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Weight gain in college females is not prevented by isoflavone-rich soy protein: a randomized controlled trial

Paige K. Berger[a], *Jessica L. Principe*[a], *Emma M. Laing*[a], *E.C. Henley*[a], *Norman K. Pollock*[b], *Ruth G. Taylor*[a], *Robert M. Blair*[c], *Clifton A. Baile*[a], *Daniel B. Hall*[d], *Richard D. Lewis*[a,*]

[a] *Department of Foods and Nutrition, The University of Georgia, Athens, GA, USA*
[b] *Department of Pediatrics, Georgia Regents University, Augusta, GA, USA*
[c] *Physicians Pharmaceuticals, Inc., Winston-Salem, NC, USA*
[d] *Department of Statistics, The University of Georgia, Athens, GA, USA*

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ABSTRACT

Human clinical trials targeted at preventing gains in body weight using soy protein and isoflavones are limited to adults and yield conflicting results. We hypothesized that daily intake of soy protein/isoflavones would attenuate gains in body weight to a greater extent than a casein-based control in 18 to 19 year-old females. To test this hypothesis, we conducted a randomized, double blind, placebo-controlled trial over 16 weeks to examine the effects of a soy protein/isoflavone-based meal replacement (experimental group) versus a casein-based meal replacement (control group) on body weight and body composition variables in female college freshmen (N = 120). Fat mass (FM), fat-free soft tissue mass (FFST), and percent body fat (%BF) were measured using dual energy X-ray absorptiometry (DXA; Delphi A). Repeated measures mixed models were used to determine the effects of treatment on anthropometric and body composition variables (body weight, waist circumference, FM, FFST, and %BF). No significant group × time interactions were observed, even when body mass index was controlled for in the analysis. Over 16 weeks, body weight, FM, FFST, and %BF significantly increased in both groups ($P < .05$). Our findings show that female college freshmen gained a significant amount of weight over the course of the 16-week study. Gains in body weight and FM were similar among participants assigned to the soy protein/isoflavone- and the casein-based meal replacements. Future research is warranted to determine the effects of soy protein/isoflavone- and casein-based meal replacements versus a non-intervention (i.e., non-protein based) control.

1. Introduction

The first, or freshman, year of college has been identified as a period of considerable weight gain in late adolescence [1–4]. On average, freshmen males and females gain three to four pounds during the first semester alone [1–3], and approximately 25% of freshmen students are five pounds heavier by the end of their first school year [2,4]. Excessive weight gain in late adolescence may lead to overweight and obesity in adulthood, placing adolescents at risk for metabolic complications later in

Abbreviations: SOY, soy protein/isoflavone-group; CAS, casein-based group; %BF, body fat percentage; FM, fat mass; FFST, fat-free soft tissue; DXA, dual energy X-ray absorptiometry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; ICCs, intraclass correlation coefficients; Soy FFQ, Seattle Soy Food Frequency Questionnaire.

* *Corresponding author.* Department of Foods and Nutrition, 279 Dawson Hall, The University of Georgia, Athens, GA 30605. Tel.: +1 706 542 4901.
E-mail address: rlewis@fcs.uga.edu (R.D. Lewis).

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life. To help prevent future health implications related to obesity, such as type-2 diabetes mellitus, hyperlipidemia, and hypertension [5–7], researchers must explore innovative and inexpensive dietary strategies to promote weight maintenance in late adolescence. Weight management interventions targeted at college freshmen present a unique opportunity to investigate the efficacy of such dietary strategies.

One dietary strategy that has garnered considerable attention, but warrants further investigation, is the use of supplements containing soy isoflavones. Isoflavones comprise a class of phytoestrogens that first gained popularity as an alternative therapy for menopausal symptoms and the prevention of bone loss. *In vitro* studies [8–10] and animal studies [11–16] support a potential role of soy isoflavones in weight loss and weight maintenance through a reduction in adipocyte accumulation. However, human studies examining the effects of soy protein-based interventions on body weight yield conflicting results [17–21].

