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Research Article

Effects of Soy Supplementation on Abdominal Obesity and Metabolic Risks on Post-Menopausal Women: A Pilot Study

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Abstract

Over two-thirds of American post-menopausal women are abdominally obese. Post-menopausal estrogen deficiency is considered a key risk factor for excess visceral fat accumulation and increased metabolic abnormalities. Although hormone replacement therapy is effective in reducing visceral fat, its adverse effects warrant research identifying safe, estrogen-mimenic phytochemicals for central obesity prevention among menopausal women.

Objectives: This pilot study investigated the effect of daily soy supplementation on abdominal fat, lipid profile and circulating inflammatory markers in early post-menopausal women.

Methods: This was a six-month double-blind placebo-controlled trial. Subjects were early post-menopausal women with menses ceased less than three years. Twelve subjects were evenly, randomly assigned to Intervention (25g soy protein + 2 capsules containing 160mg isoflavones) or Control (25g whey protein + 2 cellulose filled capsules). Study outcomes included anthropometrics, blood pressure, total and abdominal fat by dual-energy X-ray absorptiometry, lipid profile, serum levels of C-reactive protein, interleukin 6 and insulin by immunoassay.

Results: Eight completed the study, 4 in Intervention and 4 in Control. At study endpoint, compared to Control, Intervention subjects had significantly lower waist circumference (-2.2cm, $p<0.05$) and had marginally significant lower abdominal fat 1.32kg ($p=0.06$), with no body weight difference and a higher body fat content of 0.84kg. Although not statistically significant, Intervention appeared to have favorable metabolic profile, with an exception of higher triglyceride level.

Conclusion: Soy supplementation for six months appears to be effective in reducing abdominal fat and improving metabolic profiles among early post-menopausal women.

Keywords: Abdominal obesity; Post-menopausal women; Lipid profile; Soy supplementation

Abbreviations

DEXA: Dual-Energy X-ray Absorptiometry; CRP: C-Reactive Protein; IL-6: Interleukin 6; BMI: Body Mass Index; CVD: Cardiovascular Diseases; HRT: Hormone Replacement Therapy; IRB: Institutional Review Board; UTSA: The University of Texas at San Antonio; HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein; TC: Total Cholesterol; TG: Triglyceride; ELISA: Enzyme-Linked Immunosorbent Assay; Waist: Waist Circumference; HOMA-IR: Homeostasis Model Assessment-Insulin Resistance; GI: Gastrointestinal; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; GLM: General Linear Model; SE: Standard Error; MRI: Magnetic Resonance Imaging; CT: Computer Tomography; Intervention: Intervention Group; Control: Control Group

Introduction

Menopausal women are among the highest risk populations for abdominal obesity and at great risk of developing metabolic abnormalities [1-3]. It was observed that menopausal women tend to accumulate visceral fat, which is a key risk factor for metabolic syndrome [4]. The withdrawal of estrogen has negative effects on the cardiovascular system including: the transition from a gynoid to an android adipose storage pattern, reduced glucose tolerance, abnormal lipid profile, increased blood pressure, increased sympathetic tone, endothelial dysfunction, and vascular inflammation [5-8]. Among the inflammatory factors, cytokine interleukin 6 (IL-6) may lead to mediating pathways for cardiovascular diseases (CVD) [9]; while C-reactive protein (CRP) is an indicator of metabolic abnormality [10,11] and CVD in post-menopausal women [12].

Hormone replacement therapy (HRT) has been shown to be effective in reducing visceral fat and improving lipid profile in menopausal women [13,14]. However, HRT may increase risk of

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breast and uterine cancers [15]. As such, prolonged use of HRT is not recommended for CVD prevention [15]. Therefore, safe, hormone substitute compounds exerting estrogenic properties are warranted to prevent and treat estrogen deficiency related disease states, e.g. abdominal obesity and its metabolic complications.

