentrations peak at 02:00 and remains stable over waking hours when humans were fed a high fat/low carbohydrate or a high carbohydrate/low fat diet (25). Another study had leptin patterns in agreement with those of this study as leptin concentrations begin a gradual rise at 18:00 (23). For detection of changes of leptin in response to a diet, a 24-hour curve is the best measure of changes in leptin over time. However, measuring leptin concentrations at fasting gives an accurate representation of the leptin concentrations during waking hours (07:00-22:00) women and normal weight men, but does not accurately represent leptin concentrations over the day in overweight men in our study.

Adiponectin concentrations were lower in overweight men in comparison to normal weight men consuming the Atkins diet in period 1. Adiponectin concentrations are lower in obese subjects than in normal weight counterparts (9). Our data in overweight men in period 1 consuming the Atkins diet might suggest that overweight men consuming the Atkins diet would have adiponectin responses more similar to obese men than normal weight men would
(9). However, there was no difference in between overweight men and normal weight men consuming the Atkins diet in period 2 and no difference in adiponectin concentrations between overweight and normal weight men consuming the AHA diet in either period. Adiponectin concentrations responded differently in overweight and normal weight women consuming the AHA or Atkins diets in each sequence, which is different from the findings of our study in men (Chapter II Part II).

Some studies have shown that there is no effect of diet on adiponectin and that adiponectin has small changes in concentration from the fasted to postprandial states, however, in our study, adiponectin concentrations are influenced by diet, weight, timepoint, and sequence (10-12). There was a sequence and diet effect for normal weight men consuming the AHA in period 1, normal weight men consuming the Aktins in period 1 and overweight men consuming the AHA in period 1. Adiponectin concentrations were higher in all three of these groups in period 1 in comparison to period 2 after breakfast.

Growth hormone concentrations increased in normal weight women at the same time or after adiponectin concentrations appeared to be higher, but this relationship was not apparent in men (Chapter II Part II). The increase in GH concentrations observed in the men may be a result of GH stimulated FFA release from adipose tissue for use as energy (13). It is unclear why there is an increase in GH concentrations from hours 7-13 in all men in period 2. Further study into the possible relationship of GH and adiponectin between males and females is needed to understand fully the role of GH on glucose and fatty acid metabolism.

Universal dietary guidelines for men without taking into account different weight classes may not achieve optimal health. In addition, this study indicates that the order in which the diets are administered affects hormone concentrations in the blood. Overweight men do not
respond the same as normal weight men. Experimental design is critical in obtaining information on the relationship of hormones related to energy metabolism and body composition.

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REFERENCES


Table 1: Characteristics of Male Participants\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Age (yr)</th>
<th>BMI</th>
<th>Total Mass (kg)</th>
<th>Body Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Weight</td>
<td>21.25 ± 1.71</td>
<td>21.3 ± 1.7</td>
<td>62.2 ± 7.1</td>
<td>12.9 ± 1.6</td>
</tr>
<tr>
<td>Overweight</td>
<td>21.25 ± 1.26</td>
<td>27.9 ± 1.8</td>
<td>84.8 ± 9.6</td>
<td>25.1 ± 6.3</td>
</tr>
</tbody>
</table>

\(^1\)Values are means ± std. dev.

a,b Means with different superscripts are different.
Table 2: Significance of the Terms in the Model

<table>
<thead>
<tr>
<th>Term</th>
<th>Adiponectin</th>
<th>AG</th>
<th>TG</th>
<th>GH</th>
<th>Glucagon</th>
<th>Insulin</th>
<th>Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>0.025</td>
<td>0.0103</td>
<td>0.28</td>
<td>0.002</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Weight (wt)</td>
<td>0.25</td>
<td>0.51</td>
<td>0.53</td>
<td>0.035</td>
<td>0.19</td>
<td>0.19</td>
<td>0.13</td>
</tr>
<tr>
<td>Timepoint (tp)</td>
<td>&lt;0.0001</td>
<td>0.003</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.011</td>
</tr>
<tr>
<td>Sequence (seq)</td>
<td>0.88</td>
<td>0.14</td>
<td>0.98</td>
<td>0.055</td>
<td>0.97</td>
<td>0.55</td>
<td>0.8264</td>
</tr>
<tr>
<td>Diet*wt</td>
<td>0.16</td>
<td>0.0061</td>
<td>0.049</td>
<td>0.039</td>
<td>0.0203</td>
<td>0.86</td>
<td>0.019</td>
</tr>
<tr>
<td>Wt*seq</td>
<td>0.48</td>
<td>0.93</td>
<td>0.54</td>
<td>0.066</td>
<td>0.69</td>
<td>0.042</td>
<td>0.68</td>
</tr>
<tr>
<td>Tp*seq</td>
<td>0.16</td>
<td>0.104</td>
<td>0.082</td>
<td>&lt;0.0001</td>
<td>0.059</td>
<td>0.048</td>
<td>0.68</td>
</tr>
<tr>
<td>Diet<em>wt</em>tp</td>
<td>0.18</td>
<td>0.0021</td>
<td>0.001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.101</td>
</tr>
<tr>
<td>Diet<em>wt</em>seq*tp</td>
<td>&lt;0.0001</td>
<td>0.0402</td>
<td>0.0023</td>
<td>&lt;0.0001</td>
<td>0.23</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*P < 0.05 was considered significant and 0.05 > P < 0.1 was considered a trend. AG = Acylated ghrelin, TG = Total ghrelin, GH = growth hormone.*
Table 3: Metabolic Panel

