The Effect of Breakfast Protein Source on Postprandial Hunger and Glucose Response in Normal Weight and Overweight Young Women

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The Effect of Breakfast Protein Source on Postprandial Hunger and Glucose Response in Normal Weight and Overweight Young Women

An Honors Thesis submitted in partial fulfillment of the requirements for Honors Studies in the Dale Bumpers College of Agriculture, Food, and Life Science

Christina M. Crowder

Spring 2015

Nutrition and Dietetics

Dale Bumpers College of Agriculture, Food, and Life Science

University of Arkansas
Acknowledgements

I would like to thank Dr. Jamie I. Baum as my thesis mentor for her guidance and support of this project. My sincerest thanks to Brianna Neumann for all of her contributions as well as Stephanie Shouse. I would like to thank Dr. Jean-Francois Meullenet and the University of Arkansas Food Science Department for allowing me the use of their facilities. I would like to thank the Dale Bumpers College of Agriculture, Food, and Life Sciences and the University of Arkansas Honors College for funding this project. Lastly, I would like to thank Dr. Curt Rom as the Honors Program Director and Dr. Charles Rosenkrans for serving as my thesis committee.
Abstract
Skipping breakfast is associated with weight gain and obesity, as well as cardio-metabolic risk factors, such as poor glucose control. Currently, there is debate as to what the ideal macronutrient composition of breakfast should be for optimal health. Studies have shown that subjects who eat a breakfast high in protein (PRO) stay fuller throughout the day compared to subjects who consumed a carbohydrate (CHO)-based breakfast. Therefore, the objective of this study was to determine if PRO quality (animal vs plant PRO) at breakfast influences postprandial satiety and glucose response in subjects consuming a higher PRO breakfast. Normal weight (NW; n=14) and overweight women (OW; n=8) ages 18-36 were recruited to participate in the study. All subjects completed two visits in a randomized, crossover design with at least one week between visits. On each testing day, height and weight, fasting blood glucose, and baseline appetite were measured. Subjects were then served one of two breakfasts similar in caloric content: animal PRO (AP; 30% PRO, 45% CHO, 29% fat), plant PRO (PP; 28% PRO, 47% CHO, 25% fat). Blood glucose and appetite were then assessed at 15, 30, 60, and 120 min postprandial.. Subjects were instructed to keep a 1-day food record for the duration of each test day. Subjects preferred ($P < 0.05$) the appearance of the AP to the PP, and there was no difference in taste preference. There was no difference between OW and NW for satiety and glucose response. In addition, there was no difference in satiety or glucose response between AP and PP over the 2-hour postprandial period. However, subjects had a lower peak in glucose 30 min after consuming AP (36.2%) compared to PP (44%), indicating that consumption of an AP breakfast has the potential to improve postprandial glucose response. OW subjects tended to consume more calories throughout the day after the PP compared to the AP breakfast and the NW group. Caloric intake was similar between NW and OW following the AP breakfast. These data suggest protein source influences postprandial glucose response without impacting satiety.
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The Effect of Breakfast Protein Source on Postprandial Hunger and Glucose Response in Normal Weight and Overweight Young Women

Introduction

Over 68.5% of people in the United States are overweight or obese (Ogden et al., 2014). In 2013, 65.2% of adult women in the state of Arkansas were classified as either overweight or obese (CDC, 2013). This finding resulted in Arkansas being the only state in the United States to show an increase in obesity rate for 2013 and made Arkansas rank as the third most obese state (www.fasinfat.org).

Early adulthood is a vulnerable life stage for weight gain, especially among women. The average weight gain for women between the ages of twenty and thirty is 12-25 pounds. Weight gain during early adulthood increases the risk of a number of chronic health conditions such as type 2 diabetes mellitus, depression, polycystic ovary syndrome, and infertility. For example, after the age of eighteen years, women are 1.9 times more likely to develop type 2 diabetes body weight increased 10-16 pounds and 2.7 times more likely if body weight increased 16-22 pounds (Hutchesson et al., 2013).

