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Evaluation of protein source at breakfast on energy metabolism, metabolic health, and food intake: a pilot study

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Abstract

Over 30% of adults in the U.S. are obese. A primary contributor to obesity is an unhealthy diet related to imbalanced macronutrients. Diets higher in protein (PRO) rather than carbohydrate (CHO) are associated with increased energy expenditure (EE) and reduced food intake. The objective of this pilot study was to determine if protein source at breakfast influences EE in young men (n = 4; ages 18-35). Participants consumed three isocaloric (whey (WP), pea (PP), beef (BP); 275 kcal, 62% PRO, 23% CHO, 15% Fat) drinks in a randomized, crossover design study with a one-week washout period (time between the administration of each treatment to control for potential interactions). Each test day EE, appetite, and cravings were assessed at 0, 15, 30, 60, 120, 180, and 240 min following consumption. Data were analyzed using 2-way analysis of variance (ANOVA) for effects of protein source over time and one-way ANOVA for area under the curve (niAUC). Resting EE niAUC was 8% lower in BP vs PP and 5% lower vs WP. Thermic effect of feeding niAUC was 77% lower in BP vs WP; PP was 43% lower than WP. Carbohydrate oxidation was higher (31%) with PP compared to WP with no difference between BP and WP. Fat oxidation was 23% higher in WP vs BP and PP. The WP was most satiating. Participants had a higher craving for sweet foods following PP and a higher desire for snacks following BP. Food intake post-treatment was similar in calories and macronutrient distribution. Lack of significant difference among measurements suggests that protein source is not a predictor of postprandial EE, appetite response, or food intake.

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Meet the Student-Author



Lauren Cambias

I was raised in Batesville, Arkansas, I graduated with honors from Batesville High School in the spring of 2012 and moved to Fayetteville to begin college at the University of Arkansas. I then graduated from the University of Arkansas in the spring of 2016 with a Bachelor of Science in Human Environmental Sciences, majoring in Human Nutrition and Hospitality Innovation. Throughout my four years of undergraduate study I was able to serve as Hall Senate Treasurer for Holcombe Hall; be an active member of Gamma Beta Phi honors society; take part in the Student Dietetics Association; serve as Secretary, Vice-President, and President of the Registered Student Organization United Campus Ministry; and have a breathtaking study abroad experience in Rome, Italy over the summer of 2014. I would like to thank Jamie Baum for being my honors thesis mentor, for taking time to explain the research process as we went, and for meeting and corresponding with me when I needed guidance. I would like to especially thank Brianna Neumann for teaching me metabolic cart skills and all of her contributions to the research. In addition, I would like to thank Stephanie Shouse, Charlayne Mitchell, and Enela Silva for their help in conducting this research. I am excited to say I will begin a combined Dietetic Internship and Master's Degree Program at the University of Texas Medical Branch in the fall of 2016.

Introduction

More than one-third of U.S. adults—78.6 million—are obese (Ogden et al., 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).

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). Proteins eaten at earlier meals (e.g., breakfast, lunch) may have an effect on the quantity of foods chosen for consumption at later meals, decreasing the amount consumed and preventing overeating (Anderson and Moore, 2004; Lang et al., 1998; Leidy et al., 2013; Weigle et al., 2005). In addition, several studies have found that fat intake, as well as protein and carbohydrate intake, was lower after consuming high protein meals (Latner and Schwartz, 1999).

Consumption of proteins has a large metabolic effect because protein consumption increases the thermic effect of food, which increases calorie expenditure postprandially (Weigle et al., 2005; Baba et al., 1999). Thermic effect of food refers to the energy required by the digestion, absorption, metabolism, and storage of food (Nelms and Sucher, 2015). Thermic effect of food is one of three components of energy expenditure, accounting for the least amount of total energy expenditure; it is influenced by both the macronutrient (protein, carbohydrate, or fat) makeup of foods and the amount eaten, and its effects can last up to four hours postprandial (Nelms and Sucher, 2015). The macronutrient protein increases thermic effect of food 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, also referred to as resting energy expenditure, and the thermic effect of activity. Resting energy expenditure is the energy necessitated by a body at rest in order for body systems to function (Nelms and Sucher, 2015). Resting energy expenditure makes up the majority of the total energy expenditure, while thermic effect of activity 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 and Sucher, 2015).

