

The effects of whey protein, resistant starch and nutrition education on body weight

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ABSTRACT

Background: Obesity is widely recognized as one of the most critical health threats to families and children across the country. Obesity is a very serious health problem for people in Louisiana and especially in African Americans Children with 40.5% in the State classified as either overweight or obese as compared to 41.2% nationally. African American women have the highest rates of being overweight or obese (79.8%) compared to non-Hispanic whites (57.9%). In 2007, African Americans were 1.4 times as likely to be obese as whites. Therefore this study was designed to test the impact of dietary whey protein (WP) and resistant starch (RS) shakes/smoothies on reduction of body fat via increased satiety and increased energy expenditure in African Americans.

Methods: Thirty-three African American males and females aged between 21 and 43 were randomly assigned to two groups (15 controls, and 18 treatments). Twenty-eight (85%) of the participants (13 controls, 15 treatments) completed the study. For a period of 24-weeks, the treatment group consumed WP and RS shakes/smoothies for breakfast and received nutrition education. For the same period, the control group consumed the same shakes/smoothies but with starch powder and received nutrition education. The data was analyzed using SAS version 9.3.

Results: At the end of the 24 week study, the treatment group lost a mean body weight of approximately 6 kg with standard deviation of 8.38 kg ($p < 0.029$). In control group, weight did not differ significantly ($p < 0.209$) between week 0 and 24. In addition, the treatment group exhibited a significant decrease of about 6 cm in waist circumference ($p < 0.023$). There was no significant effect on mean blood pressure in treatment and control group.

Conclusion: The findings from this study suggest that a combination of WP and RS in the form of shakes/smoothies consumed for breakfast along with a nutrition education component may be an effective method in decreasing body weight, waist circumferences and cumulative food intake in African American males and females.

Key words: Whey Protein, Resistant Starch, Obesity, Breakfast shakes, Nutrition education

BACKGROUND

The incidence of overweight and obesity in the United States has reached an epidemic proportion. Obesity is widely recognized as one of the most critical health threats to families and children across the country. The direct medical costs and loss of workers' productivity due to obesity and obesity-associated chronic diseases, such as cardiovascular disease and diabetes in the United States and worldwide are staggering. According to the Center for Disease Control and Prevention (CDC), obesity prevalence has risen dramatically particularly among low income and minority groups [1]. The Prevalence of overweight and obesity for adults was 68.5% in 2011-2012 [2]. Obesity is a very serious health problem for people in Louisiana and especially in African-Americans children with 40.5% in the State classified as either overweight or obese as compared to 41.2% nationally [3]. African American women have the highest rates of being overweight or obese (79.8%) compared to non-Hispanic whites (57.9%) [4]. African American children and youth are also obese, as are Hispanics compared to whites. In 2007, African Americans were 1.4 times as likely to be obese as whites [4].

Louisiana currently ranks sixth in the nation for the rate of obesity. Over thirty three percent of Louisiana citizens are considered obese. Nearly 42 % of Louisianans who are obese are African American, and almost 33 % are Latino. One in two children in Louisiana is overweight or obese. Obesity related medical costs in the state total approximately \$1.4 billion annually [5]. Obesity reduction can be accomplished by diet and exercise and altering hunger or satiety signals. Unfortunately, the amount of weight lost is seldom as much as the dieter would like to lose, and even more seriously, the lost weight is often regained.

Citizens of Louisiana are becoming more concerned about nutritional value, quality, and affordability of their foods. A high level of consumer knowledge about the relationship of food, diet, nutrition, fitness and disease is vital to maintaining a healthy society.

Caregivers especially mothers have a significant influence on the diet of 2-5 year olds in developing, eating habits through modeling and child feeding practices [6]. It was anticipated that caregivers will incorporate knowledge gained through this research to combat adult and childhood obesity.

Appetite control with whey protein (WP): Decreased body fat as a result of dietary WP occurs at several levels of the nervous system between the brain and the gut [7]. There is evidence that a higher WP intake by humans and animals increases satiety and decreases energy intake, body fat, and food intake when compared to diets with lower protein content. This can control obesity more than egg albumin, soy protein or casein [8-10]. In human and animal studies, high protein diets consistently reduce body fat and food intake more than carbohydrate and fat which lead to a

reversal of the metabolic syndrome [11-18]. Luhovyy et al., 2007 [19] concluded that WP has potential as an added component in dietary plans and in functional foods aimed at control of appetite and body weight and in the management of metabolic consequences of excess body fat.

Diet composition can have a profound effect on gut gene expression and serum levels of peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) [20-25]. Whey protein prevents GLP-1 degradation by inactivating dipeptidyl peptidase-4 (DPP4) enzyme activity [26]. After ingestion, WP is hydrolyzed into bioactive peptides and amino acids that are sensed by the nervous system at various levels of the gastrointestinal tract and the central nervous system. These sensing systems act through satiety signals to reduce food intake.

Energy expenditure by resistant starch (RS): The term resistant starch refers to “a small fraction of starch that is resistant to hydrolysis by exhaustive amylase and pullulanase treatment in vitro and in vivo”. In humans RS is not hydrolyzed in the stomach and passes through small intestine. It reaches the large intestine where is fermented into short chain fatty acids such as butyrate [27, 28]. The scientific community believes it is the type of carbohydrate, rather than the amount, in the diet is what is important. There are four different types of resistant starches: RS-1 is found in whole grains and legumes and is entrapped in a non-digestible matrix. RS-2 is found in foods such as raw potatoes and high amylose cornstarch. The RS-3 category includes foods that have undergone “retrogradation”, which occurs when foods containing starches are cooked and then cooled (i.e., potatoes cooled after cooking and puddings). RS-4 includes chemically modified starches with the addition of ester and ether groups and cross-linking amylose strands (breads and cakes) [29, 30]. The levels of RS in human diets have been progressively decreased due to modern milling and food preparation methods [31-33]. Many Americans, especially those of lower socioeconomic status, consume 3-8 g/day of RS in their diets; a level that is lower than intake in medieval Europe (50-100 g/day) or developing countries (30-40 g/day) [33, 34]. RS is now available as an ingredient that can be incorporated into breads, cereal, dairy products and other baked goods that are acceptable to the United States (US) population. RS has general health benefits for example, several animal studies have shown that RS increases satiety or energy expenditure, decreases plasma cholesterol and triglycerides, increases insulin sensitivity, and produces anticancer effects in humans [24, 25, 35-40].