Breakfast has been defined as the first meal of the day; eaten before or at the start of daily activities (e.g., errands, travel, work, etc.); within two hours of waking; typically no later than 10:00AM; and containing an energy level between 20 and 35% of daily energy needs (Timlin and Pereira, 2007). Breakfast is often cited as the most important meal of the day for children, but this is also true for adults. There are many benefits associated with eating a healthy breakfast: improved micronutrient intake; decreased incidence of overweight and obesity; improved concentration and performance in the classroom and at work; lower cholesterol levels (Ruxton and Kirk, 1997; Pollitt and Matthews, 1998; Stanton
and Keast, 1989; Keski-Rahkonen et al., 2003). Several studies, in both adults and children, have shown that individuals who eat breakfast tend to weigh less than those who omit breakfast as eating a healthy breakfast can reduce hunger throughout the day. Studies have also shown that adults consuming ready-to-eat cereals had lower body mass index (BMI) and weighed less than those individuals who rarely or never ate breakfast cereal or ate higher-fat breakfasts (Deshmukh-Taskar et al., 2013 & Deshmukh-Taskar et al., 2010).

Consuming more protein (20-30g) at breakfast than found in the standard cereal-based breakfast (10-15g) may increase subjective feeling of fullness and satiety through neuroendocrine responses to satiety biomarkers such as ghrelin, insulin, and glucagon-like peptide 1 (GLP-1) (Blom, 2009; Veldhorst, 2009). A recent study found that when adults ate eggs for breakfast, they stayed fuller throughout the day (Vander Wal, 2008). Another study (Ratliff et al., 2010) demonstrated that eating a protein-rich breakfast reduced hunger and decreased calorie intake at lunch. And finally, a study comparing a protein-based breakfast to a carbohydrate-based breakfast found that overweight women who ate protein for breakfast five times a week for eight weeks lost 65% more weight and reduced their waist circumference by 83% more than those participants eating a carbohydrate-based breakfast (Vander Wal, 2008).

Well-established evidence in the literature documents greater satiety following protein ingestion as compared to equivalent fat or carbohydrate intake— with fat more satiating than carbohydrate (Obeid, 2005). Protein’s satiating effects are seen both acutely (immediately following a meal) and long-term (≥24 hours after a meal) in regard to subsequent food intake and feelings of hunger and fullness (Latner, 1999; Westerterp-
Plantenga and Lejeune, 2005). Recently, Ortinau (2014) found that young women who consumed a high protein snack in the afternoon reduced their food intake at dinner; had decreased feelings of hunger; and had increased fullness compared to participants that consumed a high-fat, energy dense snack. These long-term satiating effects are especially seen in the context of energy-reduced or weight loss diets (Westerterp-Plantenga et al., 2004; Due, 2004).

The use of high protein diets to reduce the amount of food consumed at the next meal is a strategy used to help maintain negative energy balance during weight loss or to maintain weight equilibrium within the day-to-day fluctuations of variable caloric consumption (Manuela, 2005). As a person decreases their body mass through a negative energy balance (food restriction and/or exercise), feelings of hunger due to increased ghrelin production increase in response to the deficit making continued weight loss or maintenance difficult (Briggs and Andrews, 2010). The comparison of high (25% total energy) and moderate protein diets (12% total energy) with fixed fat content (30%) shows increased dieter compliance, greater weight loss, and more successful weight loss maintenance over a year for high protein diet (Due, 2004). Several other studies have demonstrated the benefits of higher protein intake in aiding weight loss and fat-free mass (FFM) preservation when dieting.

Accumulating evidence also attributes the greater satiety response following protein consumption to increased thermogenesis and neuroendocrine response (Westerterp-Plantenga, 1999). The thermic effect of food, defined as the ability of a food to increase metabolic rate after consumption, is thought to contribute to the increased weight loss typically seen from high protein diets. Protein is the most thermogenic macronutrient, with
20-35% of its caloric content used in the digestion, absorption, and utilization for energy as compared to carbohydrate that only requires 5-15% for the same processes (Halton, 2004). This unique aspect of protein helps contribute to overall energy balance or furthering of an energy deficit in an individual engaging in weight loss. Further, protein-based breakfasts positively affect postprandial blood glucose homeostasis, of which tighter control is strongly associated with a lower risk of type 2 diabetes mellitus, hypertension, and cardiovascular disease. Healthy subjects as well as individuals with type 2 diabetes mellitus both respond positively to high protein breakfasts, resulting in favorably altered biomarkers including reduced HbA1C%, postprandial glucose, postprandial insulin and lower systolic blood pressure (Gannon, 2003; Rabinovitz, 2014).

