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**Effects of Dietary Macronutrients on Appetite-Related Hormones in Blood on Body Composition of Lean and Obese Rats**

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**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. Fifty-three adult male Sprague Dawley and 30 adult male Zucker Fatty rats were assigned randomly to one of five diets: Control, 75% control, American Heart Association (AHA), Atkins, or high fat (HF). Diets were fed for five weeks. Weekly plasma samples were collected and analyzed for ghrelin, leptin, insulin, and adiponectin. Terminal plasma samples were analyzed for ghrelin, leptin, insulin, glucagon, oxyntomodulin, adiponectin, and blood metabolites. Our results indicate that macronutrient composition of the diet influences appetite-related hormones differently in genetically divergent rats. For example, glucagon concentration was higher in obese rats fed the Atkins diet in comparison to obese rats fed the HF and 75% control diets (P<0.05) and tended to be higher in obese rats fed the Atkins diet in comparison with rats fed the AHA and control diets (0.06<P<0.15). In lean rats, glucagon concentration was higher in rats fed the Control diet in comparison with all other diets (P<0.03). Influence of diet and animal type on other related hormones will be presented. Our results document a relationship of appetite-related hormones with respect to diet composition in lean and obese rats as a model for humans.

**Introduction**

Ghrelin is a newly discovered hormone that acts upon the growth hormone secretagoge 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. 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. 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 synthesized in the arcuate nuclei and oxyntic glands of the stomach. Stomach ghrelin is thought to be involved in physiological effects and possibly in stimulating 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. Oxyntomodulin (OXM) is cleaved from the proglucagon hormone, which is synthesized in the oxyntic glands of the stomach. Oxyntomodulin decreases food intake and suppresses appetite in humans. Studies have been conducted to investigate the effects of OXM or ghrelin on feed intake and appetite. In this proposed study, the effects of diet composition on ghrelin and OXM concentrations in plasma of rats will be analyzed.

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, and chronic heart failure or surgical modifications of the stomach. Patients with AN have higher plasma ghrelin concentrations than do normal humans. Furthermore, plasma ghrelin in AN patients is associated negatively with body mass index (BMI) values. Patients with chronic heart failure and cachexia have high plasma ghrelin concentrations. Ghrelin administered to patients with chronic heart failure improves hemodynamic function. Ghrelin could serve two roles in chronic heart failure: creating a positive energy balance and improving 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. Lower plasma ghrelin concentrations in gastric bypass patients could be explained by the surgery. Because ghrelin is produced primarily 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.

In 2002, Beck and colleagues 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 by Monteleone and colleagues 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 2003, Weigle and colleagues showed that 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 subcutaneously and an increase in adiposity was shown. In 2002, 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. In 2004, Salbe et al. showed that ghrelin has a negative association with ad libitum feed intake. Salbe and colleagues, 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 such as the Salbe study used a total ghrelin assay rather than the active ghrelin assay. This finding 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 complexity of hormonal regulation is obvious in Figure 1. On the basis of primarily rat studies, after eating a meal, the stomach releases less ghrelin and more OXM into the blood and thus the appetite centers in the brain are stimulated less. Therefore, the animal has less stimulus to eat. As the stomach empties (fasting), ghrelin secretion increases and OXM secretion from the stomach decreases. Other hormones such as leptin, NPY, PYY, and CRH (abbreviations defined in legend of Figure 1) also signal the brain to regulate food intake.

The objective of this study is to investigate the effect of macronutrients on ghrelin concentration and expression, concentration of other hormones that regulated feed intake, and metabolites in the blood. We hypothesize that obese rats will maintain greater ghrelin concentrations in serum over a 24-hour day and that rats fed low carbohydrate, high fat diets will have lower serum ghrelin than those rats fed high carbohydrate, low fat diets.

Materials and Methods
A total of 83 adult rats were used in this 28-day experiment. Two strains of rats were utilized. Fifty-one Sprague Dawley rats served as normal control rats, and 32 Zucker fatty rats served as obesity-prone rats. The experiment was carried out in blocks. Rats were fed the control diet for the one-week adjustment period. During the second week of adjustment, the rats were assigned randomly to one of the following diets (Table 1).
Table 1: Diet Composition.