A popular way to consume fruits and vegetable in today's society is through shakes and smoothies, but health professionals often perceive that some shakes/ smoothies are relatively high in energy and sugar compared to the whole fruits [41]. Solah et al., 2010 [42] has shown that when WP based drinks are included in the breakfast meal (30g), resulted in reduced hunger or a higher satiety effect when compared to the low protein alginate based drink (2g) as long as the viscosity of the drinks was the same. Furthermore, the consumption of a high protein diet results in weight maintenance [43, 44]. Another group of researchers conducted a study with 20 young adults. They were divided in the control and the treatment group. They concluded that consumption of 48g RS over a 24-h period decreased appetite, which can be used for the management of the metabolic syndrome in humans [45].

Therefore, this study was designed to test the impact of dietary WP and RS shakes/smoothies in conjunction with nutrition education on reduction of body fat via increased satiety and increased energy expenditure in humans. The specific objective were: to determine

how to incorporate the best combination of WP and RS into shakes/smoothies and to evaluate the effects of the best combination of WP and RS in conjunction with nutrition education on body weight, body fat distribution, blood pressure, and cumulative food intake in obese African American men and women.

METHODS

Subjects: African American men and women aged 18 to 45 years with a Body Mass Index (BMI) 30-45 kg/m² were eligible for inclusion. Participants with heart disease, renal insufficiency, diabetes requiring insulin, intake of more than 14 alcoholic drinks per week, and those taking any type of drugs or medications that would affect blood pressure or nutrient metabolism were excluded. Women also were excluded if they were pregnant, breast-feeding or were less than six months postpartum. This information was collected by telephone screening, personal interviews and health information forms.

Subjects for the study were recruited through mailed brochures, printed articles, radio advertisements, and word of mouth through networking with different colleges on the Southern University campus, Head Start centers, schools, local health departments, health organizations, social, and religious groups. Telephone and personal screening interviews was used to identify potential participants. After agreeing to participate in the study, participants were given a consent form describing details of the study and were scheduled to visit Southern University Agricultural Research and Extension Center (SUAREC) for screening. The study protocol, procedures, and consent form were reviewed and approved by the SUAREC Institutional Review Board. All participants received MyPlates, pedometers, water bottles, healthy snacks and meals, and a stipend of \$300 as incentives for participating and completing the 24 week study.

The number of participants needed was estimated using power analyses with a power of 90% [46]. After the completion of the selection process, 33 participants aged between 21 and 43 were randomly assigned to two groups (15 controls, and 18 treatments). A total of 28 (85%) participants (13 controls, 15 treatments) completed the 24 week study. Two dropped from the control, and 3 from the treatment group due to job schedule changes, medical emergencies, and were lost to follow-up. Over 90% of participants were female (1 male in control and 3 males in treatment group); more than 50% single; and 80% never smoked. The treatment group consumed shakes/smoothies made with WP and RS once a day (in the morning) for 24 weeks; while the control group consumed the same products with starch powder. Breakfast was chosen because research studies have shown there is a strong correlation between breakfast consumption and body weight [47, 48]. Shake/smoothie mixes were prepared at SUAREC metabolic kitchen and participants took them home and made their own shakes/smoothies following the recipe provided by SUAREC staff. It was up to the participants to make the shake with water or other fat free milks.

Shakes/smoothies: Whey protein isolate was purchased from Kraft Foods (Glenview, IL). WP isolate was chosen because the protein is purified using a high amount of filtration and it has very low levels of carbohydrates and fat and is most exclusively pure protein (90-94%). In this study the Hi-Maze resistant starch (RS-2) was used. RS-2 was purchased from National Starch and Chemical Company (Bridgewater, NJ). All other ingredients such as milk, lactose free milk,

almond milk, chocolate powder, and different fruits were purchased from local super markets. The shakes/smoothies incorporating WP and RS were developed to the desired concentration and flavor in the metabolic kitchen at SUAREC. A shake survey was administered to random individuals to assess their preferred flavor. The participants were asked to choose their flavor of choice which consisted of chocolate, strawberry, strawberry/banana, vanilla, and cookies-n-cream. Also each participant was asked to rate each of the samples based on appearance, color, odor, overall taste, grittiness, texture, aftertaste and overall liking using the 5 point hedonic scale ranging from “dislike extremely” to “like extremely”. They were also asked to rate the acceptability and selling potential of each sample. The shake mix was 44 grams per serving.

In order to determine the calories for each shake mix, the ingredients and final shake mixes were chemically analyzed for fat, protein, moisture and ash using AOAC approved methods (983.23, 992.15, 920.15, and 985.14) with modifications [49]. Carbohydrate was determined by calculation.

Approval: Prior to commencement of this study, the requisite approval for human feeding research study, and consent forms were obtained from the Institutional Review Board (IRB) for Protection of Human Subjects.

Measurement: After the informed consents were obtained, baseline measurements were taken. Initial screening application, study specific questions and anthropometric measurements were collected. Table 1 shows the baseline information for control and treatment group.

Table 1. Subject Characteristics at baseline (means ±standard error)

	Control (n=13)	Treatment (n=15)
Age (years)	32±2	35±1
Height (cm)	166±1	165±2
Weight (kg)	126±6	125±7
BMI (kg/m ²)	43±2	43±2
Waist Circumference (cm)		
†	125±5	125±5

†The values for the waist circumference were calculated using n=11 for control and n=12 for treatment.