Protein quality is defined as the ability of protein to achieve certain metabolic actions within the digestion, absorption, and assimilation process. Two important aspects of protein quality include a) the individual protein and food matrix within which it is consumed, and b) the availability of essential and conditionally essential amino acids (Millward, 2008). One of the main concerns with protein quality is the ability to satisfy essential amino acid requirements. Plant derived protein, with the exception of soy, is considered incomplete because it lacks one or more amino acids necessary for growth and development. Animal proteins are complete proteins that contain all the necessary amino acids. Protein quality is important because although equal quantities of plant and animal protein may have the same caloric content, the digestibility and content of amino acids have notable effects on blood glucose regulation (Millward, 2008).

Studies have shown mixed results in terms of satiety as a response to protein quality. One study comparing the dose-dependent satiating effects of whey as compared to
casein and soy protein demonstrated that within both low and high protein diets (10% or 25% energy) whey has greater satiating effects due to decreases in subjective hunger shown through increases neuroendocrine markers of satiety like ghrelin, insulin, and glucagon-like peptide 1 (GLP-1) (Veldhorst, 2009). Another study compared liquid meals of egg albumin, pea protein, soy protein, casein, gelatin, and wheat gluten for satiety response in healthy subjects: no differences in satiety response to protein source within a mixed meal were noted (Lang, 1998). This finding could be attributed the addition of fat and carbohydrate from the mixed meal that delay gastric emptying, negating any post-absorption differences in the proteins. A primary concern for comparing protein sources (plant vs animal) is the vehicle through which the protein is ingested (e.g. whole food vs isolated protein).

The rates at which proteins are digested and absorbed also affect satiety. Whey protein and casein are said to be “fast” and “slow” proteins, respectively, in relation to their rate of digestion and absorption which can effect plasma amino acid concentrations and metabolic outcomes (Luhovvy, 2007). One study looked at the effect of casein and whey protein on subjective feelings of hunger and fullness and the amount of food eaten during an ad libitum meal after a 48g casein or whey preload. Subjects demonstrated less hunger and increased fullness after the whey preload and consumed significantly less food during the meal. Gastrointestinal satiety hormones were measured, and the whey protein had greater response of GLP-1, CCK, and glucose-dependent insulinotropic peptide, which are more sensitive markers of satiety as compared to VAS scales (Hall, 2003). Although whey and casein are both animal based proteins, this demonstrates the effect of digestibility on satiety and the importance of protein quality.
The objective of this study is to determine if protein quality (plant protein versus animal protein) at breakfast influences satiety, glucose response and decreases daily food intake. We hypothesize that participants will have a greater feeling of fullness and improved glycemic response (glucose values) following the animal protein-based breakfast compared to the plant protein-based breakfasts.

Materials and Methods
Subjects
Recruitment was performed between October 2014 and February 2015 through the Department of Food Science at the University of Arkansas. The study protocol was approved by the Office of Research Compliance Institutional Review Board of the University of Arkansas. Subjects were recruited into the study using the University of Arkansas Newswire. The selection was carried out with a phone interview, and exclusion criteria included the following: underweight (BMI ≤18.4), current smoker, current medication usage (except hormonal birth control), food allergies or disliked the foods served during the study, functional or metabolic disease. Subjects signed a consent form before being permitted to participate in the study. The participants were recruited on a rolling basis and assigned to a treatment group based on BMI (Normal Weight or Overweight). Subjects were compensated with a $50 gift card.

Study Design
Twenty-two healthy, female adults ages 18-36 y were enrolled in the study. Subject characteristics can be found in Table 1. Once enrolled in the study, subjects were assigned to the normal weight (NW; n =14) or overweight (OW; n = 8) group based on BMI. The study was conducted using a randomized, cross-over design in which each participant received two different breakfasts, animal protein-based (AP) and plant protein-
based (PP), with at least a one-week washout period between each test day breakfast, with no more than two weeks between testing days. The treatment groups consisted of 14 NW and 8 OW subjects. Subjects were instructed to fast for at least 8 hours overnight prior to the study days and limit their physical activity the day prior to data collection. On each data collection day, food items for breakfast were portioned, weighed, and labeled appropriately for each subject. Subjects were given 15 minutes to consume the test breakfast. The participants were asked to evaluate the taste and appearance of the breakfast on a visual analog scale (VAS). Blood glucose and appetite were analyzed at 0, 15, 30, 45, 60, 90, and 120 minutes after each test breakfast. In addition, subjects were asked and instructed to keep food records for the rest of each test day.