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Overweight Atkins</th>
<th>Normal Weight Atkins</th>
<th>Overweight Atkins</th>
<th>Normal Weight Atkins</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Period 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>73.5 ± 9.2</td>
<td>71.0 ± 19.8</td>
<td>85.5 ± 7.8</td>
<td>84.0 ± 0.0</td>
</tr>
<tr>
<td>Blood Urea Nitrogen (mg/dL)</td>
<td>32.5 ± 2.1</td>
<td>33.0 ± 1.4</td>
<td>12.0 ± 0.0</td>
<td>12.0 ± 2.8</td>
</tr>
<tr>
<td>AST (IU/mL)</td>
<td>22.5 ± 7.8</td>
<td>25.0 ± 4.2</td>
<td>24.5 ± 2.1</td>
<td>22.0 ± 4.2</td>
</tr>
<tr>
<td>ALT (IU/mL)</td>
<td>25.5 ± 7.8</td>
<td>15.5 ± 0.7</td>
<td>37.5 ± 19.1</td>
<td>18.5 ± 0.7</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>173.5 ± 33.2</td>
<td>172.5 ± 34.6</td>
<td>162.0 ± 11.3</td>
<td>152.5 ± 14.8</td>
</tr>
<tr>
<td>Triacylglycerols (mg/dL)</td>
<td>92.0 ± 2.8</td>
<td>61.5 ± 16.3</td>
<td>127.0 ± 58.0</td>
<td>120.0 ± 46.7</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dL)</td>
<td>43.0 ± 1.4</td>
<td>63.0 ± 7.1</td>
<td>39.0 ± 1.4</td>
<td>44.0 ± 1.4</td>
</tr>
<tr>
<td>LDL Cholesterol (mg/dL)</td>
<td>112.0 ± 31.1</td>
<td>97.0 ± 24.0</td>
<td>97.5 ± 2.1</td>
<td>84.5 ± 3.5</td>
</tr>
<tr>
<td>LDL/HDL ratio</td>
<td>2.59 ± 0.64</td>
<td>1.53 ± 0.21</td>
<td>2.50 ± 0.15</td>
<td>1.92 ± 0.02</td>
</tr>
<tr>
<td><strong>Period 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>79.0 ± 12.7</td>
<td>85.0 ± 8.5</td>
<td>73.0 ± 14.1</td>
<td>85.5 ± 2.1</td>
</tr>
<tr>
<td>Blood Urea Nitrogen (mg/dL)</td>
<td>29.0 ± 2.8</td>
<td>31.0 ± 5.7</td>
<td>12.0 ± 1.4</td>
<td>14.5 ± 0.7</td>
</tr>
<tr>
<td>AST (IU/mL)</td>
<td>22.5 ± 3.5</td>
<td>22.5 ± 3.5</td>
<td>29.0 ± 12.7</td>
<td>21.5 ± 2.1</td>
</tr>
<tr>
<td>ALT (IU/mL)</td>
<td>32.0 ± 17.0</td>
<td>18.0 ± 4.2</td>
<td>39.0 ± 14.1</td>
<td>18.5 ± 0.7</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>186.0 ± 0.0</td>
<td>201.5 ± 48.8</td>
<td>154.5 ± 2.1</td>
<td>141.5 ± 34.6</td>
</tr>
<tr>
<td>Triacylglycerols (mg/dL)</td>
<td>144.0 ± 53.7</td>
<td>78.5 ± 24.7</td>
<td>146.0 ± 26.9</td>
<td>84.0 ± 19.8</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dL)</td>
<td>42.0 ± 4.2</td>
<td>61.5 ± 7.8</td>
<td>35.0 ± 0.0</td>
<td>58.0 ± 15.6</td>
</tr>
<tr>
<td>LDL Cholesterol (mg/dL)</td>
<td>115.5 ± 6.4</td>
<td>124.5 ± 46.0</td>
<td>90.5 ± 3.5</td>
<td>66.5 ± 14.8</td>
</tr>
<tr>
<td>LDL/HDL ratio</td>
<td>2.76 ± 0.13</td>
<td>1.99 ± 0.50</td>
<td>2.59 ± 0.10</td>
<td>1.15 ± 0.05</td>
</tr>
</tbody>
</table>

*Values are means ± std. dev.

δ indicates difference between diets only with no effect of period or weight.

α indicates difference between weights only with no effect of period or diet.

δα indicates the difference in the main effects of weight and diet separately with no effect of period.
### Table 4: Body Composition

<table>
<thead>
<tr>
<th></th>
<th>Trunk Mass</th>
<th>Trunk Fat</th>
<th>Total Mass</th>
<th>Total Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(kg)</td>
<td>(%)</td>
<td>(kg)</td>
<td>(%)</td>
</tr>
<tr>
<td><strong>Period 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight Atkins</td>
<td>37.8 ± 2.3</td>
<td>24.5 ± 3.8</td>
<td>82.8 ± 5.2</td>
<td>23.4 ± 4.4</td>
</tr>
<tr>
<td>Normal Weight Atkins</td>
<td>29.9 ± 5.0</td>
<td>11.9 ± 1.1</td>
<td>64.2 ± 9.0</td>
<td>11.8 ± 0.1</td>
</tr>
<tr>
<td>Overweight AHA</td>
<td>45.6 ± 5.5</td>
<td>29.0 ± 6.5</td>
<td>93.9 ± 12.0</td>
<td>26.3 ± 7.6</td>
</tr>
<tr>
<td>Normal Weight AHA</td>
<td>31.9 ± 2.9</td>
<td>13.7 ± 0.7</td>
<td>69.8 ± 7.1</td>
<td>13.8 ± 0.4</td>
</tr>
<tr>
<td><strong>Period 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight Atkins</td>
<td>45.2 ± 6.3</td>
<td>27.5 ± 7.3</td>
<td>92.8 ± 12.8</td>
<td>25.4 ± 7.9</td>
</tr>
<tr>
<td>Normal Weight Atkins</td>
<td>31.4 ± 1.5</td>
<td>12.6 ± 0.8</td>
<td>69.8 ± 6.1</td>
<td>13.6 ± 0.5</td>
</tr>
<tr>
<td>Overweight AHA</td>
<td>38.2 ± 2.1</td>
<td>23.4 ± 5.1</td>
<td>83.1 ± 5.3</td>
<td>23.2 ± 4.7</td>
</tr>
<tr>
<td>Normal Weight AHA</td>
<td>29.4 ± 4.4</td>
<td>11.1 ± 1.1</td>
<td>63.7 ± 8.3</td>
<td>11.9 ± 0.4</td>
</tr>
</tbody>
</table>

1Values are means ± std. dev.  