Height: Height was measured with stadiometer twice and averaged. Height measurement to the nearest 1 cm was taken then converted to inches by a certified staff member. Participant stood on a firm, level surface that was at a right angle to the vertical board of the stadiometer.

Weight: The participants were asked to stand in the center of the scale platform, since standing off-center may affect the weight measurement. The participant stood with arms relaxed at the sides, head erect, and eyes looking straight ahead. A Digital Medical Scale was provided for weight measurements. Weight was measured before the nutrition education class. When the digital readout was stabilized, the observed weight was recorded to the nearest 0.10 kg or 0.22

pounds. All weight measurements consisted of two independent weight assessments, and were performed weekly on each participant

Waist circumferences: The subject wore little clothing obscuring the abdominal region. The subject stood erect with abdomen relaxed, the arms at the sides, and feet together. The measurer faced the subject and placed an inelastic tape around the subject, in a horizontal plane, at the level of the natural waist, which is the narrowest part of the torso, as seen from the anterior aspect. The measurement was taken at the end of a normal expiration, without the tape compressing the skin. It was recorded to the nearest 1cm.

Blood pressure: Blood pressure was measured twice using an Omron machine every week for 24 weeks. Participants were resting for 10 minutes prior to the first blood pressure measurement, and an additional 5 minutes prior to the second blood pressure measurement.

Body fat distribution: Body fat distribution was measured using a Dual-energy X-ray Absorptiometry (DXA) at the beginning and end of the 24 week study.

Nutrition education: Nutrition education intervention/classes were provided weekly for control and treatment group on how to follow a low fat diet and be physically active for 12 weeks, and once a month for the next 12 weeks. The classes were held at SUAREC metabolic kitchen dining area or one of the meeting rooms and lasted for 1hour and 30 minutes. The time for the meetings was established after the initial meeting incorporating participants input. The Expanded Food and Nutrition Education Program (EFNEP) and 2010 New Dietary Guidelines for Americans [50] were utilized to design nutrition classes for this study. EFNEP was chosen because since 1969 this program has successfully addressed nutrition and physical activity behaviors and needs of low-income families, particularly those with young children. The curriculum included various teaching methods such as food demonstrations, educational displays, group sessions, games and activities [51]. For each nutrition education class, both pretest and posttest was administered and collected.

Diet and physical activity self-monitoring: Participants monitored their food intake and physical activity by keeping a self-reported food and exercise diaries. Instructions for keeping food and exercise diaries were provided in the lesson plans. Each assigned 7-day block consisted of 5 weekdays and 2 weekend days. Food and exercise self-reported diaries were collected and distributed every week and used to provide feedback and guidance based on current recommendations to maintain a healthy weight by making healthier choices. The project director and staff evaluated and discussed the diaries at each visit to assist the participants in controlling serving sizes, their consumption of fatty foods, physical activities, and any other concerns. Each participant were advised to use household measuring items such as measuring cups and spoons etc. (was provided) to estimate amount foods consumed in each food category.

Statistical analysis: Percent ash, moisture, protein, fat and carbohydrate (proximate analysis) and caloric values of WP and RS (treatment) and control shakes were analyzed using PROC

GLM of SAS (version 9.3) [52]. Control and treatment values were compared by a single degree of freedom F test. Body weight, measured across 24 weeks, was analyzed using the PROC MIXED of SAS (version 9.3) method for comparing repeated measures using regression [46]. Orthogonal, polynomial contrasts for linear, quadratic, and higher-order regression effects indicated no significant ($p \leq 0.05$) quadratic or higher-order effects and were eliminated from the model. Also, scatter plots of body weight by week for each participant did not suggest the presence of quadratic effects. The mixed model used body weight as the dependent variable and week and treatment as fixed independent variables, with participant as a random effect. The data was fitted to the “first-order autoregressive moving average” variance-covariance structure [52]. Control intercepts and slopes were compared with treatment intercepts and slopes by single degree of freedom F tests. Body weight and waist circumference measured at 0 weeks were compared with that at 24 weeks by paired t-tests for both control and treatment groups, using PROC TTEST of SAS (version 9.3) [52].

RESULTS

Shake/ smoothie: Results from sensory survey indicated that chocolate was the most favorable choice with more than a 50% preference followed by strawberry at 26% and vanilla at 10%. Cookies-n-cream was the least favorite. There was no substantial distinction among the control (wheat starch shake mix), the treatment (the WP and RS shake mixes with sugar or with sugar substitute) and a commercial shake mix. The feedback provided by the participants indicated that most found the consistency of the WP and RS shake/ smoothie samples to be favorable and they would consume the product on a daily basis knowing that it would be beneficial to their health.

The analysis of shake mixes showed that control and treatment within each shake mix flavor differed significantly ($p < 0.01$) for each of the attributes (Table 2). For example, the amounts

Table 2. Proximate composition and caloric content in chocolate, strawberry and vanilla flavor whey protein and resistant starch (treatment) and control shakes (mean ± standard deviation)

Attributes	Chocolate		Strawberry		Vanilla	
	Control	Treatment	Control	Treatment	Control	Treatment
Ash (%)	2.7±0.3 ^{b*}	3.5±0.2 ^a	0.7±0.1 ^b	2.2±0.1 ^a	0.5±0.1 ^b	2.00.0 ^a
Moisture (%)	12.0±0.5 ^a	10.6±0.2 ^b	12.7±0.5 ^a	8.1±0.4 ^b	12.9±0.5 ^a	8.5±0.8 ^b
Protein (%)	1.2±0.2 ^b	56.2±0.3 ^a	0.0±0.0 ^b	55.9±0.7 ^a	0.0±0.0 ^b	55.3±0.6 ^a
Fat (%)	0.9±0.0 ^b	1.6±0.1 ^a	0.0±0.0 ^b	0.8±0.0 ^a	0.1±0.0 ^b	0.9±0.0 ^a
Carbohydrate (%)	83.1±0.5 ^a	28.1±0.5 ^b	86.5±0.6 ^a	33.2±0.2 ^b	86.4±0.4 ^a	33.2±0.8 ^b
Calories (Kcal)†	152.1±1.2 ^b	154.7±0.4 ^a	152.4±1.0 ^b	159.9±0.6 ^a	152.6±0.7 ^b	159.6±1.5 ^a

* Control and treatment values, within rows and shake flavors, with different super script letters differ at $p \leq 0.01$ by a single degree of freedom F test.

