Evaluation of Protein Source at Breakfast on Energy Metabolism, Metabolic Health, and Food Intake: A Pilot Study

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Evaluation of Protein Source at Breakfast on Energy Metabolism, Metabolic Health, and Food Intake: A Pilot Study

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

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Nutrition and Dietetics

Dale Bumpers College of Agricultural, Food, and Life Sciences

The University of Arkansas
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Introduction & Background

More than one-third of U.S. adults—78.6 million—are obese (CDC, 2014). As consumers grow concerned for their health, nutrition researchers endeavor to provide evidence that supports obesity prevention, weight control, and weight loss. The consumption of plant-based proteins as substitutions for and alternatives to animal-based proteins have been recommended in recent years (Douglas et al., 2015). An important goal of this research is to determine how proteins may help the public achieve their health goals, specifically through identifying differences among protein sources and their effects on metabolism and satiety.

Dietary protein may play an important role in opposing the obesity epidemic Americans currently face (CDC, 2014; Douglas et al., 2015; Millward et al., 2008; Veldhorst et al., 2008; Veldhorst et al., 2009). Protein in the diet may be beneficial for weight loss and weight maintenance due to protein’s satiating properties. Feelings of satiety between meals greatly contribute to appetite and caloric intake throughout the day (Weigle et al., 2005). Recent research has shown that the highest contributor to satiety is both the physical form (e.g. solid versus liquid) and the macronutrient content (e.g. protein, carbohydrate, and fat) of the food (Stull et al., 2008; Weigle et al., 2005; Latner & Schwartz, 1999; Anderson & Moore, 2004; Hall et al., 2003; Lang et al., 1998; Millward et al., 2008). Solid foods have been shown to suppress hunger longer than liquids, while protein-dense foods have been found to be more satiating than carbohydrate- or fat-dense foods (those containing a majority of kilocalories from protein versus those containing a majority of kilocalories from carbohydrate or fat respectively) (Stull et al., 2008; Weigle et al.,
Proteins eaten at earlier meals (e.g. breakfast, lunch) may also have an effect on the quantity of foods chosen for consumption at later meals, decreasing the amount consumed and preventing overeating (Anderson & Moore, 2004; Lang et al., 1998; Leidy et al., 2013; Weigle et al., 2005). Several studies have found that fat intake, as well as protein and carbohydrate intake, was lower after consuming high protein meals (Latner & Schwartz, 1999).

Another study reported the consumption of proteins has a large metabolic effect because protein consumption increases the thermic effect of food (TEF), which increases calorie expenditure postprandially (Weigle et al., 2005; Baba et al., 1999). TEF refers to the energy required by the digestion, absorption, metabolism, and storage of food (Nelms & Sucher, 2015). TEF is one of three components of energy expenditure, accounting for the least amount of total energy expenditure; it is influenced by both the macronutrient makeup of foods and the amount eaten, and its effects can last up to four hours postprandial (Nelms & Sucher, 2015). The macronutrient protein increases TEF through requiring more energy to facilitate digestion than fats or carbohydrates (Weigle et al., 2005). The other two forms of energy expenditure that significantly contribute to a person’s daily total energy expenditure are the resting metabolic rate (RMR), also referred to as resting energy expenditure (REE), and the thermic effect of activity (TEA). REE is the energy necessitated by a body at rest in order for body systems to function (Nelms & Sucher, 2015). REE makes up the majority of the total energy expenditure, while
TEA is the most variable contributor to total expenditure—it is the energy expended with any physical work or heat generation that requires muscular initiation (Nelms & Sucher, 2015).

