ABSTRACT

Human intervention trials aimed at reducing weight gain and body fat using soy protein and isoflavones are few in number and show equivocal results. The objective of this study was to compare the effects of a daily isoflavone-rich soy-based meal replacement versus a casein-based control on body weight and fat gain over 16 weeks in 18 to 19 year old female college freshmen (N = 120). No difference was found between groups with respect to body weight or composition measures. There was a significant increase in body weight in both groups over time (p < 0.05), and this increase is 50 to 75% less than previously reported in college freshmen. Both soy and milk-based meal replacements may be beneficial with respect to slowing weight gain in healthy premenopausal women; however, further study is needed to determine the effects of soy and casein meal replacements using a non-intervention control group.

INDEX WORDS: soy protein, soy isoflavones, casein, body weight, Freshman 15
EFFECTS OF 16 WEEKS OF ISOFLAVONE-RICH SOY PROTEIN ON WEIGHT AND FAT GAIN IN FEMALE COLLEGE STUDENTS

by

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CHAPTER 1
INTRODUCTION

Obesity is a serious medical problem, associated with increased morbidity and mortality rates, impaired health related quality of life, and reduced life expectancy.\textsuperscript{1-5} The prevalence of overweight during adolescence has increased almost three-fold since the early 1970s.\textsuperscript{6, 7} Children and adolescents who become or remain overweight in young adulthood are at increased risk for metabolic complications associated with obesity such as type II diabetes, hyperlipidemia and hypertension.\textsuperscript{6, 8, 9} Therefore, it is important to explore innovative dietary strategies to prevent and treat childhood and adolescent overweight. Although numerous dietary approaches have been employed to prevent weight gain or promote weight loss, scientific evidence of the long-term safety and efficacy of many popular diet practices is lacking.\textsuperscript{10-14}

Observational and epidemiological data report that higher soy protein and isoflavone intakes are associated with a lower body mass index (BMI)\textsuperscript{15, 16} and reduced body fat.\textsuperscript{17} Cell culture\textsuperscript{18-20} and animal studies\textsuperscript{21-23} show that soy isoflavones may reduce adipose tissue accumulation; however, results from human intervention trials are equivocal. To date, only a few clinical trials have used various combinations of soy protein and isoflavones to examine the effects of soy intake on body weight and body fat.

Three studies, two using soy-based meal replacements and one using an isoflavone supplement, support the role of soy for promoting weight and fat loss.\textsuperscript{24-26} For example, compared to overweight or obese adults receiving dietary counseling, only those that received soy-based meal replacements had greater weight and fat loss over 12 or 24 weeks.\textsuperscript{24, 25}
Furthermore, pre- and postmenopausal women receiving 100 mg soy isoflavone tablets (20.9% daidzein, 4.5% genistein, 10.5% glycitein) per day for 24 weeks had reduced body fat gain and less BMI change compared to a placebo group. Lack of dietary control groups and reported energy intakes, however, preclude assumptions about the weight reducing effects of soy protein or isoflavones in particular. Conversely, studies using a soy-based meal replacement, an isoflavone-rich soy protein isolate or 40 mg genistein found no beneficial effects on weight or fat loss on the prevention of weight gain compared to respective control groups. Due to the small number of human trials, various study designs and conflicting results, the effects of soy intake on body weight and composition in healthy premenopausal women remain largely to be determined.

The freshman year of college has been identified as a critical period for weight gain. Freshmen men and women attending Cornell University and the Albany-State University of New York (Albany-SUNY) gained 1.3-1.9 kg (2.9-4.2 lbs), on average, during the first semester at college. At the end of their first semester, more of the Albany-SUNY students were classified as overweight or obese and approximately 25% were more than 5 lbs heavier compared to the beginning of the school year. Based on these data, the freshman year of college may provide a unique opportunity for trials aimed at assessing the efficacy of dietary strategies to prevent weight gain such as consumption of an isoflavone-rich soy meal replacement.

To our knowledge, no study to date has investigated the effects of a soy meal replacement versus a casein control on weight and fat gain in young, healthy premenopausal females. The purpose of this study was to examine the effects of soy as it is commonly consumed in the United States, as an isolated protein containing isoflavones, on weight and fat
gain in normal weight and overweight young women versus a casein control. The main hypothesis was that a daily isoflavone-rich soy protein-based meal replacement would attenuate weight and fat gain in female college freshmen to a greater extent than a casein-based meal replacement over 16 weeks. The specific aims of the study were to: 1) assess total body weight and fat changes, 2) determine differences in regional fat gain and 3) to determine differences in waist circumference in women consuming soy compared with casein.
REFERENCES


CHAPTER 2
REVIEW OF THE LITERATURE

Introduction

The prevalence of overweight and obesity in the United States has risen to epidemic proportions in the last two decades. The National Center for Health Statistics estimates that approximately 30% of US adults (≥ 20 years of age) are obese, or have a body mass index (BMI) greater than or equal to 30 kg/m². The problem is not limited to adults, but affects children and adolescents as well. The prevalence of overweight in adolescents (12 to 19 years of age) increased significantly in both males and females from 14.8% in 1999 to 18.3% and 16.4%, respectively, in 2004. Overweight children and adolescents are more likely to be overweight or obese as adults and suffer related health problems. Several factors including genetics, metabolism, environment, behavior, culture, and socioeconomic status, have been linked to the development of overweight and obesity. Environment and behavior are important factors in that they afford health professionals, researchers and individuals significant opportunities for prevention and treatment. This chapter outlines popular dietary weight loss strategies, animal studies examining the weight loss effects of soy protein and isoflavones, possible mechanisms for fat loss effects of soy protein and isoflavones, and human clinical trials examining the relationship between soy protein and isoflavones and body composition.

Strategies for losing or maintaining body weight abound in the popular press, however, many claims of efficacy have not been scientifically substantiated. In an article published in the March 2001 supplement of Obesity Research, Freedman et al. found 1,214 books in a search
of http://www.Amazon.com using the key words “weight loss.” The same search performed in our laboratory in September 2006 retrieved 1,688 matches. Eighty-eight percent of top-selling diet books in 2001 had been published in the last four years, and over half had been published in the last two or three years. 7 Despite the popularity of methods such as the most recently popularized low-carbohydrate diet, peer-reviewed scientific evidence for many popular diet practices is lacking7,8 and the prevalence of overweight and obesity has continued to increase.9,10

Evidence for the prevalence of attempted weight loss and weight maintenance is not lacking, however. According to the 1996 Behavioral Risk Factor Surveillance Survey (BRFSS),11 two-thirds of US adults (≥ 18 years of age) reported trying to lose or maintain weight. Interestingly, the prevalence of trying to lose weight was not inversely associated with BMI, whereas, an inverse association was found between BMI and the prevalence of trying to maintain weight. This finding indicates that trying to maintain weight may be an effective approach for weight control.

The freshman year of college has been identified as a critical period for weight gain. A study conducted at the Albany-State University of New York (Albany-SUNY),12 found that 26% of freshman men and women gained more than 2.3 kg (5 lbs) in the first semester of college. In addition, the proportion of students classified as overweight or obese significantly increased from one-fifth to nearly one-third of the sample at the end of the semester. A study conducted at Cornell University13 had similar findings. Freshman at Cornell, on average, gained slightly more weight than freshman at Albany-SUNY, 1.9 kg (4.2 lbs) vs. 1.3 kg (2.9 lbs), respectively. Based on the results from these studies, the freshman year of college may provide a unique opportunity for trials of strategies to reduce weight gain. Strategies that are successful in this population
could be effective in the general population where persistent weight gain over time is a growing problem.\textsuperscript{13}

**Dietary Strategies to Control Weight**

Three general categories of dietary weight control strategies have been investigated at length in the scientific literature and will be reviewed here. These categories include: low-fat, low-carbohydrate and meal replacement diets.

**Low-Fat Diets**

Reducing dietary fat is a common and extensively researched weight loss strategy. The Women’s Health Initiative\textsuperscript{14} investigated the efficacy of a reduced-fat ad libitum diet for the prevention of weight gain in women 50 to 79 years of age. Women in the intervention group were instructed by study dietitians to reduce fat intake to 20\% or less of total caloric intake, increase fruit and vegetable intake to at least five servings per day and increase servings of grains to at least six servings per day. Participants were informed that the diet was not intended to promote weight loss, and energy intake was kept constant by replacing fat with fruits, vegetables and grains. The control group in this study was given a copy of the Dietary Guidelines for Americans plus other diet and health related materials, but had no contact with study dietitians. The average length of follow-up for women in this study was 7.5 years. Despite the lack of emphasis on weight loss, women in the intervention group lost an average of 2.2 kg in the first year, and showed no tendency toward weight gain over the course of follow-up. Women in the intervention group maintained lower body weight overall compared to the women in the control group, and the greatest amount of weight loss in either group occurred in those individuals who decreased percent energy intake from fat. A similar trend was observed in women who increased servings of fruits and vegetables. This study suggests that a reduced-fat ad libitum dietary
pattern achieved by replacing sources of dietary fat with complex carbohydrate sources such as fruits, vegetables and grains results in long-term weight loss and weight maintenance.

Additional support for a reduced-fat diet to promote weight loss is provided by a study conducted at the University of Minnesota, in which the effects of a low-fat, ad libitum complex-carbohydrate diet versus a low-energy diet were compared. This study was conducted in moderately obese women (120-140% ideal body weight) who were 25 to 45 years of age. Participants in this study were instructed to either 1) reduce total calorie intake and reduce fat intake to 30% of total energy (low-energy diet group), or to 2) reduce daily fat intake to 20 g while consuming an unrestricted amount of high complex-carbohydrate foods (low-fat, ad libitum diet group). All participants were encouraged to increase physical activity and had regular contact with study dietitians for the six-month study duration. Although no significant differences were found in weight loss between treatment groups, multiple regression analyses showed an independent association between weight loss and change in percent energy intake from fat in both groups. This association was not found for change in total energy intake or physical activity, and suggests a fundamental relationship between change in percent energy from fat and body weight over time.

It is well established that energy balance is crucial to weight maintenance, and weight loss is, above all, achieved by a sustained negative energy balance. Data from the 1994-1996 Continuing Survey of Food Intake by Individuals (CSFII), showed that diets high in carbohydrate (> 55%) and low to moderate in fat (≤ 30%) tend to be lower in energy than other dietary patterns including low-carbohydrate diets. In addition, high-carbohydrate diets were associated with the lowest body mass indices for individuals surveyed, while low-carbohydrate diets were associated with the highest BMIs. This could be a reflection of choice of diet strategy
according to BMI, with those individuals having higher BMIs choosing a low-carbohydrate diet pattern, and this self-selection cannot be ruled out. Vegetarian diets were also associated with low energy intakes and BMIs (1606 kcal/day; BMIs: 24.6 women, 25.2 men), similar to high-carbohydrate diets (1360 kcal/day; BMIs: 24.4 women, 25.2 men). Although the CSFII is cross-sectional, it provides useful information on the comparison of total energy and associated BMIs according to diet pattern; however, it is unclear whether macronutrient ratio or energy intake is mainly responsible for observed lower BMIs with high-carbohydrate, low-fat diets.