† Calories are calculated per serving size (44 grams).

of moisture for chocolate, strawberry and vanilla were significantly lower ($p < 0.01$) in the WP and RS shake mixes with 10.6%, 8.1% and 8.5% vs. 12.0%, 12.7%, and 12.9% for the control group, respectively. The amount of fat in WP and RS chocolate flavor (1.6%) was significantly higher ($p < 0.01$) than all samples and the lowest in control strawberry flavor (0.0%) samples. The control shake mixes had much higher amount of total carbohydrate compare to WP and RS samples. The calories per serving (44 grams) was 152.1 and 159.9 kcal for treatment and control shake mixes respectively when water was used for preparation of shakes. If fat free milk, fruits and vegetables were used in preparation of shakes/smoothies extra calories were added. This information was obtained from daily dairies.

Body weight: Table 3 illustrates that by the end of the 24 week study the treatment group lost a mean body weight of approximately 6 kg with standard deviation of 8.38 kg ($p < 0.029$).

Table 3. Comparison of body weight and waist circumference from baseline to end of study

Group	n	Week 0	Week 24	Mean difference	p > t
Body weight (kg)					
Control	13	125.72 ± 5.73	124.12 ± 6.66	1.60	0.209
Treatment	15	124.91 ± 7.03	119.16 ± 6.78	5.75	0.029
Waist circumference (cm)					
Control	11 ^a	124.69 ± 5.02	121.46 ± 5.36	3.23	0.089
Treatment	12 ^b	125.09 ± 4.82	119.38 ± 4.54	5.71	0.023

^a2 participants data missing.

^b3 participants data missing.

Body weight and waist circumference values expressed as means ± SEM.

In the control group the weight did not differ significantly ($p < 0.209$) between 0 and the end of 24 week study. Body weight decreased linearly across the 24 weeks for the treatment group with a slope of -0.236 kg per week. The slope for the control group was -0.076 kg per week. The p- value for the difference between the control and treatment slopes was 0.053 (Figure 1).

The standard deviations corresponding to the linear regression line points in Figure 1 ranged from 19.2 to 28.2 kg for control group and from 19.7 to 27.7 kg for treatment group. These standard deviations are large relative to the differences between the treatment and control group means within each week. Nevertheless, the analysis that fit the data to the ‘first-order autoregressive moving average’ variance-covariance structure resulted in a significant ($p \leq 0.0001$) slope for the treatment linear regression.

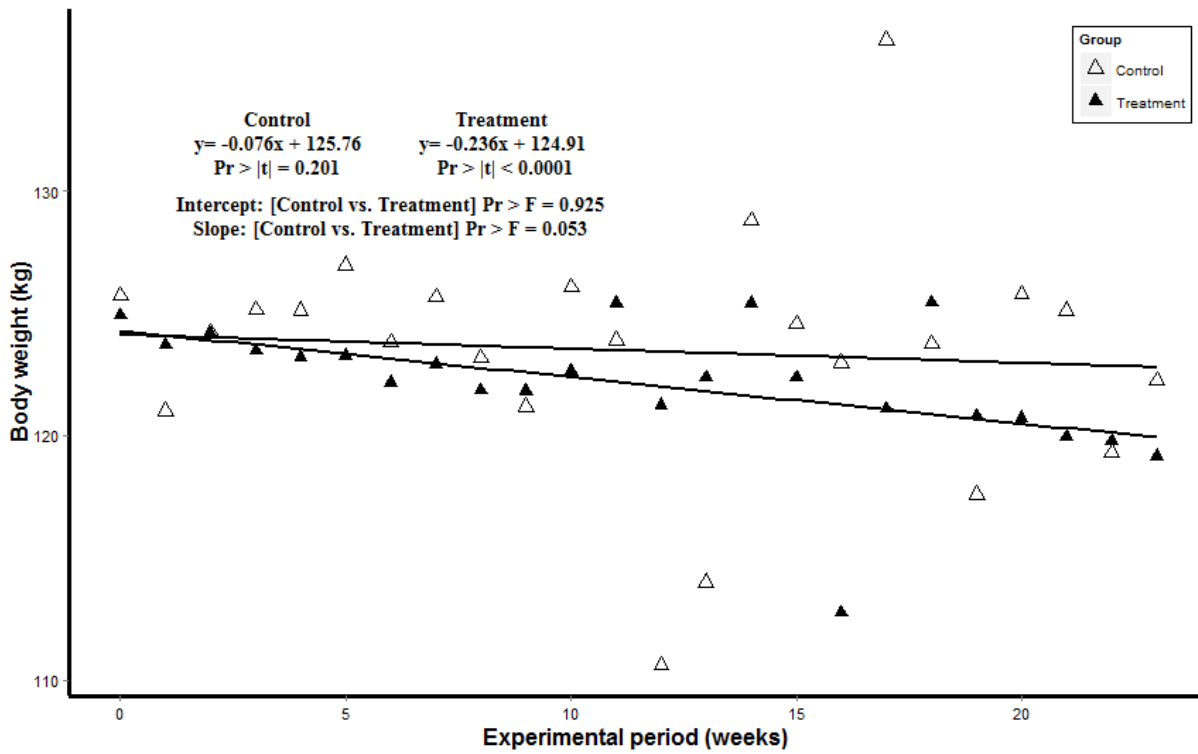


Figure 1. Linear regression of body weight of obese human participants on weeks of experimental period for duration of 24 weeks. Linear regression points are unadjusted means. Regression equations are from the PROC MIXED analysis that fit the data to the ‘first-order autoregressive moving average’ variance-covariance structure.