Metabolic and satiety differences among the macronutrients are well established, however, the impact of different sources of protein (e.g. plant versus animal) on satiety and *ad libitum* food intake is limited (Anderson & Moore, 2004; Lang et al., 1998; Veldhorst et al., 2008). Though a few studies have found that the source of a protein induces a unique metabolic response (Anderson & Moore, 2004; Millward et al., 2008; Veldhorst et al., 2008; Hall et al., 2003), evidence is not unanimously supportive; many studies have found protein sources to have minimal variations in their effects on satiety and food intake at later meals (Hall et al., 2003; Douglas et al., 2015; Lang et al., 1998; Veldhorst et al., 2008). One study suggests that the quantity of protein/amino acids given at a test meal was a greater determinant of satiety and *ad libitum* food intake than the source of the protein/amino acids (Veldhorst et al., 2009). However, significant differences discovered between the two types of milk proteins, casein and whey, strongly suggest that protein composition influences metabolism and food intake (Anderson & Moore, 2004; Veldhorst et al., 2009). Casein has been found to be less satiating, correlating with a slower gastric emptying time and slower entry of amino acids into circulation; whey was found to be more quickly digested and absorbed, which correlated with whey leading to feelings of greater satiety (Anderson & Moore, 2004; Hall et al., 2003; He & Giuseppin, 2014; Millward et al., 2008; Veldhorst et al.,
The properties that are potentially unique to each protein source (protein quality, amino acid profile, digestibility) may result in differing metabolic responses.

Protein quality describes a food protein’s content of essential amino acids as well as its digestibility, or its ability to be absorbed (Millward et al., 2008). Higher quality proteins may affect satiety to a greater degree than lower quality proteins based upon their content of essential amino acids, those involved in the regulation of protein synthesis, protein degradation, insulin secretion/synthesis, and hormone signaling, among other processes (Veldhorst et al., 2009). The branched chain amino acid leucine, found in whey, has been proposed to aid in the regulation of food intake (Anderson & Moore, 2004), while tryptophan has been suggested to influence satiety through its role in the production of serotonin, a hormone important to the regulation of mood and appetite (Veldhorst et al., 2009). The amino acid content of various proteins may contribute to food intake through neurochemical signaling (Anderson & Moore, 2004), but amino acid profile may also affect the thermic effect of feeding through the differences in the ways that various amino acids are oxidized (Veldhorst et al., 2008).

Another factor that coincides with amino acid content and can influence metabolic responses is the digestive actions of proteins (Millward et al., 2008; He & Giuseppin, 2014; Anderson & Moore, 2004). The processes that take place in the gastrointestinal tract involving proteins may affect food intake independently of their amino acid composition (Anderson & Moore, 2004; Hall et al., 2003). Digestion flows from the breakdown of proteins in the stomach to the further lysis of peptides in the small intestine (He & Giuseppin, 2014). Protein type may influence the rate of
each protein to be digested and absorbed (Lang et al., 1998), which influences the rate at which amino acids are present in circulation (He & Giuseppin, 2014), which in turn may influence feelings of satiety (Hall et al., 2003). Because of the complex multi-system interactions that regulate appetite, it is more difficult to determine how unique protein types influence satiety than to discover that correlative differences exist among protein sources and satiety, metabolic rate, and postprandial food intake (Millward et al., 2008).

The need for more research on the implications of protein sources on food intake, metabolism, and health is apparent due to the limited or conflicting current knowledge of the effects of various protein sources, as well as the mechanisms by which various protein sources act on metabolism (Anderson & Moore, 2004; Veldhorst et al., 2008; Veldhorst et al., 2009; Lang et al., 1998; Douglas et al., 2015). Aspects requiring further investigation are the ways in which amino acids individually influence satiety and the role of the digestive rate of different proteins in satiety (Lange et al., 1998; He & Giuseppin, 2014; Veldhorst et al., 2009). Also necessary is further study regarding how much protein is necessary for optimum feelings of satiety to occur and what individual human factors may cause that amount to vary among subjects (Anderson & Moore, 2004; Hall et al., 2003; Latner & Schwartz, 1999).

Ultimately, further research needs to be conducted regarding the roles of specific proteins in the diet, as few studies have compared various animal- and vegetarian-sourced proteins. Therefore, the objective of this study was to further contribute to the research pool through examining the impacts of different protein
sources on postprandial metabolism, satiety, and food intake. We hypothesize that higher-quality complete protein isolates (e.g. animal sources of protein) would be more satiating and have a higher thermic effect of food than the incomplete protein isolates (e.g. plant sources of protein).
Materials & Methods

Subject Recruitment and Participation

Subjects were recruited on a voluntary basis in fall 2015 by advertisement in University of Arkansas Newswire, on flyers in University buildings, through social media (e.g. Facebook, twitter), and by word of mouth. Eight adult males (n=8) ages 18 to 36 were recruited, however, only 4 persons were able to participate for the duration of the study as 4 subjects dropped out due to either scheduling issues or difficulties complying with the study protocol. All interested potential subjects corresponded via email and were screened by phone. The participants had no health conditions, food allergies/intolerances, and were not prescribed any medications. All participants were non-smokers, were not currently dieting, and were not participating in more than 4 hours of strenuous physical activity per week. All participants signed and submitted a participant consent form before taking part in the study. Participants were randomly assigned to treatment groups and given coded subject labels to protect participant privacy. Upon completion of the study, subjects received a gift card and a free body composition scan (DXA) as compensation for their participation. The study design was approved by the University of Arkansas’ Institutional Review Board (IRB) (protocol #15-07-005).

