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THE EFFECTS OF WHEY AND SOY PROTEIN SUPPLEMENTATION ON
APPETITE AND DIETARY QUALITY IN OVERWEIGHT AND OBESE
COLLEGE-AGED INDIVIDUALS

BY

KERRI L. ALEXANDER

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE
IN
NUTRITION AND FOOD SCIENCES

UNIVERSITY OF RHODE ISLAND

2012

MASTER OF SCIENCE THESIS

OF

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2012

ABSTRACT

Background: Over one-third of the United States population is obese. Obesity is a complicated disorder associated with many chronic diseases such as coronary artery disease, diabetes, hypertension and stroke. Many college students are overweight or obese, which may be due to lack of physical activity and unhealthy diets. Both dietary quality and satiety are important factors that may help modify obesity and associated health conditions. The impact of various interventions on these factors has not been clarified. Protein supplementation may be able to improve satiety, but research regarding improvement in diet quality in relation to this supplementation is limited.

Objective: To determine the impact of an 8-week protein supplementation intervention on dietary quality and appetite by comparing groups supplemented with whey or soy protein with each other and with an assessment only control group.

Methods: In a randomized, control trial with pre-post testing, subjects were randomized to one of three groups, whey protein, soy protein or non-treatment control. Experimental group subjects participated in 8 weeks of protein supplementation, and the control group received no treatment. Dietary quality and appetite were assessed at week 0 and week 8. The primary dietary quality outcome was total Alternate Healthy Eating Index (AHEI) score. The primary appetite outcomes were lab-assessed fasting hunger and satiety. Subjects assigned to the supplement groups were asked to ingest either the whey or the soy protein supplement, providing 21.5 grams of protein per day, on a daily basis for 8-weeks. All laboratory visits were conducted at the University of Rhode Island. Analysis of variance was used to compare within-group

and between-group differences in dietary quality and satiety for pre and post measurements.

Participants: Nine overweight and obese students (three per group) were recruited from the University of Rhode Island and surrounding areas through classroom announcements, fliers and mass emails.

Results: There were no significant differences between the groups at baseline, although, based on the visual analog scales, the control group tended to have higher fasting hunger levels (control: 54.1 ± 27.7 mm, experimental: 35.5 ± 26.1 mm) and lower fasting satiety levels (control: 35.5 ± 19.3 mm, experimental: 56.0 ± 26.5 mm). The control group also had lower energy intake (control: 2428.4 ± 1266.9 kcal, experimental: 2838.3 ± 1182.5 kcal) and lower protein intake (control: 77.3 ± 13.3 g, experimental: 99.3 ± 24.4 g) based on three day food diaries, as well as higher diet quality (control: 44.0 ± 6.6 , experimental: 35.7 ± 6.5) based on the total AHEI scores. No significant time by group or within group differences were found for AHEI scores or visual analog scales for hunger, satiety or appetite. During supplementation, the experimental groups consumed significantly more protein than the control group (experimental: 115.3 ± 25.5 grams, control: 73.7 ± 10.6 grams, $p=.033$). Measures of satiety and hunger were not significantly impacted by the intervention.

Conclusion: The addition of a protein supplement to the diet of overweight and obese young adults, with dietary counseling incorporated, did not improve diet quality or suppress fasting appetite. With a larger sample size, the effectiveness of this intervention may be measureable. Overall, this research gathered valuable information for use in interventions in the future.

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**“The effects of whey and soy protein supplementation on appetite and dietary
quality in overweight and obese college-aged individuals”**

by

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INTRODUCTION

Obesity has been on the rise in the United States since the late 1980s (Baskin, Ard, Franklin & Allison, 2005; Ogden & Carroll, 2010; Paddon-Jones et al., 2008). In 2008, over 74% of the adult population in the United States was overweight, obese or extremely obese (Ogden & Carroll, 2010). Obesity is a complicated disorder associated with many chronic diseases such as coronary artery disease, type II diabetes, hypertension, dyslipidemia, stroke, sleep apnea, and some cancers (Paddon-Jones et al., 2008). According to the National College Health Risk Behavior survey, 35% of college students are overweight or obese, which may be due to lack of physical activity and unhealthy diets (Huang, Harris, Lee, Born & Kaur, 2003). College students are within the 18-29 year old population, which is where the greatest increase in weight gain and obesity has been observed (Racette, Deusinger, Strube, Highstein & Deusinger, 2008). The college environment can be conducive to overconsumption of energy dense food (Strong, Parks, Anderson, Winett & Davy, 2008). Levitsky et al. measured weight changes from high school to the first few months of college and reported that between 58 and 71% of the total variance of weight gain was due to increased food consumption and unhealthy eating behaviors (Levitsky, Halbmaier & Mrdjenovic, 2004). Obesity is partially due to chronic positive energy balance, related to a diet lacking in balance and moderation (U.S. Department of Health and Human Services, 2010)

Diet Quality & Protein

Poor diet quality, as defined by the Healthy Eating Index (Guo, Warden, Paeratakul & Bray, 2004), is associated with obesity and overweight status (Guo, Warden, Paeratakul & Bray, 2004). Improving diet quality could influence weight status in some individuals. High dietary quality as measured by the Alternate Healthy Eating Index (AHEI), has been correlated with lower risks for major chronic diseases (McCullough et al., 2002). The AHEI targets food choices and macronutrients associated with reduced chronic disease (McCullough et al., 2002). The AHEI may be useful as a guideline for reducing the risk of chronic disease (Fung S et al., 2005). When compared to other instruments used to measure dietary quality, the AHEI appears to be more sensitive. In 2002, McCullough et al. found that, compared to the original Healthy Eating Index, the AHEI was more closely related to chronic disease risk. These researchers found that the AHEI was almost twice as predictive of chronic disease risk as the original HEI (McCullough et al., 2002). Little or no research has been conducted on the effects of protein supplementation on diet quality.

Satiety & Protein

Protein is known to be the most satiating macronutrient (Paddon-Jones et al., 2008), which makes it an effective tool for weight loss and management (Veldhorst et al., 2008). Higher protein diets have been shown to decrease appetite, as protein increases satiety and metabolic rate (Paddon-Jones et al., 2008). Most studies measuring the

effects of protein supplementation on appetite focus on healthy individuals, not the overweight and obese population. Many of these studies look at the postprandial effects of the supplementation following a laboratory meal and not during a free-living situation (Alfenas, Bressan & Paiva, 2010). This study will measure a free-living setting. In addition, different protein sources affect the body differently in regards to satiety (Veldhorst et al., 2008). In a study conducted by DeCassia et al., researchers found that soy protein increased thermogenesis, whey protein decreased respiratory quotient and casein decreased energy intake. That study only included normal weight subjects for the four 7-day experimental, crossover sessions (Alfenas, Bressan & Paiva, 2010). Some protein supplementation research has been completed in the college population, but the majority of the work looks at the effect on physical performance and examined few nutrition parameters. Soy protein supplementation has not been addressed in the college population in regards to improving dietary quality or satiety. Both whey and soy proteins have been shown to aid in weight loss, lower blood pressure, improve lipid profile, and reduce overall risk of cardiovascular disease in some populations (Fluegel et al., 2010; Zhang et al., 2003), but have not been systematically compared for their effects on satiety in overweight and obese young adults.

METHODS AND PROCEDURES

Study Design

This research was a randomized, experimental design conducted over an 8-week period examining the relationships among protein supplementation, dietary quality and appetite. Subjects were randomized to one of three groups, whey protein, soy protein or non-treatment control. Researchers assessed dietary quality and satiety before and after the intervention for all three groups. Subjects assigned to the supplement groups ingested either whey or soy protein supplements which provided 21.5 grams of protein per day, on a daily basis for the 8-week period. The researchers provided the supplements. All laboratory visits were conducted at the Human Performance Laboratory at the University of Rhode Island.

Participants

Nine college-aged men and women between the ages of 18 and 25 were recruited for the study. Subjects were recruited through flyers and classroom announcements at the University of Rhode Island- Kingston campus. This study was part of a larger study where eligibility criteria included having at least two risk factors for cardiovascular disease. Risk factors included waist circumference greater than 40 inches for men and 35 inches for women, body mass index greater than 30 kg/m^2 , sedentary lifestyle, or recent diagnosis of metabolic syndrome, hypertension or dyslipidemia by a physician.

Recent diagnosis of metabolic syndrome, hypertension and dyslipidemia was determined using a medical history questionnaire (See Appendix B). As described later, waist circumference was measured to the nearest 0.2cm using a non-stretchable tape measure at the level of the umbilicus upon exhalation, and body mass index was calculated using the following formula: body weight in kilograms / (height in meters)². Sedentary lifestyle was defined as participating in less than 30 minutes of moderate intensity physical activity less than three times per week. Exclusion criteria included pregnancy/lactation and vegetarians who consume mainly soy protein. All subjects were required to read and sign two informed consent forms (See Appendix I), approved by URI's Institutional Review Board, before participation in the study. Participants kept one copy of the informed consent form, while the other copy stayed in their participant folder.

Procedures

Subjects were asked to make three visits to the lab throughout the study, a preliminary visit, a pre-intervention test visit and a post intervention test visit. The preliminary visit involved consent, body composition measurements (height, weight, waist circumference and percent body fat), questionnaires (age, medical history, nutrition and physical activity; Appendix B-D) and a discussion of how to complete dietary records and visual analog scales (VAS) at home. Subjects were asked to complete a preliminary daily appetite profile (See Appendix E) and a 3 day food record (See Appendix E) before the second visit. They were also asked to keep a log of when they

took the supplement each day (See Appendix G) for the 8-weeks, which was provided to them by the researcher. Oral instructions were given to each subject in regards to completing the food logs and appetite profile. During the second visit, dietary records and VAS were collected and reviewed with the subject to ensure accuracy. At the beginning of each laboratory visit, subjects were asked to fill out a fasting appetite profile (See Appendix F). Subjects in the treatment groups received the powdered protein supplements during their second visit to the laboratory. Subjects and researchers were blinded about the two treatment groups. In order to maintain isocaloric diets, due to the addition of 190 kilocalories from protein supplements per day, the subjects were given individualized instruction from a trained nutrition research assistant regarding dietary adherence to reduce their usual dietary intake by around 200 kilocalories per day to avoid gaining weight during the study period.

Three-day diet records

Three day diet records were used to record subject food intake for the three days before the intervention began as well as the three final days of protein supplement intake. The form had space for subjects to record a description of the food/beverage consumed, the amount, the total calories and the time as well as any comments they had. Each participant was given instructions regarding estimating portion sizes and locating pertinent dietary information from food labels. The diet records were entered into Food Processor SQL (FP-SQL) for analysis. The three days were averaged for analysis. All data entry was double-checked for accuracy.

Dietary Quality

The AHEI was used to measure the dietary quality after data were entered into FP-SQL. The AHEI is based on 9 items, including vegetables, fruits, nut and soy, ratio of white to red meat, cereal fiber, trans fat, ratio of polyunsaturated fat to saturated fat, duration of multivitamin use and alcohol. Servings of vegetables, fruits, nuts & soy protein and alcohol were determined using the completed diet records. Ratio of white to red meat, cereal fiber, trans fat, ratio of polyunsaturated to saturated fat were determined after food logs were entered into FP-SQL. Duration of multivitamin use was determined by the nutrition and medical history questionnaires. The AHEI has a minimum score of 2.5 and a maximum score of 87.5 (Fung S et al., 2005). All items, except multivitamin use, were scored from 0-10, with 10 indicating the recommendation was met. Rationale for points system is described in detail in previous studies (McCullough et al. 2002). Points were awarded for long-term multivitamin use, 2.5 points indicating regular intake less than 5 years and 7.5 for long term use, greater than 5 years. The protein supplements were not included in the total AHEI scores, since the soy protein group would be favored by the addition of one serving of soy protein to each day they recorded during the supplementation period.