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 amino acid content of various proteins may contribute to food intake through neurochemical signaling (Anderson and Moore, 2004), but amino acid profile may also affect the thermic effect of food through the differences in the ways that the 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 and Giuseppin, 2014; Anderson and Moore, 2004). The processes that take place in the gastrointestinal tract involving proteins may affect food intake independently of their amino acid composition (Anderson and Moore, 2004; Hall et al., 2003). 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 and 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 and Moore, 2004; Veldhorst et al., 2008; Veldhorst et al., 2009; Lang et al., 1998; Douglas et al., 2015). Therefore, the objective of this study was to further contribute to the research pool through examining the impacts of different protein sources on postpran-

dial 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 and Methods

Subject Recruitment and Participation

Subjects were recruited on a voluntary basis in fall 2015 by advertisement in University of Arkansas Newswire (an e-news source for the University), on flyers in University buildings, through social media (e.g. Facebook, twitter), and by word of mouth. 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. Eight adult males (n = 8) ages 18 to 36 were recruited, however, only 4 people 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 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 (time between the administration of each treatment to control for potential interactions) 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 for compositions

Table 1. Nutrient compositions of protein drink treatments.

Nutrient	Drink Type		
	Beef	Pea	Whey
Kcal Content	275 kcal	275 kcal	275 kcal
Carbohydrate Content	15.4 g	15.5 g	14.9 g
Protein Content	42.3 g	41.2 g	43.2 g
Fat Content	04.5 g	04.3 g	04.6 g
Fiber Content	<1.0 g	03.4 g	01.7 g

and Table 2 for recipes 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, Fayetteville, Arkansas between 7:00 AM and 7:30 AM. 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 4 hours. Small amounts of water were permitted according to subjects' thirst. During the 4-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 were 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 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.

Measurements and 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 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 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. Resting metabolic rate was measured with a TrueMax[®] 2400 metabolic cart (Parvo Medics, Sandy, Utah) and used to find the thermic effect of food, the rate of carbohydrate oxidation (KCHO), and the rate of fat oxidation (KFAT). 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). Thermic effect of food was determined by assessing the difference in resting metabolic rate immediately before and 30, 60, 120, 180, and 240 minutes after 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, Ore.) 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' effects on metabolism, hunger, satiety, and cravings. In order to analyze the effects of the protein drinks across the 4-hour test period, net incremental area under the curve (niAUC) was calculated using the trapezoidal rule; niAUC was then analyzed using one-way ANOVA. GraphPad Prism Software v. 6.0 (La Jolla, Calif.) was used for all data analysis and figure production.

Results and Discussion

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.

Table 2. Recipe for protein drink treatments.

Ingredient	Drink Type		
	Beef	Pea	Whey
Water added	385.0 mL	385.0 mL	385.0 mL
Powder mix added	47.6 g	75.6 g	58.8 g
Canola oil added	4.5 g	-	2.0 g
Cane sugar added	12.0 g	-	8.0 g

Metabolic Measurements

Resting Energy Expenditure and Thermic Effect of Food. The pea treatment had a significantly higher resting energy expenditure than the beef protein treatment ($P = 0.02$, Fig. 1). The resting energy expenditure niAUC for beef was 8% lower than the niAUC for pea and 5% lower than the niAUC for whey. There were significant differences in thermic effect of food between pea and whey and between beef and whey ($P < 0.05$, Fig. 2). The niAUC for thermic effect of food found no differences among treatments, though the niAUC for whey was 77% higher than the niAUC for beef and 43% higher than pea.

Carbohydrate Oxidation and Fat Oxidation. There was no significant difference between treatments for KCHO (Fig. 3). There was a significant difference in KFAT between the rate of whey over the rate of pea ($P < 0.05$, Fig. 4).

Appetite Assessments

Perceived Hunger and Fullness. Perceived hunger increased and fullness of the participants measured by VAS scale decreased over time (Fig. 5). However, there was no difference in hunger between protein treatments. There was a significant difference in perceived fullness following the beef treatment compared to the pea and whey treatments ($P < 0.05$, Fig. 5).

Strength of Desire to Eat and Prospective Food Consumption. There was no difference in desire to eat between the three treatments. However, perceived desire for a snack was higher with beef protein compared to whey protein ($P < 0.05$, Fig. 6). For prospective amount of food desired, there was a significantly greater desire ($P < 0.05$) to eat more food following the beef protein than there were following the pea or whey protein (Fig. 7).