Study Design

The study was a randomized, crossover design. Participants received each dietary treatment with a one-week washout period between treatments. The three treatments included: a beef-sourced protein drink, a pea-sourced protein drink, and a whey-sourced protein drink (refer to Table 1 and Table 2 for compositions of test
drinks). Participants were asked to consume one treatment on each consecutive testing day spaced one week apart.

Participants were asked to refrain from eating at least 8 hours overnight prior to each test day—initial measurements were collected while participants were in a fasted state. Participants arrived at the Food Science Building at the University of Arkansas between 07:00 and 07:30. Upon arrival, standing height and weight were measured; baseline satiety values were recorded using visual analog scales (VAS). Resting energy expenditure was measured using a metabolic cart. Following baseline measurements, participants were provided with the test breakfast beverage. Participants were given 8 minutes to consume the entire beverage. After consumption, participants were asked to refrain from eating for four hours. Small amounts of water were permitted according to subjects’ thirst. During the four-hour period, participants’ appetites were assessed periodically using VAS scale surveys: at 0, 15, 30, 60, 120, 180, and 240 minutes postprandial. Data using a metabolic cart was also collected at six time points throughout the four hours: at 0, 30, 60, 120, 180, and 240 minutes postprandial. In addition, participants were also be asked to record food intake for the following 24 hours beginning at the end of the test day using a provided food diary form, for a total of 3 food records per participant.
**Table 1. Nutrient Compositions of Protein Drink Treatments**

<table>
<thead>
<tr>
<th>Drink Type</th>
<th>Beef</th>
<th>Pea</th>
<th>Whey</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kcal Content</strong></td>
<td>275 kcal</td>
<td>275 kcal</td>
<td>275 kcal</td>
</tr>
<tr>
<td>CHO Content</td>
<td>15.4 g</td>
<td>15.5 g</td>
<td>14.9 g</td>
</tr>
<tr>
<td>PRO Content</td>
<td>42.3 g</td>
<td>41.2 g</td>
<td>43.2 g</td>
</tr>
<tr>
<td>FAT Content</td>
<td>4.5 g</td>
<td>4.3 g</td>
<td>4.6 g</td>
</tr>
<tr>
<td>Fiber Content</td>
<td>&lt;1 g</td>
<td>3.4 g</td>
<td>1.7 g</td>
</tr>
</tbody>
</table>

**Table 2. Recipe for Protein Drink Treatments**

<table>
<thead>
<tr>
<th>Drink Type</th>
<th>Beef</th>
<th>Pea</th>
<th>Whey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water added</td>
<td>385 mL</td>
<td>385 mL</td>
<td>385 mL</td>
</tr>
<tr>
<td>Powder mix added</td>
<td>47.6 g</td>
<td>75.6 g</td>
<td>58.8 g</td>
</tr>
<tr>
<td>Canola oil added</td>
<td>4.5 g</td>
<td>-</td>
<td>2 g</td>
</tr>
<tr>
<td>Cane sugar added</td>
<td>12 g</td>
<td>-</td>
<td>8 g</td>
</tr>
</tbody>
</table>
Measurements & Data Analysis

*Height, Body Weight, and Body Mass Index (BMI):* The height of each participant was measured to the nearest 0.1 cm using a stadiometer while barefoot, in a freestanding position. Body weight was measured at each visit for each subject (without shoes) to the nearest 0.05 kg using calibrated balance scales. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared.

*Appetite Assessment:* Participants were asked to rate their perceived hunger, fullness, strength of desire to eat, desire for a snack, amount of prospective food desired, cravings for salty foods, and cravings for sweet foods using visual analog scales (VAS) spanning 100-mm with opposing anchors (e.g. “extremely hungry” to “not hungry at all”). Appetite was measured periodically at 0, 15, 30, 60, 120, 180, and 240 minutes postprandial.