Visual Analog Scales (VAS)

Validated VAS were used to measure levels of satiety (Flint, Raben, Blundell & Astrup, 2000). Standard procedures were followed in both the laboratory and free-

living state (Flint, Raben, Blundell & Astrup, 2000; Poppitt et al., 2011). The lab VAS was administered once at the pre- and once at the post-intervention visits to assess fasting appetite perceptions under standardized conditions. Free-living VAS were completed in conjunction with the 3-day food logs at baseline and week 8. The following questions were used: “How hungry are you right now?/How satisfied are you right now?/ How much could you eat right now?” and were anchored with “Not at all/Nothing” on the left and “Extremely/Vast quantities” on the right. Participants were asked to mark their responses by placing a slash on the 100mm horizontal line. Visual analog scale measurements were completed before breakfast and before bed for the three days of food intake recording. VAS scores were calculated by measuring millimeters from the left anchor and entered into an Excel spreadsheet for analysis. The three days were averaged for analysis. All scores were double checked for accuracy.

Body Fat Percentage

Percent body fat was determined using air displacement plethysmography (BodPod model 2000A, Life Measurement Inc, Concord, CA). Standard procedures were used. Subjects were asked to refrain from physical activity and caffeine consumption for two hours before the test. Subjects were asked to wear a swimsuit or compression suit and a swim cap during the procedure to improve the accuracy of the results. Jewelry, socks and shoes were removed. Body composition measurements occurred during the initial and post-intervention visits to the lab for each subject.

Waist Circumference

Waist circumference was measured in centimeters using a Dritz model 11036 non-stretch, traditional tape measure. The measurement was taken with excess clothing removed. The tape was placed at the level of the umbilicus. Participants were asked to take a deep breath and relax their abdomen as they exhale. Waist circumference was measured to the nearest 0.2 centimeters. Waist circumference was measured fasting, at the pre and post visit for each subject. Measurements were taken in duplicate and averaged, provided both were within $\frac{1}{4}$ centimeter of each other. Measurements were repeated until two measurements were within given tolerance range.

Height

Height was measured in centimeters using a Seca model 216 stadiometer (Hanover, MD) with excess clothing and accessories removed. The measurement was taken from the floor to the top of the head with feet together and flat on the floor. The trained research assistant ensured the participant's head, shoulders, buttocks, and heels were against the stadiometer and participant's head was in contact with the stadiometer at the Frankfort plane during measurement. The participant was asked to inhale and hold his or her breath. Height was measured to the nearest centimeter. Height was measured during the pre and post visit for each subject. Measurements were taken in duplicate and averaged provided both were within $\frac{1}{2}$ centimeter of each other. Measurements were repeated until two measurements were within given tolerance range.

Weight

Weight was measured in kilograms with an electronic weighing system (Tanita Corporation, Japan). The scale was calibrated using two known 10-kilogram weights before each participant was weighed. Participants were weighed in the center of the scale with shoes and excess clothing removed. Weight was measured to the nearest tenth of a kilogram, fasting, during the pre and post laboratory visit for each subject. Measurements were taken in duplicate and averaged.

Supplements

Supplement	Calories (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)
Whey protein concentrate	190	21.5	1.3	22.7
Soy protein isolate	190	21.5	1.3	22.7

Data Collection

The same researcher performed all measurements to help ensure uniformity in procedures. Each research assistant was trained prior to measurements. Each research assistant was required to show test-retest reliability for trials before data collection began.

Week#	Schedule
1	<ul style="list-style-type: none"> • Visit 1: Assessed eligibility, collected baseline data for treatment and control groups, distributed 3 day food records and daily appetite profile • Completed 3 day food record and daily appetite profile • Visit 2: collected additional baseline data for treatment and control groups, randomized to groups, completed fasting VAS, distributed supplement, food records and appetite profile
2-9	<ul style="list-style-type: none"> • 8 week supplementation period for treatment groups
10	<ul style="list-style-type: none"> • Visit 3: Collected post-intervention data for treatment and control groups • Completed 3 day food record and appetite profile

Data for the treatment and control groups were collected at baseline and at the end of the intervention via questionnaires, anthropometric measurements, food records and appetite profiles. The data provided by the participants was coded to preserve confidentiality.

Statistical Analysis

All numerical data were entered into an Excel spreadsheet (Microsoft Corporation, Redmond, Washington) and transferred into SPSS version 20 (IBM Corporation, Somers, New York) for statistical analysis. All data were double checked for accuracy. Mean values and standard deviations were calculated. Normality was assessed based on skewness and kurtosis. Descriptive data included gender, age, BMI and body composition at 0 and 8 weeks for each group. Diet records were entered into FoodProcessor SQL (Esha Research, Salem, Oregon) to analyze nutrient composition. Data from the three diet records were averaged for the three days to obtain a single

measurement for diet quality. Dietary quality was analyzed by calculating the AHEI score from the averaged food record. The primary dietary quality outcome was total AHEI score although intake of energy, macronutrients, fiber, fruit and vegetables also were examined. The primary appetite outcomes were lab-assessed fasting hunger and satiety, although free-living daily averages, plus fasting morning and pre-bedtime ratings were also examined. The primary analysis was a three group by two occasion repeated measures analysis of variance in order to observe changes over time within each group as well as a time by group interaction. For appetite data, two groups by two occasion repeated measures analysis of variance was analyzed to observe changes over time with each group as well as a time by group interaction comparing the 2 protein groups to the non-treatment control, since differences between the two protein groups were not found for these data. Pearson correlations of total protein intake with AHEI scores as well as appetite ratings were explored. Effect sizes were reported as partial eta squared values. Statistical significance was set at $p < 0.05$.

RESULTS

Two hundred and four individuals responded to fliers, classroom announcements and mass emails. Of the 204, 78 individuals did not meet the inclusion criteria. Fifty nine individuals had no risk factors for cardiovascular disease. Fourteen individuals had only 1 risk factor for cardiovascular disease. Three individuals were vegetarians who consumed mainly soy protein. One individual was already consuming whey protein on a regular basis. One individual did not meet the age requirements. Of the 126 who qualified, 9 participants met all inclusion criteria and completed all 3 laboratory visits. Subject characteristics by group for all analyses can be seen in Table 1. Values are presented as mean $\pm$ standard deviation. Nine participants between 18-24 years old (SP = 18.7 ± 1.2 , WP = 20.7 ± 2.0 , C= 21.7 ± 2.1) were included for data analysis. Each of the three groups was composed of 3 participants, including 2 males and 1 female. On average, participants were 34.0 ± 7.6 percent body fat (SP = 34.8 ± 10.0 , WP= 33.4 ± 10.6 , C= 33.7 ± 4.0) with a BMI of 34.4 ± 4.1 (SP= 35.0 ± 3.6 , WP= 34.5 ± 3.2 , C= 33.6 ± 6.5). No statistically significant differences were found between the groups at baseline based on independent t-tests, although the control group tended to have higher fasting hunger levels (control: 54.1 ± 27.7 mm , experimental: 35.5 ± 26.1 mm), lower fasting satiety levels (control: 35.5 ± 19.3 mm, experimental: 56.0 ± 26.5 mm), lower energy intake (control: 2428.4 ± 1266.9 kcal, experimental: 2838.3 ± 1182.5 kcal), lower protein intake (control: 77.3 ± 13.3 g, experimental: 99.3 ± 24.4 g) and higher diet quality (control: 44.0 ± 6.6 , experimental: 35.7 ± 6.5).

Visual Analog Scales

Analysis of variance was used to compare within-group and between-group differences in hunger, satiety and appetite (Table 2). No significant time by group or within group differences were found. Average laboratory measured fasting hunger responses increased for both protein groups, but decreased for the control group (SP=21.2mm, WP=15.0mm, C= -16.2mm). Average laboratory measured fasting satiety responses decreased for both protein groups, but increased slightly for the control group (SP= -21.0mm, WP= -15.5mm, C=2.8mm). Desire to eat increased for both protein groups, but decreased for the control group (SP=3.13mm, WP=20.63, C= -6.7). Average free living fasting hunger responses increased for all 3 groups (SP=5.17mm, WP=12.4mm, C=3.3mm), but was most drastic for the whey protein group. Average free living before bed hunger responses decreased for both protein groups, but increased for the control group (SP= -17.37mm, WP= -6.90mm, C= 17.93mm).

Energy Intake

No significant time by group or within group differences were found for energy intake. Analysis of variance was used to compare within-group and between-group differences in energy intake (Table 3). Average energy intake decreased for all group (SP= -181.8kcal, WP= -822.6 kcal, C= -572.5 kcal), but most drastically for the whey protein group. Although the analysis of variance did not show statistical significance

($p = 0.30$), the effect size was large (partial eta squared = 0.334) for time by group tests of between subject effects for energy intake (Table 3).

Protein Intake

Following protein supplementation, the experimental groups were consuming significantly more protein than the control group (experimental: 115.3 ± 25.5 grams, control: 73.7 ± 10.6 grams, $p = .033$). There was no significant difference between the two protein groups in terms of protein intake at post measurements. Although the analysis of variance did not show significant differences ($p = 0.28$), the effect size was large (partial eta squared = 0.346) for time by group tests of within group effects for protein intake (Table 3). Pearson correlations were also run to measure the strength of the relationship between protein intake with total AHEI scores and lab-assessed fasting appetite scores (Table 5). There were small correlations between protein intake and lab assessed satiety and desire to eat (Pearson correlations = 0.21, -0.10, $p = 0.587$, 0.799). As predicted, the correlation between protein intake and lab assessed satiety was positive and the correlation between protein intake and lab assessed desire to eat was negative. There were no correlations between protein intake and AHEI scores or between protein intake and lab assessed fasting hunger.

Dietary Quality Scores

Analysis of variance was used to compare within-group and between-group differences in total AHEI scores, fat intake, carbohydrate intake, fiber intake and vegetable intake, as well as percentage of energy intake for protein, fat and carbohydrate (Table 3). No significant time by group or within group differences were found. The average AHEI scores for each group decreased over the intervention period (SP= -1.41, WP= -5.76, C= -1.30), but the average decrease in the whey protein group was much larger when compared to the soy protein or control groups. Fiber intake decreased for all 3 groups (SP= -10.16, WP= -9.02, C= -3.79), but least considerably for the control group. Vegetable intake decreased for the soy protein group by 0.84 servings on average, while intake for the other groups increased very slightly (WP= 0.39, C= 0.22).

DISCUSSION

The purpose of this study was to determine the impact of an 8-week protein supplementation intervention on dietary quality and appetite by comparing groups supplemented with whey or soy protein with each other and with an assessment only control group. Previous protein supplementation studies have made assumptions about dietary changes without actually measuring total dietary intake during interventions. To our knowledge, this is the first study to examine the effect of whey and soy protein supplementation on dietary quality in the overweight and obese college-aged population. Overall, this study offered exploratory data and generated some insight into how to best implement a protein supplementation intervention in the college-aged population in the future.

Dietary quality and appetite measures were not significantly affected by the addition of a protein supplement for an 8 week intervention. However, there was a large effect size of the intervention, based on partial eta squared for between group effects over time energy intake (.334). Since all three groups decreased energy intake, the partial eta squared value indicates that 33.4% of the changes in energy intake are due to the protein supplementation. Based on the partial eta squared values, over half (57.4%) of the change in AHEI scores were due to the protein supplementation. Since all three groups decreased AHEI, this is a surprising value. For protein intake, percent energy from protein and carbohydrate intake, just under half (47.0%, 42.0%, 45.1%, respectively) of the change in values was due to the protein supplementation, based on the partial eta squared. Future research will need to take this into account when

creating interventions to improve diet quality and appetite in overweight and obese college students.

The VAS ratings of hunger, satiety and desire to eat were not consistent across the groups, within the groups or when comparing free living to lab assessed. Flint et al. tested the reproducibility and validity of VAS and found them to be reliable, but this research was measuring single meals and not fasting measures of appetite in the lab and in a free living situation (Flint, Raben, Blundell & Astrup, 2000). Contrary to what Mars et al. found earlier in 2012, the VAS measures of appetite did not seem to predict subsequent food consumption (Mars, Statfleu, & de Graaf, 2012). The average energy intake for each of the groups in the current study decreased, while the appetite measure did not reflect this. Similar to the findings of Flint et al, the VAS were not able to predict changes in food intake, especially for the overweight and obese population (Flint, Raben, Blundell & Astrup, 2000). The current participants may be “hedonistic” eaters, in which case the VAS measures may not be as accurate since they are used to measure physiological cues rather than responses to pleasure (Blundell, Stubbs, Golding, Croden, Alam, 2005). If individuals do not eat in response to physiological cues, it may be difficult for them to record accurate VAS measures because they may not understand exactly what they should be feeling in terms of hunger or satiety never mind be able to record those feelings. One limitation when comparing the lab assessed to the free living appetite measures, is that during the free living condition, participants woke on their own schedule. If a participant does not typically wake up until noon, and we are measuring his or her fasting hunger levels at

seven in the morning, this may have an impact on his or her ability to report accurate appetite levels.