a,b Means with different superscripts are different. Significant difference are between overweight and normal weight men in trunk mass, trunk fat, total mass and total fat. There was no difference between diets or sequence.
Figure 1. Acylated ghrelin concentrations in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet and two normal and two overweight subjects were assigned to the AHA diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid and the AHA diet contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid of a 3,000 kcal/day diet. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. Breakfast was served after hour 1, lunch after hour 4, and dinner after hour 10. Significance is $P < 0.05$ with $δ$ referring to the difference is between diets and $ω$ referring to the difference is between weights with 1 or 2 referring to the sequence when the difference is between the diets or weights, and $ζ$ referring to the difference between sequences.
Figure 2. Total ghrelin concentrations in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet and two normal and two overweight subjects were assigned to the AHA diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid and the AHA diet contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid of a 3,000 kcal/day diet. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. Breakfast was served after hour 1, lunch after hour 4, and dinner after hour 10. Significance is $P < 0.05$ with $\delta$ referring to the difference is between diets and $\sigma$ referring to the difference is between weights with 1 or 2 referring to the sequence when the difference is between the diets or weights, and $\zeta$ referring to the difference between sequences.
Figure 3. Insulin concentrations (mean ± SEM) in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet and two normal and two overweight subjects were assigned to the AHA diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid and the AHA diet contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid of a 3,000 kcal/day diet. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. Breakfast was served after hour 1, lunch after hour 4, and dinner after hour 10. Significance is $P < 0.05$ with $\delta$ referring to the difference is between diets and $\omega$ referring to the difference is between weights with 1 or 2 referring to the sequence when the difference is between the diets or weights, and $\zeta$ referring to the difference between sequences.
Figure 4. Glucagon concentrations in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet and two normal and two overweight subjects were assigned to the AHA diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid and the AHA diet contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid of a 3,000 kcal/day diet. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. Breakfast was served after hour 1, lunch after hour 4, and dinner after hour 10. Significance is $P < 0.05$ with δ referring to the difference is between diets and ω referring to the difference is between weights.
Figure 5. Leptin concentrations (mean ± SEM) in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet and two normal and two overweight subjects were assigned to the AHA diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid and the AHA diet contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid of a 3,000 kcal/day diet. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. Breakfast was served after hour 1, lunch after hour 4, and dinner after hour 10. Significance is $P < 0.05$ with $\delta$ referring to the difference is between diets and $\omega$ referring to the difference is between weights with 1 or 2 referring to the sequence when the difference is between the diets or weights, and $\zeta$ referring to the difference between sequences.
Figure 6. Adiponectin concentrations in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet and two normal and two overweight subjects were assigned to the AHA diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid and the AHA diet contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid of a 3,000 kcal/day diet. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. Breakfast was served after hour 1, lunch after hour 4, and dinner after hour 10. Significance is $P < 0.05$ with $\delta$ referring to the difference is between diets and $\alpha$ referring to the difference is between weights with 1 or 2 referring to the sequence when the difference is between the diets or weights, and $\zeta$ referring to the difference between sequences.
Figure 7. Growth Hormone concentrations in the peripheral blood of normal weight men and overweight men fed the AHA and Atkins diets. Two normal and two overweight subjects were assigned to the Atkins diet and two normal and two overweight subjects were assigned to the AHA diet. The Atkins diet contained 10% of energy as carbohydrate, 45% of energy as protein, and 45% of energy as lipid and the AHA diet contained 63% of energy as carbohydrate, 12% of energy as protein, and 25% of energy as lipid of a 3,000 kcal/day diet. Each diet was fed for 14 days, and then subjects switched to the other diet, which is indicated by period 1 and period 2. Breakfast was served after hour 1, lunch after hour 4, and dinner after hour 10. Significance is $P < 0.05$ with $\delta$ referring to the difference is between diets and $\omega$ referring to the difference is between weights with 1 or 2 referring to the sequence when the difference is between the diets or weights, and $\zeta$ referring to the difference between sequences.
Chapter IV

Plane of nutrition affects plasma ghrelin concentrations in neonatal calves

A manuscript submitted to the Journal of Nutrition

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1 These authors contributed equally to this work
2 Author disclosures: M. Bohan Brown, M. Foote, B. Nonnecke, D. Beitz, no conflicts of interest.
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5 Corresponding author: Donald C. Beitz, 313 Kildee Hall, Ames, IA 50011, dbeitz@iastate.edu
ABSTRACT

Investigating effects of different planes of nutrition on appetite-related hormones could provide knowledge into the role of these hormones on growth, malnutrition, and obesity in early life. The objective of the current study was to investigate the effects of feeding rates on ghrelin and other metabolic hormones in plasma from preruminant calves. Treatments (n = 8 per treatment) were designed to achieve three targeted daily rates of gain (No Growth = 0.0 kg, Low Growth = 0.55 kg, or High Growth = 1.2 kg) in live weight over a 7-wk period. All calves were fed a 30% crude protein, 20% fat, all-milk protein milk replacer reconstituted to 14% dry matter. Fasting acylated ghrelin concentrations were greater (P < 0.0001) in no-growth calves over the 7-wk period in comparison to low- and high-growth calves. High-growth calves had greater plasma glucagon after wk 4 than did no- and low-growth calves (P = 0.0229). High-growth calves had greater plasma insulin concentrations in comparison to no- and low-growth calves at all time points after wk 0 (P < 0.0001). Plasma insulin concentrations in high-growth calves were significantly greater than baseline over the 7-wk period (P < 0.0001). These results indicate an inverse relationship of acylated ghrelin concentration with respect to plane of nutrition and growth rate in bovine neonates. In addition, results suggest that in the growing neonate positive energy balance may be sufficient to decrease active ghrelin concentrations, suggesting the potential existence of an energy status threshold regulating acylated ghrelin concentration.

Keywords: ghrelin, nutrition, preruminant calves, caloric intake, neonate
INTRODUCTION

Ghrelin is a 28 amino acid peptide hormone that is synthesized primarily in the X/A-like endocrine cells in the fundus region of the stomach of mammals (1, 2). In ruminants, ghrelin is produced predominantly in the abomasum (3). The location of ghrelin production lends well to the sensing of nutrient intake and the regulation of energy balance (4).

Ghrelin can be classified into two major circulating forms, n-octanoyl ghrelin and des-acyl ghrelin. Both acylated and des-acyl ghrelin have roles in energy homeostasis (5, 6). Acylation of ghrelin is necessary for the orexigenic and growth hormone stimulation activities.

Plasma ghrelin concentrations are influenced by energy balance, time of feeding, and disease states in both domestic animals and humans (7). In humans, plasma ghrelin concentrations in obese individuals are lower in comparison to ghrelin concentrations in normal weight individuals (8). In 2004, Salbe and colleagues demonstrated a negative association between plasma ghrelin and ad libitum feed intake (9). As nutrients are restricted, ghrelin concentrations increase in cattle, which demonstrate the inverse relationship of ghrelin concentrations and energy balance (10-12).

The objective of the current study was to investigate the effects of three different feeding rates, resulting in three targeted growth rates, on total and acylated ghrelin, as well as on insulin and glucagon concentrations in plasma. The calf model provides a unique opportunity to investigate tightly-controlled nutrient status on metabolism and appetite regulation in neonates. Our results suggest that a high plane of nutrition increases dramatically plasma insulin concentrations, whereas protein-energy malnutrition over an extended period of time increases plasma ghrelin concentrations.
MATERIALS AND METHODS

Animals. Animal procedures were approved by the Animal Care and Use Committee of the National Animal Disease Center Ames, IA. As described previously in a companion publication (13), 24 Holstein male calves were acquired from a single Wisconsin dairy herd over a 2-wk period. All were given 3.9 L of colostrum within 6 h of birth. At birth, navels were dipped in iodine and an *Escherichia coli* vaccine (Genecol-99; Schering Plough Animal Health, Union, NJ) was administered orally. Calves were transported to the National Animal Disease Center where they were housed individually in elevated pens (152.4 cm long × 91.44 cm wide × 91.44 cm high) in a temperature-controlled (18°C) barn. Each calf was given 2 mL of iron (Fe⁺⁺) dextran (100 mg/mL; AmTech, Phoenix Scientific, Inc., St. Joseph, MO) intramuscularly, 2.5 mL of BoSe (2.19 mg/mL of sodium selenite, 50 mg of RRR-α-tocopherol/mL; Schering-Plough Animal Health, Union, NJ) intramuscularly, and 2 mL of a vitamin B complex (Phoenix Scientific, Inc., St. Joseph, MO) subcutaneously upon arrival. Calf health was monitored and recorded daily.