Dietary analysis: The food and exercise questionnaires were collected at the baseline and the end of 24 weeks. Also self-reported food diaries were collected every week. The participant recorded their daily consumption of shakes/smoothies for breakfast, lunch, dinner and snacks. They also recorded their physical activities. These diaries were used to assess the compliance of shakes/smoothies. Therefore by comparison of daily diaries, the questionnaires, pre and posttest, and personal interviews with the participants, the ones who were committed to change and improve their eating habits significantly, were detected. Seventy two percent of the participant changed their eating and physical activities to some extent. The participants that lost the most weight consumed not only the provided shakes for breakfast (recorded in the food dairies) but attended all nutrition education classes. They incorporated more fresh fruits and vegetables (from 0-1 servings to 3-4 servings per day), modified their cooking methods (from frying to baking) and substituted the sugary drinks with water.

Body composition: Weight loss was consistently higher in the treatment participants and DXA profile showed (by comparing the results visually) that the weight loss in the WP and RS group was primarily around chest and stomach area. An example of the DXA output for one of the participant, who lost the most weight, is shown in Figure 2. It must be noted that this image cannot be totally attributed to treatment effect.

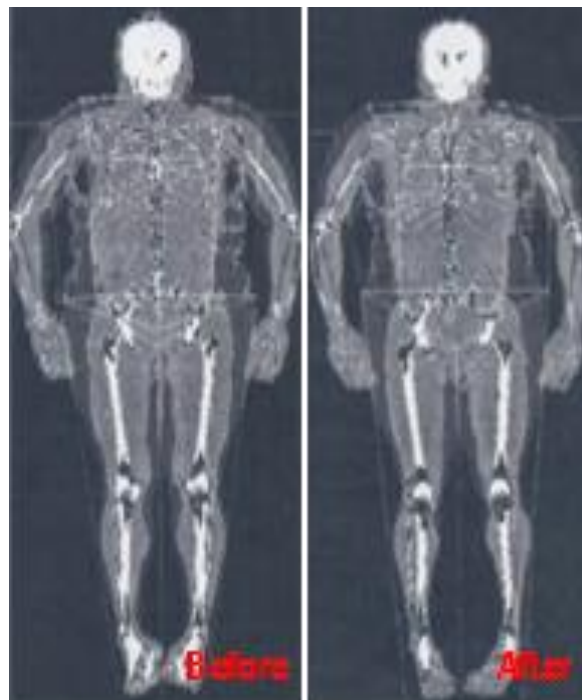


Figure 2. Comparison of Dual-energy X-ray Absorptiometry results for one of the participant who lost the most weight from the treatment group at baseline and end of 24 weeks.

Waist circumferences: The average waist circumference for the control group did not change, but the treatment group showed significant ($p < 0.023$) decrease of approximately 6 cm from week 0 to week 24 (Table 3).

Blood pressure: There were no significant differences observed in the control or treatment groups for blood pressure.

DISCUSSION

Results from this study showed that consumption of WP and RS reduced body fat waist circumferences and changed fat distribution. Furthermore, the results showed that consumption of WP and RS shakes/smoothies for breakfast in conjunction with nutrition education decreased body weight compared to control group that had no WP and RS in their shakes/smoothies over 24 weeks of study. Some of the participants in the control group lost weight too. This may be due to nutrition education intervention. Studies suggest the effectiveness of moderate exercise in conjunction with nutrition education interventions that last at least for 15 weeks, have a long lasting weight loss effect [53-56]. In the present study, the results from comparing pre and posttests, personal interviews, observing food selections (healthy vs unhealthy choices) during cooking, preparing and demonstrating food, and comparing the daily dairies, showed that up to 72% of the participants changed their eating habits. They incorporated more fresh fruits and vegetables (from 0-1 servings to 3-4 servings per day), modified their cooking methods (from frying to baking) and substituted the sugary drinks with water.

The shakes/smoothies with WP and RS combination produced synergistic effects on weight loss while remaining low in calories. The treatment shake mixes (with WP and RS) had 9% of

RS and about 68% proteins (almost half of daily intake), gum, flavors and artificial sweetener shakes compared to control shakes with wheat starch (no WP and RS) with the same amount of gum, flavors and artificial sweetener. The comparison of the 'before and after' images in Figure 2 elucidated the decrease in total body fat in a participant, who lost the most weight, especially around the waist and stomach area. A study conducted by Frestedt et al., 2008 [14], showed a 6% loss of fat mass with the consumption of WP beverages for 12 weeks. In that particular study, both the control (n=28) and treatment groups (n=31) showed significant body fat reduction mainly due to the controlled caloric intake (500 calories less per day for both groups) and the consumption of an isocratic beverage containing WP twice a day before their meals. The subjects who consumed WP supplement (Prolibra) lost significantly less lean muscle mass than control group.

Studies in animals and human suggests that the consumption of dietary fiber such as resistant starch have high impact on satiety, which in turn leads to lower caloric intake thus reduced body weight and fat [11-14, 24, 57]. In a study by Willis et al., 2009 [57], 20 healthy men and women participated in a randomized study comparing the effect of 4 fibers and a low fiber treatment on obesity. The result of their study showed that RS and corn bran had the most impact on satiety.