Most researchers have investigated the influence of soy protein/isoflavones on body weight in older adult women, not college-age women during a period associated with significant weight gain. In addition, few have compared soy isoflavones versus control treatment using meal replacements, an effective vehicle for weight management [22–24]. We hypothesized that daily intake of soy protein/isoflavones would attenuate gains in body weight to a greater extent than a casein-based control in 18 to 19 year-old females. To test this hypothesis, we conducted a randomized, double blind, placebo-controlled trial to examine the effects of a soy protein/isoflavone-based meal replacement versus a casein-based meal replacement on body weight and body composition variables in female college freshmen over 16 weeks. Sixteen weeks is a sufficient time period to observe significant changes in weight and body composition with dietary interventions [18,25,26], and also aligned with the first semester of college.

2. Methods and materials

2.1. Study design and participants

Healthy female college freshmen, 18 to 19 years of age (N = 120), participated in this 16-week, randomized, double blind, placebo-controlled, parallel-group dietary soy protein/isoflavone intervention trial. Participants were recruited beginning in the summer of 2005 from the University of Georgia through newspaper advertisements, campus fliers, and presentations given to large freshman classes. Trained study personnel administered a telephone screen to prospective participants to determine eligibility. During the telephone screen, prospective participants were informed that the purpose of the study was to investigate the effects of soy on bone health. In addition to exclusion for soy or chocolate allergies, variability among participants was reduced by excluding those who reported significant weight loss or weight gain in the previous six months (± 10% initial body weight), were vegetarians, competed in National Collegiate Athletic Association Division-I athletics, had been diagnosed with an eating disorder, experienced irregular menstruation (less than 4 out of 6 periods in the last 6 months), or took medications known to affect body weight. These exclusion criteria were determined in order to recruit a homogenous group that did not engage in extreme levels of activity or dietary practices and had the greatest likelihood of weight gain during their freshman year. All study procedures were approved by the Institutional Review Board for Human Subjects at the University of Georgia, and written informed consent was obtained from each participant.

At enrollment, eligible participants were assigned a participant ID number and scheduled for testing at baseline, 8 weeks, and 16 weeks. All testing procedures were conducted at the Bone and Body Composition Laboratory at the University of Georgia through the spring of 2006. Participants arrived at the baseline visit for a blood draw and completion of study questionnaires. Participants were randomly assigned following simple randomization procedures (random-number table) to either the soy protein/isoflavone- (SOY; n = 62) or casein-based (CAS; n = 58) groups based on their participant ID number. A non-biased individual with no direct involvement in the clinical trial was responsible for labeling shake packets with the appropriate corresponding code. All investigators, research personnel, and participants remained blinded to these codes.

All meal replacement shake packets were provided by Revival Soy (Kernersville, NC). Shakes were available in chocolate and vanilla flavors, and an equal assortment of each flavor was distributed to participants unless only one flavor was requested. Chocolate and vanilla SOY shakes were identical in taste, color, odor, and texture to corresponding flavors of CAS shakes. SOY shakes contained 20 g soy protein and 161 mg total isoflavones per serving (95 mg aglycone equivalents: 39% daidzein, 40% genistein, 21% glycitein). CAS shakes contained 20 g casein protein and were identical to the SOY shakes in kilocalories (kcals), fat, carbohydrates, fiber, and calcium content (Table 1). Trained study personnel provided information on how to prepare shakes. Participants were instructed to replace breakfast with one study shake per day and to limit soy intake to less than one serving per week.

2.2. Adherence measures

Participants were asked to return empty shake packets along with any unused products at 8 weeks and 16 weeks. Adherence was measured in all participants as percent of shakes consumed (number of shake packets/number of days in the study). Adherence was confirmed through assessment of serum isoflavones in a random subsample of participants at baseline (N = 32) and 16 weeks (N = 94). A rapid 2-minute liquid chromatography-tandem mass spectrometry (LC-MS/MS) method operating in multiple reaction ion monitoring mode was used for the measurement of daidzein, genistein, and glycitein, as described by Prasain et al. [27]. This assay demonstrated a linear response for each analyte observed over a range of 1 to 5000 ng/mL (all $R \geq 0.99$).