Table 1. Subject Characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Normal Weight</th>
<th>Overweight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>25.57±1.252</td>
<td>25.00 ± 1.626</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.00 ± 1.29</td>
<td>165.9 ± 1.769</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>60.94 ± 1.810</td>
<td>88.15 ± 7.934</td>
</tr>
<tr>
<td>BMI</td>
<td>22.35 ± 0.531</td>
<td>31.93 ± 2.664</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Caucasian</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Asian</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Indian</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Breakfast Composition
The nutrient composition of the test breakfasts can be found in Table 2. The PP and AP breakfasts were similar in calories, carbohydrates, fat, and fiber. This allows for a controlled comparison of protein source.
Table 2. Energy, protein, carbohydrate, fiber, and fat content of the two breakfast meals.

<table>
<thead>
<tr>
<th></th>
<th>Plant PRO breakfast</th>
<th>% energy</th>
<th>Animal PRO breakfast</th>
<th>% energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>375</td>
<td>28%</td>
<td>360</td>
<td>30.0%</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>26.4</td>
<td>28%</td>
<td>27.1</td>
<td>30.0%</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>43.8</td>
<td>46.6%</td>
<td>36.7</td>
<td>40.7%</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>4.1</td>
<td></td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>Fat (g)</td>
<td>10.5</td>
<td>25.3%</td>
<td>11.7</td>
<td>29.2%</td>
</tr>
</tbody>
</table>
Measurements and Data Analysis

**Body Height & Weight, and Body Mass Index (BMI)**
Body height was measured to the nearest 0.01 cm using a stadiometer (Detecto, St. Louis, MO) with subjects barefoot, in the freestanding position. Body weight was measured in the fasting state with subjects without shoes to the nearest 0.01 kg using calibrated balance scales (Detecto, St. Louis, MO). Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared.

**Dietary Assessment**
The energy and macronutrient composition of test breakfast meals and the 1-day dietary records were analyzed using the Genesis R&D diet analysis software package (Salem, OR).

**Blood Glucose**
After an overnight fast, blood glucose samples was measured via finger stick at 0, 15, 30, 45, 60, 90, and 120 minutes postprandial using a Lifescan One Touch UltraSmart System (New Brunswick, NJ).

**Appetite and Palatability Assessment**
Participants were asked to rate their perceived hunger, fullness, desire to eat, amount of food able to eat, desire for something sweet, and desire for something savory using a 100-mm visual analog scale (VAS) (Flint et al., 2000). In addition, subjects were asked to rate how much they liked the taste and appearance of the test breakfasts using a Visual Analog Scale (VAS). The VAS is a validated questionnaire incorporating a 100 mm horizontal line scale with questions worded as “how strong is your feeling of” and end anchors of “not at all” to “extremely.”

**Statistical Analysis**
In order to analyze the effect of the dietary treatments (e.g. breakfast types), Repeated Measures Analysis of Variance Two-Way (ANOVA) was used and Tukey posthoc test was used for multiple comparisons between groups. In order to analyze the effect of
each breakfast over time, AUC was calculated using the trapezoidal rule (Allison et al. 1995). Area under the curve was then analyzed using One-Way ANOVA using Bonferonni posthoc analysis for multiple comparisons between groups. In cases where no differences between body weight groups existed, the groups were combined to analyze AP versus PP by Paired t-test. These analyses were used to determine differences in blood glucose response, hunger, satiation, palatability, and 24-hour energy intake between the plant protein breakfast and animal protein breakfast. GraphPad Prism Software v6.0 (La Jolla, CA) was used for all data analysis.