In the present study (Table 3), at the end of the 24 week study, the treatment group lost a mean body weight of approximately 6 kg with standard deviation of 8.38 kg ($p < 0.029$) but in the control group the weight did not differ significantly ($p < 0.209$). The statistical comparisons of the control and treatment groups for regression slope shows that weight decreased linearly with increasing weeks, and the decrease (negative slope) was significantly ($p < 0.0001$) greater for the treatment group (Figure 1). The results from this study are in agreement with the study conducted by others [14, 42, 57] the participants in the treatment group that consumed the combination of WP and RS shakes for breakfast lost from 0 to 28 kg of their weight over 24 weeks. In a weight management study led by U.S. Department of Agriculture (USDA) scientists, 29 obese but otherwise healthy women age 20-45 participated in a 12-week weight loss program. The amount of weight that the volunteers lost was varied from 0 to 12.2 kg even though the food and caloric intake was very controlled [58]. The same pattern was observed in the present study. The participants in the treatment group lost from 0 to 28 kg. Another study has shown that consuming WP based drink /shakes (30g) for breakfast resulted in reduced hunger or higher satiety compare to low (< 2g) alginate based drink. Thirty three healthy adults aged between 18 and 24 participated in the study [42]. Bodinham et al. 2010, conducted a study with 20 young adults divided in the control and the treatment group, and concluded that consumption of 48g RS over a 24-h period resulted in decreased appetite through the management of the metabolic syndrome in humans [45].

In the treatment group in the present study, not only weight was affected by consuming the shakes, but also a significant ($p < 0.023$) decrease of approximately 6 cm in waist circumference was observed at the end of 24 week study. In a study conducted by Frestedt et al., 2008 [14] a significant ($p \leq 0.05$) reduction in waist circumferences of approximately 6 cm in control and treatment group was observed. Both groups were on a restricted diet. In the present study the control group also lost weight. This may be attributed to the nutrition education component of the study. Both groups received the same nutrition education intervention.

Therefore, the results from the current study shows that consumption of WP and RS with nutrition education intervention may reduce body fat, body fat distribution and as a result lowers BMI. This information should directly increase the public awareness and interest in consumption of foods containing sufficient amounts of WP and RS, and acceptance of milk proteins and RS as important bioactive food components. To effectively intervene, researchers and health care practitioners need to address both energy balance and a physically active lifestyle through nutrition education. The use of foods and their bioactive compounds that promote satiety and energy expenditure and thereby the control of food intake in humans, represents a noninvasive and cost-effective strategy for reducing the risk of obesity. Furthermore the information gained from this study about WP and RS synergistically reducing body fat, can assist in developing food products or formulations that include such a dietary bioactive compounds. This mechanism can be viewed as a consumer friendly and cost effective approach to combat current epidemic of adult and childhood obesity especially in African Americans.

Results from the current study also suggest that a university/academic setting may provide an effective delivery venue for testing studies of this nature. Although it was not an objective, future studies may produce greater weight loss if trained peer educators are used to implement the study which was done in previous community-based participatory research models [15]. Though the results of this study are promising for the duration of time it was conducted, to be successful in future weight loss studies several factors must be considered in order for the results to be meaningful and more generalizable: 1) increase the sample size; 2) utilize peer educators for sustainability of the intervention to be conceivable in the communities ; and 3) target more men for participation as women seemingly always far exceed their presence in weight loss research studies whether community-based or otherwise.

CONCLUSIONS

The findings from this study suggest that a combination of WP and RS in the form of shake/smoothie consumed for breakfast along with a nutrition education component may be effective in decreasing body weight, waist circumference, and cumulative food intake in African American males and females.

Abbreviations used: Whey protein, WP, ; resistant starch, RS; Southern University Agricultural Research and Extension Center, SUAREC; Pennington Biomedical Research Center, PBRC ; Body Mass Index, BMI; Peptide YY, PYY; Glucagon-like peptide-1, GLP-1; Dipeptidyl peptidase-4, DPP4; Expanded Food and Nutrition Education Program, EFNEP; Dual-energy X-ray Absorptiometry, DXA; centimeter, cm ; kilogram, kg; gram, g; meter, m.

Competing interest:

The authors declare that there is no financial interest or conflict of interest regarding the publication of this paper.

Authors' contributions: All authors listed have contributed to this study that was conducted at Southern University Agricultural Research and Extension Center in Baton Rouge, Louisiana. The Email addresses for the authors are as follow:

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REFERENCES

1. Centers for Disease Control and Prevention (CDC). Prevalence of Self-Reported Obesity Among U.S. Adults, by State and Territory [http://www.cdc.gov/obesity/data/prevalence-maps.html]
2. Ogden C. L., Carroll M. D., Kit B. K., Flegal K. M. Prevalence of childhood and adult obesity in the United States, 2011-2012. *Journal of American Medical Association*. 2014, 311:806-814.
3. The Child Policy Research Center and the Child and Adolescent Health Measurement Initiative/Data Resource Center: The Child Policy Research Center and the Child and Adolescent Health Measurement Initiative (CAHMI)/Data Resource Center Analysis. In *National Survey of Children's Health: National Institute for Children's Health Quality (NICHQ)*; 2003.
4. National Center for Health Statistics: *Health, United States, 2008: with special feature on the health of young adults*. 2009.
5. Lavizzo-Mourey R., Levi J.,: *What is the State of Obesity in America?* Project of The Trust for America's Health and the Robert Wood Johnson Foundation. [http://stateofobesity.org/letter/]
6. White A. H., Wilson J. F., Burns A., Blum-Kemelor D., Singh A., Race P. O., Soto V., Lockett A. F.: Use of qualitative research to inform development of nutrition messages for low-income mothers of preschool children. *Journal of nutrition education and behavior* 2011, 43:19-27.
7. Stanley S., Wynne K., Bloom S.: Gastrointestinal satiety signals III. Glucagon-like peptide 1, oxyntomodulin, peptide YY, and pancreatic polypeptide. *American journal of physiology* 2004, 286:G693-697.
8. Halton T. L., Hu F. B.: The effects of high protein diets on thermogenesis, satiety and weight loss: a critical review. *Journal of the American College of Nutrition* 2004, 23:373-385.
9. Moran L. J., Luscombe-Marsh N. D., Noakes M., Wittert G. A., Keogh J. B., Clifton P. M.: The satiating effect of dietary protein is unrelated to postprandial ghrelin secretion. *Journal of Clinical Endocrinol Metabolism* 2005, 90:5205-5211.
10. Weigle D. S., Breen P. A., Matthys C. C., Callahan H. S., Meeuws K. E., Burden V. R., Purnell J. Q.: A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake [http://support.sas.com/documentation/onlinedoc/stat/indexproc.html#stat132, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. *The American journal of clinical nutrition* 2005, 82:41-48.