2.3. Anthropometric measures

Height was measured using a wall-mounted stadiometer (Novel Products Inc., Rockton, IL) to the nearest 0.1 cm. Weight was measured using an electronic scale (Seca Bella 840, Columbia, MD) to the nearest 0.1 kg. Waist circumference was measured using a flexible measuring tape to capture the

Table 1 – Nutrient composition of chocolate and vanilla, SOY and CAS meal replacement shakes [1]

	Chocolate flavor	Vanilla flavor
Ingredients	Fructose, Sucrose, Dutch Processed Cocoa, Calcium Phosphate, Maltodextrin Soy Lecithin, Salt, Potassium Chloride, Modified Corn Starch Artificial Flavor, Undegraded Carrageenan, Carboxymethylcellulos, and Xanthan Gum	Fructose, Sucrose, Calcium Phosphate, Maltodextrin Soy Lecithin, Salt, Potassium Chloride, Modified Corn Starch Artificial Flavor, Undegraded Carrageenan, Carboxymethylcellulos, Xanthan Gum, and Real Vanilla Flavor
Energy (kcal)	240	220
Total fat (g)	2.5	2
Total carbohydrate (g)	36	31
Dietary fiber (g)	2	0
Calcium (mg)	500	500
	SOY	CAS
Soy protein (g)	20	–
Total isoflavones (mg)	161	–
Daidzein (mg)	37	–
Genistein (mg)	38	–
Glycitein (mg)	20	–
Casein protein (g)	–	20

[1] SOY and CAS meal replacement shakes provided by Revival Soy (Kernersville, NC).

distance around the smallest area of the trunk, below the ribcage and above the umbilicus, to the nearest 0.1 cm [28]. All anthropometric measurements were conducted twice and averaged. If the difference between two measurements was greater than 1.0 cm or 0.1 kg, a third measurement was taken and the two closest values were averaged. Body mass index (BMI) was calculated as weight (kg)/height (m^2), and BMI-for-age percentiles were determined using Epi Info 2000 and the CDC 2000 reference database [29]. One-way random effects model, single measure intraclass correlation coefficients (ICCs) were calculated in ten females, 18 to 30 years of age, and measured twice in our laboratory during a seven-day period for height, weight, and waist circumference (all $R \geq 0.99$). The estimated sample size needed for a reliability study of measuring waist circumference was 10 participants, based on the following parameters: $k = 2$ (two measurement time points), $R = 0.95$ (expected ICC), $\omega = 0.2$ (confidence interval width), and $\alpha = 0.05$ [30,31].

2.4. Body composition

Fat mass (FM; kg), fat-free soft tissue mass (FFST; kg), and % body fat (%BF) were primary outcomes measured using 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., that was composed of different thickness levels of aluminum and lucite and calibrated against stearic acid (100% fat) and water (8.6% fat). One-way random effects model, single measure ICCs were calculated in five females, 18 to 30 years of age, and scanned twice in our laboratory during a seven-day period for FM, FFST, and %BF (all $R \geq 0.87$).

2.5. Physical activity assessment and dietary intake

Physical activity assessment and dietary intake data were collected to help determine adherence to the study protocol. Participants were encouraged to maintain their usual dietary habits (with the exception of shake substitution) and physical activity regimen. All participants received the same instructions regarding diet and physical activity to reduce the possibility of experimental bias between groups. Total daily energy expenditure (kcal/day) was determined at each time point using the Stanford 7-Day Physical Activity Recall Questionnaire [32]. Dietary soy intake was assessed using the Seattle Soy Food Frequency Questionnaire (Soy FFQ) [33]. Three-day diet records (including two weekdays and one weekend day) were used to estimate average daily energy, macronutrient, and calcium intakes. Baseline diet records were returned prior to shake distribution, and therefore did not include study shakes. At 8 weeks and 16 weeks, participants were instructed to report study shake intake. Nutrient intakes generated from the 3-day diet records are highly correlated with direct observation (r = 0.78-0.94) in females 9 to 10 years of age [34]. The 3-day diet records were analyzed using Food Processor for Windows version 8.0 (ESHA Research, Salem, OR). 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 were calculated for energy (R = 0.47) and calcium (R = 0.71).