Table 3. Participant characteristics.

Characteristic	Value
Age, years ^a	21.75 ± 2.63
Height, cm	179.07 ± 7.90
Weight, kg	100.58 ± 24.67
BMI	31.57 ± 8.75
Fat Mass, kg	26.87 ± 18.87
Fat Free Mass, kg	70.46 ± 10.60
Ethnicity	
Black	2
White	1
Other	1
Total	4

^a Age, height, weight, body mass index (BMI), Fat Mass, and Fat Free Mass are expressed as mean ± SEM.

Table 4. Dietary intake following treatments.

Nutrient Data	Beef	Pea	Whey
Energy, kcal ^a	3557.20 ± 1745.70	2736.80 ± 0451.80	3071.60 ± 1740.50
CHO, g	0441.46 ± 0253.06	0325.96 ± 0078.96	0340.14 ± 0174.10
% Kcal from CHO	50%	48%	45%
PRO, g	0127.47 ± 0049.69	0126.25 ± 0035.92	0114.01 ± 0068.41
% Kcal from PRO	14%	18%	15%
FAT, g	0139.30 ± 0074.31	0102.58 ± 0027.64	0133.04 ± 0111.53
% Kcal from FAT	36%	34%	40%

^a Energy, carbohydrate (CHO), protein (PRO), and FAT are expressed as mean ± standard error of mean.

Perceived Salty/Sweet Cravings. There was no difference in cravings for salty and sweet foods between protein treatments.

Recorded Dietary Intakes

The beef protein treatment relates on average with the highest postprandial intake of calories and grams of each

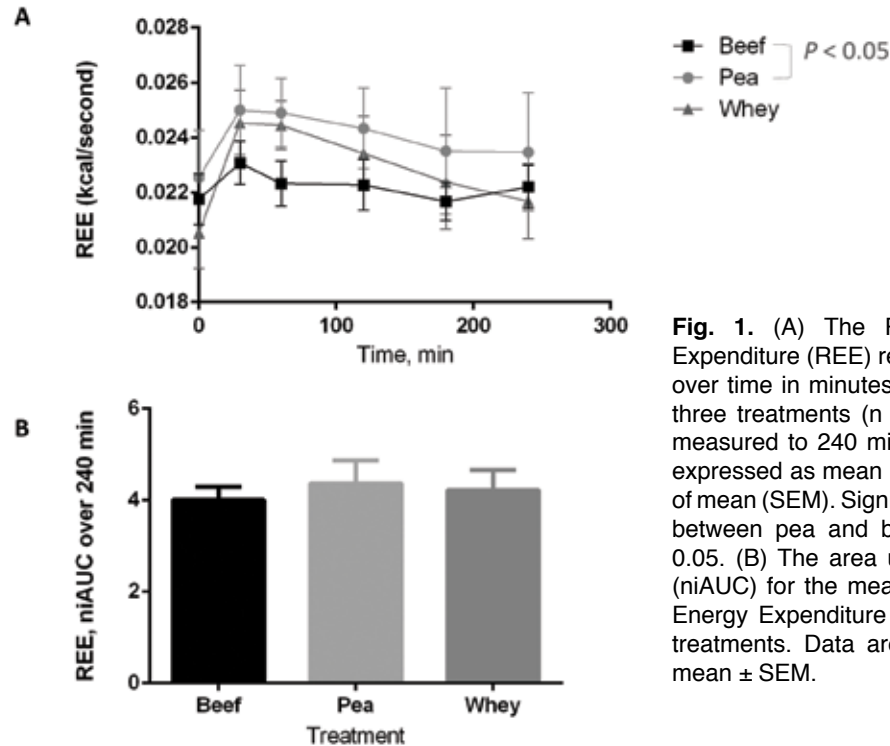


Fig. 1. (A) The Resting Energy Expenditure (REE) results averaged over time in minutes for each of the three treatments ($n = 4$). Time was measured to 240 minutes. Data are expressed as mean \pm standard error of mean (SEM). Significant difference between pea and beef where $P < 0.05$. (B) The area under the curve (niAUC) for the measure of Resting Energy Expenditure for each of the treatments. Data are expressed as mean \pm SEM.

