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Design and baseline characteristics of the Soy Phytoestrogens As Replacement Estrogen (SPARE) study — A clinical trial of the effects of soy isoflavones in menopausal women

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ABSTRACT

Following the results of the Women's Health Initiative, many women now decline estrogen replacement at the time of menopause and seek natural remedies that would treat menopausal symptoms and prevent bone loss and other long-term consequences of estrogen deficiency, but without adverse effects on the breast, uterus, and cardiovascular system. The results of most soy studies in this population have had limitations because of poor design, small sample size, or short duration. This report describes the study rationale, design, and procedures of the Soy Phytoestrogens As Replacement Estrogen (SPARE) study, which was designed to determine the efficacy of soy isoflavones in preventing spinal bone loss and menopausal symptoms in the initial years of menopause.

Women ages 45 to 60 without osteoporosis and within 5 years from menopause were randomized to receive soy isoflavones 200 mg daily or placebo for 2 years. Participants have yearly measurements of spine and hip bone density, urinary phytoestrogens, and serum lipids, thyroid stimulating hormone, and estradiol. Menopausal symptoms, mood changes, depression, and quality of life are assessed annually.

The SPARE study recruited 283 women, 66.1% were Hispanic white. With a large cohort, long duration, and large isoflavone dose, this trial will provide important, relevant, and currently unavailable information on the benefits of purified soy isoflavones in the prevention of bone loss and menopausal symptoms in the first 5 years of menopause. Given the high proportion of Hispanics participating in the study, the results of this trial will also be applicable to this minority group.

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1. Introduction

Estrogen deficiency plays a major role in menopausal bone loss, vaginal epithelium atrophy, hot flashes and mood changes; thus, until recently menopausal symptoms and the prevention of bone loss had been primarily managed with hormone therapy [1]. The findings of increased risk of breast cancer and cardiovascular complications by the Women's Health Initiative Study (WHI) resulted in the early termination of its estrogen/progestin arm [2]. In response to these well publicized results many menopausal women discontinued or did not start hormone therapy [3–5]. Consequently, there has been a vast increase in the use of herbal products containing phytoestrogens by women who believe that products containing "natural" estrogens would provide all of the benefits but none of the risks of prescription hormones [6]. Soy foods and tablets containing

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isoflavones derived from soy protein are particularly popular. This report describes the study design and baseline characteristics of the participants in the Soy Phytoestrogens as Replacement Estrogen (SPARE) study, a clinical trial designed to address the effectiveness of isoflavones extracted from soy protein in preventing bone loss and menopausal symptoms.

Clinical trials assessing the effectiveness of soy foods or isoflavones extracted from soy protein in the prevention of bone loss have had conflicting results [7,8]. Most have had serious limitations as they included a small number of participants [9–15], included pre- and postmenopausal women or women in a wide range of ages and years from menopause [11,15–20], had high drop-out rates [10,21], had a duration of 12 months or less [9,12–15,17–20,22,23], or used interventions, whether as foods or tablets containing isoflavones extracted from soy, that provided less than 100 mg of soy isoflavone daily [9–13,16,17,21–24]. Two recent clinical trials that used isoflavones extracted from soy protein in a daily dose of 120 mg and included a sufficient number of participants failed to demonstrate over 2 and 3 years, any significant effect in the prevention of bone loss in the usual fracture sites, i.e. lumbar spine and proximal femur [25,26]. Finally, none have tested these products in the US Hispanic population.

2. Study design and methods

2.1. Objectives and study design

The overall aim of the SPARE study was to establish the long-term effectiveness of 200 mg of isoflavones extracted from soy in preventing the complications and symptoms associated with declining estrogen concentrations in the first 5 years of menopause: spinal bone loss, hot flashes, and vaginal atrophy.

This single-center, double-blind, placebo-controlled, randomized clinical trial was conducted at the University of Miami Miller School of Medicine, Miami, Florida, USA. Menopausal women were recruited for a 2-year intervention that required 10 clinic visits. The SPARE study was approved by the University of Miami's Institutional Review Board.

2.2. Study population

The SPARE study recruited a multiethnic group of community-dwelling women aged 45 to 60 years who were eligible to participate if they met the following inclusion criteria: (1) did not consume soy products regularly, (2) had a bone mineral density (BMD) T-score in the lumbar spine or total hip <−2.0, and (3) had their last menstrual period at least 12 months before enrolling or at least 6 months before enrolling and a follicle stimulating hormone (FSH) >40 IU/l. Exclusions were designed to identify women who had osteoporosis or were receiving medications or had medical conditions that would influence rates of bone loss. The complete inclusion and exclusion criteria are listed in Table 1.

2.3. Recruitment strategies

The plan for participant recruitment was designed to result in the enrollment of a group of women representative of the local community. The effort initially relied on self-referral of University of Miami employees volunteering for the study, in addition to referrals from the medical center's physicians. Posters and brochures advertising the study were placed in the entrances of buildings, waiting areas, and bulletin boards. Because recruitment was slow, the original plan was revised and several other strategies were employed to extend recruitment activities to the community at large. Thereafter, many women were enrolled at different venues such as health fairs and community groups, but mostly from community-based mailings. Using a list provided by the Florida Department of Motor Vehicle, multiple mailings of bilingual postcards were sent to women ages 45–60 residing in South Florida. In addition, posters were placed in local commuting trains, community doctor's offices, and other public locations. The study employed bilingual staff who made special efforts to recruit minorities by attending health fairs and community events and by working with local newspapers and radio and television stations to produce stories about the study.

Table 1
Eligibility criteria for participation in the SPARE study.