Dietary Treatments. Before the trial, calves were fed twice daily 0.3 kg of a 20% crude protein, 20% fat milk replacer (Instant Nursing Formula: Dairy Herd & Beef Calf Milk Replacer; Land O’ Lakes, Inc., Shoreview, MN) reconstituted to 15% DM. On the first Monday after arrival (average age 9.1 ± 2.4 d; wk 0 of the experiment), calves were weighed and assigned randomly to one of three treatment groups (8 calves per treatment) designed to achieve 3 targeted daily rates of gain [no-growth (NG) = 0.0 kg/d, low-growth (LG) = 0.55 kg/d, or high-growth (HG) = 1.2 kg/d] in body weight over an 7-wk period. The NRC Nutrient Requirements of Dairy Cattle calf model computer program (14) was used to estimate the milk replacer intakes needed to achieve target growth rates. All calves were fed
a 30% crude protein, 20% fat, all-milk protein milk replacer (Table 1, 30-20 Milk Replacer, Land O’ Lakes, Inc.) reconstituted to 14% DM. The diet was formulated to ensure that protein would not be a limiting nutrient. The milk replacer composition is described by Foote et al. (13). Calves were weighed weekly, and the amount of milk replacer fed daily was adjusted on the day the weight was taken to allow for changes in BW. Because vitamin concentrations in the milk replacer were based on the DM intake of HG calves, LG and NG calves were given supplements of vitamins A, D₃, and E once weekly to compensate for lesser milk replacer consumption. Supplements were calculated to ensure that all calves received similar amounts of vitamins A, D₃, and E. Calves were bucket-fed twice a day (0700 and 1800 h) and offered water ad libitum. No starter grain was offered. The amounts of milk replacer offered and refused were recorded at each feeding.

**Preparation and Administration of Vaccines.** All calves were vaccinated subcutaneously in the right midcervical region with 10⁷ cfu of *Mycobacterium bovis, bacille Calmette Guerin (Pasteur strain)* at wk 3 of the experiment and also vaccinated subcutaneously in the left midcervical region with ovalbumin in Freund’s incomplete adjuvant at wk 3 and wk 5. Preparation of these vaccines is described by Foote et al. (13).

**Experiment 1.** The objective of experiment one was to determine the effect of plane of nutrition on fasting plasma hormones concentrations over the 7-wk period.

**Measurement of Plasma Hormones.** Blood was collected by jugular venipuncture weekly in the morning before feeding from wk 0 through wk 7. At each blood sampling, calves were fasted for 12 hr and blood was collected in EDTA-containing vacutainer tubes (BD Scientific, Franklin Lakes, NJ) and kept at 4 °C during processing. Samples were centrifuged at 3000 x g for 20 min. For acylated ghrelin samples, 50 μL of 1 N hydrochloric
acid and 10 µL of 10 mg/mL phenylmethylsulfonyl fluoride (PMSF, Sigma, St. Louis, MO) in methanol were added for every 1 mL of plasma immediately after centrifugation. For glucagon samples, 50 µL of aprotinin (5500 KIU/mL, Sigma, St. Louis, MO) were added to 1 mL of plasma with a final concentration of 550 KIU aprotinin per mL of plasma. All plasma samples were stored at -20 °C until assayed.

Total ghrelin concentrations were measured by using a commercially available radioimmunoassay kit (Linco Research, St. Charles, MO). The lower limit of detection was 93 pg/mL, and the intra-assay coefficient of variation was < 10%. Acylated ghrelin concentrations were measured by using a commercially available radioimmunoassay kit (Linco Research, St. Charles, MO). This assay has been found to be highly specific for acylated ghrelin with less than 0.1 % cross-reactivity for desoctanoyl ghrelin and no cross reactivity with ghrelin 14-28, motilin-related peptide, leptin, insulin, glucagon, or GLP-1 (7-36). The lower detection limit was 7.8 pg/mL. The intra-assay coefficient of variation was < 10%. Glucagon concentrations were measured by using a commercially available radioimmunoassay (Linco Research, St. Charles, MO). The lower limit of detection was 20 pg/mL, and the intra-assay coefficient of variation was < 10%. Insulin concentrations were measured by using a commercially available radioimmunoassay (Linco Research, St. Charles, MO). The lower limit of detection was 0.2 µU/mL, and the intra-assay coefficient of variation was < 10%.

In all hormone RIA assays, samples from calves in each treatment group were processed together so that inter-assay effects would be balanced across treatments.

**Experiment 2.** A subset of calves from Experiment 1 was used to complete Experiment 2. Twelve calves (n = 4 per treatment) were selected randomly from the 24 calves in
Experiment 1. Experiment 2 was conducted one wk after the completion of Experiment 1. The objective of experiment two was to determine the effect of plane of nutrition on plasma ghrelin concentrations during waking hours (06:30 h to 19:30 h).

**Measurement of Plasma Ghrelin.** After completion of the seven wks of feeding regimes, ghrelin concentrations during waking hours were quantified. Calves were fasted for 12 hr, and blood was collected by jugular venipuncture in the morning before feeding at 06:30 h. Subsequent blood samples were taken at 07:30, 08:30, 11:00, 13:00, 15:00, 17:30, 18:30, and 19:30 h. Calves were fed at 07:00 h and 18:00 h. All blood samples were collected in vacutainer EDTA-containing tubes (BD Scientific, Franklin Lakes, NJ) and kept at 4 °C during processing. Samples were centrifuged at 3000 x g for 20 min. For acylated ghrelin assays, samples were processed as described previously and stored at -20 °C until assayed.

**Statistical Analysis.** Hormone data were analyzed by using the MIXED procedure in SAS (Version 9.1, SAS Institute, Cary, NC). All hormone data were analyzed as a split-plot with repeated measures ANOVA. The model included fixed effects of growth rate (No, Low, or High), time (week of experiment or time-point), and treatment × time interaction, and calf was included in the model as the random effect. Significance of the interaction terms are listed in Table 2. For all tests, P < 0.05 was considered significant.

**RESULTS**

*Growth Performance.* Rate of growth was 0.11 kg/d for NG calves, 0.58 kg/d for the LG calves, and 1.16 kg/d for HG calves and were significantly different from each other throughout the study (P < 0.0001, 13).
Concentrations of Hormones in Blood

Experiment 1. The degree of caloric restriction significantly affected acylated ghrelin concentrations in the blood (Fig. 1). The interaction term in the model of treatment*timepoint was significant ($P = 0.0192$). The NG calves had greater concentrations of acylated ghrelin ($P < 0.05$) in comparison to LG and HG calves over the course of the 7-wk study. Low caloric intake to achieve no growth in growing calves resulted in a sustained high concentrations of acylated ghrelin in plasma over the 7-wk period. The LG and HG calves did have different acylated ghrelin concentrations from each other ($P < 0.05$); however, the difference between the acylated ghrelin concentrations in the LG and HG calves was not as pronounced after wk 2. Nutrient intake to achieve a low rate of gain in growing calves resulted in greater plasma acylated ghrelin concentrations for the first 2 wks of the 7-wk period and decreased plasma acylated ghrelin concentrations for the last 5 wks of the 7-wk period. The LG calves adapted to the daily amount of calories provided and maintained the selective concentrations of acylated ghrelin in the blood. Nutrient intake to achieve a high rate of gain resulted in a decrease in plasma acylated ghrelin concentrations from the beginning of the treatment until wk 4 where the concentrations of acylated ghrelin remained constant over the remaining 3 wk.

