

Nutrient Regulation of Plasma Ghrelin Concentration in Lean and Overweight Female Humans

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Michelle Bohan, graduate student of biochemistry;
Lloyd Anderson, distinguished professor of animal science;

Allen Trenkle, distinguished professor of animal science;
Donald Beitz, distinguished professor of animal science and biochemistry

Summary and Implications

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 female subjects, ages 20-30, were used in this study: four normal subjects with body mass index (BMI) of 19-24 and four overweight/obese subjects with 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 were switched to the other diet. We hypothesized that subjects consuming the Atkins' diet would have lower plasma ghrelin concentrations than subjects consuming the AHA diet. On days 6 and 20, blood was taken at one hour before and after the noon meal. Blood was taken every hour from 7 am to 9 pm on days 13 and 27 of the study. On days 14 and 28, subjects were fasted from 7 am to 12 pm and fed their meal at noon. Two blood samples were taken on days 14 and 28 at 11 am and one hour after the meal. All plasma samples were analyzed for ghrelin. Normal weight women consuming the Atkins diet had lower fasting ghrelin concentrations than did women consuming the AHA diet ($p=0.0141$). Ghrelin concentrations in overweight women were not significantly different ($p=0.8076$). These results indicate a relationship of appetite-related hormones with respect to diet composition.

Introduction

Since the discovery and isolation of ghrelin in 1999, 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. Additionally, leptin activity is controlled by ghrelin. Ghrelin is an antagonist of leptin

by acting upon the neuropeptide Y/Y1 receptor pathway. Leptin causes satiety, whereas ghrelin stimulates nutrient intake. Leptin and ghrelin thereby regulate the action of each other.

Ghrelin is primarily synthesized in the brain and 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, increased gastric acid secretion in rats, and increased gastric motility in rats.

Few 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 or humans with specific disease states such as anorexia nervosa (AN), polycystic ovary syndrome, chronic heart failure, or surgical modifications of the stomach. Patients with AN have higher plasma ghrelin concentrations than do normal human. Furthermore, plasma ghrelin in AN patients was associated negatively with body mass index (BMI) values. 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. 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 associated with gastric bypass surgery.

A study in 2002 showed that rats fed a high carbohydrate diet had higher plasma ghrelin than did rats fed a low carbohydrate diet. This study was a long-term feeding study and demonstrated the long-term effects of diet on ghrelin concentration. In a study in 2003, healthy non-obese women were fed either a high fat or high carbohydrate meal. 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. Diet-induced obesity, however, was not related directly to ghrelin concentration in juvenile rats prone to obesity. In a study conducted by Weigle and colleagues, high fat diets decrease adiposity without increasing appetite. The few studies involving ghrelin and diet composition have conflicting results, leaving the

relationship between ghrelin and diet composition unclear.

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. Cerebrospinal fluid (CSF) ghrelin is lower in obese humans than in normal weight humans. Obese humans have lower ghrelin concentrations both in plasma and CSF. This latter observation is contrary to the findings in rodents where ghrelin was injected and an increase in adiposity was shown. In 2002, a study by English and colleagues demonstrated that refeeding after fasting did not decrease the ghrelin concentrations in obese human patients. In normal weight humans, fasting ghrelin concentrations decreased after feeding. Ghrelin has a negative association with *ad libitum* feed intake. However, measured fasting (average) ghrelin as their measurement, which may not be representative of the rise in ghrelin concentration before a meal. Furthermore, many studies have used the total ghrelin assay rather than the active ghrelin assay. The use of total ghrelin assay instead of the active ghrelin assay may explain the disparity in the literature about the relationship of ghrelin, diet composition, and obesity. 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.

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

Materials and Methods

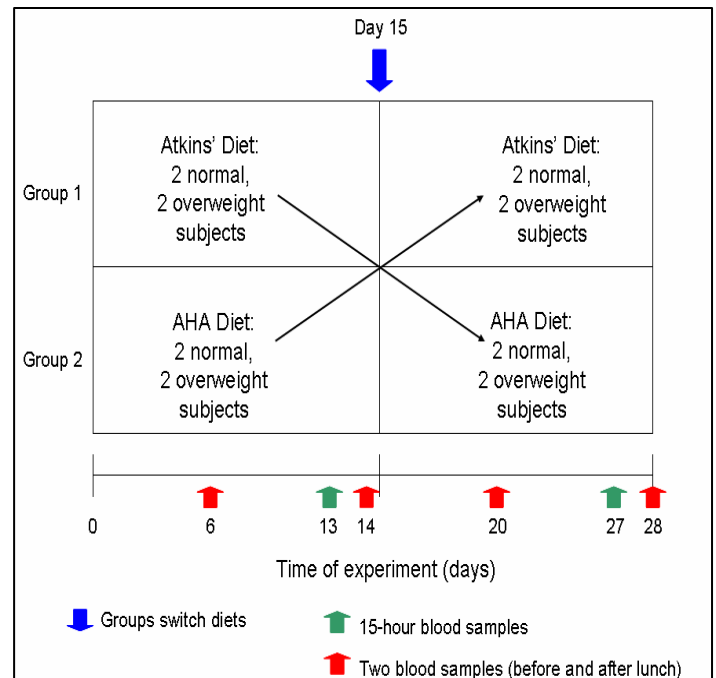
Each subject was screened for BMI, eating disorders, and major health problems through an interview and physical examination. Volunteers were excluded if they have or had an eating disorder and/or have a major health problem such as diabetes, heart conditions, and hypoglycemia. The selected subjects had a medical screening physical at the Thielen Student Health Center