Inclusion criteria
- Women 45 to 60 years old
- Absence of menses for more than 12 months but less than 5 years, or absence of menses for 6 to 12 months and FSH >40 IU/L

Exclusion criteria
- Unable to speak and/or read English or Spanish
- Unable to ambulate without assistance
- Treatment with estrogens (oral, dermal, or vaginal), progesterone (oral or topical), raloxifene, or tamoxifen during the previous 3 months
- Treatment with long-acting LHRH agonists in the previous 6 months
- Any treatment with bisphosphonates, calcitonin, fluoride for 4 weeks in the last 2 years or any treatment in the previous 3 months
- Systemic corticosteroids: a total of 84 mg of methylprednisolone or equivalent in the previous year
- Current treatment with anticonvulsants
- Use of soy/herbal supplements within the last 3 months, including DHEA
- Use of antibiotics in the preceding 6 months
- Use of prescription medication for hot flashes in the last 3 months
- Chemical menopause, including post chemotherapy
- Hyperthyroidism (TSH below the normal range), poorly controlled diabetes (glycohemoglobin >8.0% within the last 3 months), malabsorption syndromes, and chronic diseases including ulcerative colitis, Crohn's syndrome, and chronic liver disease
- BMI >32
- Low bone mineral density by DXA defined as T-score<−2.0 in lumbar spine or femoral neck
- Osteoporotic fractures (spine, wrist or hip with minimal trauma), osteomalacia, hyperparathyroidism, Paget's disease, osteogenesis imperfecta, significant scoliosis or spine abnormalities
- Abnormal mammogram
- History of cancer, except for basal/squamous cell skin cancer, in the last 10 years
- History or current psychosis
- Actively suicidal
- Current diagnosis of panic disorder or of post traumatic stress disorder (PTSD)
- Current substance abuse and/or dependence
- Current intake of 2 or more alcoholic drinks daily (1 drink: 12 oz. beer, 4 oz. wine, 1 oz. liquor)
- Unwillingness to avoid soy-containing foods and supplements in the following 2 years
- Plans not to reside the area in the next 2 years

2.4. Screening process

Potential participants followed a two-stage screening process. The first stage consisted of a brief telephone screen

designed to determine if the woman met the main eligibility criteria: age range, menopausal, and Body Mass Index (BMI) ≤ 32 kg/m^2. It also provided the opportunity to exclude women who included soy products in their daily diet and were not willing to discontinue them for the study duration. Candidates who successfully completed the telephone screening interview were scheduled for the first clinic visit.

The second phase of the screening process was conducted during a clinic visit. After signing an informed consent form, all study candidates went through the screening procedures described in Table 2. Women meeting all eligibility criteria were assigned to a 1-month, single-blind, placebo run-in period designed to identify and exclude those participants who would not comply with study requirements or be lost to follow-up early on.

2.5. Randomization

Those participants who completed the run-in period were assigned in equal proportions to the intervention or control group following the randomization sequence created by the study biostatistician. Participants were randomized in blocks of ten to ensure equal allocation to the control or treatment group.

2.6. Study medication rationale

Randomized subjects were assigned to receive either isoflavones extracted from soy protein, 200 mg daily, or a similarly-appearing placebo. Each daily dose was provided by four 50 mg tablets. Participants were instructed to take all tablets in the morning, 30 min before consuming food. The daily dose of 200 mg was selected to provide approximately twice the amount of soy isoflavones that are ingested from food sources in an Asian diet and thus ensure an effective dose [27].

The tablets were manufactured by Atrium, Inc. (Wautoma, WI) with isoflavones extracted from soy protein purchased from Archer Daniels Midland, Decatur, IL, which reported a total isoflavone content (aglycone equivalent) in each tablet of 52.9 mg, or 105% of the stated amount. Testing of the tablets at the FDA's National Center for Toxicological Research, Jefferson, AR using previously published methodology [28], showed that each tablet contained 48.5 ± 2.2 mg of total isoflavones, or 97% of the stated amount (daidzein 25.7 ± 1.1 mg and genistein 22.8 ± 1.2 mg). The placebo tablets were similarly analyzed and did not contain isoflavones. Based on their initial responses to the dietary assessment [29] and in order to provide sufficient calcium intake to all participants, subjects were provided tablets of combined calcium carbonate 500 mg plus vitamin D 200 IU. Participants received calcium 1000 mg plus vitamin D 400 IU per day if their daily calcium intake was less than 500 mg, or calcium 500 mg plus vitamin D 200 IU if their daily calcium intake was between 500 and 1000 mg.

Table 2
Overview of the SPARE study visits and measurements.

				Time from randomization in months						
	S[a]	−1[b]	0[c]	2	4	8	12	16	20	24
Informed consent	X									
Inclusion/exclusion criteria	X									
Bone mineral density	X		X				X			X
Body composition			X				X			X
Mammogram	X						X			X
Vital signs	X	X	X	X	X	X	X	X	X	X
Yale Physical Activity Survey			X				X			X
Dietary assessment	X						X			X
Vaginal cytology			X				X			X
Women's health questionnaire			X				X			X
SF-36			X				X			X
Profile mood of states			X				X			X
Beck Depression Inventory			X				X			X
Hot flash diary		X	X				X			X
CBC[d]	X						X			X
Serum chemistries	X						X			X
TSH[e]	X						X			X
Lipids			X		X		X			
NTx[f]			X				X			X
Urinary isoflavones	X						X			X
Adverse events		X	X	X	X	X	X	X	X	X
Compliance			X	X	X	X	X	X	X	X

[a] Screening visit.
[b] Enrollment and start of run-in phase of the study.
[c] Randomization.
[d] Complete blood cell count.
[e] Thyroid stimulating hormone.
[f] N-telopeptide of Type I bone collagen.