Phytoestrogens are a group of compounds with a diphenolic structure similar to steroidal estrogens [16]. Phytoestrogens may be a safe and effective alternative to reduce the risk of abdominal obesity and related chronic diseases among menopausal women. Isoflavones, a group of phytoestrogens, in soybeans, appear to have the potential as a therapeutic remedy for mitigating menopausal abdominal obesity accumulation and related chronic disease [17]. An epidemiological study showed that menopausal women who consumed a high soy diet had a lower waist-hip ratio as compared to those who consumed no soy [18]. A small-scale randomized trial has shown that a daily 70mg isoflavone supplement for six-months increased fat-free mass and muscle mass in obese-sarcopenic (a condition where in a person shows an increase in fat mass and a reduction in lean mass) post-menopausal women [19]. Another small-scale clinical trial revealed that a 3-month daily 20g soy protein + 160 mg isoflavone supplement prevented increases in subcutaneous and total abdominal fat, but not visceral fat in post-menopausal Caucasian women [17]. This same research group later reported that a daily 20g soy protein + 160mg isoflavone supplement for three months reduced abdominal fat and lowered IL-6, with no effect on CRP in both obese Caucasian and African-American post-menopausal women [20]. Similarly, Liu et al. reported that six-month supplementation of a daily dose of 15g soy protein with 100mg isoflavones had a mild effect on reducing weight and body fat in Chinese post-menopausal women [21]. However, in a randomized trial, a daily dose of 40g of soy flour supplement containing 12.8g soy protein and 49mg isoflavones for six months did not affect total body fat nor lean mass in a small sample of post-menopausal women [22]. Matvienko et al. recently reported no change in body composition after a one year daily 80mg soy isoflavone tablet supplement in healthy post-menopausal women aged 45 to 65 years [23]. Such contradictory findings from the limited number of clinical studies are likely the result of different doses and components of soy compounds, varied intervention duration, age and menopausal status, since research showed that estrogen receptor sensitivity declines with age and prolonged periods of menses cessation [24]. As such, we hypothesized that soy supplementation may be effective in preventing abdominal fat accumulation and reducing metabolic risk factors in early post-menopausal women before estrogen receptors sensitivity decline. The objective of this pilot study was to examine the effects of soy supplementation on abdominal fat and metabolic risk factors among early post-menopausal women.

Methods

This was a six-month randomized double-blind, placebo-controlled trial conducted between 2011 and 2012. Subjects were early post-menopausal women randomly assigned to either the Intervention group (Intervention) or the Control group (Control). The primary outcomes was abdominal fat by dual-energy X-ray absorptiometry (DEXA) and secondary outcome included total body fat mass, anthropometrics, and key biomarkers for metabolic abnormalities, including: high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG), insulin sensitivity, homeostasis model assessment-insulin resistance (HOMA-IR), inflammatory cytokines IL-6 and CRP. The measurements and testing were conducted at the start of the 6-month period and again at the end Study protocols were reviewed and approved by the Institutional Review Board (IRB) at The University of Texas at San Antonio. Informed consent was obtained from study subjects.

Study subjects

Study subject inclusion criteria were 1) women aged 45-60 years with menses ceased less than three years; 2) with a BMI greater than 25kg/m^2 and waist circumference (Waist) greater than 88 cm; 3) currently experiencing menopausal symptoms.

Exclusion criteria included: 1) with a prior history of reproductive tumors or other cancers; 2) allergy to soy or milk protein; 3) metabolic disorders that may affect study outcomes (e.g., hypercortisolism and hypothyroidism, non-alcoholic fatty liver disease); 4) currently receiving hormone replacement therapy or estrogen-like remedy; 5) taking medications (e.g., thyroid, cortisol/cortisone, ephedra, thermogenics, etc.) and 6) emotional or uncontrolled eaters as determined by a brief screening tool conducted in the telephone interview [25].

Subjects were recruited from the local community via flyers, bulletin board advertisements and word-of-mouth referrals. A block randomization in block intervals of 16 was used for subject randomization. A list of randomized numbers was generated by computer and assigned to each of the two groups. Research staff not directly involved in the study performed the randomization and labeled the supplements. Subjects were assigned the number series in the order of their enrollment into the trial. Both researcher and subjects were blinded to the treatment assignment until the conclusion of the trial.