Vaccination with BCG and OVA during wk 3 and OVA during wk 5 affected acylated ghrelin concentrations in the blood. On wks 4 and 6 of the study, a decrease in acylated ghrelin concentrations in all groups was observed. This decrease in ghrelin was significant in the NG calves ($P < 0.05$). This decrease, however, was not significant in the LG and HG calves.
Plane of nutrition did not affect plasma total ghrelin concentrations (Fig. 2). The interaction term in the model of treatment*timepoint was not significant ($P = 0.5083$). There was no difference in total ghrelin concentrations among all three treatments on wks 1 through 7. Total ghrelin concentrations were not different among the treatment groups during wks 1 through 7; however, total ghrelin concentrations on wks 1 through 4 were greater than wks 5 through 7 for all treatment groups.

Plasma glucagon concentrations were greater in the HG calves on wks 4, 6, and 7 ($P < 0.05$) in comparison to both the NG and LG calves (Fig. 3). On average, HG calves had greater plasma glucagon after wk 4 ($P < 0.05$) than did NG and LG calves. The HG calves had greater plasma glucagon concentrations on wk 2 and wks 4 through 7 in comparison to wk 0 ($P < 0.05$). Plasma glucagon concentrations were not significantly different between the NG and LG calves throughout the entire study. Both NG and LG calves had lower plasma glucagon concentrations on wk 1 in comparison to wk 0 ($P < 0.05$). Low Growth calves had lower plasma glucagon concentrations in comparison to wk 0 on wk 6 and wk 7 ($P < 0.05$). The interaction term in the model of treatment*timepoint was significant ($P = 0.0229$).

Feeding calves to achieve a high rate of gain affected insulin concentrations in the blood (Fig. 4). The interaction term in the model of treatment*timepoint was significant ($P < 0.0001$). High Growth calves had greater plasma insulin concentrations ($P < 0.001$) in comparison to the NG and LG calves on at all times after wk 0. Plasma insulin concentrations in the HG calves were significantly greater ($P < 0.05$) than wk 0 over the 7-wk period. Plasma insulin concentrations did not differ between the NG and LG calves throughout the study. However, insulin concentrations in LG calves were greater
numerically than insulin concentrations in the NG calves throughout the 7-wk study. No Growth calves had significantly lower plasma insulin concentrations (P < 0.05) in comparison to baseline (wk 0) on wks 2 through 7 of the study.

Experiment 2. Feed intake did not affect plasma acylated ghrelin concentrations over the course of a day (Fig. 5). No Growth calves had greater acylated ghrelin concentrations at 06:30 h (fasting) in comparison with those of the LG and HG calves. Acylated ghrelin concentrations in the NG, LG, and HG calves did not differ from each other from 06:30 h to 19:30 h. The interaction term in the model of treatment*timepoint was not significant (P = 0.6954).

Overall, feed intake did not affect total ghrelin concentrations in the blood during waking hours (06:30 h-19:30 h, Fig. 6). Total ghrelin total concentrations responded similarly in all treatment groups. The interaction term in the model of treatment*timepoint was not significant (P = 0.2559).

DISCUSSION

Amount of dietary intake significantly influenced acylated ghrelin concentrations in the blood. Our results indicate that feeding to achieve either a low or high growth rate led to a similar suppression in acylated ghrelin concentrations that persisted throughout the study. These results show that positive energy balance at either moderate (i.e. normal) or higher (overnutrition) levels is sufficient to suppress ghrelin concentrations in neonates. In contrast, feeding to achieve no-growth caused an increase in acylated ghrelin concentrations throughout the study. The greater acylated ghrelin concentrations in the NG calves in comparison to the LG and HG calves seems to be related to the amount of calories ingested
as shown by Callahan et al. (15). Greater acylated ghrelin concentrations occur in lactating dairy cows in negative energy balance and in fasted steers (10-11). In human neonates, infants with failure to thrive have elevated ghrelin concentrations and restricted growth (16).

The decrease in acylated ghrelin concentrations on wks 3 through 7 in the LG group is most likely an adaptive response to energy intake. Post-prandial acylated ghrelin suppression is associated positively with caloric intake (15). However, we observed similar concentrations of acylated ghrelin in the LG and HG calves after wk 2 despite the caloric difference between the two diets. The lack of a difference between acylated ghrelin concentrations between the LG and HG calves suggests a threshold at which acylated ghrelin concentrations is not affected by energy balance and caloric intake.

Protein malnutrition can lead to stunted growth in humans. In the NG calves, growth was stunted by the amount of calories and not the amount of protein in the diet because protein in the diet was calculated to not be a limiting factor. Acylated ghrelin in cattle is elevated during times of negative energy balance, but little is known about the effects of insufficient protein energy (11-12).

After vaccination on wk 3 and 5, acylated ghrelin concentrations in NG calves were significantly lower from baseline (wk 0) after vaccination on wks 4 and 6, and a pattern of suppression of acylated ghrelin following vaccination was present in all groups. It is possible that the immune response to vaccination with OVA in Freund’s incomplete adjuvant contributed to the decrease in acylated ghrelin. We hypothesize that cytokines may influence appetite through the suppression of acylated ghrelin concentrations in the wk after each vaccination. In disease states, several cytokines (IL-6, IL-1β, and TNF-α) are involved in the development of cachexia-anorexia syndrome (17-19). These cytokines have been shown to
play an important role in normal food intake control and energy balance (20). Ghrelin has been shown to function as a signal coupling the immune system and the metabolic axis (21-22). However, this report is the first, to our knowledge, to show how an immune challenge (i.e., vaccination) can affect circulating ghrelin concentrations \textit{in vivo}. Interestingly, the degree of suppression was dependent on nutritional status.

We observed a significant effect of caloric intake on acylated ghrelin concentrations with no significant effect of caloric intake on total ghrelin concentrations during the 7-wk period. Several studies have indicated that total ghrelin is a good marker for acylated ghrelin concentrations (23-24). In 2009, Ping and Bistrian showed that total ghrelin concentrations were not different in rats fed \textit{ad libitum}, 25%, and 50% of \textit{ad libitum} intake with normal, slowed, and stunted growth, respectively (25). In our study, NG calves had greater acylated ghrelin concentrations than did the LG and HG calves (Fig. 1); whereas total ghrelin was not significantly different in the three groups (Fig. 2). The growth rates and total ghrelin concentrations between our study and the study by Ping and Bistrian (25) had a similar response to the decrease in caloric content of the diet. The discrepancy in findings on ghrelin concentrations with respect to dietary manipulation can be explained by the methods used to analyze ghrelin concentrations and the form of ghrelin measured and reported. Our data suggest that total ghrelin is not a good marker for acylated ghrelin concentrations in neonatal calves.