2.6. Statistical analyses

A planned sample size of 58 per group, allowing for 30% attrition, and resulting in 40 per group at posttest (16 weeks), was selected to provide 80% or more power using a two-sided α level of 0.05 to detect group differences on primary outcomes (body weight, 91%; FM, 93%; %BF, 81%; and waist circumference, 94% power). This was based on results from a prior study of a soy-based meal replacement program for the treatment of obesity [18], which showed group differences in change in body weight (–7.0 kg), FM (–4.3 kg), %BF (–1.5%), and waist circumference (–6.0 cm) after 12 weeks. All statistical analyses were performed using SAS software, (version 9.1, Cary, NC) and a two-sided α level of 0.05 was set as significant. Group differences at baseline were determined using independent samples t tests.

Repeated-measures mixed models were used with maximum likelihood estimation in an intention-to-treat analysis of each outcome measure using all available data. Base models for each outcome measure included the fixed effects of the intervention group (SOY or CAS) and measurement period (baseline, 8 weeks, or 16 weeks). Participant nested

within group was considered a random effect. The modeled covariance structure between measurement periods was unstructured, which used all available measurements on the same participant, including those from participants who dropped out of the study. The primary intent was to include any characteristics that differed significantly at baseline as covariates in the primary analyses of treatment effects on the dependent variables. However, significant group differences in baseline characteristics were not detected. Therefore, covariates were not considered in the primary analyses.

Secondary analyses were conducted to consider potential effects of non-adherence. Adherence was measured as percent of shakes consumed, and validated by comparing plasma isoflavones between groups at baseline and 16 weeks using independent samples t tests. In order to adjust for adherence, subgroup analyses of the treatment effect were performed, where the subgroups were defined according to baseline characteristics that were found to predict adherence [35]. In the present study, a stepwise regression procedure found only baseline BMI to be marginally associated with total adherence ($P = 0.06$). As a result, participants were divided into three subgroups according to baseline BMI tertiles (low, or <21.2; medium; and high, or >23.3). Repeated-measures mixed models, as described above, were then conducted for each outcome measure that included BMI tertile group (low, medium, or high) as a fixed effect alongside intervention group (SOY or CAS) and measurement period (baseline, 8 weeks, or 16 weeks).

3. Results

3.1. Participants

Participant enrollment, allocation, follow-up, and analysis are presented in Fig. 1 [36]. Eighty-six participants completed the intervention. Of the 34 participants who elected to discontinue study participation, 11 participants chose to stop meal replacements but complete all testing measures (SOY: n = 6; CAS: n = 5). Reasons for discontinuing study participation and/or meal replacements included: unwillingness to consume shakes (SOY: n = 4; CAS: n = 4), taste intolerance (SOY: n = 1), illness not related to study (SOY: n = 7; CAS: n = 8), gastrointestinal distress related to shakes (SOY: n = 6; CAS: n = 2), and concern for cancer risk (SOY: n = 1; CAS: n = 1).

Baseline characteristics of participants are presented in Table 2. There were no significant differences in age, anthropometric and body composition measurements, energy