Among the 49 individuals interested in the study, 12 met the inclusion criteria and consented to take part in the study. They were randomly assigned into either Intervention or Control. One subject in the Control discontinued for undisclosed reasons, while another dropped out due to discomfort of ingesting daily protein shake. One Intervention subject discontinued for undisclosed reason. At study endpoint, five subjects remained in the Intervention, with one being excluded due to poor adherence to supplement intake (<70%). Four subjects completed the study in the Control (Figure 1).

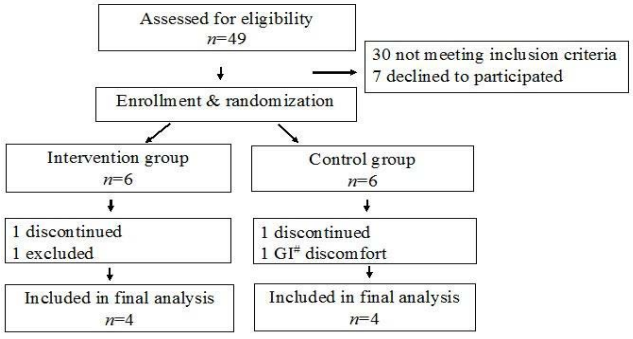

Figure 1: Randomization for trial participants. #: GI discomfort: gastrointestinal discomfort.

Table 1: Endpoint outcomes measures by treatment (Mean and SE)#.

	Intervention (*n*=4)		Control (*n*=4)		*p*
	Mean	SE	Mean	SE	
BMI (kg/m^2)	33.45	0.50	33.64	0.50	0.107
Abdominal fat (kg)	2.94	0.57	4.26	0.49	0.068
Total body fat (kg)	40.96	0.84	40.12	0.97	0.194
Waist (cm)	105.59	0.30	107.21	0.30	0.015
SBP (mm Hg)	125.41	6.11	129.99	6.64	0.314
DBP (mm Hg)	73.06	3.42	74.61	3.55	0.348
TC (mg/dl)	171.38	3.45	175.16	3.33	0.245
HDL (mg/dl)	53.69	3.13	47.66	3.043	0.216
LDL (mg/dl)	98.98	6.42	106.9	6.06	0.129
TG (mg/dl)	114.05	3.09	104.98	3.189	0.069
Glucose (mg/dl)	85.98	2.08	89.47	2.19	0.161
CRP(mg/L)	3.11	0.04	3.08	0.05	0.073
IL-6 (ng/ml)	0.10	0.05	0.04	0.06	0.239
HOMA-IR	1.62	0.42	1.62	0.51	0.497

#: Means are adjusted for baseline measure and age as covariates.
Abbreviations: Waist: Waist Circumference; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; TC: Total Chlesterol; HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein; TG: Triglyceride; CRP: C - Reactive Protein; IL-6: Interleukin 6; HOMA-IR: Homeostasis Model Assessment-Insulin Resistance; Intervention: Intervention Group; Control: Control Group; SE: Standard Error.

Intervention and supplement preparation

Intervention received a daily soy supplement of two capsules containing 160mg isoflavones and 25g of soy protein, while the Control received a daily placebo of two cellulose-filled capsules and 25g of whey protein. Frutarom Netherlands provided both the isoflavones (Soy Life 40%, containing 15% of genitein, 50% of daidzein and 35% of glycitein) and placebo capsules, which were identical in size and color. Both soy and whey protein were prepared in the form of powder and weighted 25g per package, available in vanilla and chocolate flavors. They could be mixed with water, milk and other beverages. Subjects were asked to consume two capsules and a powder packet daily, preferably at breakfast, for 6 months. All subjects were required not to take any supplements containing phytoestrogens, or other extracts known to affect outcome measures, and continue the usual dietary intake and physical activity level. Subjects returned monthly to refill their supplements. At each refill event, subjects' unconsumed capsules were counted and protein powder weighed for adherence to the study regimen.

Study measurements and data collection

The study's primary outcome was change in abdominal fat. Secondary outcomes included changes in total body fat, anthropometrics, and key biomarkers for metabolic abnormalities. All outcome data were measured at baseline (prior to the treatment) and study endpoint. Subjects' socio-demographics, menopausal status, and medical history were collected via a self-administered questionnaire. The subjects' race and ethnicity included Hispanics, White and African-American. Family income level was assessed with three options "very hard for basics", "somewhat hard for basic" and "not hard for basics".