Results

Blood Glucose
The blood glucose levels measured at 0, 15, 30, 45, 60, 90, and 120 minutes postprandial for each group on the AP and PP study days are shown in the Figure 1A. The corresponding bar graph, Figure 1B, represents the AUC analysis. There was an overall trend for the AP breakfast to generate a more stable postprandial glucose response for both NW and OW individuals compared to the PP breakfast. However, there was no significant difference for AUC for blood glucose between the breakfast or weight groups. Percent change of blood glucose levels from time 0 to the 30 min peak was not significant between the groups, but there was an overall trend for the NW and OW individuals having greater postprandial glucose values after the PP breakfast as compared to AP. Figure 1C shows at the 30 min peak, OW-PP had a 26% rise in postprandial blood glucose as compared to then OW-AP at an 18.5% increase. Likewise, the NW demonstrated a 27.2% rise in PP BG after the PP breakfast as compared to 22.2% after the AP breakfast treatment. Figure 1D shows percent change of blood glucose values from the 30 min peak to the final measure at 120 min showed significant differences between OW-PP and OW-AP (48.6% vs 25.1%).
(P=0.007) and NW-PP and NW-AP (45.2% vs 29%), (P=0.045).

**Appetite and Palatability Assessment**

*Taste and Appearance*

The perceived taste and appearance responses to each breakfast were measured at the end of the breakfast consumption period, 15 min, for each group on AP and PP study days as shown in the bar graphs of **Figure 2A and 2B**. There was no significant difference in taste among PP or AP breakfast treatment. Significant differences in appearance favoring the AP breakfast over the PP (P=0.03) were found by participants.

*Perceived Hunger and Fullness*

The perceived hunger and fullness responses measured at 0, 15, 30, 45, 60, 90, and 120 minutes postprandial for each group on AP and PP study days are shown in the line graphs of **Figure 3A and 4A**. The corresponding bar graphs, **Figure 3B** and **Figure 4B**, represents the AUC analysis. The were no significant changes apart from expected hunger reappearance over the two hour time course for both NW and OW individuals. Though not significant, the NW subjects indicated they were 9.71% hungrier after the PP as compared to the AP breakfast treatment. There were no significant increases in fullness from either group after PP or AP breakfast treatment.

*Desire to Eat and Prospective Food Consumption*

The perceived desire to eat and prospective food consumption responses completed at 0, 15, 30, 45, 60, 90, and 120 minutes postprandial are shown in the line graphs of **Figure 5A** and **Figure 6A**. The corresponding bar graphs, **Figure 5B** and **Figure 6B**, represent the AUC analysis. There was no significant difference between the breakfast and weight groups over time for perceived desire to eat. There was no significant difference in prospective food consumption between the breakfast groups (P > 0.05). But, as shown in **Figure 6B**, the NW subjects indicated a 9.13% greater prospective food consumption
desire after the PP as compared to the AP breakfast treatment.

**Perceived Cravings**
The perceived cravings of sweet and savory responses completed at 0, 15, 30, 45, 60, 90, and 120 minutes postprandial are shown in the line graphs of Figure 7A and Figure 8A. The corresponding bar graphs, Figure 7B and Figure 8B, represent the AUC analysis. The perceived sweet cravings over time between OW-PP, OW-AP, NW-PP, and NW-AP breakfast treatment groups were not significant between breakfast treatments or groups.

**Figure 7B** shows the AUC analysis of perceived sweet cravings, while not significant, the OW demonstrated 16% greater sweet cravings after the PP breakfast and NW demonstrated 28% greater sweet cravings after the AP breakfast. The OW-PP, OW-AP, NW-PP, and NW-AP breakfast treatment group showed no significance in perceived savory cravings with the consumption of AP or PP breakfast. **Figure 8B** shows the AUC analysis of perceived savory cravings, while not significant, the OW demonstrated 24% greater savory cravings after the PP breakfast.