In terms of their dietary quality based on the AHEI scores, the current participants clearly did not follow the dietary instructions. Participants were asked to take the supplement in place of less healthful items, such as sugar sweetened beverages, fast food items, fried foods, et cetera, that provided around 190 kilocalories. Based on this information, the total energy intake should have been similar at pre and post intervention if they simply replaced the 190 kilocalorie item with the 190 kilocalorie protein supplement. The energy intake decreased for all three groups. The participants decreased their fruit and vegetable intake, which in turn decreased their fiber intake. Instead of decreasing less healthful items, the participants replaced the already limited amounts of fruits and vegetables they were consuming with the protein supplement. One limitation of the AHEI is the fact that it does not account for total kilocalorie intake. Individuals who consumed more calories had higher AHEI scores just because they were consuming larger amounts of food, so there were more opportunities for that individual to consume food items that would increase their total dietary score. The AHEI does not take points away to less healthful choices, such as fried foods and sugar sweetened beverages. If two individuals had all the same food items, but one consumed just water and the other consumed just sugar-sweetened beverages, they would have the same total score because sugar intake is not included in this diet quality index. This is clearly a limitation of the total AHEI scores.

In terms of dietary quality, the current participants confirmed the findings of Racette et al., who found that college students do not meet the guidelines for dietary

patterns (Racette, Deusinger, Strube, Highstein & Deusinger, 2008). For fruit and vegetable intake, the average participant was consuming 0.5 and 1.59 serving respectively. These numbers clearly do not meet the recommendations set forth by the Centers for Disease Control. On average, the female participants should be consuming around 2 cups of fruit and 2.5 cups of vegetables each day. On average, the male participants should be consuming around 2 cups of fruit and 3.5 cups of vegetables (CDC Nutrition for Everyone, 2012). Lack of fruits and vegetables put these individuals at greater risk for chronic diseases in the future (McCullough et al, 2002). The current participants clearly follow a more Western style dietary pattern with large consumptions of processed meats, sugar sweetened beverages, sweets, refined carbohydrate and potatoes. The current study confirms the findings of Sofi et al. that determined the Western style dietary pattern leads to lower quality diets (Sofi, Abbate, Gensini & Casini, 2010). Future research should determine how to best improve the dietary patterns of overweight and obese college students.

While the amount of protein did increase for all individuals in the experimental groups, it did not have a significant impact. There may not have been a great enough increase in protein intake. Weigle et al. found that adults decreased their energy intake (-441 ± 63 kcal/day) when protein was increased from 15% to 30% of total energy intake (Weigle et al, 2005). The current participants in the experimental groups were consuming $19 \pm 0.04\%$ of energy from protein at the end of the intervention period. Paddon-Jones et al. found that metabolic profile is improved once protein intake reached greater than 25% of total energy intake (Paddon-Jones et al, 2008). These

studies did not examine the effects of a protein supplement, but rather used food items to increase the subject's protein intake.

There are some limitations that are worth mentioning. This study's greatest limitation was the small group sizes. Recruitment of more subjects and a more diverse population are needed for future studies. Significance for some of the results may have been achieved with a larger sample size. Also, subjects were all overweight or obese individuals. Previous research suggests that overweight and obese individuals are most likely to underreport food consumption, especially in the free-living environment. Some of the reasons for underreporting include trying to please the researcher, forgetting, undereating on test days or not recording what they actually ate (de Castro, 2004). This may have occurred in our study resulting in lower recorded energy intakes. However, all subjects reported energy intakes above the cutoff criteria established by Goldberg et al. (1991) in previous studies suggesting that the reported energy intakes of these participants are plausible values. Another limitation is the use of food logs. Subjects were instructed to record the three days prior to their laboratory visit. This resulted in the majority of subjects recording 3 weekdays. Typically 3 day food logs include 2 weekdays and 1 weekend day to create a better overall picture of the participant's normal eating habits. Future studies may want to use more accurate methods for measuring energy intake. Multiple pass 24-hour recalls with the Nutrition Data System for Research, may be more accurate and reliable than 3 day diet records (Conway, Ingwersen & Moshfegh, 2004), but that has not been confirmed in the overweight or obese college aged population.

There are also some strengths of this study. All subjects were tested alone to minimize outside influences. This also allowed for personalized dietary information to be given to each participant. The three groups contained the same number of people as well as the same percentage of males and females. Also, a control group was used to compare both within and between group data. The same researchers conducted all of the participant visits to ensure uniformity in measurement techniques. Data were collected during both the fall and spring semesters.

If this study were to be repeated, there are a few changes that should be made. Clearly, more subjects would need to be recruited to increase the likelihood of finding statistical significance. Participant visits should be based around the participant's typical schedule, instead of bringing all subjects in first thing in the morning. More accurate measures of energy intake should be used. Overall, this research gathered valuable observations of this intervention in the overweight and obese college-aged population. A protein supplement may not be able to improve the diet quality or appetite levels of overweight or obese individuals, even with dietary counseling, but this is important information to bring to future research studies. Ideally with a larger sample size, detailed insight into the effectiveness of a protein supplementation intervention can be assessed.

TABLES

Table 1: Descriptive Characteristics of 9 Participants at Baseline			
Characteristics	Mean ± standard deviation (range)		
	Soy Protein (n=3)	Whey Protein (n=3)	Control (n=3)
Age (years)	18.7±1.2(18-20)	20.7±2.1(19-23)	21.7±2.1(20-24)
Height (m)	1.74±0.09(1.65-1.83)	1.75±0.13(1.60-1.85)	1.73±0.1 (1.63-1.82)
Weight (kg)	107.0±19.7(94.7-129.7)	105.6±14.7(90.7-120.1)	101.9±30.5 (77.8-136.2)
BMI (kg/m ²)	35.0±3.6(31.6-38.7)	34.5±3.1(31.0-37.1)	33.6±6.5 (29.3-41.1)
Waist Circumference (cm)	111.3±15.8(101.5-129.5)	106.7±11.1(99.0-119.4)	99.8±16.8 (85.0-118.0)
Body fat (%)	34.8±10.0(24.3-44.3)	33.4±10.6(22.0-42.9)	33.7±4.0 (29.1-36.3)
Lab assessed fasting Hunger (mm)	32.8±17.5 (15.0-50.0)	38.2±37.1 (0.0-74.0)	54.2±27.7 (23.0-76.0)
Lab assessed Satiety (mm)	54.0±19.7 (36.0-75.0)	58.0±36.9 (31.0-100.0)	35.5±19.3 (16.0-54.5)
Lab assessed Desire to eat(mm)	38.7±10.0 (31.0-50.0)	33.7±30.4 (0.0-59.0)	60.2±28.8 (39.5-93.0)
Free living fasting Hunger (mm)	40.0±24.0 (13.0-59.0)	33.3±30.3 (2.8-63.3)	31.6±28.6 (13.3-64.5)
Free living Hunger before bed (mm)	59.4±24.8 (44.8-99.0)	55.0±29.7 (20.8-73.5)	24.0±4.9 (18.3-27.0)
Energy Intake (kcal)	2206.5±1290.8 (1188.9-3658.5)	3470.1±795.0 (2857.8-4368.6)	2428.4±1266.8 (1667.6-3890.0)
Protein Intake (grams)	87.7±32.4 (57.2-1221.7)	110.9±5.7 (104.4-115.2)	77.33±13.3 (62.8-89.0)
Percent energy from protein	16.0±5.0 (10.0-19.0)	12.0±3.0 (9.0-15.0)	16.0±3.0 (13.0-19.0)
Fat Intake (grams)	94.0±59.9 (46.3-161.2)	112.5±27.5 (82.0-135.4)	100.9±41.9 (72.1-148.9)
Percent energy from fat	34.0±5.0 (28.0-38.0)	34.0±11.0 (23.0-44.0)	0.38 ±.01 (0.38-0.40)
Carbohydrate Intake (grams)	256.6±160.0 (140.0-439.8)	524.5±181.7 (334.4-696.4)	317.7±227.4 (181.5-580.2)
Percent energy from carbohydrate	52.0±11.0 (44.0-64.0)	57.0±12.0 (44.0-68.0)	46.0±2.0 (44.0-48.0)
Total AHEI score	31.6±2.5 (29-33.9)	39.7±7.2 (32.2-46.5)	44.0±6.6 (36.5-49.0)
Vegetables (servings)	2.39±0.19 (2.17-2.50)	1.61±0.82 (0.67-2.17)	0.78±0.84 (0.0-1.67)
Fruit (servings)	0.83±1.04 (0.0-2.0)	0.33±0.58 (0.0-1.0)	0.33±0.58 (0.0-1.0)
Fiber (grams)	22.7±20.9 (9.7-46.8)	23.2±18.7 (10.1-44.6)	18.8±4.6 (14.1-23.2)

Table 2: Analysis of variance for fasting appetite measures of 9 subjects from pre to post measurements

variable	group	pre	post	within subject effect		between subject effect	
		mean $\pm$ SD	mean $\pm$ SD	p	partial eta sq.	p	partial eta sq.
Lab-assessed hunger (mm)	SP	32.83 $\pm$ 17.51	54.00 $\pm$ 37.59	0.481	0.216	0.993	0.002
	WP	38.17 $\pm$ 37.06	53.17 $\pm$ 47.78				
	C	54.17 $\pm$ 27.71	38.00 $\pm$ 35.54				
Lab-assessed satiety (mm)	SP	54.00 $\pm$ 19.67	33.00 $\pm$ 26.02	0.525	0.193	0.826	0.062
	WP	58.00 $\pm$ 36.86	42.50 $\pm$ 41.14				
	C	35.50 $\pm$ 19.25	38.33 $\pm$ 23.03				
Lab-assessed desire to eat (mm)	SP	38.67 $\pm$ 10.02	41.83 $\pm$ 13.48	0.280	0.346	0.724	0.102
	WP	33.67 $\pm$ 30.37	54.33 $\pm$ 29.30				
	C	60.17 $\pm$ 28.75	53.33 $\pm$ 40.00				
Free-living fasting hunger (mm)	SP	40.00 $\pm$ 24.02	45.17 $\pm$ 17.96	0.895	0.036	0.887	0.039
	WP	33.27 $\pm$ 30.25	45.67 $\pm$ 35.64				
	C	31.60 $\pm$ 28.55	34.90 $\pm$ 18.53				
Free-living hunger before bed (mm)	SP	59.37 $\pm$ 24.80	42.00 $\pm$ 8.85	0.246	0.374	0.319	0.317
	WP	55.03 $\pm$ 29.68	48.13 $\pm$ 12.99				
	C	24.00 $\pm$ 4.94	41.93 $\pm$ 22.07				

Table 3: Analysis of variance for diet quality of 9 subjects at pre and post measurements							
Variable	group	pre mean ± SD	post mean ± SD	within subject effect p partial eta sq.		between subject effect p partial eta sq.	
AHEI total score	SP	31.64±2.46	30.23±1.85	0.253	0.368	0.077	0.574
	WP	39.67±7.17	33.91±3.68				
	C	44.00±6.62	42.70±8.37				
Energy Intake (kcal)	SP	2206.53±1290.82	2024.77±812.72	0.707	0.109	0.295	0.334
	WP	3470.10±795.02	2647.53±364.06				
	C	2428.37±1147.68	1855.90±181.40				
Protein Intake (grams)	SP	87.67±32.40	113.63±33.91	0.166	0.451	0.149	0.470
	WP	110.93±5.75	116.93±21.47				
	C	77.33±13.33	73.73±10.61				
%EI from protein	SP	16.0±5.0	22.0±4.0	0.355	0.292	0.196	0.420
	WP	12.0±3.0	16.0±2.0				
	C	16.0±3.0	18.0±2.0				
Fat Intake (grams)	SP	93.97±59.90	70.50±36.46	0.979	0.007	0.819	0.064
	WP	112.53±27.51	83.73±8.22				
	C	100.87±41.87	73.00±7.45				
%EI from fat	SP	34.0±5.0	30.0±6.0	0.180	0.435	0.856	0.051
	WP	34.0±1.0	34.0±1.0				
	C	39.0±1.0	31.0±2.0				
Carbohydrate Intake (grams)	SP	257.57±160.02	234.63±95.19	0.620	0.147	0.165	0.451
	WP	524.47±181.68	355.93±66.68				
	C	317.73±227.36	233.87±48.63				
%EI from Carbohydrate	SP	52.0±11.0	47.0±8.0	0.207	0.408	0.649	0.134
	WP	57.0±12.0	51.0±7.0				
	C	46.0±2.0	53.0±5.0				
Fiber Intake (grams)	SP	22.71±20.92	12.55±2.36	0.882	0.041	0.966	0.011
	WP	23.22±18.65	14.20±1.27				
	C	18.83±4.56	15.04±2.65				
Vegetable Intake (servings)	SP	2.39±0.19	1.55±0.39	0.050	0.631	0.148	0.471
	WP	1.61±0.82	2.00±1.00				
	C	0.78±0.84	1.00±0.33				