and dual-energy x-ray absorptiometry (DXA) scans at the ISU Human Metabolic Unit. After successful completion of the screening, the female subjects were assigned randomly to treatment diets.

Diets consisted of breakfast, lunch, and dinner. In this study, each subject received both treatment 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. All diets were prepared and served by personnel at the Metabolic Unit. Each diet was fed for 14 days, and then subjects switched to the other diet. Meals were served at 8 am, 12 pm, and 5 pm in the dining area of Human Metabolic Unit.

There were three DXA scans during the study per subject. The first scan was the Friday before the feeding begins. The second scan was on day 14 of the study. The final scan was on day 28, which is the final day of the study. The DXA measurements were used to compare the effects of diet composition in lean and overweight women body composition. Each DXA scan will expose the subject to 1.5 mRem, which is a small dose of radiation in comparison to natural background and dental x-rays (300 mRem).

Figure 1. Experimental design of the human feeding study.



On days 6 and 20, blood was taken at one hour before and after the noon meal. A total of 10 mL of blood was taken each day. Plasma was analyzed for ghrelin (active and total), glucagon, adiponectin, insulin, leptin, growth hormone concentrations. Blood was taken every hour from 7 am to 9 pm on day 13 and 27 of the study. A total of 75 mL of blood was taken each day. Plasma was collected and analyzed for ghrelin (active and total), glucagon, adiponectin, insulin, leptin, growth hormone, glucose, and cholesterol. Additionally, the plasma will be analyzed for oxyntomodulin (OXM). On days 14 and 28, subjects were fasted from 7 am to 12 pm and then fed their meal at 12 pm. Two blood samples were taken on days 14 and 28 at 11 am and one hour after the meal. Plasma was analyzed for ghrelin (active and total), glucagon, adiponectin, insulin, leptin, growth hormone concentrations.

Statistics

Data were analyzed using the mixed model procedure of SAS. The model used for all hormones was the following:

$$\text{Hormone} = \text{diet period day BA weight weight*diet weight*seq}$$

Where:

BA = before or after

Weight = lean or overweight

Seq = diet*period

Results

Hormone concentrations responded differently to diet, weight, day, period, and interactions of these variables (Table 1). The fasting ghrelin active concentrations in normal and overweight women fed the AHA and Atkins diets are shown in Figure 1. Normal weight women consuming the Atkins diet had lower fasting ghrelin active concentrations than did women consuming the AHA diet ($p=0.0141$). However, ghrelin active concentrations in overweight women were not significantly different when fed the Atkins or AHA diets ($p=0.8076$). Ghrelin active concentrations tended to be lower in overweight women fed the AHA diet than normal weight women fed the AHA diet ($p = 0.166$). Women who were overweight had a tendency to have higher fasting ghrelin active concentrations when consuming the Atkins diet than did normal weight women

consuming the Atkins diet ($p = 0.0685$). Ghrelin concentration was not significantly different in women fed the Atkins diet than women fed the AHA diet one hour before lunch without fasting ($p > 0.1$, data not shown).

Lastly, the effect of the sequence in which the diets were fed was tested. The sequence in which the diet were fed tended to alter fasting ghrelin active concentrations ($p = 0.129$). Women who started on the Atkins diet had lower fasting ghrelin concentrations when fed the AHA diet than women who started on the AHA diet. The fasting ghrelin concentrations in women fed the Atkins diet did not differ between each weight classification.

Conclusions and Implications

Dietary composition did not significantly alter ghrelin concentrations in a nonfasting state in either normal or overweight women when fed isocaloric diets. This study supports the hypothesis that ghrelin is controlled by caloric content not macronutrient content of the diet in a nonfasted state. In the fasted state, diet composition did not alter ghrelin active concentrations in overweight women. However, normal weight women fed the Atkins diet had lower ghrelin active concentration than did women fed the AHA diet. This study supports the hypothesis that ghrelin is controlled by caloric content not macronutrient content of the diet in overweight women in a fasted state but not normal weight women in fasted state. These results indicate that universal dietary guidelines may not be appropriate for all humans to achieve optimal health.