*Resting Metabolic Rate and Thermic Effect of Feeding (TEF):* Resting metabolic rate was measured with a TrueMax 2400 metabolic cart (Parvomedics, Sandy, UT). Indirect calorimetry, using the ventilation hood technique, was measured in 15-second increments after rest periods while in the supine, reclined position. A canopy hood was placed over each participant and breath-by-breath analysis was conducted for 30 minutes (at time point 0) or for 20 minutes (at each of the following time points across 240 minutes). TEF was determined by assessing the difference in resting metabolic rate (RMR) immediately before and periodically after (30, 60, 120, 180, and 240 minutes) the consumption of the test protein drinks.
Dietary Assessment: The energy and macronutrient composition of test drinks and 24-hour dietary records were analyzed for each participant using Genesis R&D nutrient analysis software (ESHA Research, Salem, OR) and information was organized by test drink.

Statistical Analysis: Repeated measures Analysis of Variance (ANOVA), two-Way ANOVA and t-tests, were used to compare the differences among the three protein treatments’ affects on metabolism, hunger, satiation, and cravings. In order to analyze the affects of the protein drinks across the 4-hour test period, net incremental Area Under the Curve (niAUC) was calculated using the trapezoidal rule (Allison et al. 1995); niAUC was then analyzed using one-Way ANOVA. GraphPad Prism Software v6.0 (La Jolla, CA) was used for all data analysis and figure production.
Results

Participant Characteristics

A total of four participants completed the study in its entirety. Table 3 shows the baseline anthropometric measurements and other specific characteristics of participants in terms of age, height, weight, BMI, body fat mass, fat free mass, and ethnicity. Height, weight, BMI, body fat mass, and fat free mass were gathered from each participants’ DXA scan taken immediately before beginning the study.

Table 3. Participant Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>21.75 ± 2.63</td>
</tr>
<tr>
<td>Height, cm</td>
<td>179.07 ± 7.90</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>100.58 ± 24.67</td>
</tr>
<tr>
<td>BMI</td>
<td>31.57 ± 8.75</td>
</tr>
<tr>
<td>Fat Mass, kg</td>
<td>26.87 ± 18.87</td>
</tr>
<tr>
<td>Fat Free Mass, kg</td>
<td>70.46 ± 10.60</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>2</td>
</tr>
<tr>
<td>White</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
</tr>
</tbody>
</table>

Data expressed as ± SEM
Metabolic Measurements

REE and TEF:

Resting Energy Expenditure (REE) was measured at 0, 30, 60, 120, 180, and 240 minutes following the ingestion of a protein treatment. The average REE over time is shown in Figure 1A for beef, pea, and whey. The average thermic effect of food (TEF) calculated from the REE at time points 0, 30, 60, 120, 180, and 240 minutes post-treatment are shown in Figure 2A for beef, pea, and whey. Figure 1B and Figure 2B are bar graphs representing the area under the curve (niAUC) analysis for each treatment regarding REE and TEF.

Results showed that the pea treatment had a significantly higher REE than the beef protein treatment in two-way ANOVA of REE adjacent to fat free mass (p = 0.0245), and it was found to be significant through an unpaired t-test of pea versus beef (p < 0.05). There was no significant difference between pea and whey or whey and beef. A one-way ANOVA of the niAUC for REE found no difference among rates, though the niAUC for beef was 8% lower than the niAUC for pea and 5% lower than the niAUC for whey. Two-way ANOVA among the TEF values for the treatments found significant differences between pea and whey and between beef and whey (p < 0.05). There was no difference between pea and beef in a two-way ANOVA. In addition, an unpaired t-test of pea versus whey found no difference in TEF, while an unpaired t-test of beef versus whey found the differences to be significant (p < 0.05). The one-way ANOVA of the niAUC for TEF found no differences among treatments, though the niAUC for whey was 77% higher than the niAUC for beef and 43% higher than pea.
Figure 1

**Figure 1A:** A line graph of the Resting Energy Expenditure results averaged over time in minutes for each of the three treatments; time was measured to 240 minutes. Data is expressed as ± SEM.