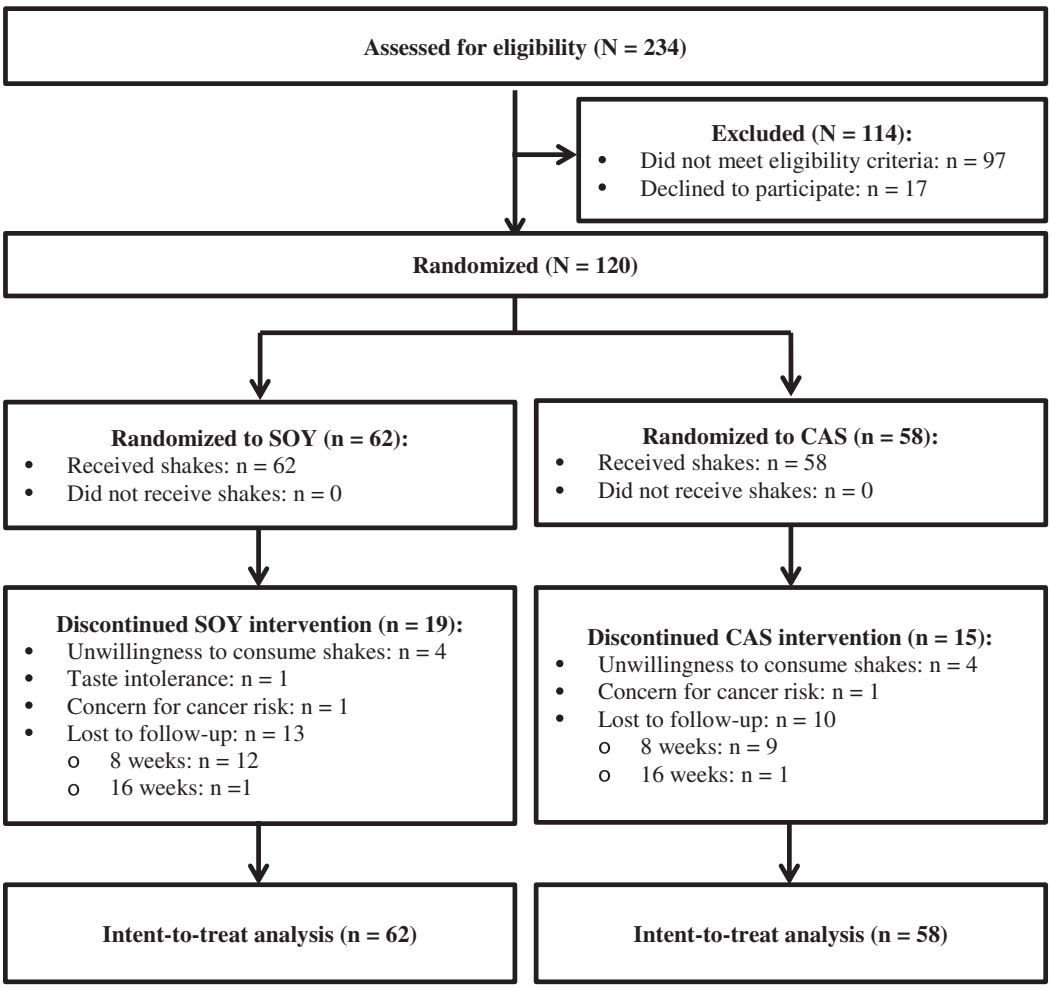

Fig. 1 – CONSORT 2010 flow diagram of subject enrollment, allocation, follow-up, and analysis. 546 Modified from Schultz et al. [36].

Table 2 – Baseline descriptive characteristics of participants

Variables	SOY (n = 62)	CAS (n = 58)	P value for difference [1]	Both groups (n = 120)
Age (years)	18.3 (0.4)	18.2 (0.4)	0.278	18.2 (0.4)
Height (cm)	165 (6.3)	163 (5.5)	0.136	164 (5.9)
Weight (kg)	60.8 (8.4)	59.6 (7.1)	0.415	60.2 (7.8)
BMI (kg/m^2)	22.4 (2.9)	22.4 (2.1)	0.975	22.4 (2.6)
Fat mass (kg)	18.3 (5.1)	17.4 (4.3)	0.324	17.9 (4.7)
Fat-free soft tissue (kg)	41.5 (4.4)	41.0 (4.3)	0.520	41.2 (4.3)
Waist circumference (cm)	71.9 (6.4)	71.6 (4.6)	0.800	71.8 (5.6)
Energy intake (kcal/d)	1824 (476)	1777 (414)	0.565	1801 (445)
Energy expenditure (kcal/d)	2105 (328)	2078 (292)	0.646	2085 (311)
Calcium intake (mg/d)	751 (381)	656 (213)	0.098	705 (313)
Soy intake (g/wk) [2]	111 (136)	153 (249)	0.289	131 (198)
Soy intake (servings/wk) [2]	1.4 (1.7)	2.0 (2.5)	0.185	1.7 (2.1)
Isoflavone intake (mg/wk) [2]	19.9 (33.3)	29.9 (56.6)	0.274	24.7 (46.1)
Daidzein (mg/wk) [2]	7.63 (13.0)	11.0 (20.0)	0.313	9.23 (16.8)
Genistein (mg/wk) [2]	10.2 (17.0)	16.3 (34.4)	0.249	13.1 (26.9)
Glycitein (mg/wk) [2]	2.10 (3.32)	2.58 (4.34)	0.514	2.32 (3.84)