In addition, this study indicates that the order in which the diets are administered may affect hormone concentrations in the blood. Experimental design is critical in obtaining information on the relationship of hormones related to appetite and body composition.

Diet composition affects plasma hormone concentrations differently in normal and overweight female humans. These data provide us with more insight into how diet composition influences hormone concentrations. Future research is needed to further investigate the role diet composition plays in hormone secretion.

Acknowledgements

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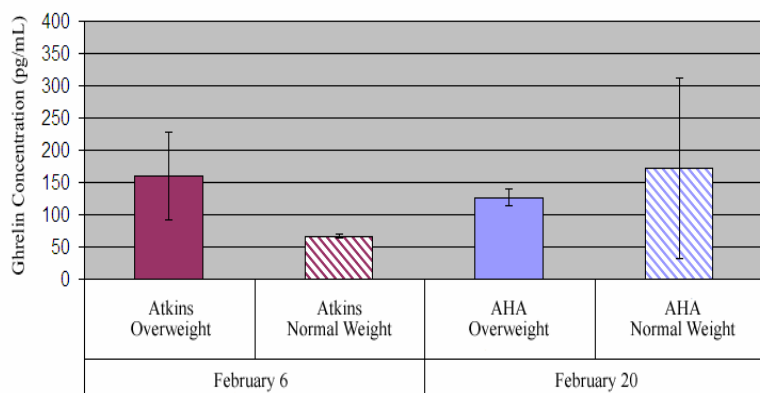
Table 1. Analysis of Factors that Effect Plasma Hormone Concentrations.

	Adiponectin ($\mu\text{g/mL}$)	Ghrelin Active (pg/mL)	Ghrelin Total (pg/mL)	Glucagon (pg/mL)	Growth Hormone (ng/mL)	Insulin ($\mu\text{U/mL}$)	Leptin (ng/mL)	Ghrelin Active/Ghrelin Total
Diet	<i>0.0676</i>	0.9204	0.2401	<i><0.0001</i>	0.9903	<i>0.0005</i>	0.5140	0.9097
Weight	0.2487	0.3923	0.6895	0.3154	0.1376	0.3375	<i>0.0158</i>	0.1621
Weight*Seq	0.3378	0.9254	0.9312	<i>0.1015</i>	0.7955	<i>0.0414</i>	<i>0.0399</i>	0.9926
Period	<i>0.1029</i>	0.4154	<i>0.0015</i>	0.9992	0.7595	0.7380	0.9726	0.9828
Day	<i><0.0001</i>	0.9553	0.2912	<i>0.0473</i>	<i>0.0758</i>	0.7958	0.4267	0.5461
BA	0.8202	<i>0.0079</i>	0.4236	0.1236	<i>0.0002</i>	<i><0.0001</i>	0.9079	<i>0.0125</i>
Weight*Diet	0.2584	<i>0.0131</i>	0.9142	0.5921	0.5731	0.9471	0.3750	<i>0.0179</i>
Seq	0.9454	<i>0.0656</i>	0.5916	0.1693	0.8588	0.3175	0.4980	<i>0.0518</i>

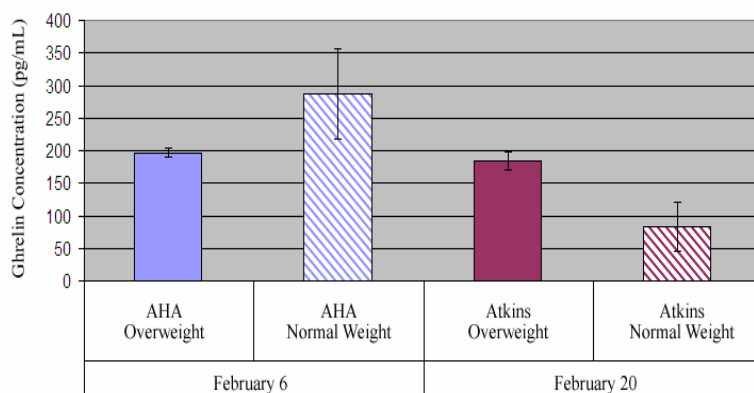
Bold, highlighted p-values are considered significant ($p < 0.05$). Bold, italicized p-values indicate a trend ($0.05 < p < 0.20$).

Figure 1.

Effects of Atkins and AHA Diets on Fasting Ghrelin Active Concentration
Group 1



Effects of Atkins and AHA Diets on Fasting Ghrelin Active Concentration
Group 2



All means \pm SD

Group 1: Started on Atkins diet for two weeks then switched to AHA diet

Group 2: Started on AHA diet for two weeks then switched to Atkins diet