Table 4: Anthropometric measures for all groups at both time points (mean ± standard deviation)							
variable	group	pre	post	within subject effect		between subject effect	
		mean±SD	mean±SD				
		p	partial eta sq.	p	partial eta sq.		
weight (kg)	SP	107.03±19.66	107.00±21.23	0.508	0.202	0.959	0.014
	WP	105.63±14.71	103.83±13.84				
	C	101.90±30.51	101.50±29.15				
BMI (kg/m2)	SP	35.03±3.56	35.07±3.09	0.438	0.241	0.922	0.027
	WP	34.50±3.15	33.90±2.57				
	C	33.57±6.54	33.63±5.94				
WC (cm)	SP	111.33±15.75	114.50±14.65	0.088	0.555	0.587	0.163
	WP	106.67±11.10	102.20±13.51				
	C	99.83±16.75	100.83±15.46				
body fat (%)	SP	34.83±10.04	35.30±8.03	0.230	0.387	0.915	0.029
	WP	33.37±10.57	31.60±8.84				
	C	33.70±4.00	35.43±6.50				

Table 5: Correlations of protein intake (grams) with total AHEI and lab assessed appetite

variable	Pearson Correlation	Sig (2-tailed)
total AHEI score	0.035	0.928
lab assessed fasting hunger	-0.05	0.898
lab assessed fasting satiety	0.21	0.587
lab assessed desire to eat	-0.1	0.799

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APPENDICES

Appendix A: Review of Literature

Introduction

The United States has seen a substantial increase in obesity, cardiovascular disease, and diabetes partially related to an unhealthy diet. Both dietary quality and satiety are important factors that can help modify obesity and associated health conditions, but the impact of various interventions on dietary quality and satiety has not been clarified. Protein supplementation has been shown to improve satiety in overweight and obese individuals, but research regarding improvement in diet quality is lacking. Further, little work has explored the relative influence that different types of proteins may have on such outcomes.

Obesity

Obesity has been on the rise in the United States since the late 1980s (Baskin, Ard, Franklin & Allison, 2005; Ogden & Carrol, 2010; Paddon-Jones et al., 2008). In 2008, over 74% of the adult population in the United States was overweight, obese or extremely obese (Ogden & Carrol, 2010). According to the Centers for Disease Control, over 78 million adults were obese in 2009-2010 (Ogden & Carrol, 2010). More specifically, in Rhode Island, 26% of adults are obese and 37.5% are overweight. These statistics put the majority of Rhode Island at risk for numerous weight-related health issues (CDC Office of Surveillance, 2010). Rates of overweight and obesity are only expected to rise.

According to Wang et al, 75% of the United States population will be overweight or obese by 2015 (Wang & Beydoun, 2007).

Obesity is a complicated disorder associated with many chronic diseases such as coronary artery disease, type II diabetes, hypertension, dyslipidemia, stroke, sleep apnea, and some cancers (Paddon-Jones et al., 2008; Veldhorst et al., 2008). Obesity is also extremely expensive. According to the Centers for Disease Control, obesity costs \$147 billion in obesity-related medical care annually (Ogden & Carroll, 2010). Also, overweight and obese individuals may find themselves with lower health-related quality of life, compared to their normal weight counterparts (Kushner & Foster, 2000). Obesity is partially due to chronic positive energy balance (Veldhorst et al., 2008), related to a diet lacking in balance, variety and moderation (U.S. Department of Health and Human Services, 2010). Since obesity has such a negative impact on individuals and society as a whole, it is important for researchers to explore approaches to improve this current epidemic.

Health Risks of Obesity

Obesity is related to many health problems including as coronary artery disease, type II diabetes, hypertension, dyslipidemia, stroke, sleep apnea, depression and some cancers (Paddon-Jones et al., 2008; Veldhorst et al., 2008). Previous research has shown that overweight individuals live shorter lives (Adams et al., 2006). Lloyd-Jones et al. found that being overweight or obese by the age of 40 results in a 3.3 year decrease in lifespan for women and 3.1 year decrease for men (Lloyd-Jones et al., 2009). Adams et al. found similar results in

a study of over 500,00 individuals between the ages of 50 and 71 (Adams et al., 2006). These results clearly indicate that individuals who are overweight or obese are more likely to die early than individuals who are within a healthy weight range.

In 2004, Lloyd-Jones et al, determined that 13% of all cardiovascular disease related deaths could be related to obesity (Lloyd-Jones et al., 2009). Obesity also leads to greater risk of coronary artery disease, as well as elevated total cholesterol and hypertension (Pal & Ellis, 2010). While looking at the relationship between BMI before age 30 and coronary heart disease, Owen et al. found that a one kgm^{-2} rise in BMI was positively associated with an 8% increase in the risk for coronary heart disease (Owen et al., 2009).

Obesity also impacts quality of life due to increased incidence of body pain, fatigue and physical limitations (Kushner & Foster, 2000). Obesity is associated with higher rates of depression and lower self esteem (Pal & Ellis, 2010). Also, reproductive function is reduced with obesity (Kushner & Foster, 2000).

Obesity in College-Aged Individuals

According to the National College Health Risk Behavior survey, 35% of college students are overweight or obese, which may be due to lack of physical activity and unhealthy diets (Huang, Harris, Lee, Born & Kaur, 2003). College students are within the 18-29 year old population, which is where the greatest increase in weight gain and obesity has been observed (Racette, Deusinger, Strube, Highstein & Deusinger, 2008). The college environment can be

conducive to overconsumption of energy dense food (Strong, Parks, Anderson, Winett & Davy, 2008). Levitsky et al. measured weight changes from high school to the first few months of college and reported that between 58% and 71% of the total variance of weight gain was due to increased food consumption and unhealthy eating behaviors (Levitsky, Halbmaier & Mrdjenovic, 2004). Levitsky et al. identified the following as factors contributing to unhealthy weight gains: ‘all you can eat’ facilities, high fat food consumption, evening snacks, and increased consumption of energy dense foods with low nutrient density (Levitsky, Halbmaier & Mrdjenovic, 2004).

Similarly, data from the CARDIA study, a prospective, epidemiologic investigation of cardiovascular risk in 5,115 young adults between the ages of 18-30 years, found that all groups experienced significant increase in prevalence for all overweight categories ($BMI \geq 25\text{kg/m}^2$) and also decreases in the normal weight category from baseline to year 10 (Lewis et al., 2000). These subjects were studied from 1985-1996. These young adults gained an average of .06-1.19kg/year over the ten years. This study shows that weight gain is much more common than weight stability or weight loss and the largest weight gains occur more often among individuals who were overweight at baseline compared with those of normal weight (Lewis et al., 2000).

Another study found obesity rates increased from 14.7% to 17.8% in females during their freshman year in college. Seventy percent of the 382 study participants gained 1.6 kilograms on average (Lloyd-Richardson, Bailey, Fava & Wing, 2009). Zagorsky et al found that college females gain 4 kilograms on

average during a four year program (Zagorsky & Smith, 2011). Racette et al found that college students do not meet the guidelines for dietary patterns or physical activity while they are in school (Racette, Deusinger, Strube, Highstein & Deusinger, 2008). Increased levels of stress, increased alcohol consumption and changes to familial support have all been added to the list of changes in college that may lead to weight gain (Lloyd-Richardson, Bailey, Fava & Wing, 2009). A large portion of the college population are currently overweight or obese. It is important for researchers and health care professionals to take this into consideration when planning future weight related studies.

Appetite

Appetite is what drives an individual to find, choose and consume food (de Graaf, Blom, Smeets, Stafleu & Hendriks, 2004). There are numerous ways to measure appetite, including subjective ratings, food intake, or physiological markers (de Graaf, Blom, Smeets, Stafleu & Hendriks, 2004). Under normal circumstances, satiety occurs following food ingestion to inhibit further consumption. Between eating episodes, satiety typically prevents food intake and delays the start of the next meal or snack (Halford & Harrold, 2012). Satiety can be affected by many factors, including energy density, weight, volume, macronutrient composition, appearance, satisfaction and palatability of certain foods or meals (Solah et al., 2010). In terms of public health and controlling obesity, satiety is an important factor to consider (Solah et al., 2010).

Individuals consume food for a number of reasons. From a physiological standpoint, the body needs food and these needs must be satisfied for survival.

When energy availability is low, the body will respond by increasing motivation to eat or appetite (Halford & Harrold, 2012). One problem with homeostatic controls of appetite is that they may be overtaken by hedonic drivers of consumption.

Individuals who are considered “hedonistic” eaters may consume food in response to pleasure rather than the physiological cues the VAS are intended to measure (Blundell, Stubbs, Golding, Croden F & Alam, 2005). Some non-physiological factors that influence energy intake are social circumstance, dietary restraint, availability, properties of the food, and subjective judgment of the food (Bellisle, 2003).

Measuring Appetite

VAS are one method used to measure appetite. Previous research has shown that changes in appetite can be detected by VAS (Halford & Harrold, 2012). Mars et al found that changes in VAS can predict subsequent food consumption more accurately than any biomarkers of appetite (Mars, Statfleu & de Graaf, 2012). Halford et al. determined that much of the current literature regarding appetite measurements is not long term, does not use adequate measures of food intake and only has the participants consuming the product on a limited number of occasions (Halford & Harrold, 2012). Other research has shown that changing eating behavior is much more complicated than just improving subjective appetite scores (Poppitt et al., 2011). Visual analog scales do not always predict changes in food intake, especially for the overweight and

obese population (Flint, Raben, Blundell & Astrup, 2000; Poppitt et al., 2011). The debate is still out on whether or not VAS can predict energy intake.

Diet Quality

More recent diet and health related epidemiologic studies have switched their focus from single nutrients to overall indicators of diet quality and patterns (Guo, Warden, Paeratakul & Bray, 2004). At this point, there is no universally accepted measurement of dietary quality, nor is there a universally accepted definition. According to Fung et al, the purpose of diet quality indices are to measure and guide people toward a dietary intake that will promote health and prevent disease (Fung S et al., 2005). Dietary quality has been measured using various indices including the Healthy Eating Index (HEI), Diet Quality Index, Recommended Food Score, Dietary Variety Score and the Alternate Healthy Eating Index (AHEI) (Fung S et al., 2005). Researchers will need to continue to look for the ideal combination of dietary factors and also the best way to assess whether the population is adhering to this ideal combination.

Diet Quality & Chronic Disease

Poor diet quality, as defined by the Healthy Eating Index, is associated with obesity and overweight status. Guo et al. found that average HEI scores computed from 24 hour food recalls were significantly lower for obese individuals compared to their normal weight counterparts. On average, obese individuals scored 62.3, while normal weight individuals scored 63.6. This was found to be statistically significant ($p=.04$) This study was a cross sectional analysis of 10,930 adults who completed the Third National Health and Nutrition

Examination Survey (Guo, Warden, Paeratakul & Bray, 2004). Improving diet quality could influence weight status in some individuals (McCullough et al., 2002). High dietary quality as measured by the AHEI, has been correlated with lower risks for major chronic diseases (McCullough et al., 2002). Akbaraly et al. found that individuals in the top tertile for AHEI score had about a 25% lower chance of all-cause mortality and 40% lower chance of mortality from cardiovascular disease, compared with individuals who scored in the bottom tertile for AHEI score (Akbaraly et al., 2011). This study included semi-quantitative food frequency questionnaires for 7319 participants as well as an 18 year follow up.