**Low-Carbohydrate Diets**

Low-carbohydrate diets may promote greater weight loss in the short-term compared to low-fat diets. In a randomized trial of moderately obese women (BMI range 30-35), a low-carbohydrate ad libitum diet (≤20 g carbohydrate/day for the first two weeks, 40-60 g carbohydrate/day after the first two weeks) produced significantly greater weight loss than a calorie-restricted moderately low-fat diet (55% carbohydrate, 15% protein, 30% fat) over a period of six months. Weight loss in the low-carbohydrate group averaged 8.5 kg versus 3.9 kg in the low-fat group. There were no differences observed in percent weight lost from fat or lean mass between groups. Despite significant differences in weight loss, calorie-restriction between the two groups was similar and somewhat greater in the low-fat diet group, with each group decreasing caloric intake by 300-460 kcal from baseline. The macronutrient composition of the two diets differed dramatically, however. At three months, the low-carbohydrate group diet composition was 15% carbohydrate, 28% protein and 57% fat, whereas, the low-fat group diet composition was 54% carbohydrate, 18% protein and 28% fat. Moreover, the low-carbohydrate diet group consumed significantly more protein, total fat, saturated fat, monounsaturated fat, polyunsaturated fat, and cholesterol than the low fat diet group at three months; however, all
subjects showed reduced total cholesterol, LDL cholesterol and triglycerides at three months, and increased HDL at six months. Greater weight loss in the low-carbohydrate group may have prevented the expected adverse changes in lipid levels with increased saturated fat intake. The long-term effects of a low-carbohydrate diet on lipid levels and body weight maintenance remain unknown.

A 12-month randomized trial in obese men and women found significantly greater weight loss with the low-carbohydrate (Atkins) diet in the first six months compared with a high-carbohydrate, low-fat, calorie-restricted (conventional) diet, but no difference in weight loss after 12 months. The authors attributed the enhanced weight loss in the low-carbohydrate group to a greater energy deficit, presumably resulting from diet simplicity or monotony and factors related to satiety, although neither total energy nor satiety was reported for either group. The precise mechanism for increased weight loss in the first six months on a low-carbohydrate diet is still unclear.

A meta-analysis of low-carbohydrate diets provides some insight into the efficacy and safety of this type of diet. A total of 94 studies published between January 1, 1966 and February 15, 2003 were included in the analysis. Length of studies ranged from four days to one year, and total carbohydrate intake ranged from 0-901 g/day. Total caloric intake ranged from 525-4629 kcal/day. Overall weight loss in obese patients was not associated with reduced carbohydrate intake, but with longer diet duration and restriction of energy intake. No significant adverse effects on serum lipids, fasting serum glucose, fasting serum insulin, or blood pressure were observed. Due to limitations in study duration, carbohydrate dose and age of subjects, the authors were unable to draw conclusions regarding the safety and efficacy of low-carbohydrate diets in individuals over 50 years of age, use of low-carbohydrate diets for greater
than 90 days and use of diets providing less than 20 g carbohydrate/day. These results suggest that low-carbohydrate diets may be a safe and effective method of reducing calorie intake in the short-term (90 days or less), and thus promoting weight loss; however, further study is needed to demonstrate the safety of this type of diet in the long-term, with more extreme carbohydrate restriction, and in older individuals.

**Meal Replacement Diets**

The use of meal replacements is a fourth common method of weight management designed to help with calorie control. Typically in the form of a milk- or soy-based “shake”, some diet plans use solid meal replacement bars or entrees in place of liquid forms. Meal replacements may provide additional benefit particularly with regard to weight maintenance. A five-year weight control study using milk-based meal replacements found that, following successful weight loss, participants who used meal replacements to self-manage their weight weighed approximately four to five kilograms less than at baseline, while age, sex, race, and BMI-matched controls weighed approximately 6.5 kilograms more than at baseline.20 A meta-and pooling analysis of studies using meal replacements found significantly greater weight loss at three months in subjects treated with partial meal replacements compared with subjects treated with reduced calorie diets.21 A partial meal replacement program was defined as a plan that included one or more meals replaced by a commercially available, calorie-reduced product fortified with vitamins and minerals and at least one meal per day consisting of regular foods. The total calories provided by such a program ranged from 800-1600 kcal/day. Study duration ranged from three to 51 months, with the most common duration being 12 weeks. At the one-year follow-up for all studies for which data were available, meta- and pooling analyses found consistently greater overall weight loss in subjects who had been treated with a partial meal
replacement plan. The average amount of weight loss at one year was similar to that at three months, indicating effective weight maintenance in both groups; however greater weight loss was maintained in the partial meal replacement group. Data from this analysis provide support for the use of meal replacements as an effective tool for weight loss and weight maintenance.

A meta-analysis of weight loss programs evaluated the effects of soy and non-soy meal replacement diets (SOYs and MRs), energy-restricted (>1500 kcal/day; ERDs), low-energy (800-1500 kcal/day; LEDs), and very low-energy (<800 kcal/day; VLEDs) diets for a period of 24 weeks. The soy meal replacement diets (SOYs) were also very low in energy (<800 kcal/day). Similar weight loss effects were observed with the SOYs and VLEDs with the exception of the first four weeks, when soy meal replacement diets promoted significantly greater weight loss than any other diet program. After eight weeks, however, VLEDs surpassed SOYs in continuing to promote weight loss. Soy meal replacement diets reported significantly higher compliance than any other diet program, which is of note considering the very low energy content of the diets. Good compliance with soy meal replacement products may predict enhanced long-term results. Results of this meta-analysis suggest that soy-based meal replacements are comparable to non-soy-based products and may promote compliance with a very low calorie diet to promote long-term weight loss.

**Soy Protein**

Comparable to animal protein in quality and digestibility, soy protein has a Protein Digestibility Corrected Amino Acid Score (PDCAAS) of 1.0. A PDCAAS score of 1.0 indicates that a protein provides 100% of the indispensable amino acids per gram, corrected for digestibility, to meet the protein requirements for the two to five year old, and is considered a high-quality protein that meets essential amino acid requirements if consumed as the sole source.
of protein in the diet of a child or an adult.\textsuperscript{26,27} Soy is the only vegetable protein source with a PDCAAS of 1.0, making it uniquely a “complete vegetable protein.”\textsuperscript{24} Additionally, the amino acids, arginine and glycine, are present in soy in significantly larger amounts compared to animal proteins, and this may lead to a reduced insulin:glucagon ratio \textit{in vivo}.\textsuperscript{28,29} A major component of soy protein is the peptone β-conglycinin, which has been shown to suppress food intake and inhibit gastric emptying in rats by increasing plasma cholecystokinin.\textsuperscript{30} β-conglycinin has also been shown to induce β-oxidation, down-regulate fatty acid synthase and inhibit triglyceride absorption in genetically obese mice.\textsuperscript{31} These data suggest that components of soy protein may offer added benefits with regard to weight control; however, further study in humans is necessary.

\textbf{Soy Protein and Body Weight: Animal Studies}

In animal models of obesity, reduced-calorie soy protein diets have been shown to reduce body fat and body weight to a greater extent than milk proteins (casein or whey). Body fat content in mice fed a diet containing soy protein isolate was significantly lower than mice receiving an isocaloric diet with casein as the protein source.\textsuperscript{32} Aoyama et al.\textsuperscript{33} found significantly greater body fat reduction in diet-induced yellow KK obese mice fed a 60% calorie-restricted diet (corresponding to a 1200-1600 kcal human diet) containing ~42% soy protein isolate (~0.1 g soy protein isolate/g body weight/day) compared with a diet containing ~46% whey protein isolate. Soy and whey protein hydrolysates were also used as protein sources, and mice fed diets containing ~42% soy protein hydrolysate lost more body fat than mice fed ~46% whey protein isolate or hydrolysate. In a separate study, Aoyama et al.\textsuperscript{34} found reduced body fat in diet-induced obese rats and genetically obese mice fed diets containing soy protein isolate or hydrolysate versus casein protein isolate or hydrolysate. The animal literature provides strong
support for a body fat-reducing effect of high-protein, reduced-calorie diets containing soy protein isolate in place of casein or whey.

**Soy Isoflavones**

The soybean is one of the most abundant natural sources of the isoflavone glycosides, genistin, daidzin and glycitin. In nature, soy isoflavones exist predominantly in biologically inactive glycosylated forms until, upon ingestion, they are hydrolyzed and fermented in the intestines. Bacterial β-glucosidases in the small intestine are responsible for hydrolysis into the aglycones, genistein, daidzein and glycitein. Fermentation of aglycones into various metabolites occurs in the colon. Daidzein can be metabolized into equol, O-desmethylandalensin (O-DMA), or dihydrodaidzein, and distinct variation in these processes exists among individuals. Genistein can be metabolized into p-ethylphenol. The major isoflavones detected in blood and urine are daidzein, genistein, equol, and O-DMA. Aglycone forms of isoflavones and their metabolites exert potent biological effects in humans. Often referred to as phytoestrogens, soy isoflavones possess estrogenic properties resulting from structural similarity to the hormone 17β-estradiol. Genistein has the greatest estrogen receptor (ER) binding affinity at roughly 100 times lower than that of 17β-estradiol, and daidzein has a binding affinity roughly 10-15 times lower than genistein. Equol, a metabolite of daidzein, has a binding affinity comparable to that of genistein. Despite lower binding affinity, plasma isoflavone concentrations in soyfood-consuming adults can exceed endogenous estradiol concentrations by 10,000-20,000 fold, thereby allowing competitive ER binding due to abundance. Both estrogenic and anti-estrogenic effects of soy isoflavones have been documented in vivo, and it is thought that estrogen status of an individual may play a role in modulating these effects.
In order to facilitate accurate interpretation and comparison of research results, the preferred method of reporting isoflavone intakes is in aglycone equivalents. The aglycone composes ~60% of a glycosylated isoflavone, so a conversion factor of 0.60 can be used to estimate aglycone equivalents from total isoflavone content.\textsuperscript{50} It is also helpful to report percentage of individual isoflavones in a mixture, as diverse biological effects of genistein and daidzein have been documented and those of glycitein are relatively unknown.\textsuperscript{50}

**Soy Isoflavones and Body Weight: Animal Studies**

Evidence in the animal literature suggests that soy isoflavones may reduce fat gain. Obese and lean rats fed diets containing a 0.1% soy isoflavone mixture had significantly reduced fat pad and body weights compared with rats fed an isoflavone-free diet.\textsuperscript{51} Growing-finishing pigs also gained significantly less ham fat and had lower carcass fat percentage when fed diets containing soy protein concentrate with isoflavones compared with diets containing soy protein concentrate without isoflavones.\textsuperscript{52} When isoflavones were added to diets in amounts two or five times greater than those found in typical soybean meal, no added benefit was found.\textsuperscript{52} Pigs implanted with estrogen and estrogen-like compounds have also shown significant reductions in fat content. From these studies it is thought that soy isoflavones exert estrogenic effects on fat tissue at levels typically consumed in soybean meal, and may exert anti-estrogen effects at higher levels due to enhanced competitive ER binding and weaker modulation compared to estradiol.\textsuperscript{52,53}

**Possible Mechanisms**

Several mechanisms are postulated for the apparent fat-reducing effects of soy protein and isoflavones. Suggested mechanisms can be categorized into three general areas: isoflavone effects on fat cells, amino acid modulation of insulin concentration and regulation of food intake
by soy protein fractions. Possibly the largest amount of research has been conducted in the area of the isoflavone effects, specifically genistein, on fat cell development and metabolism.