11. Anderson G. H., Moore S. E.: Dietary proteins in the regulation of food intake and body weight in humans. *The Journal of nutrition* 2004, 134:974S-979S.
12. Lacroix M., Gaudichon C., Martin A., Morens C., Mathé V., Tomé D., Huneau J. F.: A long-term high-protein diet markedly reduces adipose tissue without major side effects in Wistar male rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 2004, 287:R934-R942.
13. Pichon L., Huneau J. F., Fromentin G., Tome D.: A high-protein, high-fat, carbohydrate-free diet reduces energy intake, hepatic lipogenesis, and adiposity in rats. *Journal of Nutrition* 2006, 136:1256-1260.
14. Frestedt, J.L., J.L. Zenk, M.A. Kuskowski, L.S. Ward, and E.D. Bastian, A whey-protein supplement increases fat loss and spares lean muscle in obese subjects: a randomized human clinical study. *Nutrition and Metabolism (London, UK)*, 2008. 5(1): p. 8.
15. Kennedy, B.M., P.T. Katzmarzyk, W.D. Johnson, G.S. Johnson, B.B. McGee, C.M. Champagne, D.W. Harsha, T. Crawford, and D.H. Ryan, People United to Sustain Health (PUSH): a community-based participatory research study. *Clin Transl Sci*, 2014. 7(2): p. 108-14.
16. Lagiou, P., S. Sandin, M. Lof, D. Trichopoulos, H.O. Adami, and E. Weiderpass, Low carbohydrate-high protein diet and incidence of cardiovascular diseases in Swedish women: prospective cohort study. *Bmj*, 2012. 344: p. e4026.
17. Westerterp-Plantenga, M.S., S.G. Lemmens, and K.R. Westerterp, Dietary protein—its role in satiety, energetics, weight loss and health. *British journal of nutrition*, 2012. 108(S2): p. S105-S112.
18. Wycherley, T.P., L.J. Moran, P.M. Clifton, M. Noakes, and G.D. Brinkworth, Effects of energy-restricted high-protein, low-fat compared with standard-protein, low-fat diets: a meta-analysis of randomized controlled trials. *Am J Clin Nutr*, 2012. 96(6): p. 1281-98.
19. Luhovyy B. L., Akhavan T., Anderson G. H.: Whey proteins in the regulation of food intake and satiety. *Journal of the American College of Nutrition* 2007, 26:704S-712S.
20. Adams S. H., Lei C., Jodka C. M., Nikoulina S. E., Hoyt J. A., Gedulin B., Mack C. M., Kendall E. S.: PYY[3-36] administration decreases the respiratory quotient and reduces adiposity in diet-induced obese mice. *The Journal of nutrition* 2006, 136:195-201.
21. Batterham R. L., Cohen M. A., Ellis S. M., Le Roux C. W., Withers D. J., Frost G. S., Ghatei M. A., Bloom S. R.: Inhibition of food intake in obese subjects by peptide YY3-36. *The New England Journal of Medicine* 2003, 349:941-948.
22. Davis H. R., Jr., Mullins D. E., Pines J. M., Hoos L. M., France C. F., Compton D. S., Graziano M. P., Sybertz E. J., Strader C. D., Van Heek M.: Effect of chronic central administration of glucagon-like peptide-1 (7-36) amide on food consumption and body weight in normal and obese rats. *Obesity research* 1998, 6:147-156.
23. Flint A., Raben A., Ersboll A. K., Holst J. J., Astrup A.: The effect of physiological levels of glucagon-like peptide-1 on appetite, gastric emptying, energy and substrate metabolism in obesity. *International journal of obesity and related metabolic disorders* 2001, 25:781-792.
24. Keenan M. J., Zhou J., McCutcheon K. L., Raggio A. M., Bateman H. G., Todd E., Jones C. K., Tulley R. T., Melton S., Martin R. J., Hegsted M.: Effects of resistant