**Dietary Intake**
The total caloric consumption and calories from each macronutrient are shown in Table 3. Both the OW and AP breakfast treatment groups consumed a within group consistent amount of food after the PP and AP breakfasts, and the OW subjects ate on average 50-200 kcals more than NW. The OW group consumed on average 45% of kcals from carbohydrate, 37% kcals from fat, and 16% from protein after each breakfast. The NW group consumed on average 50% of kcals from carbohydrate, 30% kcals from fat, and 20% from protein. Overall, the OW group tended to eat a greater portion of post-meal kcals from fat as compared to the NW group, averaging about 7% more fat. The OW group also ate less protein than the NW group, 16.4% compared to 21.5%. The NW group ate more carbohydrates than the OW group, 49% compared to 45%.
Table 3

<table>
<thead>
<tr>
<th></th>
<th>OW-PP</th>
<th>OW-AP</th>
<th>NW-PP</th>
<th>NW-AP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy (Kcal)</strong></td>
<td>2345 ± 339</td>
<td>2239 ± 103</td>
<td>2021 ± 144</td>
<td>2204 ± 115</td>
</tr>
<tr>
<td><strong>Carbohydrate (g)</strong></td>
<td>258.6 ± 52.4</td>
<td>252.3 ± 17.8</td>
<td>239.7 ± 17.2</td>
<td>259.5 ± 15.7</td>
</tr>
<tr>
<td><strong>Carbohydrate %</strong></td>
<td>44.7 ± 4.9</td>
<td>44.83 ± 1.96</td>
<td>48.6 ± 2.8</td>
<td>47.7 ± 2.4</td>
</tr>
<tr>
<td><strong>Fat (g)</strong></td>
<td>93.51 ± 15.26</td>
<td>97.47 ± 4.63</td>
<td>68.98 ± 7.67</td>
<td>74.76 ± 9.35</td>
</tr>
<tr>
<td><strong>Fat %</strong></td>
<td>36.95 ± 0.05</td>
<td>39.42 ± 1.83</td>
<td>30.87 ± 2.48</td>
<td>30.16 ± 2.77</td>
</tr>
<tr>
<td><strong>Protein (g)</strong></td>
<td>91.21 ± 15.56</td>
<td>92.04 ± 5.55</td>
<td>113.0 ± 20.2</td>
<td>120.4 ± 18.99</td>
</tr>
<tr>
<td><strong>Protein %</strong></td>
<td>16.39 ± 0.02</td>
<td>16.47 ± 0.09</td>
<td>21.55 ± 2.47</td>
<td>21.65 ± 3.04</td>
</tr>
</tbody>
</table>
Figure 1. The comparison of blood glucose levels over time between OW-PP, OW-AP, NW-PP, and NW-AP breakfast treatment groups. Figure 1A shows no significant effect of breakfast consumption on blood glucose, but trends supporting lower values for the AP breakfast in both OW and NW. Figure 1B shows the AUC analysis for blood glucose levels. Figure 1C shows percent change of postprandial glucose from time 0 to 30 min, with the AP breakfasts with smaller margins. Figure 1D shows significant differences between AP and PP percent change of postprandial blood glucose in both groups from 30 min to 120 min, with (*P=0.045, **P=0.007)
Figure 2. The comparison of taste (Figure 2A) appearance (Figure 2B) between breakfasts. There was no significant difference between the taste of PP or AP breakfast (P>0.05). There was a significant difference in appearance, with the AP preferred over the PP breakfast (P=0.023). VAS-Visual Analog Scale.
Figure 3. The comparison of hunger responses over time between OW-PP, OW-AP, NW-PP, and NW-AP breakfast treatment groups. **Figure 3A** shows no significant changes in expected hunger over 2 hours with the consumption of the AP or PP breakfast. **Figure 3B** shows the hunger AUC analysis, while not significant, the NW group was 9.71% hungrier after the AP than PP breakfast VAS- Visual Analog Scale.
Figure 4

Figure 4 shows no significant increase in fullness with the consumption of the AP or PP breakfast. Figure 4A shows the fullness AUC analysis. VAS- Visual Analog Scale.
Figure 5. The perceived desire to eat over time between OW-PP, OW-AP, NW-PP, and NW-AP breakfast treatment groups. Figure 5A shows no significant decrease in the perceived desire to eat with the consumption of breakfast. Figure 5B shows the AUC analysis of perceived desire to eat, no significance.
Figure 6

Figure 6. The perceived prospective food consumption over time between OW-PP, OW-AP, NW-PP, and NW-AP breakfast treatment groups. Figure 6A shows no significant decrease in the perceived prospective food consumption with the AP or PP breakfast. Figure 6B shows the AUC analysis of perceived prospective food consumption, while not significant, the NW subjects indicated a 9.13% greater desire to consume more food after the PP breakfast. VAS- Visual Analog Scale.
Figure 7

The perceived sweet cravings over time between OW-PP, OW-AP, NW-PP, and NW-AP breakfast treatment groups. Figure 7A shows no significant differences between the AP or PP groups. Figure 7B shows the AUC analysis of perceived sweet cravings, while not significant, the OW demonstrated 16% greater sweet cravings after the PP breakfast and NW demonstrated 28% greater sweet cravings after the AP breakfast.