2.7. Compliance with study medication

Drug compliance was calculated for each subject at every visit using the following formula: (number of tablets dispensed − number of tablets returned/number of tablets expected to take) × 100. In addition, to validate compliance results obtained by pill-count, urinary isoflavone concentrations were determined at baseline and at each annual visit.

2.8. Outcomes, procedures and schedule of study visits

Each subject made 10 visits over a period of 2 years. All endpoints were measured at baseline, and after 12 and 24 months, with the exception of lipids that were assessed after a 12-hour fast at baseline and month 12. A complete physical exam was performed in all participants before randomization. Questionnaires were administered in either English or Spanish, depending upon the participants' preference [30]. An overview of the schedule of study visits and assessments is shown in Table 2.

Main outcomes included 2-year changes in lumbar spine BMD and in urinary NTx. BMD was measured by dual X-ray absorptiometry (DXA) using a Lunar Prodigy densitometer (Lunar, Madison, WI). Using the same equipment, a total body scan for analysis of body composition was performed in those participants who agreed. NTx was tested in a fasting second-morning urine sample by an enhanced chemiluminescence assay (Quest Labs, San Juan de Capistrano, CA) [31].

Secondary outcomes consisted of changes total hip and femoral neck BMD, vaginal maturation index and maturation value, thyroid stimulation hormone, thyroid peroxidase antibodies, serum lipids, menopausal symptoms, mood, depression, and quality of life. To determine the degree of estrogenization of the vaginal epithelium, vaginal cytology was performed from samples obtained by gently brushing vaginal side walls with a

Rover's Cervex-Brush™ and processed with the Surepath™ preparation system for gynecologic cytology. The vaginal maturation index (MI) is expressed as MI: % parabasal cells: % intermediate cells: % superficial cells [32]. The vaginal maturation value (MV) is calculated as MV: 0.2 (number of parabasal cells) + 0.6 (number of intermediate cells) + number of superficial cells [33]. Serum ultrasensitive estradiol and the total isoflavones, genistein, daidzein, and equol, were tested in a fasting spot urine sample at baseline and at each annual visit by using validated LC/MS/MS–isotope dilution methodology, as previously described [34]. All samples were stored frozen at −80 °C until tested at the end of the study. Calcium and vitamin D intake were estimated using the Block Dietary Data Systems [29], physical activity was evaluated using the Yale Physical Activity Survey (YPAS) [35], menopausal symptoms were assessed using the Women's Health Questionnaire (WHQ) [36], quality of life with the Short Form of 36 questions (SF-36) [37], depression with the Beck Depression Inventory (BDI) [38], and mood with the Profile Mood of States (POMS) [39]. Participants were also asked to complete a Hot Flashes Diary during the week preceding each study visit.

2.9. Data and participant safety

Safety measures included a serum chemistry panel, complete cell blood count, and mammogram at baseline and at each yearly visit. Study design, recruitment strategies, and participant and data safety were monitored semi-annually by a Data Safety and Monitoring Board (DSMB) established for this purpose by the funding agency, the National Institutes of Arthritis, Musculoskeletal and Skin Diseases (NIAMS). Twice a year, the DSMB reviewed study data concerning recruitment, treatment effects, and adverse events by group. Participants were notified of abnormal laboratory, BMD, and mammogram results of clinical significance.

Excessive bone loss was defined as >8% loss from baseline or the previous study, or reaching a T-score of –2.5 or below. Bone loss greater than 8% required the participant to be repositioned and rescanned. The average of the two scans was then used to recalculate the rate of change. Participants who experienced excessive bone loss or non-traumatic fractures were referred to their physician for medical management and asked to permanently discontinue study medication but continue with scheduled study visits.

2.10. Statistical analyses

The sample size/power calculation was based on testing the primary hypothesis that soy isoflavones tablets prevent bone loss among women in the first years of menopause. Bone loss will be measured by BMD of the lumbar spine. The trial will test the hypothesis that treatment with soy isoflavones tablets may maintain bone density values at 2 years (no additional bone loss) as compared to a placebo control. Based on the data provided by Lunar (Lunar, Madison, WI), the mean BMD in women aged 50 years without intervention is 1.149 g/cm^2 with SD deviation = 0.120 g/cm^2. It is well documented that women are expected to experience 4–7% bone loss in the first 2 years of menopause [40]. Thus, with a sample size of 130 in each group (two-tailed, $\alpha = 0.05$), the study has >80% power to detect a 4% or greater difference in BMD of the lumbar spine with the assumption that the mean BMD in the control group will lose 4–5% bone mass. Assuming a 15% attrition rate, the target total sample size is 306.

Univariate descriptive statistics, including boxplots and graphs, will be used to describe patterns of data to a) ensure that the scales have distributional properties that do not violate assumptions underlying statistical procedures, b) determine missing data, and c) detect outliers. For variables that are not approximately normally distributed, transformations (log and square root) will be tried first to test the normality; otherwise nonparametric techniques will be used for analysis of these variables. For outliers, original data will be checked to exclude any data entry errors. If needed, a description of the data will provide for the appropriate use for transformations of outliers.

To test the hypothesis that soy isoflavones decrease hot flash incidents in menopausal women, generalized linear models will be fitted to the data. Also random effect models will be used. These models which represent a general approach to the problem of modeling repeated measurements with fairly general error structures, can allow for missing observations, serial correlations, time-varying covariates, and irregular measurement occasions. The number of hot flashes will be treated as the dependent variable in the model. The treatment vs. placebo will be included in the model as an indicator variable. Demographic variables as well as pertinent clinical variables including (e.g., use of antidepressants and/or sleep aids) will be included in the model as covariates.

For vaginal cytology, chi-square tests will be used to compare the percentage of basal, intermediate, and superficial squamous cells or MI between treatment group and controls at a given point of time. For the description of the data, the association of covariates with MI at a given point of time will be assessed using logistic regression models.