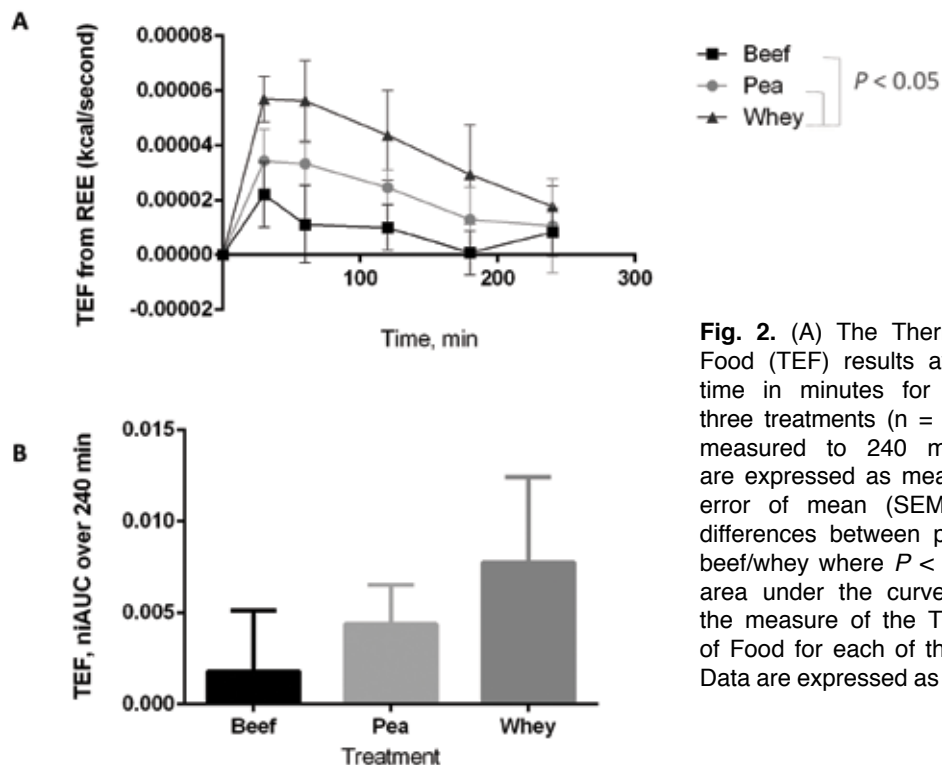


Fig. 2. (A) The Thermal Effect of Food (TEF) results averaged over time in minutes for each of the three treatments ($n = 4$). Time was measured to 240 minutes. Data are expressed as mean \pm standard error of mean (SEM). Significant differences between pea/whey and beef/whey where $P < 0.05$. (B) The area under the curve (niAUC) for the measure of the Thermal Effect of Food for each of the treatments. Data are expressed as mean \pm SEM.

macronutrient (Table 4 shows the average consumption of kcal, carbohydrate, protein, and fat in the 24-hour period following each protein treatment and the percentage of kcal from each macronutrient within each treatment