Figure 7
Figure 8. The perceived savory cravings over time between OW-PP, OW-AP, NW-PP, and NW-AP breakfast treatment groups. Figure 8A shows no significance in perceived savory cravings with the consumption of AP or PP breakfast. Figure 8B shows the AUC analysis of perceived savory cravings, while not significant, the OW demonstrated 24% greater savory cravings after the PP breakfast.
Discussion

This is one of the first studies to examine the effect of complete, isocaloric meals consisting of plant protein versus animal protein, on satiety and postprandial glucose response in normal weight and overweight individuals. The present study led to the conclusion that there is no difference in the effect of protein source (animal versus plant) on satiety, cravings, or daily food intake. However, protein source may influence postprandial glucose response.

Although no difference in postprandial satiety response between animal or plant protein was detected, these results were not unexpected. Several studies have compared the effect of protein source on satiety within a mixed meal (Veldhorst et al., 2009; Lang et al., 1998; Lang et al., 1999; Marsset-Baglieri et al., 2015; Douglas et al., 2015), demonstrating equal satiety responses to plant and animal proteins within higher protein meals (>22% protein). In addition, a majority of studies have demonstrated no overall difference in satiety response to pure proteins, aside from some minor variations that were related to rate of absorption (Veldhorst et al., 2009; Luhovvy, 2007). At lower meal concentrations (10% protein) whey protein (an animal source of protein) seems to exert a greater satiating effect, perhaps due to branched-chain amino acid concentration, but this concentration is much lower than the concentration of animal protein tested in the current study (Veldhorst et al., 2009). This study used test meals similar in caloric content with matched macronutrient compositions, therefore, we did not expect to find large variations in postprandial satiety response between test meals.

This study appears to be the first to examine how protein source influences food cravings. The OW subjects had lower cravings for sweet and savory foods following the AP breakfast; however, the same response was not observed in NW group. This indicates that
protein source may influence postprandial food preference differently in OW and NW individuals. However, more research is needed. One study by Hoertel et al. (2014) found that subjects consuming a high protein diet had lower sweet and savory cravings than subjects who consumed normal protein or skipped breakfast (Hoertel et al., 2014). This study supports the data from our study in terms of cravings. However, no differences were seen in *ad libitum* food intake between diets, although the OW group ate more calories and more fat compared to the NW group. The specific “sweet or savory” qualities of the foods consumed post-breakfast were not recorded, but this data could be further investigated with subsequent studies.

An increase in protein intake throughout the day, starting with breakfast, may help an individual to feel more satisfied and respond to neural signals of satiety and blood glucose regulation (Woods, 2009). The OW subjects consumed fewer calories after the AP breakfast. Additionally, the OW subject consumed less protein on average but consumed more calories than the NW over the remaining 24-hour period. The underlying mechanism is still unknown, but high protein diets seem to spontaneously reduce food intake in individuals and could be attributed to protein’s satiating effect (Anderson and Moore, 2004).

Despite no statistically significant differences between overall glucose response between meals or subjects, there was a trend for more stable postprandial glucose response following the AP breakfast for both NW and OW groups. The control of postprandial glucose levels is important for HbA1C% levels and diabetes risk (Leiter et al., 2005; Boden et al., 2005) and minimizing cardiovascular disease risk and pathogenesis. Both eucaloric and hypocaloric diets with increased protein in general lead to more stable postprandial glucose levels with lesser peak excursions and incremental area under the curve (O’Keefe et al., 2008; Farnsworth et al., 2003; Layman et al., 2003; Gannon and
Nuttall, 2006). There is uncertainty as to why there were greater postprandial glucose levels for both NW and OW following the PP breakfast, but this could be attributed to the slight disparity in breakfast carbohydrate content or differing amino acid profiles. It has been observed that healthy individuals and those with postprandial glucose levels on the higher end of normal may do better with a high animal protein based breakfast, with high protein in general preferred over low protein/carbohydrate based breakfast (Leidy et al., 2014). Overall, in our study the OW subjects had greater postprandial glucose levels, and could benefit most from a high protein breakfast for better glucose control.