A random effects model will be used to analyze the impact of the treatment on lipid concentrations including total cholesterol, total triglycerides, HDL-cholesterol, LDL, and VLDL. Potential confounding factors and effect modifiers discussed above will be adjusted for in the models.

Repeated measures analysis of variance will be performed to determine whether soy isoflavones tablets improve quality of life (measured by SF-36) and mental (measured by BDI) and emotional health (measured by POMS). The analysis is essentially the same as the analysis for BMD and NTx using a Group × Time analysis of variance after controlling for covariates. Post hoc analyses will be tested if Group × Time interaction is significant.

Because during menopause and during the trial many subjects may be prescribed antidepressants, lipid-lower agents, sleeping aids, or other medication, each of these will have an impact on quality of life, mental and/or emotional health, these variables will be treated as covariates and controlled for in the repeated measures ANOVA.

3. Results

3.1. Study recruitment

All data in this report represent the baseline information collected at the time of initial consent for the women who

were screened and for those who were later randomized to soy isoflavone or placebo tablets. The recruitment, enrollment, and randomization study scheme is presented in Fig. 1. Approximately 4200 women participated in the initial telephone screening process. The main reasons for disqualifying at this stage were frequent use of soy products, recent or current hormone therapy, BMI >32 kg/cm^2, and treatment with bisphosphonates. The 956 women who qualified were scheduled for a screening visit, of which 524 attended the visit and consented to participate. Among the 192 women who did not qualify for participation after the screening procedures, 88% had only one reason for not qualifying and 11% had two or more reasons. The main reasons for not qualifying were low BMD (26%), abnormal mammogram (22%), incomplete screening visit (8%), and a high BMI (7%). Of the 333 women who qualified for participation after the screening visit, 50 chose not to enroll in the study and all of the 282 who enrolled received placebo for 4 weeks. After the placebo run-in phase, 87% of study candidates had >80% compliance with study medication. Citing mostly either lack of interest or refusal to comply with study requirements, 34 women declined randomization. The remaining 248 participants were randomized to tablets containing isoflavones extracted from soy protein.

3.2. Demographic characteristics of the screened and randomized population

Table 3 summarizes baseline demographic characteristics, presented as frequency and mean distributions ± SD, for all the women who were screened, the women who did not qualify, those who qualified but declined randomization, and for those who were randomized.

Demographic baseline characteristics did not differ among the various subgroups. The age of the study candidates who were screened was 52.3 ± 3.4 years; 69.8% were white Hispanic, 19.8% white non-Hispanic, 8.6% black, and 1.7% Asian, reflecting the current make-up of the local population as reported by the US Census Bureau, which is 61.3% white Hispanic, 18.3% white non-Hispanic, and 20.0% black. Among the screened women, the mean number of years of education was 14.3 ± 3.4; 84% had at least a high school education and 37% a college degree or higher. The women who were randomized had comparable characteristics; their age was 52.5 ± 3.3, 66.1% were Hispanic, 22.6% white non-Hispanic, 9.7% black, and 1.6% Asian. Among those randomized, the mean number of years of education was 14.5 ± 3.3; 86 % had at least a high school education and 40% a college degree or higher.

3.3. Clinical characteristics of the screened and randomized population

Table 4 summarizes baseline clinical characteristics, presented as frequency and mean distributions ± SD, for all the women who were screened, the women who did not qualify, those who qualified but declined randomization, and for those who were randomized.

As expected, mean BMD was higher in the population that qualified for the study than in the screened population, which included several women with osteoporosis who were subsequently excluded from participation. None of the participants had osteoporosis (T-score ≤ −2.5), compared to 17.7% of those who did not qualify. Other clinical characteristics were similar among the women who were screened and those who qualified for the study, as shown in Table 4.

Despite all attempts to identify obese women during the initial telephone screen, 17% study candidates who attended a screening visit had BMI >30 kg/m^2. Mean BMI in all the women who were screened was 26.3 ± 3.5 kg/m^2; only 36% of the screened women had normal BMI (18.5 to 24.9 kg/m^2), 44% were overweight (BMI 25 to 29 kg/m^2), and 17% had BMI >30 kg/m^2. BMI in participants who were randomized was 26.3 ± 3.2 kg/m^2, 35% had normal BMI, 47% were overweight, and 17% had BMI >30 kg/m^2.

Blood pressure was normal in 37% of the screened candidates, 38% were pre-hypertensive (systolic blood pressure (SBP) ≥120 or <140, or diastolic blood pressure (DBP) ≥80 or <90 mm Hg), and 22% were hypertensive (SBP ≥ 140 or DBP ≥90 mm Hg). Among the randomized women, 40% had

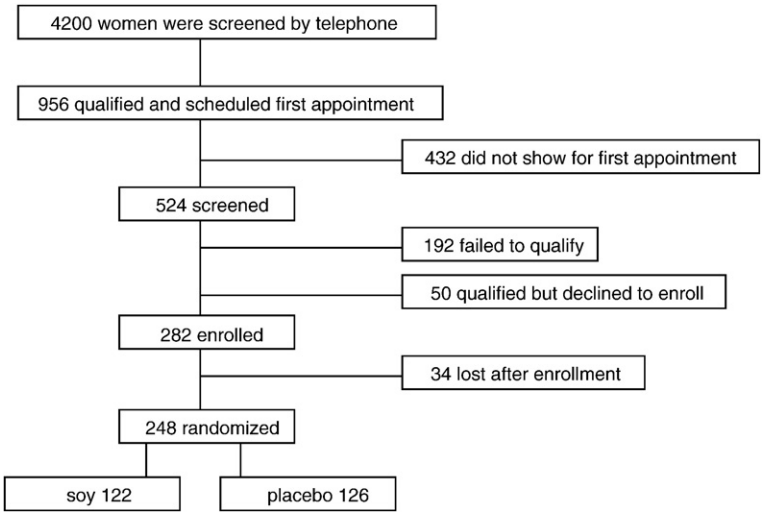

Fig. 1. Participant flow in the soy phytoestrogen as replacement estrogen (SPARE) study.