Abdominal fat and total body fat mass: The DEXA (Hologic QDR Discovery A, Bedford, MA) was used to quantify abdominal fat and total fat mass using the Hologic software (version 12.5). The subject lied supine on the scanning bed with their arms at their sides during the scan. The scanner was calibrated daily with a spine phantom and its performance was monitored using a quality assurance protocol. Abdominal fat measures were calculated from the midpoint of the intervertebral space between the T12 and L1 vertebra to the midpoint of the intervertebral space between the L4 and L5 vertebra [26]. The output for the abdominal region was fat mass in kilograms (kg).

Anthropometrics and blood pressure measurement: Subjects' waist circumference was measured midway between the iliac crest and bottom of the rib cage. Body weight was measured using Tanita Medical Scale (BWB-627A) to the nearest of 0.1kg. Subjects' height was measured using a Seca 214 Stadiometer to the nearest 0.1cm. BMI (kg/m^2) was calculated using the equation of weight (kg) over height (m) squared. Subjects' resting systolic and diastolic blood pressure (SBP and DBP, mmHg) was measured with an electronic sphygmomanometer (Omron, USA). All parameters were measured twice and the mean of the two measures was calculated and utilized for data analysis.

Lipid profile, metabolic and inflammatory biomarker measurement: Subjects' fasting serum and plasma were collected and stored at -80°C until subsequent analysis. Lipid profile, including total cholesterol (TC), HDL cholesterol, LDL cholesterol, TC/HDL ratio, and total TG levels, was measured using enzymatic methods (Cholestech LDX System, USA). Serum glucose and plasma insulin level were measured using an Insulin ELISA kit (Labor Diagnostika Nord, USA). Serum IL-6 and CRP levels were measured using Assay Max Human IL-6 ELISA kit (ASSAPro, USA) and Assay Max Human C-Reactive Protein ELISA kit (ASSAPro, USA) following the manufacturer's instructions. Triplicate samples and standards were measured. Means were calculated and utilized for data analysis. HOMA-IR was calculated with the following formula: (fasting plasma insulin (μ U/ml))×fasting serum glucose (mg/dL))/405 [27,28].

Statistical analysis

Statistical analysis was performed with the SPSS 19.0 software (USA). The level of significance was set at 0.05. Independent t-test was performed to compare baseline characteristics between groups. The GLM Univariate procedure was used to determine intervention effects. The models included outcome measures as the dependent variable, treatment condition as the fixed factor (1=control; 2=intervention), and corresponding baseline outcome measures and age were covariates. The intervention effects were reported as the differences in outcome measures at endpoint with and without intervention, controlling for baseline values, age and menopausal status.

Results

Subjects' characteristics at baseline

All subjects were early post-menopausal women aged 54.3± 4.4 yr (Intervention) and 53.7± 4.0 yr (Control). Intervention subjects' race was 50% Hispanics and 50% African-Americans. Control subjects' race was 50% Hispanics, 25% Whites and 25% African-Americans.