One of the limitations of this study is the short postprandial data collection period following breakfast consumption. Two hours postprandial may not be enough time to fully capture the postprandial satiety response, as meals are generally four to five hours apart and initiated by habit or hunger (Woods, 1991). Many studies take measurements for four hours following treatment to ensure subjects return to baseline (Leidy and Racki, 2010; Leidy et al., 2014; Douglas et al., 2015). The small discrepancy in caloric values of the meals may have been why we see small changes in postprandial blood glucose. We do not think these differences are significant enough to affect any of the glucose values, but we cannot ignore the possibility that the difference produced some effect because the meals were not isocaloric. In addition, food records have been proven inaccurate in terms of self-report energy intake, but funding limitations inhibit the ability to use more advanced methods. Dhurandhar and colleagues present a strong case for the discontinuance of subjective energy intake reporting methods, but until more advanced reporting methods are developed and accessible the 24-hour energy intake will have to suffice (Dhurandhar et al., 2014). Additionally, assays for ghrelin, GLP-1, and serum insulin could be used for objective satiety measurements along with subject visual analog
scales (VAS).

Overall, there was no difference in the response between normal and overweight subjects in response to either the animal or protein breakfasts. Subjects had a greater glucose response at 30 min following the plant protein breakfast. There was less fluctuation in blood glucose following the animal protein breakfast. There was no difference in postprandial satiety response between breakfasts. Overweight subjects tended to consume more calories following both breakfasts and more calories from fat compared to normal weight subjects. Normal weight subjects consumed more calories from protein. With these findings, our recommendations are for both healthy and metabolically compromised individuals to consume high quality, high protein breakfasts derived from lean muscle proteins, eggs, dairy, or soy with preference to animal proteins.
References


following ingestion of high-carbohydrate, high-fat, and high-protein meals in males. Annals of Nutrition & Metabolism, 50(3), 260-269.


Ratlliff, J., et al., Consuming eggs for breakfast influences plasma glucose and ghrelin, while reducing energy intake during the next 24 hours in adult men. Nutr Res, 2010. 30(2): p. 96-103


energy intake, including amino acid and 'satiety'hormone responses. European Journal of Nutrition, 48(2), 92-100.


Appendix I: IRB Approval Letter

September 2, 2014

MEMORANDUM

TO: Jamie Baum, Stephanie Shoue
Dallas Johnson, Amy Dunn
Christina Crowder, Shelby Payne
Brianna Neumann, Mune Abadalhida

FROM: Ro Windwalker, IRB Coordinator

RE: New Protocol Approval

IRB Protocol #: 14-07-028

Protocol Title: The Effect of Breakfast Composition on Safety, Glucose Response and 24-Hour Food Intake

Review Type: ☑ EXEMPT ☐ EXPEDITED ☑ FULL IRB

Approved Project Period: Start Date: 09/02/2014 Expiration Date: 08/12/2015

Your protocol has been approved by the IRB. Protocols are approved for a maximum period of one year. If you wish to continue the project past the approved project period (see above), you must submit a request, using the form Continuing Review for IRB Approved Projects, prior to the expiration date. This form is available from the IRB Coordinator or on the Research Compliance website (http://pre.d.uark.edu/210.php). As a courtesy, you will be sent a reminder two months in advance of that date. However, failure to receive a reminder does not negate your obligation to make the request in sufficient time for review and approval. Federal regulations prohibit retroactive approval of continuation. Failure to receive approval to continue the project prior to the expiration date will result in termination of the protocol approval. The IRB Coordinator can give you guidance on submission times.

This protocol has been approved for 20 participants. If you wish to make any modifications in the approved protocol, including enrolling more than this number, you must seek approval prior to implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

The IRB determined and documented that the risk is no greater than minimal and this protocol may be reviewed under expedited review procedure for future continuing reviews.

If you have questions or need any assistance from the IRB, please contact me at 210 Administration Building, 5-2208, or irb@uark.edu.