Table 3
Demographic characteristics of the women who were screened, those who failed to qualify, qualified but did not agree to be randomized, and qualified and were randomized to the SPARE Study.

Characteristic	Screened	Failed to qualify	Qualified but not randomized	Qualified and randomized
	($n=524$)	($n=192$)	($n=84$)	($n=248$)
Age (years), *n* (%)				
45–49	116 (22.1)	45 (23.4)	21 (25.0)	50 (20.2)
50–54	263 (50.2)	96 (50.0)	38 (45.2)	129 (52.0)
55–60	145 (27.7)	51 (26.6)	25 (29.8)	69 (27.8)
Age, years, mean ± SD	52.3 ± 3.4	52.1 ± 3.3	52.5 ± 3.3	52.5 ± 3.3
Race/ethnicity, *n* (%)				
White Hispanic	366 (69.8)	139 (72.4)	63 (75.0)	164 (66.1)
White non-Hispanic	104 (19.8)	33 (17.2)	15 (17.9)	56 (22.6)
Black	45 (8.6)	17 (8.9)	4 (4.8)	24 (9.7)
Asian	9 (1.7)	3 (1.6)	2 (2.4)	4 (1.6)
Education, *n* (%)				
Less than high school	84 (16.0)	33 (17.2)	16 (19.0)	35 (14.1)
High school	117 (22.4)	51 (26.6)	16 (19.0)	50 (20.2)
Part college	117 (22.3)	33 (17.2)	20 (23.9)	64 (25.8)
College	109 (20.8)	34 (17.7)	16 (19.0)	59 (23.8)
Post college	86 (16.4)	30 (15.6)	16 (19.0)	40 (16.0)
Unknown	11 (2.1)	11 (5.7)	0	0
Education, years, mean ± SD	14.3 ± 3.4	13.9 ± 3.6	14.3 ± 3.2	14.5 ± 3.3
Marital status, *n* (%)				
Married or significant other	297 (56.7)	97 (50.5)	47 (56.0)	153 (61.7)
Divorced/separated	156 (29.8)	62 (32.3)	29 (34.5)	65 (26.2)
Widowed	17 (3.2)	5 (2.6)	3 (3.6)	9 (3.6)
Never married	44 (8.4)	18 (9.4)	5 (6.0)	21 (8.5)
Unknown	10 (1.9)	10 (5.2)	0	0

Table 4
Clinical characteristics of the women who were screened, those who failed to qualify, qualified but did not agree to be randomized, and qualified and were randomized to the SPARE study.

Characteristic	Screened	Failed to qualify	Qualified but not randomized	Qualified and randomized
	($n=524$)	($n=192$)	($n=84$)	($n=248$)
BMI, *n* (%)				
Underweight (BMI <18.5)	2 (0.4)	2 (1.0)	0	0
Normal (BMI ≥18.5–24.9)	190 (36.3)	73 (38.0)	29 (34.5)	90 (35.8)
Overweight (BMI 25–29)	231 (44.1)	74 (38.5)	41 (48.8)	116 (47.2)
Obese (BMI ≥30)	90 (17.2)	34 (17.7)	14 (16.7)	42 (17.1)
Unknown	9 (1.7) (2.1)	9 (4.7)	0	0
BMI, kg/m^2, mean ± SD	26.3 ± 3.5	26.2 ± 4.0	26.3 ± 3.2	26.3 ± 3.2
Waist to hip ratio	0.835 ± 0.4	0.825 ± 0.1	0.816 ± 0.1	0.85 ± 0.5
BP (mm Hg), (mean ± SD) ($n=511$)				
Mean systolic BP	125.9 ± 18.3	127.8 ± 19.2	123.4 ± 17.5	125.3 ± 17.8
Mean diastolic BP	78.4 ± 9.4	78.7 ± 9.7	78.5 ± 9.6	78.1 ± 9.1
Blood pressure status, *n* (%)				
Normal blood pressure [a]	194 (37.0)	63 (32.8)	32 (38.1)	99 (39.9)
Pre-hypertensive [b]	199 (38.0)	76 (39.6)	38 (45.2)	85 (34.3)
Hypertension [c]	118 (22.5)	42 (21.9)	14 (16.7)	62 (25.0)
Unknown	13 (2.5)	11 (5.7)	0	2 (0.8)
BMD (T-score), mean ± SD ($n=491$)				
Lumbar spine	−0.7 ± 1.2	−1.2 ± 1.3	−0.6 ± 0.9	−0.3 ± 1.0
Total hip	−0.4 ± 0.9	−0.7 ± 1.2	−0.3 ± 0.9	−0.2 ± 0.9
Femoral neck	−0.9 ± 0.9	−1.1 ± 0.9	−0.9 ± 0.8	−0.7 ± 0.8
BMD category, *n* (%)				
Met inclusion criteria (T-score ≥−2.0)	430 (88.0)	102 (53.1)	84 (100)	248 (100)
Normal (T-score ≥−1.0)	219 (41.8)	51 (26.6)	37 (44.0)	134 (54.0)
Osteopenia (T-score <−1.0 to >−2.5)	237 (45.2)	76 (36.9)	47 (56.0)	114 (46.0)
Osteoporosis (T-score ≤ −2.5)	34 (6.5)	34 (17.7)	–	–
Unknown (BMD not done)	34 (6.5)	31 (16.1)	–	–
Number of menopausal symptoms [d]	6.3 ± 2.8	6.28 ± 2.9	6.51 ± 2.75*	6.20 ± 2.84

BMI: Body Mass Index; BP: blood pressure; BMD: bone mineral density.
[a] Normal blood pressure: systolic BP (SBP) <120 and diastolic BP (DBP) <80.
[b] Pre-hypertensive: SBP ≥120 to <140 *or* DBP ≥80 to <90.
[c] Hypertension: SBP ≥140 *or* DBP ≥90.
[d] Number of menopausal symptoms (hot flashes, insomnia, vaginal dryness, etc.) reported by participants.

normal blood pressure, 34% were pre-hypertensive, and 25% hypertensive.