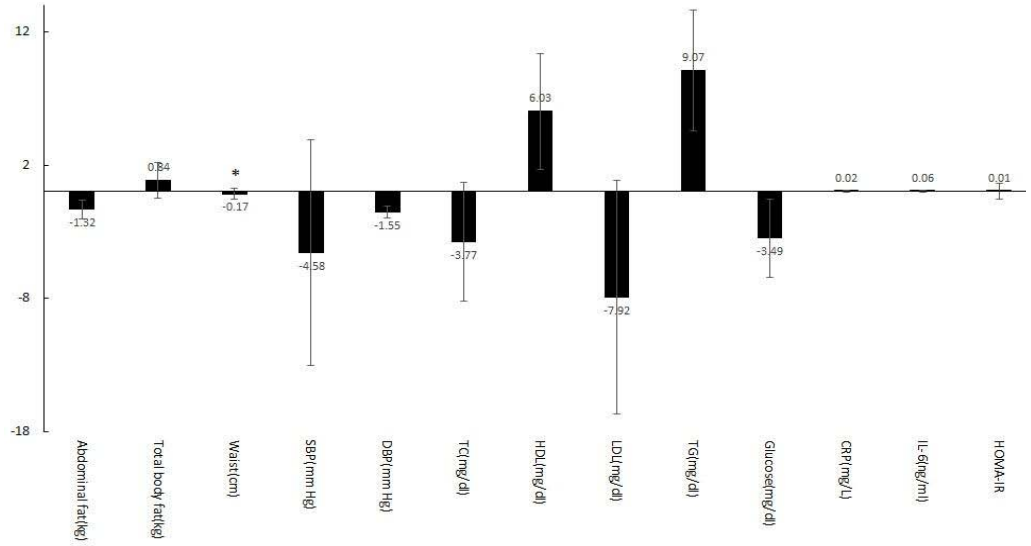

Figure 2: Intervention versus control at study endpoint: outcomes measures adjusted for baseline measure and age as covariates. *: $p<0.05$.

All subjects had a full-time job. Seventy-five percent of subjects were below college education and 25% had a college or graduate degree in both groups. All Intervention subjects reported family income being "not hard for basics"; while 50% of control subjects reported "somewhat hard" and "not hard for basics". No significant differences were found between groups on subjects' baseline demographic profile, abdominal fat, total body fat, waist circumference and blood pressure.

Intervention effects on primary and secondary outcomes

Table **1** shows intervention effects on outcome measures. At study endpoint, subjects in Intervention had a significant lower waist circumference as compared to Control ($p<0.05$). Intervention also has 1.32kg less abdominal fat content ($p=0.06$), although no difference in body weight and 0.84kg higher in total body fat content than Control (Figure 2). Further illustrates the outcome differences of Intervention over the Control. Although not statistically significant, compared to Control, the Intervention showed a favorable profile of blood pressure, TC, HDL, LDL and fasting glucose level, with the exception of higher TG level. No significant differences were found for HOMA-IR, CRP and IL-6 between groups.

Discussion

The objective of this pilot study was to investigate the effect of daily soy supplementation on abdominal fat, lipid profile and circulating inflammatory markers in early post-menopausal women. It appears that soy supplementation was associated with a favorable effect on lowering abdominal fat and waist circumference, and improving blood pressure and lipid profile in the study sample.

Soy supplementation may improve body composition by attenuating visceral fat deposit in early stage post-menopausal women. Menopause is a naturally occurring process which has been associated with increased central adiposity, reduced energy expenditure during rest and physical activity, and accelerated loss of fat-free mass [8]. Many intervention trials have provided evidence that HRT improves body composition by preventing the increase in abdominal adiposity in post-menopausal women [29,30]. Due to its estrogenic properties, soy isoflavones and soy supplementation are studied in menopausal women as one of the estrogen alternatives. In previous studies, the dose, components of the soy supplements used and intervention duration all varied. Additionally, the subjects' age and menopausal status were regarded as factors contributing to the contradictory results of soy supplementation in menopausal women. Liu et al.'s study suggested, with the subgroup analysis, a more pronounced soy protein effect in menopausal women in early stages of menopause (<4 years of menopause) [21], indicating that early post-menopause may be a critical time for isoflavones to exert their estrogenic role to prevent excess abdominal fat accumulation and redistribution. As such, the outcome data in the current study was adjusted for their corresponding baseline value and age as covariates and menopause status as random factors when analyzing the data between groups. Although no significant difference was found on body weight reduction between groups, Control's body weight was lower than Intervention's by 1.27kg. Nonetheless, we found that compared to Control, soy supplementation decreased abdominal fat at 1.32kg which might directly cause significant decrease in waist circumference. These findings support that soy supplementation may have more favorable effects on preventing visceral adiposity in early, post-menopausal, obese women, and may limit adipose tissue accumulation or re-distribution during the menopause transition. Our findings coincided with Liu et al.'s study, in which 15g soy protein and 100mg isoflavone supplement for six-months was associated with mildly favorable effects on body composition and waist circumference in post-menopausal women with mild hyperglycemia [21]. It has also been reported that daily supplementation with 20g soy protein plus 160mg isoflavones mitigated increases in subcutaneous and total abdominal fat using CT-scan when compared to a casein placebo in post-menopausal women, although no difference in weight and body composition changes [17]. Our inability to detect statistically significant difference in abdominal fat between treatment groups may be limited by the small sample size and insufficient power.