In cell culture studies, genistein was shown to act via ER and non-ER-dependent pathways to affect adipogenesis and adipocyte differentiation. One mechanism through which genistein was shown to regulate adipogenesis is via ER-dependent peroxisome proliferator-activated receptor γ (PPARγ) regulation. During adipocyte differentiation, genistein blocked DNA binding and transcriptional activity of certain proteins leading to a down-regulation of PPARγ, reduction in adipocyte number and reduced lipid accumulation.\textsuperscript{54,55} Genistein may act via another ER-dependent mechanism to up-regulate transforming growth factor β-1 leading to a reduction in proliferation rate of precursor cells.\textsuperscript{55,56} Genistein may also act via a non-ER-dependent mechanism by inhibiting tyrosine phosphorylation of certain proteins during adipocyte differentiation, further limiting adipogenesis.\textsuperscript{54}

It is important to consider the estrogenic effects of genistein and daidzein as tissue and concentration-specific. At concentrations ≤1μM in mesenchymal progenitor cells, genistein was shown to activate ERα or ERβ to down-regulate PPARγ transcriptional activity, thus leading to a down regulation of adipogenesis.\textsuperscript{57} At concentrations >1μM, genistein demonstrated opposite effects. The balance between the activation of two factors, ERs and PPARγ may help to explain the effects of genistein in different tissues, with different serum concentrations, in the presence and absence of estrogen, and at different stages of the lifecycle.

Both genistein and daidzein were shown to enhance lipolysis and inhibit lipogenesis in adipose tissue via non-ER dependent mechanisms. In isolated rat adipocytes, genistein was shown to reduce glucose conversion to total lipids at 0.01, 0.3, and 0.6 mM concentrations in both the presence and absence of insulin.\textsuperscript{58} Genistein also restricted the conversion of acetate to
lipids at 0.01, 0.1, and 1.0 mM concentrations. Estradiol at the same concentrations resulted in less marked effects. These results suggest that genistein may alter glucose transport and metabolism as well as fatty acid synthesis and esterification. Genistein also has a significant effect on basal lipolysis. At concentrations of 0.1 and 1.0 mM, genistein was shown to augment basal lipolysis, possibly due to inhibition of cAMP phosphodiesterase. Estradiol has not been shown to enhance basal lipolysis, except in the presence of epinephrine.

Similarly to genistein, daidzein was also shown to affect lipogenesis and lipolysis in isolated rat adipocytes. Daidzein inhibited glucose conversion to lipids at 0.1 mM and 1.0 mM concentrations in the absence of insulin and at 0.01 mM, 0.1 mM and 1.0 mM concentrations in the presence of insulin by inhibiting glucose metabolism to acetyl CoA. Unlike genistein, however, daidzein does not inhibit glucose transport via glucose transporter protein 1. At concentrations of 0.1 mM and 1.0 mM daidzein augmented basal triglyceride breakdown, possibly via activation of protein kinase A; however, in the presence of epinephrine, daidzein at 1.0 mM reduced lipolysis, possibly by restricting action of hormone sensitive lipase. Isoflavone concentration and endogenous hormone status significantly determine effects of isoflavones on fat cell metabolism.

Adipocyte apoptosis is another mechanism through which genistein may act directly on fat cells to reduce body fat. Genistein was shown to induce apoptosis in several types of cancer cells, and recently was shown to induce apoptosis in mature adipocytes. At a concentration of 400 µmol/L, genistein induced apoptosis and reduced viability of differentiated 3T3-L1 adipocytes. At 10 µmol/L and 100 µmol/L concentrations, apoptosis was induced but cell viability was not significantly affected. Genistein had no apoptotic effect on preadipocytes. In vivo, 1500 mg genistein/kg of diet increased DNA fragmentation in the inguinal fat pads of
ovariectomized adult mice by 290%, but no effect was observed in parametrial and retroperitoneal fat pads. No effects were observed in mice fed 150 mg genistein/kg of diet. Apoptotic effects of genistein may be fat pad specific, as in the case of leptin or tumor necrosis factor-α, and dependent on age, gender, and tissue-related ER-sensitivity. It was demonstrated that serum genistein concentrations in mice fed 1500 mg genistein/kg of diet reach between 1-4 μmol/L depending on the assay used. Serum concentrations in humans consuming soy-based foods typically range between 0-5 μmol/L. Therefore, effects similar to those seen in animals may translate to humans while augmented effects seen in some cell culture studies may not.

A second area in which soy may affect body weight is through serum insulin modulation with soy protein intake. Insulin acts to enhance fat storage by facilitating the entry of glucose into fat cells, activating enzyme systems to form de novo lipids from acetyl CoA and promoting esterification of lipids for storage. The hormone glucagon counteracts the effects of insulin, promoting mobilization and utilization of body fat. In humans, soy protein intake is associated with lower postprandial insulin/glucagon ratio than casein. In rats, casein intake leads to a higher insulin/glucagon ratio and increased activity of lipogenic enzymes in the liver. Rats fed a diet containing 18% soy protein had lower serum insulin concentrations than rats fed an 18% casein protein diet, and over a longer period of time rats on the casein diet became hyperinsulinemic, while rats fed soy protein maintained normal insulin concentrations. The amino acids arginine and glycine, present in soy protein at twice the amounts found in casein, are thought to be responsible for insulin-modulating effects. Humans receiving a diet high in soy protein have elevated fasting concentrations of arginine and glucagon. Arginine, at levels typically found in dietary proteins, is associated with a reduction in circulating insulin and the
The product used in this study contained 7.6 mg arginine per gram of protein, compared with casein, which contains 3.7 mg arginine per gram of protein (Manufacturer’s Data). Supplementing casein with arginine and glycine up to the amounts found in soy protein results in elevated plasma arginine and glycine levels and lower postprandial serum insulin/glucagon ratio comparable to levels observed after feeding a soy protein test meal. These results may be attributed to a hyperinsulinemic effect of casein rather than an insulin-lowering effect of soy. Lysine, leucine and phenylalanine have been shown to stimulate the release of insulin, and may play a role in the hyperinsulinemic effect of casein.

Soy protein intake may also play a role in food intake regulation. Intraduodenal infusion of the peptide β-conglycinin, found in soy, was shown to suppress food intake and inhibit gastric emptying by increasing plasma cholecystokinin (CCK) in rats compared with distilled water. A soy protein isolate hydrolysate was shown to stimulate CCK release from isolated rat intestinal mucosal cells to a greater extent than hydrolysates of casein, egg white and gluten. It is thought that CCK acts as a short-term satiety signal, and stimulation of CCK release may indicate an increase in short-term satiety, which may lead to earlier meal termination when soy peptides are consumed. The mechanism for protein hydrolysate stimulation of CCK release is unknown, but is thought to be independent of luminal trypsin activity, and may be related to guanidyl or arginine residues in the peptides. In humans, varying the protein source in a mixed meal did not affect food behavior or postprandial plasma insulin response; however, the lack of an effect with soy protein may be a result of the processing methods used to produce the soy protein concentrate, including the degradation of certain peptides.
Human Clinical Trials

Clinical trials designed to promote weight loss in humans using soy-based meal replacements showed positive results, albeit with some methodological flaws. In two studies, soy-based meal replacements plus calorie restriction promoted greater weight and fat loss in overweight and obese adults compared with lifestyle education or counseling.\textsuperscript{89,90} In these studies, energy intake was not reported, so it is difficult to identify the mechanism driving the observed weight loss, and the lack of a dietary control group precludes assumptions about weight reducing effects of soy in particular. In another study, soy-based meal replacements offered no additional benefit compared to milk-based meal replacements with regard to weight loss in overweight or obese men and women (ages 18 to 65 years).\textsuperscript{91} Lack of statistical power or sex differences between groups may have contributed to the lack of significant difference between treatments. Further study is needed to determine whether soy protein confers any additional benefit with regard to weight loss in humans.

Some evidence suggests that soy isoflavones may play a role in the prevention of fat gain in humans as well. A study by Mori et al.,\textsuperscript{92} examined body fat and BMI in normal weight pre- and postmenopausal women (aged 40 to 60 years) taking a 100 mg total isoflavones tablet (20.9% daidzein, 4.5% genistein and 10.5% glycitein) daily for 24 weeks. Compared with a placebo containing vitamins C and E, soy isoflavone intake resulted in significantly greater reduction in body fat (-6% change vs. -2% change) and BMI (-0.3% change vs. +1% change).\textsuperscript{92} Conversely, premenopausal women (aged 25 to 55 years) consuming tablets containing 40 mg genistein per day for 12 weeks experienced significant weight gain compared to women consuming a placebo.\textsuperscript{93} In perimenopausal women, Moeller et al.\textsuperscript{94} found intakes of 20 g soy protein isolate, containing 80.4 mg isoflavone aglycone equivalents, per day for 24 weeks
diminished thigh fat gain, while neither isoflavone-poor soy protein (4.4 mg aglycone equivalents) nor whey protein intake had any body fat-reducing effect. Heterogeneity of participants with respect to baseline 17B-estradiol levels may have contributed to this unexpected result and may complicate the interpretation of these findings. The studies summarized above provide preliminary evidence for a beneficial effect of soy protein containing native isoflavones in preventing fat gain in humans; however, more research is needed to determine effects in younger women.

A stipulation exists when comparing data from animal and human studies with soy isoflavones, and that is the fact that all rodents are capable of metabolizing daidzein to equol, whereas only 30-50% of humans have this capability. If results are less consistent in human versus animal weight loss studies using soy, the variability in equol production among subjects may be a key contributor. In the enantiomeric form in which it is exclusively produced by human intestinal bacterial flora, S-equol has a high binding affinity for estrogen receptor ERβ and a weak affinity for ERα. Equol’s affinity for ERβ is greater than that of its precursor daidzein. Therefore, if equol is indeed responsible in large part for the beneficial effects of soy on body weight, these effects will only be detected in an expected 30-50% of a human sample.

**Conclusion**

Recent animal and cell culture studies are supportive of the effects of soy protein and isoflavones in reducing body fat via several different mechanisms. Human intervention studies, however, have been few in number and limitations in study design have led to equivocal findings. Many intervention studies have been designed with weight loss as a primary outcome, and have included calorie reduction in one or more treatment arms. No study has investigated the effects of adding a soy-based meal replacement to an otherwise
unmodified American diet in young, healthy premenopausal females. Previous animal and human studies have been conducted in ovariectomized models or postmenopausal women, respectively, and have shown fat-reducing effects of soy isoflavones in an estrogen-deficient physiological environment.\textsuperscript{92, 94, 98} Soy isoflavones may have significantly different, even opposite effects in an estrogen-replete physiological environment, such as that of premenopausal females. The freshman year of college provides a unique opportunity for studying approaches to reduce weight gain.\textsuperscript{12, 13} The purpose of this study (described in Chapter 3) was to assess changes in body weight and composition in 18 to 19 year old female college freshmen consuming a daily isoflavone-rich soy-based meal replacement compared with a casein-based control.
REFERENCES


CHAPTER 3

EFFECTS OF 16 WEEKS OF ISOFLAVONE-RICH SOY PROTEIN ON WEIGHT AND FAT GAIN IN FEMALE COLLEGE STUDENTS

ABSTRACT

Background: Human intervention trials aimed at reducing weight gain and body fat using soy protein and isoflavones are few in number and show equivocal results.

Objective: To compare the effects of a daily isoflavone-rich soy-based meal replacement versus a casein-based control on body weight and fat gain over 16 weeks in 18 to 19 year old female college freshmen (N = 120).

Design: Participants were randomized to treatment group (soy or casein) in this double-blind, placebo-controlled trial. Fat mass (FM), fat-free soft tissue mass (FFST) and % body fat (%BF) were measured using dual energy X-ray absorptiometry (Delphi 4500). A 2 x 3 repeated measures ANOVA was used to measure the response of the dependent variables (body weight, FM, FFST, %BF, and waist circumference) to the intervention.