In response to questions regarding menopausal symptoms, 81% of the screened participants reported hot flashes and almost 50% vaginal dryness. Regardless of the subgroup, women reported approximately 6 different menopausal symptoms at the time of screening. Among the women who were randomized, 46% had low but measurable urinary concentrations of soy isoflavone metabolites at baseline.

3.4. Participant bias

In order to ascertain the potential of participation bias, demographic and clinical characteristics of participants who qualified and were randomized were compared to those of women who qualified but chose not to continue into the treatment phase (Tables 3, 4). No significant differences between the two groups were detected for any of the demographic variables. However, participants who were randomized had significantly higher lumbar spine BMD than those who qualified but chose not to participate ($p=0.028$). Results of the screening tests were shared with all study participants, thus suggesting that although none had osteoporosis, a lower BMD might have prompted some of them to seek medical treatment.

3.5. Randomized group by race and ethnicity

Demographic, clinical and laboratory characteristics of randomized participants according to treatment group are summarized in Tables 6 and 7. Although we randomized a smaller percentage of blacks than expected, our study group adequately reflects the high proportion of Hispanics in the South Florida population. Because of the small number of Asian participants, this group was excluded from further statistical analyses. There were significant differences among the three main race/ethnic groups (Table 5). White non-Hispanics reported more years of education than did both white Hispanics ($p=0.001$) and blacks ($p=0.04$). White non-Hispanic participants had significantly lower mean BMI than black participants (25.6 ± 3.2 vs. 27.8 ± 2.9, $p=0.024$). In addition, black women had a significantly higher mean systolic blood pressure than both white Hispanic and white non-Hispanic women ($p=0.001$, $p=0.003$, respectively), and significantly higher bone density than either group ($p=0.006$ and $p=0.016$, respectively). White non-Hispanic participants reported significantly higher daily calcium intake at baseline than black participants ($1{,}080\pm734$ mg vs. 692 ± 451 mg, $p=0.016$).

4. Discussion

The main purpose of the SPARE study was to determine the effectiveness of soy isoflavones, 200 mg daily, in attenuating or preventing the rapid bone loss that usually occurs in the first years of menopause. A total of the 248 women were randomized into this trial, which includes a large proportion of white Hispanic women.

Although persons volunteering for a clinical trial represent a self-selected group, we believe the characteristics of our participants represent those of the general local population. Clinical characteristics found during the screening of this multiethnic group of recently menopausal women anticipate potential medical problems that might arise as they age. Remarkably, almost 18% of the women who failed the screening process did so because of osteoporosis; 61% of the women screened were either overweight or obese, and 25% were hypertensive. In addition, 47% of the women participating in the study had measurable isoflavone concentrations in urine at baseline. Although the concentrations were low, they reflect the ubiquitous presence of soy isoflavones in the food supply [41].

Table 5
Clinical characteristics of participants randomized in the SPARE study by race and ethnicity.

	Total	White Hispanic	White non-Hispanic	Black
	($n=244$) [a]	($n=164$)	($n=56$)	($n=24$)
Age, mean ± SD	52.5 ± 3.3	52.2 ± 3.2	53.2 ± 3.4	52.4 ± 3.4
Education, mean ± SD	14.5 ± 3.3	13.9+ ± 3.4 [b]	16.1 ± 2.2	14.6 ± 3.1
Menopausal symptoms, mean ± SD	6.2 ± 2.8	6.1 ± 2.9	6.5 ± 2.9	5.7 ± 2.8
BMI (kg/m^2), mean ± SD	26.3 ± 3.2	26.3 ± 3.2	25.7 ± 3.2 [c]	27.4 ± 2.9
Systolic BP (mm Hg), mean ± SD	125.3 ± 17.9	124.1 ± 17.4	123.8 ± 17.3	136.8 ± 18.9 [d]
Diastolic BP (mm Hg), mean ± SD	78.1 ± 9.2	77.8 ± 9.1	77.6 ± 8.9	81.5 ± 9.8
Normal blood pressure, *n* (%)	99 (40.9)	73 (44.8)	234 (41.8)	3 (12.5)
Pre–hypertension, *n* (%)	81 (33.5)	49 (30.1)	21 (38.2)	11 (45.8) [e]
Hypertension, *n* (%)	62 (25.6)	41 (25.2)	11 (20.0)	10 (41.7) [e]
BMD spine (T-score), mean ± SD	−0.346 ± 1.04	−0.461 ± 1.06	−0.305 ± 0.866	0.345 ± 1.07
BMD femoral neck (T-score), mean ± SD	−0.711 ± 0.80	−0.787 ± 0.83	−0.664 ± 0.667	−0.300 ± 0.78 [f]
BMD total hip (T-score), mean ± SD	−0.162 ± 0.85	−0.213 ± 0.87	−0.205 ± 0.760	0.292 ± 0.81 [f]
Normal BMD [g], *n* (%)	132 (54.1)	76 (46.3)	36 (64.3)	20 (83.3)

[a] Four Asian participants not included in this analysis.
[b] White Hispanics significantly have fewer years of education than White non-Hispanics ($p=0.00$).
[c] White non-Hispanic participants have significantly lower BMI than blacks ($p=0.024$).
[d] Black women have significantly higher systolic blood pressure (BP) than both white Hispanics and white non-Hispanic participants ($p=0.04$).
[e] A higher proportion of black women are both pre-hypertensive and hypertensive than white Hispanic ($p=0.001$) and white non-Hispanic women ($p=0.003$).
[f] Black women have significantly higher T-scores at all skeletal sites than both white Hispanic and white non-Hispanic participants ($p=0.006$ and $p=0.016$, respectively).
[g] Normal BMD according to WHO definition (T-score ≥ -1.0).