McCullough and Willett collected dietary intake data from two large cohorts of men and women between 1984-1990 and found that participants whose dietary intakes were closest to meeting the AHEI goals had a 20% and 11% lower risk of major chronic disease. More specifically, these individuals in the top quintile for AHEI score, had significantly lower risk for cardiovascular (39% in men, 28% in women), compared to the lowest quintile (McCullough & Willett, 2006).

Fung et al. compared diet quality scores of 660 females using the Healthy Eating Index, Alternate Healthy Eating Index, Diet Quality Index revised, Recommended Food Score and the alternate Mediterranean Diet Index (aMed). They found that all of the diet quality scores were significantly correlated to each other. Also, once researchers adjusted for age, body mass index, alcohol intake, physical activity, smoking status, and energy intake, only the AHEI and aMed

scores were associated with significantly lower concentrations of biomarkers of inflammation and endothelial dysfunction. Both endothelial dysfunction and inflammation are related to diseases such as atherosclerosis and diabetes. The researchers believed the AHEI and aMED scores were associated with lower concentrations of inflammatory and endothelial dysfunction biomarkers because of their focus on high consumption of fruits and vegetables, whole grains, nuts, and fish and moderate alcohol (Fung S et al., 2005).

Alternate Healthy Eating Index

The Alternate Healthy Eating Index was created to improve the Healthy Eating Index, by targeting food choices and macronutrient sources that have been shown to decrease chronic disease risk (McCullough et al., 2002). The AHEI focuses on vegetables, fruits, nuts & soy, ratio of white to red meat, fiber, trans fat, ratio of polyunsaturated to saturated fat, multivitamin use and alcohol consumption.

Each of the components of the AHEI were included because of their ability to decrease risk for cardiovascular disease. Adequate vegetable intake has been linked to decreases in chronic disease risk (Steinmetz & Potter, 1991). All vegetables except for potatoes are included in the Alternate Healthy Eating Index. Five servings of vegetables per day were found to be ideal based on the current dietary guidelines (Appel et al., 1997; McCullough et al., 2002). Fruit intake has been linked to decreases in cardiovascular disease (Steinmetz & Potter, 1991). Four servings of fruit per day were found to be ideal based on the current dietary guidelines (Appel et al., 1997). Nuts and soy protein have both

been associated with lower risk of cardiovascular disease (Hu et al., 1998). McCullough et al. found one serving of nuts and soy protein per day to be ideal (McCullough et al., 2002). The white to red meat ratio was included based on the knowledge that fish and poultry have been linked to lower risks of coronary heart disease and cancer, while red meat and processed meats have been shown to increase these risks (Ascherio, Rimm, Stampfer, Giovannucci & Willett, 1995). Fiber has been associated with decreased risks of coronary heart disease and also stroke (Jacobs, Meyer, Kushi & Folsom, 1998). Trans fatty acids have been shown to raise LDL cholesterol, lower HDL cholesterol, and increase coronary heart disease risk (Willet, Stamper & Manson, 1993). A high polyunsaturated fat intake, compared to saturated fat intake has been shown to lower coronary heart disease (Willet, 1990). Long-term folate intake and supplementation have been associated with decreased coronary heart disease and cancer. Folate is typically found in multivitamins. (Rimm et al., 1998). Moderate alcohol consumption has also been associated with a lower risk for cardiovascular disease. McCullough et al. defined moderate intake as 1.5-2.5 drinks per day for men and 0.5-1.5 drinks per day for women (McCullough et al., 2002).

Diet Quality of College Students

Previous research has shown that college students as a whole, do not comply with recommendations for healthy diet practices (Wengreen & Moncur, 2009). Another study observed a sample of students at a large university to evaluate their nutrition knowledge, beliefs and practices. The study found that most of the students had a good understanding of basic nutrition, but the majority

(69%) of them consumed less than one serving of fruit per day and about half (43%) of the students consumed less than one serving of vegetables per day (Melby, Femra & Sciacca, 1986). More research is necessary to determine what causes these habits. One researcher identified skipping breakfast, snacking on chips or sweets, consuming sweetened beverages and consuming fast food when short on time, as behaviors related to weight management in college students that need to be addressed (Wengreen & Moncur, 2009). College students are introduced to a large variety of energy dense, less healthful foods, and new dietary patterns when they transition from high school to college. It is important for researchers and healthcare professionals to determine the causes of these unhealthful behavior changes and find ways to improve them.

Improving Diet Quality

Previous research has shown that dietary patterns following the Mediterranean-style diet result in higher dietary quality. These dietary patterns include more whole grains, vegetables and fruits. The Mediterranean diet also typically includes higher levels of fat intake from plant sources. The current Western-style dietary pattern, which is composed of more red meat, processed meat, sugar sweetened beverages, sweets, refined carbohydrate and potatoes leads to lower quality diets and obesity (Sofi, Abbate, Gensini & Casini, 2010).

A study conducted by Lee and Chang evaluated the impact of nutrition education promoting a high protein, low carbohydrate and high fiber diet for college students over an 8 week weight management program. Forty six of the 69 participants experienced a small, but clinically significant weight loss (1.3 kg) as

well statistically significant increases in diet quality scores (71.1 to 75.3) based on the Dietary Quality Index (Lee & Chang, 2007). Future work should focus on improving the diet quality of overweight and obese college aged individuals through nutrition education and restructuring current dietary patterns.

Protein Intake

Benefits of protein intake

Protein intake greater than the recommended 10-15% of total energy intake maybe a strategy for successful weight loss and also the prevention of weight gain following weight loss (Westerterp-Plantenga, 2008). Some of the additional benefits, beyond improved satiety, include increased thermogenesis and maintenance or accretion of fat-free mass (Paddon-Jones et al., 2008).

Improved thermogenesis may improve energy expenditure. When protein intake is greater than 25% of energy intake, some individuals retain lean muscle mass and improve metabolic profile possibly due to improved muscle protein anabolism (Paddon-Jones et al., 2008).

Protein Intake & Satiety

Individuals who find difficulty in controlling their appetite may find satiety-enhancing foods beneficial to help decrease the urge for consumption (Halford & Harrold, 2012). Protein is known to be the most satiating macronutrient (Paddon-Jones et al., 2008), related to increased diet induced thermogenesis (Westerterp-Plantenga, 2008). This makes protein intake an important factor in the context of weight loss and management (Veldhorst et al., 2008). Higher protein diets have been shown to decrease appetite, as protein

increases satiety and metabolic rate (Paddon-Jones et al., 2008). Previous research has shown that protein-induced satiety from high protein ad libitum meals can last from 1 to 6 days, up to 6 months (Veldhorst et al., 2008).

In a study conducted by Dougkas et al, a morning snack with an average of 12 grams of protein decreased appetite and subsequent lunch intake compared to the control group (Dougkas & Minihane, 2012). In a similar study, additional benefits were seen when the protein snacks contained 24 grams of protein (Douglas, Ortinau, Hoertel & Leidy, 2012). Researchers concluded that a small, high protein snack consumed most days may delay or prevent snacking or over-eating for the latter part of the day (Douglas, Ortinau, Hoertel & Leidy, 2012). Both Douglas and Dougkas, used yogurt snacks and recruited only healthy women between the ages of 18 and 50.

In a study conducted by Poppitt et al, researchers discovered that having overweight and obese women consume a protein-enriched water beverage containing 5 to 20 grams of protein prior to an ad libitum buffet lunch, would result in improved feelings of fullness and less hunger for the six hours following the preload compared to a water control condition. Forty-six women participated in the double-blind cross over study for each of the four beverage conditions, including a water control, 1%, 2% and 4% protein by weight beverage conditions. Researchers did not see a significant change in energy intake (Poppitt et al., 2011).

On the other hand, Wiegler et al, found that by increasing protein from 15% to 30% of total energy intake in 19 healthy adults, average energy intake

decreased (-441 ± 63 kcal/day) while satiety levels were maintained during a 12 week intervention, compared to baseline food logs and visual analog scales (Weigle et al., 2005). Most studies measuring the effects of protein supplementation on appetite focus on healthy individuals, not the overweight and obese population. Many of these studies look at the postprandial effects of the supplementation following a laboratory meal and not during a free-living situation (Alfenas, Bressan & Paiva, 2010). Future research should focus on the ability of protein to improve satiety in a free living environment, rather than just in the laboratory. Also, further work should examine effects in overweight and obesity population.

Satiety & Protein Source

In addition, different protein sources may affect the body differently in regards to satiety (Alfenas, Bressan & Paiva, 2010; Veldhorst et al., 2008). In a study conducted by Alfenas et al. (2008), researchers found that soy protein increased thermogenesis, whey protein decreased respiratory quotient and casein decreased energy intake. This study only included normal weight subjects for the four 7-day experimental, crossover sessions. One strength of this study was that the amount of protein added to the diet was based on the individual's weight in kilograms. The researchers added 2 grams of protein per kilogram of bodyweight to the test meal of milkshakes with crackers, cookies or cake (Alfenas, Bressan & Paiva, 2010). Both whey and soy proteins have been shown to aid in weight loss, lower blood pressure, improve lipid profile, and reduce overall risk of cardiovascular disease in some populations (Fluegel, Shultz, Power et al., 2010;

Zhang et al., 2003), but have not been systematically compared for their effects on satiety in overweight and obese young adults.

In contrast to the above studies, Lang et al. did not observe any differences in satiety when comparing protein source, which included egg albumin, casein, gelatin, soy protein, pea protein and wheat gluten. Participants included 12 healthy subjects who each consumed the 6 protein-manipulated lunches (Lang et al., 1998). This may be due to differences in methodology. Lang et al. added the protein directly into the lunch meal, while other studies incorporated protein as a preload to an ad libitum lunch meal. Also, the researchers noted that fiber was not controlled during some of the previous studies, which could mask the satiating capacity of the protein intake (Lang et al., 1998)

Protein Supplementation

Some protein supplementation research has been completed in the college population (Candow, Burke, Smith-Palmer & Burke, 2006), but the majority of the work looks at the effect on physical performance in healthy subjects and examined few nutrition parameters (Fluegel, Shultz, Powers et al., 2010).

Researchers have assumed that the diets of their subjects either stay the same or improve when they add a protein supplement, which may not be accurate. Neither soy nor whey protein supplementation has not been addressed in the college population in regards to improving dietary quality or satiety.

Obesity & Reporting Energy Intake

Underreporting of energy intake is a reoccurring challenge in nutrition, especially when self-report assessment methods are utilized. Underreporting occurs when people report estimated food intakes that are lower than their true energy intake. Poslusna et al. define underreporting as the discrepancy between reported energy intake and measured energy expenditure without any change in body mass during the observation or reference period (Poslusna, Ruprich, de Vries, Jakubikova & Veer, 2009). Researchers have suggested that reported energy intake can be used to assess an individual's reported energy intake compared to their energy requirement (Goldberg, Black & Jebb, 1991).

According to previous research, overweight and obese individuals are more likely to underreport their energy intake than their normal weight counterparts. Pietiläinen et al found that obese individuals significantly ($p=0.036$) underreported their energy intake by an average of 764 kilocalories (Pietiläinen et al., 2010). Another study, conducted by Buhl et al, found similar results. This study used doubly labeled water and reported that each of their ten overweight participants underreported energy intake. This was used to explain the participants inability to lose weight after being placed on energy restrictive diets and not reporting any weight loss. All participants had previously reported consuming less than 1200 kilocalories per day (Buhl, Gallagher, Hoy, Matthews & Heymsfield, 1995).

Another study found that obese individuals underreport their energy intake by 20-50% (Vansant & Hulens, 2006). The higher the BMI of the participant, the

greater the risk of underreporting of energy intake at any given meal (Mirmiran, Hajifaraji, Bahadoran, Sarvghadi & Azizi, 2012). Researchers have not been able to determine how to prevent this underreporting. Clearly, accurate methods of determining free living energy intake for overweight and obese individuals still needs to be discovered.