Results: No significant group by time interactions were observed, even when controlling for body mass index, a significant predictor of compliance. Body weight, FM, FFST, and %BF increased significantly in both groups over time (i.e., time effects; p<0.05).

Conclusions: Both soy and milk-based meal replacements may be beneficial with respect to slowing weight gain in young premenopausal women, as the average weight gain in the present study (0.5 kg) was 50-75% less than previously reported in other studies of college freshmen; however, further study is needed to determine the effects of soy and casein meal replacements using a non-intervention control group.

Key Words: soy protein, soy isoflavones, casein, body weight, Freshman 15
INTRODUCTION

Obesity is a serious medical problem, associated with increased morbidity and mortality rates, impaired health related quality of life, and reduced life expectancy.\textsuperscript{1-5} It is alarming that the prevalence of overweight during adolescence has increased almost three-fold since the early 1970s.\textsuperscript{6, 7} Children and adolescents who become or remain overweight in young adulthood are at increased risk for metabolic complications associated with obesity such as type II diabetes, hyperlipidemia and hypertension.\textsuperscript{6, 8, 9} Therefore, it is important to explore innovative dietary strategies to prevent and treat childhood and adolescent overweight. Although numerous dietary approaches have been employed to prevent weight gain or promote weight loss, scientific evidence of the long-term safety and efficacy of many popular diet practices is lacking.\textsuperscript{10-14}

Observational and epidemiological data show that higher soy protein and isoflavone intakes are associated with a lower body mass index (BMI)\textsuperscript{15, 16} and reduced body fat.\textsuperscript{17} Cell culture\textsuperscript{18-20} and animal studies\textsuperscript{21-23} provide evidence that soy isoflavones may reduce adipose tissue accumulation; however, results from human intervention trials have been equivocal. To date, only a few clinical trials have used various combinations of soy protein and isoflavones to examine the effects of soy intake on body weight and body fat.

Three studies, two using soy-based meal replacements and one using an isoflavone supplement, support the role of soy for promoting weight and fat loss.\textsuperscript{26} For example, compared to overweight or obese adults receiving dietary counseling, those that received both dietary counseling and soy-based meal replacements had greater weight and fat loss over 12 or 24 weeks.\textsuperscript{24, 25} Furthermore, pre- and postmenopausal women receiving 100 mg soy isoflavone tablets (20.9% daidzein, 4.5% genistein, 10.5% glycitein) per day for 24 weeks had reduced body fat gain and less BMI change compared to a placebo group.\textsuperscript{26} Lack of dietary control
groups and reported energy intakes, however, preclude assumptions about weight reducing
effects of soy protein plus isoflavones in particular. Conversely, studies using a soy-based meal
replacement,\textsuperscript{27} an isoflavone-rich soy protein isolate\textsuperscript{28} or 40 mg genistein\textsuperscript{29} found no beneficial
effects on weight or fat loss or prevention of weight gain compared to respective control groups.
Due to the small number of human trials, various study designs and conflicting results, the
effects of soy intake on body weight and composition in healthy premenopausal women remain
largely to be determined.

The freshman year of college has been identified as a critical period for weight gain.
Freshmen men and women attending Cornell University and the Albany-State University of New
York (Albany-SUNY) gained 1.3-1.9 kg (2.9-4.2 lbs), on average, during the first semester at
college.\textsuperscript{30,31} At the end of their first semester, more of the Albany-SUNY students were
classified as overweight or obese and approximately 25\% were more than 5 lbs heavier
compared to the beginning of the school year.\textsuperscript{31} Based on these data, the freshman year of
college may provide a unique opportunity for trials aimed at assessing the efficacy of dietary
strategies to prevent weight gain\textsuperscript{30} such as consumption of an isoflavone-rich soy meal
replacement.

To our knowledge, no study to date has investigated the effects of a soy meal
replacement versus a casein control on weight and fat gain in young, healthy premenopausal
females. The purpose of this study was to examine the effects of soy as it is commonly
consumed in the United States, as an isolated protein containing isoflavones, on weight and fat
gain in normal and overweight young women versus a casein control. The main hypothesis was
that a daily isoflavone-rich soy protein-based meal replacement will attenuate weight and fat
gain in female college freshmen to a greater extent than a casein-based meal replacement over 16
weeks. The specific aims of the study were to: 1) assess total body weight and fat changes, 2) determine differences in regional fat gain and 3) to determine differences in waist circumference in women consuming soy compared with casein.

SUBJECTS AND METHODS

Study design and participants

Healthy female college freshmen, 18 to 19 years of age (N = 120), were recruited for this 16-week, randomized, double-blind, placebo-controlled, dietary soy intervention trial. Participants were recruited through advertisements in a university newspaper, on campus fliers and on campus buses, as well as presentations in large freshman classes. Telephone pre-screening was used to determine eligibility. Women who had experienced significant weight loss or gain in the previous 6 months (± 10% initial BW), vegetarians, NCAA Division I athletes, women with eating disorders, irregular menstruation (less than four out of six periods in the last 6 months), soy or chocolate allergy, and those taking medications or herbal supplements known to affect body weight were excluded. Participants were randomized to either the soy (SOY) or casein (CAS) treatment group, and were tested at baseline, 8 and 16 weeks. Eighty-six women completed the intervention. Twenty-three women elected to discontinue study participation (13 SOY and 10 CAS.) Eleven participants (six SOY and five CAS) elected to stop taking the meal replacements, but agreed to complete all testing measures. Reasons for discontinuing study treatment included: 1) unwillingness to take shakes (four SOY and four CAS); 2) taste intolerance (one SOY); 3) illness not related to study (seven SOY and eight CAS); 4) gastrointestinal distress related to shakes (six SOY and two CAS), and 5) concern for cancer risk (one SOY and one CAS). The Institutional Review Board for Human Subjects at UGA approved all procedures for this study, and written informed consent was obtained from each subject.
Testing Protocol

All testing procedures took place at The University of Georgia Bone and Body Composition Laboratory in Athens, Georgia, USA. Following a successful telephone screen, during which potential subjects were informed that the purpose of the study was to investigate the effects of soy on bone health, participants arrived at the lab in the early morning following an overnight fast for a baseline blood draw and signing of consent forms. Subjects were given several study questionnaires and asked to return these at their second visit, which was scheduled within one week for baseline body composition scans, and distribution of study supplements. Participants were individually instructed by trained staff to consume one study shake each day and to limit soy intake to less than one serving per week. Participants were given verbal and written instructions for mixing shakes and were counseled on substituting shakes for a caloric equivalent of food in their diets. Baseline 3-day diet records were used in order to individualize dietary counseling. Other than the shake substitution, participants were encouraged to maintain usual dietary and physical activity habits. Each participant was given an 8-week supply of shake packets plus three extra if needed at baseline and 8 weeks.

Meal replacement shakes

All shake packets were provided by Revival® Soy. The shakes were available in two flavors: chocolate and vanilla. Chocolate shakes contained 240 kcal, 2.5 g total fat, 36 g total carbohydrate, 2 g dietary fiber, and 500 mg calcium per serving. Vanilla shakes contained 220 kcal, 2 g total fat, 31 g total carbohydrate, 0 g dietary fiber, and 500 mg calcium per serving. The soy-based shakes contained 20 g soy protein and 161.19 mg total isoflavones (95 mg aglycone equivalents: 39% daidzein, 40% genistein, 21% glycitein) per serving. The control shakes contained 20 g casein protein and were identical to the soy shakes in calorie, fat,
carbohydrate, fiber, and calcium content. Subjects received an equal assortment of both flavors unless one single flavor was requested. Subjects were asked to return empty shake packets along with any unused product at 8 and 16 weeks to be used for measures of compliance.

**Anthropometry**

Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Novel Products Inc., Rockton, IL). Weight was measured to the nearest 0.1 kg using an electronic scale (Seca Bella 840, Columbia, MD). Waist circumference was measured to the nearest 0.1 cm with a flexible measuring tape as the distance around the smallest area of the trunk below the rib cage and above the umbilicus at the end of a normal exhalation.\(^{32}\) Height, weight and waist circumference measurements were made twice at each visit and average measures were used in the analysis. If two measurements differed by greater than 1.0 cm or 0.1 kg, three measurements were taken and the two closest values were averaged. For each subject, BMI was calculated as weight (kg)/height (m)\(^2\) and BMI-for-age percentiles were calculated using Epi Info 2000 and the CDC 2000 reference database.\(^{33}\)

**Body Composition**

Fat mass (FM; kg), fat-free soft tissue mass (FFST; kg), and % body fat (%BF) were measured at baseline, 8 and 16 weeks with dual energy X-ray absorptiometry (DXA; Delphi A; S/N 70467; Hologic Inc., Bedford, MA). The same technician conducted and analyzed all scans using Whole Body Analysis software, Hologic Inc., version 11.2. Quality assurance for FM, FFST, and %BF measured by DXA was carried out by calibration against a three-step soft tissue wedge provided by Hologic, Inc., composed of different thickness levels of aluminum and lucite, calibrated against stearic acid (100% fat) and water (8.6% fat). One-way random effects model,
single measure ICCs were calculated in five females, aged 18 to 30 years, scanned twice in our lab during a seven day period for FM, FFST, and %BF (all $R \geq 0.87$).

**Physical Activity**

Total daily energy expenditure (kcal/day) was estimated at baseline, 8 and 16 weeks using the validated Stanford Seven-Day Physical Activity Recall Questionnaire. The questionnaire distinguishes between activities by probing for specific physical activities and verifying statements regarding type, intensity and duration.

**Dietary Intake**

*Soy intake*

Dietary soy intake was assessed at baseline, 8 and 16 weeks using the validated Seattle Soy Food Frequency Questionnaire (Soy FFQ). The Soy FFQ estimated frequency of consumption and serving sizes of soy foods and food products, excluding study shakes, prior to and during the study.

*Energy, macronutrients and calcium*

Three-day diet records were used to estimate average daily energy, macronutrient and calcium intake. The three days included two weekdays and one weekend day. Baseline records were returned prior to shake distribution, and therefore did not include study shakes. At 8 and 16 weeks, participants were instructed to report study shake intake. Nutrient intakes generated from the 3-day diet records have been shown to correlate highly with direct observation ($r = 0.78-0.94$) in 9- to 10-year-old females. The 3-day diet records were analyzed by Food Processor for Windows version 8.0 (ESHA Research, Salem, OR). In our laboratory, one-way random effects model, average measure (i.e., 3 days) ICCs were conducted for dietary intake estimates in
females 6 to 10 years of age (N = 10), whose 3-day diet records were completed twice in a 2-week period, and are calculated for energy (R = 0.47) and calcium (R = 0.71).