category). 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

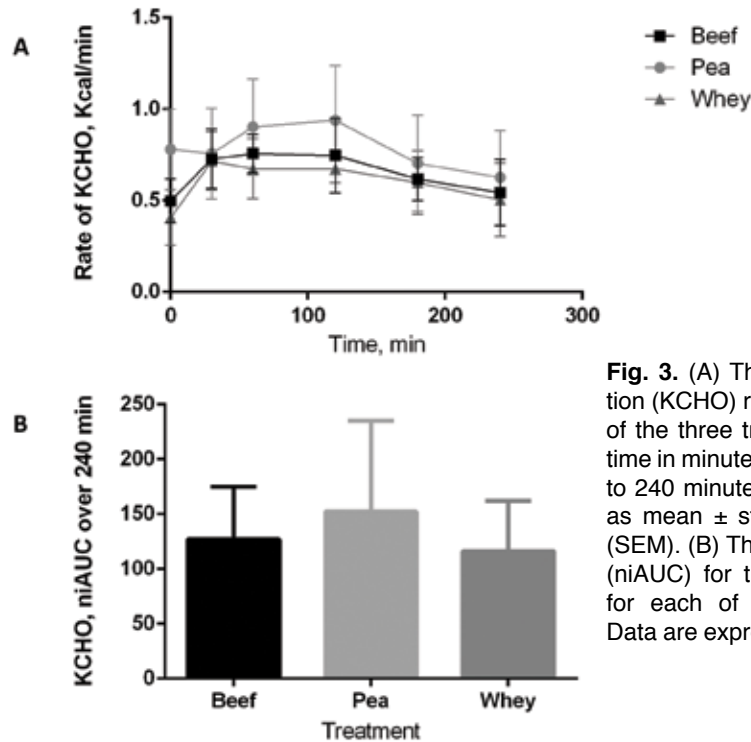


Fig. 3. (A) The carbohydrate oxidation (KCHO) rates averaged for each of the three treatments ($n = 4$) over time in minutes. Time was measured to 240 minutes. Data are expressed as mean \pm standard error of mean (SEM). (B) The area under the curve (niAUC) for the measure of KCHO for each of the three treatments. Data are expressed as mean \pm SEM.

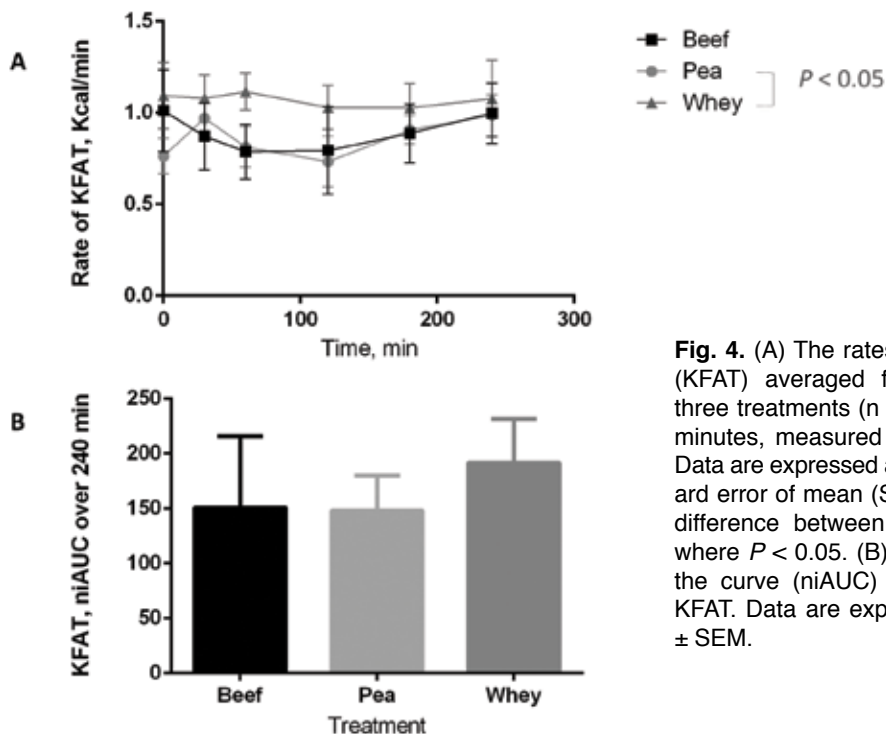


Fig. 4. (A) The rates of fat oxidation (KFAT) averaged for each of the three treatments ($n = 4$) over time in minutes, measured to 240 minutes. Data are expressed as mean \pm standard error of mean (SEM). Significant difference between whey and pea where $P < 0.05$. (B) The area under the curve (niAUC) for the rates of KFAT. Data are expressed as mean \pm SEM.