Conclusions

Overweight and obesity are preventable causes of morbidity and mortality that affect a vast majority of the United States adult population (Ogden & Carroll, 2010). The United States has seen a substantial increase in obesity, cardiovascular disease, and diabetes partially related to an unhealthy diet. Both dietary quality and satiety are important factors that can help modify obesity and associated health conditions, but the impact of various interventions on dietary quality and satiety has not been clarified. Protein supplementation has been shown to improve satiety in overweight and obese individuals (Mirmiran, Hajifaraji, Bahadoran, Sarvghadi & Azizi, 2012), but research regarding improvement in diet quality is lacking. Further, little work has explored the relative influence that different types of proteins may have on such outcomes. The literature supports the fact that more research involving larger samples and broader demographics needs to be conducted in order to show the absolute effects of protein supplementation on dietary quality and appetite in overweight and obese college aged subjects. More protein supplementation research needs to be conducted in free living conditions.

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Appendix B: Medical History Questionnaire

HUMAN PERFORMANCE LABORATORY MEDICAL HISTORY QUESTIONNAIRE

Study _____ Subject # _____
Name _____ Sex _____ Age _____ DOB _____
Street _____
City _____ e _____ Zip _____ Phone _____
Email _____

PLEASE ANSWER ALL OF THE FOLLOWING QUESTIONS AND PROVIDE DETAILS FOR ALL "YES" ANSWERS IN THE SPACES AT THE BOTTOM OF THE FORM.

Y	N																															
		1 Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?																														
		2 Has your doctor ever denied or restricted your participation in sports or exercise for any reason?																														
		3 Do you ever feel discomfort, pressure, or pain in your chest when you do physical activity?																														
		4 In the past month, have you had chest pain when you were not doing physical activity?																														
		5 Do you lose your balance because of dizziness or do you ever lose consciousness?																														
		6 Does your heart race or skip beats during exercise?																														
		7 Has a doctor ever ordered a test for you heart? (i.e. EKG, echocardiogram)																														
		8 Has anyone in your family died for no apparent reason or died from heart problems or sudden death before the age of 50?																														
		9 Have you ever had to spend the night in a hospital?																														
		10 Have you ever had surgery?																														
		11 Please check the box next to any of the following illnesses with which you have ever been diagnosed or for which you have been treated.																														
		<table border="1"> <tbody> <tr> <td>☐</td> <td>High blood pressure</td> <td>☐</td> <td>Elevated cholesterol</td> <td>☐</td> <td>Diabetes</td> </tr> <tr> <td>☐</td> <td>Asthma</td> <td>☐</td> <td>Epilepsy (seizures)</td> <td>☐</td> <td>Kidney problems</td> </tr> <tr> <td>☐</td> <td>Bladder Problems</td> <td>☐</td> <td>Anemia</td> <td>☐</td> <td>Heart problems</td> </tr> <tr> <td>☐</td> <td>Coronary artery disease</td> <td>☐</td> <td>Lung problems</td> <td>☐</td> <td>Chronic headaches</td> </tr> <tr> <td>☐</td> <td>Metabolic Syndrome</td> <td></td> <td></td> <td></td> <td></td> </tr> </tbody> </table>	☐	High blood pressure	☐	Elevated cholesterol	☐	Diabetes	☐	Asthma	☐	Epilepsy (seizures)	☐	Kidney problems	☐	Bladder Problems	☐	Anemia	☐	Heart problems	☐	Coronary artery disease	☐	Lung problems	☐	Chronic headaches	☐	Metabolic Syndrome				
☐	High blood pressure	☐	Elevated cholesterol	☐	Diabetes																											
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☐	Bladder Problems	☐	Anemia	☐	Heart problems																											
☐	Coronary artery disease	☐	Lung problems	☐	Chronic headaches																											
☐	Metabolic Syndrome																															
		12 Have you ever gotten sick because of exercising in the heat? (i.e. cramps, heat exhaustion, heat stroke)																														
		13 Have you had any other significant illnesses not listed above?																														
		14 Do you currently have any illness?																														
		15 Do you know of <u>any other reason</u> why you should not do physical activity?																														
		16 Please list all medications you are currently taking. Make sure to include over-the-counter medications, birth control pills, and medications to treat diabetes or high cholesterol.																														

DETAILS:

17 Please list all allergies you have.

		Substance	Reaction

Y	N	18 Have you smoked?	If yes, #/day	Age Started	If you've quit, what age?
		Cigarettes			
		Cigars			
		Pipes			

19 Do you have a family history of any of the following problems? If yes, note who in the space provided.

☐	High blood pressure		☐	Heart disease	
☐	High cholesterol		☐	Kidney disease	
☐	Diabetes		☐	Thyroid disease	

20 Please check the box next to any of the following body parts you have injured in the past and provide details.

Y	N	21 Have you ever had a stress fracture?	22 Have you ever had a disc injury in your back?	23 Has a doctor ever restricted your exercise because of an injury?	24 Do you currently have any injuries that are bothering you?
		☐ Head	☐ Hip	☐ Calf/shin	
		☐ Neck	☐ Thigh	☐ Shoulder	
		☐ Upper back	☐ Knee	☐ Upper arm	
		☐ Lower back	☐ Ankle	☐ Elbow	
		☐ Chest	☐ Foot	☐ Hand/fingers	

25 Do you consider your occupation as?

☐	Sedentary (no exercise)
☐	Inactive-occasional light activity (walking)
☐	Active-regular light activity and/or occasional vigorous activity (heavy lifting, running, etc.)
☐	Heavy Work-regular vigorous activity

26 List your regular physical activities

Activity	How often do you do it?	How long do you do it?	How long ago did you start?

ADDITIONAL

DETAILS:

Appendix C: Nutrition History Questionnaire

Nutritional History Questionnaire

1. Do you drink caffeinated coffee or colas? _____ Yes _____ No
If yes, how many per week? _____
2. Are you now or have you ever been on a diet? _____ Yes _____ No
If yes, please explain _____

3. Do you consider yourself overweight? _____ Yes _____ No
Do you consider yourself underweight? _____ Yes _____ No
4. Number of meals you usually eat per day: _____
5. Do you usually eat breakfast? _____ Yes _____ No
6. Number of times per week you usually eat the following:
_____ Beef _____ Fish _____ Pork _____ Chicken
_____ Desserts _____ Fried Foods _____ Fast Food
7. Do you regularly use any of the following? (please circle)
Butter Sugar Sweeteners Salt Whole Milk
8. How would you describe your nutrition habits? (please circle)
good fair poor
9. Please describe your knowledge of nutrition. (please circle)
very knowledgeable knowledgeable no knowledge
10. Do you regularly use any vitamin or mineral supplementation?
_____ Yes _____ No
If yes, please list: _____

11. Do you use any other form of supplementation (i.e. herbal remedies, food supplements, performance enhancing drugs, etc?)
_____ Yes _____ No
If yes, please list: _____

12. Have you ever taken protein, casein protein, soy protein, sports nutrition bars, protein shakes or smoothies?

_____ Yes _____ No

If yes, are you currently using these products now on a regular (weekly) basis?

_____ Yes _____ No

Please list the manufacture and name of any of these products you have ever had: _____

If you are currently using any of these products, how many times per week do you eat/drink each product?: _____

If you aren't currently taking any of these product, but have in the past, how often did you use them?: _____

When was the last time you used them and which type did you have?: _____

Appendix D: Physical Activity Questionnaire

Physical Activity Questionnaire

Cardiovascular/Aerobic Exercise

Do you currently engage in cardiovascular exercise on a regular basis?

_____ Yes _____ No

If yes, what mode(s) of exercise do you perform? _____

How many times per week do you perform this/these exercise(s)? _____

What is the duration and intensity of exercise (ex: 30 minutes at 3.2 mph, 3.0% grade on the treadmill)? _____

For how many years have you been performing this type of exercise? _____

Have you participated in any other physical activity (other than resistance exercise) on a regular basis in the last 5 years?

_____ Yes _____ No

If yes, what type? _____

Resistance Exercise (Weight Training)

Do you currently engage in resistance exercise on a regular basis?

_____ Yes _____ No

If yes, how many days per week? _____

List some examples of common exercises you perform. _____

Appendix E: Daily Appetite Profile Packet

VISIT NUMBER	
--------------	--

Subject Initials			Subject Number				Visit Date		

DAILY APPETITE PROFILE (DAP)

Please fill out this form for the 3 days *prior* to your next scheduled visit. For example, if you are scheduled for an appointment on Wednesday, please fill out this form on Sunday, Monday and Tuesday.

We ask that on each day, you evaluate your hunger and satiety on the provided scales during the following times: *immediately before breakfast, immediately after breakfast, midmorning, immediately before lunch, immediately after lunch, mid afternoon, immediately before dinner, immediately after dinner and before bed.*

In the attached pages, questions will be asked in the following format and you should give your answers by putting an "X" on the horizontal line.

NOTE: If you put an "X" at the left end of the line, i.e.

How hungry are you right now?

Not at all

Extremely

Then you are indicating you are not hungry.

If you put an "X" at the right end of the line, i.e.

How hungry are you right now?

Not at all

Extremely

Then you are indicating you are extremely hungry.

Remember: 1. The further you place the "X" to the right, the more hunger/satiety you are experiencing.

2. The further you place the "X" to the left, the less hunger/satiety you are experiencing.

3. Please do not place the "X" outside the end markers.

NOTE: Once you have completed one day, proceed to the next. Please do not look back to your previous answers.

DAY 1

Date:

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

Not at all Extremely

2. How satisfied are you right now?

Not at all Extremely

3. How much could you eat right now?

Nothing Vast Quantities

AFTER BREAKFAST

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

Not at all Extremely

2. How satisfied are you right now?

Not at all Extremely

3. How much could you eat right now?

Nothing Vast Quantities

4. How does the amount you ate compare to your normal breakfast?

MID-MORNING

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

2. How satisfied are you right now?

3. How much could you eat right now?

BEFORE LUNCH

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

2. How satisfied are you right now?

3. How much could you eat right now?

AFTER LUNCH

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

Not at all

Extremely

2. How satisfied are you right now?

Not at all

Extremely

3. How much could you eat right now?

Nothing

Vast Quantities

4. How does the amount you ate compare to your normal lunch?

Much less

Much more

MID-AFTERNOON

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

Not at all

Extremely

2. How satisfied are you right now?

Not at all

Extremely

3. How much could you eat right now?

Nothing

Vast Quantities

BEFORE DINNER

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

2. How satisfied are you right now?

3. How much could you eat right now?

AFTER DINNER

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

2. How satisfied are you right now?

3. How much could you eat right now?

4. How does the amount you ate compare to your normal dinner?

BEFORE BED

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

Not at all Extremely

2. How satisfied are you right now?

Not at all Extremely

3. How much could you eat right now?

Nothing Vast Quantities

4. How does the amount you ate today compare to your normal day?

Much less Much more

5. Overall, how hungry have you been today compared to your normal day?

Much less Much more

Thank you for completing today's Daily Appetite Profile!

DAY 2
BEFORE BREAKFAST

Date: _____
Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

5. How satisfied are you right now?

6. How much could you eat right now?

AFTER BREAKFAST

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

2. How satisfied are you right now?

3. How much could you eat right now?

4. How does the amount you ate compare to your normal breakfast?

MID-MORNING

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

Not at all Extremely

2. How satisfied are you right now?

Not at all Extremely

3. How much could you eat right now?

Nothing Vast Quantities

BEFORE LUNCH

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

Not at all Extremely

2. How satisfied are you right now?

Not at all Extremely

3. How much could you eat right now?

Nothing Vast Quantities

AFTER LUNCH

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

2. How satisfied are you right now?

3. How much could you eat right now?

4. How does the amount you ate compare to your normal lunch?

MID-AFTERNOON

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

2. How satisfied are you right now?

3. How much could you eat right now?

BEFORE DINNER

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

Not at all Extremely

2. How satisfied are you right now?

Not at all Extremely

3. How much could you eat right now?

Nothing Vast Quantities

AFTER DINNER

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

Not at all Extremely

2. How satisfied are you right now?

Not at all Extremely

3. How much could you eat right now?

Nothing Vast Quantities

4. How does the amount you ate compare to your normal dinner?

BEFORE BED

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

Not at all Extremely

2. How satisfied are you right now?

Not at all Extremely

3. How much could you eat right now?

Nothing Vast Quantities

4. How does the amount you ate today compare to your normal day?

Much less Much more

5. Overall, how hungry have you been today compared to your normal day?

Much less Much more

Thank you for completing today's Daily Appetite Profile!