Values are means (SD).
[1] Tests of significance between treatment groups at baseline are based on two-tailed independent samples t tests ($P < .05$).
[2] Dietary soy and isoflavone intakes are based on the Seattle Soy Food Frequency Questionnaire [33].

expenditure, energy intake, calcium intake, and soy and isoflavone intakes (Table 2). Percent adherence was not significantly different between groups over the course of the study: adherence was 72% and 77% at 8 weeks, and 69% and 73% at 16 weeks for SOY and CAS groups, respectively. Percent adherence was validated by assessment of plasma isoflavones presented in Table 3. At baseline, plasma isoflavones were not significantly different between groups. At 16 weeks, the SOY group exhibited greater plasma daidzein ($P < .02$), genistein ($P < .07$), and glycitein ($P < .04$) compared to the CAS group.

3.2. *Anthropometric measurements and body composition*

Mean values for anthropometric and body composition measurements of participants are listed in Table 4. Changes in anthropometric and body composition variables were not significantly different between groups, even after adjusting for BMI. There was a significant increase in weight, FM, FFST, and %BF in both groups over time ($P < .05$; Table 4). Post-hoc pairwise comparisons revealed a significant increase in weight and FM from baseline to 8 weeks and from baseline to 16 weeks ($P < .05$). There was a significant increase in %BF and percent trunk fat from baseline to 8 weeks ($P < .05$). In contrast, there was a significant decrease in waist circumference from baseline to 8 weeks and from baseline to 16 weeks. There were no significant differences in percent leg fat and percent arm fat over time.

3.3. *Physical activity assessment and dietary intake*

Mean values for energy expenditure and dietary intake are shown in Table 5. No group x time interaction was observed for any of these variables. Participants reported significantly greater energy expenditure and significantly lower energy intake over time. Moreover, both SOY and CAS groups reported a decrease in percent energy intake from fat and an increase in percent energy intake from protein and total calcium (all $P < .05$). There were no significant changes in percent energy intake from carbohydrate or total soy over time.

4. Discussion

Female college freshmen gained a significant amount of weight over the course of the 16-week study. Weight gain was similar among participants assigned to either the soy protein/isoflavone- or the casein-based meal replacements. Therefore, we reject the research hypothesis. To our knowledge, this is the first study to investigate the effects of soy protein/isoflavones on weight and body composition in late adolescent females. By recruiting normal-weight female college freshmen, our group was able to examine the effects of soy protein/isoflavones on weight maintenance or prevention of weight gain rather than weight loss.

Table 3 – Plasma isoflavones (daidzein, genistein, and glycitein) at baseline and 16 weeks

Plasma isoflavones (nmol/L)	Baseline			16 weeks		
	SOY (n = 16)	CAS (n = 16)	P value	SOY (n = 47)	CAS (n = 47)	P value
Daidzein	68.5 (200)	14.4 (38.7)	0.297	172 (358.4)	36.4 (113)	0.016
Genistein	20.9 (30.0)	25.7 (83.4)	0.829	62.0 (101)	31.9 (46.1)	0.068
Glycitein	5.9 (16.4)	13.4 (53.8)	0.596	32.0 (89.4)	3.8 (14.0)	0.038

Values are means (SD). Tests of significance between treatment groups at baseline and at 16 weeks are based on two-tailed independent samples t tests ($P < .05$).

Table 4 – Body weight and body composition in soy and casein meal replacement groups at baseline, 8 and 16 weeks