Table 6
Demographic characteristics of participants randomized in the SPARE study by treatment arm.

Characteristic	All	Soy	Placebo
	($n=248$)	($n=122$)	($n=126$)
Age (years), *n* (%)			
45–49	50 (20.2)	23 (18.9)	27 (21.4)
50–54	129 (52.0)	60 (49.2)	69 (54.8)
55–60	69 (27.8)	39 (32.0)	30 (23.8)
Age, years, mean ± SD	52.5 ± 3.3	52.9 ± 3.3	52.1 ± 3.3*
Race/ethnicity, *n* (%)			
White Hispanic	164 (66.1)	76 (62.3)	88 (69.8)
White non-Hispanic	56 (22.6)	33 (27.0)	23 (18.3)
African American	24 (9.7)	12 (9.8)	12 (9.5)
Asian	4 (1.6)	1 (0.8)	3 (2.4)
Education, *n* (%)			
Less than high school	35 (14.1)	12 (9.8)	23 (18.3)
High school	114 (46.0)	54 (44.3)	60 (47.6)
College	59 (23.8)	33 (27.1)	26 (20.6)
Post-graduate	40 (16.1)	23 (18.8)	17 (13.5)
Education, years, mean ± SD	14.5 ± 3.3	14.8 ± 3.1	14.3 ± 3.4
Marital status, *n* (%)			
Married or Significant other	153 (61.7)	72 (59.0)	81 (64.3)
Divorced/separated	65 (26.2)	33 (27.1)	32 (25.4)
Widowed	9 (3.6)	5 (4.1)	4 (3.2)
Never married	21 (8.5)	12 (9.8)	9 (7.1)
Unknown	0	0	0

*$p=0.03$.

Table 7
Clinical and laboratory characteristics of participants in the SPARE study by treatment arm.

Characteristic	All	Soy	Placebo
	($n=248$)	($n=122$)	($n=126$)
BMI, *n* (%)			
Underweight (BMI <18.5)	1 (0.4)	–	1 (0.8)
Normal (BMI ≥18.5–24.9)	89 (35.9)	46 (37.7)	43 (34.1)
Overweight (BMI 25–29)	116 (46.8)	60 (49.2)	56 (44.4)
Obese (BMI ≥30)	42 (16.9)	16 (13.1)	26 (20.6)
BMI (kg/m^2), mean ± SD	26.29 ± 3.2	25.99 ± 3.2	26.57 ± 3.2
Waist to hip ratio	0.848 ± 0.5	0.82 ± 0.1	0.82 ± 0.1
BP (mm Hg), mean ± SD			
Mean systolic BP	125.3 ± 17.8	121.4 ± 16.8	118.6 ± 14.9
Mean diastolic BP	78.1 ± 9.1	76.4 ± 9.5	75.5 ± 8.8
Blood pressure status, *n* (%)			
Normal blood pressure [a]	99 (39.9)	44 (36.1)	55 (43.7)
Pre-hypertensive [b]	85 (34.3)	44 (36.1)	41 (32.5)
Hypertension [c]	62 (25.0)	33 (27.0)	29 (23.0)
BMD (g/cm^2), mean ± SD			
Lumbar spine	1.135 ± 0.126	1.146 ± 0.125	1.132 ± 0.124
Total hip	0.984 ± 0.107	0.990 ± 0.110	0.982 ± 0.104
Femoral neck	0.936 ± 0.108	0.940 ± 0.119	0.937 ± 0.100
BMD category n (%)			
Normal (T-score ≥−1.0)	133 (53.8)	66 (54.1)	68 (54.0)
Osteopenia (T-score <−1.0 to >−2.0)	114 (46.2)	56 (45.9)	58 (46.0)
Menopausal symptoms, mean ± SD	12.58 ± 6.87	12.15 ± 6.61	12.95 ± 7.10
Vaginal maturation value, mean ± SD	41.7 ± 19.0	40. 7 ± 18.9	43.0 ± 19.3
Estradiol (pg/mL), mean ± SD	16.22 ± 25.79	13.85 ± 21.08	18.95 ± 30.22
Genistein, urine (pmol/μL), mean ± SD	0.43 ± 1.40	0.53 ± 1.84	0.32 ± 0.59
Daidzein, urine (pmol/μL), mean ± SD	0.94 ± 3.28	1.23 ± 4.42	0.62 ± 0.94
Equol, urine (pmol/μL), mean ± SD	0.19 ± 0.95	0.25 ± 1.27	0.12 ± 0.33
TSH (μIU/L), mean ± SD	2.35 ± 4.82	2.01 ± 1.3	2.74 ± 7.0
Positive TPO Ab (%) [d]	13.19	10.10	16.87
Total cholesterol (mg/dL), mean ± SD	214.0 ± 36.7	215.4 ± 38.4	212.5 ± 34.8
HDL cholesterol (mg/dL), mean ± SD	56.3 ± 14.6	56.9 ± 15.5	55.5 ± 13.7
LDL cholesterol (mg/dL), mean ± SD	132.4 ± 33.0	132.3 ± 35.9	132.5 ± 29.6
Triglycerides(mg/dL), mean ± SD	128.3 ± 76.5	133.5 ± 89.7	122.1 ± 57.4

BMI: Body Mass Index; BP: blood pressure; and BMD: bone mineral density.
[a] Normal blood pressure: systolic BP (SBP) <120 and diastolic BP (DBP) <80.
[b] Pre-hypertensive: SBP ≥120 to <140 *or* DBP ≥80 to <90.
[c] Hyypertension: SBP ≥140 *or* DBP ≥90.
[d] Positive TPO antibodies: >35 IU/mL.