Despite elevated blood glucose concentrations (13), HG calves have greater plasma glucagon concentrations over the last 4 wks of the study in comparison with that of the LG and NG calves. In neonates, plasma glucagon concentrations are not suppressed by glucose concentrations in the blood (26). Neonatal insulin response to glucose concentrations in the
blood is not as well developed as the insulin response in adults (27). The lack of suppression of glucagon concentrations in the HG calves could be the result of the poor insulinogenic effect of glucose (27).

Insulin regulates plasma glucose concentrations and energy balance (28). Insulin concentrations increased in the HG group over the 7-wk period. In addition, HG calves had increasing weight gain over the 7-wk period (13). Blood glucose concentrations in the HG calves were higher than blood glucose concentrations of NG and LG calves (13). The rise in insulin is partially in response to the high blood glucose concentrations (27). However, blood glucose does not decrease after wk 2 (13), even though insulin concentrations are still increasing over wks 2 through 7. In human neonates, the response of insulin to rising glucose concentrations in the blood is not as well developed as in adults (27). The poor insulinogenic effect of glucose and the lack of suppression of glucagon may explain the increasing concentrations of insulin and elevated glucose concentrations over the 7-wk period in the HG calves.

The number of children diagnosed with Type II diabetes has been increasing as the rates of obesity in children has been increasing (29). The cause in insulin resistance is unclear. Obesity is thought to be a predictor of insulin resistance in children (30). However, not all obese children develop insulin resistance (31). In contrast, the fetal origins hypothesis indicates undernutrition in fetal development and infancy can lead to Type II diabetes later in life (32). High growth calves may be developing insulin resistance as a result of increased adiposity. However, NG calves also may be at risk for developing insulin resistance as well.

An inverse relationship between ghrelin and insulin has been described in cattle (12, 33). Acylated ghrelin concentrations in the LG group were greater than the HG group only during
the first few weeks of life and were the same as the HG group for the remainder of the study, whereas insulin concentrations in the LG calves were lower than insulin concentrations in the HG calves from wks 1 to 7. These data suggest that insulin may be a signal of adiposity in calves fed to achieve a high growth rate and may play a role in regulating food intake.

In meal-fed sheep, plasma acylated ghrelin concentrations increased pre-prandially and decreased post-prandially (34). However, we did not observe a pre-prandial increase and post-prandial decrease in acylated or total ghrelin concentrations. These results are consistent with pre- and post-prandial effects observed in 3-mo-old calves (35). In human neonates and young children, total ghrelin concentrations did not differ pre and postprandially, whereas total ghrelin in adult humans decreased post-prandially (36-37). Several factors may be influencing these results. These discrepancies may be attributed to developmental age, sampling time, or food matrix (liquid versus solid) (38).

Acylated ghrelin concentrations in growing preruminant calves differ from acylated ghrelin concentrations in adult ruminants, but do not differ from acylated ghrelin concentrations in growing ruminant calves. In growing ruminant calves, acylated ghrelin concentrations are lower than concentrations in mature cows and are not influenced by meals to the extent they are in mature cows (35). Ghrelin concentrations are lower in infants fed breast milk/formula in comparison to infants fed solid foods (38). Ghrelin mRNA in the digestive tract of adult ruminants is predominantly produced in the abomasum with smaller amounts produced in the small intestine (Wertz-Lutz, SDSU, Brookings, SD, personal communication). Moreover, the response of ghrelin in our experiment is similar to the response of ghrelin in rodents and humans (39). Because ghrelin is not produced in significant amounts in the rumen of cattle, the type of feed and frequency of feeding may be
influencing ghrelin concentrations between mature and preruminant cattle rather than developmental state of the ruminant digestive tract.

In conclusion, our results indicate that ghrelin and insulin concentrations are regulated by plane of nutrition in the neonatal calf. In addition, our results suggest a threshold at which acylated ghrelin concentrations are not affected by energy balance and caloric intake. The preruminant calf provides a unique tool to investigate the effects of nutrient status on metabolism and appetite regulation in neonates. The effect of immune challenge on the circulation of appetite-regulating hormones warrants further investigation.

LITERATURE CITED


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### Table 2. Significance of terms in the statistical model

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*P < 0.05 was considered significant and 0.05 > P < 0.1 was considered a trend.

*NA = not available as glucagon and insulin were not measured.
Figure 1. Acylated ghrelin concentrations (mean ± SEM) in the peripheral blood of calves fed milk replacer to achieve no, low, and high growth rates (n = 8 per treatment). Dietary treatments were initiated at wk 0, and all calves were vaccinated with *Mycobacterium bovis*, Bacillus Calmette Guerin at wk 3 and with ovalbumin at wk 3 and 5. a–c Treatment means with different superscripts at specific times differ, $P < 0.05$. *Means within a treatment group differ from the wk 0 value, $P < 0.05$. 
Figure 2. Total ghrelin concentrations (mean ± SEM) in the peripheral blood of calves fed milk replacer to achieve no, low, and high growth rates (n = 8 per treatment). Dietary treatments were initiated at wk 0, and all calves were vaccinated with *Mycobacterium bovis*, Bacillus Calmette Guerin at wk 3 and with ovalbumin at wk 3 and 5. a–c Treatment means with different superscripts at specific times differ, $P < 0.05$. *Means within a treatment group differ from the wk 0 value, $P < 0.05$. 
Figure 3. Glucagon concentrations (mean ± SEM) in the peripheral blood of calves fed milk replacer to achieve no, low, and high growth rates (n = 8 per treatment). Dietary treatments were initiated at wk 0, and all calves were vaccinated with *Mycobacterium bovis*, Bacillus Calmette Guerin at wk 3 and with ovalbumin at wk 3 and 5. a–c Treatment means with different superscripts at specific times differ, $P < 0.05$. *Means within a treatment group differ from the wk 0 value, $P < 0.05$. 
Figure 4. Insulin concentrations (mean ± SEM) in the peripheral blood of calves fed milk replacer to achieve no, low, and high growth rates (n = 8 per treatment). Dietary treatments were initiated at wk 0, and all calves were vaccinated with *Mycobacterium bovis*, Bacillus Calmette Guerin at wk 3 and with ovalbumin at wk 3 and 5. a–c Treatment means with different superscripts at specific times differ, $P < 0.05$. *Means within a treatment group differ from the wk 0 value, $P < 0.05$. 
**Figure 5.** Acylated ghrelin concentrations (mean ± SEM) in the peripheral blood of calves fed milk replacer to achieve no, low, and high growth rates (n = 4 per treatment). Calves were 8 wk of age. Calves were fed at 07:00 and 18:00 indicated by vertical lines. a–c Treatment means with different superscripts at specific times differ, *P* < 0.05. *Means within a treatment group differ from the baseline (06:30) value, *P* < 0.05.
Figure 6. Effect of growth rate on total ghrelin concentrations (mean ± SEM) in the peripheral blood of calves fed milk replacer to achieve no, low, and high growth rates (n = 4 per treatment). Calves were 8 wk of age. Calves were fed at 07:00 and 18:00 indicated by vertical lines. a–c Treatment means with different superscripts at specific times differ, $P < 0.05$. *Means within a treatment group differ from the baseline (06:30) value, $P < 0.05$. 
Chapter V
General Summary and Conclusions

Summary

Chapters II and III: Part I

In this study, we investigated the effects of consuming breakfast on hormone concentrations changes before and after lunch. Consumption of breakfast may be a key element to successful weight loss. In this study, visual analog scale of hunger and fullness were not taken but informally reported feeling less hunger and increased fullness when consuming the Atkins diet versus the AHA diet in both men and women.