	SOY			CAS			Fixed effects (P value)		
Primary variables	Baseline	8 weeks	12 weeks	Baseline	8 weeks	12 weeks	Time	Group	Time × group
Weight (kg)	60.8 (1.0)	61.2 (1.0)	61.4 (1.0)	59.6 (1.0)	59.9 (1.0)	60.0 (1.0)	0.023	0.367	0.793
Fat mass (kg)	18.3 (0.6)	18.6 (0.6)	18.7 (0.6)	17.4 (0.6)	17.7 (0.7)	17.6 (0.6)	0.017	0.286	0.392
Fat-free soft tissue (kg)	41.5 (0.5)	41.3 (0.6)	41.7 (0.6)	41.0 (0.6)	41.2 (0.6)	41.4 (0.6)	0.015	0.708	0.439
Body fat (%)	29.1 (0.6)	29.6 (0.6)	29.5 (0.6)	28.6 (0.6)	28.7 (0.6)	28.5 (0.6)	0.035	0.328	0.280
Waist circumference (cm)	71.9 (0.7)	71.2 (0.7)	71.5 (0.7)	71.6 (0.7)	70.9 (0.7)	71.0 (0.8)	0.000	0.742	0.760
Secondary variables									
Trunk fat (%)	24.8 (0.7)	25.4 (0.7)	25.4 (0.7)	23.8 (0.7)	24.2 (0.8)	23.9 (0.8)	0.007	0.242	0.273
Leg fat (%)	35.4 (0.6)	35.7 (0.6)	35.6 (0.6)	35.2 (0.6)	35.3 (0.6)	35.0 (0.6)	0.070	0.664	0.465
Arm fat (%)	33.8 (0.7)	33.7 (0.7)	34.1 (0.7)	32.9 (0.8)	32.8 (0.8)	33.1 (0.8)	0.101	0.341	0.971

Values are estimated means (± SE). Tests of significance between treatment groups over 16 weeks are based on mixed models ($P < .05$).

Other studies examining the relationship between soy protein/isoflavones and body weight have been conducted in healthy adult females and yield conflicting results. Kumar et al. [37] found that women 25 to 55 years of age assigned to 12 weeks of supplementation with soy isoflavones (40 mg/d of genistein) gained more weight compared to those assigned to a milk-protein placebo (0.77 kg versus 0.5 kg, respectively; $P < .04$). Liu et al. [38] found that post-menopausal females randomized to 6 months of supplementation with soy protein/isoflavones (100 mg/d) versus a milk-protein placebo exhibited a small but significant decrease in body weight ($P < .05$). Moreover, a recent meta-analysis of randomized controlled trials in post-menopausal women found that supplementation with soy isoflavones was most effective in significantly reducing body weight among normal weight women (BMI <30 kg/m^2) receiving a low dose of soy isoflavones (<100 mg) for a short duration (<6 months) [39]. Our results are in agreement with those by Moeller et al. [40], who found that peri-menopausal women receiving 24 weeks of supplementation with soy protein/isoflavones versus a whey-based placebo exhibited similar gains in regional FM measured by DXA. Ultimately, it is difficult to determine the overall effects of soy isoflavones on weight maintenance by comparing existing studies with varying doses of proteins, isoflavones, study durations, and ages of participants. However, given the lack of studies targeted at college-age females, our findings provide valuable insight into this relationship in a population subject to weight gain [1–4].

It is possible that the lack of significant differences between the SOY group and the CAS group was related to adherence with supplements. However, percent adherence (measured as percent of shakes consumed) was not significantly different between groups over the course of the study. The percent of shakes consumed was also similar to what has been reported in other studies using meal replacements [25,41–43]. Moreover, adherence was validated by comparing plasma isoflavones between groups at baseline and at 16 weeks.

Although supplementation with soy protein/isoflavones versus a casein-based placebo did not prevent weight gain, we found that normal weight college freshmen in both SOY and CAS groups exhibited lower average weight gain than college freshmen in previous studies [1–4]. In the present study, average weight gain was 0.5 kg, which is approximately 50 to 75% lower than what has been reported in other studies of college freshmen [1–4]. Both groups reported increases in percent energy from protein and decreases in percent energy from fat, a shift in macronutrient intake that has been associated with weight loss and maintenance [44–46]. In addition, both soy

Table 5 – Dietary intake and energy expenditure in soy and casein meal replacement groups at baseline, 8 and 16 weeks