**Figure 1B:** A bar graph of the area under the curve for the measure of Resting Energy Expenditure for each of the treatments. Data is expressed as ± SEM.
**Figure 2**

**Figure 2A**: A line graph of the Thermal Effect of Food results averaged over time in minutes for each of the three treatments. Time was measured to 240 minutes. Data is expressed as ± SEM.

**Figure 2B**: A bar graph of the area under the curve for the measure of the Thermal Effect of Food for each of the treatments. Data is expressed as ± SEM.
**RQ, KCHO & KFAT:**

Respiratory quotient (RQ), the ratio of carbon dioxide expelled to oxygen taken in, was measured at 0, 30, 60, 120, 180, and 240 minutes post-treatment. The number of kilocalories of carbohydrate expended per minute (KCHO) and the number of kilocalories of fat expended per minute (KFAT) were found from the measure of RQ and analyzed. The resulting line graph of KCHO over time is displayed in Figure 3A and the line graph of KFAT over time is displayed in Figure 4A. The bar graphs for the niAUC of KCHO and KFAT are shown in Figure 3B and Figure 4B.

Pea protein consistently had a higher KCHO mean rate than whey or beef treatments, however, the two-way ANOVA of KCHO and the one-way ANOVA of niAUC for KCHO revealed there was no difference among treatments. Upon observation of the graphs of KFAT, it can be noticed that whey protein maintains the highest rate of KFAT during the four-hour fasting period. A two-way ANOVA of KFAT found that there was statistical significance between the rate of whey over the rate of pea (p < 0.05). A t-test of KFAT for pea versus whey found no difference. Upon running a one-way ANOVA of the graph of niAUC for KFAT, the test found no statistical significance in the apparent rate of KFAT of whey being increased over the KFAT of pea and beef.
Figure 3

Figure 3A: A line graph of the KCHO rates averaged for each of the three treatments over time in minutes. Time was measured to 240 minutes. Data is expressed as ± SEM.

Figure 1B: A bar graph of the area under the curve for the measure of KCHO for each of the three treatments. Data is expressed as ± SEM.
**Figure 4**

![Figure 4A](image1)

**Figure 4A**: A line graph of the rates of KFAT averaged for each of the three treatments over time in minutes, measured to 240 minutes. Data is expressed as ± SEM.

![Figure 4B](image2)

**Figure 4B**: A bar graph of the area under the curve for the rates of KFAT. Data is expressed as ± SEM.
**Appetite Assessments**

**Perceived Hunger and Fullness:**

The perceived hunger and fullness of the participants measured by VAS scale at 0, 15, 30, 60, 120, 180, and 240 minutes postprandial for each of the three protein treatments was assessed and is shown in Figure 5A and Figure 6A. Bar graphs Figure 5B and Figure 6B show the niAUC for hunger and fullness responses. The graphs show that hunger increases as time increases for each of the three protein treatments, while fullness decreases as time increases. A two-way ANOVA of hunger showed that there was no difference among the treatments’ effects. A one-way ANOVA of niAUC for perceived hunger similarly found that there were no distinctions among the proteins. Feelings of hunger were similar following the consumption of each of the treatments. A two-way ANOVA of perceived fullness found that there was a significant difference between perceived fullness following the beef treatment versus perceived fullness following the treatments pea and whey (p < 0.05). Beef protein correlated with lesser feelings of fullness than pea or whey proteins. In the one-way ANOVA of niAUC for fullness there was no significant difference found, though it was visible in Figure 6B for beef protein to create lesser feelings of fullness than pea protein and whey protein.
**Figure 5**

**Figure 5A:** A line graph of the VAS scales’ measure of participants’ appetites over the four hour fasting period. Time was measured in minutes. Data is expressed as ± SEM.

**Figure 5B:** A bar graph of the niAUC of the VAS scales’ measure of participants’ appetites over the four hour fasting period. Data is expressed as ± SEM.
**Figure 6**

**Figure 6A:** A line graph of the VAS scales’ measure of participants’ degree of fullness over the four hour fasting period. Time was measured in minutes. Data is expressed as ± SEM.