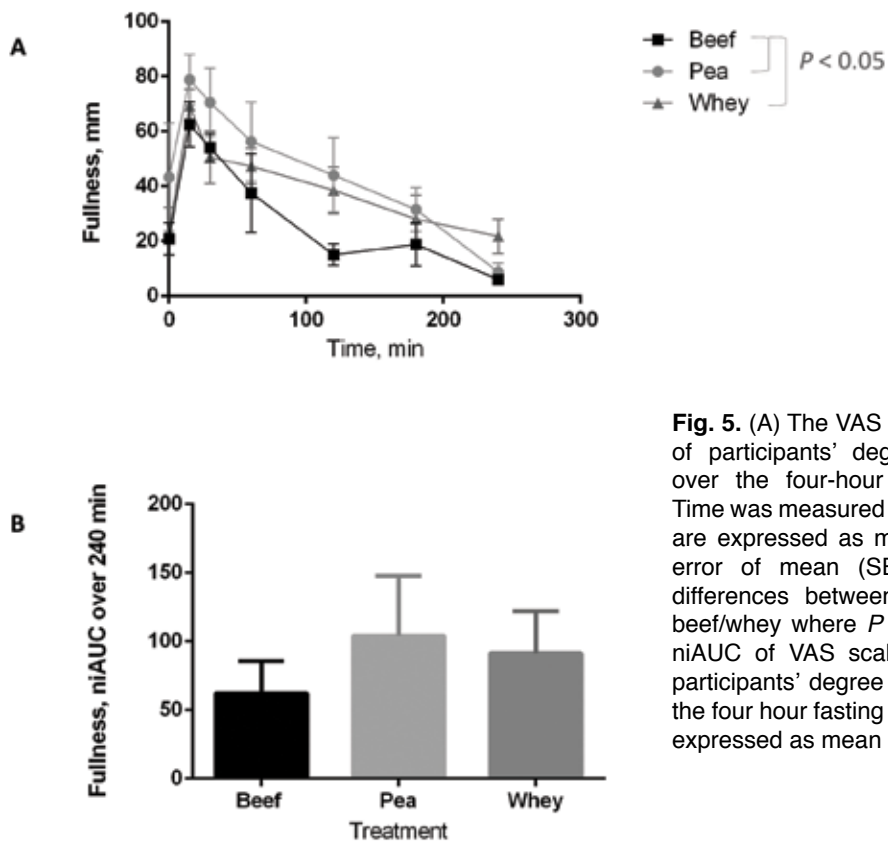


Fig. 5. (A) The VAS scales' measure of participants' degree of fullness over the four-hour fasting period. Time was measured in minutes. Data are expressed as mean \pm standard error of mean (SEM). Significant differences between beef/pea and beef/whey where $P < 0.05$. (B) The niAUC of VAS scales' measure of participants' degree of fullness over the four hour fasting period. Data are expressed as mean \pm SEM.

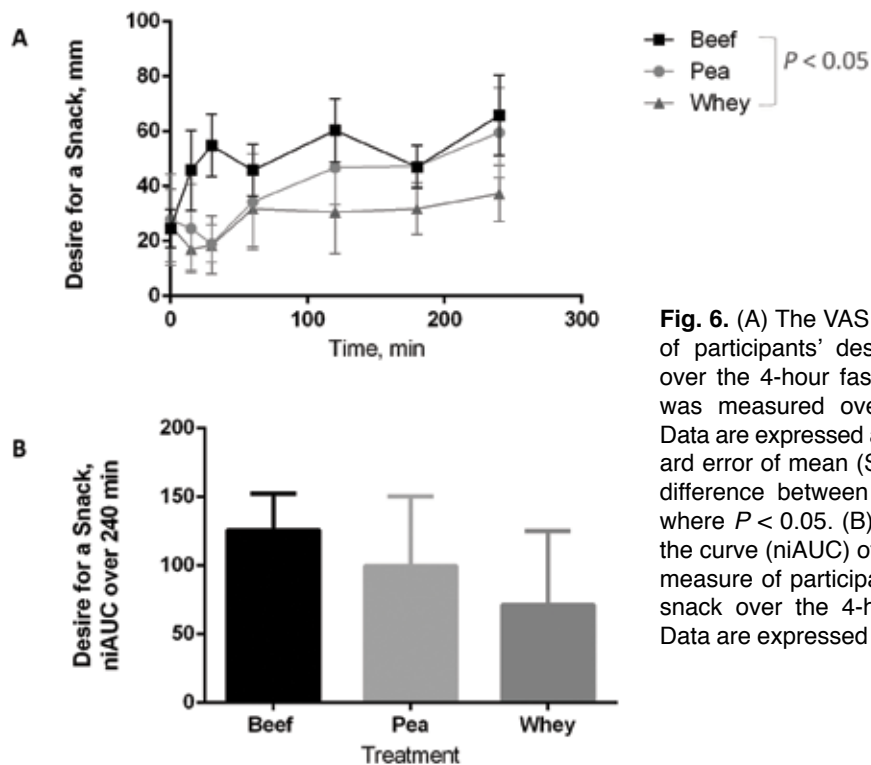


Fig. 6. (A) The VAS scales' measure of participants' desire for a snack over the 4-hour fasting period; time was measured over 240 minutes. Data are expressed as mean \pm standard error of mean (SEM). Significant difference between beef and whey where $P < 0.05$. (B) The area under the curve (niAUC) of the VAS scales' measure of participants' desire for a snack over the 4-hour test period. Data are expressed as mean \pm SEM.