Variables	SOY			CAS			Fixed effects (P value)		
	Baseline	8 weeks	12 weeks	Baseline	8 weeks	12 weeks	Time	Group	Time × group
Energy intake (kcal/d) [1]	1824 (57)	1701 (74)	1651 (61)	1777 (59)	1676 (73)	1661 (63)	0.020	0.769	0.860
Energy expenditure (kcal/d)	2105 (40)	2150 (39)	2155 (55)	2078 (41)	2131 (40)	2175 (56)	0.010	0.879	0.712
Fat (% of kcal/d) [1]	33.2 (0.9)	30.6 (0.9)	31.3 (0.9)	32.9 (0.9)	28.8 (0.9)	29.9 (0.9)	0.000	0.222	0.569
Protein (% of kcal/d) [1]	15.3 (0.4)	18.4 (0.6)	18.4 (0.6)	15.6 (0.4)	19.9 (0.6)	18.9 (0.6)	0.000	0.191	0.220
Carbohydrate (% of kcal/d) [1]	51.7 (1.0)	52.4 (1.1)	51.5 (1.1)	52.2 (1.0)	52.2 (1.0)	52.6 (1.1)	0.895	0.698	0.682
Calcium intake (mg/d) [1]	751 (40)	1079 (49)	1000 (47)	656 (41)	1089 (49)	1031 (48)	0.000	0.710	0.167
Soy intake (g/wk) [2]	111 (27)	206 (91)	129 (40)	153 (28)	213 (86)	80 (42)	0.264	0.992	0.354
Soy intake (servings/wk) [2]	1.4 (0.3)	1.3 (0.4)	1.0 (0.3)	2.0 (0.3)	2.1 (0.4)	2.0 (0.4)	0.168	0.122	0.934
Isoflavone intake (mg/wk) [2]	19.7 (6.2)	13.4 (7.5)	14.4 (6.4)	29.3 (6.4)	28.5 (7.3)	21.2 (6.8)	0.397	0.170	0.680
Daidzein (mg/wk) [2]	7.6 (2.3)	4.8 (2.8)	5.6 (2.2)	10.8 (2.4)	10.6 (2.7)	7.7 (2.4)	0.388	0.167	0.598
Genistein (mg/wk) [2]	10.1 (3.6)	7.1 (4.2)	7.0 (3.9)	15.9 (3.7)	15.3 (4.1)	12.1 (4.1)	0.449	0.173	0.819
Glycitein (mg/wk) [2]	2.1 (0.5)	1.5 (0.7)	1.8 (0.5)	2.6 (0.5)	2.6 (0.6)	1.4 (0.5)	0.285	0.422	0.295

Values are estimated means (± SE). Tests of significance between treatement groups over 16 weeks are based on mixed models ($P < .05$).

[1] Energy, macronutrient, and calcium intakes include study shakes at 8 and 16 weeks.

[2] Dietary soy and isoflavone intakes are based on the Seattle Soy Food Frequency Questionnaire [33].

protein/isoflavone- and casein-based meal replacements contained 500 mg of calcium, which has been reported to play a role in the regulation of adipocyte metabolism [47–49].

The shift in nutrient intake with meal replacement shakes may also help explain the significant decrease in waist circumference observed in both SOY and CAS groups over time [44–49]. Waist circumference is a predictor of disease risk and an indicator of abdominal fat, and studies of obese adult females support a relationship between central adiposity and intake of soy isoflavones. Sites et al. [50] reported a significant reduction in total ($P < .005$) and subcutaneous abdominal ($P < .01$) fat measured by computed tomography in overweight post-menopausal participants assigned to a soy protein/isoflavone-based shake versus an energy-equivalent casein-based shake. Christie et al. [21] reported a significant decrease in abdominal fat and interleukin-6 in obese participants receiving supplementation with soy isoflavones versus a casein placebo without isoflavones. Additional research is needed to determine whether soy isoflavones are effective in weight loss and weight maintenance related to regional fat deposition.

A limitation of the present study was the use of a protein-based placebo, which made it difficult to determine the overall effects of isoflavones on body weight. In addition, this study only reported total body fat and did not examine regional adiposity using other imaging technology. This information has the potential to provide greater insight into the effects of treatment, given the significant decrease in waist circumference among participants.

In conclusion, female college freshmen assigned to soy protein/isoflavone-based meal replacements versus casein-based meal replacements exhibited similar gains in body weight. Future studies should compare college-age participants receiving isoflavones alone versus a non-protein placebo to better identify the components required for the prevention of weight gain.

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