<table>
<thead>
<tr>
<th>Type of Diet</th>
<th>Composition of diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carbohydrate (%)</td>
</tr>
<tr>
<td></td>
<td>Lipid (%)</td>
</tr>
<tr>
<td></td>
<td>Protein (%)</td>
</tr>
<tr>
<td>Control</td>
<td>70</td>
</tr>
<tr>
<td>High Fat</td>
<td>20</td>
</tr>
<tr>
<td>Atkins</td>
<td>10</td>
</tr>
<tr>
<td>AHA</td>
<td>56</td>
</tr>
<tr>
<td>75% calories of Control</td>
<td>70</td>
</tr>
</tbody>
</table>

During the second week of adjustment, caloric intakes of each group were measured. All rats were fed the caloric intake of the lowest consuming group so that all rats were fed isocalorically. At the end of the adjustment period, the following blood sampling schedule was followed:

Rats were restrained by using rodent restraint cones. Blood was collected from the saphenous vein on days 1, 7, 14, 21, and 28. Rats were euthanized using CO2 anesthesia. Terminal blood (day 35) was collected by cardiac puncture. Weekly plasma samples were analyzed for ghrelin, leptin, insulin, and adiponectin. Terminal plasma samples were analyzed for concentrations of ghrelin, insulin, leptin, adiponectin, glucagon, growth hormone. All hormone concentrations were determined by radioimmunoassay (RIA, Linco Scientific). Carcass lipid and protein content were determined for body composition. Carcass lipids were measured using modified Folch wet tissue lipid extraction. Carcass protein was determined by micro Kjeldahl assay.

**Results and Discussion**

**Body Composition**

Sprague Dawley rats fed the 75% calories of control diet had lower carcass lipid and higher carcass protein in comparison to rats fed all other diets (Table 2). In Zucker fatty rats, carcass lipid was higher in rats fed the high fat diet in comparison to AHA, Atkins, and 75% calories of control diet. Zucker fatty rats fed the control diet were not significantly different from any of the other treatment groups with respect to carcass lipid percentage. There was no significant effect of any of the diets on carcass protein of Zucker fatty rats. In lean rats, decreasing caloric intake by 25% of regular intake decreased body lipid percentage and increased body protein, which would be expected. When calorie content was held constant, there was no significant difference in body composition amongst rats fed the AHA, Atkins, control and high fat diets. The underlying physiology of this observation may explain the change body composition seen by humans using these diets, which may likely result from the number of calories consumed and not the actual composition of the diet. However, in obese rats, the same results were not observed. Obese rats fed the high fat diet had a higher percentage of carcass lipid in comparison to obese rats fed all other diets. These results suggest that obese rats may partition nutrients differently than do lean rats.

**Hormone Concentrations**

**Adiponectin**

Sprague Dawley rats fed the 75% calories of control diet had significantly higher plasma adiponectin concentration in comparison to rats fed all other diets (Figure 2). However, Zucker fatty rats fed the AHA diet had significantly higher plasma adiponectin concentration in comparison to rats fed all other diets (Figure 3). Both calorie restriction and the AHA diet have been shown to have positive health benefits in humans. In lean rats, calorie restriction led to the highest concentration of plasma adiponectin whereas in obese rats, the AHA diet led to the highest concentration of plasma adiponectin. Plasma adiponectin improves insulin resistance. The different response to diet composition between lean and obese rats is not clear.

**Ghrelin**

There was no significant difference in plasma ghrelin concentration of Sprague Dawley rats fed any of the five diets (data not shown). Zucker fatty rats fed the AHA and high fat diets had significantly lower plasma ghrelin concentration in comparison to the rats fed 75% calories of control. The ghrelin concentrations of obese rats fed the Atkins and control diets were not significantly different from those of rats fed the AHA and high fat diets or the 75% calories of control diet (Figure 4). Higher ghrelin in the 75% calories of control group is a likely result of the negative energy balance caused by the calorie restriction. The ghrelin responses in both the lean and obese group are not as dramatic as one might expect. This might be because the plasma samples analyzed were taken in the morning while the rats were in the light phase (resting). The differences in ghrelin may be more pronounced right before the beginning of the dark phase (active).

**Glucagon**

Plasma glucagon concentration was higher in Sprague Dawley rats fed the control diet in comparison with the concentration in rats fed all other diets (Figure 5). Plasma glucagon concentration was higher in Zucker fatty rats fed the Atkins diet in comparison to that in obese rats fed AHA diet. Obese rats fed the control, 75% calories of control,
and high fat diets did not differ from rats fed either the Atkins or AHA diet (Figure 6).

**Growth hormone**

There was no significant effect of diet composition on growth hormone concentration in either the Sprague Dawley or Zucker fatty rats (data not shown).

**Insulin**

Plasma insulin concentration were higher in Sprague Dawley rats fed the Atkins, control, and high fat diet in comparison with those in rats fed the AHA and 75% calories of control diets (Figure 7). Plasma insulin concentration was significantly lower in rats fed the 75% calories of control diet in comparison to those in rats fed all other diets (Figure 8).