The timing of soy supplementation on abdominal obesity management may be critical. It is worth noting that not all clinical

trials observed favorable effects of soy supplementation on abdominal obesity among postmenopausal women. For example, a randomized trial using a daily dose of 40g soy protein supplement for six months had no effect on total body fat nor lean mass in a small sample of postmenopausal women [22]. Liu et al noticed a more pronounced soy protein effect on abdominal obesity in menopausal women in early stages of menopause (<4 years of menopause) [21], indicating that early post-menopause may be a critical time for isoflavones to exert their estrogenic role to prevent excess abdominal fat accumulation and redistribution. Our findings support that soy supplementation may have more favorable effects on preventing visceral adiposity in early, post-menopausal, obese women, and may limit adipose tissue accumulation or re-distribution during the menopause transition.

The appropriateness of placebo should be an important consideration of any clinical trials. The current study using whey protein as a placebo to soy protein may have comprised the observation of study outcomes. The lack of significant difference in BMI between treatment groups in the current study might be related to appetite suppression and decreased food intake, higher levels of satiation, and thermogenic effects associated with a high-protein diet. A recent double-blind, randomized trial found that 23-week whey protein, but not soy protein supplementation resulted in significantly lower body weight and fat than isoenergetic amount of carbohydrate in free-living overweight and obese subjects [31]. In fact, whey has been shown to be the strongest appetize suppressant among all proteins [32,33]. As such, whey protein may not be an ideal placebo control in clinical trials evaluating soy's effects on body fat and related metabolic abnormalities in menopausal women.

There has been convincing evidence that a diet high in soy protein improved lipid profile [34]. A meta-analysis of 38 studies concluded that high dietary soy protein consumption resulted in decreased TC, LDL, and TG without significantly affecting HDL concentrations in men and women [34]. Subsequently, the FDA allowed a health claim on food labels to state that "25 grams of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease" [35]. It is important to note that this meta-analysis's study population included both men and women consuming a high soy protein diet with little animal based protein. The cholesterol lowering effect may be related to a healthier plant-based diet, as compared to the regular western diet higher in animal foods. In addition, some of these studies used commercial isolated soy protein products with most of the naturally occurring isoflavones being washed out by ethanol during the manufacturing process [36,37]. Thus, the nature of these studies is different from the current study in which isolated soy protein and isoflavone capsules were used to mimic whole soy supplementation specifically in post-menopausal women. Although not statistically significant, we observed that soy supplementation increased HDL while lowering LDL. This is consistent with those studies using soy supplementation containing isoflavones among menopausal females [38,39]. Although we did not modify subjects' diets in the current study, soy supplementation's effects on improved lipoprotein profile maybe the result of soy protein and isoflavones working synergistically to reduce abdominal fat, a key risk factor to metabolic abnormalities.

Our study has several limitations. First, the small sample size may have prevented finding statistically significant differences between groups. Second, the placebo in the current, i.e., whey protein, may not be a true placebo, as whey protein has been found to have favorable effects on weight management, lipid profile and inflammatory biomarkers [40]. As such, whey used as placebo control might have partially masked the favorable effects of soy supplementation on improving lipid profile in the current study. Isoenergetic amount of carbohydrate may be more suitable for study of this nature. Third, the DEXA abdominal fat measurement includes both subcutaneous and visceral fat in the abdominal cavity, preventing assessment of visceral adiposity alone. As such, future large scale randomized control trials are warranted to further investigate the effect of soy supplementation on attenuation of visceral fat accumulation using isoenergetic amount of carbohydrate as placebo and more sophisticated measurement such as MRI or CT-scan.

Conclusion

The current randomized, double-blind pilot study showed that compared to whey, soy supplementation appears effective in reducing abdominal fat and improving metabolic profiles in early post-menopausal women.

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