BEFORE BREAKFAST

Clock time: _____ ☐ am ☐ pm

- Not at all Extremely

-
- Not at all Extremely

-
- Nothing Vast Quantities

Clock time: _____ ☐ am ☐ pm

- Not at all Extremely

- Not at all Extremely

-
- Nothing Vast Quantities

-

MID-MORNING

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

2. How satisfied are you right now?

3. How much could you eat right now?

BEFORE LUNCH

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

2. How satisfied are you right now?

3. How much could you eat right now?

AFTER LUNCH

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

2. How satisfied are you right now?

3. How much could you eat right now?

4. How does the amount you ate compare to your normal lunch?

MID-AFTERNOON

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

2. How satisfied are you right now?

3. How much could you eat right now?

BEFORE DINNER

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

2. How satisfied are you right now?

3. How much could you eat right now?

AFTER DINNER

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

2. How satisfied are you right now?

3. How much could you eat right now?

4. How does the amount you ate compare to your normal dinner?

BEFORE BED

Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

Not at all Extremely

2. How satisfied are you right now?

Not at all Extremely

3. How much could you eat right now?

Nothing Vast Quantities

4. How does the amount you ate today compare to your normal day?

Much less Much more

5. Overall, how hungry have you been today compared to your normal day?

Much less Much more

Thank you for completing your 3-day Daily Appetite Profile!

DIETARY FOOD RECORD INSTRUCTIONS

- Please record dietary intake for 3 days.
- Follow the guidelines for recording foods, beverages, and supplements provided with this packet.

HELPFUL HINTS:

- **ALL foods and beverages (INCLUDING WATER)** that are consumed should be recorded.
- Be very specific in your description of the type, the preparation method, and the amount of each food/beverage you consume.
- Use the label on foods to help you determine portion sizes.
- Save labels from packages and return them with your food record forms (this will greatly assist and enhance our analysis of your true nutrient intake).
- Use nutrient descriptors (e.g., low-fat, fat-free, light, reduced calorie, etc.) and brand names (e.g., Kraft, Nabisco, Planters, etc.) to describe foods.
- Record food/beverage consumption after each meal/snack instead of waiting until the end of the day.

MEATS/CHEESES

Description: Include description of the type, cut, and preparation method.

Portion Sizes:

List cooked (not raw) amounts of meats.

Determine amounts by weighing when possible.

Three ounces of cooked meat is equivalent to approximately a deck of cards or the palm of your hand.

**Listed below are examples of how to document foods*

3oz. Skinless, boneless, chicken breast-roasted

3oz. Ground beef round-fried

3oz. Deli turkey breast slices

3oz. Atlantic cod-baked

3oz. Sirloin steak-grilled

1/2 cup cubed beef stew meat

1 slice ham, 3" x 4" x 1/4"

1 oz colby cheese

1 piece cheddar cheese, 3" x 2" x 1"

STARCH/BREAD (CEREALS, BREADS, PASTAS, RICES, BEANS)

Description: Include a complete description of the starch/bread including preparation method and brand name if applicable.

Portion Sizes:

List cooked (not raw) amounts of starch/bread products.

Generally, a measuring cup will suffice for cereals, rices, pastas, and beans.

1/2 cup brown rice (Uncle Bens)

2 slices rye bread-toasted

2 cups spaghetti noodles-boiled

1 1/2 cups dry cereal (Cheerios)

1 cup oatmeal (Quaker Oats)-microwaved

8 animal crackers

Blueberry muffin, small

1/2 cup canned baked beans

1 corn tortilla, 6" across

FRUITS/VEGETABLES

Description: Include description of fruit/vegetable and whether it was fresh, frozen, or canned.

Include preparation method (e.g., steamed, fried, etc.)

Portion Sizes:

For whole pieces of fruit or vegetables, you may use small, medium, or large.

For many fruits/vegetables, cups may be used also.

- 1 medium Granny Smith apple
- $\frac{1}{2}$ of a large tomato-fresh
- 5 small strawberries-fresh
- $\frac{1}{2}$ cup canned pineapple-canned in water
- 1 cup frozen peas-steamed
- $\frac{3}{4}$ cup frozen mixed vegetables
- 3 spears steamed broccoli
- 2 medium raw carrots

COMBINATION DISHES

For standard mixed dishes, it is generally acceptable to list the type of dish without trying to list the ingredients separately. If the food is modified (e.g., low-fat), indicate this and try to describe how the food was modified. Provide enough detail to explain the composition of the dish.

For tossed salad, list the individual ingredients paying careful attention to salad dressings and other caloric-dense toppings (bacon bits, cheese, ham, chopped egg, etc.).

- 1 cup bean chili w/o meat
- 3 slices thin crust large cheese pizza with pepperoni-frozen
- $\frac{1}{2}$ cup potato salad
- 1 cup tuna casserole
- 1 cup macaroni and cheese (Kraft)
- 1 slice angel food cake
- 2 cups tossed salad
 - 2 cups lettuce greens
 - 3 slices cucumber
 - 3 slices tomato
 - 1 T shredded cheddar cheese
 - 1 T shredded carrots
 - 2 T fat-free Italian dressing

BEVERAGES/FLUIDS

Description: Include ALL BEVERAGES INCLUDING WATER complete description of the beverage

Portion Sizes: Use fluid ounces, liters, cups, or tablespoons.

- 6 oz regular coffee, brewed
- 12 oz Diet Pepsi
- 1 cup 2% milk
- 16 oz unsweetened iced tea
- 4 oz red table wine
- 6 oz orange juice (from concentrate)
- 2 T light olive oil

MISCELLANEOUS

Remember to list all condiments and additions to foods and beverages, such as cream, sugar, butter, jelly, lemon, salad dressing, artificial sweeteners, catsup, etc.

- 3 T low-fat french salad dressing
- 2 tsp black raspberry jam
- 1 packet Sweet'n Low
- 1 T cream (half and half)
- 2 tsp margarine spread (Country Crock)
- 3 T fat-free ranch salad dressing (Kraft)

Date: _____ Name: _____

Time	FOOD/BEVERAGE DESCRIPTION	AMOUNT	Total kcal <i>(from label)</i>

Comments:

Date: _____ Name: _____

Time	FOOD/BEVERAGE DESCRIPTION	AMOUNT	Total kcal <i>(from label)</i>

Comments:

Date: _____ Name: _____

Time	FOOD/BEVERAGE DESCRIPTION	AMOUNT	Total kcal <i>(from label)</i>

Comments:

Appendix F: Lab-Assessed Visual Analog Scales

VISIT NUMBER	
--------------	--

Subject Initials			Subject Number				Visit Date		

FASTING APPETITE PROFILE

NOTE: If you put an "X" at the left end of the line, then you are indicating you are not hungry. If you put an "X" at the right end of the line, then you are indicating you are extremely hungry.

How hungry are you right now?

Not at all		Extremely

Remember:

1. The further you place the "X" to the right, the more hunger/satiety you are experiencing.
2. The further you place the "X" to the left, the less hunger/satiety you are experiencing.
3. Please do not place the "X" outside the end markers.

BEFORE VISIT ONE

Date: _____
Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

2. How satisfied are you right now?

3. How much could you eat right now?

BEFORE VISIT TWO

Date: _____
Clock time: _____ ☐ am ☐ pm

1. How hungry are you right now?

Not at all Extremely

2. How satisfied are you right now?

Not at all Extremely

3. How much could you eat right now?

Nothing Vast Quantities

Appendix G: Supplement Log

Daily Supplement Log	
Week 1	
Date	Time that you took your supplement

Daily Supplement Log	
Week 2	
Date	Time that you took your supplement

Daily Supplement Log	
Week 3	
Date	Time that you took your supplement

Daily Supplement Log	
Week 4	
Date	Time that you took your supplement

Daily Supplement Log	
Week 5	
Date	Time that you took your supplement

Daily Supplement Log	
Week 6	
Date	Time that you took your supplement

Daily Supplement Log	
Week 7	
Date	Time that you took your supplement

Daily Supplement Log	
Week 8	
Date	Time that you took your supplement

The Human Performance Laboratory at the University of Rhode Island

is seeking

Men and Women

(between 18-25 years old)

to participate in a

Protein Supplement Study

Examining the effects of whey and soy protein on
markers of cardiovascular disease

You will receive **\$25.00** upon study completion!

Protein Supplement Study
Call Jon C. at 401-874-2980
whey.soy.study@gmail.com

Appendix I: Consent Form for Research

The University of Rhode Island
Department of Kinesiology
Independence Square III
25 West Independence Way, Ste. P
Kingston, RI 02881

The effects of whey and soy protein supplementation on endothelial function and markers of coronary artery disease

CONSENT FORM FOR RESEARCH

You have been invited to take part in a research project described below. The researcher will explain the project to you in detail. You should feel free to ask questions. If you have more questions later, Jon Conca-Petit at 401-874-2980 or Dr. Hatfield at 874-5183, the person mainly responsible for this study, will discuss them with you. You must be at least 18 years old to be in this research project.

Description of the project:

The purpose of this investigation will be to assess the effects of whey and soy protein supplementation on risk factors of heart disease in college-aged individuals (ages 18-25). You may only take part in this study if you are not on a vegetarian diet in which soy protein is your primary source of protein or regularly ingest soy products as a replacement for other protein sources. In addition, you should also have at least two factors for heart disease. The risk factors for heart disease are:

1. Waist circumference of over 40 inches in men and over 35 inches in women or a body mass index (BMI) of 30 or greater.
2. A sedentary lifestyle, defined as not having participated in 30 minutes or more of moderate intensity activity 3 days per week.
3. Hypertension, defined as a blood pressure of 140/90 mm Hg or more.
4. Dyslipidemia as diagnosed by a physician (an LDL cholesterol of above 130 mg/dl, and HDL cholesterol of below 40 mg/dl or total cholesterol of above 200 mg/dl.)
5. Recent diagnosis of metabolic syndrome or high cholesterol by a physician.
6. A family history in which your mother, father, brother, or sister died from a heart attack or other heart disease related event before the age of 45 in men and 55 in women.

If you choose to take part in this study and meet the criteria above, you will be randomly divided into one of three groups: a whey protein supplement group, a soy protein supplement group, or a control group. The study will last 8 weeks. Immediately before and immediately after the 8-weeks, you will have an ultrasound of your brachial artery (located in the upper arm). During the ultrasound, we will slow down blood flow to the arm briefly (for less than 2 minutes) with a blood pressure cuff and measure blood flow velocity, artery vessel size, and how quickly the artery returns to its original size after the

cuff is released. These measures are very accurate measures of heart disease risk, and we will use these to assess the effects of whey or soy protein supplementation on cardiovascular disease risk.

What will be done:

If you decide to take part in this study here is what will happen:

You will be asked to visit the Human Performance Laboratory at the beginning and end of the 8-week study. During the first session you will complete a medical history questionnaire, a nutritional and physical activity questionnaire, answer questions about how hungry you feel, and we will measure your percentage of body fat. Your body fat will be tested using a device called the BodPod. We will also measure some types of fats and sugar in drops of blood from a finger stick in your finger and ask you some questions about how hungry you feel. In addition, we will measure your metabolic rate (how many calories you burn) and take an ultrasound of your upper arm while a blood pressure cuff is inflated on your arm before and after the 8 weeks.