Adiponectin has not been shown to be influenced by dietary composition, and we did not observe a difference in adiponectin concentrations in women between the AHA and Atkins diets. Adiponectin concentrations were different between the fasted and fed states. In contrast, plasma adiponectin concentrations before lunch in men were influenced by diet, weight class, sequence and the consumption of breakfast. In the female study, there was no significant change in adiponectin concentrations from the fasted or fed pre-prandial state to the post-prandial state. We did not observe a significant difference in adiponectin concentrations between normal weight and overweight women, which may indicate that overweight women do not respond similarly to obese women. In the male study, dietary composition affected adiponectin concentrations before lunch and the change in adiponectin concentrations. The influence of the dietary composition was different between overweight men and normal weight men.
In our study, overweight women tended to have lower ghrelin than did the normal weight women when consuming a high carbohydrate diet. There was no difference in ghrelin concentrations between normal and overweight women when a low carbohydrate, high protein diet was consumed. The macronutrient content of the diet influenced the acylated ghrelin concentrations in normal weight women consuming the AHA but not normal weight women consuming the Atkins diet, nor overweight women consuming the AHA or Atkins diets. Normal weight and overweight women consuming the Atkins diet had no change in acylated ghrelin from the preprandial to postprandial state. Weight class may be less important than diet in determining the response of acylated ghrelin in women. Men had higher acylated ghrelin in the fasted state in comparison to the fed state. We observed a difference in acylated ghrelin concentrations between the Atkins and AHA diets before lunch. In the fed state, normal weight women fed the Atkins diet had higher total ghrelin concentrations than did normal weight women fed the AHA diet. There was no change in total ghrelin concentrations from pre-prandial to postprandial state in women.

In women, GH concentrations were higher in the fasted state in comparison to the fed state. In addition, the change in GH concentrations was greater in the fasted state in comparison to the fed state. In men, GH concentrations may not have been high enough to regulate ghrelin concentrations as seen in women under the same conditions. Men consuming the AHA diet had an increase in acylated ghrelin concentrations over the lunch period; whereas, there was no change in acylated ghrelin concentrations in men consuming the Atkins diet.
Glucagon concentrations were influenced by dietary composition in women. Women consuming the Atkins diet had higher glucagon in comparison to women consuming the AHA diet. However, in men, glucagon concentrations were influenced by the dietary composition, weight class, sequence and physiological state.

Insulin concentrations were influenced by weight, diet, and physiological state in women. Insulin concentrations were lower in overweight women consuming the Atkins diet in comparison to the AHA diet. Insulin concentrations were higher in the fed state versus the fasted state in overweight women consuming the AHA diet. The increase in insulin concentrations was higher in normal weight women in comparison to overweight women consuming the AHA diet in the fed state and was seen in overweight women but only when consuming the AHA diet, which indicates that consuming a higher protein/lower carbohydrate diet such as the Atkins diet results in lower insulin concentrations. Overweight women consuming the AHA diet had a decrease in insulin concentrations over lunch in the fed state in comparison to an increase in insulin concentrations in the fasted state over lunch. However, in normal weight women fed the AHA diet, insulin concentrations increased in the women who were fasted or who had consumed breakfast. Breakfast did not have the same effect in normal weight women as consuming breakfast did in overweight women.

Insulin concentrations were higher in men who had consumed breakfast in comparison to men who were fasted. In addition, men consuming the Atkins diet had lower insulin concentrations in comparison the men consuming the AHA diet. The change in insulin concentrations over lunch were influenced by the interaction weight, diet, and physiological
state. Fasted overweight men had an increase in insulin concentration over lunch when consuming the AHA diet in comparison fasted overweight men consuming the Atkins diet.

Overweight women had higher leptin concentrations in comparison to normal weight women in both physiological states when fed the Atkins diet and in the fasted state when fed the AHA diet. In addition, leptin concentrations were higher in overweight men in comparison to normal weight men when consuming the AHA diet when the AHA diet was given before the Atkins diet. Consuming the Atkins diet before the AHA diet may have influenced the response of leptin to the AHA diet. There were no differences in the change in leptin concentrations in women. However, leptin concentrations increased over lunch when overweight men had consumed breakfast whereas leptin concentrations decreased in overweight men over lunch when breakfast was not eaten.

**Chapters II and III: Part II**

There was an effect of sequence (period*diet) in all hormones with the exception of glucagon in men. In this exploratory study, we did not have an adjustment period before period 1 and food logs were not taken. Hormones react relatively quickly to changes in diet, however, it is not known how long humans take to adjust to a meal schedule and set amount of calories per day when coming from varied diet and prior habits. We demonstrated that there are confounding factors that play into differences in hormone concentrations. These confounding factors may explain the discrepancy in results related to studies of single macronutrient effects on hormone concentrations.
Our data on overweight men in period 1 consuming the Atkins diet might suggest that overweight men consuming the Atkins diet would have adiponectin responses more similar to obese men than normal weight men would. However, there was no difference in between overweight men and normal weight men consuming the Atkins diet in period 2 and no difference in adiponectin concentrations between overweight and normal weight men consuming the AHA diet in either period. Adiponectin concentrations responded differently in overweight and normal weight women consuming the AHA or Atkins diets in each sequence, which is different from the findings of our study in men. Growth hormone concentrations increased in normal weight women at the same time or after adiponectin concentrations appeared to be higher, but this relationship was not apparent in men.

There were no differences in acylated ghrelin concentrations between the Atkins and AHA diet in normal weight women when the AHA diet was given before the Atkins diet. We did see higher acylated ghrelin concentrations in normal weight women fed the AHA diet in comparison the Atkins diet when the Atkins diet was given before the AHA diet. In this study, there were no clear preprandial increases in acylated ghrelin concentrations before meals. However, in our study with men, there is a dramatic increase in acylated ghrelin concentrations in normal weight and overweight men consuming the AHA diet when the Atkins diet was given before the AHA diet. The consumption of the Atkins diet before the AHA diet may have altered the response of the body to the AHA diet in period 2. Total ghrelin concentrations increased in men consuming the AHA diet in period 2 and the peak is at the same time as the increase in acylated ghrelin concentrations. Total ghrelin
concentrations differed after breakfast to one hour after lunch in normal weight women starting on the AHA diet and in all overweight women.