It is estimated that about 40% of all women will suffer an osteoporotic fracture in their lifetime [42]. Women experience the most severe menopausal symptoms immediately following the last menses and more than 10% of their bone mass can be lost in the initial 5 years of menopause [40,43–45]. Thus, until recently estrogen therapy was the first-line treatment for prevention of menopausal symptoms and early postmenopausal bone loss. The beneficial skeletal effects of estrogens have been extensively documented in observational studies and in prospective studies assessing surrogate markers for fracture risk, such as BMD and markers of bone turnover. The WHI was the first large-scale prospective clinical trial to assess the effectiveness of estrogen, with and without progestin, in preventing fractures. Although the WHI did not address specifically early postmenopausal bone loss, the study showed a 24% decreased risk of fractures in menopausal women on estrogen therapy, with or without progestin [46]. After a mean use of hormone therapy for 5.2 years, the trial also demonstrated a 37% decreased risk of colon cancer, but a 26% increased risk of breast cancer and a 22% increase in cardiovascular events. Because of these concerns, many menopausal women stopped using estrogens and searched for "natural" alternatives that would provide skeletal benefits and relief from menopausal symptoms similarly to estrogens but none of the potential adverse effects [6].

Soy products have been actively sought by menopausal women because of their isoflavone content. In addition to protein, the soy bean contains isoflavones, natural phytoestrogens that activate estrogen receptors. The two major isoflavone glucosides in the soybean are genistin and daidzin. Once ingested, isoflavone glucosides are metabolized in the bowel by β-glucosidases of microbial and intestinal origin into the aglycones genistein and daidzein, respectively [47]. These compounds are absorbed as free isoflavones and are also metabolized by the intestinal flora into other metabolites, such as equol which is derived from daidzein and is the most potent soy isoflavone metabolite [48].

It is still not clear how isoflavones influence bone remodeling in humans and evidence suggests that it might be through different mechanisms than estradiol [49–51]. At the time of menopause, low estrogen concentrations result in increased osteoclastic activity and rapid bone loss. Estrogen therapy prevents bone loss by decreasing the function and life span and inducing apoptosis of the osteoclast. Studies suggest that

isoflavones act on both osteoclasts and osteoblasts [52,53]. Genistein appears to have an anabolic effect on bone, by acting directly on osteoblasts and some of these effects might not be through the ER [51]. The relative affinities of these compounds for the ER vary and are lower than for estradiol, i.e., genistein has greater affinity for ER-beta than ER-alpha and equol has 10- to 100-fold greater affinity to ERs than daidzein [49,50].

Epidemiological studies strongly suggest that phytoestrogens have a beneficial skeletal effect. Among Chinese women, those on diets with higher soy isoflavone content have higher BMD and lower markers of bone turnover [54]. A prospective population-based study of Chinese women reported an inverse relationship between the consumption of soy foods and fracture risk [55]. Postmenopausal Japanese women and Japanese–American premenopausal women also show a significant positive association between isoflavone intake and BMD [56,57]. The consumption of soy products among women in the US varies significantly and is generally very low [57].

Although animal studies demonstrate a clear skeletal benefit, prospective trials of soy isoflavones on bone in postmenopausal women, whether using soy foods or tablets containing isoflavones purified from soy protein, show conflicting results. Problems with most of these trials include their study design, small sample size, short duration (12 months or less), use of low doses, variation in isoflavone formulation, and high drop-out rates. In addition, some trials have enrolled women who had recent treatment with bone-active drugs and most included women in a wide age range or who were several years into menopause, not women in the initial postmenopausal years, the most likely users of soy products [10,20]. Two recently completed trials in mostly Caucasian women utilized a daily dose of 120 mg of soy isoflavones in tablet form and addressed some of these concerns, but neither showed any reduction in bone loss in common fracture sites. One was a 2-year intervention in 403 women, mean 6.7 years since menopause [25], and the other a 3-year intervention in 224 women, mean 2.8 years since menopause [26]. In contrast, the SPARE, study which also includes a large sample size, enrolled a multiethnic group of women in the first 5 years of menopause, has a 2-year duration, utilizes a dose of soy isoflavones equivalent to twice the amount found in a typical Asian diet, and measures isoflavone metabolites to serve as an objective indicator of compliance. The results of the SPARE study will provide a wide range of information that is particularly important to a growing number of women, a number that continues to rise as the "baby boom" generation reaches menopause. Strengths of this study include its multiethnic population which will allow us to evaluate differences and similarities in the response to soy isoflavones among several racial and ethnic groups, and that to ensure an effective dose, it utilized a daily dose of 200 mg, much larger than what has been used in previous studies. A limitation of the study is the relatively high BMI of the study population which could impact the rate of bone loss over the course of the trial [40].

5. Conclusions

Soy supplements are increasingly popular among women who expect these products to prevent bone loss and symptoms associated with menopause. Although animal and human studies suggest a protective role of soy isoflavones, these studies have had several limitations. The SPARE Study is a double-blind randomized trial that recruited 248 women for a 2-year intervention with 200 mg of soy isoflavones or placebo. Given its large sample size and long duration, this study will provide much needed information about the skeletal and other benefits of soy isoflavone supplementation in the first 5 years of menopause.

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