**Figure 6B:** A bar graph of the niAUC of VAS scales’ measure of participants’ degree of fullness over the four hour fasting period. Data is expressed as ± SEM.
**Strength of desire to eat and prospective food consumption:**

The perceived strength of desire to eat, the desire for a snack, and the prospective amount of food desired was measured using VAS scales at 0, 15, 30, 60, 120, 180, and 240 minutes postprandial. Line graphs over time are shown in Figure 7A, Figure 8A, and Figure 9A. Bar graphs of the niAUC for each are shown in Figure 7B, Figure 8B, and Figure 9B. The strength of desire to eat generally increases as time increases. Similarly, as time increases, the estimated amount of food able to be consumed increases. The two-way ANOVA of the perceived strength of desire to eat showed there was no difference among the three treatments, and the one-way ANOVA of the niAUC for perceived strength of desire for food found there was no difference. The two-way ANOVA of perceived desire for a snack found beef protein to correlate with a significantly higher desire for a snack than whey protein (p < 0.05). A one-way ANOVA of the data for the niAUC of perceived desire for a snack found there to be no significant difference between whey protein and beef protein. For prospective amount of food desired, the two-way ANOVA showed that there was a significantly greater desire (p < 0.05) to eat more food following the beef protein test drink than there were following the pea or whey test drinks. The one-way ANOVA of the niAUC found no difference among the three protein drinks. When unpaired t-tests of the niAUC for prospective food consumption were analyzed, no difference was found between beef and whey treatments, or between beef and pea treatments.
**Figure 7**

**Figure 7A:** A line graph of the VAS scales' measure of participants' desire to eat over the four hour fasting period; time was measured to 240 minutes. Data is expressed as ± SEM.

**Figure 7B:** A bar graph of the niAUC of VAS scales' measure of participants' desire to eat over the four hour fasting period. Data is expressed as ± SEM.
**Figure 8**

**Figure 8A:** A line graph of the VAS scales' measure of participants' desire for a snack over the four hour fasting period, time was measured over 240 minutes. Data is expressed as ± SEM.

**Figure 8B:** A bar graph of the niAUC of the VAS scales' measure of participants’ desire for a snack over the four hour test period. Data is expressed as ± SEM.
**Figure 9**

**Figure 9A:** A line graph of the VAS scales’ measure of participants’ estimations for the amount of food they could eat at points over the four-hour fasting period. Time was measured in minutes. Data is expressed as ± SEM.

**Figure 9B:** A bar graph of the niAUC of VAS scales’ measure of participants’ estimations for the amount of food they desired to eat over the test period. Data is expressed as ± SEM.
Perceived Salty/Sweet Cravings:

The perceived cravings for salty and sweet-flavored foods were measured using VAS scales at 0, 15, 30, 60, 120, 180, and 240 minutes postprandial. Line graphs over time of cravings are shown in Figure 10A and Figure 11A. Bar graphs of the niAUC for both cravings are shown in Figure 10B and Figure 11B. There was a pattern for cravings to increase in strength over time, but there were no discernable correlations among test drinks and cravings. A two-way ANOVA of salty food cravings found no significant differences among the three treatments. A one-way ANOVA of the niAUC of salty cravings also found there to be no difference. For sweet cravings, a two-way ANOVA of the data found there to be no significant differences among ratings. A one-way ANOVA of the data also found no difference. Despite no significant correlations, the pea protein treatment was associated with the lowest cravings over time for salty foods, while pea protein was associated with the highest cravings over time for sweet foods. Salty cravings were rated higher overall than the sweet cravings on the VAS scales.
Figure 10

Figure 10A: Above is a line graph of the perceived craving for salty foods over the 240-minute test period. Data is expressed as ± SEM.

Figure 10B: A bar graph is shown above of the niAUC for the perceived cravings for salty foods. Data is expressed as ± SEM.
Graph 11A: Above is a line graph of the perceived craving for sweet foods over the 240 minute test period. Data is expressed as ± SEM.

Graph 11B: A bar graph is shown above of the niAUC for the perceived cravings for sweet foods. Data is expressed as ± SEM.
Recorded Dietary Intakes

Food intake over the 24 hours following each study day was recorded before being examined using Genesis R&D software. The total amount of calories consumed following each protein treatment and the breakdown of the amount of each macronutrient in grams of carbohydrate, fat, or protein, is shown below in Table 4. The beef protein treatment correlates on average with the highest postprandial intake of calories and grams of each macronutrient. The beef protein treatment was followed, on average, by an intake of 485 more calories than the whey treatment and 820 more calories than the pea treatment, though the standard deviations from the means were large. Fat intake following the beef protein contributed an average of nearly 35% of calories from fat while the intake of calories from fat after ingestion of the pea and whey proteins were similarly 34% and 36% respectively. The postprandial intake of participants following each of the three protein treatments was similar.
Table 4: Dietary Intake Following Treatments