During the course of the study, we will ask you to record everything you eat for 3 days at the beginning, middle, and end of the eight weeks. If you are in one of the supplement groups, we will ask you ingest either the whey or soy protein supplement (it will be provided to you) daily, and record the time of day you took the supplement on a calendar signifying that you did take the supplement that day. A more complete explanation of what each testing day entails is below:

The preliminary visit will involve the following:

1. **BODY COMPOSITION MEASUREMENTS:** The following physical characteristics will be recorded: age, height, and body weight. Your percentage of body fat will be determined by something called air displacement plethysmography. For this measurement, you will be asked to sit inside the BodPod machine and comfortably rest for 2-5 minutes while your body fat percentage is estimated. For this test, we will ask you to bring a bathing suit to wear while you are in the BodPod. This simply minimizes the chances that bulky clothing will affect the reading. We will provide you with a swimming cap to place over your hair, this also minimizes the chances that air that is caught around your scalp will affect the reading. Your body fat is estimated in this fashion by calculating the amount of air you displace inside the known area of the BodPod. This test should take less than 30 minutes.
2. **DIETARY RECORDS:** You will be given some dietary logs to complete. During the 3 days prior to starting your supplement (if you are in a supplement group) or your starting in the control group you will be required to write down everything that you eat or drink. In addition, we will copy your first dietary log and return it to you for the last trial and ask you to repeat it as closely as you can. This ensures that you have a similar diet intake for both trials. If you are in one of the supplement groups, we will ask you to record the time of day you took the supplement on a calendar signifying that you did take the supplement that day. This should take 20 minutes per day on the days you have

to complete the dietary logs and less than a minute to signify you took your supplement if you are in a supplement group.

3. **QUESTIONNAIRES:** We will ask you to complete a medical health history questionnaire and a nutrition/activity profile to ensure that you don't have any known medical conditions that would prohibit you from taking part in this study. These medical conditions include kidney disorders, and other conditions that require medications that affect insulin and cholesterol levels such as diabetes medications and cholesterol lowering medications. These forms should take about 30 minutes to complete. We will also give you some satiety logs for 3 days at the beginning, middle, and end of the 8 weeks; these logs rate how hungry you feel. These should take you less than 5 minutes per day to complete, and we will ask that you fill them out at the start, the middle, and the end of the 8 week study.

After this initial visit will we schedule a second testing visit. This next visit must take place very early in the morning (prior to 7:00 A.M.). The second visit will involve the following.

1. **BASAL METABOLIC RATE:** You will be asked report to the lab very early in the morning. We will ask that you have a roommate or friend drive you to the lab. We would like you to be as relaxed and as close to a sleeping state as possible. We will ask that when you wake up, you immediately get into the car and come to the lab. We will greet you at the door with a wheelchair and wheel you into the lab. You will be asked to lie quietly with a large plastic hood over your head for about 30 minutes. This hood measures the amount of air you are breathing in and out. The hood is see-through and will not obstruct your breathing in any way, or be uncomfortable. This information can be used to assess the amount of calories you need.
2. **BRACHIAL ARTERY ULTRASOUND:** After your metabolic rate test, we ask you to lie quietly why we place an ultrasound probe on your upper arm and a blood pressure cuff on your forearm. We will inflate the blood pressure cuff for two minutes and measure your blood flow and artery size with the ultrasound. With the ultrasound probe still on, we will deflate the blood pressure cuff and measure how quickly your artery returns to the size it was before we inflated the cuff. The blood pressure cuff is the same one that is commonly used in doctor's offices. Before placing the ultrasound probe on your arm, we will put about two tablespoons of a gel substance in order to get a better picture. This gel is hypo-allergenic and will wipe off easily. It may feel slightly cold. You will only feel a small touch from the placement of the ultrasound on your arm. This test will only take about 10 minutes total.
3. **FINGER STICK:** We will clean your finger prior to taking a few drops of blood with a small lancet. We will apply a bandage to the area after test. These pin-pricks usually only yield a few drops before they close spontaneously, even without any treatment. The blood draw is done in order to assess your cholesterol and triglyceride levels. These measures are markers of cardiovascular disease. We may also measure the amount of

glucose in your blood so we can assess your risk of metabolic diseases such as diabetes and metabolic syndrome.

During the eight-week study period, you will maintain your normal activity and eating habits. If you are in one of the supplement groups, we will ask you to ingest a daily supplement that contains approximately 20 grams of protein (or about a ¼ of a cup) that we will provide. This protein will be in powder form, and you can mix it with juice or water.

You may not exercise or use any nicotine, alcohol or drugs 48 hours before to each research trial session. This includes over-the-counter anti-inflammatory (such as ibuprofen), herbal remedies, supplements, topical analgesics (such as Icy Hot), or prescription drugs that may affect the results of the study (such as narcotic-containing drugs.) You must also refrain from caffeine for 2 days prior to each trial. This includes all caffeine containing beverages and foods (chocolate, cocoa, tea, caffeinated soda, energy drinks). You must also refrain from all strenuous physical activity such as jogging, working out, or playing basketball 48 hours prior to testing. For instance, walking to and from class, light housework, and other normal daily activities are acceptable. You should refrain from recreational activities such as basketball, jogging, softball, etc. for this period of time.

Risks or discomfort:

There are minimal risks for the following procedures: height, weight, and BodPod measurements.

There are minimal risks to ingesting either soy or whey protein in most people. If you have a known dairy or soy allergy you may not participate in the study. Although whey protein is a milk product, it is lactose free and is tolerated well by lactose-intolerant individuals.

The inflation of the blood pressure cuff can be uncomfortable, but this is the same discomfort you would feel having your blood pressure taken in the doctor's office. The plastic hood used when assessing your metabolic rate may make you feel a bit claustrophobic. If you experience this feeling at any time, we will discontinue the test.

The discomforts associated with blood collection are minimal. Even though experienced personnel will perform the blood draw using sterile technique, it is possible that bruising and infection may occur.

If during the course of the analysis, any incidental findings emerge that indicate a health risk to you (such as high insulin or cholesterol levels), you will be informed and will be advised to consult with your personal physician.

Benefits of this study:

After your participation in the study, you will be given your individual body composition data, the results from your blood tests, and your brachial artery ultrasound results. This information is useful to you as it may indicate your risk for cardiovascular disease. It is important to note that your results are not diagnostic, and you should see your physician for an actual diagnosis. In addition, the information that is gathered in this study will be very useful for researchers and will add to the research literature about potential benefits of whey and/or soy. You will also be paid \$25.00 upon completion of the entire study.

Confidentiality:

Any information obtained from you during the study will remain confidential and you will not be identified by name in any publication or reports that result from this study. All records will be coded and stored by subject identification codes. Records of codes will be locked and stored in a file cabinet in Dr. Disa Hatfield's office in 25 West Independence Way at the University of Rhode Island. The researchers will be the only people to have access to these records. Records will be kept for 3 years and then destroyed. Any data entered into a computer program will contain only subject codes to ensure anonymity and no names will be published. After 3 years, the data files will be destroyed. Each subject will receive a random numerical code (ie odd numbers for women, even for men.) Only one document will exist that links these subject codes to their names, and will be kept in Dr. Hatfield's personal computer (which is password protected.) These file will be deleted after 3 years.

In case there is any injury to the subject:

It is not the policy of the University of Rhode Island to compensate subjects in the event that a research procedure results in physical or psychological injury. The University of Rhode Island will, however, make its best effort to refer you to appropriate services, upon request, if injury does occur. You may discuss this question with Disa Hatfield at (401) 874-5183. You may also call the office of the Vice President for Research, 70 Lower College Road, University of Rhode Island, Kingston, Rhode Island, telephone: (401) 874-4328. However, if you experience any problems related to this study you should contact your personal physician.

Decision to quit at any time:

The decision whether or not to take part in this study is entirely up to you. You do not have to participate in this study. If you decide to take part in this study, you may quit at any time. Whatever you decide will be accepted and there is no way you will be penalized. Your decision to participate and/or terminate your participation will not affect your grades in any classes. If you wish to quit, simply inform Jon Conca-Petit at 401-874-2980 or Disa Hatfield at (401)-874-5183 of your decision. We may terminate your participation in this study at any time if you show obvious signs of non-compliance with the study protocols (ie not regularly ingesting your supplement if you are in a supplement group.)

Rights and Complaints:

If you are not satisfied with the way this study is performed, you may discuss your complaints with Jon Conca-Petit at 401-874-2980 or Disa Hatfield (401)-874-5183, anonymously if you choose. In addition, if you have questions about your rights as a research participant, you may contact the office of the Vice President for Research, 70 Lower College Road, Suite 2, University of Rhode Island, Kingston, Rhode Island, telephone: (401) 874-4328.

I, the undersigned, have received, in my opinion, an adequate explanation of the nature, duration and purpose of this research investigation, the means by which the study will be conducted, and any possible inconvenience, discomforts, risks or adverse effects on my health which could result from my participation.

You have read the Consent Form. Your questions have been answered. Your signature on this form means that you understand the information and you agree to participate in this study.

Signature of Participant

Signature of Researcher

Typed/printed Name

Typed/printed name

Date

Date

Please sign both consent forms, keeping one for yourself

Appendix J: Supplemental Tables

Two group analysis of variance for diet quality of 9 subjects at pre and post

variable	group	pre	post	within subject effect		between subject effect	
		mean±SD	mean±SD	p	partial eta sq.	p	partial eta sq.
AHEI total score	P	35.65±6.51	32.07±3.30	0.409	0.099	0.490	0.447
	C	44.00±6.61	42.70±8.37				
Energy Intake (kcal)	P	2838.32±1182.49	2336.15±658.46	0.916	0.002	0.471	0.076
	C	2428.37±1266.85	1855.90±580.41				
Protein Intake (grams)	P	99.30±24.40	115.28±25.45	0.167	0.253	0.061	0.416
	C	77.33±13.33	73.73±10.62				
%EI from protein	P	14.0±4.0	19.0±4.0	0.204	0.219	0.974	0.000
	C	16.0±3.0	18.0±2.0				
Fat Intake (grams)	P	103.25±42.91	77.12±24.73	0.940	0.001	0.882	0.003
	C	100.87±41.87	73.00±7.45				
%EI from fat	P	34.0±7.0	32.0±8.0	0.103	0.335	0.674	0.027
	C	39.0±1.0	31.0±2.0				
Carbohydrate Intake (grams)	P	391.02±211.70	295.28±99.08	0.927	0.001	0.517	0.062
	C	317.73±227.36	233.87±48.63				
%EI from Carbohydrate	P	55.0±11.0	49.0±7.0	0.103	0.335	0.674	0.027
	C	46.0±2.0	53.0±5.0				
Fiber Intake (grams)	P	22.97±17.73	13.37±1.92	0.607	0.04	0.827	0.007
	C	18.83±4.56	15.04±2.65				
Vegetable Intake (servings)	P	2.00±0.68	1.78±0.72	0.421	0.095	0.440	0.461
	C	0.78±0.84	1.00±0.33				
Fruit Intake (servings)	P	0.58±0.80	0.38±0.64	0.700	0.023	0.732	0.018
	C	0.33±0.58	0.39±0.35				

Two group analysis of variance for appetite measures of 9 subjects at pre and post

variable	pre		post		within subject effect		between subject effect	
	group	mean±SD	mean±SD		p	partial eta sq.	p	partial eta sq.
Lab assessed fasting hunger	P	35.50±26.08	53.58±38.45		0.213	0.211	0.939	0.001
	C	54.17±27.71	38.00±35.54					
Lab assessed fasting satiety	P	56.00±26.52	37.75±31.22		0.249	0.184	0.579	0.046
	C	35.50±19.25	38.33±23.03					
Lab assessed fasting desire to eat	P	36.17±20.41	48.08±21.52		0.216	0.209	0.413	0.097
	C	60.17±28.75	53.33±40.00					
Free living fasting hunger	P	36.63±24.71	45.42±25.24		0.748	0.016	0.630	0.035
	C	31.60±28.55	34.90±18.53					
Free living hunger before bed	P	57.20±24.57	45.07±10.49		0.980	0.342	0.115	0.316
	C	24.00±4.94	41.93±22.07					

Two group analysis of variance for anthropometric measures for all groups at both time points

variable	group	pre	post	within subject effect		between subject effect	
		mean±SD	mean±SD	p	partial eta sq.	p	partial eta sq.
weight (kg)	A	106.33±15.54	105.42±16.12	0.712	0.021	0.785	0.011
	C	101.90±30.51	101.50±29.15				
BMI (kg/m2)	A	34.77±3.02	34.48±2.62	0.493	0.07	0.734	0.018
	C	33.57±6.54	33.63±5.94				
WC (cm)	A	109.00±12.45	108.35±14.29	0.642	0.033	0.428	0.092
	C	99.83±16.75	100.83±15.46				
body fat (%)	A	34.10±9.26	33.45±7.82	0.188	0.233	0.884	0.003
	C	33.70±3.99	35.43±3.52				

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