The increased glucagon concentrations were for a longer duration in women consuming the Atkins diet in when the AHA diet was given before the Atkins diet in comparison to when the Atkins diet was given before the AHA diet. The increased glucagon concentrations from hours 7-14 were higher in overweight men at hours 8 and 14 in comparison to normal weight men consuming the Atkins diet. Unlike our previous study with women, sequence did not affect the response of glucagon concentrations to diet and weight.

Overall, overweight and normal weight men consuming the Atkins diet had lower insulin concentrations in comparison to overweight and normal weight men consuming the AHA diet. There were differences in the response of insulin to the diet in the different sequences and weight classes around meals. Normal weight and overweight men responded differently to the AHA or Atkins diet when the sequence in which the diets were administered was different. Overweight and normal weight women consuming the Atkins diet had lower insulin concentrations in comparison to overweight and normal weight women consuming the AHA diet after breakfast and after dinner. There were differences in the response of insulin to the diet in the different sequences around lunchtime and before and right after dinner. When the AHA diet was consumed, insulin concentrations had more profound spikes after meals in comparison to when the Atkins diet was consumed.
Leptin concentrations remained relatively stable over the 14-hour period in men and women, which agrees with the previous findings of the 24-hour pattern of leptin concentrations. Overweight women consuming the Atkins and the AHA diets when the Atkins diet was given before the AHA had higher leptin concentrations in comparison to normal weight women and overweight women in the opposite sequence. However, overweight men in period 1 had a decrease in leptin concentrations from hours 10-14 which has not been previously reported.

**Chapter IV: Caloric Effects**

Amount of dietary intake significantly influenced acylated ghrelin concentrations in the blood. Our results indicate that feeding to achieve either a low or high growth rate led to a similar suppression in acylated ghrelin concentrations that persisted throughout the study. The lack of a difference between acylated ghrelin concentrations between the LG and HG calves suggests a threshold at which acylated ghrelin concentrations is not affected by energy balance and caloric intake.

We observed a significant effect of caloric intake on acylated ghrelin concentrations with no significant effect of caloric intake on total ghrelin concentrations during the 7-wk period. Despite elevated blood glucose concentrations, HG calves have greater plasma glucagon concentrations over the last 4 wks of the study in comparison with that of the LG and NG calves. Insulin concentrations increased in the HG group over the 7-wk period. In addition, HG calves had increasing weight gain over the 7-wk period. Blood glucose concentrations in the HG calves were higher than blood glucose concentrations of NG and LG calves.
General Conclusions

In Part I of the human studies, we investigated the effects of consuming breakfast on hormone concentrations changes before and after lunch. In this study, visual analog scale of hunger and fullness were not taken but informally reported feeling less hunger and increased fullness when consuming the Atkins diet versus the AHA diet. Consuming breakfast may improve the insulin response to the lunch meal and decrease insulin resistance whereas skipping breakfast increases insulin concentrations over the lunch period when consuming a high carbohydrate diet such as the AHA diet.

In Part II, the response of the select hormones was measured over 14 hours. From this part of the study, we can conclude that overweight and normal weight men respond differently to the dietary interventions. In addition, sequence affected the concentrations of hormones in the blood. More research is needed to determine the effects of switching diets on the concentrations of hormones in the blood and human health. In this study, we measured multiple hormones from the same subjects at the same time. By doing this, we have gained insight into the interplay between hormone concentrations in response to the diet and develop studies to further test the relationship of these hormones. In women, we have shown a possible relationship of GH and adiponectin, which warrants further study.

Our results from the calf study indicate that ghrelin and insulin concentrations are regulated by plane of nutrition in the neonatal calf. In addition, our results suggest a threshold at which acylated ghrelin concentrations are not affected by energy balance and caloric intake. High growth calves may be developing insulin resistance because of
increased adiposity. However, NG calves also may be at risk for developing insulin resistance as well. The preruminant calf provides a unique tool to investigate the effects of nutrient status on metabolism and appetite regulation in neonates. The effect of immune challenge on the circulation of appetite-regulating hormones warrants further investigation.

Universal dietary guidelines may not be appropriate for all humans to achieve optimal health. Grouping normal weight, overweight, and obese women and men would not be appropriate based on results in this study and previous research. Weight class affected some hormone concentrations differently and thus women and men in overweight and normal weight classes should be analyzed separately. In addition, dietary composition influences hormone concentrations. High protein diets affected hormone concentrations differently in comparison to a high carbohydrate diet. The differences in findings between studies lie with the total dietary composition. Several high protein diets increased the percent of calories from protein by lowering the percent calories from lipid or by lowering calories from both carbohydrate and lipid. Each class of macronutrients influences hormone concentrations, care should be taken when drawing conclusions about the general effects of high protein diets, and more generally, any diet based on changing a single macronutrient. Experimental design is critical in obtaining information on the relationship of hormones related to appetite and body composition.
**Future Research**

1. Conduct the above human studies again with modifications

In the above exploratory study, one should note the importance of schedule and prior dietary habits on the effects of macronutrient concentrations. I would modify the above study in the following ways.

a) I would have more involved screening for the selection of participants. I would study African American, Hispanic/Latino, Asians, and Caucasians separately. In addition, I would pick individuals who have lived in the United States for an extended period as the lifestyle and dietary habits of individuals from other countries may influence the response of the participant to the dietary intervention. As well as a baseline sample would be taken and all of the hormones in the study measured to screen for abnormal concentrations or patterns. Body fat mass would be taken by bioelectrical impedance (BIA) prior to selection.

b) One month prior to the dietary treatments, I would have the individuals begin eating their meals on the schedule that they would follow during the dietary treatments.

c) I would obtain daily exercise and sleep logs from the participants.

d) During all blood testing, visual analogs scales of hunger, satiety, and overall health would taken.

e) Normal weight, overweight, and obese individuals would be studied in both sexes.
f) The 14-hour samples would be taken the Saturday before the beginning of the
dietary treatments in addition to the current study.

2. Conduct a free-living self-reporting study in conjunction with number 1. These
individuals would undergo the same blood-sampling schedule but would be counseled
on the diets and given guidelines on how to prepare the diets themselves. They would
be subject to all of the other measurements of the participants above with the
exception of having all meals prepared for them and having to consume those meals
in a research unit.

3. Conduct research with the AHA and Atkins diets in different age groups. Age plays
a role in the concentration of many hormones. Diets would be modified to provide
necessary nutrition for the selected age groups. Ideally, children prior to puberty,
children after the onset of puberty, young adult of reproductive age, and men and
postmenopausal women would be studied. Each of these groups has different
metabolic and hormone profiles and the effects of diet should be studied in each
group to assess the response to macronutrient compositions.
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