**Statistical Analyses**

Data were analyzed using Statistical Analysis Software (SAS, Cary, NC, Version 9.1). Response of the dependent variables to the intervention (soy meal replacement or casein) was analyzed using a 2 x 3 repeated measures ANOVA assuming an unstructured variance-covariance matrix for repeated measures on the same subject. In addition, independent samples t-tests were used to test whether the treatment groups differed at baseline with respect to age, height, body weight, BMI, FM, FFST, waist circumference, energy expenditure, and soy, energy, macronutrient or calcium intake. Compliance was measured as percent of shakes consumed (# of empty shake packets/# of days in study). To adjust for compliance we followed the procedure recommended by Rochon\textsuperscript{37} in which we performed subgroup analyses of the treatment effect where the subgroups were defined based upon baseline characteristics of the subjects that were found to be predictive of compliance. In this case, a stepwise regression procedure found only baseline BMI to be marginally significantly associated with total compliance (p = 0.06). Therefore, we defined subgroups of subjects according to low, medium, and high baseline BMI, and reran the repeated measures ANOVA (2 treatments by 3 BMI levels by 3 time points) to determine whether there were different treatment effects based on baseline BMI group, which was predictive of compliance. In all group comparisons, we employed the intent-to-treat principle in which participant data were analyzed at each time point for which data were available according to the treatment group into which they were randomized, without regard to actual compliance or missing data due to study withdrawal after baseline.
RESULTS

Participant Characteristics

Baseline characteristics of the participants are presented in Table 1. The SOY and CAS groups did not differ significantly with respect to initial age, height, weight, BMI, FM, FFST, waist circumference, energy intake, energy expenditure, calcium, soy, or isoflavone intakes (Table 1). At 8 and 16 weeks, percent compliance was 72.1% and 68.6% in the soy group and 76.9% and 72.5% in the casein group, respectively. Percent compliance did not differ significantly between groups during the course of the study.

Body weight and composition

Mean values for body weight and composition are listed in Table 2. Response of body weight and composition variables to treatment did not differ significantly between groups (i.e., no group by time interaction), even when controlling for BMI, a significant predictor of compliance. However, body weight, FM, FFST, and %BF increased significantly over time in both groups (i.e., time effects p < 0.05; Table 2). Based on post-hoc pairwise comparisons, body weight and FM significantly increased from baseline to 8 weeks and from baseline to 16 weeks (p <0.05). From baseline to 8 weeks, %BF and percent trunk fat significantly increased (p <0.05). In contrast, waist circumference decreased from baseline to 8 weeks and from baseline to 16 weeks. No significant time effects were observed for percent leg fat or percent arm fat.

Dietary intake and energy expenditure

Mean values for dietary intake and energy expenditure are shown in Table 3. No group by time interactions were observed for any of the variables. Over time, participants in both groups reported significantly lower energy intake and greater energy expenditure. Moreover, both groups reported a decrease in percent total calorie intake from fat, and an increase in both...
percent energy intake from protein and overall calcium intake. Post-hoc pairwise comparisons demonstrated significant changes over time from baseline to 8 weeks and baseline to 16 weeks among these variables. No changes were observed over time in percent energy intake from carbohydrate or soy intake.

DISCUSSION

The primary finding of this randomized controlled trial was that consuming an isoflavone-rich soy protein-based meal replacement did not attenuate the small, but significant, weight and fat gains to a greater extent than a casein-based meal replacement in female college freshmen. Because the human trials to date that have investigated the effects of soy on body weight and composition differ with respect to study designs, subject age, gender, BMI, and importantly, the soy products used in the interventions, caution must be employed when making comparisons between the findings from this investigation and others.

The similar body weight and fat gain responses in the SOY and CAS groups are in agreement with findings from prior research using either a soy-based meal replacement,27 40 mg genistein29 or a soy protein isolate28 in older peri-and premenopausal women. Like participants in the present study, the mean BMI values for participants in two of these studies were considered in the normal range. The two trials demonstrating a weight-reducing effect with soy meal replacements included subjects who were classified as obese.24,25 Importantly, the control groups in these two trials did not receive a comparable placebo product, but rather, dietary counseling. Hence, the initial body weight or BMI of participants, the specific aim of the study (i.e., weight loss versus prevention of weight gain) and appropriate product control are likely important considerations with respect to addressing the impact of soy intake on body weight and composition.
The present study was unique in that the participants were late adolescent females within a narrow age range and similar menstrual status, and that the aim of the study was to prevent weight gain, not to promote weight loss. A strength of this study design was that all participants were blinded to the weight-gain prevention aim of the study. Compliance was 70.3% for SOY and 74.7% for CAS, similar to what has been documented in other meal replacement studies.38-41

Soy isoflavone intakes among participants, excluding study shakes, was higher than expected based on published data on U.S. soy intake. Data collected between 1991 and 1994 in the Framingham Study shows the dietary intake of phytoestrogens in healthy Caucasian postmenopausal women to be less than 1 mg per day.42 Subjects in the present study reported average intakes of 3.1-3.5 mg soy isoflavones per day. While this is higher than the average intake of women in the Framingham study, it is similar to more recent intakes reported in non-Asian women living in California of 2.9 mg per day.43 Some subjects in the present study reported consuming up to 40 mg soy isoflavones per day, comparable to intakes seen in some Asian populations.44 The higher levels and large variation in soy isoflavone intakes in the present study may have contributed to the lack of significant differences between isoflavone-rich and isoflavone-poor treatment groups.

Average weight gain among participants in this study (0.5 kg) was lower than previously reported in studies of first-year college students.30,31 Male and female students have been shown to gain 1.3 to 1.9 kg during their first semester in college. Therefore, it is plausible that both the soy and casein meal replacements used in the present study were equally effective in preventing additional weight gain among the freshmen college students. Both the SOY and CAS groups showed significant increases in percent energy intake from protein, and significant decreases in percent energy intake from fat over the course of the study. Decreasing percent energy intake
from fat has been shown to correlate strongly with weight loss and weight maintenance.\textsuperscript{10, 11} Therefore, the shift in macronutrient distribution may have contributed to an attenuation in weight gain.

The SOY and CAS shakes each contained 500 mg of calcium and mean calcium intakes, as expected, increased over the 16 weeks for both groups. It is possible that the higher calcium intakes impacted body weight and composition gains over the course of the study.\textsuperscript{45-47} Moreover, several reports have shown that keeping a prospective diet record can promote weight loss.\textsuperscript{48} This practice of completing three separate 3-day diet records may have also contributed to reduced weight gain in the present study. Self-selection bias may have existed among study participants in that women who chose to participate in a research study during their first year of college are more motivated to not gain weight, despite being blinded to the fact that this was a weight study. However, the only way to definitively determine the efficacy of soy in preventing body weight and fat gains would have been to include two additional groups to the existing study design: a non-protein, non-isoflavone group and a non-protein, high-isoflavone group.

It was surprising that participants in this study gained weight despite reporting decreased energy intakes and increased energy expenditures. It is possible that the protein content in the study shakes may have increased satiety or promoted a reduced glycemic effect for the meal, thereby contributing to a reduced energy intake in both groups at 8 and 16 weeks.\textsuperscript{49, 50} However, it is also possible that both energy intake and energy expenditure were under- and over-reported, respectively.\textsuperscript{51, 52} For example, energy intakes have been shown to be underreported in women by as much as 20-30\%.\textsuperscript{51} Energy expenditure may be over-reported when using the 7-day physical activity recall by as much as 31\%.\textsuperscript{52} Adjusting for underreporting of energy intake and
over-reporting of energy expenditure by as much as 30% for either measure, or 15% for both measures, could help to explain the discrepancies found in our data.

In summary, body weight and fat gains in SOY and CAS participants in the present study were similar between groups and were less than expected based on prior studies of college freshmen. Milk products containing casein have been reported to offer unique benefits with regard to weight loss. Results from this study do not suggest greater efficacy of either soy or casein-based meal replacements with regard to weight gain prevention. Soy- and casein-based meal replacement shakes were well tolerated by female college freshmen students and may be beneficial with respect to weight control, although further studies using non-protein, non-isoflavone and non-protein, high isoflavone control groups are warranted.

ACKNOWLEDGEMENTS

RDL, EML, ECH, RMB, CAB, and JLP were responsible for the study concept and design. RMB provided the soy and casein meal replacements. RDL, RAG, and JLP were responsible for the acquisition of the data. DBH conducted the statistical analyses. RDL, EML, NKP, and JLP were responsible for the interpretation of the data and drafting the manuscript. All authors contributed to the revision of the manuscript. This research was supported by The University of Georgia Research Foundation.
REFERENCES


**TABLE 1** Baseline descriptive characteristics of participants.

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<th>Variable</th>
<th>SOY (n = 62)</th>
<th>CAS (n = 58)</th>
<th>P value for difference¹</th>
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<td>18.2 (0.4)</td>
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<td>18.2 (0.4)</td>
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<td>Height (cm)</td>
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</tr>
<tr>
<td>Fat mass (kg)</td>
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<td>17.4 (4.3)</td>
<td>0.324</td>
<td>17.9 (4.7)</td>
</tr>
<tr>
<td>Fat-free soft tissue (kg)</td>
<td>41.5 (4.4)</td>
<td>41.0 (4.3)</td>
<td>0.520</td>
<td>41.2 (4.3)</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>71.9 (6.4)</td>
<td>71.6 (4.6)</td>
<td>0.800</td>
<td>71.8 (5.6)</td>
</tr>
<tr>
<td>Energy intake (kcal/d)</td>
<td>1824 (476)</td>
<td>1777 (414)</td>
<td>0.565</td>
<td>1801 (445)</td>
</tr>
<tr>
<td>Energy expenditure (kcal/d)</td>
<td>2105 (328)</td>
<td>2078 (292)</td>
<td>0.646</td>
<td>2085 (311)</td>
</tr>
<tr>
<td>Calcium intake (mg/d)</td>
<td>751 (381)</td>
<td>656 (213)</td>
<td>0.098</td>
<td>705 (313)</td>
</tr>
<tr>
<td>Soy intake (g/wk)²</td>
<td>111 (136)</td>
<td>153 (249)</td>
<td>0.289</td>
<td>131 (198)</td>
</tr>
<tr>
<td>Soy intake (servings/wk)²</td>
<td>1.4 (1.7)</td>
<td>2.0 (2.5)</td>
<td>0.185</td>
<td>1.7 (2.1)</td>
</tr>
<tr>
<td>Isoflavone intake (mg/wk)²</td>
<td>19.9 (33.3)</td>
<td>29.9 (56.6)</td>
<td>0.274</td>
<td>24.7 (46.1)</td>
</tr>
<tr>
<td>Daidzein (mg/wk)³</td>
<td>7.63 (13.0)</td>
<td>11.0 (20.0)</td>
<td>0.313</td>
<td>9.23 (16.8)</td>
</tr>
<tr>
<td>Genistein (mg/wk)²</td>
<td>10.2 (17.0)</td>
<td>16.3 (34.4)</td>
<td>0.249</td>
<td>13.1 (26.9)</td>
</tr>
<tr>
<td>Glycitein (mg/wk)²</td>
<td>2.10 (3.32)</td>
<td>2.58 (4.34)</td>
<td>0.514</td>
<td>2.32 (3.84)</td>
</tr>
</tbody>
</table>

Values are means (±SD).

¹ Tests of significance between treatment groups at baseline are based on two-tailed independent samples t-tests.

² Dietary soy and isoflavone intakes are based on the Seattle Soy Food Frequency Questionnaire.³⁵
**TABLE 2** Estimated mean body weight and composition values in soy and casein meal replacement groups at baseline, 8 and 16 weeks.