<table>
<thead>
<tr>
<th></th>
<th>Beef</th>
<th>Pea</th>
<th>Whey</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kcal Consumed</strong></td>
<td>3,557.2 ± 1,745.7</td>
<td>2,736.8 ± 451.8</td>
<td>3,071.6 ± 1,740.5</td>
</tr>
<tr>
<td><strong>CHO, g</strong></td>
<td>441.46 ± 253.06</td>
<td>325.96 ± 78.96</td>
<td>340.14 ± 174.10</td>
</tr>
<tr>
<td>% Kcal from CHO</td>
<td>50%</td>
<td>48%</td>
<td>45%</td>
</tr>
<tr>
<td><strong>PRO, g</strong></td>
<td>127.47 ± 49.69</td>
<td>126.25 ± 35.92</td>
<td>114.01 ± 68.41</td>
</tr>
<tr>
<td>% Kcal from PRO</td>
<td>14%</td>
<td>18%</td>
<td>15%</td>
</tr>
<tr>
<td><strong>FAT, g</strong></td>
<td>139.30 ± 74.31</td>
<td>102.58 ± 27.64</td>
<td>133.04 ± 111.53</td>
</tr>
<tr>
<td>% Kcal from FAT</td>
<td>36%</td>
<td>34%</td>
<td>40%</td>
</tr>
</tbody>
</table>

Table 4: A table showing the average consumption of kcal, carbohydrate, fat, and protein in the 24-hour period following each protein treatment. The percentage of kcal from each macronutrient within each treatment category is given. Values preceding “±” represent statistical means; values following the symbol represent standard deviations from the mean.
Discussion

The large range of protein choices commercially available and the great variation in food selection, dietary supplementation, and overall protein intake among modern consumers, normal weight or otherwise, support our research interest in determining the metabolic effects of different protein sources (Hall et al., 2003).

This study explored the potential for several varying effects among individual protein sources consumed as isocaloric test drinks (comprised of near identical macronutrients), on the metabolisms of healthy young adult males. It was our hypothesis that “complete” protein would have the greatest metabolic effect regarding REE and TEF based upon current research (Millward et al., 2008), and “incomplete” protein would be less satiating than “complete” protein (Millward et al., 2008). Results from this study revealed that beef protein overall was less satiating and increased metabolic rate to a lesser degree than whey or pea proteins. However, minimal significant differences among beef, pea, and whey isolate proteins were found, though correlations were detected that could have larger implications in a more expansive study.

The measures of REE and TEF were affected by protein source, though the treatments would need a repeat testing to look for greater significance as there were discrepancies present. TEF seemed to be significantly affected by whey over pea and beef in some tests, and REE was significantly raised with pea consumption above the consumption of beef protein in few but not all tests as well. In a recent study, whey was the leading protein found to increase energy expenditure through
REE and TEF to a greater degree than casein or soy (Acheson et al., 2011). The perception of fullness was significantly affected by protein source in our study, with beef being significantly less satiating than pea or whey, while the reciprocal measure of perceived hunger found no significant differences, though overall beef correlated with greater feelings of hunger and lesser feelings of fullness. In a similar satiety studies comparing milk/soy proteins and amount of protein, a whey treatment was found to correlate with the greatest feelings of hunger and least feelings of fullness (Acheson et al., 2011), while a higher amount of protein lead to the greatest feelings of fullness (Leidy et al, 2013).

Protein source could also be an important factor when considering connections between the physiological/neural responses post-ingestion. The differences in perceived strength of desire for food showed no statistical significance, but the perceived desire for a snack and the amount of prospective food consumption in our study were significantly greater following the beef treatment than following the whey treatment (or the pea treatment for the amount of prospective food consumption). Similar protein studies have found prospective food consumption to be greatest following ingestion of whey protein compared to casein and soy proteins (Acheson et al., 2011).