**Leptin**

There was no significant difference in plasma leptin concentration of Zucker fatty rats fed any of the five diets (data not shown). Zucker fatty rats have a mutation in the leptin receptor gene, which leads to high plasma leptin concentrations, and is the cause of their obesity. Hence all of these rats had high plasma leptin concentrations, as expected for Zucker rats. Plasma leptin concentration was higher in Sprague Dawley rats fed the AHA in comparison to the Atkins, control, and 75% calories of control diets fed to Sprague Dawley rats (Figure 9). Sprague Dawley rats fed the 75% calories of the control diet had less plasma leptin, which is a result of the calorie restriction. The calorie restriction causes a negative energy balance, which suppresses the secretion of leptin which deceases satiety.

**Implications**

Diet composition affects plasma hormone concentrations and body composition differently in lean and obese rats. This study compared lean rats with genetically obese rats. The results from this study indicate that lean rats respond differently to diet composition than do genetically obese rats. Those data provides us with more insight into how diet composition influences hormone concentrations and body composition. Future research is needed to further investigate the role diet composition plays in hormone secretion.

**Acknowledgements**

Financial support for this experiment was provided by the Iowa State University Burroughs Endowment.

### Table 2: Body Composition

<table>
<thead>
<tr>
<th>Value</th>
<th>Rat Breed</th>
<th>AHA</th>
<th>High Fat</th>
<th>75% Control</th>
<th>Atkins</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcass</td>
<td>SD</td>
<td>9.4 ± 1.0a</td>
<td>10.4 ± 1.0a</td>
<td>5.2 ± 1.0b</td>
<td>9.3 ± 1.0a</td>
<td>6.9 ± 1.0f</td>
</tr>
<tr>
<td>Lipid* (%)</td>
<td>ZF</td>
<td>58.7 ± 2.6a</td>
<td>68.0 ± 2.6b</td>
<td>59.6 ± 2.6a</td>
<td>59.1 ± 2.6a</td>
<td>64.2 ± 2.6ab</td>
</tr>
<tr>
<td>Carcass</td>
<td>SD</td>
<td>18.4 ± 0.6a</td>
<td>19.6 ± 0.6ab</td>
<td>20.3 ± 0.6b</td>
<td>19.6 ± 0.6ab</td>
<td>19.0 ± 0.6ab</td>
</tr>
<tr>
<td>Protein* (%)</td>
<td>ZF</td>
<td>9.4 ± 0.7a</td>
<td>7.3 ± 0.7a</td>
<td>8.9 ± 0.7a</td>
<td>8.1 ± 0.7a</td>
<td>7.8 ± 0.7a</td>
</tr>
</tbody>
</table>

Mean ± SEM
a-c Means with different superscripts differ
*Per gram wet tissue

SD = Sprague Dawley
ZF = Zucker Fatty
Figure 2. Plasma Adiponectin Concentration in Sprague Dawley Rats

Mean ± SEM Significant p<0.05
a-c Columns with different letters differ

Figure 3. Plasma Adiponectin Concentration in Zucker Fatty Rats

Mean ± SEM Significant p<0.05
a-c Columns with different letters differ

Figure 4. Plasma Ghrelin Concentration in Zucker Fatty Rats

Mean ± SEM Significant p<0.05
a-c Columns with different letters differ

Figure 5. Plasma Glucagon Concentration in Sprague Dawley Rats

Mean ± SEM Significant p<0.05
a-c Columns with different letters differ

Figure 6. Plasma Glucagon Concentration in Zucker Fatty Rats

Mean ± SEM Significant p<0.05
a-c Columns with different letters differ

Figure 7. Plasma Insulin Concentration in Sprague Dawley Rats

Mean ± SEM Significant p<0.05
a-c Columns with different letters differ
**Figure 8.**

Plasma Insulin Concentration in Zucker Fatty Rats

<table>
<thead>
<tr>
<th></th>
<th>AHA</th>
<th>High Fat</th>
<th>75% Control</th>
<th>Atkins</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin Concentration (ng/ml)</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

Mean ± SEM Significant p<0.05

a-c Columns with different letters differ

**Figure 9.**

Plasma Leptin Concentration in Sprague Dawley Rats

<table>
<thead>
<tr>
<th></th>
<th>AHA</th>
<th>High Fat</th>
<th>75% Control</th>
<th>Atkins</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin Concentration (pg/ml)</td>
<td>a</td>
<td>ab</td>
<td>b</td>
<td>c</td>
<td>bc</td>
</tr>
</tbody>
</table>

Mean ± SEM Significant p<0.05

a-c Columns with different letters differ