<table>
<thead>
<tr>
<th>Variable</th>
<th>SOY (n = 62)</th>
<th>CAS (n = 58)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>8 weeks</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>8 weeks</td>
</tr>
<tr>
<td>Body weight (kg)(^1,2,3)</td>
<td>60.8 (1.0)</td>
<td>61.2 (1.0)</td>
</tr>
<tr>
<td>Fat mass (kg)(^1,2,3)</td>
<td>18.3 (0.6)</td>
<td>18.6 (0.6)</td>
</tr>
<tr>
<td>Fat-free soft tissue (kg)(^1)</td>
<td>41.5 (0.5)</td>
<td>41.3 (0.6)</td>
</tr>
<tr>
<td>Body fat (%)(^1,2)</td>
<td>29.1 (0.6)</td>
<td>29.6 (0.6)</td>
</tr>
<tr>
<td>Trunk fat (%)(^1,2)</td>
<td>24.8 (0.7)</td>
<td>25.4 (0.7)</td>
</tr>
<tr>
<td>Leg fat (%)</td>
<td>35.4 (0.6)</td>
<td>35.7 (0.6)</td>
</tr>
<tr>
<td>Arm fat %</td>
<td>33.8 (0.7)</td>
<td>33.7 (0.7)</td>
</tr>
<tr>
<td>Waist circumference (cm)(^1,2,3)</td>
<td>71.9 (0.7)</td>
<td>71.2 (0.7)</td>
</tr>
</tbody>
</table>

Values are estimated means (SE). Tests of significance between treatment groups over 16 weeks are based on repeated measures ANOVA.

\(^1\) There is a significant time effect (P < 0.05).

\(^2\) There is a significant time effect from baseline to 8 weeks (P < 0.05).

\(^3\) There is a significant time effect from baseline to 16 weeks (P < 0.05).
TABLE 3  Estimated mean dietary intake and energy expenditure values in soy and casein meal replacement groups at baseline, 8 and 16 weeks.

<table>
<thead>
<tr>
<th>Variable</th>
<th>SOY (n = 62)</th>
<th></th>
<th></th>
<th>CAS (n = 58)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>8 weeks</td>
<td>16 weeks</td>
<td>Baseline</td>
<td>8 weeks</td>
<td>16 weeks</td>
</tr>
<tr>
<td>Energy intake (kcal/d)¹²³⁵</td>
<td>1824 (57)</td>
<td>1701 (74)</td>
<td>1651 (61)</td>
<td>1777 (59)</td>
<td>1676 (73)</td>
<td>1661 (63)</td>
</tr>
<tr>
<td>Energy expenditure (kcal/d)¹²³</td>
<td>2105 (40)</td>
<td>2150 (39)</td>
<td>2155 (55)</td>
<td>2078 (41)</td>
<td>2131 (40)</td>
<td>2175 (56)</td>
</tr>
<tr>
<td>Fat (% of kcal/d)¹²³⁵</td>
<td>33.2 (0.9)</td>
<td>30.6 (0.9)</td>
<td>31.3 (0.9)</td>
<td>32.9 (0.9)</td>
<td>28.8 (0.9)</td>
<td>29.9 (0.9)</td>
</tr>
<tr>
<td>Protein (% of kcal/d)¹²³⁵</td>
<td>15.3 (0.4)</td>
<td>18.4 (0.6)</td>
<td>18.4 (0.6)</td>
<td>15.6 (0.4)</td>
<td>19.9 (0.6)</td>
<td>18.9 (0.6)</td>
</tr>
<tr>
<td>Carbohydrate (% of kcal/d)⁴</td>
<td>51.7 (1.0)</td>
<td>52.4 (1.0)</td>
<td>51.5 (1.1)</td>
<td>52.2 (1.0)</td>
<td>52.2 (1.0)</td>
<td>52.6 (1.1)</td>
</tr>
<tr>
<td>Calcium intake (mg/d)¹²³⁵</td>
<td>751 (40)</td>
<td>1079 (49)</td>
<td>1000 (47)</td>
<td>656 (41)</td>
<td>1089 (49)</td>
<td>1031 (48)</td>
</tr>
<tr>
<td>Soy intake (g/wk)⁴</td>
<td>111 (136)</td>
<td>212 (526)</td>
<td>131 (305)</td>
<td>153 (249)</td>
<td>235 (499)</td>
<td>102 (106)</td>
</tr>
<tr>
<td>Soy intake (servings/wk)⁴</td>
<td>1.4 (1.7)</td>
<td>1.4 (1.8)</td>
<td>1.1 (1.9)</td>
<td>2.0 (2.5)</td>
<td>2.2 (3.1)</td>
<td>2.0 (2.5)</td>
</tr>
<tr>
<td>Isoflavone intake (mg/wk)⁴</td>
<td>19.9 (33.3)</td>
<td>15.0 (23.6)</td>
<td>16.5 (29.4)</td>
<td>29.9 (56.6)</td>
<td>31.3 (60.2)</td>
<td>27.7 (53.9)</td>
</tr>
<tr>
<td>Daidzein (mg/wk)⁴</td>
<td>7.6 (13.0)</td>
<td>5.4 (8.1)</td>
<td>6.3 (10.9)</td>
<td>11.0 (20.0)</td>
<td>11.5 (21.9)</td>
<td>9.7 (17.5)</td>
</tr>
<tr>
<td>Genistein (mg/wk)⁴</td>
<td>10.2 (17.0)</td>
<td>8.0 (13.2)</td>
<td>8.3 (15.2)</td>
<td>16.3 (34.4)</td>
<td>17.0 (36.2)</td>
<td>16.2 (36.4)</td>
</tr>
<tr>
<td>Glycitein (mg/wk)⁴</td>
<td>2.1 (3.3)</td>
<td>1.6 (2.4)</td>
<td>1.9 (3.5)</td>
<td>2.58 (4.34)</td>
<td>2.8 (4.7)</td>
<td>1.7 (1.5)</td>
</tr>
</tbody>
</table>

Values are estimated means (SE). Tests of significance between treatment groups over 16 weeks are based on repeated measures ANOVA.

¹ There is a significant time effect (P < 0.05).

² There is a significant time effect from baseline to 8 weeks (P < 0.05).

³ There is a significant time effect from baseline to 16 weeks (P < 0.05).

⁴ Dietary soy and isoflavone intakes are based on the Seattle Soy Food Frequency Questionnaire,³⁵ and do not include study shakes.

⁵ Energy, macronutrient, and calcium intakes include study shakes at 8 and 16 weeks.
CHAPTER 4
SUMMARY AND CONCLUSIONS

Late adolescence is a significant period of human growth and maturation, where unique changes in body weight and composition are occurring and many adult patterns are established. The freshman year of college has been identified as a critical period during which a significant increase in overweight occurs among young adults, placing these individuals at greater risk for adverse health effects. Therefore, it is important to explore innovative dietary strategies to prevent and treat childhood and adolescent overweight.

The view that dietary soy intake may be a useful strategy for preventing or attenuating weight gain is largely based on animal, cell culture, and observational data. Human intervention trials examining the effects of soy protein and isoflavones on body weight and composition are limited due to the wide variety of study designs, control groups and soy treatments used. The present 16-week study was conducted in late adolescent females using isoflavone-rich soy protein (SOY) and casein (CAS) as a control in order to assess changes in body weight and composition between groups during the freshman year of college (Chapter 3). The primary finding of this study was that the soy protein meal replacement did not attenuate weight and fat gain to a greater extent than the casein-based meal replacement.

When attempting to determine if gains in the present study were different within groups over time, it appeared that both SOY and CAS showed similar increases in body weight, fat mass, fat-free soft tissue, percent body fat, and percent trunk fat throughout the 16-week time period. The possibility exists that both soy and casein are effective in preventing excess weight
gain, as average weight gain in the present study was less than in other studies in first-year college students. However, due the lack of a non-protein control, conclusions regarding overall effects of soy intake on weight gain during the freshman year of college cannot be made.

No differences were observed between groups for height, weight or BMI during the 16-week testing period, nor were differences observed when assessing energy intake, energy expenditure or calcium intake. The SOY and CAS groups showed significant increases in percent energy intake from protein, and decreases in percent energy intake from fat over the course of the study. Decreasing percent energy intake from fat has been shown to correlate strongly with weight loss and weight maintenance, and this shift in macronutrient distribution may have contributed to an attenuation in weight gain.

The SOY and CAS shakes each contained 500 mg of calcium and mean calcium intakes, as expected, increased over the 16 weeks for both groups. It is possible that the higher calcium intakes impacted body weight and composition gains over the course of the study. Although average calcium intake in both groups increased over time, amounts consumed were below the AI (1300 mg per day).

It was surprising that participants in this study gained weight despite reporting decreased energy intakes and increased energy expenditures, and it is not unlikely that both energy intake and energy expenditure were under- and over-reported, respectively. Adjusting for underreporting of energy intake and over-reporting of energy expenditure by as much as 30% for either measure, or 15% for both measures, could help to explain the discrepancies found in our data.

In conclusion, body weight and fat gains in SOY and CAS participants in the current study were similar among groups and were less than expected based on prior studies of college
freshmen. Soy-and casein-based meal replacement shakes were well tolerated by female college freshmen and may be beneficial with respect to weight control in this population. If overweight can be prevented during critical periods such as the freshman year of college, this may translate into a substantial reduction in adult obesity risk. In order to assess the role of soy protein and isoflavone intake on body weight and composition changes, future studies using non-intervention and non-protein, high isoflavone control groups are warranted.
APPENDIX A: TELEPHONE SCREENING QUESTIONNAIRE
Soy, Bone and Health in College Females

Telephone Screening Questionnaire
This interview should only take approximately ten minutes:

Date: ____________ Time: ____________ Screen completed by: ____________

Participant’s name: ______________________________________________________

Address: ________________________________________________________________

Zip Code: ________________________________________________________________

Daytime Phone Number: _________________________________________________

How did you hear about the study? _________________________________________


2. Age _____years DOB: _____ / _____ / ______
   mm dd yy

3. What year are you in school? ________________

4. Height ____ft____ in

5. Weight___________ lbs

6. Have you lost or gained weight in the past 3 months? YES or NO; circle one
   If yes, how much? ____________ lbs

7. Are you a student athlete? YES or NO; circle one
   if the answer to #7 is YES, please tell the potential volunteer that she does not qualify
   for the study.

8. How physically active are you? ________ mins/day, _________day/week

9. Number of periods in last 6 months______________

10. Are you taking any medications? YES or NO; circle one
    If yes, what medication(s)? _____________________________________________
        (check specifically for birth control pills, Adderall, Ritalin, and steroid medications)
How long have you been taking the above medication(s)? ______________

11. Do you have any food allergies? ________________________________
   (check specifically for soy, milk and chocolate) - if the answer to #11 includes
   allergies to soy, milk or chocolate, please tell the potential volunteer that she does not
   qualify for the study.

12. Do you have any of the following diseases or conditions:
   if the answer to any of the questions from 12a-12h is YES, please stop and tell the
   potential volunteer that she does not qualify for the study.

   a) Diabetes YES ___ NO _______
   b) Thyroid Disease YES ___ NO _______
   c) Soy Allergies YES ___ NO _______
   d) Pregnancy YES ___ NO _______
   e) Gall bladder Disease YES ___ NO _______
   f) Kidney Disease YES ___ NO _______
   g) Osteoporosis YES ___ NO _______
   h) Psychological Illness YES ___ NO _______

13. Are you taking an herbal or dietary supplement? YES or NO; circle one
    If yes, what supplement(s), how much and how often? __________________________
    __________________________

    If yes, would you be willing to stop taking the supplement? YES or NO; circle one

14. Are you a vegetarian? YES or NO; circle one

15. Are you soy food consumer? YES or NO; circle one

    How many servings per week? ____________________________
    If the answer is YES to #15, ask if she would be willing to limit her soy intake to less
    than one serving per week for the 16-week duration of the study. If the reply is yes,
    proceed to the next question. If the reply is no, please tell the potential volunteer that
    she does not qualify for the study.