With regard to the dietary intake of study participants following each study day, participants on average consumed a similar amount of calories, carbohydrates, protein, and fat in the 24 hours following the treatment of beef protein as the treatments of pea and whey proteins. Current research has also found protein breakfasts of varying protein amounts and sources to have similar daily intakes,
though high fat snacks were more limited when test breakfasts were higher in protein (Leidy et al., 2013), reinforcing the idea that the presence of protein at breakfast may be more influential than the amount or type of protein.

KCHO and KFAT rates among the treatments were not of statistical significance. However, the rate of KCHO following the pea test drink was consistently higher than the rates of KCHO after consumption of beef protein or whey protein. Though the test drinks were nearly identical in all macronutrient content, carbohydrate metabolism was elevated in this study following pea protein ingestion. This finding (among others) may be attributed to the unequal distribution of the fiber content of the test drinks, a value greatest in the pea treatment (Douglas et al., 2015; Lang et al., 1998; Latner & Schwartz, 1999). If fiber content is correlated to the elevated rate of KCHO, it is interesting to note how such small differences in fiber may have manipulated the observed rates. For KFAT rates, fat metabolism was consistently highest after the whey treatment with only a slight difference between the rates of pea and beef seen graphically (Figure 4A, Figure 4B). The elevated rate of KFAT following the whey treatment is consistent with recent research that found the rate of KFAT to be significantly higher following a whey treatment than after treatments of casein and soy proteins (Acheson et al., 2011).

Cravings for salty versus sweet foods throughout the fasting period showed no statistical significance among the different proteins, suggesting that the taste of food desired following protein ingestion may not be as affected as the type of macronutrient desired. However, it was interesting to note that the recorded cravings for salty foods were higher in general than the recorded cravings for sweet
foods. Sweet tasting foods frequently contain significant amounts of fat as well as refined sugars. Further testing of cravings may support the current evidence that consuming high amounts of protein reduces cravings for fatty foods and cravings for food in general (Latner & Schwartz, 1999).

Limitations of the study include the small sample population (n=4). Had more young adult males been able to participate within the window of the study, the correlations that polarized the beef, pea, and whey protein treatments might have been more statistically significant. Also, food records as a quantitative way of assessing postprandial caloric and macronutrient intake are often found to be inaccurate due to their self-assessing nature. In addition, this study focused on testing proteins that were in isolate powdered form and ingested as a drink. Studies testing non-isolate proteins, solid foods, individual amino acids, or mixed meals may have varying metabolic results (Douglas et al., 2015). The amino acid profiles of the tested proteins (beef, pea, whey) may have greatly attributed to our results, as well as the amount of protein tested (Douglas et al., 2015). Lastly, generalizations across genders, ages, and BMI categories for our observations cannot be made since the population examined was limited to young adult males (He & Giuseppin, 2014).

Across all measurements of the study, the observation of beef protein to be less satiating and to have a lesser effect on raising metabolism, as well as the observation for whey protein to be more satiating, is prevalent, but not significant. This data suggests that protein source (animal versus plant) is not a predictor of postprandial EE and appetite response. As statistically significant differences were not common despite clearly observed graphical differences within our small, tested
sample, it is recommended that protein sources related to degrees of satiation and rates of energy expenditure should be more extensively studied, with particular attention to beef/whey proteins and fiber content. Other unstudied isolate proteins at different protein loads are in need of testing, as well as individually ingested amino acids. Further research of potential correlations among specific proteins and their subsequent effects on energy metabolism, satiety, and postprandial food intake is essential to understanding the unique metabolic properties of particular protein sources and their role in promoting healthy appetites and active metabolisms.


MEMORANDUM

TO: Jamie Baum  
    Brianna Neumann  
    Stephanie Shouse  
    Lauren Cambias  
    Enelia Silva  
    Charlayne Mitchell

FROM: Ro Windwalker  
       IRB Coordinator

RE: New Protocol Approval

IRB Protocol #: 15-07-005

Protocol Title: Evaluation of Protein Source on Energy Metabolism, Metabolic Health, and Food Intake in Young versus Older Adults

Review Type: ☒ EXEMPT   ☐ EXPEDITED   ☒ FULL IRB

Approved Project Period: Start Date: 09/09/2015 Expiration Date: 07/22/2016

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 (https://vprud.uark.edu/units/rscp/index.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 160 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.

If you have questions or need any assistance from the IRB, please contact me at 109 MLKG Building 5, 575-2208, or irb@uark.edu.