- starch, a non-digestible fermentable fiber, on reducing body fat. *Obesity* 2006, 14:1523-1534.
25. Zhou J., Hegsted M., McCutcheon K. L., Keenan M. J., Xi X., Raggio A. M., Martin R. J.: Peptide YY and proglucagon mRNA expression patterns and regulation in the gut. *Obesity* 2006, 14:683-689.
 26. Gunnarsson P. T., Winzell M. S., Deacon C. F., Larsen M. O., Jelic K., Carr R. D., Ahren B.: Glucose-induced incretin hormone release and inactivation are differently modulated by oral fat and protein in mice. *Endocrinology* 2006, 147:3173-3180.
 27. Englyst H., Wiggins H. S., Cummings J. H.: Determination of the non-starch polysaccharides in plant foods by gas-liquid chromatography of constituent sugars as alditol acetates. *Analyst* 1982, 107:307-318.
 28. Englyst H. N., Kingman S. M., Cummings J. H.: Classification and measurement of nutritionally important starch fractions. *European journal of clinical nutrition* 1992, 46 Suppl 2:S33-50.
 29. Sajilata M.G., Singhal R. S., Kulkarni P. R.: Resistant starch—a review. *Comprehensive reviews in food science and food safety* 2006, 5:1-17.
 30. Topping D. L., Fukushima M., Bird A. R.: Resistant starch as a prebiotic and synbiotic: state of the art. *Proceedings of the Nutrition Society* 2003, 62:171-176.
 31. Baghurst K. I., Baghurst P. A., Record S. J.: Demographic and dietary profiles of high and low fat consumers in Australia. *Journal of Epidemiology and Community Health* 1994, 48:26-32.
 32. Brighenti F., Casiraghi M. C., Baggio C.: Resistant starch in the Italian diet. *The British Journal of Nutrition* 1998, 80:333-341.
 33. Dysseler P., Hoffem D.: Estimation of resistant starch intake in Europe. In *Proceedings of the concluding plenary meeting of EURESTA*. Wageningen The Netherlands; 1994: 84-86.
 34. Birkett A.M.: Dietary Resistant Starch Interacts with Non-starch Polysaccharide and Protein to Influence Colonic Protein Fermentation, with Possible Implications for Colon Cancer Risk in Humans. Faculty of Health Behavioural Sciences, University, Victoria; 1997.
 35. Brown I. L.: Applications and uses of resistant starch. *Journal of AOAC International* 2004, 87:727-732.
 36. Cassidy A., Bingham S. A., Cummings J. H.: Starch intake and colorectal cancer risk: an international comparison. *British Journal of Cancer* 1994, 69:937-942.
 37. de Deckere E. A., Kloots W. J., van Amelsvoort J. M.: Resistant starch decreases serum total cholesterol and triacylglycerol concentrations in rats. *The Journal of nutrition* 1993, 123:2142-2151.
 38. Higgins J. A.: Resistant starch: metabolic effects and potential health benefits. *Journal of AOAC International* 2004, 87:761-768.
 39. Robertson M. D., Bickerton A. S., Dennis A. L., Vidal H., Frayn K. N.: Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. *The American journal of clinical nutrition* 2005, 82:559-567.

40. Robertson M. D., Currie J. M., Morgan L. M., Jewell D. P., Frayn K. N.: Prior short-term consumption of resistant starch enhances postprandial insulin sensitivity in healthy subjects. *Diabetologia* 2003, 46:659-665.
41. Ruxton C. H. S.: Smoothies: one portion or two? *Nutrition Bulletin* 2008, 33:129-132.
42. Solah V. A., Kerr D. A., Adikara C. D., Meng X., Binns C.W, Zhu K., Devine A., Prince R. L.: Differences in satiety effects of alginate-and whey protein-based foods. *Appetite* 2010, 54:485-491.
43. Lejeune M. P., Kovacs E. M., Westerterp-Plantenga M. S.: Additional protein intake limits weight regain after weight loss in humans. *The British Journal of Nutrition* 2005, 93:281-289.
44. Westerterp-Plantenga M. S., Lejeune M. P. G. M., Nijs I., van Ooijen M., Kovacs E. M. R.: High protein intake sustains weight maintenance after body weight loss in humans. *International journal of obesity and related metabolic disorders* 2003, 28:57-64.
45. Bodinham C. L., Frost G. S., Robertson M. D.: Acute ingestion of resistant starch reduces food intake in healthy adults. *The British Journal of Nutrition* 2010, 103:917-922.
46. Littell Ramon C, Stroup Walter W, Milliken George A, Wolfinger Russell D, Schabenberger Oliver: SAS for mixed models. SAS institute; 2006.
47. Heijden Amber AWA, Hu Frank B., Rimm Eric B., Dam Rob M.: A prospective study of breakfast consumption and weight gain among US men. *Obesity* 2007, 15:2463-2469.
48. Veltsista Alexandra, Laitinen Jaana, Sovio Ulla, Roma Eleftheria, Järvelin Marjo-Ritta, Bakoula Chryssa: Relationship between eating behavior, breakfast consumption, and obesity among Finnish and Greek adolescents. *Journal of nutrition education and behavior* 2010, 42:417-421.
49. Nielsen S Suzanne: United States Government regulations and international standards related to food analysis. Springer; 2010.
50. McGuire, S., US Department of Agriculture and US Department of Health and Human Services, Dietary Guidelines for Americans, 2010. Washington, DC: US Government Printing Office, January 2011. *Advances in Nutrition: An International Review Journal*, 2011. 2(3): p. 293-294.
51. USDA-Expanded Food and Nutrition Education Program (EFNEP)
<http://nifa.usda.gov/program/expanded-food-and-nutrition-education-program-efnep>.
52. Institute SAS: SAS/STAT @13.2 User's Guide- procedure
[<http://support.sas.com/documentation/onlinedoc/stat/indexproc.html#stat132>]
53. Miller Wayne C, Koceja DM, Hamilton EJ: A meta-analysis of the past 25 years of weight loss research using diet, exercise or diet plus exercise intervention. *International journal of obesity* 1997, 21:941-947.
54. Hamman R. F., Wing R. R., Edelstein S. L., Lachin J. M., Bray G. A., Delahanty L., Hoskin M., Kriska A. M., Mayer-Davis E. J., Pi-Sunyer X., et al: Effect of weight loss with lifestyle intervention on risk of diabetes. *Diabetes care* 2006, 29:2102-2107.
55. Contento, I.R., J.S. Randell, and C.E. Basch, Review and analysis of evaluation measures used in nutrition education intervention research. *Journal of nutrition education and behavior*, 2002. 34(1): p. 2-25.

56. Martin, P. and E. Ninos, Sustainability: A Natural Vehicle for Nutrition Education and Outreach at College. *Journal of nutrition education and behavior*, 2015. 47(4): p. S19.
57. Willis H. J., Eldridge A. L., Beiseigel J., Thomas W., Slavin J. L.: Greater satiety response with resistant starch and corn bran in human subjects. *Nutrition Research* 2009, 29:100-105. 58.
58. Wood, M., Weight Loss, Cortisol, and Your Brain Scientists Explore Connections. *Agricultural Research*, 2013. 61(3): p. 4