16. In this study, all participants must provide blood and urine samples 3 times (at the
    start, at 8 weeks, and at 16 weeks), and each testing session could last up to 1 ½-2 ½
    hours. Are you willing to do this? YES or NO; circle one

17. If selected to participate, what mornings during the week would you be available to
    come to the UGA Bone and Body Composition Lab, located in Dawson Hall, for testing?
    M____ T____ W_____ Th_____ F_____ S _____
Morning testing will begin as early as 7 am, will involve a fasting blood draw and will take approximately 1 hour and 30 minutes.

“That’s the end of our telephone screening. We will review this and determine your eligibility for the study. We will get back to you within a week to let you know the status of your eligibility. Do you have any additional questions for me?”

Make sure the potential volunteer has contact numbers for future questions.
Consent Form

I agree to participate in the research study entitled "Soy, Bone and Health in College Females," which is being conducted by Dr. Richard Lewis, Department of Foods and Nutrition, The University of Georgia. Dr. Richard Lewis can be reached at (706) 542-4901.

The purpose of this study is to determine the effect of isoflavone-rich soy protein on bone parameters in college-age women. In order to make this study a valid one, some information about my participation will be withheld until after the study. Upon completion of the final testing procedures at the 16-week testing session, the Investigator will inform me about this information.

The following points have been explained to me:

1) The reason for this research is to determine if isoflavone-rich soy protein, eaten daily as a food for 16 weeks will safely promote bone health in college-aged women. The benefits that I may expect from my participation are to receive payments of $25 for completing each of three visits to the UGA Clinical and Sports Nutrition Lab for measurements that have been explained to me. The meal supplement will be provided without cost to me. I will also learn about my bone density, diet and activity habits without cost. However, if abnormalities are found in bone density measurements, I will be notified and advised to contact my physician.

2) The procedures are as follows:

(Single Center Trials)
I will be one of approximately 120 participants to be asked to participate in this trial. To qualify for the study, I must:

- Be 18-19 years of age, and a college female;
- Not be underweight based on my body weight and height;
- Have had regular menses for the past 6 months;
- Be non-vegan (consumes animal foods);
- Be in good health (e.g. no evidence of thyroid, gall bladder, kidney, or liver disease; no history of psychological illness; no history of bone fractures; no established osteoporosis; no diabetes);
- Not be on any dietary, exercise, or drug treatment for high blood cholesterol;
- Agree not to begin any dietary, drug, or exercise treatment during the 16-week study period unless it is medically necessary;
- Must agree to come to the Clinical and Sports Nutrition Laboratory at the University of Georgia for blood work, bone density testing and to complete several questionnaires;
- Must agree to not eat any food after dinner in the evening before coming to the laboratory for blood tests.
- During my visit the following procedures will be done:
  - My weight, height, and vital signs will be taken;
A second void urine sample will be collected at baseline, 8-weeks, and 16-weeks; 
A blood sample (up to 50 mL) will be taken from an arm vein; 
The total amount of bone in my body will be measured with a bone-scanning machine that will take approximately 20 minutes. The measurement will be within the normal ranges based on young adult women; 
I will complete the following questionnaires:
  - Three-day Diet Record to estimate my energy intake;
  - A Soy Food Questionnaire to document my soy intake;
  - A Menstrual Cycle Questionnaire to document my menstrual activity;
  - A Physical Activity Questionnaire to document my physical activity;
  - A Satiety Questionnaire to document my satiety after eating the meal supplement;
  - A Beck Depression Inventory, a Profile of Mood States and a Daily Symptom Report to document my feelings throughout the study.

If any of the results from the blood test are not within the normal ranges, I will not be enrolled in the study. If I qualify for the study, I will receive a 8-week supply of meal supplements. I will be asked to drink the daily meal supplement once a day for a period of 16 weeks. I will be educated by the Investigator on how to replace part or all of my food choices with this meal supplement, in order to eat the same number of calories that I would usually eat at breakfast during these 16 weeks.

I will return to the Laboratory in 8 weeks to pick up a new supply of meal supplements. All the supplements will be provided free of charge. Bone measurements will be repeated on me at my 16-week visit, and clinical blood chemistries will be repeated on me at my 8-week and 16-week visits. I will complete the soy food and physical activity questionnaires and the Lifestyle/Health Questionnaire during my 8 and 16-week visits. I will complete a 3-Day Food Diary 3 times during the course of the study. I also understand that the placebo supplement will not contain any soy protein or isoflavones. I will have no choice on the supplement I receive. I will be assigned randomly to receive either the placebo or soy and I will not be told which supplement I am receiving during the study.

3) The following discomforts and risks have been explained in detail to me:

Other studies with soy isoflavones lasting 6 months did not show any adverse effects, but the long-term effect is not known. The amount of isoflavones to be used in this study is less than 1/4 of that used in these studies.

The total bone scan takes approximately 50 minutes. I will be exposed to 15-65.61 μSv of radiation during each measurement. The radiation exposure of an adult chest X-ray is approximately 500 to 800 μSv. However, any illness or injury not related to the study is not the responsibility of the investigator or the University of Georgia. If I am pregnant...
or could possibly become pregnant, I realize that the radiation involved in this study could be harmful to a fetus. I have no intentions of becoming pregnant during this study. If I am not sure about potential pregnancy, I will notify the Investigator and be provided a pregnancy test to take in the privacy of my own home. If I do become pregnant, I may maintain confidentiality by electing not to notify the Investigator, however I will voluntarily withdraw from the study. If I do become pregnant and decide to notify the Investigator, I understand that I would be told that I cannot participate in the study, and I will receive information about and a referral to the UGA Health Center’s Women’s Clinic.

No risk is expected, but I may experience some discomfort or stress when my blood is drawn. The risks of drawing blood from my arm include the unlikely possibilities of a small bruise or localized infection, bleeding, and fainting. These risks will be reduced in the following ways: my blood will be drawn only by a qualified and experienced phlebotomist who will follow standard sterile techniques, who will observe me after the needle is withdrawn, and who will apply pressure to the blood-draw site. In the event that I have any health problems associated with the blood draws, my insurance or I will be responsible for any related medical expenses. My blood will not be tested for HIV-AIDS. I understand that these questions and blood tests are not for diagnostic purposes. If I have questions about my test results I should see a physician.

Less than 1% of American adults have allergies to soy protein. Allergy symptoms are those associated with most food allergies such as abdominal discomfort (nausea, diarrhea, or constipation), and/or a mild skin rash. Allergy symptoms cease with discontinuation of soy products. I may withdraw from the study at any time if I experience food allergy symptoms.

It is possible that the completion of the depression questionnaire may pose some undue stress or psychological harm. I realize that if the results of the depression questionnaire warrants a referral to a mental health professional, I will receive information about and a referral to the UGA Health Center’s Counseling and Psychological Services and a list of local mental healthcare providers. I understand that the results of the depression questionnaire and potential referral to a mental health professional will remain confidential.

The risks of participating in this study are minimal and the data will increase our knowledge on how safe and effective soy protein and isoflavones are in increasing bone health in college-age women. Therefore, the benefits of the study are believed to equal or outweigh the very minimal risk.

If I have questions, Dr. Lewis, the research coordinator, or graduate student will be available to talk with me.

4) The results of this participation will be confidential, and will not be released in any individually identifiable form without my prior consent, unless otherwise required by law. The data generated from my tests will be stored on the computers in the lab room 279,
Dawson Hall. All data will be associated with a code number and not my name. There will be a list of participant’s names and corresponding code numbers, but no data with that list. My data will be destroyed on or before September 1, 2010. The information collected will be used for research purposes.

I have been informed that there may be unknown risks/discomforts involved, and that I will receive any new information discovered during the course of the study concerning significant treatment findings that may affect my willingness to continue to participate.

In the event of injury resulting from this research, the University of Georgia and/or the laboratory of Dr. Richard Lewis are not able to offer financial compensation or to absorb the costs of medical treatment. However, necessary facilities, emergency treatment and professional services will be available to research participants, just as they are to the community in general. My signature below acknowledges my voluntary participation in this research project. Such participation does not release the investigator(s), institution(s), sponsor(s), or granting agency(ies) from their professional and ethical responsibility to me.

My participation is voluntary and I may refuse to participate or may discontinue my participation AT ANY TIME, without penalty, loss of benefits, or change in my present or future care. The investigator has the right to withdraw me from the study at any time. My withdrawal from the study may be for reasons related solely to me (e.g. not following study-related directions from the Investigator; a serious adverse event reaction) or because the entire study has been terminated. The Sponsor has the right to terminate the study or the Investigator’s participation in the study at any time.

The investigator or his designee has answered all of my questions. If I have additional questions during the course of this study about the research or my rights as a research participant, I may address them to the University of Georgia Review Board for Human Subject Research Office at (706) 542-3199. In the event of a research-related injury or if any other problems arise, I may contact Richard D. Lewis, Ph.D., at 706-542-4901.

I HAVE READ THE INFORMATION PROVIDED ABOVE (OR HAVE HAD IT READ TO ME) AND HAD MY QUESTIONS ANSWERED TO MY SATISFACTION. I VOLUNTARILY AGREE TO PARTICIPATE IN THIS STUDY. I WILL RECEIVE A COPY OF THIS CONSENT FORM.

________________________  ___________
Signature of Investigator   Date
Richard D. Lewis
706-542-4901
rlewis@fcs.uga.edu

________________________  ___________
Signature of Participant   Date

PLEASE SIGN BOTH COPIES OF THIS FORM. KEEP ONE AND RETURN THE OTHER TO THE INVESTIGATOR.

Additional questions or problems regarding your rights as a research participant should be addressed to The Chairperson, Institutional Review Board, University of Georgia, 612 Boyd Graduate Studies Research Center, Athens, Georgia 30602-7411; Telephone (706) 542-3199; E-Mail Address IRB@uga.edu
Soy, Bone and Health in College Females

Consent Form for the Use of the Hologic Delphi A X-Ray Bone Densitometer and XCT 2000 pQCT

Are you pregnant or do you think you might be pregnant?  YES  NO
*If yes, please do not participate in this study using the Delphi A bone densitometer and the XCT 2000 pQCT.

I, _________________________________, am hereby giving my consent to be used for research conducted by Dr. Richard D. Lewis, University of Georgia, Foods and Nutrition Department, 279 Dawson Hall.

I understand that by giving my consent I am agreeing to be scanned on the Hologic Bone Delphi A X-Ray Densitometer and on the XCT 2000 peripheral Quantitative Computer Tomography machine. Both of these instruments use a low dose x-ray to determine bone mineral density and body composition.

I understand that the Hologic Delphi A X-Ray Bone Densitometer uses a very low level of x-ray and that under most operating conditions, the entrance dose to the patient is 0.5mRem-10mRem. This equals about 3% to 30% of the exposure of a standard chest x-ray and is of no danger to me.

I understand that the XCT 2000 pQCT uses a very low level of x-ray and that under most operating conditions, the maximum entrance dose to the patient is less than 1 mRem.

I understand that The University of Georgia is responsible for my safety during my participation in this study. However, any illness or injury not related to this study is not the responsibility of the investigator or the University of Georgia.

I understand that my participation is entirely voluntary. I can withdraw my consent at any time without penalty and have the results of my participation returned to me, removed from records or destroyed.