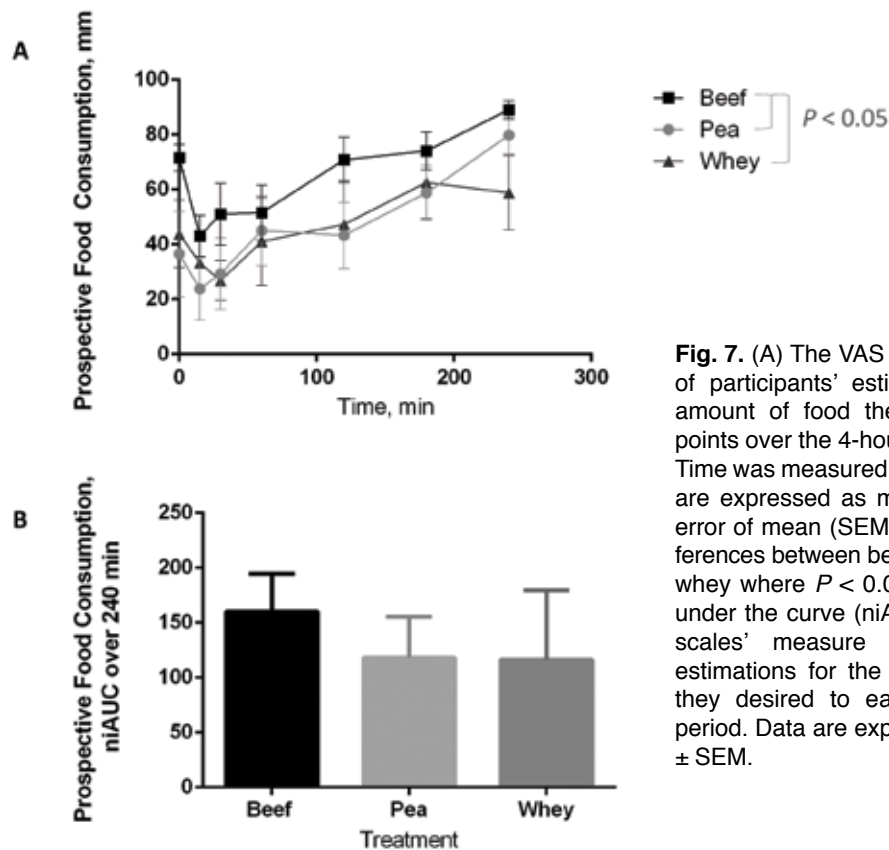


Fig. 7. (A) The VAS scales' measure of participants' estimations for the amount of food they could eat at points over the 4-hour fasting period. Time was measured in minutes. Data are expressed as mean \pm standard error of mean (SEM). Significant differences between beef/pea and beef/whey where $P < 0.05$. (B) The area under the curve (niAUC) of the VAS scales' measure of participants' estimations for the amount of food they desired to eat over the test period. Data are expressed as mean \pm SEM.

large. Fat intake following the beef protein contributed an average of nearly 36% of calories from fat while the intake of calories from fat after ingestion of the pea and whey proteins were similarly 34% and 40%, respectively. The postprandial intake of participants following each of the three protein treatments was statistically similar.

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 resting energy expenditure and thermic effect of food 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 relationships were detected that could have larger implications in a more expansive study.

The measures of resting energy expenditure and thermic effect of food were affected by protein source, though the treatments would need a repeat testing to look for greater significance as there were discrepancies present. Thermic effect of food seemed to be significantly affected by whey over pea and beef in some tests, and resting energy expenditure 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 resting energy expenditure and thermic effect of food 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. 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 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 led to the greatest feelings of fullness (Leidy et al., 2013).

Protein source could also be an important factor when considering connections between 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.

The 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 and 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 (Fig. 4a,b). 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 and 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 and 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. These data suggest 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.

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