________________________________________________________________________

Signature of Investigator          Date          Signature of Participant          Date
APPENDIX C: SUPPLEMENT INSTRUCTIONS
**FOCUS Study Supplement Instructions**

Use the shaker cup or a blender to mix this supplement with 8 oz or more of water or another calorie-free liquid, or simply add it to something you already consume.

*BE SURE TO REMOVE ANOTHER 240 KCAL ITEM FROM YOUR DIET.*

Examples of calorie-free liquids that can be mixed with supplement:
- Water (or ice)
- Diet soda
- Crystal Light

Examples of things you may already consume that can be mixed with supplement:
- Oatmeal
- Cream of Wheat
- Fruit juice or low-calorie fruit juice
- Milk
- NOT Soymilk

- If you forget to drink a shake one day, **do not** drink two the next day.
- Save all empty packets and bring them back to us at your next visit.
- Bring all packets you did not consume back at your next visit.

If you have any questions or concerns, please call the UGA Bone and Body Composition Lab at 542-4918.

Thank you for participating!
APPENDIX D: 3-DAY DIET RECORD
QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.
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are needed to see this picture.
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QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.
SOY FOOD QUESTIONNAIRE

Participant ID ____________________________
Date ____________________________
Time Period ____________________________

This section is about how often you usually eat specific soy foods. Please think about what you usually ate during the last 3 months.

First: Mark the column to show how often, on the average, you ate the food.
Second: Mark your usual serving size as small, medium or large.

Please note:
- A small serving is about one-half (½) the medium serving size, or less.
- A large serving is about one-and-a-half (1 ½) times the medium serving size, or more.
- If you never ate a food, mark "Never or less than once per month," and omit the serving size.
- Please do not skip any foods.

Sample: This person ate a veggie soy burger about twice per month and never ate vegetarian cold cuts.

<table>
<thead>
<tr>
<th>TYPE OF FOOD</th>
<th>HOW OFTEN YOU ATE THE FOOD OVER THE LAST 3 MONTHS</th>
<th>AMOUNT</th>
<th>MEDIUM SERVING SIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NEVER or less than once per month</td>
<td>1 per month</td>
<td>2 - 3 per month</td>
</tr>
<tr>
<td>Veggie soy burger</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetarian cold cuts</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MAIN DISHES, LUNCH ITEMS</th>
<th>HOW OFTEN YOU ATE THE FOOD OVER THE LAST 3 MONTHS</th>
<th>AMOUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NEVER or less than once per month</td>
<td>1 per month</td>
</tr>
<tr>
<td>Tofu (all types), including low-fat, flavored, marinated, smoked</td>
<td>4 oz. (4 1” chunks)</td>
<td></td>
</tr>
<tr>
<td>Tempeh, all types</td>
<td>4 oz. (4 1” chunks)</td>
<td></td>
</tr>
<tr>
<td>Tofu or soy breakfast sausage, bacon, or other breakfast meat</td>
<td>2 links or 3 strips</td>
<td></td>
</tr>
<tr>
<td>Tofu or soy cold cuts, hot dogs, or other deli meat substitutes</td>
<td>2 dogs or 2 slices</td>
<td></td>
</tr>
<tr>
<td>Veggie soy or tofu burger, ground meat substitute (TVP), soy or tofu chicken or turkey</td>
<td>4 oz.</td>
<td></td>
</tr>
<tr>
<td>Packaged mixed dishes with soy or tofu, such as lasagna, burritos, or stir fry</td>
<td>1 meal</td>
<td></td>
</tr>
<tr>
<td>Miso soup</td>
<td>1 cup or 1 med. Bowl</td>
<td></td>
</tr>
<tr>
<td>SOY MILK AND SOY &quot;DAIRY&quot; PRODUCTS</td>
<td>HOW OFTEN YOU ATE THE FOOD OVER THE LAST 3 MONTHS</td>
<td>AMOUNT</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-----------------------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>Soy milk, regular or low-fat, plain or flavored</td>
<td>NEVER or less than one per month</td>
<td>1 cup</td>
</tr>
<tr>
<td>Soy cheese, such as cheddar, mozzarella, cream cheese or parmesan. Include foods made with soy cheese.</td>
<td>1 slice or 1 oz.</td>
<td></td>
</tr>
<tr>
<td>Soy yogurt, all types</td>
<td>1 cup</td>
<td></td>
</tr>
<tr>
<td>Soy ice cream, tofu, or other soy desserts</td>
<td>½ cup</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>OTHER SOY FOODS</th>
<th>AMOUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooked soybeans or edamame (green soybeans)</td>
<td>½ cup</td>
</tr>
<tr>
<td>Roasted soy nuts</td>
<td>2 Tbs. or 1 handful</td>
</tr>
<tr>
<td>Soy sauce, tamari, teriyaki sauce, Szechwan sauce, hoisin</td>
<td>1 Tablespoon</td>
</tr>
<tr>
<td>Natto</td>
<td>1 Tablespoon</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SUPPLEMENTS</th>
<th>AMOUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid nutrition drinks containing soy or soy protein, such as Odwalla Future Shake, Ensure Plus</td>
<td>1 cup</td>
</tr>
<tr>
<td>Soy protein powders, such as performance or body builder powders</td>
<td>1-2 scoops</td>
</tr>
<tr>
<td>High energy bars or diet bars containing soy or soy protein</td>
<td>1 bar</td>
</tr>
<tr>
<td>Pills containing soy, isoflavones, or &quot;natural&quot; estrogen</td>
<td>1 pill</td>
</tr>
</tbody>
</table>

**Other (Please write other soy foods you have eaten; enter amount you ate in Medium Serving Size column)**

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APPENDIX F: 7-DAY PHYSICAL ACTIVITY RECALL
7-DAY PHYSICAL ACTIVITY RECALL QUESTIONNAIRE

1. On the average, how many hours did you sleep each night during the last 5 weekday nights (Sunday-Thursday)? Record to nearest quarter-hour.

   Hours: _________   Minutes: _________

2. On the average, how many hours did you sleep each night last Friday and Saturday nights?

   Hours: _________   Minutes: _________

3. First let’s consider moderate activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these moderate activities or others like them? Please tell me to the nearest half-hour.

   Hours: _________   Minutes: _________

4. Last Saturday and Sunday, how many hours did you spend on moderate activities and what did you do? (Can you think of any other sport, job, or household activities that would fit in this category?)

   Hours: _________   Minutes: _________

5. Now let’s look at hard activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these hard activities or others like them? Please tell me to the nearest half-hour.

   Hours: _________   Minutes: _________

6. Last Saturday and Sunday, how many hours did you spend on hard activities and what did you do? (Can you think of any other sport, job, or household activities that would fit in this category?)

   Hours: _________   Minutes: _________

7. Now let’s look at very hard activities. What activities did you do and how many total hours did you spend during the last 5 weekdays doing these very hard activities or others like them? Please tell me to the nearest half-hour.

   Hours: _________   Minutes: _________

8. Last Saturday and Sunday, how many hours did you spend on very hard activities and what did you do? (Can you think of other sport, job, or household activities that would fit in this category?)

   Hours: _________   Minutes: _________
Physical Activity List

**Moderate Activities**

*Occupational Tasks:*
9. Delivering mail or patrolling on foot
10. House painting
11. Truck driving (making deliveries – lifting and carrying light objects)

*Household activities:*
1. Raking the lawn
2. Sweeping and mopping
3. Mowing the lawn with a power mower
4. Cleaning windows

*Sports Activities (Actual playing time)*
1. Volleyball
2. Ping pong
3. Brisk walking for pleasure or to work (3 mph or 20 min/mile)
4. Golf-walking and pulling or carrying clubs
5. Calisthenic exercises

**Hard Activities**

*Occupational Tasks:*
1. Heavy carpentry
2. Construction work – doing physical labor

*Household Tasks:*
1. Scrubbing floors

*Sports Activities (Actual playing time):*
1. Doubles tennis
2. Disco, Square, or Folk dancing

**Very Hard Activity**

*Occupational Tasks:*
1. Very Hard physical labor – digging or chopping with heavy tools
2. Carrying heavy loads, such as bricks or lumber

*Sports Activities (Actual playing time):*
1. Jogging or swimming
2. Singles tennis
3. Racquetball
4. Soccer
5. Aerobics
6. Stair climbing
7. Weight training
8. Gymnastics
<table>
<thead>
<tr>
<th>Activity</th>
<th>Time Spent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
Worksheet for Calculating Daily Energy Expenditure

1. Add up all the hours of sleep and naps you had.
2. Multiply the total number of hours of sleep and naps (line 1) by 1.
   \[ X = \]
3. Add up the total number of hours spent in moderate activity.
4. Multiply the hours spent in moderate activity (line 3) by 4.
   \[ X = \]
5. Add up the total number of hours spent in hard activity.
6. Multiply the hours spent in hard activity (line 5) by 6.
   \[ X = \]
7. Add up the total number of hours spent in very hard activity.
8. Multiply the hours spent in very hard activity (line 7) by 10.
   \[ X = \]
9. Add up the figures in lines 1, 3, 5, and 7.
   \[ (1 + 3 + 5 + 7) = \]
10. Hours spent in light activity is equal to 24 hours minus the hours in lines 1, 3, 5, and 7.
    \[ 24 - (1 + 3 + 5 + 7) = \]
11. Multiply the figure in line 10 by 1.5.
    \[ X = \]
12. Add up the figures in lines 2, 4, 6, 8, and 11.
    \[ (2 + 4 + 6 + 8 + 11) = \]
13. The figure you arrived at in line 12 is the total kilocalories per kilogram of body weight expended per day.
    \[ \text{cal} \cdot \text{kg}^{-1} \cdot \text{day}^{-1} = \]
14. To calculate the total number of calories you expended in one day, multiply your total body weight in kilograms (weight in pounds ÷ 2.2046 = kilograms) by the figure in line 13. Body weight (kg) \( X \text{cal} \cdot \text{kg}^{-1} \cdot \text{day}^{-1} = \text{total calories expended} = \)

The following are some average kcal \( \text{kg}^{-1} \cdot \text{day}^{-1} \) for individuals of different ages:

- **17-19 years**
  - male = 44
  - female = 35

- **20-29 years**
  - male = 40
  - female = 35

- **30-39 years**
  - male = 38
  - female = 33

- **40-49 years**
  - male = 37
  - female = 31

- **50-59 years**
  - male = 36
  - female = 30

- **60-69 years**
  - male = 34
  - female = 29
APPENDIX G: ANTHROPOMETRIC DATA RECORDING SHEET
Subject Information Sheet Anthropometrics/DXA

Project: FOCUS Isoflavone Study

Subject ID: ___________ Visit Number:_______Visit Date:_________

Race/Ethnicity: ________

DOB: Month ________ Day ________ Year ________

Weight (kg):

Measure 1   Measure 2   Average of 1 and 2

Height (cm):

Measure 1   Measure 2   Average of 1 and 2

Waist Circumference (cm):

Measure 1   Measure 2   Average of 1 and 2

BMI:

<table>
<thead>
<tr>
<th>DXA operator use</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ WB</td>
</tr>
<tr>
<td>□ Hip</td>
</tr>
<tr>
<td>□ AP Spine</td>
</tr>
</tbody>
</table>

Scan date: 
Completed by: 
initials of operator

Comments:________________
Soy, Bone and Health in College Females

Debriefing Statement

One of the primary goals of this study was to determine the effects of soy intake on body weight and body composition. We did not inform you of this at the beginning of the study because of the possibility that your knowledge may have caused you to alter your behavior in a way that would have affected the results of the study.